Thyoid Cancer

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Abstract: Cancer is the cells that grow out of control. Cancer cells can also invade other tissues. Growing out of control and invading other tissues are what makes a cell a cancer cell. Involved in more than 100 diseases, the cancer can cause serious illness and death.Normally, the cells become cancer cells because of DNA damage. This material is a literature collection of the researches on the thyoid cancer.
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Keywords: cancer; thyoid; biology; life; disease; research; literature

1. Introduction

Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries.

Abbosh, P. H., X. Li, et al. (2007). "A conditionally replicative, Wnt/beta-catenin pathway-based adenovirus therapy for anaplastic thyroid cancer." Cancer Gene Ther **14**(4): 399-408.

Thyroid cancer affects between 10,000 and 15,000 people per year in the US. Typically, this disease can be controlled with surgical resection and radioiodide treatment. However, resistance to these conventional therapies is observed in some patients, who develop intractable anaplastic thyroid cancer (ATC), for which no effective therapies exist. Recently, a sizable fraction of undifferentiated or poorly differentiated thyroid cancers were shown to contain mutations in beta-catenin, an oncogenic protein involved in the etiology of cancers of many tissues. We developed a conditionally replicative adenovirus (named 'HILMI') which, by virtue of TCF response elements drives E1A and E1B expression, replicates specifically in cells with an active Wnt/beta-catenin pathway. We show that several thyroid cancer cell lines, derived from undifferentiated or anaplastic tissues and possessing an active Wnt/beta-catenin pathway, are susceptible to cell killing by HILMI. Furthermore, viral replication in ATC cells as xenograft tumors in nude mice was observed, and prolonged survival of mice with ATC tumors was observed following administration of the HILMI therapeutic vector. The results warrant further development of this therapeutic approach for ATC patients.

Abdelhakim, A., A. Barlier, et al. (2009). "RET genetic screening in patients with medullary thyroid cancer: the Moroccan experience." J Cancer Res Ther **5**(3): 198-202.

BACKGROUND: Germline RET gene mutations are well known to be the genetic causes of multiple endocrine neoplasia type 2 (MEN2) and may be identified by genetic screening. AIM: The purpose of the present study was to screen nine MTC patients for RET sequence changes. MATERIALS AND METHODS: In this study, our sample was composed of 30 individuals: 9 index patients with medullary thyroid carcinoma (MTC) corresponding either to 3 subjects with clinical evidence of MEN2, 6 with apparently sporadic MTC (sMTC), and 21 relatives have been investigated for RET mutations. After DNA extraction from peripheral blood leukocytes, RET exons 8, 10, 11, 13-16 and exon/intron boundaries were analyzed by direct PCR sequencing. RESULTS: Three different known RET germline mutations in exon 11 (codon 634), p.Cys634Arg (c1900 T-->C) (de novo case), p.Cys634Phe (c1901 G-->T), p.Cys634Trp (c1902 C-->G), were detected in three individuals with MEN2 phenotype. Of the 21 relatives, 2 cases presented mutation. Among the six probands with sMTC, none was found to carry mutation. There was no difference between RET polymorphisms detected among both MEN2 and sMTC patients. CONCLUSIONS: These preliminary data suggest that the RET mutation spectra observed in Moroccan patients with MEN2 are similar to those previously reported in other countries.

Abu-Amero, K. K., A. S. Alzahrani, et al. (2005). "High frequency of somatic mitochondrial DNA mutations in human thyroid carcinomas and complex I respiratory defect in thyroid cancer cell lines." Oncogene **24**(8): 1455-60.

Significant progress has been made to elucidate the molecular mechanisms that determine thyroid tumor development and progression. However, most investigations have mainly focused on the genetic alterations of nuclear DNA. The potential role of mitochondrial DNA (mtDNA) mutations in thyroid tumorigenesis is not well defined. In the present study, we investigated the frequency of mtDNA mutations in 24 thyroid tumor specimens (19 primary papillary thyroid carcinomas (PTC), one follicular thyroid carcinoma, and four multinodular hyperplasias) and four thyroid cancer cell lines by sequencing the entire coding regions of mitochondrial genome. Among the 19 PTC samples tested, seven (36.8%) had somatic mutations. Somatic mtDNA mutations were also detected in one of four multinodular hyperplasias examined. All the thyroid tumor cell lines carried sequence variations that change amino acid and have not been reported previously as normal sequence variants. Flow cytometry analysis of mitochondria respiratory function in the thyroid tumor cell lines revealed a severe defect in mitochondrial complex I activity. The majority of the mutations was involved in genes located in the complex I of the mitochondrial genome. The mutations were either A --> G or C --> T transitions, often resulting in a change of a moderately or highly conserved amino acid of their corresponding protein. These data suggest that mtDNA mutations may play an important role in the thyroid tumorigenesis. Given that mtDNA mutation is present in the benign multinodular hyperplasia, it might be involved in the early stage of tumor development.

Abubaker, J., Z. Jehan, et al. (2008). "Clinicopathological analysis of papillary thyroid cancer with PIK3CA alterations in a Middle Eastern population." J Clin Endocrinol Metab **93**(2): 611-8.

CONTEXT: Genetic aberration in phosphatidylinositol 3-kinase (PI3K)/AKT pathway has been detected in numerous and diverse human cancers. PIK3CA, which encodes for the catalytic subunit of p110alpha of PI3K, is amplified in some cases of papillary thyroid cancer (PTC). Mutations in the PIK3CA have also been identified in thyroid cancers and, although relatively common in anaplastic thyroid carcinoma, are uncommon in PTC. OBJECTIVE: The objective of the study was to investigate genetic alterations like PIK3CA gene mutation, PIK3CA amplification, RAS, and RAF mutations and to further explore the relationship of these genetic alterations with various clinicopathological characteristics in Middle Eastern PTC. DESIGN: We used the fluorescence in situ hybridization technique for analysis of PIK3CA amplification from 536 PTC cases, and selected amplified samples were further validated by real-time quantitative PCR. Mutation analysis was done by direct DNA sequencing of PIK3CA, N2-RAS, and BRAF genes. RESULTS: PIK3CA amplification was seen in 265 of 499 PTC cases analyzed (53.1%); PIK3CA gene mutations in four of 207 PTC (1.9%); N2-RAS mutations in 16 of 265 PTC (6%); and BRAF mutations in 153 of 296 PTC (51.7%). N-RAS mutations were-associated with an early stage (P = 0.0465) and lower incidence of extrathyroidal extension (P = 0.027), whereas BRAF mutations were-associated with metastasis (P = 0.0274) and poor disease-free survival (P = 0.0121) in PTCs. CONCLUSION: A higher incidence of PIK3CA alterations and the possible synergistic effect of PIK3CA alterations and BRAF mutations suggest their major role in Middle Eastern PTC tumorigenesis and argue for therapeutic targeting of PI3K/AKT and MAPK pathways.

Aiello, A., G. Pandini, et al. (2006). "Peroxisomal proliferator-activated receptor-gamma agonists induce partial reversion of epithelial-mesenchymal transition in anaplastic thyroid cancer cells." Endocrinology **147**(9): 4463-75.

Anaplastic thyroid cancer (ATC) is an extremely aggressive tumor characterized by marked epithelial mesenchymal transition, which leads, almost invariably, to death. Peroxisomal proliferator-activated receptor (PPAR)-gamma agonists have recently emerged as potential antineoplastic drugs. To establish whether ATC could be a target of PPAR gamma agonists, we first examined PPAR gamma protein expression in a panel of six ATC cell lines and then studied the biologic effects of two PPAR gamma agonists, ciglitazone and rosiglitazone, that belong to the class of thiazolidonediones. PPAR gamma protein was present and functional in all ATC cell lines. Both ciglitazone and rosiglitazone showed complex biological effects in ATC cells, including inhibition of anchorage-dependent and -independent growth and migration, and increased apoptosis rate. Rosiglitazone-induced growth inhibition was associated with cell cycle arrest and changes in cell cycle regulators, such as an increase of cyclin-dependent kinases inhibitors p21(cip1) and p27(kip1), a decrease of cyclin D1, and inactivation of Rb protein. Rosiglitazone-induced apoptosis was associated with a decrease of Bcl-X(L) expression and caspase-3 and -7 activation. Moreover, rosiglitazone antagonized IGF-I biological effects by up-regulating phosphatase and tensin homolog deleted from chromosome 10 with subsequent inhibition of the phosphatidylinositol 3-kinase/Akt signaling pathway. Finally, rosiglitazone increased the expression of thyroid-specific differentiation markers. In

conclusions, these data suggest that PPAR gamma agonists induce a partial reversion of the epithelial mesenchymal transition in ATC cells by multiple mechanisms. PPAR gamma agonists may, therefore, have a role in the multimodal therapy currently used to slow down ATC growth and dissemination.

Ainahi, A., M. Kebbou, et al. (2006). "Study of the RET gene and his implication in thyroid cancer: Morocco case family." Indian J Cancer **43**(3): 122-6.

BACKGROUND: Multiple endocrine neoplasia type 2A (MEN 2A) is an autosomal dominant inherited cancer syndrome that affects multiple tissues derived from the neural crest. Inheritance of MTC is related to the presence of specific mutations in the RET proto-oncogene. Almost all mutations in MEN 2A involve one of the cysteines in the extracellular domain of the RET receptor. AIMS: The objective of the present study was the biochemical and molecular characterization of the first Moroccan clinically established MEN 2A patient and at-risk family members. SETTINGS AND DESIGN: This is a study on a family presented with MTC referred to our institute in 2004. MATERIALS AND METHODS: Peripheral blood leukocyte DNA samples were isolated and amplified by polymerase chain reaction followed by restriction enzyme analysis and DNA sequencing. RESULTS: We identified a heterozygous germ line missense mutation at codon 634 of exon 11 in the RET gene that causes a cysteine to arginine amino acid substitution in the DNA of the proband; this mutation was not found in the DNA of the parents or relatives. CONCLUSIONS: The detection of mutated MEN 2A gene carriers enables us to differentiate high-risk members from those who bear the wild-type gene. Occasionally, application of RET proto-oncogene testing may lead to the detection of unexpected de novo mutation that could be transmitted to children.

Akaishi, J., M. Onda, et al. (2006). "Down-regulation of transcription elogation factor A (SII) like 4 (TCEAL4) in anaplastic thyroid cancer." BMC Cancer **6**: 260.

BACKGROUND: Anaplastic thyroid cancer (ATC) is one of the most aggressive human malignancies and appears to arise mainly from transformation of pre-existing differentiated thyroid cancer (DTC). However, the carcinogenic mechanism of anaplastic transformation remains unclear. Previously, we investigated specific genes related to ATC based on gene expression profiling using cDNA microarray analysis. One of these genes, transcription elongation factor A (SII)-like 4 (TCEAL4), encodes a member of the transcription elongation factor A (SII)-like gene family. The detailed function of TCEAL4 has not been described nor has any association between this gene and human cancers been reported previously. METHODS: To investigate the role of TCEAL4 in ATC carcinogenesis, we examined expression levels of TCEAL4 in ACLs as well as in other types of thyroid cancers and normal human tissue. RESULTS: Expression of TCEAL4 was down-regulated in all 11 ACLs as compared to either normal thyroid tissues or papillary and follicular thyroid cancerous tissues. TCEAL4 was expressed ubiquitously in all normal human tissues tested. CONCLUSION: To our knowledge, this is the first report of altered TCEAL4 expression in human cancers. We suggest that loss of TCEAL4 expression might be associated with development of ATC from DTC. Further functional studies are required.

Akaishi, J., M. Onda, et al. (2007). "Down-regulation of an inhibitor of cell growth, transmembrane protein 34 (TMEM34), in anaplastic thyroid cancer." J Cancer Res Clin Oncol **133**(4): 213-8.

PURPOSE: Ansaplastic thyroid cancer (ATC) is one of the most lethal malignancies, but the carcinogenic mechanism of ATC has not been clarified. Recently, we performed a cDNA microarray analysis and identified transmembrane protein 34 (TMEM34) that down-regulated in anaplastic thyroid cancer cell lines (ACL)s as compared to normal thyroid tissues. METHODS: To investigate the role of TMEM34 in ATC carcinogenesis, we examined expression levels of TMEM34 in ACLs as well as differentiated thyroid cancers (DTC)s and normal human tissues. To explore the effect of TMEM34 in ATC development, cell-growth assays with KTA2 cells were performed. RESULTS: Expression of TMEM34 was down-regulated in all 11 ACLs, as compared to either normal thyroid tissues or cell lines derived from papillary or follicular thyroid cancers. TMEM34 was expressed ubiquitously in normal human tissues tested. Transfection of TMEM34 into KTA2 cells led to inhibition of cell growth. CONCLUSIONS: Our findings suggest that TMEM34 might be a tumor suppressor gene, associated with the development of ATC from DTC.

Akeno-Stuart, N., M. Croyle, et al. (2007). "The RET kinase inhibitor NVP-AST487 blocks growth and calcitonin gene expression through distinct mechanisms in medullary thyroid cancer cells." Cancer Res **67**(14): 6956-64.

The RET kinase has emerged as a promising target for the therapy of medullary thyroid cancers (MTC) and of a subset of papillary thyroid cancers. NVP-AST487, a N,N'-diphenyl urea with an IC(50) of 0.88 mumol/L on RET kinase, inhibited RET autophosphorylation and activation of downstream

effectors, and potently inhibited the growth of human thyroid cancer cell lines with activating mutations of RET but not of lines without RET mutations. NVP-AST487 induced a dose-dependent growth inhibition of xenografts of NIH3T3 cells expressing oncogenic RET, and of the MTC cell line TT in nude mice. MTCs secrete calcitonin, a useful indicator of tumor burden. Human plasma calcitonin levels derived from the TT cell xenografts were inhibited shortly after treatment, when tumor volume was still unchanged, indicating that the effects of RET kinase inhibition on calcitonin secretion were temporally dissociated from its tumor-inhibitory properties. Accordingly, NVP-AST487 inhibited calcitonin gene expression in vitro in TT cells, in part, through decreased gene transcription. These data point to a previously unknown physiologic role of RET signaling on calcitonin gene expression. Indeed, the RET ligands persephin and GDNF robustly stimulated calcitonin mRNA, which was blocked by pretreatment with NVP-AST487. Antagonists of RET kinase activity in patients with MTC may result in effects on plasma calcitonin that are either disproportionate or dissociated from the effects on tumor burden, because RET kinase mediates a physiologic pathway controlling calcitonin secretion. The role of traditional tumor biomarkers may need to be reassessed as targeted therapies designed against oncoproteins with key roles in pathogenesis are implemented.

Ambroziak, M., J. Pachucki, et al. (2005). "Disturbed expression of type 1 and type 2 iodothyronine deiodinase as well as titf1/nkx2-1 and pax-8 transcription factor genes in papillary thyroid cancer." Thyroid **15**(10): 1137-46.

Type 1 and type 2 iodothyronine 5' deiodinases (D1 and D2, respectively) catalyze the conversion of thyroxine (T(4)) to triiodothyronine (T(3)). Similar to other genes crucial for T(3) generation, D1 and D2 expression might be disturbed in papillary thyroid cancer (PTC) possible as a result of impairments in thyroid transcription factors Titf1/Nkx2-1 and Pax-8. The aim of the study was to investigate changes in the expression of D1 and D2 in PTC compared to changes in the expression of Titf1/Nkx2-1 and Pax-8. Although D1 and D2 activities were decreased in tumor samples (PTC) compared to control C samples (tissues from a nontumorous part of the gland), the differences were not statistically significant. Contrary to that, their mRNA levels were significantly decreased in PTC samples compared to C samples ($p = 0.017$ and $p = 0.012$, respectively). Interestingly there was clear discrepancy between enzymatic activity and mRNA level of both deiodinases. There was a statistically significant correlation between D1 and Pax-8 ($r = 0.464$, $p = 0.039$), D2 and Pax-8 ($r = 0.461$, $p = 0.041$), D2 and Titf1/Nkx2-1 mRNA levels ($r = 0.526$, $p = 0.017$). Our results show that changes in D1 and D2 expression in PTC, including the discrepancy between deiodinases activity and mRNA level, might possibly related to impaired Titf1/Nkx2-1 and Pax-8 action.

Aranda, A., O. Martinez-Iglesias, et al. (2009). "Thyroid receptor: roles in cancer." Trends Endocrinol Metab **20**(7): 318-24.

The thyroid hormone receptors, encoded by the TRalpha and TRbeta genes, are ligand-dependent transcription factors that belong to the nuclear receptor superfamily. In addition to the role of these receptors in growth, development and metabolism, there is increasing evidence that they also inhibit transformation and act as tumor suppressors. Aberrant TR action, as well as receptor silencing, are common events in human cancer, and TRs also have an important role in tumor progression in experimental animal models, suggesting that these receptors constitute a novel therapeutic target in cancer. This review highlights recent studies on mechanisms by which loss of expression and/or function of these receptors results in a selective advantage for cellular transformation, tumor development and metastatic growth.

Asai, N., M. Jijiwa, et al. (2006). "RET receptor signaling: dysfunction in thyroid cancer and Hirschsprung's disease." Pathol Int **56**(4): 164-72.

Gain-of-function mutations within the receptor tyrosine kinase gene RET cause inherited and non-inherited thyroid cancer. Somatic gene rearrangements of RET have been found in papillary thyroid carcinoma and germline point mutations in multiple endocrine neoplasia (MEN) types 2A and 2B and familial medullary thyroid carcinoma (FMTC). Conversely, loss-of-function mutations are responsible for the development of Hirschsprung's disease, a congenital malformation of the enteric nervous system. Comparison between normal RET signaling activated by the RET ligand glial cell line-derived neurotrophic factor (GDNF) and abnormal RET signaling caused by various mutations has led to a deeper understanding of disease mechanisms. The focus of the present review is on recent progress in the study of RET signaling dysfunction in human diseases.

Baida, A., M. Akdi, et al. (2008). "Strong association of chromosome 1p12 loci with thyroid cancer susceptibility." Cancer Epidemiol Biomarkers Prev **17**(6): 1499-504.

Several genes directly related to thyroid cancer development have been described; nevertheless, the genetic pathways of this tumorigenesis process are unknown. Together with environmental factors, susceptibility genes could have an important role in thyroid cancer. Our previous studies suggest that the chromosome 1p12-13 is related to thyroid cancer incidence. Here, we extend the analysis with a case-control association study in a Spanish population. Thus, six single-nucleotide polymorphisms were genotyped, covering 2.4 Mb of the 1p12-13 region. A statistically significant association between thyroid cancer incidence and the rs2145418 and rs4658973 polymorphisms was found ($P < 0.0001$). No association was detected for the other four polymorphisms studied. The rs2145418 marker showed a significant odds ratio of 5.0 [95% confidence interval (95% CI), 2.85-8.83] and 9.2 (95% CI, 4.50-21.6) for heterozygous and homozygous G-variant alleles, respectively. For rs4658973, the odds ratios were 0.40 (95% CI, 0.26-0.62) and 0.07 (95% CI, 0.03-0.18) for heterozygous and homozygous G-variant alleles, respectively. These markers map into the 1p12 region, and no linkage disequilibrium was found between them, indicating an independent relation of these polymorphisms with thyroid cancer susceptibility. Our data provide evidence of a strong association of the chromosome 1p12 with thyroid cancer risk, and it is the first study describing susceptibility loci for thyroid cancer in this region.

Baida, A., S. M. Farrington, et al. (2005). "Thyroid cancer susceptibility and THRA1 and BAT-40 repeats polymorphisms." Cancer Epidemiol Biomarkers Prev **14**(3): 638-42.

Although genetic and environmental factors have been identified in the etiology of thyroid cancer, the specific genetic implications in sporadic thyroid tumors are poorly understood but, as in other common cancers, low-penetrance susceptibility genes are believed to be crucial in the tumorigenesis processes. Here, we have carried out a case-control study to investigate whether there is an association between THRA1 CA repeat or BAT-40 A repeat polymorphisms and thyroid cancer risk. The THRA1 repeat resides in the thyroid hormone receptor-alpha1 gene, which is associated with thyroid cancer and whose expression depends on the THRA1 repeat size. We also analyzed the BAT-40 repeat that maps to chromosome 1, a region known to be involved in thyroid cancer. This repeat is located in the 3-beta-hydroxysteroid dehydrogenase gene that is associated with prostate cancer susceptibility. The THRA1 repeat was genotyped in 212 thyroid cancer patients and 141 controls of a Spanish population. From these individuals, 207 patients and 138 controls were also analyzed for the BAT-40 marker. No significant difference in the THRA1 allele distribution between patients and controls was found, although short alleles (<128 bp) might have some protective effect on thyroid cancer risk of carriers (odds ratio, 0.50; 95% confidence interval, 0.22-1.13; $P = 0.094$). By contrast, the BAT-40 allele distribution in patients was significantly different with respect to control ($P = 0.035$). Essentially, the difference were found in the genotypes involving the 111- to 115-bp allele range, which seem to be associated with a protective effect on thyroid cancer susceptibility in the studied population (odds ratio, 0.18; 95% confidence interval, 0.01-0.57; $P = 0.02$). Therefore, our results indicate that the BAT-40 containing region and to a less extend the thyroid hormone receptor-alpha1 gene are related to thyroid cancer susceptibility. To our knowledge, this is the first study reporting the identification of genetic factors for thyroid cancer susceptibility.

Bakhsh, A., G. Kirov, et al. (2006). "A new form of familial multi-nodular goitre with progression to differentiated thyroid cancer." Endocr Relat Cancer **13**(2): 475-83.

We report a kindred with euthyroid multi-nodular goitre (MNG) of adolescent onset. Two of the seven subjects with MNG have progressed to papillary thyroid cancer. One affected male had nodular kidney disease, and breast cancer occurred in one affected female. Genes that were candidates on the basis of the associated kidney (PAX8) and breast diseases (sodium iodide symporter (NIS)), were sequenced. No mutations were found in the coding region, intron/exon splice sites or in the promoter sequences (from -1248 relative to the translation initiation codon) of PAX8. Similar results were obtained for NIS. Subsequently, microsatellite analyses were performed on 14 informative family members. We used 2 to 3 markers per locus for 6 loci (on chromosomes 1,2,3,14,19,X) previously reported to predispose to MNG and/or familial non-medullary thyroid cancer (FNMTC). On the basis of non-significant logarithm of the odds ratio (LOD) scores or inheritance of different alleles in affected individuals, all loci have been excluded. Thyroidectomy specimens from three members of the kindred show multiple benign lesions, with papillary cancer in two. The morphological features do not resemble those seen in familial adenomatous polyposis, Cowden syndrome, or in multiple oxyphil lesions. From these findings and from the absence of any linkage to any of the known loci associated with MNG or FNMTC, we suggest that this represents a

new form of inherited MNG with a significant risk of progression to papillary carcinoma.

Ball, D. W., N. Jin, et al. (2007). "Selective growth inhibition in BRAF mutant thyroid cancer by the mitogen-activated protein kinase kinase 1/2 inhibitor AZD6244." J Clin Endocrinol Metab **92**(12): 4712-8.

CONTEXT: Activating mutations in the BRAF gene, primarily at V600E, are associated with poorer outcomes in patients with papillary thyroid cancer. MAPK kinase (MEK), immediately downstream of BRAF, is a promising target for ras-raf-MEK-ERK pathway inhibition. OBJECTIVE: The objective of the investigation was to study the efficacy of a MEK1/2 inhibitor in thyroid cancer preclinical models with defined BRAF mutation status. EXPERIMENTAL DESIGN: After treatment with the potent MEK 1/2 inhibitor AZD6244, MEK inhibition and cell growth were examined in four BRAF mutant (V600E) and two BRAF wild-type thyroid cancer cell lines and in xenografts from a BRAF mutant cell line. RESULTS: AZD6244 potently inhibited MEK 1/2 activity in thyroid cancer cell lines regardless of BRAF mutation status, as evidenced by reduced ERK phosphorylation. Four BRAF mutant lines exhibited growth inhibition at low doses of the drug, with GI50 concentrations ranging from 14 to 50 nm, predominantly via a G0/G1 arrest, comparable with findings in a sensitive BRAF mutant melanoma cell line. In contrast, two BRAF wild-type lines were significantly less sensitive, with GI50 values greater than 200 nm. Nude mouse xenograft tumors derived from the BRAF mutant line ARO exhibited dose-dependent growth inhibition by AZD6244, with effective treatment at 10 mg/kg by oral gavage. This effect was primarily cytostatic and associated with marked inhibition of ERK phosphorylation. CONCLUSION: AZD6244 inhibits the MEK-ERK pathway across a spectrum of thyroid cancer cells. MEK inhibition is cytostatic in papillary thyroid cancer and anaplastic thyroid cancer cells bearing a BRAF mutation and may have less impact on thyroid cancer cells lacking this mutation.

Balthasar, S., J. Samulin, et al. (2006). "Sphingosine 1-phosphate receptor expression profile and regulation of migration in human thyroid cancer cells." Biochem J **398**(3): 547-56.

S1P (sphingosine 1-phosphate) receptor expression and the effects of S1P on migration were studied in one papillary (NPA), two follicular (ML-1, WRO) and two anaplastic (FRO, ARO) thyroid cancer cell lines, as well as in human thyroid cells in primary culture. Additionally, the effects of S1P on proliferation, adhesion and calcium signalling were addressed in ML-1 and FRO cells. All cell types expressed multiple S1P receptors. S1P evoked intracellular calcium signalling in primary cultures, ML-1 cells and FRO cells. Neither proliferation nor migration was affected in primary cultures, whereas S1P partly inhibited proliferation in ML-1 and FRO cells. Low nanomolar concentrations of S1P inhibited migration in FRO, WRO and ARO cells, but stimulated ML-1 cell migration. Consistently, S1P1 and S1P3, which mediate migratory responses, were strongly expressed in ML-1 cells, and S1P2, which inhibits migration, was the dominating receptor in the other cell lines. The migratory effect in ML-1 cells was mediated by G(i) and phosphatidylinositol 3-kinase. Both S1P and the S1P1-specific agonist SEW-2871 induced Akt phosphorylation at Ser473. However, SEW-2871 failed to stimulate migration, whereas the S1P1/S1P3 antagonist VPC 23019 inhibited S1P-induced migration. The results suggest that aberrant S1P receptor expression may enhance thyroid cancer cell migration and thus contribute to the metastatic behaviour of some thyroid tumours.

Barbosa, G. F. and M. Milas (2008). "Peripheral thyrotropin receptor mRNA as a novel marker for differentiated thyroid cancer diagnosis and surveillance." Expert Rev Anticancer Ther **8**(9): 1415-24.

Thyroid cancer is the most prevalent endocrine cancer whose incidence rates, particularly among women, have increased over the last decade. Although survival outcomes following surgery (with or without radioactive iodine ablation treatment) remain favorable, a significant proportion of patients are at lifetime risk of locoregional lymph node recurrence and distant metastasis. Serum thyroglobulin (Tg) has been the only circulating marker in routine use for detecting thyroid cancer recurrence, but it lacks sensitivity and is unreliable when Tg antibodies are present. New molecular markers for thyroid cancer have been investigated, with most based on detection in thyroid nodule or tumor tissue specimens. Recently, it has become possible to detect thyroid cancer cells in peripheral blood by measuring the mRNA of thyroid-specific genes, such as the mRNA of Tg and thyrotropin receptor. These have become promising new circulating markers for thyroid cancer. This review highlights the progress in this field from the perspective of improved initial cancer diagnosis and enhanced ability to monitor thyroid cancer recurrence.

Bastos, H. N., M. R. Antao, et al. (2009). "Association of polymorphisms in genes of the homologous recombination DNA repair pathway and thyroid cancer risk." Thyroid **19**(10): 1067-75.

BACKGROUND: Ionizing radiation exposure has been pointed out as a risk factor for thyroid cancer. The double-strand breaks induced by this carcinogen are usually repaired by homologous recombination repair pathway, a pathway that includes several polymorphic genes. Since there is a scarcity of data about the involvement of these gene polymorphisms in thyroid cancer susceptibility, we carried out a case-control study in a Caucasian Portuguese population. METHODS: We genotyped 109 patients and 217 controls for the XRCC3 T241M, XRCC2 R188H, NBS1 E185Q, and RAD51 Ex1-59G>T polymorphisms to evaluate their potential main effects on risk for this pathology. RESULTS: The results obtained showed that for the RAD51 Ex1-59G>T polymorphism, the homozigosity for the variant allele was associated with an almost significant increase of the odds ratio (OR) (adjusted OR = 1.9; confidence interval 95%: 1.0-3.5; p = 0.057). Additionaly, when the XRCC3 T241M data were analyzed concerning the presence of at least one wild-type allele, we observed that individuals homozygous for the variant allele had a higher risk for thyroid cancer (adjusted OR = 2.0; confidence interval 95%: 1.1-3.6; p = 0.026). When the data were analyzed according to the number of RAD51 Ex1-59G>T and XRCC3 T241M variant alleles, the coexistence of three or more variant alleles in either gene was associated to a significant higher risk (three variant alleles: adjusted OR = 2.9, p = 0.036; four variant alleles: adjusted OR = 8.0, p = 0.006). CONCLUSIONS: Since XRCC3 is involved in the assembly and stabilization of RAD51 protein multimers at double-strand break sites, we cannot exclude that the interaction of both polymorphisms can lead to a decreased DNA repair capacity and consequently increased risk for thyroid cancer.

Bauer, J., J. Weng, et al. (2009). "Germline variation of the melanocortin-1 receptor does not explain shared risk for melanoma and thyroid cancer." Exp Dermatol **18**(6): 548-52.

BACKGROUND: Recently, germline variants of the melanocortin-1 receptor (MC1R) have been shown to be associated with an increased risk for BRAF mutant but not BRAF wild-type cutaneous melanoma. Similar to melanoma, BRAF mutations are also commonly found in papillary thyroid carcinomas. Furthermore, patients with melanoma have an increased risk for thyroid carcinoma and vice versa. METHODS: To determine whether MC1R variation also represents a risk factor for BRAF mutant thyroid carcinomas, we sequenced BRAF and MC1R in two separate case-control cohorts. RESULTS: We demonstrate that MC1R is expressed in normal and neoplastic thyroid epithelial cells, albeit at lower levels than in melanocytes. In the first cohort of 66 follicular and 62 papillary thyroid carcinomas (PTC), and 128 matched controls from the San Francisco Bay Area we found no association between the number of MC1R variant alleles and thyroid cancer. Patients with BRAF-mutated tumors had a higher frequency of MC1R variant alleles than their matched controls (P = 0.039). However, contrary to the findings in melanoma, the odds ratio for having a BRAF mutant cancer decreased from 3.9 for carriers of one MC1R allele to 1.5 for carriers of two or more alleles. As the frequency of MC1R alleles varies highly among different ethnic populations, we analysed a second, ethnically more homogeneous cohort from Spain and Portugal, and found no association with PTC nor with BRAF-mutated PTC. CONCLUSION: Our data indicate that the strong association between BRAF mutations and MC1R variants previously found in melanoma does not extend to thyroid cancer.

Bergant, D., M. Hocevar, et al. (2006). "Hereditary medullary thyroid cancer in Slovenia--genotype-phenotype correlations." Wien Klin Wochenschr **118**(13-14): 411-6.

BACKGROUND: Medullary thyroid cancer (MTC) is a rare endocrine tumor that may be sporadic or inherited in settings of MEN2A, MEN2B and FMTC. Germline point mutations in the RET proto-oncogene are responsible for tumor occurrence, inheritance and great clinical variability. The aim of this study was to correlate the genotype and phenotype of patients with hereditary MTC (age at diagnosis, sex, TNM classification and clinical features). PATIENTS: Between 1997 and 2003 genetic testing was performed in 69 out of 98 patients with "sporadic" MTC. Carriage of mutation was found in 14 (20.2%) patients (index patients) and in 16 out of 31 (51.6%) of their relatives. One patient with MEN2B and codon 918 mutation was excluded from further analysis. METHODS: Genomic DNA was isolated from peripheral blood leukocytes. Exons 10, 11, 13, 14, 15 and 16 of the RET proto-oncogene were amplified in polymerase chain reactions. Point mutations of the RET gene were detected with single-strand conformation analysis and DNA sequencing. Detected mutations were confirmed with restriction enzyme analysis. RESULTS: Codon 634 mutations were detected in 15 patients (50%; aged 18-76 years; 6 families), codon 618 in nine patients (30%; aged 12-65 years; 4 families) and codon 790 in five patients (16.6%; aged 16-74 years; 3 families). The median age at diagnosis was 31 +/- 17.3, 33 +/- 15.9 and 36 +/- 23.8 years for patients with codon 618, 634 and 790 mutations. Selected by sex, females with codon mutations 618 and 634 versus 790 had median age at diagnosis of 34.5 +/- 15.6 years and 43.5 +/- 22.9

years, whereas the inverse result was observed in males (26.5 +/- 18.0 versus 16 years). The male/female ratio was 1:2 for patients with codon 618 and 634 mutations and 1:4 for patients with codon 790 mutations. Some of the data suggested correlation between specific genotypes, tumor size, stage of MTC and age at diagnosis. Pheochromocytoma (12 out of 15 patients) and primary hyperparathyroidism (6 out of 15 patients) were diagnosed solely in patients with codon 634 mutations. One patient with FMTC and Hirschprung disease was found in a family with codon 618 mutations. CONCLUSION: Correlation between tumor size, stage of MTC at diagnosis in view of patient's age, and specific genotype were indicated in our limited series and were more evident in female patients with codon 790 mutations. Later onset and a probably less aggressive course of MTC in these patients than in patients with other mutations should be considered in planning prophylactic thyroid surgery. MEN2A syndrome was related solely to codon 634 mutations.

Bufalo, N. E., J. L. Leite, et al. (2006). "Smoking and susceptibility to thyroid cancer: an inverse association with CYP1A1 allelic variants." Endocr Relat Cancer **13**(4): 1185-93.

In contrast to most human malignancies, epidemiologic studies have frequently reported a reduced risk of differentiated thyroid cancer in tobacco consumers. Cytochrome P4501A1 (CYP1A1) gene variants may be related to an increased capacity to activate polycyclic aromatic hydrocarbons, producing highly reactive electrophilic intermediates that might damage DNA. Hence, the germline inheritance of a wild-type CYP1A1 gene may decrease the susceptibility for thyroid cancer. The present study was designed to investigate CYP1A1 (m1 and m2) role in thyroid tumorigenesis and its connection with GSTM1, GSTT1, GSTP1, GSTO1, and codon 72 of p53 genotypes. A total of 248 patients with thyroid nodules, including 67 benign goiters, 13 follicular adenomas, 136 papillary carcinomas, and 32 follicular carcinomas, and 277 controls with similar ethnic backgrounds were interviewed on their lifetime dietary and occupational histories, smoking habit, previous diseases, and other anamnestic data. DNA was extracted from a blood sample and submitted to PCR-restriction fragment length polymorphism assays. The wild-type CYP1A1m1 genotype was more frequent among papillary carcinoma patients (74.26%) than in the control population (62.45%; P=0.0147), reducing the risk for this type of cancer (odds ratio=0.564; 95% confidence interval=0.357-0.894). A multiple logistic regression analysis showed an inverse correlation between cigarette smoking (P=0.0385) and CYP1A1 germline inheritance (P=0.0237) with the susceptibility to papillary carcinomas. We were not able to find any correlation between smoking, clinical features, parameters of aggressiveness at diagnosis or during follow-up, and any of the GST or CYP genotypes considered separately or in different combinations. We suggest that CYP1A1 genotype might be associated with the reported reduced risk to papillary carcinomas among smokers.

Capezzone, M., S. Cantara, et al. (2008). "Short telomeres, telomerase reverse transcriptase gene amplification, and increased telomerase activity in the blood of familial papillary thyroid cancer patients." J Clin Endocrinol Metab **93**(10): 3950-7.

BACKGROUND: Differentiated papillary thyroid cancer is mostly sporadic, but the recurrence of the familial form has been reported. Short or dysfunctional telomeres have been associated with familial benign diseases and familial breast cancer. OBJECTIVE: The aim of our work was to study the telomere-telomerase complex in the peripheral blood of patients with familial papillary thyroid cancer (FPTC), including the measurement of relative telomere length (RTL), telomerase reverse transcriptase (hTERT) gene amplification, hTERT mRNA expression, telomerase protein activity, and search of hTERT or telomerase RNA component gene mutations. PATIENTS: Cumulating a series of patients seen at the University of Siena and a series at the University of Rome, the experiments were conducted in 47 FPTC patients, 75 sporadic papillary thyroid cancer (PTC) patients, 20 patients with nodular goiter, 19 healthy subjects, and 20 unaffected siblings of FPTC patients. RESULTS: RTL, measured by quantitative PCR, was significantly ($P < 0.0001$) shorter in the blood of FPTC patients, compared with sporadic PTCs, healthy subjects, nodular goiter subjects, and unaffected siblings. Also by fluorescence in situ hybridization analysis, the results confirmed shorter telomere lengths in FPTC patients ($P = 0.01$). hTERT gene amplification was significantly ($P < 0.0001$) higher in FPTC patients, compared with the other groups, and in particular, it was significantly ($P = 0.03$) greater in offspring with respect to parents. hTERT mRNA expression, as well as telomerase activity, was significantly higher ($P = 0.0003$ and $P < 0.0001$, respectively) in FPTC patients, compared with sporadic PTCs. RTL, measured in cancer tissues, was shorter ($P < 0.0001$) in FPTC patients, compared with sporadic PTCs. No mutations of the telomerase RNA component and hTERT genes were found. CONCLUSION: Our study demonstrates that patients with FPTC display an imbalance of the telomere-telomerase complex in the peripheral blood, characterized by short telomeres,

hTERT gene amplification, and expression. These features may be implicated in the inherited predisposition to develop FPTC.

Carvalho, D. P. and A. C. Ferreira (2007). "The importance of sodium/iodide symporter (NIS) for thyroid cancer management." Arq Bras Endocrinol Metabol **51**(5): 672-82.

The thyroid gland has the ability to uptake and concentrate iodide, which is a fundamental step in thyroid hormone biosynthesis. Radioiodine has been used as a diagnostic and therapeutic tool for several years. However, the studies related to the mechanisms of iodide transport were only possible after the cloning of the gene that encodes the sodium/iodide symporter (NIS). The studies about the regulation of NIS expression and the possibility of gene therapy with the aim of transferring NIS gene to cells that normally do not express the symporter have also become possible. In the majority of hypofunctioning thyroid nodules, both benign and malignant, NIS gene expression is maintained, but NIS protein is retained in the intracellular compartment. The expression of NIS in non-thyroid tumoral cells in vivo has been possible through the transfer of NIS gene under the control of tissue-specific promoters. Apart from its therapeutic use, NIS has also been used for the localization of metastases by scintigraphy or PET-scan with 124I. In conclusion, NIS gene cloning led to an important development in the field of thyroid pathophysiology, and has also been fundamental to extend the use of radioiodine for the management of non-thyroid tumors.

Catalano, M. G., N. Fortunati, et al. (2005). "Valproic acid induces apoptosis and cell cycle arrest in poorly differentiated thyroid cancer cells." J Clin Endocrinol Metab **90**(3): 1383-9.

Poorly differentiated thyroid carcinoma is an aggressive human cancer that is resistant to conventional therapy. Histone deacetylase inhibitors are a promising class of drugs, acting as antiproliferative agents by promoting differentiation, as well as inducing apoptosis and cell cycle arrest. Valproic acid (VPA), a class I selective histone deacetylase inhibitor widely used as an anticonvulsant, promotes differentiation in poorly differentiated thyroid cancer cells by inducing Na(+)/I(-) symporter and increasing iodine uptake. Here, we show that it is also highly effective at suppressing growth in poorly differentiated thyroid cancer cell lines (N-PA and BHT-101). Apoptosis induction and cell cycle arrest are the underlying mechanisms of VPA's effect on cell growth. It induces apoptosis by activating the intrinsic pathway; caspases 3 and 9 are activated but not caspase 8. Cell cycle is selectively arrested in G(1) and is associated with the increased expression of p21 and the reduced expression of cyclin A. Both apoptosis and cell cycle arrest are induced by treatment with 1 mm VPA, a dose that promotes cell redifferentiation and that is slightly above the serum concentration reached in patients treated for epilepsy. These multifaceted properties make VPA of clinical interest as a new approach to treating poorly differentiated thyroid cancer.

Cengic, N., C. H. Baker, et al. (2005). "A novel therapeutic strategy for medullary thyroid cancer based on radioiodine therapy following tissue-specific sodium iodide symporter gene expression." J Clin Endocrinol Metab **90**(8): 4457-64.

CONTEXT: In contrast to papillary and follicular thyroid cancer, medullary thyroid cancer (MTC) remains difficult to treat due to its unresponsiveness to radioiodine therapy and its limited responsiveness to chemo- and radiotherapy. OBJECTIVE: To investigate an alternative therapeutic approach, we examined the feasibility of radioiodine therapy of MTC after human sodium iodide symporter (hNIS) gene transfer using the calcitonin promoter to target hNIS gene expression to MTC cells (TT). DESIGN: TT cells were stably transfected with an expression vector, in which hNIS cDNA was coupled to the calcitonin promoter. Functional hNIS expression was confirmed by iodide accumulation assays, Northern and Western blot analysis, immunostaining, and in vitro clonogenic assay. RESULTS: hNIS-transfected TT cells showed perchlorate-sensitive iodide uptake, accumulating 125-I about 12-fold in vitro with organification of 4% of accumulated iodide resulting in a significant decrease in iodide efflux. NIS protein expression was confirmed by Western blot analysis using a monoclonal hNIS-specific antibody, which revealed a major band of a molecular mass of 80-90 kDa. In addition, immunostaining of hNIS-transfected TT cells revealed hNIS-specific immunoreactivity, which was primarily membrane associated. In an in vitro clonogenic assay, 84% of NIS-transfected TT cells were killed by exposure to 131-I, whereas only about 0.6% of control cells were killed. CONCLUSIONS: A therapeutic effect of 131-I has been demonstrated in MTC cells after induction of tissue-specific iodide uptake activity by calcitonin promoter-directed hNIS expression. This study demonstrates the potential of NIS as a therapeutic gene, allowing radioiodine therapy of MTC after tissue-specific NIS gene transfer.

Chakravarty, G., A. A. Santillan, et al. (2009). "Phosphorylated insulin like growth factor-I receptor

expression and its clinico-pathological significance in histologic subtypes of human thyroid cancer." Exp Biol Med (Maywood) **234**(4): 372-86.

Overexpression of insulin-like growth factor-I receptor (IGF-IR) is seen in a multitude of human thyroid cancers and correlates with poor prognosis. However, recent studies suggest that low phospho-IGF-IR (pIGF-IR) expression rather than its overexpression may be an indicator of poorly differentiated disease. No previous study has evaluated the expression of pIGF-IR to determine if activation or loss of expression of this receptor is associated with thyroid tumor progression. Accordingly, a quantitative immunohistochemical (IHC) method was used to evaluate the clinico-pathological significance of pIGF-IR expression in archival samples of human thyroid carcinomas. Quantitative analysis of pIGF-IR levels revealed a significant difference in the median index of pIGF-IR between different histological subtypes of thyroid cancer ($P < 0.001$). Specifically, the median pIGF-IR index of differentiated thyroid cancers was significantly higher than the median index of other poorly differentiated thyroid cancer ($P < 0.001$). This was further confirmed in individual tumor sections of thyroid carcinoma where anaplastic and differentiated components co-existed. No significant difference was noted in the pIGF-IR index of tumors grouped by size or stage but a trend towards lower mean pIGF-IR index was noted in older patients. Our data indicates that pIGF-IR is upregulated in a majority of follicular thyroid carcinomas, suggesting it may be a potential target for therapy for patients with this disease. In addition, since low pIGF-IR expression was found to correlate with aggressive human thyroid carcinoma, it also suggests that IGF-IR may not be needed for progression of anaplastic thyroid carcinoma possibly because other cell signaling pathways are activated, obviating the need for IGF-IR signaling. However, more mechanistic studies would be necessary to substantiate the possibility that pIGF-IR may be important for differentiation of thyroid tissues and is lost with disease progression.

Chan, I. H. and M. L. Privalsky (2006). "Thyroid hormone receptors mutated in liver cancer function as distorted antimorphs." Oncogene **25**(25): 3576-88.

Aberrant thyroid hormone receptors (TRs) are found in over 70% of the human hepatocellular carcinomas (HCCs) analysed. To better understand the role(s) of these TR mutants in this neoplasia, we analysed a panel of HCC mutant receptors for their molecular properties. Virtually all HCC-associated TR mutants tested retained the ability to repress target genes in the absence of T3, yet were impaired in T3-driven gene activation and functioned as dominant-negative inhibitors of wild-type TR activity. Intriguingly, the HCC TRalpha1 mutants exerted dominant-negative interference at all T3 concentrations tested, whereas the HCC TRbeta1 mutants were dominant-negatives only at low and intermediate T3 concentrations, reverting to transcriptional activators at higher hormone levels. The relative affinity for the SMRT versus N-CoR corepressors was detectably altered for several of the HCC mutant TRs, suggesting changes in corepressor preference and recruitment compared to wild type. Several of the TRalpha HCC mutations also altered the DNA recognition properties of the encoded receptors, indicating that these HCC TR mutants may regulate a distinct set of target genes from those regulated by wild-type TRs. Finally, whereas wild-type TRs interfere with c-Jun/AP-1 function in a T3-dependent fashion and suppress anchorage-independent growth when ectopically expressed in HepG2 cells, at least certain of the HCC mutants did not exert these inhibitory properties. These alterations in transcriptional regulation and DNA recognition appear likely to contribute to oncogenesis by reprogramming the differentiation and proliferative properties of the hepatocytes in which the mutant TRs are expressed.

Cheng, S., W. Liu, et al. (2009). "Expression of the melanoma-associated antigen is associated with progression of human thyroid cancer." Endocr Relat Cancer **16**(2): 455-66.

Thyroid cancer exhibits a spectrum from relatively indolent tumors to tumors that are invasive, metastatic, or progress to poorly differentiated carcinoma. Microarray expression analysis of thyroid cancer cell lines has implicated a member of the melanoma-associated (MAGE) family of cancer-testis antigens in thyroid cancer development and progression. We performed this study to validate the role of MAGE in human thyroid cancers. A tissue microarray (TMA) of samples from 375 patients with thyroid cancer was analyzed with immunohistochemistry (IHC) to localize MAGE. Western blotting of fractionated proteins from MAGE-transfected cells was used to confirm intracellular localization of proteins. Automated analysis of TMA samples was evaluated and subjected to statistical analysis. MAGE immunoreactivity was identified in nuclear and cytoplasmic compartments of normal and malignant tissues. Specificity of staining was proved by fractionation studies that confirmed MAGE expression in nucleus and cytoplasm. Normal thyroid tissue exhibited weak cytoplasmic and strong nuclear MAGE reactivity. Tumors exhibited an increase in cytoplasmic MAGE scores that correlated with clinical behavior: larger tumors had higher

MAGE scores, and there was a positive and significant correlation between MAGE cytoplasmic score and the number of histologically proven lymph node metastases. There was a statistically significant negative correlation between cytoplasmic MAGE and the percentage of p53-positive nuclei. Our data confirm gene-profiling evidence that members of the MAGE family play a role in thyroid cancer progression. The use of TMA analyses identifies IHC techniques that are translatable to the clinical setting for prognostic assessment of patients with thyroid cancer.

Chiacchio, S., A. Lorenzoni, et al. (2008). "Anaplastic thyroid cancer: prevalence, diagnosis and treatment." Minerva Endocrinol **33**(4): 341-57.

Anaplastic thyroid cancer (ATC) is a rare aggressive tumor arising from the follicular cells of the thyroid gland (as does well differentiated thyroid cancer, WDTC), but ATC cells do not retain any of the biological features of the original follicular cells, such as uptake of iodine and synthesis of thyroglobulin. Prognosis is almost invariably fatal. In this article the Authors review the pathology, epidemiology, clinical presentation, diagnosis and treatment options of ATC. ATC incidence typically peaks at the 6-7th decade of life (mean age at diagnosis 55-65 years), women representing 55-77% of all patients. ATC represents 2-5% of all thyroid tumors, with a decreasing trend with respect to the incidence of WDTC. The histologic patterns of ATC include giant-cell, spindle-cell and squamoid-cell tumors; these subtypes frequently coexist and are not predictive of patients' outcome. Immuno-cyto-chemistry for thyroglobulin is usually negative or weakly positive and some cases are also negative for keratin, particularly in the spindle-cell areas. ATC may arise de novo, but in most cases it develops from a pre-existing WDTC, especially the follicular subtype. Most ATC patients complain of local compressive symptoms, such as dysphagia, dysphonia, stridor and dyspnea in addition to neck pain and tenderness; in over 70% of the patients the tumor infiltrates surrounding tissues, such as fat, trachea, muscle, esophagus, and larynx. The clinical course of a rapidly enlarging mass that is firm and fixed to surrounding structures in an elderly patient is quite suggestive for ATC. Diagnosis can be confirmed by fine needle aspiration cytology or, in doubtful cases, by histology on core biopsy. Computed tomography (CT) scan and magnetic resonance imaging (MRI) are useful for defining the local extent of disease and for identifying distant metastases, as is also positron-emission tomography (PET) with [(18)F]FDG. Tracheoscopy and esophagoscopy should be performed every two months, or whenever patients refer the appearance or worsening of local symptoms. Bone scintigraphy may be included in the follow-up of patients with a longer survival and relatively good health. Because of its aggressive behavior, the latest American Joint Committee on Cancer Staging Manual classifies all ATCs as T4 and Stage IV tumors, regardless of their actual overall tumor burden. Treatment of ATC has not been standardized because it is not clear whether or not therapy is effective in prolonging survival; most patients die within six momths from diagnosis, primarily because of asphyxiation caused by local tumor invasion. When employed alone, surgery, radiotherapy, or chemotherapy are seldom adequate to achieve overall control of the disease, but a combination of these treatments may improve local control. Surgical treatment of local disease offers the best opportunity for prolonged survival if the tumor is intrathyroidal. When the tumor is extrathyroidal, the surgical approach to ATC is controversial. Some favourable results have recently been reported with newly developed chemotherapy agents and hyper-fractioned radiation therapy. Tracheostomy should be performed in patients with impending airway obstruction when death is not imminent from other sites of disease, and if patients are not candidates for local resection or chemoradiation. Interventional bronchoscopy, including Nd-YAG laser and airways stenting are alternatives to surgery in inoperable ATC-induced tracheal obstruction. Gene therapy is under investigation. Although very rare, ATC is a highly aggressive tumor that belongs to the group of killer tumors with median survival time not longer than 6-8 months. Surgery, chemotherapy and radiotherapy are the conventional therapeutic strategies performed in the attempt to improve survival. Unfortunately, very often they do not succeed any clinical benefit but only palliative RESULTS: New therapeutic strategies based on molecular approaches are desirable.

Chiappetta, G., C. De Marco, et al. (2007). "Overexpression of the S-phase kinase-associated protein 2 in thyroid cancer." Endocr Relat Cancer **14**(2): 405-20.

Loss of expression of the cyclin-dependent kinase inhibitor p27 through enhanced protein degradation frequently occurs in human cancer. Degradation of p27 requires ubiquitination by the S-phase kinase-associated protein 2 (Skp2), a member of the F-box family of Skp1-Cullin-F-box protein ubiquitin ligases. In the present study, we have investigated the role of Skp2 in human thyroid tumours. Immunohistochemistry analysis showed that Skp2 was overexpressed significantly in thyroid carcinomas (26 out of 51) compared with goitres (0 out of 12, $P<0.001$) or adenomas (1 out of 10,

P<0.05), and that high Skp2 expression was detected more often in anaplastic thyroid (ATC; 83%, n=12) than follicular thyroid (FTC; 40%, n=20) or papillary thyroid (PTC; 42%, n=19) carcinomas (P<0.05). Thyroid cancer cell lines and tissues with high levels of Skp2 protein presented high p27 degradation activity and there was an inverse correlation between Skp2 and p27 expression in thyroid cancer tissues (n=68; P<0.05). In most cases, the observed overexpression of Skp2 protein was paralleled by an increase in the levels of Skp2 mRNA, and we observed Skp2 gene amplification at 5p13 in 2 out of 6 cell lines and in 9 out of 23 primary tumours (six out of eight ATCs, two out of nine PTCs and one out of six FTCs) using Q-PCR and/or fluorescence in situ hybridization analysis. Finally, in vitro experiments demonstrated that suppression of Skp2 expression drastically reduced proliferation of thyroid cancer cells and, conversely, forced expression of Skp2 circumvented serum dependency and contact inhibition in Skp2-negative cells by promoting p27 degradation. These findings indicate that Skp2 plays an important role for the development of thyroid cancer.

Cho, M. A., M. K. Lee, et al. (2007). "Expression and role of estrogen receptor alpha and beta in medullary thyroid carcinoma: different roles in cancer growth and apoptosis." J Endocrinol **195**(2): 255-63.

Medullary thyroid carcinoma (MTC) originates from parafollicular C cells. Estrogen receptor beta(ERbeta) expressionwas detected in normal parafollicular C cells and MTC tumor tissue, but ERalpha expression in MTC tumors still remains undetermined. The appearance and loss of ERalpha or ERbeta expression has been known to play a role in the development and progression of many human cancers. We performed immunohistochemical studies of ERalpha, ERbeta, and Ki67, a mitotic index, in 11 human MTC tissue samples. ERalpha was detected in 10 cases (91%), and ERbeta expression was observed in 8 cases (72.7%). A majority (8/10) of ERalpha-positive tumors showing ERbeta Ki67 expression was detected in three cases (27.3%). Neither clinical parameters nor tumor node metastasis (TNM) tumor staging was correlated with the positivity for ERs or Ki67. To investigate the biological role of each ER, we used ER-negative MTC TT cells and adenoviral vectors carrying ERalpha (Ad-ERalpha), ERbeta (Ad-ERbeta), estrogen response element (ERE)-Luc (Ad-ERE-Luc), and activator protein 1 (AP1)-Luc (Ad-AP1-Luc). Estrogen stimulated and anti-estrogen, ICI 182 780, suppressed ERE reporter activity in TT cells expressing ERalpha or ERbeta, suggesting that both ERs use the same classical ERE-mediated pathway. Ad-ERalpha infection stimulated TT cell growth; in contrast, Ad-ERbeta infection suppressed their growth. Apoptosis was detected in Ad-ERbeta-infected TT cells. Estrogen and anti-estrogen suppressed AP1 activity in Ad-ERalpha-infected cells, whereas upon Ad-ERbeta infection estrogen further stimulated AP1 activity which in turn is suppressed by anti-estrogen, suggesting that each ER acts differently through a non-ERE-mediated pathway. Our results suggest that ERalpha and ERbeta may play different roles in MTC tumor growth and progression.

Ciampi, R., J. A. Knauf, et al. (2005). "Oncogenic AKAP9-BRAF fusion is a novel mechanism of MAPK pathway activation in thyroid cancer." J Clin Invest **115**(1): 94-101.

Genes crucial for cancer development can be mutated via various mechanisms, which may reflect the nature of the mutagen. In thyroid papillary carcinomas, mutations of genes coding for effectors along the MAPK pathway are central for transformation. BRAF point mutation is most common in sporadic tumors. By contrast, radiation-induced tumors are associated with paracentric inversions activating the receptor tyrosine kinases RET and NTRK1. We report here a rearrangement of BRAF via paracentric inversion of chromosome 7q resulting in an in-frame fusion between exons 1-8 of the AKAP9 gene and exons 9-18 of BRAF. The fusion protein contains the protein kinase domain and lacks the autoinhibitory N-terminal portion of BRAF. It has elevated kinase activity and transforms NIH3T3 cells, which provides evidence, for the first time to our knowledge, of in vivo activation of an intracellular effector along the MAPK pathway by recombination. The AKAP9-BRAF fusion was preferentially found in radiation-induced papillary carcinomas developing after a short latency, whereas BRAF point mutations were absent in this group. These data indicate that in thyroid cancer, radiation activates components of the MAPK pathway primarily through chromosomal paracentric inversions, whereas in sporadic forms of the disease, effectors along the same pathway are activated predominantly by point mutations.

Ciampi, R., J. A. Knauf, et al. (2005). "BRAF kinase activation via chromosomal rearrangement in radiation-induced and sporadic thyroid cancer." Cell Cycle **4**(4): 547-8.

Activating point mutations of the BRAF gene have been recently described in a variety of human tumors. In a study published in the Journal of Clinical Investigation, we reported a novel mechanism of activation of this gene via paracentric inversion of chromosome 7q. The fusion protein, AKAP9-BRAF, contains the intact kinase domain and lacks the

autoinhibitory N-terminal portion of BRAF. It exhibited constitutive activation of BRAF kinase and was transforming for NIH3T3 cells. This finding represents the first demonstration of RAF activation by chromosomal rearrangement in human tumors. AKAP9-BRAF was more common in radiation-induced thyroid tumors, whereas point mutations of BRAF predominated in sporadic tumors of the same type, demonstrating the association between environmental factors and specific mechanisms of BRAF activation.

Ciampolillo, A., C. De Tullio, et al. (2005). "The IGF-I/IGF-I receptor pathway: Implications in the Pathophysiology of Thyroid Cancer." Curr Med Chem **12**(24): 2881-91.

The biological actions of the insulin-like growth factor(IGF)-I are mediated by its activation of the IGF-I receptor (IGF-I R), a transmembrane tyrosine kinase linked to the Akt and ras-raf-MAPK cascades. A functional IGF-I R is required for the cell to progress through the cell cycle. Most importantly, cells lacking this receptor cannot be transformed by any of a number of dominant oncogenes, a finding that proves that the presence of the IGF-I R is important for the development of a malignant phenotype. Consistent with this role, it has been well established that IGF-I can protect cells from apoptosis under a variety of circumstances. For example, IGF-I prevents apoptosis induced by overexpression of c-myc in fibroblasts, by interleukin-3 withdrawal in interleukin-3-dependent hemopoietic cells, etoposide, a topoisomerase I inhibitor, anti-cancer drugs, UV-B irradiations, and serum deprivation. While the anti-apoptotic effect of IGF-I has been clearly demonstrated, the molecular mechanisms by which IGF-I inhibits apoptosis induced by these various stimuli remain unknown. We have previously documented increased IGF-I and IGF-I receptor immunoreactivity in human thyroid carcinomas with a corresponding up-regulation of IGF-I mRNA. Immunoreactivity for IGF-I and IGF-I receptor positively correlated with tumor diameter, but not with the occurrence of lymph node metastases. Several recent studies have identified new signaling pathways emanating from the IGF-I receptor that affect cancer cell proliferation, adhesion, migration and apoptosis, which represent critical functions for cancer cell survival and metastasizing capacity. In this review, various aspects of the IGF-I/IGF-I R pathway and its relationship to thyroid cancer are discussed.

Cirello, V., M. P. Recalcati, et al. (2008). "Fetal cell microchimerism in papillary thyroid cancer: a possible role in tumor damage and tissue repair." Cancer Res **68**(20): 8482-8.

Fetal cells enter the maternal circulation during pregnancy and can persist in the maternal blood or tissues for decades, creating a physiologic microchimerism. Because papillary thyroid cancer (PTC) is more frequent in women, the role of persisting fetal male cells in this tumor has been investigated. Tumor tissue specimens were obtained from 63 women with PTC who had a male pregnancy before the diagnosis. Male cells, identified by PCR amplification of a male-specific gene, the sex-determining region Y, was detected in 47.5% of women. By fluorescence in situ hybridization (FISH) analyses, the total number of microchimeric cells was significantly higher in neoplastic tissue than in controlateral normal sections. By combined FISH and immunohistochemistry (immuno-FISH), male cells expressing thyroglobulin were found in tumor and normal tissues, whereas male microchimeric cells stained with the CD45 antigen were detected only in tumor sections. Microchimeric cells negative for either marker were detected both in tumor and normal tissues. Moreover, both CD45(+) and Tg(+) fetal cells did not express MHC II antigens. In conclusion, fetal microchimerism has been documented in a high proportion of women with PTC. The immuno-FISH studies indicate that CD45(+)/MHC II(-) male cells found in neoplastic tissues might be committed to destroy tumor cells, whereas Tg(+)/MHC II(-) cells could have a repair function. Finally, microchimeric cells negative for either CD45 or Tg could have "progenitor-like" properties able to transdifferentiate in different cellular types. Although a pathogenetic mechanism cannot be excluded, the whole of the present results indicates a protective role of microchimerism in thyroid cancer.

Couto, J. P., H. Prazeres, et al. (2009). "How molecular pathology is changing and will change the therapeutics of patients with follicular cell-derived thyroid cancer." J Clin Pathol **62**(5): 414-21.

Well-differentiated thyroid carcinomas comprise two well-defined histological types: papillary and follicular (PTCs and FTCs, respectively). Despite being derived from the same cell (thyroid follicular cell), these two types of tumour accumulate distinct genetic abnormalities during progression. The molecular pathology of thyroid cancer is now better understood because of our ability to identify RET/PTC rearrangements and BRAF mutations in the aetiopathogenesis of the large majority of PTCs and the high prevalence of RAS mutations and PAX8/PPARgamma rearrangements in follicular patterned carcinomas (FTCs and follicular variant of PTCs). This review summarises most of the molecular alterations currently used as targets for new biological treatments and looks at some of the changes

that are already occurring or may occur in the treatment of patients with thyroid cancer. For simplicity, the review is divided up according to the major genetic alterations identified in well-differentiated thyroid carcinomas (RET/PTC rearrangements, BRAF mutations, RAS mutations and mitochondrial DNA deletions and mutations) and their respective treatments.

Cras, A., D. Darsin-Bettinger, et al. (2007). "Epigenetic patterns of the retinoic acid receptor beta2 promoter in retinoic acid-resistant thyroid cancer cells." Oncogene **26**(27): 4018-24.

Treatment with retinoic acid (RA) is effective to restore radioactive iodine uptake in metastases of a small fraction of thyroid cancer patients. In order to find predictive markers of response, we took advantage of two thyroid cancer cell lines, FTC133 and FTC238, with low RA-receptor (RAR)beta expression but differing in their response to RA. We report that in both cell lines, RA signalling pathways are functional, as transactivation of an exogenous RARbeta2 promoter is effective in the presence of pharmacological concentrations of all-trans RA, and enhanced in RA-resistant FTC238 cells after ectopical expression of RARbeta, suggesting a defective endogenous RARbeta2 promoter in these cells. Further analyses show that whereas the RARbeta2 promoter is in an unmethylated permissive status in both FTC133 and FTC238 cells, it failed to be associated with acetylated forms of histones H3 or H4 in FTC238 cells upon RA treatment. Incubation with a histone deacetylase inhibitor, alone or in combination with RA, restored histone acetylation levels and reactivated RARbeta and differentiation marker Na+/I- symporter gene expression. Thus, histone modification patterns may explain RA-refractoriness in differentiated thyroid cancer patients and suggest a potential benefit of combined transcriptional and differentiation therapies.

Dardano, A., S. Falzoni, et al. (2009). "1513A>C polymorphism in the P2X7 receptor gene in patients with papillary thyroid cancer: correlation with histological variants and clinical parameters." J Clin Endocrinol Metab **94**(2): 695-8.

INTRODUCTION: The modulation of the purinergic receptor P2X7 may be implicated in human carcinogenesis. The 1513A>C and 489C>T polymorphisms of P2X7R gene induce loss of function and gain of function, respectively. AIM: The aim of the study was to assess the frequency of both 1513A>C and 489C>T polymorphisms in patients with papillary thyroid carcinoma (PTC) and to evaluate the possible association with clinical and histological features. PATIENTS AND METHODS: P2X7R analysis was performed in lymphocytes from 121 PTC patients (100 women, 21 men; aged 43.4 +/- 13.6 yr), 100 matched healthy subjects, and 80 patients with nodular goiter. RESULTS: The minor allele frequency for 1513A>C polymorphism in PTC patients with the classical variant was similar to controls (0.21 and 0.20, respectively), whereas it resulted in a significant increase in patients with the follicular variant (0.36; P = 0.01 vs. classical variant, and P = 0.005 vs. controls). In detail, 13.6% of patients with PTC follicular variant were homozygous for the 1513C allele, compared to 2.6% of patients with the classical variant and 2% of controls. Moreover, a positive relationship between 1513A>C polymorphism and either cancer diameter (Rho = 0.22; P = 0.02) or TNM stage (Rho = 0.38; P < 0.001) was found. No significant difference in the genotype frequency of 489C>T polymorphism between PTC patients and healthy controls was observed (0.42 and 0.47, respectively). CONCLUSIONS: Our data show, for the first time, a strong association between 1513A>C polymorphism of P2X7R gene and the follicular variant of PTC. Further studies are needed to confirm the possible role of this polymorphism as a novel clinical marker of PTC follicular variant and its usefulness in selecting patients with different clinical outcome.

de Groot, J. W., T. P. Links, et al. (2006). "An introduction to managing medullary thyroid cancer." Hered Cancer Clin Pract **4**(3): 115-25.

MTC is a rare neuroendocrine thyroid tumour accounting for 3% to 10% of all thyroid malignancies. It can occur in a sporadic and a hereditary clinical setting. Hereditary MTC may either occur alone (familial MTC, FMTC) or as part of multiple endocrine neoplasia (MEN) type 2A, or MEN 2B. These disorders are due to germline mutations in the RET (REarranged during Transfection) gene. In carriers of MEN 2B-associated RET mutations, prophylactic thyroidectomy is indicated before the first year of life. In the case of MEN 2A-associated germline RET mutations with a high-risk profile, total thyroidectomy is warranted before the age of 2 years and certainly before the age of 4 years. At that age the risk of invasive MTC and metastases is acceptably low. Depending on the type of RET mutation, thyroidectomy can take place at an older age in patients with a lower risk profile. In case of elevated basal or stimulated serum calcitonin, preventive surgery including total thyroidectomy and central compartment dissection should be performed regardless of age. When MTC presents as a palpable tumour, total thyroidectomy should be combined with extensive lymph node dissection of levels II-V on

both sides and level VI to prevent locoregional recurrences.

de Groot, J. W., I. Plaza Menacho, et al. (2006). "Cellular effects of imatinib on medullary thyroid cancer cells harboring multiple endocrine neoplasia Type 2A and 2B associated RET mutations." Surgery **139**(6): 806-14.

BACKGROUND: Activating mutations in the RET gene, which encodes a tyrosine kinase receptor, often cause medullary thyroid carcinoma (MTC). Surgical resection is the only curative treatment; no effective systemic treatment is available. We evaluated imatinib, a tyrosine kinase inhibitor currently used to treat chronic myelogenous leukemia and gastrointestinal stromal tumors, as a potential drug for systemic treatment of MTC, in 2 MTC-derived cell lines expressing multiple endocrine neoplasia-associated mutant RET receptors. METHODS: We determined RET expression and Y1062 phosphorylation using Western blot analysis and quantitative polymerase chain reaction. We determined the effects on cell proliferation by a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay, and we used fluorescence-activated cell sorter analysis with annexin V/propidium iodide staining to study imatinib-induced cell-cycle arrest, apoptosis, and cell death. RESULTS: Imatinib inhibited RET Y1062 phosphorylation in a dose-dependent manner after 1.5 hours of exposure. After 16 hours both RET Y1062 phosphorylation and protein expression levels were affected. Dose-dependent decreases in cell proliferation of both cell lines after exposure to imatinib with inhibitory concentration of 50% levels of 23 +/- 2 micromol/L and 25 +/- 4 micromol/L were seen. These values are high, compared with those for chronic myelogenous leukemia and gastrointestinal stromal tumors. We further could show that imatinib induced cell-cycle arrest, and apoptotic and nonapoptotic cell death. CONCLUSIONS: Imatinib inhibits RET-mediated MTC cell growth affecting RET protein levels in vitro in a dose-dependent manner. The concentration of imatinib necessary to inhibit RET in vitro, however, makes it impossible to conclude that imatinib monotherapy will be a good option for systemic therapy of MTC.

de Melo Martins, P. C., O. Parise Junior, et al. (2007). "C8orf4/TC-1 (thyroid cancer-1) gene expression in thyroid cancer and goiter." ORL J Otorhinolaryngol Relat Spec **69**(2): 127-30.

BACKGROUND: The expression of the thyroid cancer-1(TC-1) gene seems to be related with malignant transformation in the thyroid tissue. OBJECTIVE: We evaluated the potential use of TC-1 gene expression as a marker of malignancy in thyroid nodules. METHODS: A total of 92 frozen thyroid samples were studied, including 46 samples from thyroid nodules (19 papillary carcinomas, 1 follicular carcinoma, 24 adenomatous goiters, and 2 follicular adenomas) and 46 samples from normal surrounding thyroid tissue. Total RNA was extracted and TC-1 expression was assessed by semiquantitative Multiplex PCR. Results were verified using real-time RT-PCR in some of the samples. RESULTS: Overall mean TC-1 gene expression (normalized by the ABL gene) was 1.73 +/- 1.67 (0.33-9.33). There was a significant difference ($p < 0.001$) between TC-1 gene expression in benign thyroid lesions (1.07 +/- 0.10) and carcinomas (2.73 +/- 0.51). Conclusion: Our results suggest that TC-1 gene expression may be useful in the differential diagnosis of goiters and thyroid papillary carcinomas.

Dias, E. P., F. J. Pimenta, et al. (2007). "Association between decreased WWOX protein expression and thyroid cancer development." Thyroid **17**(11): 1055-9.

CONTEXT: Chromosomal fragile sites are often related to cancer development. The WW domain-containing oxidoreductase gene (WWOX) spans the second most common chromosomal fragile site (FRA16D) and encodes an important proapoptotic protein. OBJECTIVE: To verify our hypothesis that underexpression of WWOX could contribute to malignant transformation of the thyroid cells. METHOD: We compared WWOX expression among follicular adenomas (FAs) and differentiated thyroid carcinomas [follicular thyroid carcinomas (FTCs) and papillary thyroid carcinomas (PTCs)] in 53 thyroid tumors resected from patients submitted to total thyroidectomy. DESIGN: Multiple fields of tumor areas of FAs, FTCs, and PTCs as well as normal thyroid tissue were stained with WWOX antiserum, and classified by the extent of staining (percentage of cells staining) and staining intensity. MAIN OUTCOME: PTCs showed a significantly decreased expression of WWOX when compared to FAs and FTCs. Further, using a unique model of comparison in patients in whom FAs and PTCs were concomitantly present, we detected the same result (i.e., no expression in PTCs). CONCLUSION: We conclude that WWOX underexpression is an important step that might increase the vulnerability to the carcinogenesis process in PTCs.

Drosten, M. and B. M. Putzer (2006). "Mechanisms of Disease: cancer targeting and the impact of oncogenic RET for medullary thyroid carcinoma therapy." Nat Clin Pract Oncol **3**(10): 564-74.

Growing evidence supports the concept of oncogene dependence for cancer development;

inhibition of the initiating oncogene can result in revertion of the neoplastic phenotype. The outstanding role of the RET proto-oncogene in the development of medullary thyroid carcinoma (MTC) is well established. With the emerging knowledge concerning the signal transduction pathways leading to subsequent neoplastic transformation, oncogenic activated RET becomes a highly attractive target for selective cancer therapy. A variety of novel approaches that target RET directly or indirectly have recently emerged and an increasing number are currently being assessed in clinical trials. In view of these findings, it becomes strikingly obvious that inhibition of RET oncogene function can be a viable option for the treatment of MTC. We summarize the current evidence for RET involvement in the etiology of MTC, and the therapeutic targeting of this process in preclinical and clinical studies.

Du, Z. X., H. Y. Zhang, et al. (2009). "Role of oxidative stress and intracellular glutathione in the sensitivity to apoptosis induced by proteasome inhibitor in thyroid cancer cells." BMC Cancer **9**: 56.

BACKGROUND: The proteasome inhibitor bortezomib has shown impressive clinical activity alone and in combination with conventional and other novel agents for the treatment of multiple myeloma (MM) and some solid cancers. Although bortezomib is known to be a selective proteasome inhibitor, the downstream mechanisms of cytotoxicity and drug resistance are poorly understood. METHODS: Proteasome activity, intracellular glutathione (GSH) and ROS levels, as well as activities of GSH synthesis enzymes were measured using spectrophotometric methods. Cell death was analyzed using flow cytometry and caspase activity assay. The expression level of GSH synthesis enzymes were measured using real-time RT-PCR. RESULTS: At concentrations that effectively inhibited proteasome activity, bortezomib induced apoptosis in FRO cells, but not in ARO cells. Bortezomib elevated the amount of glutathione (GSH) and the treatment with bortezomib increased the level of mRNA for GCL, a rate-limiting enzyme in glutathione synthesis. Furthermore, depletion of GSH increases apoptosis induced by bortezomib, in contrast, repletion of GSH decreases bortezomib-mediated cell death. CONCLUSION: GSH protects cells from proteasome inhibition-induced oxidative stress and glutathione-dependent redox system might play an important role in the sensitivity to proteasome inhibition-induced apoptosis.

Duncan, R. E. and M. C. Archer (2006). "Farnesol induces thyroid hormone receptor (THR) beta1 but inhibits THR-mediated signaling in MCF-7 human breast cancer cells." Biochem Biophys Res Commun **343**(1): 239-43.

Anti-cancer effects of farnesol are well established, although mechanisms mediating these effects are not fully understood. Since farnesol has been shown to regulate gene transcription through activation of the farnesoid X receptor and the peroxisome proliferator-activated receptors-alpha and -gamma, we hypothesized that farnesol may also mediate some of its effects through other nuclear hormone receptors. Here we showed that in MCF-7 human breast cancer cells, farnesol induced the expression of thyroid hormone receptor (THR) beta1 mRNA and protein at concentrations that inhibited cell growth. Changes in the expression of THR responsive genes, however, suggested that farnesol inhibits THR-mediated signaling. Protein extracts from cells treated with farnesol displayed decreased binding to oligodeoxynucleotides containing a consensus sequence for the THR response element, despite the higher THRbeta1 content, providing a mechanism to explain the decreased transcriptional activity of cellular THRs.

Duntas, L. and B. M. Grab-Duntas (2006). "Risk and prognostic factors for differentiated thyroid cancer." Hell J Nucl Med **9**(3): 156-62.

Papillary and follicular carcinomas, commonly referred to as follicular cell-derived differentiated thyroid carcinomas (DTC), account for 90% of all thyroid carcinomas. The prognosis of DTC is generally good, depending on the biologic behavior of the tumor and on the appropriate initial treatment which includes total thyroidectomy and ablation by radioiodine-131. However, a considerable number of patients, approximately 30%, as shown after 30 years of follow-up, have recurrent disease. It is thus of utmost importance to evaluate the prognostic factors, as derived from retrospective studies, and identify high risk patients. Age of more than 45 years or less than 25 years is a particularly strong independent prognostic factor; on the contrary gender is a poor prognostic factor. Histological type of the cancer especially tall cancer cells and columnar cancer cells, as well as increased vascular invasion of the tumor, lymph-node and distant metastases, are all considered as risk factors that can lead to poor prognosis. Combined prognostic factors have been used to form scoring systems (SS) such as AGES, MACIS, AMES, EORTC and TNM for a more precise description of high or low risk patients. However, prognostic significance of the SS is limited, since they do not take into consideration the clinical status or the treatment procedure during the course of the disease. Molecular factors such as rearrangements of genes RET/PTC, RAS mutations and fusion of, paired box

and 8/peroxisome proliferator-activated receptor gamma (PAX8/PPARgamma) are also involved in thyroid cancer prognosis, while some others: human Pituitary- Tumor Transforming Gene (e.g. MIB-1, hPTTG) have been reported as additional prognostic factors. In this review we describe the risk and the prognostic factors of DTC as related to management and the outcome of DTC.

Durand, S., C. Ferraro-Peyret, et al. (2008). "Evaluation of gene expression profiles in thyroid nodule biopsy material to diagnose thyroid cancer." J Clin Endocrinol Metab **93**(4): 1195-202.

CONTEXT: Detection of thyroid cancer among benign nodules on fine-needle aspiration biopsies (FNAB), which presently relies on cytological examination, is expected to be improved by new diagnostic tests set up from genomic data. OBJECTIVE: The aim of the study was to use a set of genes discriminating benign from malignant tumors, on the basis of their expression levels, to build tumor classifiers and evaluate their capacity to predict malignancy on FNAB. DESIGN: We analyzed the level of expression of 200 potentially informative genes in 56 thyroid tissue samples (benign or malignant tumors and paired normal tissue) using nylon macroarrays. Gene expression data were subjected to a weighted voting algorithm to generate tumor classifiers. The performances of the classifiers were evaluated on a series of 26 sham FNAB, i.e. FNAB carried out on thyroid nodules after surgical resection. RESULTS: A series of 19 genes with a similar expression in follicular adenomas and normal tissue and discriminating follicular adenomas+normal tissue from the following: 1) follicular thyroid carcinomas (FTCs), 2) papillary thyroid carcinomas (PTCs), or 3) both FTCs and PTCs. These were used to generate four classifiers, the FTCs, PTCs, common (FTC+PTCs), and global classifiers. In 23 of the 26 sham FNAB, the four classifiers yielded a diagnosis in agreement with the diagnosis of the pathologist used as reference; in the three other cases, the correct diagnosis was given by three of four classifiers. CONCLUSIONS: We developed a procedure of molecular diagnosis of benign vs. malignant tumors applicable to the material collected by FNAB. The molecular test complied with a preclinical validation stage; it must be now evaluated on ultrasound-guided FNAB in a large-scale prospective study.

Elisei, R., B. Cosci, et al. (2008). "Prognostic significance of somatic RET oncogene mutations in sporadic medullary thyroid cancer: a 10-year follow-up study." J Clin Endocrinol Metab **93**(3): 682-7.

BACKGROUND: Medullary thyroid carcinoma (MTC) is a well-differentiated thyroid tumor that maintains the typical features of C cells. An advanced stage and the presence of lymph node metastases at diagnosis have been demonstrated to be the most important bad prognostic factors. Somatic RET mutations have been found in 40-50% of MTCs. Although a relationship between somatic mutations and bad prognosis has been described, data are controversial and have been performed in small series with short-term follow ups. The aim of this study was to verify the prognostic value of somatic RET mutations in a large series of MTCs with a long follow up. METHODS: We studied 100 sporadic MTC patients with a 10.2 yr mean follow-up. RET gene exons 10-11 and 13-16 were analyzed. The correlation between the presence/absence of a somatic RET mutation, clinical/pathological features, and outcome of MTC patients was evaluated. RESULTS: A somatic RET mutation was found in 43 of 100 (43%) sporadic MTCs. The most frequent mutation (34 of 43, 79%) was M918T. RET mutation occurrence was more frequent in larger tumors (P=0.03), and in MTC with node and distant metastases (P<0.0001 and P=0.02, respectively), thus, a significant correlation was found with a more advanced stage at diagnosis (P=0.004). A worse outcome was also significantly correlated with the presence of a somatic RET mutation (P=0.002). Among all prognostic factors found to be correlated with a worse outcome, at multivariate analysis only the advanced stage at diagnosis and the presence of a RET mutation showed an independent correlation (P<0.0001 and P=0.01, respectively). Finally, the survival curves of MTC patients showed a significantly lower percentage of surviving patients in the group with RET mutations (P=0.006). CONCLUSIONS: We demonstrated that the presence of a somatic RET mutation correlates with a worse outcome of MTC patients, not only for the highest probability to have persistence of the disease, but also for a lower survival rate in a long-term follow up. More interestingly, the presence of a somatic RET mutation correlates with the presence of lymph node metastases at diagnosis, which is a known bad prognostic factor for the definitive cure of MTC patients.

Elisei, R., C. Romei, et al. (2007). "RET genetic screening in patients with medullary thyroid cancer and their relatives: experience with 807 individuals at one center." J Clin Endocrinol Metab **92**(12): 4725-9.

BACKGROUND: Germline RET gene mutations are causative of multiple endocrine neoplasia (MEN) 2 and may be identified by genetic screening. Three different syndromes are distinguished: MEN 2A, when medullary thyroid carcinoma (MTC) is associated with

pheochromocytoma and/or parathyroid adenomas; MEN 2B, when accompanied by a marfanoid habitus and/or pheochromocytoma; and familial medullary thyroid carcinoma (FMTC), when only MTC is present. PATIENTS AND METHODS: During the last 13 yr, we performed RET genetic screening in 807 subjects: 481 with apparently sporadic MTC, 37 with clinical evidence of MEN 2, and 289 relatives. Genomic DNA was extracted from the blood of all subjects, and exons 10, 11, 13, 14, 15, and 16 were analyzed by direct sequencing after PCR. RESULTS: We unexpectedly discovered a germline RET mutation in 35 of 481 (7.3%) apparently sporadic MTC patients. A germline RET mutation was also found in 36 of 37 patients with clinical evidence of hereditary MTC. The distribution of RET mutations in cysteine and noncysteine encoding codons was significantly different in the two groups of patients, with the prevalence of RET mutations in noncysteine codons being higher in MTC that presented as apparently sporadic ($P < 0.0001$). A total of 34 FMTCs (75.5% of all FMTC) arrived with apparent sporadic MTC, with no familial history of other MTC cases. According to genetic screening and clinical data, our 72 families were classified as follows: 45 FMTC (62.5%), 22 MEN 2A (30.5%), and five MEN 2B (7%). CONCLUSIONS: In this large series of MTC, hereditary forms, mainly FMTC, were clinically unsuspected in 7.3% of apparently sporadic cases. As a consequence, the prevalence of FMTC in our series is higher than that previously reported (60 vs. 10%). In these cases, RET mutations were more prevalently located in noncysteine codons. Data derived from our series helped elucidate the role of RET genetic screening for the identification of all forms of MEN 2, and especially for FMTC, which are frequently clinically misdiagnosed as nonheritable, sporadic cases.

Elisei, R., A. Vivaldi, et al. (2005). "All-trans-retinoic acid treatment inhibits the growth of retinoic acid receptor beta messenger ribonucleic acid expressing thyroid cancer cell lines but does not reinduce the expression of thyroid-specific genes." J Clin Endocrinol Metab **90**(4): 2403-11.

Conventional chemotherapy and radiotherapy are ineffective for the treatment of advanced thyroid tumors like poorly differentiated papillary, anaplastic, and medullary thyroid cancer. In the attempt to evaluate the possibility of using retinoic acid (RA) in the treatment of thyroid cancer refractory to conventional therapy, we studied the effect of all-trans-RA treatment on five human thyroid cancer cell lines. We found that WRO and NPA, derived from follicular and poorly differentiated human thyroid carcinoma, respectively, showed a growth inhibition after 25 and 21 d of RA treatment. Both apoptosis and a decrease in DNA synthesis were observed as mechanisms of growth inhibition. In the NPA cell line, a delay of cell-cycle progression has also been observed. On the contrary, we did not observe any recovery of mRNA expression of thyroid-specific genes and in particular of the sodium iodide symporter gene. The lack of recovery of radioiodide uptake after all-trans-RA treatment confirmed the inability to reexpress sodium iodide symporter mRNA. The main difference between the all-trans-RA responding cells (WRO and NPA) and the nonresponding cells [ARO, FRO (derived from human anaplastic thyroid tumors) and TT (derived from human medullary thyroid tumor)] was the basal and all-trans-RA induced RA receptor (RAR)beta mRNA expression. Interestingly, 14 thyroid tumors (10 papillary and four anaplastic) showed a significant lower expression of RARbeta mRNA when compared with normal thyroid tissues. In agreement with this result, only 30% of papillary thyroid carcinomas analyzed were positive for RARbeta protein expression with a degree of expression that was much lower than that found in normal thyroid tissue. In conclusion we found that all-trans-RA treatment can determine a significant in vitro growth inhibition especially in differentiated thyroid tumor-derived cell lines but it seems unable to reinduce the expression of thyroid-specific genes and in particular to reinduce the ability to take up iodine. The growth inhibition is likely due to apoptosis in an early phase and to a decrease of DNA synthesis later. In some cases, a delay of the cell-cycle progression also may be responsible for the growth inhibition. The finding of a basal and RA-induced RARbeta mRNA expression only in cell lines responding to all-trans-RA suggests that the growth inhibition might be mediated by RARbeta.

Elisei, R., A. Vivaldi, et al. (2006). "Treatment with drugs able to reduce iodine efflux significantly increases the intracellular retention time in thyroid cancer cells stably transfected with sodium iodide symporter complementary deoxyribonucleic acid." J Clin Endocrinol Metab **91**(6): 2389-95.

CONTEXT: One of the major limits of gene therapy with sodium iodide symporter (NIS), which enables cells to be subjected to radioiodine therapy, is that NIS-transfected cells rapidly release the intracellular iodine. METHODS: We transfected human anaplastic (FRO) and medullary (TT) thyroid cancer-derived cell lines that were unable to take up iodine with human NIS cDNA. The possibility of increasing the iodine retention time by treating the transfected clones with myricetin, lithium, 17-(allylamino)-17-demethoxygeldanamycin (17-AAG), and 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid

(DIDS) was explored. RESULTS: We obtained 19 FRO and 16 TT clones stably transfected with NIS. Twelve of 19 FRO and nine of 16 TT clones expressed the full-length NIS mRNA; 11 of 12 FRO and four of nine TT clones were able to take up radioiodine and correctly expressed NIS protein on the plasma membrane. Kinetic analysis of iodide uptake in the two clones (FRO-19 and TT-2) with the highest uptaking activity revealed that the plateau was reached after 30 min by FRO-19 and after 60 min by TT-2. The t(1/2) of the iodide efflux was 9 min in FRO-19 and 20 min in TT-2. The treatment of the two cell lines with four different drugs revealed that DIDS and 17-AAG, but not myricetin and lithium, significantly increased the intracellular iodide retention time in FRO-19, but not in TT-2. CONCLUSIONS: We showed that 17-AAG and DIDS prolong the retention time of (131)I in NIS-transfected thyroid tumoral cells, thus reinforcing the hope of using this approach for future clinical application, especially in patients with thyroid carcinoma who are no longer responsive to conventional therapy.

Erdogan, M., M. Karadeniz, et al. (2007). "Fas/Fas ligand gene polymorphism in patients with papillary thyroid cancer in the Turkish population." J Endocrinol Invest **30**(5): 411-6.

OBJECTIVE: Fas ligand (FasL) is an apoptotic agent and a member of tumor necrosis factor (TNF) family. FasL exists in cytotoxic T lymphocyte (CTL) and natural killer (NK) cells, and it is increased in tumor cell membrane. On the contrary, CTL and NK are bound to Fas on the surfaces of cell membrane; this triggers apoptosis in cytotoxic cells and leads to their death. This system plays an important role in eliminating viral infections and cancer cells. Malfunction of this system results in the development and spread of the malignancy. This study aims at evaluating the influence of Fas and FasL gene polymorphism in papillary thyroid cancer (PTC) in the Turkish population. RESEARCH DESIGN AND METHODS: Forty-five patients with PTC and 100 healthy controls were included in this study. The diagnosis of PTC was confirmed by histopathologic examination after surgery. The evaluation of genotype for Fas 670 A/G and FasL 843 C/T gene polymorphism was performed using the PCR-restriction fragment length polymorphism (RFLP) method. RESULTS: The evaluation of Fas/FasL genotype and gene allele frequency did not show statistically significant differences between the patient and control group ($p>0.05$). In addition, the univariate analysis did not reveal a statistically significant relationship between the size of the nodule and the Fas/FasL gene polymorphism in patients with PTC. CONCLUSIONS: As in other types of malignancy, genetic factors in the pathogenesis of PTC may also show changes in different populations. Fas/FasL gene polymorphysms are possible that different mechanisms function in apoptosis balance in PTC development.

Erdogan, M., M. Karadeniz, et al. (2008). "Interleukin-10 gene polymorphism in patients with papillary thyroid cancer in Turkish population." J Endocrinol Invest **31**(9): 750-4.

OBJECTIVE: Interleukin-10 (IL-10) is a major anti-inflammatory cytokine that plays a crucial role in the regulation of the immune system. Chronic inflammation has been reported to be a risk factor for thyroid neoplasia. The propensity to mount an inflammatory response is modified by germ line variation in cytokine and other inflammation-related genes. We hypothesized that a proinflammatory genotype would be positively associated with thyroid cancer. We aimed to evaluate the relation between the genotypic and allelic frequencies of the IL-10(-1082 G/A), IL-10(-592 A/C), and IL-10(-819 C/T) polymorphisms, and their association with the risk of developing papillary thyroid cancer (PTC) in the Turkish population. RESEARCH DESIGN AND METHODS: Forty-two patients with PTC and 113 healthy controls were included in this study. The diagnosis of PTC was confirmed by histopathologic examination after surgery. The evaluation of genotype for IL-10 gene polymorphism was performed using PCR-restriction fragment length polymorphism method. RESULTS: Statistically significant difference IL-10(-1082 G/A) gene polymorphism was determined between 2 (PTC and control) groups. No difference was determined with respect to IL-10(-592 A/C) and IL-10(-819 C/T) gene polymorphisms, and IL-10(-1082 G/A), IL-10(-592 A/C), and IL-10(-819 C/T) allele frequencies of participating between the control group and the patients with PTC ($p>0.05$). CONCLUSIONS: The polymorphism of IL-10(-1082 G/A) gene was significantly associated with the occurrence of PTC. Such studies will contribute significantly to our understanding of the biological role of IL-10(-1082 G/A) gene polymorphism in PTC development. In conclusion, IL-10(-1082 G/A) gene polymorphism may affect the survival of papillary thyroid carcinoma.

Espinosa, A. V., L. Porchia, et al. (2007). "Targeting BRAF in thyroid cancer." Br J Cancer **96**(1): 16-20.

Activating mutations in the gene encoding BRAF are the most commonly identified oncogenic abnormalities in papillary thyroid cancer. In vitro and in vivo models have demonstrated that overexpression of activated BRAF induces malignant transformation

and aggressive tumour behaviour. BRAF and other RAF kinases are frequently activated by other thyroid oncogenes and are important mediators of their biological effects including dedifferentiation and proliferation. Because current therapeutic options for patients with thyroid cancers that are aggressive and/or do not respond to standard therapies are limited, BRAF and its downstream effectors represent attractive therapeutic targets. In this review, data supporting a role for BRAF activation in thyroid cancer development and establishing the potential therapeutic efficacy of BRAF-targeted agents in patients with thyroid cancer will be reviewed.

Fagin, J. A. (2005). "Genetics of papillary thyroid cancer initiation: implications for therapy." Trans Am Clin Climatol Assoc **116**: 259-69; discussion 269-71.

Papillary thyroid cancers are the most common thyroid malignancy. They usually carry a favorable prognosis, although patients with invasive or metastatic tumors that no longer trap radioiodine do less well. There is mounting experimental support for a central role of mutations leading to constitutive activation of MAP kinase effectors in the pathogenesis ofthis disease. Thus activating mutations of the tyrosine receptor kinases RET and NTRK, and of the intracellular signaling effectors RAS and BRAF are present in a mutually exclusive fashion in more than 70% of cases. These mutations are believed to arise at early stages of cancer development, and may be important in tumor maintenance. Hence, compounds that inhibit kinase activity of effectors signaling distally along this pathway may prove effective in treating advanced forms of the disease.

Fagin, J. A. and N. Mitsiades (2008). "Molecular pathology of thyroid cancer: diagnostic and clinical implications." Best Pract Res Clin Endocrinol Metab **22**(6): 955-69.

There is now a reasonably good understanding of the key oncogenic events involved in the initiation and progression of thyroid cancer. Many of these are characteristic of certain tumor types, and their presence conveys diagnostic and prognostic information. It is not yet clear how this information will be applied to clinical practice. Based on preclinical evidence, mutations of genes encoding certain kinases may also predict response to specific tyrosine kinase inhibitors, although this has not yet been explored systematically in clinical trials.

Francipane, M. G., V. Eterno, et al. (2009). "Suppressor of cytokine signaling 3 sensitizes anaplastic thyroid cancer to standard chemotherapy." Cancer Res **69**(15): 6141-8.

We previously showed that cancer cells from papillary, follicular, and anaplastic thyroid carcinomas produce interleukin-4 and interleukin-10, which counteract the cytotoxic activity of conventional chemotherapy through the up-regulation of antiapoptotic molecules. Here, we identify Janus kinase/signal transducers and activators of transcription (STAT) and phosphatidyl inositol 3-kinase (PI3K)/AKT as the down-stream pathways through which these cytokines confer resistance to cell death in thyroid cancer. We found that the absence of suppressors of cytokine signaling (SOCS) molecules allows the propagation of the survival signaling. Exogenous expression of SOCS1, SOCS3, and SOCS5 in the highly aggressive anaplastic thyroid cancer cells reduces or abolishes STAT3 and 6 phosphorylation and PI3K/Akt pathway activation resulting in alteration in the balance of proapoptotic and antiapoptotic molecules and sensitization to chemotherapeutic drugs in vitro. Likewise, exogenous expression of SOCS3 significantly reduces tumor growth and potently enhances the efficacy of chemotherapy in vivo. Our results indicate that SOCS3 regulation of cytokines-prosurvival programs might represent a new strategy to overcome the resistance to chemotherapy-induced cell death of thyroid cancer.

Frohlich, E., I. Fink, et al. (2009). "Is transketolase like 1 a target for the treatment of differentiated thyroid carcinoma? A study on thyroid cancer cell lines." Invest New Drugs **27**(4): 297-303.

Radioactive iodine-refractory [(18)F] fluorodeoxy-glucose-positron emission tomography-positive thyroid carcinomas represent especially aggressive tumors. Targeting glucose metabolism by the transketolase isoenzyme transketolase like 1 (TKTL-1) which is over-expressed in various neoplasms, may be effective. The correlation of TKTL-1 expression and the response to oxythiamine as the currently best-characterized inhibitor of transketolases was studied in differentiated thyroid cancer cell lines. We determined TKTL-1 expression, proliferation, glucose uptake and GLUT-1 expression in non-treated thyroid cells and recorded the effect of oxythiamine on iodide uptake and on thymidine uptake. TKTL 1 was highest expressed in cell lines derived from more invasive tumors but the expression level was not strongly correlated to proliferation rate, to GLUT-1 expression or to the response to oxythiamine. Oxythiamine showed only a weak effect in the TKTL-1 expressing cell lines. Over-expression of TKTL-1 is not an indicator for responsiveness to oxythiamine. More specific inhibitors should be tested.

Fugazzola, L., M. Muzza, et al. (2008). "RET genotypes in sporadic medullary thyroid cancer: studies in a large Italian series." Clin Endocrinol (Oxf) **69**(3): 418-25.

BACKGROUND: Highly discrepant data about the different distribution of RET germline single nucleotide polymorphisms (SNPs) among patients with sporadic medullary thyroid cancer (sMTC) and controls are available. DESIGN AND PATIENTS: In the present case-control study, a wide panel of seven RET SNPs has been tested in the largest sMTC series and in a matched control group. RESULTS: None of the investigated polymorphisms show a significantly different distribution in patients with sMTC when compared to controls. Twenty haplotypes and 57 genotypes were generated, and their association with the disease and with the clinical features were statistically evaluated. Interestingly, 14 genotypes were found to be unique to sMTC patients and 25 to controls. Two haplotypes and three genotypes, all including the intronic variants IVS1-126 and IVS14-24, were significantly associated with sMTC patients and with a higher tumour aggression. The functional activity of the only nonsynonymous RET variant (c.2071C > A, G691S) was tested for the first time. Interestingly, Western blot analyses showed that the fraction of Ret9-G691S protein located at the plasma membrane level was overrepresented when compared to Ret9-WT, suggesting facilitated targeting at the cell membrane for this variant. However, no transforming activity was shown in a focus formation assay on cells carrying the Ret9-G691S, against a possible oncogenic role of G691S variant. CONCLUSIONS: RET genotypes including two intronic RET variants were associated with the risk of developing sMTC and to more aggressive behaviour. Further studies are warranted to elucidate whether these RET genotypes are in linkage disequilibrium with another susceptibility gene or whether these variants could play a role in the genesis of sMTC per se.

Furuya, F., C. Lu, et al. (2007). "Inhibition of phosphatidylinositol 3-kinase delays tumor progression and blocks metastatic spread in a mouse model of thyroid cancer." Carcinogenesis **28**(12): 2451-8.

Aberrant activation of the phosphatidylinositol 3-kinase (PI3K)-AKT/protein kinase B-signaling pathway has been associated with multiple human cancers, including thyroid cancer. Recently, we showed that, similar to human thyroid cancer, the PI3K-AKT pathway is overactivated in both the thyroid and metastatic lesions of a mouse model of follicular thyroid carcinoma (TRbeta(PV/PV) mice). This TRbeta(PV/PV) mouse harbors a knockin mutant thyroid hormone receptor beta gene (TRbetaPV mutant) that spontaneously develops thyroid cancer and distant metastasis similar to human follicular thyroid cancer. That the activation of the PI3K-AKT signaling contributes to thyroid carcinogenesis raised the possibility that this pathway could be a potential therapeutic target in follicular thyroid carcinoma. The present study tested this possibility by treating TRbeta(PV/PV) mice with LY294002 (LY), a potent and specific PI3K inhibitor, and evaluating the effect of LY on the spontaneous development of thyroid cancer. LY treatment inhibited the AKT-mammalian target of rapamycin (mTOR)-p70(S6K) signaling, and it decreased cyclin D1 and increased p27(Kip1) expression to inhibit thyroid tumor growth and reduce tumor cell proliferation. LY treatment increased caspase 3 and decreased phosphorylated-BAD to induce apoptosis. In addition, LY treatment reduced the AKT-matrix metalloproteinase 2 signaling to decrease cell motility to block metastatic spread of thyroid tumors. Thus, these altered signaling pathways converged effectively to prolong survival of TRbeta (PV/PV) mice treated with LY. No significant adverse effects were observed for wild-type mice treated similarly with LY. The present study provides the first preclinical evidence for the in vivo efficacy for LY in the treatment of follicular thyroid cancer.

Fusco, A. and M. Santoro (2007). "20 years of RET/PTC in thyroid cancer: clinico-pathological correlations." Arq Bras Endocrinol Metabol **51**(5): 731-5.

The RET/PTC oncogene has been isolated almost twenty years ago. During these years, the research has given a final answer to several questions. In fact, it has been demonstrated that: a) RET/PTC is an early event in the process of thyroid carcinogenesis and has a critical role in the generation of the papillary carcinoma; b) RET/PTC activation is essentially restricted to the papillary histotype and to the Hurthle thyroid tumors; c) its incidence increases after exposure to radiations. However, some questions have not found a final answer yet: a) which is the real frequency of RET/PTC activation? Likely it is around 20%, but this point is still questionable; b) which other gene modifications are required to lead a thyroid cell carrying a RET/PTC oncogene to the malignant phenotype?, and c) is there any correlation between RET/PTC activation and clinical parameters? We hope that these questions will have a clear answer in the near future.

Gandhi, M. S., J. R. Stringer, et al. (2009). "Gene position within chromosome territories correlates with their involvement in distinct rearrangement types in

thyroid cancer cells." Genes Chromosomes Cancer **48**(3): 222-8.

Chromosomal rearrangements in human cancers are of two types, interchromosomal, which are rearrangements that involve exchange between loci located on different chromosomes, and intrachromosomal, which are rearrangements that involve loci located on the same chromosome. The type of rearrangement that typically activates a specific oncogene may be influenced by its nuclear location and that of its partner. In interphase nuclei, each chromosome occupies a distinct three-dimensional (3D) territory that tends to not overlap the territories of other chromosomes. It is also known that after double strand breaks in the genome, mobility of free DNA ends is limited. These considerations suggest that loci located deep within a chromosomal territory might not participate in interchromosomal rearrangements as readily as in intrachromosomal rearrangements. To test this hypothesis, we used fluorescence in situ hybridization with 3D high-resolution confocal microscopy to analyze the positions of six oncogenes known to be activated by recombination in human cancer cells. We found that loci involved in interchromosomal rearrangements were located closer to the periphery of chromosome territories as compared with the loci that were involved in intrachromosomal inversions. The results of this study provide evidence suggesting that nuclear architecture and location of specific genetic loci within chromosome territories may influence their participation in intrachromosomal or interchromosomal rearrangements in human thyroid cells.

Garcia-Rostan, G., A. M. Costa, et al. (2005). "Mutation of the PIK3CA gene in anaplastic thyroid cancer." Cancer Res **65**(22): 10199-207.

The phosphatidylinositol 3'-kinase (PI3K) pathway is frequently activated in thyroid carcinomas through the constitutive activation of stimulatory molecules (e.g., Ras) and/or the loss of expression and/or function of the inhibitory PTEN protein that results in Akt activation. Recently, it has been reported that somatic mutations within the PI3K catalytic subunit, PIK3CA, are common (25-40%) among colorectal, gastric, breast, ovarian cancers, and high-grade brain tumors. Moreover, PIK3CA mutations have a tendency to cluster within the helical (exon 9) and the kinase (exon 20) domains. In this study, 13 thyroid cancer cell lines, 80 well-differentiated thyroid carcinomas of follicular (WDFC) and papillary (WDPC) type, and 70 anaplastic thyroid carcinomas (ATC) were investigated, by PCR-direct sequencing, for activating PIK3CA mutations at exons 9 and 20. Nonsynonymous somatic mutations were found in 16 ATC (23%), two WDFC (8%), and one WDPC (2%). In 18 of the 20 ATC cases showing coexisting differentiated carcinoma, mutations, when present, were restricted to the ATC component and located primarily within the kinase domain. Three cell lines of papillary and follicular lineage (K1, K2, and K5) were also found mutated. In addition, activation of Akt was observed in most of the ATC harboring PIK3CA mutations. These findings indicate that mutant PIK3CA is likely to function as an oncogene among ATC and less frequently well-differentiated thyroid carcinomas. The data also argue for a role of PIK3CA targeting in the treatment of ATC patients.

Garg, M., D. Kanojia, et al. (2009). "Sperm-associated antigen 9: a novel diagnostic marker for thyroid cancer." J Clin Endocrinol Metab **94**(11): 4613-8.

CONTEXT: Cancer-testis antigens are the unique class of testis proteins expressed in tumor but not healthy tissue except testis and might represent ideal targets for the development of novel diagnostics and therapeutic methods in thyroid cancer, which is the most common malignancy of the endocrine system. OBJECTIVE: Our objective was to investigate the clinical relevance of cancer-testis antigen sperm-associated antigen 9 (SPAG9) as early diagnostic and therapeutic target in thyroid cancer. DESIGN, SETTING, AND SUBJECTS: SPAG9 gene and protein expression was determined in thyroid cancer cell lines in 138 thyroid tumor specimens, 60 adjacent noncancerous tissues (ANCT), 22 multinodular goiters (nonneoplastic hyperplasia), and 20 follicular adenoma tissue samples by RT-PCR, in situ RNA hybridization, and immunohistochemistry. Gene silencing approach was used to examine the effects of suppression of SPAG9 protein on cellular growth and colony formation. Humoral immune response against SPAG9 in thyroid cancer patients was analyzed using ELISA. RESULTS: SPAG9 mRNA and protein expression was detected in 78% of the thyroid cancer patients but not multiple goiters and follicular adenoma disease patients. It is interesting to note that majority of early-stage (T1) thyroid cancer patients exhibited higher antibody response against SPAG9. Small interfering RNA-mediated knockdown of SPAG9 expression in thyroid cancer cell significantly reduced cellular growth and colony formation. CONCLUSIONS: SPAG9 expression may play a role in cellular growth and thyroid carcinogenesis. These findings support a potential role for SPAG9 as diagnostic biomarker as well as a possible therapeutic target in thyroid cancer treatment.

Ghoneim, C., M. Soula-Rothhut, et al. (2007). "Activating transcription factor-1-mediated

hepatocyte growth factor-induced down-regulation of thrombospondin-1 expression leads to thyroid cancer cell invasion." J Biol Chem **282**(21): 15490-7.

Hepatocyte growth factor (HGF) plays a major role in the pathogenesis of a variety of human epithelial tumors including papillary carcinoma of the thyroid. Previous reports demonstrated that HGF, acting through the Met receptor, repressed thrombospondin-1 (TSP-1) expression. To study the mechanisms by which HGF down-regulated TSP-1 expression, we transiently transfected a panel of deleted human TSP-1 promoter reporter plasmids into papillary thyroid carcinoma cells. We identified a region between -1210 and -1123 bp relative to the transcription start site that is responsive to HGF treatment and harbors a cAMP-responsive element (CRE) at position -1199 (TGACGTCC). Overexpression of various members of the CRE-binding protein family identified activating transcription factor-1 (ATF-1) as the transcription factor responsible for HGF-induced repression of TSP-1 promoter activity. This inhibition was associated with a concomitant increase in the abundance of nuclear ATF-1 protein. Gel shift and antibody supershift studies indicated that ATF-1 was involved in DNA binding to the TSP-1-CRE site. Finally, we utilized small hairpin RNA to target ATF-1 and showed that these small interfering RNA constructs significantly inhibited ATF-1 expression at both the RNA and the protein level. ATF-1 knockdown prevented HGF-induced down-regulation of TSP-1 promoter activity and protein expression and also reduced HGF-dependent tumor cell invasion. Taken together, our results indicate that HGF-induced down-regulation of TSP-1 expression is mediated by the interaction of ATF-1 with the CRE binding site in the TSP-1 promoter and that this transcription factor plays a crucial role for tumor invasiveness in papillary carcinoma of the thyroid triggered by HGF.

Greco, A., M. G. Borrello, et al. (2009). "Molecular pathology of differentiated thyroid cancer." Q J Nucl Med Mol Imaging **53**(5): 440-53.

Thyroid cancer is the most common endocrine malignancy; it accounts for approximately 1% of all new case of cancer each year, and its incidence has increased significantly over the last few decades. The majority of thyroid tumors originate from follicular epithelial cells. Among them, papillary (PTC) and follicular carcinomas (FTC) represent the most common forms of differentiated thyroid cancer and account for approximately 80% and 15% of all cases, respectively. Specific genetic lesions are associated to each thyroid tumor histotype: BRAF mutations and RET/PTC and TRK oncogenes have been detected in PTC, whereas FTC is characterized by PAX8/PPARgamma rearrangements and RAS mutations. In this review we summarize studies on the molecular biology of the differentiated thyroid tumors, with particular interest in the associated genetic lesions and their role in thyroid carcinogenesis. We also report recent findings on gene expression and miRNA profiles of PTC and FTC.

Griffith, O. L., A. Melck, et al. (2006). "Meta-analysis and meta-review of thyroid cancer gene expression profiling studies identifies important diagnostic biomarkers." J Clin Oncol **24**(31): 5043-51.

PURPOSE: An estimated 4% to 7% of the population will develop a clinically significant thyroid nodule during their lifetime. In many cases, preoperative diagnoses by needle biopsy are inconclusive. Thus, there is a clear need for improved diagnostic tests to distinguish malignant from benign thyroid tumors. The recent development of high-throughput molecular analytic techniques should allow the rapid evaluation of new diagnostic markers. However, researchers are faced with an overwhelming number of potential markers from numerous thyroid cancer expression profiling studies. MATERIALS AND METHODS: To address this challenge, we have carried out a comprehensive meta-review of thyroid cancer biomarkers from 21 published studies. A gene ranking system that considers the number of comparisons in agreement, total number of samples, average fold-change and direction of change was devised. RESULTS: We have observed that genes are consistently reported by multiple studies at a highly significant rate ($P < .05$). Comparison with a meta-analysis of studies reprocessed from raw data showed strong concordance with our method. CONCLUSION: Our approach represents a useful method for identifying consistent gene expression markers when raw data are unavailable. A review of the top 12 candidates revealed well known thyroid cancer markers such as MET, TFF3, SERPINA1, TIMP1, FN1, and TPO as well as relatively novel or uncharacterized genes such as TGFA, QPCT, CRABP1, FCGBP, EPS8 and PROS1. These candidates should help to develop a panel of markers with sufficient sensitivity and specificity for the diagnosis of thyroid tumors in a clinical setting.

Guan, H., M. Ji, et al. (2009). "Association of high iodine intake with the T1799A BRAF mutation in papillary thyroid cancer." J Clin Endocrinol Metab **94**(5): 1612-7.

CONTEXT: Epidemiological studies have indicated that high iodine intake might be a risk factor for papillary thyroid cancer (PTC), which commonly harbors the oncogenic T1799A BRAF mutation. OBJECTIVE: The objective of the study was to

investigate the relationship between BRAF mutation in PTC and iodine intake in patients. SUBJECTS AND METHODS: We analyzed and compared the prevalences of the T1799A BRAF mutation in classical PTC of 1032 patients from five regions in China that uniquely harbor different iodine contents in natural drinking water, ranging from normal (10-21 microg/liter) to high (104-287 microg/liter). The BRAF mutation was identified by direct DNA sequencing. RESULTS: The prevalence of BRAF mutation was significantly higher in any of the regions with high iodine content than any of the regions with normal iodine content. Overall, BRAF mutation was found in 387 of 559 PTC with high iodine content (69%) vs. 252 of 473 PTC with normal iodine content (53%), with an odds ratio of 1.97 (95% confidence interval 1.53-2.55) for the association of BRAF mutation with high iodine content ($P < 0.0001$). In addition, clinicopathological correlation analysis, the largest one of its type ever, showed that BRAF mutation was significantly associated with extrathyroidal invasion, lymph node metastasis, and advanced tumor stages of PTC. CONCLUSIONS: High iodine intake seems to be a significant risk factor for the occurrence of BRAF mutation in thyroid gland and may therefore be a risk factor for the development of PTC. This large study also confirmed the association of BRAF mutation with poorer clinicopathological outcomes of PTC.

Guan, H., M. Ji, et al. (2008). "Hypermethylation of the DNA mismatch repair gene hMLH1 and its association with lymph node metastasis and T1799A BRAF mutation in patients with papillary thyroid cancer." Cancer **113**(2): 247-55.

BACKGROUND: It remains to be investigated whether the aberrant methylation of DNA repair genes plays a pathogenic role in BRAF mutation-promoted tumorigenesis of papillary thyroid cancer (PTC). METHODS: In the current study, the promoter methylation status of 23 DNA repair genes in relation to clinicopathologic characteristics and BRAF mutation was examined in PTC tumors using methylation-specific polymerase chain reaction. RESULTS: Among the 38 PTC tumors examined, 3 of 23 DNA repair genes were hypermethylated, including the hMLH1 gene in 8 of 38 samples (21%), the PCNA gene in 5 of 38 samples (13%), and the OGG1 gene in 2 of 38 samples (5%). Methylation of these genes was also found in some thyroid cancer cell lines. Methylation of the hMLH1 gene in particular was found to be associated with lymph node metastasis of PTC (5 of 8 samples [63%] in the methylation group vs 3 of 30 samples [10%] in the nonmethylation group; P = .0049). Methylation of the hMLH1 gene was also found to be associated with the T1799A BRAF mutation in PTC (6 of 19 samples (32%) in the BRAF mutation-positive group vs 2 of 19 samples (11%) in the BRAF mutation-negative group; P = .042). CONCLUSIONS: The data from the current study suggest that, as shown previously in colon cancer, aberrant methylation of the hMLH1 gene may play a role in BRAF mutation-promoted thyroid tumorigenesis.

Gudmundsson, J., P. Sulem, et al. (2009). "Common variants on 9q22.33 and 14q13.3 predispose to thyroid cancer in European populations." Nat Genet **41**(4): 460-4.

In order to search for sequence variants conferring risk of thyroid cancer we conducted a genome-wide association study in 192 and 37,196 Icelandic cases and controls, respectively, followed by a replication study in individuals of European descent. Here we show that two common variants, located on 9q22.33 and 14q13.3, are associated with the disease. Overall, the strongest association signals were observed for rs965513 on 9q22.33 (OR = 1.75; P = 1.7 x 10(-27)) and rs944289 on 14q13.3 (OR = 1.37; P = 2.0 x 10(-9)). The gene nearest to the 9q22.33 locus is FOXE1 (TTF2) and NKX2-1 (TTF1) is among the genes located at the 14q13.3 locus. Both variants contribute to an increased risk of both papillary and follicular thyroid cancer. Approximately 3.7% of individuals are homozygous for both variants, and their estimated risk of thyroid cancer is 5.7-fold greater than that of noncarriers. In a study on a large sample set from the general population, both risk alleles are associated with low concentrations of thyroid stimulating hormone (TSH), and the 9q22.33 allele is associated with low concentration of thyroxin (T(4)) and high concentration of triiodothyronine (T(3)).

Guilhen, A. C., N. E. Bufalo, et al. (2009). "Role of the N-acetyltransferase 2 detoxification system in thyroid cancer susceptibility." Clin Cancer Res **15**(1): 406-12.

PURPOSE: Genetic polymorphisms in genes encoding for enzymes involved in the biotransformation of carcinogens have been shown to be relevant as risk for cancer and may be of considerable importance from a public health point of view. Considering that N-acetyltransferase 2 (NAT2) polymorphisms modulate the response to ionizing radiation, the strongest risk factor recognized to cause differentiated thyroid cancer (DTC) thus far, we sought to determine the influence of NAT2 detoxification system on thyroid cancer susceptibility. EXPERIMENTAL DESIGN: We conducted a prospective case-control study, comparing 195 patients presenting with DTC that were previously

genotyped for GSTT1, GSTM1, GSTP1, and CYP1A1, comprising 164 papillary carcinomas and 31 follicular carcinomas, with 196 control individuals paired for gender, age, ethnicity, diet routine, lifetime occupational history, smoking history, general health conditions, and previous diseases. We used PCR-RFLP assays and the combination of 6 variant alleles to define 18 NAT2 haplotypes that characterized slow, intermediate, or rapid phenotypes. RESULTS: A multivariate logistic regression analysis identified the presence of *12A and the absence of *12B, *13, *14B, *14D, *6A, and *7A NAT2 haplotypes as risk factors for DTC. The inheritance of a rapid acetylation phenotype doubled the risk for a papillary carcinoma (odds ratio, 2.024; 95% confidence interval, 1.252-3.272). We found no relationship between genotypes and clinical, pathologic, or laboratory features of patients or between genotypes and outcome. CONCLUSIONS: We showed that NAT2 genotypes and the NAT2 rapid acetylation phenotype are important susceptibility factors for DTC, suggesting that NAT2 detoxification system is involved in this tumor pathogenesis.

Gupta, M. and S. Y. Chia (2007). "Circulating thyroid cancer markers." Curr Opin Endocrinol Diabetes Obes **14**(5): 383-8.

PURPOSE OF REVIEW: To describe the progress in the field of circulating markers of thyroid cancer. RECENT FINDINGS: Thyroid cancer cells in the circulation can be detected by measuring the mRNA of thyroid-specific genes. Among these, thyroglobulin, and more recently thyroid-stimulating hormone receptor mRNAs' provide high diagnostic sensitivity and specificity for thyroid cancer detection. These markers can be used in synergy with current diagnostic modalities, i.e. fine-needle aspiration and ultrasound, for preoperative diagnosis and serum thyroglobulin measurement for monitoring. SUMMARY: For the detection of recurrent/residual thyroid cancer, serum thyroglobulin remains the sole circulating marker, but lacks sensitivity and is unreliable in the presence of antithyroglobulin antibodies. The measurement of thyroid-specific mRNA in blood may provide sensitive/specific markers, but significant variability exists among various studies for thyroglobulin mRNA in particular, questioning the validity of this marker. Recent studies have demonstrated the high sensitivity and specificity of thyroid-stimulating hormone receptor mRNA in detecting recurrent/residual disease even in the presence of thyroglobulin antibodies. Fine-needle aspiration biopsy is currently the sole method for evaluating thyroid nodules. Indeterminate fine-needle aspiration cytology is found in approximately 15-30% of specimens. Thyroid-stimulating hormone receptor mRNA measurement in patients with indeterminate fine-needle aspiration may enhance cancer detection and save unnecessary surgeries.

Hall, L. C., E. P. Salazar, et al. (2008). "Effects of thyroid hormones on human breast cancer cell proliferation." J Steroid Biochem Mol Biol **109**(1-2): 57-66.

The involvement of estrogens in breast cancer development and growth has been well established. However, the effects of thyroid hormones and their combined effects with estrogens are not well studied. We investigated the response of human breast cancer cells to thyroid hormone, particularly the role of T3 in mediating cell proliferation and gene expression. We demonstrated that 17beta-estradiol (E2) or triiodothyronine (T3) promoted cell proliferation in a dose-dependent manner in both MCF-7 and T47-D cell lines. The E2- or T3-dependent cell proliferation was suppressed by co-administration of the ER antagonist ICI. We also demonstrated that T3 could enhance the effect of E2 on cell proliferation in T47-D cells. Using an estrogen response element (ERE)-mediated luciferase assay, we determined that T3 was able to induce the activation of ERE-mediated gene expression in MCF-7 cells, although the effects were much weaker than that induced by E2. These results suggest that T3 can promote breast cancer cell proliferation and increase the effect of E2 on cell proliferation in some breast cancer cell lines and thus that T3 may play a role in breast cancer development and progression.

Hamatani, K., H. Eguchi, et al. (2008). "RET/PTC rearrangements preferentially occurred in papillary thyroid cancer among atomic bomb survivors exposed to high radiation dose." Cancer Res **68**(17): 7176-82.

A major early event in papillary thyroid carcinogenesis is constitutive activation of the mitogen-activated protein kinase signaling pathway caused by alterations of a single gene, typically rearrangements of the RET and NTRK1 genes or point mutations in the BRAF and RAS genes. In childhood papillary thyroid cancer, regardless of history of radiation exposure, RET/PTC rearrangements are a major event. Conversely, in adult-onset papillary thyroid cancer among the general population, the most common molecular event is BRAF(V600E) point mutation, not RET/PTC rearrangements. To clarify which gene alteration, chromosome aberration, or point mutation preferentially occurs in radiation-associated adult-onset papillary thyroid cancer, we have performed molecular analyses on RET/PTC rearrangements and BRAF(V600E) mutation in 71 papillary thyroid cancer cases among atomic bomb survivors (including

21 cases not exposed to atomic bomb radiation), in relation to radiation dose as well as time elapsed since atomic bomb radiation exposure. RET/PTC rearrangements showed significantly increased frequency with increased radiation dose (P(trend) = 0.002). In contrast, BRAF(V600E) mutation was less frequent in cases exposed to higher radiation dose (P(trend) < 0.001). Papillary thyroid cancer subjects harboring RET/PTC rearrangements developed this cancer earlier than did cases with BRAF(V600E) mutation (P = 0.03). These findings were confirmed by multivariate logistic regression analysis. These results suggest that RET/PTC rearrangements play an important role in radiation-associated thyroid carcinogenesis.

Hassan, I., A. Wunderlich, et al. (2006). "Antisense p53 oligonucleotides inhibit proliferation and induce chemosensitivity in follicular thyroid cancer cells." Anticancer Res **26**(2A): 1171-6.

BACKGROUND: The potential of MTp53 knockout by oligodesoxyribonucleotide phosphothioates (ODNs) to affect proliferation, apoptosis and chemosensitivity in undifferentiated thyroid cancer (UTC) cells with a recessive MTp53 mutation was evaluated. MATERIALS AND METHODS: Transient transfections with ODNs complementary to p53 and control ODN (HIV-RT) were carried out in FTC 133 cells. In vitro proliferation was evaluated by cell counting of 10 random fields and by the MTT assay. A single pulse of 100 microg/ml Cytarabine was added to each well and the cells were incubated for an additional day. Chemosensitivity was calculated as the ratio of apoptotic and necrotic cells versus viable cells by flow cytometry (FACS). RESULTS: Transfection of UTC cells with ODN decreased the cell number by up to 70% ($p < 0.002$). The proliferation rate also decreased up to 35% ($p < 0.03$), without inducing apoptosis. ODNs rendered FTC cells sensitive to treatment with Cytarabine, inducing apoptosis in 35% of cells, as compared to 17% of cells transfected with the reverse transcriptase gene of HIV (ODN-HIV) and less than 10% of non-transfected cells ($p < 0.05$). CONCLUSION: Transient MTp53 knockout with ODNs complementary to p53 nucleotide sequences inhibited proliferation and increased chemosensitivity in the UTC cell line FTC133.

Hassan, I., A. Wunderlich, et al. (2006). "Antisense p53 decreases production of VEGF in follicular thyroid cancer cells." Endocrine **29**(3): 409-12.

Inactivating mutations of wild-type p53 (WTp53) tumor suppressor gene are common in anaplastic thyroid cancer (ATC) and are associated with poor prognosis. Mutated p53 (MTp53) has been implicated with angiogenesis. Therefore, the potential of MTp53 knockout by oligodeoxyribonucleotide phosphorothioates (ODNs) to affect VEGF production of undifferentiated thyroid cancer cells with a recessive MTp53 mutation was evaluated. Transient transfection with 20 bp ODNs complementary to portions of exon 10 of p53 and a negative control ODN (HIV-RT) were carried out in FTC-133 cells. In vitro secretion of VEGF protein was quantified by EIA and correlated to cell numbers, which was evaluated by in vitro MTT assay. Transfection of undifferentiated thyroid cancer cells with ODN reduced VEGF secretion of FTC-133 cells following transfection by 34% as compared to the negative control (cells transfected with ODN-HIV; $p = 0.03$). These results suggest that transient MTp53 knockout with ODNs complementary to p53 nucleotide sequences impair secretion of VEGF in the undifferentiated thyroid cancer cell line FTC-133.

Henderson, Y. C., M. J. Fredrick, et al. (2007). "Differential responses of human papillary thyroid cancer cell lines carrying the RET/PTC1 rearrangement or a BRAF mutation to MEK1/2 inhibitors." Arch Otolaryngol Head Neck Surg **133**(8): 810-5.

OBJECTIVES: To examine the effects of 2 mitogen-activated protein kinase kinase (MEK1/2) inhibitors on papillary thyroid carcinoma (PTC) cell lines carrying the RET/PTC1 rearrangement or a BRAF mutation. In PTC, RET/PTC1 rearrangement or BRAF mutations results in constitutional activation of RET kinase or BRAF, respectively. Along the RET or BRAF signaling cascades, the activated RET kinase or BRAF activates MEK1/2, and then mitogen-activated protein kinases (extracellular signal-related kinase 1/2 [ERK1/2]) is activated. Activated ERK1/2 enters the nucleus and phosphorylates a variety of transcription factors, resulting in cancer cell proliferation. The MEK1/2 inhibitors, PD98059 and U0126, have been shown to inhibit cell growth in other cancers. DESIGN: In vitro study. SUBJECTS: Papillary thyroid carcinoma cell lines carrying the RET/PTC1 rearrangement (BHP2-7) or a BRAF mutation (BHP5-16). INTERVENTION: We treated PTC cells carrying the RET/PTC1 rearrangement or a BRAF mutation with 2 MEK1/2 inhibitors (PD98059 and U0126). MAIN OUTCOME MEASURES: Using Western blot analysis, we detected the expression of phosphorylated ERK1/2 and expression of cleaved poly(ADP-ribose) polymerase (PARP) in cells after treatment with either inhibitors. Growth inhibition was monitored by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. RESULTS: Using Western blot analysis, we detected the dephosphorylation of ERK1/2 in PTC cells

carrying the RET/PTC1 rearrangement or a BRAF mutation after treating the cells with 2 MEK1/2 inhibitors (PD98059 and U0126). In addition, both PD98059 and U0126 completely inhibited the growth of the PTC cells carrying a BRAF mutation but partially inhibited the growth of the PTC cells carrying the RET/PTC1 rearrangement. Finally, we observed PARP cleavage only in cells with a BRAF mutation in the Western blot analysis. CONCLUSION: These data suggested that treatment with MEK1/2 inhibitors can be used as tools for inhibiting the growth of PTC cells.

Hernandez, A., N. Xamena, et al. (2008). "Role of GST and NAT2 polymorphisms in thyroid cancer." J Endocrinol Invest **31**(11): 1025-31.

Genetic polymorphisms have shown to be susceptibility factors playing an important role in the development of most cancers. Nevertheless, as far as we know, only few studies have been conducted linking thyroid cancer incidence and GST polymorphisms, and no data are available on the possible association between NAT2 polymorphisms and thyroid cancer risk. The possible relationship between polymorphism at the GSTM1, GSTT1, GSTP1, and NAT2 genes and increased susceptibility to thyroid cancer has been evaluated in 176 thyroid cancer patients and 167 healthy controls, all from the urban district of Barcelona (Spain). The results indicate a clear role of the C481T change, present in several NAT2*5 alleles [odds ratio (OR)=0.58; 95% confidence interval (95% CI)=0.35-0.98]. Thus, those individuals carrying this change are less prone to develop thyroid cancer, mainly of the papillary type. In addition, there is a tendency towards the over-representation of the GSTM1 null genotype among thyroid cancer patients, particularly in those patients with papillary type tumor. The same is observed for the GSTM1 and GSTT1 null genotypes combination, and for other combinations with different NAT2 polymorphisms. The combinations involving the NAT2*6 and NAT2*7 genotypes showed the most important effect, and individuals carrying both alleles present a higher risk of thyroid cancer (OR=7.36; 95% CI=0.85-63.47), mainly for the follicular type (OR=17.94; 95% CI=1.34-238.70). The combination of NAT2*5 with NAT2*7 was also found to increase 5.26 (95% CI=1.07-25.76) times the risk of thyroid cancer. In conclusion, our results show that NAT2 polymorphisms play a significant role in thyroid cancer risk modulation.

Hishinuma, A., S. Fukata, et al. (2005). "High incidence of thyroid cancer in long-standing goiters with thyroglobulin mutations." Thyroid **15**(9): 1079-84.

In this paper, we report the high prevalence of thyroid malignancy in patients with thyroglobulin mutations in Japan. Mutations of the thyroglobulin gene cause defective thyroid hormone synthesis, resulting in congenital hypothyroidism. Since our report in 1999 on the thyroglobulin mutations C1264R and C1996S, we have identified 14 adult patients (7 males and 7 females) from 9 unrelated families. They visited hospitals for treatment of huge goiters that first appeared in childhood. Persistent growth of the thyroid gland, probably caused by thyrotropin (TSH) stimulation, partially compensated thyroid hormone production, resulting in lowered serum TSH concentrations in turn. Consequently, many patients had to undergo multiple operations. Of 11 patients who had undergone surgery, 7 had thyroid cancers. Of five patients whose thyroid tissue was available, we found a heterozygous activating mutation, either V599E or K600E, in cancerous tissue from each of 2 patients. From these observations, we conclude that goiter resulting from thyroglobulin mutations is associated with thyroid cancer.

Hoffmann, S., K. Maschuw, et al. (2006). "Functional thyrotropin receptor attenuates malignant phenotype of follicular thyroid cancer cells." Endocrine **30**(1): 129-38.

Thyrotropin (TSH) is a thyroid-specific growth factor inducing differentiated function and growth of thyrocytes in vitro. In thyroid cancer, loss of TSH-receptor (TSHR) expression is a sign of de-differentiation and is believed to contribute to the malignant phenotype. The present studies aimed to determine the in vitro and in vivo effects of functioning TSHR in the follicular thyroid cancer cell line HTC, a subclone of FTC133 cells, lacking endogenous expression of TSHR, and HTCtshr+ cells transfected with human TSHR-cDNA. HTCtshr+ cells grew faster in vitro (doubling time 1.15 vs 1.56 d, $p < 0.05$) and TSH caused a dose-dependent growth response. Adhesion to and invasion through reconstituted basement membrane were reduced in HTCtshr+ cells, but when stimulated with TSH increased to levels comparable to naive HTC cells. In vivo, tumor latency was 11 d for naive HTC as compared to 21 d for HTCtshr+ xenografts. Smaller tumor volumes were registered for HTCtshr+ cells (250 +/- 217 vs 869 +/- 427 mm3, $p < 0.05$). Angiogenesis, as determined by vascular surface density (VSD) of experimental tumors, was enhanced in naive HTC tumors (VSD 0.87 +/- 0.1 microm-1 vs 0.55 +/- 0.2 microm-1 in HTCtshr+, $p < 0.05$). VEGF secretion was more pronounced in naive HTC cells stimulated with EGF, than in HTCtshr+ cells stimulated with either TSH or EGF. In conclusion, regained expression of functional TSHR in the

follicular thyroid cancer cell line HTC alters in vitro features commonly associated with the malignant phenotype. Smaller tumors and reduced angiogenesis of xenotransplanted HTC cells with functioning TSHR suggest a less aggressive in vivo phenotype. The present data highlight the pivotal role of TSHR to affect transformed thyrocytes in vitro and in vivo. They also suggest a role for EGF as a modulator of angiogenesis in thyrocytes devoid of TSHR.

Horkko, T. T., K. Tuppurainen, et al. (2006). "Thyroid hormone receptor beta1 in normal colon and colorectal cancer-association with differentiation, polypoid growth type and K-ras mutations." Int J Cancer **118**(7): 1653-9.

The precursors for colorectal cancer include polypoid (conventional), flat and serrated adenomas. Polypoid growth in polypoid adenomas and serrated adenomas is associated with K-ras mutations. The regulation of polypoid or nonpolypoid growth is not well known, but could be related to trophic stimuli, such as thyroid hormones. Hence, we investigated the expression pattern of thyroid hormone receptor TRbeta1 in colorectal mucosa and in colorectal tumours and its relationship to tumour growth type. One hundred fourteen colorectal carcinoma specimens were evaluated for TRbeta1. Normal mucosa, adjacent adenomatous component (N = 46) and lymph node metastases (N = 28) were analysed when present, and the results were confirmed by Western blot analysis in selected cases. Nuclear TRbeta1 was almost always present in normal epithelium (96%), but less frequent in adenomas (83%) and in cancer (68%; $p < 0.001$ and $p < 0.001$, respectively). TRbeta1 was associated with polypoid growth, presence of K-ras mutations and also with a higher WHO histological grade and advanced Dukes' stage. Cytoplasmic expression of TRbeta1 was observed in nonneoplastic and neoplastic epithelium. In Western blot analysis, a 58 kDa band corresponding to TRbeta1 was expressed in normal mucosa and in colorectal cancer specimens with positive immunohistochemistry. Association of TRbeta1 expression with growth pattern and the presence of K-ras mutations suggest that abnormalities in thyroid hormone signalling involving TRbeta1 play a role in the development of some types of colorectal adenocarcinomas.

Hou, P., D. Liu, et al. (2007). "Genetic alterations and their relationship in the phosphatidylinositol 3-kinase/Akt pathway in thyroid cancer." Clin Cancer Res **13**(4): 1161-70.

PURPOSE: To investigate the overall occurrence and relationship of genetic alterations in the phosphatidylinositol 3-kinase (PI3K)/Akt pathway in thyroid tumors and explore the scope of this pathway as a therapeutic target for thyroid cancer. EXPERIMENTAL DESIGN: We examined collectively the major genetic alterations and their relationship in this pathway, including PIK3CA copy number gain and mutation, Ras mutation, and PTEN mutation, in a large series of primary thyroid tumors. RESULTS: Occurrence of any of these genetic alterations was found in 25 of 81 (31%) benign thyroid adenoma (BTA), 47 of 86 (55%) follicular thyroid cancer (FTC), 21 of 86 (24%) papillary thyroid cancer (PTC), and 29 of 50 (58%) anaplastic thyroid cancer (ATC), with FTC and ATC most frequently harboring these genetic alterations. PIK3CA copy gain was associated with increased PIK3CA protein expression. A mutual exclusivity among these genetic alterations was seen in BTA, FTC, and PTC, suggesting an independent role of each of them through the PI3K/Akt pathway in the tumorigenesis of the differentiated thyroid tumors. However, coexistence of these genetic alterations was increasingly seen with progression from differentiated tumor to undifferentiated ATC. Their coexistence with BRAF mutation was also frequent in PTC and ATC. CONCLUSIONS: The data provide strong genetic implication that aberrant activation of PI3K/Akt pathway plays an extensive role in thyroid tumorigenesis, particularly in FTC and ATC, and promotes progression of BTA to FTC and to ATC as the genetic alterations of this pathway accumulate. Progression of PTC to ATC may be facilitated by coexistence of PI3K/Akt pathway-related genetic alterations and BRAF mutation. The PI3K/Akt pathway may thus be a major therapeutic target in thyroid cancers.

Hsiao, P. J., M. Y. Lu, et al. (2007). "Vascular endothelial growth factor gene polymorphisms in thyroid cancer." J Endocrinol **195**(2): 265-70.

Vascular endothelial growth factor (VEGF) is a potent stimulator for angiogenesis. It has been implicated in growth and metastasis of thyroid cancer. Three functional single nucleotide polymorphisms (SNPs) of VEGF (-2578C/A, -634G/C, and +936C/T) are known to be related with VEGF expression. We conducted a case-control study to evaluate the genetic effects of these three functional SNPs on the development of thyroid cancer and lymph node metastasis. A total of 332 cases and 261 controls were recruited for this study. The genotypes were determined by the TaqMan 5'-nuclease assay. Hardy-Weinberg equilibrium (HWE) was tested for each SNP, and genetic effects were evaluated by the chi(2)-test and multiple logistic regression. We used Bonferroni correction to account for multiple testing, and a two-tailed P value <0.017 was considered statistically significant. All three SNPs were in HWE.

The A allele of -2578C/A (i.e. SNP rs699947) increased a risk for thyroid cancer (adjusted OR=136, 95% CI=1.02-1.81, P=0.039). Haplotype analysis yielded a less significant result (an empirical P value of 0.07). There was a tendency of increasing the frequency of the risk allele from controls, patients without lymph node metastasis to patients with lymph node metastasis (P(trend)=0.019). Further analysis showed that the genetic effect was only in men (adjusted OR=1.97, 95% CI=1.16-3.37, P=0.013) but not in women (adjusted OR=1.15, 95% CI=0.81-1.62, P=0.435). The other two SNPs did not show significant results. The A allele of the SNP rs699947 increased the risk of thyroid cancer development and regional lymph node metastasis in men.

Hu, S., M. Ewertz, et al. (2006). "Detection of serum deoxyribonucleic acid methylation markers: a novel diagnostic tool for thyroid cancer." J Clin Endocrinol Metab **91**(1): 98-104.

CONTEXT: Serum DNA methylation markers may potentially be useful in diagnosing thyroid cancer and monitoring its recurrence. OBJECTIVE: The objective of the study was to assess the utility of serum DNA methylation as a diagnostic test for patients with thyroid nodules and a monitoring test to detect thyroid cancer recurrence in previously treated patients. DESIGN, SETTING, AND SUBJECTS: Using real-time quantitative methylation-specific PCR, we analyzed the methylation status of five genes (CALCA, CDH1, TIMP3, DAPK, and RARbeta2) on 96 bisulfite-treated serum DNA samples isolated preoperatively from either solid thyroid nodule patients or patients in follow-up for history of treated thyroid cancer. MAIN OUTCOME MEASURE: Diagnostic sensitivity, specificity, and accuracy of serum DNA methylation marker for thyroid cancer were measured. RESULTS: For the patients with thyroid nodules, when a positive result was defined by a serum methylation level above the appropriately chosen cutoff value for any one of the five genes, the preoperative diagnostic sensitivity for thyroid cancer was 68% (26 of 38), the specificity was 95% (18 of 19), and the overall preoperative diagnostic accuracy was 77%, with positive and negative predictive values of 96 and 60%, respectively. In a subset of patients with cytologically indeterminate thyroid nodules, serum DNA methylation testing could correctly diagnose eight of 11 (73%) cancers and four of four (100%) benign tumors, with a diagnostic accuracy of 80%. We also analyzed these serum DNA methylation markers in 39 previously treated thyroid cancer patients. Among the 10 patients proved to have recurrent disease by conventional measures, seven (70%) were positive on methylation testing. Among the 29 patients who had no corroboration of residual or recurrent disease by conventional studies, six (21%) were positive for serum DNA methylation markers. CONCLUSIONS: We have demonstrated the potential usefulness of serum DNA methylation markers as a novel tool for differential diagnosis of solid thyroid nodules and thyroid cancer recurrence monitoring.

Hu, S., D. Liu, et al. (2006). "Association of aberrant methylation of tumor suppressor genes with tumor aggressiveness and BRAF mutation in papillary thyroid cancer." Int J Cancer **119**(10): 2322-9.

The role of aberrant tumor suppressor gene methylation in the aggressiveness of papillary thyroid cancer (PTC) has not been documented. By showing promoter methylation-induced gene silencing in PTC-derived cell lines, we first demonstrated the functional consequence of methylation of several recently identified tumor suppressor genes, including those for tissue inhibitor of metalloproteinase-3 (TIMP3), SLC5A8, death-associated protein kinase (DAPK) and retinoic acid receptor beta2 (RARbeta2). We then investigated the role of methylation of these genes in the aggressiveness of PTC by examining the relationship of their aberrant methylation to clinicopathological characteristics and BRAF mutation in 231 primary PTC tumors. Methylation of TIMP3, SLC5A8 and DAPK was significantly associated with several aggressive features of PTC, including extrathyroidal invasion, lymph node metastasis, multifocality and advanced tumor stages. Methylation of these genes was also significantly associated with BRAF mutation in PTC, either individually or collectively in various combinations. Methylation of these genes, either individually or collectively, occurred more frequently in more aggressive classical and tall-cell PTC subtypes than in less aggressive follicular-variant PTC, with the latter known to infrequently harbor BRAF mutation. Several other tumor suppressor genes investigated were not methylated. These results suggest that aberrant methylation and hence silencing of TIMP3, SLC5A8, DAPK and RARbeta2, in association with BRAF mutation, may be an important step in PTC tumorigenesis and progression.

Jarzab, B., M. Wiench, et al. (2005). "Gene expression profile of papillary thyroid cancer: sources of variability and diagnostic implications." Cancer Res **65**(4): 1587-97.

The study looked for an optimal set of genes differentiating between papillary thyroid cancer (PTC) and normal thyroid tissue and assessed the sources of variability in gene expression profiles. The analysis was done by oligonucleotide microarrays (GeneChip HG-U133A) in 50 tissue samples taken

intraoperatively from 33 patients (23 PTC patients and 10 patients with other thyroid disease). In the initial group of 16 PTC and 16 normal samples, we assessed the sources of variability in the gene expression profile by singular value decomposition which specified three major patterns of variability. The first and the most distinct mode grouped transcripts differentiating between tumor and normal tissues. Two consecutive modes contained a large proportion of immunity-related genes. To generate a multigene classifier for tumor-normal difference, we used support vector machines-based technique (recursive feature replacement). It included the following 19 genes: DPP4, GJB3, ST14, SERPINA1, LRP4, MET, EVA1, SPUVE, LGALS3, HBB, MKRN2, MRC2, IGSF1, KIAA0830, RXRG, P4HA2, CDH3, IL13RA1, and MTMR4, and correctly discriminated 17 of 18 additional PTC/normal thyroid samples and all 16 samples published in a previous microarray study. Selected novel genes (LRP4, EVA1, TMPRSS4, QPCT, and SLC34A2) were confirmed by Q-PCR. Our results prove that the gene expression signal of PTC is easily detectable even when cancer cells do not prevail over tumor stroma. We indicate and separate the confounding variability related to the immune response. Finally, we propose a potent molecular classifier able to discriminate between PTC and nonmalignant thyroid in more than 90% of investigated samples.

Jazdzewski, K., S. Liyanarachchi, et al. (2009). "Polymorphic mature microRNAs from passenger strand of pre-miR-146a contribute to thyroid cancer." Proc Natl Acad Sci U S A **106**(5): 1502-5.

Prior work has shown that heterozygosity G/C of single nucleotide polymorphism (SNP rs2910164) within the precursor of microRNA-146a predisposes to PTC (odds ratio = 1.62, P = 0.000007) although the mechanism was unclear. Here, we show that GC heterozygotes differ from both GG and CC homozygotes by producing 3 mature microRNAs: 1 from the leading strand (miR-146a), and 2 from the passenger strand (miR-146a*G and miR-146a*C), each with its distinct set of target genes. TaqMan analysis of paired tumor/normal samples revealed 1.5- to 2.6-fold overexpression of polymorphic miR-146a* in 7 of 8 tumors compared with the unaffected part of the same gland. The microarray data showed that widely different transcriptomes occurred in the tumors and in unaffected parts of the thyroid from GC and GG patients. The modulated genes are mainly involved in regulation of apoptosis leading to exaggerated DNA-damage response in heterozygotes potentially explaining the predisposition to cancer. We propose that contrary to previously held views transcripts from the passenger strand of miRs can profoundly affect the downstream effects. Heterozygosity for polymorphisms within the premiR sequence can cause epistasis through the production of additional mature miRs. We propose that mature miRs from the passenger strand may regulate many genetic processes.

Jonklaas, J., H. Nsouli-Maktabi, et al. (2008). "Endogenous thyrotropin and triiodothyronine concentrations in individuals with thyroid cancer." Thyroid **18**(9): 943-52.

BACKGROUND: Thyroid hormone suppression therapy is associated with decreased recurrence rates and improved survival in patients with differentiated thyroid cancer. Recently higher baseline thyrotropin (TSH) levels have been found to be associated with a postoperative diagnosis of differentiated thyroid cancer. Our objective was to confirm whether preoperative TSH levels were higher in patients who were diagnosed with differentiated thyroid cancer after undergoing thyroidectomy, compared with patients who were found to have benign disease. We also sought to determine whether thyroid hormone levels were lower in the patients with malignancy. METHODS: The study was a retrospective analysis of a prospective study. The study setting was the General Clinical Research Center of an Academic Medical Center. Participants were 50 euthyroid patients undergoing thyroidectomy. Thyroxine, triiodothyronine (T(3)), and TSH levels were documented in patients prior to their scheduled thyroidectomy. Following thyroidectomy, patients were divided into those with a histologic diagnosis of either differentiated thyroid cancer or benign disease. Preoperative thyroid profiles were correlated with patients' postoperative diagnoses. RESULTS: All patients had a normal serum TSH concentration preoperatively. One-third of the group was diagnosed with thyroid cancer as a result of their thyroidectomy. These patients had a higher serum TSH level (mean = 1.50 mIU/L, CI 1.22-1.78 mIU/L) than patients with benign disease (mean = 1.01 mIU/mL, CI 0.84-1.18 mIU/L). There was a greater risk of having thyroid cancer in patients with TSH levels in the upper three quartiles of TSH values, compared with patients with TSH concentrations in the lowest quartile of TSH values (odd ratio = 8.7, CI 2.2-33.7). Patients with a thyroid cancer diagnosis also had lower T(3) concentrations measured by liquid chromatography tandem mass spectrometry (mean = 112.6 ng/dL, CI 103.8-121.4 ng/dL) than did patients with a benign diagnosis (mean 129.9 ng/dL, CI 121.4-138.4 ng/dL). CONCLUSION: These data confirm that higher TSH concentrations, even within the normal range, are associated with a subsequent diagnosis of thyroid cancer in individuals with thyroid abnormalities. This

further supports the hypothesis that TSH stimulates the growth or development of thyroid malignancy during its early or preclinical phase. We also show for the first time that patients with thyroid cancer also have lower T(3) levels than patients with benign disease.

Kebebew, E. (2008). "Hereditary non-medullary thyroid cancer." World J Surg **32**(5): 678-82.

An estimated 5% of all non-medullary thyroid cancers are hereditary. If three or more first-degree relatives are affected, there is a greater than 94% chance that these cases are hereditary non-medullary thyroid cancer (HNMTC). Although, the susceptibility gene(s) for HNMTC has not been identified, there are enough epidemiologic studies and kindreds reported to suggest a hereditary predisposition to thyroid cancer. Until the susceptibility genes are identified, clinicians will have to rely on comprehensive history taking to identify at-risk families and clinically screen at-risk family members. When families are at risk for HNMTC, it is unclear whether neck examination and or neck ultrasound is most effective for screening. Hereditary non-medullary thyroid cancer is associated with more aggressive disease than sporadic HNMTC, especially in index cases, with higher rates of multicentric tumors, lymph node metastasis, and extrathyroidal invasion. Aggressive screening may benefit other members of the affected kindred because the outcome for the non-index cases is better. Although no studies have demonstrated any difference in mortality in patients with HNMTC versus sporadic disease, disease-free survival is shorter in HNMTC. Aggressive surgical and postoperative medical therapy is warranted in patients with HNMTC. It is likely that emerging molecular approaches may help identify the gene or genes involved in HNMTC which would have important clinical ramifications.

Kebebew, E., M. Peng, et al. (2006). "A phase II trial of rosiglitazone in patients with thyroglobulin-positive and radioiodine-negative differentiated thyroid cancer." Surgery **140**(6): 960-6; discussion 966-7.

BACKGROUND: Rosiglitazone is a peroxisome proliferator-activated receptor gamma (PPARgamma) agonist that has been shown to induce differentiation, cell cycle arrest, and apoptosis in a variety of human cancers including thyroid cancer. METHODS: Ten patients with differentiated thyroid cancer were enrolled in an open-label, phase II trial of oral rosiglitazone treatment (4 mg daily for 1 week, then 8 mg daily for 7 weeks). The levels of PPARgamma receptor mRNA and protein expression were determined in the patient's neoplasm. RESULTS: Of 10 patients, 4 had positive radioiodine scans after rosiglitazone therapy with uptake in the neck in 3 patients and in the pelvis in 1 patient. After treatment, the serum thyroglobulin level decreased in 2 patients, increased in 5 patients, and was stable in 3 patients. No patient developed clinically important toxicity associated with rosiglitazone treatment. We found no relationship in the level of PPARgamma mRNA and protein expression in patients who had radioiodine uptake compared with those who did not. CONCLUSIONS: Our findings suggest that rosiglitazone treatment may induce radioiodine uptake in some patients with thyroglobulin-positive and radioiodine-negative differentiated thyroid cancer. We found no relationship between the expression level of the PPARgamma mRNA and protein in the neoplasm and radioiodine uptake status after rosiglitazone therapy, questioning the potential pathway of effect.

Kesmodel, S., I. Prabakaran, et al. (2005). "Virus-mediated oncolysis of thyroid cancer by a replication-selective adenovirus driven by a thyroglobulin promoter-enhancer region." J Clin Endocrinol Metab **90**(6): 3440-8.

CONTEXT: Currently, there is no effective treatment for iodine-resistant thyroid cancers. OBJECTIVE: As a new approach to treatment, the efficacy of replication-selective, human thyroglobulin (TG) enhancer and promoter-driven, adenovirus (AdhTGEP)-mediated oncolysis was investigated using two well-differentiated thyroid cancer cell lines, XTC (TG positive) and FTC-133 (TG negative), and other control tumor and nontumor cell lines (all TG negative). DESIGN: A cohort study design was used. SETTING: The study setting was laboratory bench-top experiments. SUBJECTS/PARTICIPANTS: In vitro TG-expressing and nonexpressing thyroid cell culture lines, nonthyroid tumor cell lines, as well as preclinical thyroid tumor-bearing mice were studied. INTERVENTION: Adenoviral infection of cell lines was determined by immunohistochemistry, selective replication by one-step growth assays, and cytotoxicity by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tet rozolium (MTS) assay. In vivo tumor growth inhibition was determined by a single intratumoral injection of 1 x 10(9) plaque-forming units AdhTGEP, AdLacZ (control virus), or PBS to 50- to 75-mm(3) tumors. XTC cells showed intense immunohistochemical staining, whereas FTC-133 and all other control cell lines showed minimal staining for viral infection with AdhTGEP. MAIN OUTCOME MEASURES: Cell survival and tumor growth inhibition after adenoviral infection were the main outcome measures. RESULTS: One-step growth assays showed at least a more than 60-fold titer of AdhTGEP in XTC than in FTC-133 cells.

Cytotoxicity assays showed approximately 68% cell kill in XTC and minimal cell kill in FTC-133 and all other control cell lines at a multiplicity of infection of 250. There was significant in vivo growth inhibition of AdhTGEP-treated XTC tumors (67 +/- 49 mm(3)) compared with AdLacZ-treated XTC (228 +/- 45 mm(3); P < 0.01), PBS-treated XTC (372 +/- 70 mm(3); P < 0.001), or AdhTGEP-treated FTC-133 tumors (598 +/- 168 mm(3)). CONCLUSION: Replication-selective virus-mediated oncolysis is a potential therapy for recurrent, well-differentiated, TG-secreting thyroid cancer that is unresponsive to standard treatment.

Kim, C. S., H. Ying, et al. (2007). "The pituitary tumor-transforming gene promotes angiogenesis in a mouse model of follicular thyroid cancer." Carcinogenesis **28**(5): 932-9.

Overexpression of the pituitary tumor-transforming gene (PTTG) has been associated with tumorigenesis. In a mouse model that spontaneously develops follicular thyroid cancer (FTC) with distant metastasis (TRbetaPV mouse), PTTG is overexpressed, similar to human thyroid cancer. To evaluate the role of PTTG in thyroid carcinogenesis, we studied the offspring of TRbetaPV mice with mice lacking PTTG (PTTG(-/-) mice). The thyroids of TRbeta(PV/PV) PTTG(-/-) mice were significantly smaller than TRbeta(PV/PV) mice. Ki-67 staining showed a decrease in thyroid proliferation in TRbeta(PV/PV) PTTG(-/-) mice. Our evaluation of the Rb-E2F pathway, a central mediator of cell growth, found that TRbeta(PV/PV) PTTG(-/-) mice exhibited a decrease in protein levels of phosphorylated Rb along with an elevation of the cdk inhibitor p21. Histological examination documented no difference in FTC occurrence between TRbeta(PV/PV) and TRbeta(PV/PV) PTTG(-/-) mice, which indicates that PTTG removal does not prevent the initiation of FTC. However, TRbeta(PV/PV) PTTG(-/-) mice had a significant decrease in vascular invasion and less development of lung metastasis as they progressively aged. CD31 staining also showed a decrease in vessel density in TRbeta(PV/PV) PTTG(-/-) versus TRbeta(PV/PV) thyroids. Given the decreased vascular invasion in the PTTG knockout mice, we studied genes involved in angiogenesis. Real-time reverse transcription-polymerase chain reaction showed a consistent decrease in pro-angiogenic factors, fibroblast growth factor (FGF2), its receptor FGFR1 and vascular endothelial growth factor. Our results highlight the dual roles of PTTG as a regulator of thyroid growth and contributor to tumor progression. The separation of the pathways regulating cell proliferation, tumor initiation and tumor progression should direct future therapeutic options.

Kim, C. S. and X. Zhu (2009). "Lessons from mouse models of thyroid cancer." Thyroid **19**(12): 1317-31.

BACKGROUND: Thyroid cancer is the most common endocrine tumor and is increasing in incidence. The aim of this study was to review mouse models of differentiated thyroid cancer and how they elucidate human thyroid cancer biology. SUMMARY: Differentiated thyroid cancer, primarily papillary and follicular, comprises the majority of thyroid cancers. There has been tremendous growth in the cross-talk between basic science and clinical practice for thyroid cancer management. Insight into the framework of genes responsible for differentiated thyroid cancer has been gained through the use of mouse models. Common genetic alterations found in human papillary thyroid cancer such as RET/PTC rearrangements or the BRAF(V600E) mutation have genetically modified mouse counterparts. These and other preclinical mouse models have validated the importance of the cyclic adenosine monophosphate (cAMP)/protein kinase A and mitogen-activated protein kinase (MAPK) signaling pathways in papillary thyroid cancer (PTC). RAS mutations have a role in both papillary and follicular thyroid cancer development. Mice with overactivation of the phosphatidylinol-3-kinase (PI3K)-AKT and/or thyrotropin-regulated signaling pathways have been found to develop follicular thyroid cancer. Additional mouse models of thyroid cancer that utilize inducible expression systems are in development or are being characterized and will better reflect the majority of human thyroid cancers which are non-hereditary. Advances in in vivo imaging of mice allow for earlier detection of metastasis and the ability to follow tumor growth or regression which may be used in evaluation of pharmaceutical agents. CONCLUSIONS: Mouse models have expanded our understanding of the altered signaling pathways that contribute to thyroid cancer tumorigenesis and provide a powerful tool to develop novel diagnostic approaches and therapies.

Kim, D. S., J. A. Franklyn, et al. (2006). "Pituitary tumor-transforming gene regulates multiple downstream angiogenic genes in thyroid cancer." J Clin Endocrinol Metab **91**(3): 1119-28.

CONTEXT: Pituitary tumor-transforming gene (PTTG) is a multifunctional protein involved in several tumorigenic mechanisms, including angiogenesis. PTTG has been shown to promote angiogenesis, a key rate-limiting step in tumor progression, by up-regulation of fibroblast growth factor-2 and vascular endothelial growth factor. OBJECTIVE: To investigate whether PTTG regulates

other angiogenic genes in thyroid cells, we performed angiogenesis-specific cDNA arrays after PTTG transfection. Two of the genes [inhibitor of DNA binding-3 (ID3) and thrombospondin-1 (TSP-1)] which showed differential expression in primary thyroid cells were validated in vitro and in vivo. RESULTS: TSP-1 showed a 2.5-fold reduction and ID3 showed a 3.5-fold induction in expression in response to PTTG overexpression in vitro. Conversely, suppression of PTTG with small interfering RNA was associated with a 2-fold induction of TSP-1 and a 2.2-fold reduction in ID3 expression. When we examined TSP-1 and ID3 expression in 34 differentiated thyroid cancers, ID3 was significantly increased in tumors compared with normal thyroid tissue. Furthermore, ID3 expression was significantly higher in follicular thyroid tumors than in papillary tumors. Although mean TSP-1 expression was not altered in cancers compared with normal thyroids, we observed a significant independent association between TSP-1 expression and early tumor recurrence, with recurrent tumors demonstrating 4.2-fold lower TSP-1 expression than normal thyroid tissues. CONCLUSION: We have identified ID3 and TSP-1 as two new downstream targets of PTTG in thyroid cancer. We propose that PTTG may promote angiogenesis by regulating the expression of multiple genes with both pro- and antiangiogenic properties and may thus be a key gene in triggering the angiogenic switch in thyroid tumorigenesis.

Kim, T. H., S. Y. Lee, et al. (2009). "Mutant p53 (G199V) gains antiapoptotic function through signal transducer and activator of transcription 3 in anaplastic thyroid cancer cells." Mol Cancer Res **7**(10): 1645-54.

In the present study, we identified a missense mutation (G199V) in KAT-18 cell line established from primary cultures of anaplastic thyroid cancer (ATC). Notably, knockdown of this mutant (mt) p53 reduced cell viability and exerted antitumor activity equivalent to high doses of several chemotherapeutic agents. We showed that p53 knockdown had an antitumor effect via the induction of apoptosis. We further examined the underlying mechanism by which mt p53 (G199V) gains antiapoptotic function in KAT-18 cells. Microarray analysis revealed that p53 knockdown modified the expression of numerous apoptosis-related genes. Importantly, p53 knockdown led to downregulation of signal transducer and activator of transcription-3 (STAT3) gene expression. We further observed that p53 knockdown induced the downregulation of STAT3 protein. We also observed that a STAT3 inhibitor augmented the reduction of cell viability induced by p53 knockdown, whereas interleukin-6 treatment alleviated this effect. In addition, overexpression of STAT3 protected ATC cells against cell death induced by p53 knockdown. Taken together, these data show that mt p53 (G199V) gains antiapoptotic function mediated by STAT3 in ATC cells. Inhibition of the function of mt p53 (G199V) could be a novel and useful therapeutic strategy for decreasing the extent and severity of toxicity due to chemotherapeutic agents.

Kimmel, R. R., L. P. Zhao, et al. (2006). "Microarray comparative genomic hybridization reveals genome-wide patterns of DNA gains and losses in post-Chernobyl thyroid cancer." Radiat Res **166**(3): 519-31.

Genetic gains and losses resulting from DNA strand breakage by ionizing radiation have been demonstrated in vitro and suspected in radiation-associated thyroid cancer. We hypothesized that copy number deviations might be more prevalent, and/or occur in genomic patterns, in tumors associated with presumptive DNA strand breakage from radiation exposure than in their spontaneous counterparts. We used cDNA microarray-based comparative genome hybridization to obtain genome-wide, high-resolution copy number profiles at 14,573 genomic loci in 23 post-Chernobyl and 20 spontaneous thyroid cancers. The prevalence of DNA gains in tumors from cases in exposed individuals was two- to fourfold higher than for cases in unexposed individuals and up to 10-fold higher for the subset of recurrent gains. DNA losses for all cases were low and more prevalent in spontaneous cases. We identified unique patterns of copy variation (mostly gains) that depended on a history of radiation exposure. Exposed cases, especially the young, harbored more recurrent gains that covered more of the genome. The largest regions, spanning 1.2 to 4.9 Mbp, were located at 1p36.32-.33, 2p23.2-.3, 3p21.1-.31, 6p22.1-.2, 7q36.1, 8q24.3, 9q34.11, 9q34.3, 11p15.5, 11q13.2-12.3, 14q32.33, 16p13.3, 16p11.2, 16q21-q12.2, 17q25.1, 19p13.31-qter, 22q11.21 and 22q13.2. Copy number changes, particularly gains, in post-Chernobyl thyroid cancer are influenced by radiation exposure and age at exposure, in addition to the neoplastic process.

Knauf, J. A. and J. A. Fagin (2009). "Role of MAPK pathway oncoproteins in thyroid cancer pathogenesis and as drug targets." Curr Opin Cell Biol **21**(2): 296-303.

Constitutive activation of MAPK in cancer occurs through activating mutations or overexpression of upstream effectors in the pathway, primarily of genes encoding receptor tyrosine kinases, RAS and BRAF. Arguably, the evidence for MAPK activation is most compelling in thyroid cancers and in

melanomas. In this review we discuss the mechanisms of tumor development by oncogenic BRAF in these two cancer cell lineages, since this kinase signals preferentially through this pathway. We describe recent information on the mediators of BRAF-induced tumor initiation and escape from senescence. In addition, we review the biochemical events implicated in cellular growth triggered by oncogenic BRAF and the determinants of oncogene addiction. The biology of thyroid cancers induced by oncogenic BRAF is quite distinct, both in humans and in mice. There is great interest in using these insights to design rational new therapies, for which it will become crucial to understand the determinants of sensitivity and resistance to compounds designed to block the pathway. In thyroid cancer, this interest is further heightened by new information on the role of activated BRAF and MAPK pathway activation in disrupting iodine transport and thyroid hormonogenesis.

Knostman, K. A., S. M. Jhiang, et al. (2007). "Genetic alterations in thyroid cancer: the role of mouse models." Vet Pathol **44**(1): 1-14.

Thyroid carcinomas are the most common endocrine neoplasms in humans, with a globally increasing incidence. Thyroid follicular cells and neuroendocrine (parafollicular) C cells are each susceptible to neoplastic transformation, resulting in thyroid cancers of differing phenotypes with unique associated genetic mutations and clinical outcomes. Over the past 15 years, several sophisticated genetically engineered mouse models of thyroid cancer have been created to further our understanding of the genetic events leading to thyroid carcinogenesis in vivo. The most significant mouse models of papillary, follicular, anaplastic, and medullary thyroid carcinoma are highlighted, with particular emphasis on the relationship between the relevant oncogenes in these models and genetic events in the naturally occurring human disease. Limitations of each model are presented, and the need for additional models to better recapitulate certain aspects of the human disease is discussed.

Kodama, Y., N. Asai, et al. (2005). "The RET proto-oncogene: a molecular therapeutic target in thyroid cancer." Cancer Sci **96**(3): 143-8.

The RET proto-oncogene is responsible for the development of several human inherited and non-inherited diseases. Germline point mutations were identified in multiple endocrine neoplasia types 2A and 2B, and familial medullary thyroid carcinoma. More than 10 rearranged forms of RET, referred to as RET/PTC 1-9, ELKS/RET and RFP/RET, have been cloned from sporadic and radiation-associated papillary thyroid carcinomas. These mutations induced oncogenic activation of RET tyrosine kinase by different mechanisms. To date, various kinds of therapeutic approaches have been developed for the treatment of RET-associated cancers, including tyrosine kinase inhibitors, gene therapy with dominant negative RET mutants, and RNA interference to abrogate oncogenic mutant RET expression. RET and some signaling molecules that function downstream of RET could be potential targets for the development of selective cancer therapeutics.

Kogai, T., S. Sajid-Crockett, et al. (2008). "Phosphoinositide-3-kinase inhibition induces sodium/iodide symporter expression in rat thyroid cells and human papillary thyroid cancer cells." J Endocrinol **199**(2): 243-52.

TSH stimulation of sodium iodide symporter (NIS) expression in thyroid cancer promotes radioiodine uptake and is required to deliver an effective treatment dose. Activation of the insulin/phosphoinositide-3-kinase (PI3K) signaling pathway in TSH-stimulated thyroid cells reduces NIS expression at the transcriptional level. We, therefore, investigated the effects of PI3K pathway inhibition on iodide uptake and NIS expression in rat thyroid cell lines and human papillary thyroid cancer cells. A PI3K inhibitor, LY294002, significantly enhanced iodide uptake in two rat thyroid cell lines, FRTL-5 and PCCL3. The induction of Nis mRNA by LY294002 occurred 6 h after treatment, and was abolished by a translation inhibitor, cycloheximide. Expression of the transcription factor, Pax8, which stimulates NIS expression, was significantly increased in PCCL3 cells after LY294002 treatment. Removal of insulin abrogated the stimulatory effects of LY294002 on NIS mRNA and protein expression, but not on iodide uptake. These findings suggest that PI3K pathway inhibition results in post-translational stimulation of NIS. Inhibition of the PI3K pathway also significantly increased iodide uptake (approximately 3.5-fold) in BHP 2-7 papillary thyroid cancer cells (Ret/PTC1 positive), engineered to constitutively express NIS. Pharmacological inhibition of Akt, a factor stimulated by the PI3K pathway, increased exogenous NIS expression in BHP 2-7 as was seen with LY294002, but not increase the endogenous NIS expression in FRTL-5 cells. PI3K pathway inhibition increases functional NIS expression in rat thyroid cells and some papillary thyroid cancer cells by several mechanisms. PI3K inhibitors have the potential to increase radioiodide accumulation in some differentiated thyroid cancer.

Kogai, T., K. Taki, et al. (2006). "Enhancement of sodium/iodide symporter expression in thyroid and breast cancer." Endocr Relat Cancer **13**(3): 797-826.

The sodium/iodide symporter (NIS) mediates iodide uptake in the thyroid gland and lactating breast. NIS mRNA and protein expression are detected in most thyroid cancer specimens, although functional iodide uptake is usually reduced resulting in the characteristic finding of a 'cold' or non-functioning lesion on a radioiodine image. Iodide uptake after thyroid stimulating hormone (TSH) stimulation, however, is sufficient in most differentiated thyroid cancer to utilize beta-emitting radioactive iodide for the treatment of residual and metastatic disease. Elevated serum TSH, achieved by thyroid hormone withdrawal in athyreotic patients or after recombinant human thyrotropin administration, directly stimulates NIS gene expression and/or NIS trafficking to the plasma membrane, increasing radioiodide uptake. Approximately 10-20% differentiated thyroid cancers, however, do not express the NIS gene despite TSH stimulation. These tumors are generally associated with a poor prognosis. Reduced NIS gene expression in thyroid cancer is likely due in part, to impaired trans-activation at the proximal promoter and/or the upstream enhancer. Basal NIS gene expression is detected in about 80% breast cancer specimens, but the fraction with functional iodide transport is relatively low. Lactogenic hormones and various nuclear hormone receptor ligands increase iodide uptake in breast cancer cells in vitro, but TSH has no effect. A wide range of 'differentiation' agents have been utilized to stimulate NIS expression in thyroid and breast cancer using in vitro and in vivo models, and a few have been used in clinical studies. Retinoic acid has been used to stimulate NIS expression in both thyroid and breast cancer. There are similarities and differences in NIS gene regulation and expression in thyroid and breast cancer. The various agents used to enhance NIS expression in thyroid and breast cancer will be reviewed with a focus on the mechanism of action. Agents that promote tumor differentiation, or directly stimulate NIS gene expression, may result in iodine concentration in 'scan-negative' thyroid cancer and some breast cancer.

Kondo, T., X. Zhu, et al. (2007). "The cancer/testis antigen melanoma-associated antigen-A3/A6 is a novel target of fibroblast growth factor receptor 2-IIIb through histone H3 modifications in thyroid cancer." Clin Cancer Res **13**(16): 4713-20.

PURPOSE: Fibroblast growth factor (FGF) signals play fundamental roles in development and tumorigenesis. Thyroid cancer is an example of a tumor with nonoverlapping genetic mutations that up-regulate mitogen-activated protein kinase. We reported recently that FGF receptor 2 (FGFR2) is down-regulated through extensive DNA promoter methylation in thyroid cancer. Reexpression of the FGFR2-IIIb isoform impedes signaling upstream of the BRAF/mitogen-activated protein kinase pathway to interrupt tumor progression. In this analysis, we examined a novel target of FGFR2-IIIb signaling, melanoma-associated antigen-A3 and A6 (MAGE-A3/6). EXPERIMENTAL DESIGN: cDNA microarray analysis was done on human WRO thyroid cancer cells transfected with FGFR2-IIIb or empty vector. Identified gene target was confirmed by reverse transcription-PCR and Western blotting. Gene regulation was examined by treatment of WRO cells with the methylation inhibitor 5'-azacytidine followed by methylation-specific PCR and reverse transcription-PCR and by chromatin immunoprecipitation. RESULTS: Gene expression profiling identified the cancer/testis antigen MAGE-A3/6 as a novel target of FGFR2-IIIb signaling. MAGE-A3/6 regulation was mediated through DNA methylation and chromatin modifications. In particular, FGF7/FGFR2-IIIb activation resulted in histone 3 methylation and deacetylation associated with the MAGE-A3/6 promoter to down-regulate gene expression. CONCLUSIONS: These data unmask a complex repertoire of epigenetically controlled signals that govern FGFR2-IIIb and MAGE-A3/6 expression. Our findings provide insights into the interrelationship between novel tumor markers that may also represent overlapping therapeutic targets.

Kunnimalaiyaan, M., M. Ndiaye, et al. (2006). "Apoptosis-mediated medullary thyroid cancer growth suppression by the PI3K inhibitor LY294002." Surgery **140**(6): 1009-14; discussion 1014-5.

BACKGROUND: Medullary thyroid cancer (MTC) cells exhibit frequent activation of the PI3K pathway as evidenced by the presence of hyperactivation of Akt kinases and overexpression of neuroendocrine (NE) markers. We hypothesized that the inhibition of the PI3K pathway in MTC may lead to a reduction in cell growth and NE tumor marker production. METHODS: Human MTC-TT cells were treated with the PI3K inhibitor LY294002 (0-60 micromol/L) for 8 days, and cellular growth was measured. Further, TT cells were treated with nontoxic concentrations of LY294002 for 2 days, and Western blot analyses were performed for phospho-Akt, total Akt, and the NE tumor markers CgA and human achaete-scute homolog1 (ASCL1). RESULTS: Treatment of TT cells with LY294002 significantly suppressed levels of phospho-Akt. Notably, a dose-dependent reduction in cellular proliferation was also observed. Importantly, NE marker production was also reduced. Mechanistically, we show that cell

growth inhibition by PI3K inactivation is mediated by apoptosis attributable to an increase in the levels of cleaved poly(ADP-ribose) polymerase and caspase-3. CONCLUSIONS: MTC cell growth and NE marker production appear to depend on activation of the PI3K-signaling cascade. Inhibition of this important signal transduction pathway may lead to a possible therapeutic strategy to treat patients with MTC.

Kunnimalaiyaan, M., A. M. Vaccaro, et al. (2006). "Overexpression of the NOTCH1 intracellular domain inhibits cell proliferation and alters the neuroendocrine phenotype of medullary thyroid cancer cells." J Biol Chem **281**(52): 39819-30.

The role of NOTCH1 as an oncogene or tumor suppressor appears to be cell type-specific. Medullary thyroid cancer (MTC) cells characteristically express the transcription factor ASCL1 (achaete-scute complex-like 1) as well as high levels of the neuroendocrine (NE) markers calcitonin and chromogranin A (CgA). In this study, we show that the active NOTCH1 intracellular domain is absent in human MTC tumor tissue samples and MTC-TT cells. To determine the effects of NOTCH1 expression, we created a doxycycline-inducible NOTCH1 intracellular domain in MTC cells (TT-NOTCH cells). Treatment of TT-NOTCH cells with doxycycline led to dose-dependent induction of NOTCH1 protein with corresponding decreases in ASCL1 protein and NE hormones. ASCL1 promoter-reporter assay and Northern analysis revealed that ASCL1 reduction by NOTCH1 activation is predominantly via silencing of ASCL1 gene transcription. Overexpression of ASCL1 in MTC cells indicated that CgA expression is highly dependent on the levels of ASCL1. This was further confirmed by experiments using small interfering RNA against ASCL1, in which reduction in ASCL1 led to reduction in both CgA and calcitonin. Furthermore, we demonstrate that NOTCH1 signaling activation leads to ERK1/2 phosphorylation, but that reduction in NE markers is independent of ERK1/2 activation. Activation of NOTCH1 resulted in significant MTC cell growth inhibition. Notably, reduction in MTC cell growth was dependent on the level of NOTCH1 protein present. Moreover, no increase in growth upon expression of ASCL1 in NOTCH1-activated cells was observed, indicating that the growth suppression observed upon NOTCH1 activation is independent of ASCL1 reduction. Mechanistically, we show that MTC cell growth inhibition by NOTCH1 is mediated by cell cycle arrest associated with up-regulation of p21.

Kwan, J., A. Baumgartner, et al. (2009). "BAC-FISH assays delineate complex chromosomal rearrangements in a case of post-Chernobyl childhood thyroid cancer." Folia Histochem Cytobiol **47**(2): 135-42.

Structural chromosome aberrations are known hallmarks of many solid tumors. In the papillary form of thyroid cancer (PTC), for example, activation of the receptor tyrosine kinase (RTK) genes, RET and neurotrophic tyrosine kinase receptor type I (NTRK1) by intra- and interchromosomal rearrangements has been suggested as a cause of the disease. However, many phenotypically similar tumors do not carry an activated RET or NTRK-1 gene or express abnormal ret or NTRK-1 transcripts. Thus, we hypothesize that other cellular RTK-type genes are aberrantly expressed in these tumors. Using fluorescence in situ hybridization-based methods, we are studying karyotype changes in a relatively rare subgroup of PTCs, i.e., tumors that arose in children following the 1986 nuclear accident in Chernobyl, Ukraine. Here, we report our technical developments and progress in deciphering complex chromosome aberrations in case S48TK, an aggressively growing PTC cell line, which shows an unusual high number of unbalanced translocations.

Lai, L., L. Yuan, et al. (2008). "The Galphai and Galphaq proteins mediate the effects of melatonin on steroid/thyroid hormone receptor transcriptional activity and breast cancer cell proliferation." J Pineal Res **45**(4): 476-88.

Melatonin, via its MT1 receptor, but not the MT2 receptor, can modulate the transcriptional activity of various nuclear receptors - estrogen receptor alpha (ERalpha) and retinoic acid receptor alpha (RARalpha), but not ERbeta- in MCF-7, T47D, and ZR-75-1 human breast cancer cell lines. The anti-proliferative and nuclear receptor modulatory actions of melatonin are mediated via the MT1 G protein-coupled receptor expressed in human breast cancer cells. However, the specific G proteins and associated pathways involved in the nuclear receptor transcriptional regulation by melatonin are not yet clear. Upon activation, the MT1 receptor specifically couples to the G(alphai2), G(alphai3), G(alphaq), and G(alphall) proteins, and via activation of G(alphai2) proteins, melatonin suppresses forskolin-induced 3',5'-cyclic adenosine monophosphate production, while melatonin activation of G(alphaq), is able to inhibit phospholipid hydrolysis and ATP's induction of inositol triphosphate production in MCF-7 breast cancer cells. Employing dominant-negative and dominant-positive) forms of these G proteins, we demonstrate that G(alphai2) proteins mediate the suppression of estrogen-induced ERalpha transcriptional activity by melatonin, while the G(q) protein mediates the enhancement of retinoid-induced

RARalpha transcriptional activity by melatonin. However, the growth-inhibitory actions of melatonin are mediated via both G(alphai2) and G(alphaq) proteins.

Landa, I., S. Ruiz-Llorente, et al. (2009). "The variant rs1867277 in FOXE1 gene confers thyroid cancer susceptibility through the recruitment of USF1/USF2 transcription factors." PLoS Genet **5**(9): e1000637.

In order to identify genetic factors related to thyroid cancer susceptibility, we adopted a candidate gene approach. We studied tag- and putative functional SNPs in genes involved in thyroid cell differentiation and proliferation, and in genes found to be differentially expressed in thyroid carcinoma. A total of 768 SNPs in 97 genes were genotyped in a Spanish series of 615 cases and 525 controls, the former comprising the largest collection of patients with this pathology from a single population studied to date. SNPs in an LD block spanning the entire FOXE1 gene showed the strongest evidence of association with papillary thyroid carcinoma susceptibility. This association was validated in a second stage of the study that included an independent Italian series of 482 patients and 532 controls. The strongest association results were observed for rs1867277 (OR[per-allele] = 1.49; 95%CI = 1.30-1.70; P = 5.9x10(-9)). Functional assays of rs1867277 (NM_004473.3:c.-283G>A) within the FOXE1 5' UTR suggested that this variant affects FOXE1 transcription. DNA-binding assays demonstrated that, exclusively, the sequence containing the A allele recruited the USF1/USF2 transcription factors, while both alleles formed a complex in which DREAM/CREB/alphaCREM participated. Transfection studies showed an allele-dependent transcriptional regulation of FOXE1. We propose a FOXE1 regulation model dependent on the rs1867277 genotype, indicating that this SNP is a causal variant in thyroid cancer susceptibility. Our results constitute the first functional explanation for an association identified by a GWAS and thereby elucidate a mechanism of thyroid cancer susceptibility. They also attest to the efficacy of candidate gene approaches in the GWAS era.

Lanzi, C., G. Cassinelli, et al. (2009). "Targeting RET for thyroid cancer therapy." Biochem Pharmacol **77**(3): 297-309.

The limited efficacy of conventional treatments in progressive thyroid carcinomas indicates the need for new therapeutic options. Activating mutations of the receptor tyrosine kinase-encoding RET gene have been identified as driving oncogenic events in subsets of papillary (PTC) and medullary (MTC) thyroid carcinomas suggesting the interest of targeted therapy. The role of RET oncogenes and the encoded constitutively active oncoproteins as potential targets has been investigated by different strategies including gene therapy and pharmacological approaches, but targeted treatment for RET-driven cancers is not clinically available in current therapy. Small molecule tyrosine kinase inhibitors, including sorafenib, sunitinib, motesanib and vandetanib, which have already shown efficacy against other neoplastic diseases, are being evaluated in clinical trials for treatment of thyroid carcinomas. Most of them, also described as Ret inhibitors, are multi-kinase inhibitors with antiangiogenic activity related to inhibition of receptor tyrosine kinases, such as the vascular endothelial growth factor receptors. Preclinical evidence supports the relevance of Ret oncoproteins as therapeutic targets for a subset of thyroid neoplastic diseases and, although targeting the original causal genetic change may not be sufficient to control the disease efficiently, the available knowledge outlines therapeutic opportunities for exploiting Ret inhibition.

Lapouge, G., R. Millon, et al. (2005). "Cisplatin-induced genes as potential markers for thyroid cancer." Cell Mol Life Sci **62**(1): 53-64.

Despite the uncontested role of p53 in cycle arrest/cell death after cisplatin treatment, to date the question whether wild-type p53 confers a resistant or sensitive status on the cell is still a matter of debate. Isogenic and isophenotypic human thyroid papillary carcinoma cell line variants for p53 differently expressed cycle genes after cisplatin treatment. Seven genes (CDC6-related protein, CCNC, GAS1, TFDP2, MAPK10/JNK3, WEE1, RPA1) selected after expression on an Atlas human cell cycle array were analyzed by quantitative real-time PCR. While cisplatin treatment increased their expression in p53 wild-type cells it decreased it in cells with inactivated p53 and had no or less effect on cells with mutated p53. These results show that in a well-defined system, different alterations of p53 can lead to a different regulation of genes and hence to either resistance or sensitivity to cisplatin. Moreover for the first time, MAPK10/JNK3 was identified in human thyroid cells and tissue. Four of the genes (CDC6-related protein, CCNC, GAS1 and TFDP2) were decreased in human papillary carcinoma tissues. Relevance of these genes (especially a decrease in GAS1 in thyroid papillary carcinoma) in various malignant pathologies has already been shown. These genes may be explored as new markers in advanced thyroid cancer such as metastatic and anaplastic forms displaying p53 alterations.

Leboeuf, R., J. E. Baumgartner, et al. (2008). "BRAFV600E mutation is associated with preferential

sensitivity to mitogen-activated protein kinase kinase inhibition in thyroid cancer cell lines." J Clin Endocrinol Metab **93**(6): 2194-201.

CONTEXT: Mutually exclusive mutations of RET, RAS, or BRAF are present in about 70% of papillary thyroid carcinomas, whereas only the latter two are seen in poorly differentiated and anaplastic cancers. Although the signal output common to these oncoproteins is ERK, a recent report showed that only BRAF mutations consistently predicted responsiveness to MAPK kinase (MEK) inhibitors. OBJECTIVES: Here we investigated whether sensitivity to MEK inhibition was determined by oncogene status in 13 human thyroid cancer cell lines: four with BRAF mutations, four RAS, one RET/PTC1, and four wild type. RESULTS: Growth of BRAF (+) cells was inhibited by the MEK antagonist PD0325901 with an IC(50) of less than 5 nm. By contrast, RAS, RET/PTC1, or wild-type cells had IC(50) of 4 nm to greater than 1000 nm. Sensitivity was not predicted by coexisting mutations in PIK3CA or by PTEN status. Similar effects were obtained with the MEK inhibitor AZD6244. PD0325901 induced a sustained G1/S arrest in BRAF (+) but not BRAF (-) lines. PD0325901 was equipotent at inhibiting pERK1/2 after 2 h, regardless of genetic background, but pERK rebounded at 24 h in most lines. MEK inhibitor resistance was associated with partial refractoriness of pERK to further inhibition by the compounds. AZD6244 was more potent at inhibiting growth of NPA (BRAF +) than Cal62 (KRAS +) xenografts. CONCLUSION: Thyroid cancers with BRAF mutation are preferentially sensitive to MEK inhibitors, whereas tumors with other MEK-ERK effector pathway gene mutations have variable responses, either because they are only partially dependent on ERK and/or because feedback responses elicit partial refractoriness to MEK inhibition.

Lee, J. J., J. Geli, et al. (2008). "Gene-specific promoter hypermethylation without global hypomethylation in follicular thyroid cancer." Int J Oncol **33**(4): 861-9.

Genome-wide hypomethylation and hypermethylation at CpG promoters are common in cancer. To date, little is known about global methylation changes in follicular thyroid cancer (FTC). Two independent quantitative methods, bisulphite Pyrosequencing of Long Interspersed Nucleotide Elements-1 (LINE-1) and LUminometric Methylation Assay (LUMA) were used to quantify genome-wide methylation in 21 FTC and corresponding normal thyroid tissues. Unexpectedly global methylation was not found significantly altered in tumors compared to normal thyroid by either LINE-1 (p=0.57) or LUMA (p=0.42), whilst the promoter of a tumor suppressor that is often epigenetically dysregulated, RASSF1A was found to be significantly hypermethylated by Pyrosequencing (p=0.0001). Moreover, allelic imbalance at the RASSF1A locus was observed in 15/21 of the tumors. mRNA expression of RASSF1A was significantly lower in tumors compared to corresponding normal tissues (p=0.0002). In summary, the epigenetic inactivation of RASSF1A is a frequent event in FTC, but is not coupled to changes in global methylation.

Lee, J. J., C. Larsson, et al. (2006). "A dog pedigree with familial medullary thyroid cancer." Int J Oncol **29**(5): 1173-82.

Multiple endocrine neoplasia (MEN) is defined as concurrent neoplasia or hyperplasia in more than one endocrine gland. MEN is well known in humans and has also been reported in small animals. We report on a dog family of a mixed breed with Alaskan malamute as a major influence, where three members developed thyroid carcinomas and another dog had clinical signs mimicking the other three but without a confirmed diagnosis. The age of onset of the tumour was between 96-109 months. Clinical, biochemical and immunohistochemical examinations revealed that the affected individuals typically demonstrated symptoms including calcitonin positive thyroid cancer, hypothyroidism and chronic dermatitis. In addition, elevated serum calcium and multinodular adrenocortical hyperplasia were demonstrated in a single member. The diagnosis observed is similar to the familial form of medullary thyroid carcinoma (FMTC) in human. This is the first report of FMTC in dog. Up to 95% of FMTC and MEN2 is known to be caused by activating mutations in the RET gene. The dog Ret gene was analysed as a candidate in this pedigree. The complete dog Ret genomic sequence was predicted in silico. The lack of demonstratable Ret mutation suggests the involvement of alternative predisposing mutation in this pedigree. The unique occurrence of familial MTC makes this potentially an important model in further defining the genetic basis of MTC.

Leite, K. R., C. A. Mitteldorf, et al. (2008). "Cdx2, cytokeratin 20, thyroid transcription factor 1, and prostate-specific antigen expression in unusual subtypes of prostate cancer." Ann Diagn Pathol **12**(4): 260-6.

There are some unusual histologic variants of prostate carcinoma, including mucinous, signet-ring cells, and ductal carcinomas that can metastasize in a problematic way and simulate lung, colorectal, or bladder primaries. Currently, antibodies that are organ-specific have been used in the routine surgical pathology practice. Our aim is to study the profile of

expression of Cdx2, thyroid transcription factor 1 (TTF1), and cytokeratin 20 (CK20) in prostate cancer with unusual histologic finding. Twenty-nine prostate adenocarcinomas with unusual histologic findings were submitted to immunohistochemistry with prostate-specific antigen (PSA), CK20, Cdx2, and TTF1 antibodies. There were 7 mucinous, 5 ductal, 2 signet-ring cells, and 15 usual acinar adenocarcinomas with focal mucinous differentiation. To compare the results with usual acinar adenocarcinomas, we studied 10 primary and their respective lymph node metastases in a tissue microarray, 2 unusual metastatic adenocarcinomas, and 6 usual acinar high-grade carcinomas. For tumors with special histologic finding, Cdx2 was expressed by 9 (31.0%) mucinous, signet-cell, or with focal mucinous differentiation. Thyroid transcription factor 1 was moderately positive in mucinous differentiation areas of 2 (6.9%) adenocarcinomas. Cytokeratin 20 was expressed by 9 (31.0%) tumors, among them, 3 ductal adenocarcinomas. Prostate-specific antigen was positive in 28 (96.6%) cases and negative in 1 ductal adenocarcinoma. There was only 1 worrisome ductal adenocarcinoma that was strongly CK20 positive and PSA negative. Almost one third of mucinous prostate carcinomas express Cdx2. Cytokeratin 20 can be positive also in one third of prostate carcinomas, especially the ductal type. Pathologist should be alert when evaluating immunohistochemical profiles of unusual histologic findings of prostate cancer, mostly in distant sites.

Lemos, M. C., F. Carrilho, et al. (2007). "Genetic polymorphism of CYP2D6 influences susceptibility to papillary thyroid cancer." Clin Endocrinol (Oxf) **67**(2): 180-3.

OBJECTIVE: Xenobiotic-metabolizing enzymes are widely polymorphic and confer interindividual variation in the ability to detoxify carcinogens or to activate pro-carcinogens. A common polymorphism of cytochrome P450 2D6 (CYP2D6) results in lack of enzyme activity and has been associated with an altered susceptibility to several cancers. The aim of this study was to investigate the association between the CYP2D6 poor metaboliser genotype and the risk of papillary thyroid cancer (PTC). DESIGN: Retrospective case-control study. PATIENTS: One hundred and eighty-seven patients with PTC and 256 controls. MEASUREMENTS: Genotyping was performed by PCR and restriction enzyme analysis to detect the presence of the common CYP2D6*4 poor metaboliser allele. RESULTS: The frequency of individuals with the homozygous poor metaboliser genotype was lower in the patient group [1.6 vs. 5.5%, P = 0.037, OR = 0.28 (95% CI 0.09-0.93)]. The CYP2D6*4 allele frequency was also lower in the patient group [13.4 vs. 21.7%, P = 0.002, OR = 0.56 (95% CI 0.39-0.80)]. CONCLUSIONS: The results suggest that the poor metaboliser genotype is associated with a protective effect against PTC. This could be explained by a possible role of CYP2D6 on the metabolic activation of putative environmental chemical thyroid carcinogens or by linkage to another cancer-causing gene. Further research may allow the identification of metabolic risk factors and contribute towards understanding the molecular mechanisms involved in thyroid carcinogenesis.

Lemos, M. C., E. Coutinho, et al. (2008). "Combined GSTM1 and GSTT1 null genotypes are associated with a lower risk of papillary thyroid cancer." J Endocrinol Invest **31**(6): 542-5.

Individual susceptibility to cancer is influenced by polymorphisms of genes encoding drug-metabolizing enzymes such as the glutathione S-transferases (GST). The null polymorphisms of the GSTM1 and GSTT1 genes have been associated to a modified risk of several cancers but studies of thyroid cancer have produced conflicting results. The aim of this study was to investigate the relationship between these polymorphisms and the risk of papillary thyroid cancer (PTC). A total of 188 patients with PTC and 247 controls were genotyped using a PCR-based assay. Odds ratios (OR) and 95% confidence intervals (CI) for each homozygous null genotype were determined. The frequency of each of the GSTM1 and GSTT1 null genotypes did not differ significantly between patients and controls (OR=0.83, 95%CI: 0.56-1.21; p=0.328; and OR=0.66, 95%CI: 0.39-1.12; p=0.123, respectively), but the frequency of individuals that had the combined GSTM1 null/GSTT1 null genotypes was significantly lower in the patient group (OR=0.50, 95%CI: 0.26-0.97; p=0.040). The GSTM1 null genotype was associated with a lower risk of advanced cancer stages (III/IV) (OR=0.50, 95%CI: 0.26-0.96; p=0.036) and the GSTT1 null genotype was associated with a lower risk of the follicular variant of PTC (OR=0.31, 95%CI: 0.10-0.97; p=0.044). These results suggest that GSTM1 and GSTT1 null genotypes are weak, yet possible, modifiers of the risk of PTC. This protective effect may be due to a role of the GSTM1 and GSTT1 encoded enzymes in the metabolic activation of putative thyroid carcinogens or in other pathways involved in thyroid carcinogenesis.

Lin, H. Y., H. Y. Tang, et al. (2007). "Thyroid hormone is a MAPK-dependent growth factor for thyroid cancer cells and is anti-apoptotic." Steroids **72**(2): 180-7.

Thyroid hormone (l-thyroxine, T(4), or 3,5,3'-triiodo-l-thyronine, T(3)) treatment of human papillary and follicular thyroid cancer cell lines resulted in enhanced cell proliferation, measured by proliferating cell nuclear antigen (PCNA). Thyroid hormone also induced activation of the Ras/MAPK (ERK1/2) signal transduction pathway. ERK1/2 activation and cell proliferation caused by thyroid hormone were blocked by an iodothyronine analogue, tetraiodothyroacetic acid (tetrac), that inhibits binding of iodothyronines to the cell surface receptor for thyroid hormone on integrin alphaVbeta3. A MAPK cascade inhibitor at MEK, PD 98059, also blocked hormone-induced cell proliferation. We then assessed the possibility that thyroid hormone is anti-apoptotic. We first established that resveratrol (10 microM), a pro-apoptotic agent in other cancer cells, induced p53-dependent apoptosis and c-fos, c-jun and p21 gene expression in both papillary and follicular thyroid cancer cells. Induction of apoptosis by the stilbene required Ser-15 phosphorylation of p53. Resveratrol-induced gene expression and apoptosis were inhibited more than 50% by physiological concentrations of T(4). T(4) activated MAPK in the absence of resveratrol, caused minimal Ser-15 phosphorylation of p53 and did not affect c-fos, c-jun and p21 mRNA abundance. Thus, plasma membrane-initiated activation of the MAPK cascade by thyroid hormone promotes papillary and follicular thyroid cancer cell proliferation in vitro.

Lin, J. C., W. R. Kuo, et al. (2009). "Glutathione peroxidase 3 gene polymorphisms and risk of differentiated thyroid cancer." Surgery **145**(5): 508-13.

BACKGROUND: The antioxidant enzyme glutathione peroxidase 3 (GPX3) can lessen the oxidative stress in the thyroid gland. We tested for the association between tagging single nucleotide polymorphisms (tSNPs) of the GPX3 gene and the risk of differentiated thyroid cancer (DTC). METHODS: A total of 6 tSNPs (rs3763013, rs8177412, rs3805435, rs3828599, rs3792796, and rs2070593) of GPX3 were genotyped in Chinese DTC cases (n = 268) and controls (n = 378). Multivariate logistic regression was performed to estimate the genetic effect with adjustment for age and sex. RESULTS: There was no significant finding of genotype analysis in each tSNP associated with DTC, papillary thyroid carcinoma, or patients with regional lymph node metastasis. In the older group (age > or =45 years), subjects who carried at least 1 G allele of rs3805435 had a decreased risk of DTC compared with patients of AA homozygote (sex- and age-adjusted odds ratio [OR] = 0.50, P = .009). An individual with at least 1 T allele of rs3828599 had a decreased risk of DTC compared with an individual of the CC homozygote (sex- and age-adjusted OR = 0.53, P = .018). An individual carrying the TC genotype of rs8177412 in the older group had an increased risk of DTC than controls (sex- and age-adjusted OR = 1.73, P = .037). CONCLUSION: We found that the G allele of rs3805435 or the T allele of rs3828599 may exert a protective effect for DTC in the older population, whereas the C allele of rs8177412 confers an increased risk effect for DTC.

Lin, J. D. (2008). "Thyroglobulin and human thyroid cancer." Clin Chim Acta **388**(1-2): 15-21.

Thyroglobulin (Tg) is a large molecule containing 2750 amino acids with a molecular weight of 330 kD and twenty putative N-linked glycosylation sites. Tg gene expression is regulated by thyroid transcription factor 1 (TTF-1) and human paired box 8 (Pax-8). Iodinated Tg is stored in the lumen of the thyroid follicles and is released in response to specific hormonal stimulation by thyroid stimulating hormone (TSH). Following Tg reabsorption by thyrocytes and subsequent degradation, thyroid hormones triiodothryronine (T(3)) and thyroxine (T(4)) are secreted in the bloodstream. Mutations within the Tg gene cause defective thyroid hormone synthesis, resulting in congenital hypothyroidism. Thyroid carcinoma may develop from dyshormonogenic goiters due to Tg mutation. Post-thyroidectomy Tg levels are apparently associated with prognosis of papillary and follicular thyroid carcinomas and may predict tumor recurrence and metastatic potential. The detection of Tg by biochemical and molecular means has important diagnostic significance due to its pleiotropic roles in identification of tissue of thyroid origin, differentiation, and post-operative follow-up.

Lin, S. F., S. P. Gao, et al. (2008). "Synergy of a herpes oncolytic virus and paclitaxel for anaplastic thyroid cancer." Clin Cancer Res **14**(5): 1519-28.

PURPOSE: Novel therapeutic regimens are needed to improve the dismal outcomes of patients with anaplastic thyroid cancer (ATC). Oncolytic herpes simplex virus have shown promising activity against human ATC. We studied the application of oncolytic herpes simplex virus (G207 and NV1023) in combination with currently used chemotherapeutic drugs (paclitaxel and doxorubicin) for the treatment of ATC. EXPERIMENTAL DESIGN AND RESULTS: All four agents showed dose-response cytotoxicity in vitro for the human ATC cell lines KAT4 and DRO90-1. G207, combined with paclitaxel, showed synergistic cytotoxicity. Chou-Talalay combination indices ranged from 0.56 to 0.66 for KAT4, and 0.68 to 0.74 for DRO90-1 at higher affected fractions. Paclitaxel did not enhance G207 viral entry and early

gene expression or G207 viral replication. Paclitaxel combined with G207 compared with single-agent treatment or controls showed significantly increased microtubule acetylation, mitotic arrest, aberrant chromatid separation, inhibition of metaphase to anaphase progression, and apoptosis. A single i.t. injection of G207 combined with biweekly i.p. paclitaxel injections in athymic nude mice bearing KAT4 flank tumors showed significantly reduced mean tumor volume (74 +/- 38 mm(3)) compared with G207 alone (388 +/- 109 mm(3)), paclitaxel alone (439 +/- 137 mm(3)), and control (520 +/- 160 mm(3)) groups at 16 days. There was no morbidity in vivo attributable to therapy. CONCLUSIONS: Mechanisms of paclitaxel antitumoral activity, including microtubule acetylation, mitotic block, and apoptosis, were enhanced by G207, which also has direct oncolytic effects. Combination of G207 and paclitaxel therapy is synergistic in treating ATC and holds promise for patients with this fatal disease.

Lin, S. F., D. L. Price, et al. (2008). "Oncolytic vaccinia virotherapy of anaplastic thyroid cancer in vivo." J Clin Endocrinol Metab **93**(11): 4403-7.

CONTEXT: Anaplastic thyroid carcinoma (ATC) is a fatal disease with a median survival of only 6 months. Novel therapies are needed to improve dismal outcomes. OBJECTIVE: A mutated, replication-competent, vaccinia virus (GLV-1h68) has oncolytic effects on human ATC cell lines in vitro. We assessed the utility of GLV-1h68 in treating anaplastic thyroid cancer in vivo. DESIGN: Athymic nude mice with xenograft flank tumors of human ATCs (8505C and DRO90-1) were treated with a single intratumoral injection of GLV-1h68 at low dose (5x10(5) plaque-forming unit), high dose (5x10(6) plaque-forming unit), or PBS. Virus-mediated marker gene expression (luciferase, green fluorescent protein, and beta-galactosidase), viral biodistribution, and flank tumor volumes were measured. RESULTS: Luciferase expression was detected 2 d after injection. Continuous viral replication within tumors was reflected by increasing luciferase activity to d 9. At d 10, tumor viral recovery was increased more than 50-fold as compared with the injected dose, and minimal virus was recovered from the lung, liver, brain, heart, spleen, and kidneys. High-dose virus directly injected into normal tissues was undetectable at d 10. The mean volume of control 8505C tumors increased 50.8-fold by d 45, in contrast to 10.5-fold (low dose) and 2.1-fold (high dose; P=0.028) increases for treated tumors. DRO90-1 tumors also showed significant growth inhibition by high-dose virus. No virus-related toxicity was observed throughout the study. CONCLUSIONS: GLV-1h68 efficiently infects, expresses transgenes within, and inhibits the growth of ATC in vivo. These promising findings support future clinical trials for patients with ATC.

Liu, D., Z. Liu, et al. (2007). "BRAF V600E maintains proliferation, transformation, and tumorigenicity of BRAF-mutant papillary thyroid cancer cells." J Clin Endocrinol Metab **92**(6): 2264-71.

CONTEXT: Although the BRAF V600E mutant can initiate the formation of papillary thyroid cancer (PTC), it is unclear whether it is required to maintain cell proliferation, transformation, and tumor growth of BRAF mutation-harboring PTC. OBJECTIVE: The aim of the study was to investigate whether BRAF V600E is required for the proliferation, transformation, and tumorigenicity of BRAF mutation-harboring PTC cells. DESIGN: We addressed this issue using BRAF small interference RNA (siRNA) to transfect stably several BRAF mutation-harboring PTC cell lines, isolated clones with stable suppression of BRAF, and assessed their ability to proliferate, transform, and grow xenograft tumors in nude mice. RESULTS: PTC cell proliferation and transformation were suppressed in specific BRAF siRNA clones, but not in control scrambled siRNA clones. Specifically, taking the advantage of stable BRAF knockdown, we were able to show continued suppression of PTC cell proliferation and transformation, or anchorage-independent colony formation in soft agar, after long-term culture. Moreover, we also demonstrated that in vivo tumorigenicity and growth of tumors from the specific BRAF siRNA cell clones in nude mice were suppressed compared with control clones. CONCLUSIONS: BRAF V600E is not only an initiator of PTC as demonstrated previously but is also a maintainer of proliferation, transformation, and tumorigenicity of PTC cells harboring BRAF mutation, and growth of tumors derived from such cells continues to depend on BRAF V600E. These results provide further support for potentially effective therapy targeted at BRAF for BRAF mutation-harboring PTC.

Liu, D., Z. Liu, et al. (2007). "Inhibitory effects of the mitogen-activated protein kinase kinase inhibitor CI-1040 on the proliferation and tumor growth of thyroid cancer cells with BRAF or RAS mutations." J Clin Endocrinol Metab **92**(12): 4686-95.

CONTEXT: Targeting MAPK kinase (MEK) in the MAPK pathway is a potentially effective therapeutic strategy for thyroid cancer. OBJECTIVE: The objective of the study was to investigate genotype-dependent therapeutic potential of the MEK inhibitor CI-1040 for thyroid cancer. EXPERIMENTAL DESIGN: We examined the

effects of CI-1040 on proliferation, apoptosis, transformation, thyroid gene reexpression, and xenograft tumor growth with respect to genotypes in 10 thyroid tumor cell lines. RESULTS: Cell proliferation was potently inhibited by CI-1040 in cells harboring BRAF or RAS mutations but not in cells harboring RET/PTC rearrangement or wild-type alleles. For example, the IC50 values for BRAF mutation-harboring KAT10 cells and DRO cells and H-RAS mutation-harboring C643 cells were 0.365, 0.031, and 0.429 microm, respectively, whereas the IC50 values for RET/PTC1-harboring TPC1 cells and the wild-type MRO and WRO cells were 44, 46, and 278 microm, respectively. Proapoptotic effect of CI-1040 was seen in DRO cells, and cytostatic effect was seen in other cells. Down-regulation of cyclin D1 and reexpression of some thyroid genes were induced by CI-1040 in some BRAF mutation-harboring cells, and transformation was inhibited in all cells. CI-1040 also inhibited the growth of xenograft tumors in nude mice derived from KAT10 or C643 cells but not that derived from MRO cells. CONCLUSIONS: We for the first time demonstrated potent inhibitory effects of a MEK inhibitor, CI-1040, on thyroid cancer cells, some of which, particularly cell proliferation and tumor growth, seemed to be BRAF mutation or RAS mutation selective. Our data encourage a clinical trial on CI-1040 in thyroid cancer patients.

Liu, R., Z. Li, et al. (2009). "Mechanism of cancer cell adaptation to metabolic stress: proteomics identification of a novel thyroid hormone-mediated gastric carcinogenic signaling pathway." Mol Cell Proteomics **8**(1): 70-85.

Gastric cancer is the second most common cancer worldwide and has a poor prognosis. To determine the mechanism of adaptation to metabolic stress in cancer cells, we used gastric cancer as a model system to reveal the potential signaling pathways involved. Two-dimensional polyacrylamide gel electrophoresis coupled with ESI-Q-TOF MS/MS analysis was used to identify differentially expressed proteins between gastric tumor tissues and the corresponding noncancerous tissues. In total, 107 spots with significant alteration (+/-over 2-fold, $p < 0.05$) were positively identified by MS/MS analysis. Altered expression of representative proteins was validated by RT-PCR and Western blotting. Cluster analysis of the changed proteins revealed an interesting group of metabolic proteins, which suggested accumulation of triiodothyronine (T(3); the major functional component of thyroid hormone) and overexpression of hypoxia-induced factor (HIF) in gastric carcinoma. These observations were further confirmed by electrochemiluminescence immunoassay and immunohistochemistry. T(3)-induced expression of HIF1-alpha and vascular endothelial growth factor was further verified using a gastric cancer cell line and in vivo mouse model. Because the early accumulation of HIF1-alpha was found to be independent of de novo transcription, we also found that the cytosolic cascade phosphatidylinositol 3-kinase/Akt pathway sensitive to T(3) stimulus was involved. Furthermore we demonstrated that T(3)-induced overexpression of HIF1-alpha was mediated by fumarate accumulation and could be enhanced by fumarate hydratase inactivation but inhibited by 2-oxoglutarate. These results provide evidence for alteration of metabolic proteins and dysfunction of thyroid hormone regulation in gastric tumors, and a novel thyroid hormone-mediated tumorigenic signaling pathway is proposed. Our findings are considered a significant step toward a better understanding of adaptations to metabolic stress in gastric carcinogenesis.

Liu, W., W. Wei, et al. (2007). "CEACAM1 impedes thyroid cancer growth but promotes invasiveness: a putative mechanism for early metastases." Oncogene **26**(19): 2747-58.

CEACAM1, also known as biliary glycoprotein (BGP), CD66a, pp120 and C-CAM1, is a member of the CEA immunoglobulin superfamily. CEACAM1 is a putative tumor suppressor based on diminished expression in some solid neoplasms such as colorectal carcinoma. However, CEACAM1 is overexpressed in some tumors such as non-small cell lung cancer. To clarify the mechanism of action of this cell adhesion molecule, we studied thyroid carcinoma that has a spectrum of morphologies and variable behavior allowing separation of proliferation from invasion and metastasis. CEACAM1 is expressed in thyroid carcinoma cell lines derived from tumors that exhibit aggressive behavior. Introduction of CEACAM1 into endogenously deficient WRO cells resulted in reduced cell cycle progression associated with p21 upregulation and diminished Rb phosphorylation. Forced CEACAM1 expression enhanced cell-matrix adhesion and migration and promoted tumor invasiveness. Conversely, small interfering RNA (siRNA)-mediated downregulation of CEACAM1 expression in MRO cells accelerated cell cycle progression and significantly enhanced tumor size in xenografted mice. CEACAM1 is not appreciably expressed in normal thyroid tissue or benign thyroid tumors. In a human thyroid tissue array, CEACAM1 reactivity was associated with metastatic spread but not with increased tumor size. These findings identify CEACAM1 as a unique mediator that restricts tumor growth whereas increasing metastatic potential. Our data highlight a complex repertoire of actions providing a putative

mechanism underlying the spectrum of biologic behaviors associated with thyroid cancer.

Liu, Z. M., C. A. Hasselt, et al. (2009). "Expression of functional metallothionein isoforms in papillary thyroid cancer." Mol Cell Endocrinol **302**(1): 92-8.

Metallothionein (MT) isoforms have not been studied in papillary thyroid cancer. We examined how the functional MT1 and MT2 isoforms were expressed in papillary thyroid cancer (KAT5) cells. We demonstrated that KAT5 cells expressed eight functional MT1 and MT2 isoforms induced by cadmium. Elevated calcium and activated ERK1/2 predated MT expression. The inhibition of either calcium or ERK1/2 significantly blocked the isoform expression. The induction of these isoforms accompanied an increased progression of cell cycle from G0/G1 to G2-M. The alternation in cell cycle disappeared when the expression of MT isoforms was blocked by calcium inhibitor or ERK1/2 inhibitor. Collectively, KAT5 cells express eight functional MT1 and MT2 isoforms in a pathway controlled by calcium and ERK1/2. The elevation of the MT isoforms contributes to the decreased G0/G1 but increased G2-M phase. These results reveal a novel pathway for the expression of the functional MT in papillary thyroid cancer.

Lonn, S., P. Bhatti, et al. (2007). "Papillary thyroid cancer and polymorphic variants in TSHR- and RET-related genes: a nested case-control study within a cohort of U.S. radiologic technologists." Cancer Epidemiol Biomarkers Prev **16**(1): 174-7.

Several variants in the TSHR and RET signaling pathways genes have been reported to be related to cancer risk. We hypothesized that polymorphic variants in these genes are associated with the risk of papillary thyroid cancer. A nested case-control study was conducted within the U.S. Radiologic Technologists cohort. Eligible validated papillary thyroid cancer cases (n = 167) and frequency-matched (by sex and birth year) controls (n = 491) donated blood for analysis. There were no statistically significant associations between papillary thyroid cancer and 10 selected polymorphic variants in analyses of men and women combined. A borderline significant increasing risk was found for RET G691S (P(trend) = 0.05) and was especially pronounced among young women. For women under 38 years (the median age at diagnosis), the odds ratios were 2.1 (95% confidence interval, 1.2-3.7) for those heterozygous for the RET G691S polymorphism and 3.7 (95% confidence interval, 1.1-11.8) for those who were homozygous (P(trend) = 0.001). Our data provide limited evidence that TSHR- and RET-related genes are related to papillary thyroid cancer risk.

Lopez, J. P., J. Wang-Rodriguez, et al. (2007). "Gefitinib inhibition of drug resistance to doxorubicin by inactivating ABCG2 in thyroid cancer cell lines." Arch Otolaryngol Head Neck Surg **133**(10): 1022-7.

OBJECTIVE: To investigate the regulation of the breast cancer resistance protein ABCG2/BRCP1 drug transporter by epidermal growth factor receptor (EGFR) kinase activity, and to determine whether gefitinib, an EGFR small molecule inhibitor, will modulate the effects of doxorubicin hydrochloride by inhibiting its extrusion from thyroid cancer cells. DESIGN: Extrusion assays using flow cytometry analysis were used to determine the ability of thyroid cancer cells to extrude the chemotherapy drug, doxorubicin, via the ABCG2 drug transporter in the presence or absence of gefitinib. Immunofluorescence was employed to determine the cellular expression of ABCG2. The ABCG2 expression in ARO and WRO cell lines was analyzed by Western blot analysis. Inactivation of EGFR kinase by gefitinib was analyzed by Western blot analysis and immunofluorescence. A terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling assay was performed to demonstrate ABCG2-mediated apoptosis in the presence of doxorubicin. Colony formation assays were performed to determine the effect of gefitinib on thyroid cancer cell survival in response to gefitinib, doxorubicin, or the combination of both drugs. RESULTS: Inhibition of EGFR kinase activity by gefitinib causes the translocation of the ABCG2 drug transporter away from the plasma membrane, resulting in a concomitant decrease in doxorubicin extrusion in thyroid cancer cell lines. Both ARO and WRO demonstrated differential ABCG2 expression, whereas both were sensitized to doxorubicin-induced apoptosis on ABCG2 knockdown with short interfering RNA. The addition of gefitinib increases doxorubicin-induced cell death in thyroid cancer cells as measured by colony formation assay. CONCLUSIONS: Epidermal growth factor receptor regulates the function of the drug transporter ABCG2/BCRP1 and correlates with ABCG2 protein expression levels. Inactivation of the EGFR kinase by gefitinib potentiates the cytotoxic effect of doxorubicin in thyroid cancer, most likely by decreasing the ability of the cell to extrude doxorubicin. The expression of ABCG2 may explain in part the ineffectiveness of doxorubicin as a single modality treatment for anaplastic thyroid cancer or for treatment of metastatic follicular thyroid cancer. Use of this combination treatment of gefitinib and doxorubicin may be a promising therapy for anaplastic thyroid and metastatic follicular thyroid cancer and needs to be investigated further.

Luong, Q. T., J. O'Kelly, et al. (2006). "Antitumor activity of suberoylanilide hydroxamic acid against thyroid cancer cell lines in vitro and in vivo." Clin Cancer Res **12**(18): 5570-7.

PURPOSE: The histone deacetylase inhibitor, suberoylanilide hydroxamic acid (SAHA), has multiple antitumor effects against a variety of human cancers. EXPERIMENTAL DESIGN: We treated several anaplastic and papillary thyroid cancer cell lines with SAHA to determine if it could inhibit the growth of these cells in vitro and in vivo. RESULTS: SAHA effectively inhibited 50% clonal growth of the anaplastic thyroid cancer cell lines, ARO and FRO, and the papillary thyroid cancer cell line, BHP 7-13, at 1.3x10(-7) to 5x10(-7) mol/L, doses that are achievable in patients. In concert with growth inhibition, SAHA down-regulated the expression of cyclin D1 and up-regulated levels of p21WAF1. Annexin V and cleavage of poly(ADP)ribose polymerase were both increased by exposure of the thyroid cancer cells to SAHA. Expression of the death receptor 5 (DR5) gene was also increased by SAHA, but the combination of the DR5 ligand, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), with SAHA had little effect compared with SAHA alone. Of note, the combination of paclitaxel, doxorubicin, or paraplatin with SAHA enhanced cell killing of the thyroid cancer cells. In addition, murine studies showed that SAHA administered daily by i.p. injection at 100 mg/kg inhibited the growth of human thyroid tumor cells. CONCLUSION: Our data indicate that SAHA is a plausible adjuvant therapy for thyroid cancers.

Malaguarnera, R., A. Mandarino, et al. (2005). "The p53-homologue p63 may promote thyroid cancer progression." Endocr Relat Cancer **12**(4): 953-71.

Inactivation of p53 and p73 is known to promote thyroid cancer progression. We now describe p63 expression and function in human thyroid cancer. TAp63alpha is expressed in most thyroid cancer specimens and cell lines, but not in normal thyrocytes. However, in thyroid cancer cells TAp63alpha fails to induce the target genes (p21Cip1, Bax, MDM2) and, as a consequence, cell cycle arrest and apoptosis occur. Moreover, TAp63alpha antagonizes the effect of p53 on target genes, cell viability and foci formation, and p63 gene silencing by small interfering (si) RNA results in improved p53 activity. This unusual effect of TAp63alpha depends on the protein C-terminus, since TAp63beta and TAp63gamma isoforms, which have a different arrangement of their C-terminus, are still able to induce the target genes and to exert tumour-restraining effects in thyroid cancer cells. Our data outline the existence of a complex network among p53 family members, where TAp63alpha may promote thyroid tumour progression by inactivating the tumour suppressor activity of p53.

Malaguarnera, R., V. Vella, et al. (2008). "TAp73 alpha increases p53 tumor suppressor activity in thyroid cancer cells via the inhibition of Mdm2-mediated degradation." Mol Cancer Res **6**(1): 64-77.

p53 family proteins include p53 tumor suppressor, p63, and p73. Despite the high similarity in structure and function with p53, p63, and p73 function in tumor suppression is still controversial. Here, we show that TAp73alpha, a transcriptionally active p73 isoform, is able to synergize p53 tumor suppressor function in thyroid cancer cells. Indeed, depletion of p73 by small interfering RNA in thyroid cancer cells resulted in a reduced transcriptional activity of p53. Ectopic coexpression of both p53 and TAp73alpha in thyroid cancer cells resulted in increased transcription and tumor suppressor function compared with p53 or TAp73alpha alone, as well as in increased p53 protein levels. The enhancing effect of TAp73alpha on p53 activity is Mdm2 dependent because it is prevented by Mdm2 depletion by small interfering RNA. At least two mechanisms may explain the interference of TAp73alpha with p53 function. First, in thyroid cancer cells, TAp73alpha inhibits the effect of p53 on Mdm2 induction by antagonizing p53 at the Mdm2 promoter level. Second, a TAp73alpha mutant (G264W), which is devoid of DNA binding capability, is still able to increase p53 protein levels by competing with p53 for Mdm2 protein binding. Taken together, these results indicate that in thyroid cancer cells, TAp73alpha is able to increase p53 protein level and function by interfering with Mdm2-mediated p53 degradation. These results may be useful for designing gene therapies aimed at restoring a normal p53 function in thyroid cancer cells.

Malaguarnera, R., V. Vella, et al. (2007). "p53 family proteins in thyroid cancer." Endocr Relat Cancer **14**(1): 43-60.

At variance with other human malignancies, p53 mutations are not frequent in thyroid cancer and are believed to be responsible mainly for cancer progression to poorly differentiated and aggressive phenotype. p63 and p73, two proteins with a high degree of homology with p53, are overexpressed in thyroid cancer, but their role in cancer initiation or progression is controversial. Regulation of p53 family protein function depends on: (1) the balance between the expression of transcriptionally active (p53, TAp63, and TAp73) and inactive isoforms (DeltaNp63 and DeltaNp73); (2) their interaction and competition at DNA-responsive elements; (3) their

interaction with regulatory proteins, either inhibitory or activating. In thyroid cancer, therefore, although mutations of the p53 oncosuppressor protein family are rare, other mechanisms are present, including aberrant expression of p53 family dominant negative isoforms, up-regulation of inhibitory proteins, and functional inhibition of activating proteins. The overall result is a defective oncosuppressor activity. These inactivating mechanisms may be present in the early stages of thyroid cancer and in different cancer histotypes. A better understanding of this complex network may not only ameliorate our comprehension of cancer biology, but also open the possibility of innovative diagnostic procedures and the development of targeted therapies.

Marlow, L. A., L. A. Reynolds, et al. (2009). "Reactivation of suppressed RhoB is a critical step for the inhibition of anaplastic thyroid cancer growth." Cancer Res **69**(4): 1536-44.

Anaplastic thyroid carcinoma (ATC) is a highly aggressive form of the disease for which new therapeutic options are desperately needed. Previously, we showed that the high-affinity peroxisome proliferator-activated receptor gamma (PPARgamma) agonist, RS5444, inhibits cell proliferation of ATC cells via induction of the cyclin-dependent kinase inhibitor p21(WAF1/CIP1) (p21). We show here that up-regulation of RhoB is a critical step in PPARgamma-mediated activation of p21-induced cell stasis. Using multiple independently derived ATC cell lines, we found that treatment with RS5444 leads to the up-regulation of RhoB and subsequent activation of p21, and that silencing of RhoB by RNAi blocks the ability of RS5444 to induce p21 and to inhibit cell proliferation. Our results show that transcriptional regulation of RhoB by the nuclear transcription factor PPARgamma is responsible for the induction of p21 mRNA and protein. We further implicate RhoB as a key signaling effector for the growth inhibition of ATC, as treatment with a histone deacetylase inhibitor shown to increase RhoB expression in lung cancer cells caused the up-regulation of RhoB in ATC cells accompanied by increased expression of p21 and inhibition of cell proliferation; this effect occurred even in ATC cells that were unresponsive to RS5444 due to a lack of expression of PPARgamma. Our results implicate RhoB as a novel intermediate in critical signaling pathways and as an additional target for therapeutic intervention in ATC.

McCall, K. D., N. Harii, et al. (2007). "High basal levels of functional toll-like receptor 3 (TLR3) and noncanonical Wnt5a are expressed in papillary thyroid cancer and are coordinately decreased by phenylmethimazole together with cell proliferation and migration." Endocrinology **148**(9): 4226-37.

High basal levels of TLR3 and Wnt5a RNA are present in papillary thyroid carcinoma (PTC) cell lines consistent with their overexpression and colocalization in PTC cells in vivo. This is not the case in thyrocytes from normal tissue and in follicular carcinoma (FC) or anaplastic carcinoma (AC) cells or tissues. The basally expressed TLR3 are functional in PTC cells as evidenced by the ability of double-strand RNA (polyinosine-polycytidylic acid) to significantly increase the activity of transfected NF-kappaB and IFN-beta luciferase reporter genes and the levels of two end products of TLR3 signaling, IFN-beta and CXCL10. Phenylmethimazole (C10), a drug that decreases TLR3 expression and signaling in FRTL-5 thyrocytes, decreases TLR3 levels and signaling in PTC cells in a concentration-dependent manner. C10 also decreased Wnt5a RNA levels coordinate with decreases in TLR3. E-cadherin RNA levels, whose suppression may be associated with high Wnt5a, increased with C10 treatment. C10 simultaneously decreased PTC proliferation and cell migration but had no effect on the growth and migration of FC, AC, or FRTL-5 cells. C10 decreases high basal phosphorylation of Tyr705 and Ser727 on Stat3 in PTC cells and inhibits IL-6-induced Stat3 phosphorylation. IL-6-induced Stat3 phosphorylation is important both in up-regulating Wnt5a levels and in cell growth. In sum, high Wnt5a levels in PTC cells may be related to high TLR3 levels and signaling; and the ability of phenylmethimazole (C10) to decrease growth and migration of PTC cells may be related to its suppressive effect on TLR3 and Wnt5a signaling, particularly Stat3 activation.

Melillo, R. M., M. D. Castellone, et al. (2005). "The RET/PTC-RAS-BRAF linear signaling cascade mediates the motile and mitogenic phenotype of thyroid cancer cells." J Clin Invest **115**(4): 1068-81.

In papillary thyroid carcinomas (PTCs), rearrangements of the RET receptor (RET/PTC) and activating mutations in the BRAF or RAS oncogenes are mutually exclusive. Here we show that the 3 proteins function along a linear oncogenic signaling cascade in which RET/PTC induces RAS-dependent BRAF activation and RAS- and BRAF-dependent ERK activation. Adoptive activation of the RET/PTC-RAS-BRAF axis induced cell proliferation and Matrigel invasion of thyroid follicular cells. Gene expression profiling revealed that the 3 oncogenes activate a common transcriptional program in thyroid cells that includes upregulation of the CXCL1 and CXCL10 chemokines, which in turn stimulate proliferation and invasion. Thus, motile and mitogenic properties are intrinsic to transformed thyroid cells

and are governed by an epistatic oncogenic signaling cascade.

Meng, Z., N. Mitsutake, et al. (2008). "Dehydroxymethylepoxyquinomicin, a novel nuclear Factor-kappaB inhibitor, enhances antitumor activity of taxanes in anaplastic thyroid cancer cells." Endocrinology **149**(11): 5357-65.

Nuclear factor kappaB (NF-kappaB), as an antiapoptotic factor, crucially affects the outcomes of cancer treatments, being one of the major culprits of resistance to chemotherapy. In this study, we investigated whether dehydroxymethylepoxyquinomicin (DHMEQ), a novel NF-kappaB inhibitor, can enhance antitumor activities of taxanes in anaplastic thyroid cancer (ATC) cells. Taxanes induced NF-kappaB activation in ATC cells, which could compromise the therapeutic effect of the drugs. However, DHMEQ, by inhibiting the nuclear translocation of NF-kappaB, completely suppressed the DNA binding capacities of NF-kappaB and lowered the levels of nuclear NF-kappaB protein. Compared with single treatment (either taxane or DHMEQ), the combined treatment strongly potentiated apoptosis, confirmed by cell survival assay; Western blotting for poly (ADP-ribose) polymerase, caspase 3, X-linked inhibitor of apoptosis, and survivin; and flow cytometry for annexin V. Furthermore, we also demonstrate for the first time that the combined treatment showed significantly greater inhibitory effect on tumor growth in a nude mice xenograft model. These findings suggest that taxanes are able to induce NF-kappaB activation in ATC cells, which could attenuate antitumor activities of the drugs, but inhibition of NF-kappaB by DHMEQ creates a chemosensitive environment and greatly enhances apoptosis in taxanes-treated ATC cells in vitro and in vivo. Thus, DHMEQ may emerge as an attractive therapeutic strategy to enhance the response to taxanes in ATCs.

Menon, M. M. and M. R. Simha (2005). "RET mutation status in medullary thyroid cancer(MTC) patients and the significance of genetic screening for mutations in their immediate relatives--a preliminary report." Indian J Pathol Microbiol **48**(2): 161-5.

Multiple Endocrine Neoplasia (MEN) 2A is an inherited disease characterized by the development of medullary thyroid carcinoma (MTC), pheochromocytoma(PHCH) and hyperparathyroidism(HPT). It has recently been shown to be associated with germline mutations in the RET proto-oncogene. Genetic testing for RET mutations will, therefore allow the identification of people with asymptomatic MEN 2 who can be offered prophylactic thyroidectomy and biochemical screening as preventive measures. No genetic study based on RET mutation detection has been available in India so far. The aim of the present study is to detect the proportion of MTC cases having inherited germline or somatic RET mutations and to identify family members at risk for MEN and, thereby the feasibility of screening for MEN. DNA extracted from the peripheral blood and somatic (tumor) tissues were subjected to PCR using primers for exons 10,11 and 16. A few samples were subjected to direct sequencing. Germline mutations were identified in 3 of 4 MEN 2A patients, 18 of 24 sporadic MTC(SMTC), 2 of 4 children of MEN2A and 8 relatives of SMTC. Common mutation was in exon 10 and 11 (c634). It is recommended that RET mutation analysis and counseling of patients and their immediate relatives be introduced on a regular basis to identify gene carriers.

Milano, A., M. G. Chiofalo, et al. (2006). "New molecular targeted therapies in thyroid cancer." Anticancer Drugs **17**(8): 869-79.

Carcinoma of the thyroid gland is the most common malignancy of the endocrine system. Differentiated tumors are often curable with surgical resection and radioactive iodine. A small percentage of such patients, however, do not undergo remission and need new therapeutic approaches. Both anaplastic and medullary thyroid carcinomas exhibit aggressive behavior and are usually resistant to current therapeutic modalities. Thyroid carcinoma represents a fascinating model and a particularly promising paradigm for targeted therapy because some of the key oncogenic events are activating mutations of genes coding for tyrosine kinases, and these occur early in cancer development. A prototype is the RET proto-oncogene, a receptor tyrosine kinase, which is a key regulator of development and a 'hotspot' for oncogenic mutations. Mutations in the RET proto-oncogene have been identified as causative for papillary carcinoma and familial medullary thyroid carcinoma, making it an attractive target for selective inhibition in these subtypes. ZD 6474 has shown promising activity in preclinical models against RET kinase, and its contemporary inhibition of vascular endothelial growth factor and epidermal growth factor pathways renders it a very attractive drug for clinical trials in thyroid cancer. Activating point mutation of B-RAF can occur early in the development of papillary carcinoma. Moreover, papillary carcinomas with these mutations have more aggressive properties and are diagnosed more often at an advanced stage. Clinical evaluation of B-RAF-targeting drugs is undergoing and trials in thyroid cancer are planned. Agents that restore radioiodine uptake, such as histone deacetylase inhibitors and retinoids, represent another

exciting field in new drug development in thyroid cancer.

Mitsiades, C. S., V. Poulaki, et al. (2005). "Novel histone deacetylase inhibitors in the treatment of thyroid cancer." Clin Cancer Res **11**(10): 3958-65.

Histone deacetylases (HDAC) and histone acetyltransferases exert opposing enzymatic activities that modulate the degree of acetylation of histones and other intracellular molecular targets, thereby regulating gene expression, cellular differentiation, and survival. HDAC inhibition results in accumulation of acetylated histones and induces differentiation and/or apoptosis in transformed cells. In this study, we characterized the effect of two HDAC inhibitors, suberoylanilide hydroxamic acid (SAHA) and m-carboxycinnamic acid bis-hydroxamide, on thyroid carcinoma cell lines, including lines originating from anaplastic and medullary carcinomas. In these models, both SAHA and m-carboxycinnamic acid bis-hydroxamide induced growth arrest and caspase-mediated apoptosis and increased p21 protein levels, retinoblastoma hypophosphorylation, BH3-interacting domain death agonist cleavage, Bax up-regulation, down-regulation of Bcl-2, A1, and Bcl-x(L) expression, and cleavage of poly(ADP-ribose) polymerase and caspase-8, -9, -3, -7, and -2. Transfection of Bcl-2 cDNA partially suppressed SAHA-induced cell death. SAHA down-regulated the expression of the apoptosis inhibitors FLIP and cIAP-2 and sensitized tumor cells to cytotoxic chemotherapy and death receptor activation. Our studies provide insight into the tumor type-specific mechanisms of antitumor effects of HDAC inhibitors and a framework for future clinical applications of HDAC inhibitors in patients with thyroid cancer, including histologic subtypes (e.g., anaplastic and medullary thyroid carcinomas) for which limited, if any, therapeutic options are available.

Mitsutake, N., A. Iwao, et al. (2007). "Characterization of side population in thyroid cancer cell lines: cancer stem-like cells are enriched partly but not exclusively." Endocrinology **148**(4): 1797-803.

There is increasing evidence that cancers contain their own stem-like cells called cancer stem cells (CSCs). A small subset of cells, termed side population (SP), has been identified using flow cytometric analysis. The SP cells have the ability to exclude the DNA binding dye, Hoechst33342, and are highly enriched for stem cells in many kinds of normal tissues. Because CSCs are thought to be drug resistant, SP cells in cancers might contain CSCs. We initially examined the presence of SP cells in several human thyroid cancer cell lines. A small percentage of SP cells were found in ARO (0.25%), FRO (0.1%), NPA (0.06%), and WRO (0.02%) cells but not TPC1 cells. After sorting, the SP cells generated both SP and non-SP cells in culture. The clonogenic ability of SP cells was significantly higher than that of non-SP cells. Moreover, the SP prevalence was dependent on cell density in culture, suggesting that SP cells preferentially survived at lower cell density. Microarray experiment revealed differential gene expression profile between SP and non-SP cells, and several genes related to stemness were up-regulated. However, non-SP population also contained cells that were tumorigenic in nude mice, and non-SP cells generated a small number of SP cells. These results suggest that cancer stem-like cells are partly, but not exclusively, enriched in SP population. Clarifying the key tumorigenic population might contribute to the establishment of a novel therapy for thyroid cancer.

Motti, M. L., D. Califano, et al. (2005). "Complex regulation of the cyclin-dependent kinase inhibitor p27kip1 in thyroid cancer cells by the PI3K/AKT pathway: regulation of p27kip1 expression and localization." Am J Pathol **166**(3): 737-49.

Functional inactivation of the tumor suppressor p27(kip1) in human cancer occurs either through loss of expression or through phosphorylation-dependent cytoplasmic sequestration. Here we demonstrate that dysregulation of the PI3K/AKT pathway is important in thyroid carcinogenesis and that p27(kip1) is a key target of the growth-regulatory activity exerted by this pathway in thyroid cancer cells. Using specific PI3K inhibitors (LY294002, wortmannin, and PTEN) and a dominant active AKT construct (myrAKT), we demonstrated that the PI3K/AKT pathway controlled thyroid cell proliferation by regulating the expression and subcellular localization of p27. Results obtained with phospho-specific antibodies and with transfection of nonphosphorylable p27(kip1) mutant constructs demonstrated that PI3K/AKT-dependent regulation of p27(kip1) mislocalization in thyroid cancer cells occurred via phosphorylation of p27(kip1) at T157 and T198 (but not at S10 or T187). Finally, we evaluated whether these results were applicable to human tumors. Analysis of 100 thyroid carcinomas indicated that p27(kip1) phosphorylation at T157/T198 and cytoplasmic mislocalization were preferentially associated with activation of the PI3K/AKT pathway. Thus the PI3/AKT pathway and its effector p27(kip1) play major roles in thyroid carcinogenesis.

Nakashima, M., N. Takamura, et al. (2007). "RET oncogene amplification in thyroid cancer: correlations

with radiation-associated and high-grade malignancy." Hum Pathol **38**(4): 621-8.

A radiation etiology is well known in thyroid carcinogenesis. RET oncogene rearrangement is the most common oncogenic alteration in Chernobyl-related papillary thyroid cancer (PTC). To find the characteristic alteration associated with RET rearrangements in radiation-induced thyroid cancers, we analyzed the RET oncogene by fluorescence in situ hybridization. The fluorescence in situ hybridization technique has the possibility of detecting RET rearrangements at a single-cell level regardless of the specific fusion partner involved and directly reveals RET copy number on a per-cell basis. Our study demonstrated RET amplification in all 3 cases of radiation-associated thyroid cancers but not in sporadic well-differentiated PTC (n = 11). Furthermore, RET amplification was observed in all 6 cases of sporadic anaplastic thyroid cancers (ATCs). The frequency of RET amplification-positive cells was higher in ATC (7.2%-24.1%) than in PTC (1.5%-2.7%). The highest frequency of RET amplification-positive cells was observed among ATC cases with a strong p53 immunoreactivity. In conclusion, we found RET amplification, which is a rare oncogenic aberration, in thyroid cancer. This report is the first one to suggest the presence of RET amplification in PTC and ATC. RET amplification was correlated with radiation-associated, high-grade malignant potency, and p53 accumulation, suggesting genomic instability. RET amplification might be induced by a high level of genomic instability in connection with progression of thyroid carcinogenesis and, subsequently, be associated with radiation-induced and/or high-grade malignant cases.

Namba, H., V. Saenko, et al. (2007). "Nuclear factor-kB in thyroid carcinogenesis and progression: a novel therapeutic target for advanced thyroid cancer." Arq Bras Endocrinol Metabol **51**(5): 843-51.

Apoptosis is an essential physiological process of elimination of destined cells during the development and differentiation or after damage from external stresses such as ionizing radiation or chemotherapeutic agents. Disruption of apoptosis is proved to cause various diseases including cancer. Among numerous molecules involved in diverse anti- or pro-apoptotic signaling pathways, NF-kappaB is one of the key factors controlling anti-apoptotic responses. Its anti-apoptotic effect is thought to be mediated through not only transcriptional activation of dependent genes but also by crosstalking with the JNK pathway. Oncogenic proteins such as Ret/PTC, Ras and BRAF can induce NF-kappaB activation making it an important change in thyroid cancer. A number of specific or non-specific NF-kappaB inhibitors have been tried to take over the cascade in in vitro and in vivo experiments. These agents can induce massive apoptosis especially in combination with radio- or chemotherapy. Current results suggest that the inhibition of the NF-kappaB may be a promising strategy for advanced thyroid cancer treatment but further investigations are warranted to develop specific and clinically effective NF-kappaB inhibitors in future.

Niedzwiecki, S., T. Stepien, et al. (2008). "Serum levels of interleukin-1 receptor antagonist (IL-1ra) in thyroid cancer patients." Langenbecks Arch Surg **393**(3): 275-80.

BACKGROUND AND AIMS: There is growing evidence that cytokines and their antagonists are important in the pathogenesis of various malignancies. While there are several reports on interleukin-1 receptor antagonist (IL-1ra) gene polymorphism and tissue expression, there is only little data available on the impact of IL-1ra serum levels. Therefore, we performed a prospective study, analyzing IL-1ra in thyroid cancer patients. MATERIALS AND METHODS: We measured preoperative IL-1ra serum levels of 52 consecutive patients with thyroid cancer, 15 with benign adenoma and 27 healthy volunteers. The final histological diagnosis revealed 21 patients with papillary and 8 patients with follicular carcinoma (FTC), while 12 cases of medullary and 11 cases of anaplastic carcinoma (ATC) were observed. RESULTS: Compared to the control group, serum concentrations of IL-1ra were significantly higher in ATC and FTC patients. Concerning gender differences, this effect reached significance only in women with ATC and FTC. Except for the stage IV disease in ATC, there was no correlation between IL-1ra levels and International Union Against Cancer staging. CONCLUSION: The findings of our study indicate that IL-1ra may play an important role in the development of ATC and FTC. Future efforts should focus on the possible application of IL-1ra as a biomarker for the above-mentioned thyroid malignancies.

Onda, M., J. Akaishi, et al. (2005). "Decreased expression of haemoglobin beta (HBB) gene in anaplastic thyroid cancer and recovery of its expression inhibits cell growth." Br J Cancer **92**(12): 2216-24.

Anaplastic thyroid cancer (ATC) is one of the most fulminant and foetal diseases in human malignancies. However, the genetic alterations and carcinogenic mechanisms of ATC are still unclear. Recently, we investigated the gene expression profile of 11 anaplastic thyroid cancer cell lines (ACL) and

significant decreased expression of haemoglobin beta (HBB) gene in ACL. Haemoglobin beta is located at 11p15.5, where loss of heterozygosity (LOH) was reported in various kinds of cancers, including ATC, and it has been suggested that novel tumour suppressor genes might exist in this region. In order to clarify the meaning of decreased expression of HBB in ATC, the expression status of HBB was investigated with ACL, ATC, papillary thyroid cancer (PTC) and normal human tissues. Haemoglobin beta showed significant decreased expression in ACLs and ATCs; however, in PTC, HBB expressed equal to the normal thyroid gland. In addition, HBB expressed in normal human tissues ubiquitously. To validate the tumour-suppressor function of HBB, cell growth assay was performed. Forced expression of HBB in KTA2 cell, which is a kind of ACL, significantly suppressed KTA2 growth. The mechanism of downregulation of HBB in ATC is still unclear; however, our results suggested the possibility of HBB as a novel tumour-suppressor gene.

Pacifico, F., M. Paolillo, et al. (2007). "RbAp48 is a target of nuclear factor-kappaB activity in thyroid cancer." J Clin Endocrinol Metab **92**(4): 1458-66.

CONTEXT: We have recently shown that nuclear factor (NF)-kappaB activity is constitutively elevated in anaplastic human thyroid carcinomas. The inhibition of NF-kappaB in the anaplastic thyroid carcinoma cell line (FRO) leads to increased susceptibility to apoptosis induced by chemotherapeutic drugs and to the block of oncogenic activity. OBJECTIVES: To understand better the molecular mechanisms played by NF-kappaB in thyroid oncogenesis, we performed a differential proteomic analysis between FRO transfected with a superrepressor form of inhibitor of kappaBalpha (IkappaBalphaM) and the parental counterpart (FRO Neo cells). RESULTS: Differential proteomic analysis revealed that the retinoblastoma-associated protein 48 (RbAp48) is down-regulated in the absence of functional NF-kappaB. Immunohistochemical analysis of normal and pathological human thyroid specimens confirmed that RbAp48 is strongly overexpressed in primary human carcinomas. Reduction of RbAp48 expression using small interfering RNA determined the suppression of tumorigenicity, very likely due to the decrease of their growth rate rather than to an increased susceptibility to apoptosis. In addition, we showed that NF-kappaB, at least in part, transcriptionally controls RbAp 48. A functional NF-kappaB consensus sequence was located within the promoter region of RbAp48 human gene, and embryonic fibroblasts isolated from the p65 knockout mouse (murine embryonic fibroblasts p65-/-) showed decreased expression of RbAp48. CONCLUSION: Our results show that RbAp48 is a NF-kappaB-regulated gene playing an important role in thyroid cancer cell autonomous proliferation.

Pallante, P., A. Federico, et al. (2008). "Loss of the CBX7 gene expression correlates with a highly malignant phenotype in thyroid cancer." Cancer Res **68**(16): 6770-8.

Using gene expression profiling, we found that the CBX7 gene was drastically down-regulated in six thyroid carcinoma cell lines versus control cells. The aims of this study were to determine whether CBX7 is related to the thyroid cancer phenotype and to try to identify new tools for the diagnosis and prognosis of thyroid cancer. We thus evaluated CBX7 expression in various snap-frozen and paraffin-embedded thyroid carcinoma tissues of different degrees of malignancy by quantitative reverse transcription-PCR and immunohistochemistry, respectively. CBX7 expression progressively decreased with malignancy grade and neoplasia stage. Indeed, it decreased in an increasing percentage of cases going from benign adenomas to papillary (PTC), follicular, and anaplastic (ATC) thyroid carcinomas. This finding coincides with results obtained in rat and mouse models of thyroid carcinogenesis. CBX7 loss of heterozygosity occurred in 36.8% of PTC and in 68.7% of ATC. Restoration of CBX7 expression in thyroid cancer cells reduced growth rate, with a retention in the G(1) phase of the cell cycle, suggesting that CBX7 can contribute to the proliferation of the transformed thyroid cells. In conclusion, loss of CBX7 expression correlates with a highly malignant phenotype in thyroid cancer patients.

Papadopoulou, F. and E. Efthimiou (2009). "Thyroid cancer after external or internal ionizing irradiation." Hell J Nucl Med **12**(3): 266-70.

It has been known for 50 years that thyroid exposure to high doses of ionizing radiation in childhood and adolescence induces an appreciable cancer risk. Epidemiological studies in children treated with external radiotherapy for benign or malignant lesions in the head and neck have also shown the induction of thyroid cancer. The World Health Organization (WHO) has reported that the risk for developing thyroid cancer due to the Chernobyl accident is greatest in newborns and children below the age of 5, less in adolescents and negligible in adults. As reported, during the first 15 years after the accident, the increase in thyroid cancer cases in Belarus was 87.8 fold in children, 12.7 fold in adolescents and 4.5 fold in adults more than expected. Papillary thyroid cancer with a relative risk incidence of approximately 80% per se is typical in childhood and adolescence. We refer to the differences between

adult and childhood papillary thyroid cancers. Gene mutations in thyroid tumors induced after Chernobyl accident have been studied extensively. The treatment comprises thyroid surgery, suppressive doses of thyroxine and radioiodine. It is noteworthy that the thyroid gland can be protected from the intake of radioactive iodine by oral administration of potassium iodide.

Park, J. W., R. Zarnegar, et al. (2005). "Troglitazone, the peroxisome proliferator-activated receptor-gamma agonist, induces antiproliferation and redifferentiation in human thyroid cancer cell lines." Thyroid **15**(3): 222-31.

Troglitazone is a potent agonist for the peroxisome proliferator-activated receptor-gamma (PPARgamma) that is a ligand-activated transcription factor regulating cell differentiation and growth. PPARgamma may play a role in thyroid carcinogenesis since PAX8-PPARgamma1 chromosomal translocations are commonly found in follicular thyroid cancers. We investigated the antiproliferative and redifferentiation effects of troglitazone in 6 human thyroid cancer cell lines: TPC-1 (papillary), FTC-133, FTC-236, FTC-238 (follicular), XTC-1 (Hurthle cell), and ARO82-1 (anaplastic) cell lines. PPARgamma was expressed variably in these cell lines. FTC-236 and FTC-238 had a rearranged chromosome at 3p25, possibly implicating the involvement of the PPARgamma encoding gene whereas the other cell lines did not. Troglitazone significantly inhibited cell growth by cell cycle arrest and apoptotic cell death. PPARgamma overexpression did not appear to be a prerequisite for a response to treatment with troglitazone. Troglitazone also downregulated surface expression of CD97, a novel dedifferentiation marker, in FTC-133 cells and upregulated sodium iodide symporter (NIS) mRNA in TPC-1 and FTC-133 cells. Our investigations document that human thyroid cancer cell lines commonly express PPARgamma, but chromosomal translocations involving PPARgamma are uncommon. Troglitazone, a PPARgamma agonist, induced antiproliferation and redifferentiation in thyroid cancer cell lines. PPARgamma agonists may therefore be effective therapeutic agents for the treatment of patients with thyroid cancer that fails to respond to traditional treatments.

Pellegriti, G., F. De Vathaire, et al. (2009). "Papillary thyroid cancer incidence in the volcanic area of Sicily." J Natl Cancer Inst **101**(22): 1575-83.

BACKGROUND: The steadily increasing incidence of thyroid cancer has been attributed mostly to more sensitive thyroid nodule screening. However, various environmental factors, such as those associated with volcanic areas, cannot be excluded as risk factors. We evaluated thyroid cancer incidence in Sicily, which has a homogenous population and a province (Catania) that includes the Mt Etna volcanic area. METHODS: In a register-based epidemiological survey, we collected all incident thyroid cancers in Sicily from January 1, 2002, through December 31, 2004. The age-standardized incidence rate for the world population (ASR(w)) was calculated and expressed as the number of thyroid cancer diagnoses per 100 000 residents per year. The association of thyroid cancer incidence rate with sex, age, tumor histotype, and various environmental factors was evaluated by modeling the variation of the ASR(w). All statistical tests were two-sided. RESULTS: In 2002-2004, 1950 incident thyroid cancers were identified in Sicily (among women, ASR(w) = 17.8, 95% confidence interval [CI] = 16.9 to 18.7; and among men, ASR(w) = 3.7, 95% CI = 3.3 to 4.1). Although the percentage of thyroid cancers that were microcarcinomas (ie, < or = 10 mm) and ratio of men to women with thyroid cancer were similar in all nine Sicilian provinces, thyroid cancer incidence was statistically significantly higher in the province of Catania (among women, ASR(w) = 31.7, 95% CI = 29.1 to 34.3; and among men, ASR(w) = 6.4, 95% CI = 5.2 to 7.5) than in the rest of Sicily (among women, ASR(w) = 14.1, 95% CI = 13.2 to 15.0; and among men, ASR(w) = 3.0, 95% CI = 2.6 to 3.4) (all P values < .001). Incidence of papillary, but not follicular or medullary, cancers was statistically significantly increased in Catania province, and papillary tumors from patients in Catania more frequently carried the BRAF V600E gene mutation (55 [52%] of 106 tumors) than tumors from patients elsewhere in Sicily (68 [33%] of 205 tumors) (relative risk = 1.7, 95% CI = 1.0 to 2.8, P = .02). Cancer incidence was statistically significantly lower in rural areas than in urban areas of Sicily (P = .003). No association with mild iodine deficiency or industrial installations was found. Levels of many elements (including boron, iron, manganese, and vanadium) in the drinking water of Catania province often exceeded maximum admissible concentrations, in contrast to water in the rest of Sicily. CONCLUSION: Residents of Catania province with its volcanic region appear to have a higher incidence of papillary thyroid cancer than elsewhere in Sicily.

Penko, K., J. Livezey, et al. (2005). "BRAF mutations are uncommon in papillary thyroid cancer of young patients." Thyroid **15**(4): 320-5.

Mortality is low for young patients (younger than 21 years) with papillary thyroid cancer (PTC), and different mutations might contribute to this. Previous studies detected ret/PTC rearrangements

more frequently in PTC from children than adults, and recent reports describe a high incidence of BRAF T1796A transversion in adult PTC. However, BRAF mutations have not been adequately studied in PTC from young patients. We amplified and sequenced segments of the BRAF gene spanning the T1796A transversion site in 14 PTC from patients 10-21 years of age (mean, 17.5 +/- 3.5 years). The PTC (7 = class 1; 5 = class 2; 1 = class 3) ranged from 0.7-2.9 cm in diameter (mean, 1.4 +/- 0.75 cm). None of them (0/14) contained BRAF T1796A and none recurred (mean follow-up, 66 +/- 40 months). This incidence of BRAF T1796A is significantly less than that reported for adult PTC (270/699, 38.6%, p = 0.0015) in several series. None of our PTC (0/10) contained ras mutations, but 7/12 (58%) contained ret/PTC rearrangements. We conclude that BRAF mutations are less common in PTC from young patients, and ret/PTC rearrangements were the most common mutation found in these childhood PTC.

Petrella, A., M. Festa, et al. (2006). "Annexin-1 downregulation in thyroid cancer correlates to the degree of tumor differentiation." Cancer Biol Ther **5**(6): 643-7.

We investigated the expression of annexin-1 (ANXA1) in thyroid carcinoma cell lines and in thyroid cancers with a different degree of differentiation. The highest level of ANXA1 expression examined by Western blotting was detected in the papillary carcinoma cells (NPA) and in the follicular cells (WRO). On the other hand, the most undifferentiated thyroid carcinoma cells (ARO and FRO) presented the lowest level of ANXA1 expression. In surgical tissue specimens from 32 patients with thyroid cancers, we found high immunoreactivity for ANXA1 in papillary (PTC) and follicular (FTC) thyroid cancers while in undifferentiated thyroid cancers (UTC) the expression of the protein was barely detectable. Control thyroid tissue resulted positive for ANXA1. In summary, 70% of UTC examined weakly expressed ANXA1, whereas 65% of PTC or FTC specimens tested showed high expression of the protein. Thus ANXA1 expression may correlate with the tumorigenesis suggesting that the protein may represent an effective differentiation marker in thyroid cancer.

Pilli, T., K. V. Prasad, et al. (2009). "Potential utility and limitations of thyroid cancer cell lines as models for studying thyroid cancer." Thyroid **19**(12): 1333-42.

BACKGROUND: Tumor-derived cell lines are widely used to study the mechanisms involved in thyroid carcinogenesis but recent studies have reported redundancy among thyroid cancer cell lines and identification of some "thyroid cell lines" that are likely not of thyroid origin. SUMMARY: In this review, we have summarized the uses, the limitations, and the existing problems associated with the available follicular cell-derived thyroid cancer cell lines. There are some limitations to the use of cell lines as a model to "mimic" in vivo tumors. Based on the gene expression profiles of thyroid cell lines originating from tumors of different types it has become apparent that some of the cell lines are closely related to each other and to those of undifferentiated carcinomas. Further, many cell lines have lost the expression of thyroid-specific genes and have altered karyotypes, while they exhibit activation of several oncogenes (BRAF, v-raf murine sarcoma viral oncogene homolog B1; RAS, rat sarcoma; and RET/PTC, rearranged in transformation/papillary thyroid carcinoma) and inactivation of tumor suppressor gene (TP53) which is known to be important for thyroid tumorigenesis. CONCLUSIONS: A careful selection of thyroid cancer cell lines that reflect the major characteristics of a particular type of thyroid cancer being investigated could be used as a good model system to analyze the signaling pathways that may be important in thyroid carcinogenesis. Further, the review of literature also suggests that some of the limitations can be overcome by using multiple cell lines derived from the same type of tumor.

Prante, O., S. Maschauer, et al. (2009). "Regulation of uptake of 18F-FDG by a follicular human thyroid cancer cell line with mutation-activated K-ras." J Nucl Med **50**(8): 1364-70.

Dedifferentiation of thyroid carcinoma is accompanied by increased accumulation of the PET tracer (18)F-FDG. The molecular mechanisms responsible for this phenomenon are poorly understood. Therefore, we studied the regulation of (18)F-FDG uptake by the human follicular thyroid carcinoma cell line ML-1 and the as-yet-unknown oncogene expression of that cell line. The data obtained in ML-1 were compared with those of a well-differentiated thyroid cell line of rat origin (FRTL-5). METHODS: The expression of the thyroid-stimulating hormone (TSH) receptor was investigated by immunocytochemistry, and the expression of the glucose transporters (GLUTs) was determined by Western blotting. Mutation analysis of ML-1 was performed for K-ras codons 12 and 13. The effect of TSH on intracellular cAMP levels was determined by a competitive enzyme immunoassay. Cells were incubated with (18)F-FDG (0.5-1.0 MBq/mL) for 1 h, and tracer uptake was related to protein concentration. The effects of bovine TSH, the cAMP analog (Bu)(2)cAMP, and the

phosphatidylinositol-3-kinase (PI3-kinase) inhibitor LY294002 on (18)F-FDG uptake were investigated. RESULTS: The TSH receptor was present in both cell lines. FRTL-5 clearly expressed GLUT-1 and also GLUT-4. In ML-1 only, the expression of GLUT-3 was detected. TSH and (Bu)(2)cAMP had a significant effect on (18)F-FDG uptake or GLUT-1 expression in FRTL-5, but not in ML-1 cells. PI3-kinase inhibition by LY294002 downregulated (18)F-FDG uptake in FRTL-5 by 58% +/- 9% (n = 6) and in ML-1 by 26% +/- 5% (n = 42, both P < 0.05). Mutation analysis of ML-1 cells revealed a Gly12Ser point mutation at codon 12 of the K-ras gene. CONCLUSION: (18)F-FDG uptake in the thyroid carcinoma cell line ML-1 is no longer regulated by TSH or cAMP or mediated by GLUT-1. However, in this cell line, this variable is still governed to some extent by PI3-kinase located downstream to the constitutively active K-ras in the Ras-PI3-kinase-Akt pathway. These data suggest that increases in (18)F-FDG uptake in thyroid carcinomas observed in vivo by PET may reflect activation of intracellular signal transduction cascades by oncogenes.

Presta, I., F. Arturi, et al. (2005). "Recovery of NIS expression in thyroid cancer cells by overexpression of Pax8 gene." BMC Cancer **5**: 80.

BACKGROUND: Recovery of iodide uptake in thyroid cancer cells by means of obtaining the functional expression of the sodium/iodide symporter (NIS) represents an innovative strategy for the treatment of poorly differentiated thyroid cancer. However, the NIS gene expression alone is not always sufficient to restore radioiodine concentration ability in these tumour cells. METHODS: In this study, the anaplastic thyroid carcinoma ARO cells were stably transfected with a Pax8 gene expression vector. A quantitative RT-PCR was performed to assess the thyroid specific gene expression in selected clones. The presence of NIS protein was detected by Western blot and localized by immunofluorescence. A iodide uptake assay was also performed to verify the functional effect of NIS induction and differentiation switch. RESULTS: The clones overexpressing Pax8 showed the re-activation of several thyroid specific genes including NIS, Pendrin, Thyroglobulin, TPO and TTF1. In ARO-Pax8 clones NIS protein was also localized both in cell cytoplasm and membrane. Thus, the ability to uptake the radioiodine was partially restored, associated to a high rate of efflux. In addition, ARO cells expressing Pax8 presented a lower rate of cell growth. CONCLUSION: These finding demonstrate that induction of Pax8 expression may determine a re-differentiation of thyroid cancer cells, including a partial recovery of iodide uptake, fundamental requisite for a radioiodine-based therapeutic approach for thyroid tumours.

Prodosmo, A., S. Giglio, et al. (2008). "Analysis of human MDM4 variants in papillary thyroid carcinomas reveals new potential markers of cancer properties." J Mol Med (Berl) **86**(5): 585-96.

A wild-type (wt) p53 gene characterizes thyroid tumors, except for the rare anaplastic histotype. Because p53 inactivation is a prerequisite for tumor development, alterations of p53 regulators represent an alternative way to impair p53 function. Indeed, murine double minute 2 (MDM2), the main p53 negative regulator, is overexpressed in many tumor histotypes including those of the thyroid. A new p53 regulator, MDM4 (a.k.a. MDMX or HDMX) an analog of MDM2, represents a new oncogene although its impact on tumor properties remains largely unexplored. We estimated levels of MDM2, MDM4, and its variants, MDM4-S (originally HDMX-S) and MDM4-211 (originally HDMX211), in a group of 57 papillary thyroid carcinomas (PTC), characterized by wt tumor protein 53, in comparison to matched contra-lateral lobe normal tissue. Further, we evaluated the association between expression levels of these genes and the histopathological features of tumors. Quantitative real-time polymerase chain reaction revealed a highly significant downregulation of MDM4 mRNA in tumor tissue compared to control tissue ($P<0.0001$), a finding confirmed by western blot on a subset of 20 tissue pairs. Moreover, the tumor-to-normal ratio of MDM4 levels for each individual was significantly lower in late tumor stages, suggesting a specific downregulation of MDM4 expression with tumor progression. In comparison, MDM2 messenger RNA (mRNA) and protein levels were frequently upregulated with no correlation with MDM4 levels. Lastly, we frequently detected overexpression of MDM4-S mRNA and presence of the aberrant form, MDM4-211 in this tumor group. These findings indicate that MDM4 alterations are a frequent event in PTC. It is worthy to note that the significant downregulation of full-length MDM4 in PTC reveals a novel status of this factor in human cancer that counsels careful evaluation of its role in human tumorigenesis and of its potential as therapeutic target.

Puxeddu, E., G. Zhao, et al. (2005). "Characterization of novel non-clonal intrachromosomal rearrangements between the H4 and PTEN genes (H4/PTEN) in human thyroid cell lines and papillary thyroid cancer specimens." Mutat Res **570**(1): 17-32.

The two main forms of RET rearrangement in papillary thyroid carcinomas (PTC) arise from intrachromosomal inversions fusing the tyrosine

kinase domain of RET with either the H4 (RET/PTC1) or the ELE1/RFG genes (RET/PTC3). PTEN codes for a dual-specificity phosphatase and maps to chromosome 10q22-23. Germline mutations confer susceptibility to Cowden syndrome whereas somatic mutations or deletions are common in several sporadic human tumors. Decreased PTEN expression has been implicated in thyroid cancer development. We report the characterization of a new chromosome 10 rearrangement involving H4 and PTEN. The initial H4/PTEN rearrangement was discovered as a non-specific product of RT-PCR for RET/PTC1 in irradiated thyroid cell lines. Sequencing revealed a transcript consisting of exon 1 and 2 of H4 fused with exons 3-6 of PTEN. Nested RT-PCR with specific primers bracketing the breakpoints confirmed the H4/PTEN rearrangements in irradiated KAT-1 and KAT-50 cells. Additional H4/PTEN variants, generated by recombination of either exon 1 or exon 2 of H4 with exon 6 of PTEN, were found in non-irradiated KAK-1, KAT-50, ARO and NPA cells. Their origin through chromosomal recombination was confirmed by detection of the reciprocal PTEN/H4 product. H4/PTEN recombination was not a clonal event in any of the cell lines, as Southern blots with appropriate probes failed to demonstrate aberrant bands, and multicolor FISH of KAK1 cells with BAC probes for H4 and PTEN did not show a signal overlap in all cells. Based on PCR of serially diluted samples, the minimal frequency of spontaneous recombination between these loci was estimated to be approximately 1/10(6) cells. H4/PTEN products were found by nested RT-PCR in 4/14 normal thyroid tissues (28%) and 14/18 PTC (78%) ($P<0.01$). H4/PTEN is another example of recombination involving the H4 locus, and points to the high susceptibility of thyroid cells to intrachromosomal gene rearrangements. As this also represents a plausible mechanism for loss-of-function of PTEN, other thyroid neoplastic phenotypes and eventually other cancer types need to be screened for clonal H4/PTEN rearrangements.

Raitila, A., M. Georgitsi, et al. (2009). "Aryl hydrocarbon receptor interacting protein mutations seem not to associate with familial non-medullary thyroid cancer." J Endocrinol Invest **32**(5): 426-9.

BACKGROUND: Over 95% of all thyroid malignancies are non-medullary thyroid carcinomas (NMTC). Familial NMTC are more aggressive and mortality is higher as compared with sporadic carcinomas. Known genetic factors do not explain all familial NMTC. Recently, thyroid disorders have been observed in families with germline mutations in aryl hydrocarbon receptor interacting protein (AIP) but, due to frequent occurrence of these conditions in the population, the significance of this co-occurrence is not clear. AIM, SUBJECTS AND METHODS: To examine whether AIP is involved in familial NMTC, we performed AIP mutation screening in 93 familial NMTC cases. In addition, the AIP status was studied in one follicular thyroid adenoma patient with a known AIP mutation from an additional cohort. RESULTS: No potentially pathogenic changes were identified, but two likely rare polymorphisms were detected. AIP mutation-positive patient's follicular thyroid adenoma showed no loss of heterozygosity or lack of immunohistochemical AIP staining. CONCLUSION: Our study indicates that germline AIP mutations are rare or do not exist in familial NMTC.

Rao, A. S., N. Kremenevskaja, et al. (2006). "Wnt/beta-catenin signaling mediates antineoplastic effects of imatinib mesylate (gleevec) in anaplastic thyroid cancer." J Clin Endocrinol Metab **91**(1): 159-68.

CONTEXT: Dysregulation of Wnt signaling is a key step in neoplastic thyrocyte proliferation. However, it is unclear whether the selective tyrosine kinase (TK) inhibitor, imatinib mesylate, is linked to the Wnt/beta-catenin cascade and is able to modulate the pathway. OBJECTIVE: Conflicting data are reported on the therapeutic effects of imatinib in anaplastic thyroid carcinomas (ATCs), but the molecular mechanism of action is unclear. Here, we further delineated the antitumor effects and the potential efficacy of imatinib in dedifferentiated thyroid carcinomas. RESULTS: Tissue microarray of histologically proven ATCs (n = 12) demonstrated that six of 12 tumors expressed at least one of the imatinib-sensitive TKs. Similarily, imatinib-sensitive TKs were detected in seven of 10 thyroid cancer cell lines derived from metastatic papillary, follicular, and ATCs. Coimmunoprecipitation in ARO cells demonstrated a direct link between c-abl and beta-catenin. Imatinib (10 microM for 48 h) drastically reduced beta-catenin expression and redistributed it from the nucleus to the cell membrane. It stabilized adherens junctions by increasing beta-catenin/E-cadherin binding and reduced the invasive potential of thyroid cancer. Furthermore, imatinib (10 microM for 48 h) attenuated T cell factor/lymphoid enhancer factor activity, reduced cyclin D1 levels and dose-dependently suppressed thyrocyte proliferation by half without affecting apoptosis. CONCLUSION: Our data provide a molecular mechanism for the antitumor activity of imatinib that may help to develop it as a therapeutic option in a subset of ATC patients.

Rebai, M., I. Kallel, et al. (2009). "Association of polymorphisms in estrogen and thyroid hormone

receptors with thyroid cancer risk." J Recept Signal Transduct Res **29**(2): 113-8.

The receptors for thyroid hormone (THR) and oestrogen (ESR) are prototypes of nuclear transcription factors that regulate the expression of target genes. Genetic alterations in the genes of these receptors were found to be involved in cancer development. In this study we investigated the association of one SNP (rs2228480, T594T) and one microsatellite marker (D6S440) within the ESR1 gene and a dinucleotide repeat (D17S2189) within the THRA gene, with thyroid cancer risk. A case-control association study was conducted with 299 healthy individuals and 106 patients with thyroid cancer. Genotypic and allelic frequencies for the dinucleotide repeat in the ESR1 gene were similar between thyroid cancer patients and controls. For the AC repeat in the THRA gene, a slightly significant difference was found for the genotype 18/20 between the two groups (P = 0.034), which suggests that alleles with less than 20 repeats might have a protective effect in thyroid cancer risk. For the SNP T594T, the A allele was much more prevalent in patients than in controls and was highly associated with the risk of thyroid cancer (OR: 4,56; IC: 3.23-6.44; P < 10(-18)) and seems to have an additive mode of action. In conclusion, our data suggest that the SNP T594T but not the D6S440 and D17S189 is associated with thyroid cancer risk.

Rebai, M., I. Kallel, et al. (2009). "Association of EGFR and HER2 polymorphisms with risk and clinical features of thyroid cancer." Genet Test Mol Biomarkers **13**(6): 779-84.

The epidermal growth factor receptor family plays a critical role in the control of many physiological processes. Genetic alterations and/or variations in the gene encoding these receptors have been implicated in a variety of human cancers. In this study we evaluate the association of two single-nucleotide polymorphisms (SNP), R497K and I655V, of the EGFR and HER2 genes, respectively, with thyroid cancer risk. The analysis was performed with 302 healthy individuals and 106 thyroid cancer patients. No significant difference was found in the allelic and genotypic frequency distribution of the SNP R497K between the control and patient groups. While for the SNP I655V, the allele G is more frequent in patients than in controls and was associated with an increased risk of thyroid cancer (odds ratio = 1.88; 95% confidence intervals: 1.18-3.01; p = 0.007). We have also investigated the relationship between these two polymorphic sites and clinicopathological characteristics such as thyroid-stimulating hormone level, off-thyroxin, serum thyroglobulin, tumor histology, metastasis, tumor status, tumor stage, and survival. No significant association was observed. Tumor status was found significantly associated with HER2 I655V as well as with two previously studied markers in the thyroid hormone receptor A and estrogen receptor 1 (ESR1) genes (D17S2189 and D6S440, respectively). We also report a correlation between thyroglobulin level and genotypes for SNP rs2228480 in exon 8 of the ESR1 gene. In conclusion, our results suggest that the SNP HER2 I655V, but not the EGFR R497K, was associated with thyroid cancer risk.

Rebbaa, A., F. Chu, et al. (2008). "Novel function of the thyroid hormone analog tetraiodothyroacetic acid: a cancer chemosensitizing and anti-cancer agent." Angiogenesis **11**(3): 269-76.

Previous studies from our laboratory have demonstrated that thyroid hormones play a key role in cancer progression. In addition, a deaminated form, tetraiodothyroacetic acid (tetrac), that antagonizes the proliferative action of these hormones was found to possess anti-cancer functions through its ability to inhibit cellular proliferation and angiogenesis. The present study was undertaken to investigate whether tetrac could also suppress the development of drug resistance, known as a causative factor of disease relapse. Tetrac was shown to enhance cellular response in vitro to doxorubicin, etoposide, cisplatin, and trichostatin A in resistant tumor cell lines derived from neuroblastoma, osteosarcoma, and breast cancer. The mechanism of action of tetrac did not involve expression of classical drug resistance genes. However, radiolabeled doxorubicin uptake in cells was enhanced by tetrac, suggesting that one or more export mechanisms for chemotherapeutic agents are inhibited. Tetrac was also found to enhance cellular susceptibility to senescence and apoptosis, suggesting that the agent may target multiple drug resistance mechanisms. Tetrac has previously been shown to inhibit tumor cell proliferation in vitro. In vivo studies reported here revealed that tetrac in a pulsed-dose regimen was effective in suppressing the growth of a doxorubicin-resistant human breast tumor in the nude mouse. In this paradigm, doxorubicin-sensitivity was not restored, indicating that (1) the in vitro restoration of drug sensitivity by tetrac may not correlate with in vivo resistance phenomena and (2) tetrac is an effective chemotherapeutic agent in doxorubicin-resistant cells.

Reis, E. M., E. P. Ojopi, et al. (2005). "Large-scale transcriptome analyses reveal new genetic marker candidates of head, neck, and thyroid cancer." Cancer Res **65**(5): 1693-9.

A detailed genome mapping analysis of 213,636 expressed sequence tags (EST) derived from nontumor and tumor tissues of the oral cavity, larynx,

pharynx, and thyroid was done. Transcripts matching known human genes were identified; potential new splice variants were flagged and subjected to manual curation, pointing to 788 putatively new alternative splicing isoforms, the majority (75%) being insertion events. A subset of 34 new splicing isoforms (5% of 788 events) was selected and 23 (68%) were confirmed by reverse transcription-PCR and DNA sequencing. Putative new genes were revealed, including six transcripts mapped to well-studied chromosomes such as 22, as well as transcripts that mapped to 253 intergenic regions. In addition, 2,251 noncoding intronic RNAs, eventually involved in transcriptional regulation, were found. A set of 250 candidate markers for loss of heterozygosis or gene amplification was selected by identifying transcripts that mapped to genomic regions previously known to be frequently amplified or deleted in head, neck, and thyroid tumors. Three of these markers were evaluated by quantitative reverse transcription-PCR in an independent set of individual samples. Along with detailed clinical data about tumor origin, the information reported here is now publicly available on a dedicated Web site as a resource for further biological investigation. This first in silico reconstruction of the head, neck, and thyroid transcriptomes points to a wealth of new candidate markers that can be used for future studies on the molecular basis of these tumors. Similar analysis is warranted for a number of other tumors for which large EST data sets are available.

Rhoden, K. J., K. Unger, et al. (2006). "RET/papillary thyroid cancer rearrangement in nonneoplastic thyrocytes: follicular cells of Hashimoto's thyroiditis share low-level recombination events with a subset of papillary carcinoma." J Clin Endocrinol Metab **91**(6): 2414-23.

CONTEXT: RET/papillary thyroid cancer (PTC) is a marker for papillary thyroid carcinoma, but its specificity has been questioned because of the disputed identification of RET/PTC in Hashimoto's thyroiditis (HT), oncocytic tumors, and other thyroid lesions. OBJECTIVE: The objective of this study was to determine 1) whether RET/PTC occurs in nonneoplastic follicular cells of HT, and 2) its recombination rate in thyroid tumors. DESIGN/PATIENTS: Forty-three samples from 31 cases of HT were examined using interphase fluorescence in situ hybridization (FISH) with RET probes spanning the breakpoint region; real-time RT-PCR to quantify RET/PTC1, RET/PTC3, and c-RET transcripts; and RT-PCR after laser capture microdissection to enrich samples for follicular cells. The results were compared with those similarly obtained in 34 papillary carcinomas, eight thyroid oncocytic tumors, and 21 normal thyroids. RESULTS: Normal samples showed no RET rearrangement. Sixty-eight percent (15 of 22) of HT were positive by FISH; in all thyroiditis, signals were localized to rare nonneoplastic follicular cells; low-level RET/PTC was identified in 17% (five of 29) of thyroiditis cases by real-time RT-PCR and in an additional six of 11 real-time negative cases after increasing sensitivity with laser capture microdissection. Low RET/PTC1 levels were detected in 26% (nine of 34) of papillary carcinomas with an expression pattern and proportion of FISH-positive cells similar to those of the thyroiditis. Forty-seven percent (16 of 34) of papillary carcinomas and one oncocytic carcinoma expressed high RET/PTC1 mRNA levels. CONCLUSIONS: Low-level RET/PTC recombination occurs in nonneoplastic follicular cells in HT and in a subset of papillary thyroid carcinomas. RET/PTC expression variability should be taken into account for the molecular diagnosis of thyroid lesions. Overlapping molecular mechanisms may govern early stages of tumor development and inflammation in the thyroid.

Ricarte-Filho, J. C., C. S. Fuziwara, et al. (2009). "Effects of let-7 microRNA on Cell Growth and Differentiation of Papillary Thyroid Cancer." Transl Oncol **2**(4): 236-41.

Papillary thyroid carcinoma (PTC) is the most common endocrine malignancy and RET/PTC rearrangements represent key genetic events frequently associated to this cancer, enhancing proliferation and dedifferentiation by activation of the RET/PTC-RAS-BRAF-mitogen-activated protein kinase (MAPK) pathway. Recently, let-7 microRNA was found to reduce RAS levels in lung cancer, acting as a tumor suppressor gene. Here, we report that RET/PTC3 oncogenic activation in PCCL3 rat thyroid cells markedly reduces let-7f expression. Moreover, stable transfection of let-7 microRNA in TPC-1 cells, which harbor RET/PTC1 rearrangement, inhibits MAPK activation. As a result, let-7f was capable of reducing TPC-1 cell growth, and this might be explained, at least in part, by decreased messenger RNA (mRNA) expression of cell cycle stimulators such as MYC and CCND1 (cyclin D1) and increased P21 cell cycle inhibitor mRNA. In addition, let-7 enhanced transcriptional expression of molecular markers of thyroid differentiation such as TITF1 and TG. Thus, reduced expression of let-7f might be an essential molecular event in RET/PTC malignant transformation. Moreover, let-7f effects on thyroid growth and differentiation might attenuate neoplastic process of RET/PTC papillary thyroid oncogenesis through impairment of MAPK signaling pathway activation. This is the first functional demonstration of

an association of let-7 with thyroid cancer cell growth and differentiation.

Riesco-Eizaguirre, G., I. Rodriguez, et al. (2009). "The BRAFV600E oncogene induces transforming growth factor beta secretion leading to sodium iodide symporter repression and increased malignancy in thyroid cancer." Cancer Res **69**(21): 8317-25.

The activating mutation BRAF(V600E) is a frequent genetic event in papillary thyroid carcinomas (PTC) that predicts a poor prognosis, leading to loss of sodium/iodide symporter (NIS) expression and subsequent radioiodide-refractory metastatic disease. The molecular basis of such an aggressive behavior induced by BRAF remains unclear. Here, we show a mechanism through which BRAF induces NIS repression and promotes epithelial to mesenchimal transition and invasion based on the operation of an autocrine transforming growth factor (TGF)beta loop. BRAF induces secretion of functional TGFbeta and blocking TGFbeta/Smad signaling at multiple levels rescues BRAF-induced NIS repression. Although this mechanism is MAP/extracellular signal-regulated kinase (ERK) kinase (MEK)-ERK independent, secreted TGFbeta cooperates with MEK-ERK signaling in BRAF-induced cell migration, Matrigel invasion, and EMT. Consistent with this process, TGFbeta and other key components of TGFbeta signaling, such as TbetaRII and pSmad2, are overexpressed in human PTC, suggesting a widespread activation of this pathway by locally released TGFbeta. Moreover, this high TGFbeta/Smad activity is associated with PTC invasion, nodal metastasis, and BRAF status. Interestingly, TGFbeta is overexpressed in the invasive front, whereas NIS is preferentially expressed in the central regions of the tumors, suggesting that this negative correlation between TGFbeta and NIS occurs locally inside the tumor. Our study describes a novel mechanism of NIS repression in thyroid cancer and provides evidence that TGFbeta may play a key role in promoting radioiodide resistance and tumor invasion during PTC progression.

Riesco-Eizaguirre, G. and P. Santisteban (2007). "New insights in thyroid follicular cell biology and its impact in thyroid cancer therapy." Endocr Relat Cancer **14**(4): 957-77.

Well-differentiated thyroid cancer has in general terms a very good outcome. It has a very slow growth rate and, although it metastasizes at a relatively high frequency, it has very high survival rates. Whereas the prevalence of nodular thyroid disease worldwide is high, malignant conversion from benign thyroid nodules is rare. Treatment of thyroid cancer is usually successful, but we still do not have effective therapies for patients with invasive or metastatic thyroid cancer if the disease does not concentrate radioiodine and it is not surgically resectable. On the other hand, from the same thyroid cell, one of the most aggressive human tumours can arise--undifferentiated or anaplastic thyroid carcinoma--leading to death in a few months. What features of this malignancy account for such paradoxical behaviour? The most common type of thyroid cancer--papillary thyroid carcinoma--stands out among solid tumours because many of the tumour-initiating events have been identified. All of them function in a single pathway--the RTK/RAS/RAF/MAPK pathway--and obey an 'exclusivity principle': one and only one component of the pathway is mutated in a single tumour. This highlights the requirement of this signal transduction pathway for the transformation to thyroid cancer and paves the way to targeted therapies against a tumour with a mutation in a known gene or any gene upstream of the target. However, it is also interesting to underscore the differences among the tumours arising from the different mutations. Studies in vitro and in vivo, including genomic profiling and genetically engineered mouse models, have clearly shown that each oncoprotein exerts its own oncogenic drive, conferring a distinct biological behaviour on thyroid tumours. In this review, we attempt to summarise the most recent advances in thyroid follicular cell-derived cancers research and their potential clinical impact that may change the management of thyroid cancer in the near future.

Rigual, N. R., G. R. Anderson, et al. (2005). "Molecular prognosticators and genomic instability in papillary thyroid cancer." Laryngoscope **115**(8): 1479-85.

OBJECTIVES/HYPOTHESIS: Tumor progression has been attributed to the accumulation of DNA damage concurrent with selection of advantageous mutations; this DNA damage may result from failure to maintain genomic integrity or from susceptibility to carcinogens. Glutathione S-transferases (GSTs), enzymes that metabolize many carcinogens, may play a role in preserving genome integrity. The objectives of this study are to assess the relationship of GST genotypes with prognosis, clinicopathologic parameters, and genomic instability in papillary thyroid cancer. STUDY DESIGN: Prospective analysis. METHODS: GSTM1 and GSTT1 genotypes of 35 matched normal and papillary thyroid cancer specimens were determined by polymerase chain reaction (PCR) using primers specific for the coding sequences of each gene. Genomic instability was measured by intersimple sequence repeat PCR for each tumor/normal pair and

compared with the GAMES prognostic scoring system and clinicopathologic parameters including age, extrathyroidal extension, tumor grade, size, stage metastasis, sex, and smoking history. RESULTS: GSTM1 and GSTT1 null genotypes were found in the normal tissues of 46% and 45%, respectively. No gene losses were detected in the tumor specimens. A significant association between the GSTM1 null genotype and increased risk of recurrence and death was observed. Elevated GII correlated with smoking and tumor stage but not with GST genotype. CONCLUSION: The association of GSTM1 null genotype with intermediate and high risk GAMES categories suggests that GSTM1 provides some protection against disease progression. However, this protection does not confer resistance to disease onset. GST genotyping may be a useful adjunct prognosticator with GAMES.

Ringel, M. D. (2009). "Molecular markers of aggressiveness of thyroid cancer." Curr Opin Endocrinol Diabetes Obes **16**(5): 361-6.

PURPOSE OF REVIEW: To review recent progress at defining molecular markers that predict the biological behavior of thyroid cancer. RECENT FINDINGS: Thyroid cancer behavior is defined by the effects of the initiating oncogene as well as secondary events in tumor cells and the tumor microenvironment that are both genetic and epigenetic. Over the past several years, there has been intense focus on identifying molecular markers to better predict the aggressiveness of thyroid cancers and also to define therapeutic targets. The results of recent articles in this area of work are summarized with a focus of differentiated follicular-cell-derived forms of thyroid cancer. SUMMARY: Clinical staging predicts tumor behavior in many cases, but does not allow true 'personalization' of initial therapy or identify potential therapeutic targets for patients with progressive disease that does not respond to standard therapies. Recent data point to several new opportunities to refine thyroid cancer treatment based on molecular information. Several highlighted articles have begun to apply this information with clinical intent.

Rogounovitch, T. I., V. A. Saenko, et al. (2006). "TP53 codon 72 polymorphism in radiation-associated human papillary thyroid cancer." Oncol Rep **15**(4): 949-56.

The study investigated an association between the germline polymorphism at TP53 codon 72 and the development of papillary thyroid cancer (PTC) following exposure to radiation from the Chernobyl accident. TP53 genotype was examined in 48 pediatric/adolescent (age at diagnosis <18 years) and 68 adult post-Chernobyl patient with PTC, 53 adult patients with sporadic PTC and 313 healthy individuals from Russian-Ukrainian population. In addition, we evaluated loss of heterozygosity for TP53 and the allele expression ratio. The genotype of the patients was correlated with clinicopathological data. Arg TP53 homozygotes were found to be significantly underrepresented among adults with post-Chernobyl PTC, but not in children and adolescents when compared with sporadic PTC cases and the general population. In the tumors, cell transformation did not lead to allelic loss or biased TP53 allele expression in heterozygous individuals. None of TP53 genotypes specifically associated with tumor stage and morphology, however there were particular correlations with lymph node status in certain age groups of radiation-associated cases not seen in sporadic PTCs. The findings suggest TP53 allele combinations other than Arg/Arg may contribute to the risk of development of PTC in individuals exposed to radiation during their late childhood, adolescence or in young adulthood.

Roman, S., P. Mehta, et al. (2009). "Medullary thyroid cancer: early detection and novel treatments." Curr Opin Oncol **21**(1): 5-10.

PURPOSE OF REVIEW: Medullary thyroid cancer (MTC) is derived from the parafollicular cells of the thyroid. Understanding the molecular biology behind specific mutations of the RET gene and their prognostic implications have led to the establishment of tailored treatment modalities for certain patients. We review the most recent studies on the molecular biology, calcitonin screening, diagnosis, imaging, and treatment of MTC. RECENT FINDINGS: Newly identified rearranged during transfection point mutations have helped with MTC prognosis and have resulted in the establishment of new treatment guidelines. Screening for MTC in the United States with basal serum calcitonin for patients with thyroid nodules would cost $11,793 per life-year saved (LYS), compared with colonoscopy and mammography screening. For metastatic or recurrent disease, neck ultrasound, chest computed tomography scan, liver MRI, bone scintigraphy, and axial skeleton MRI have been proven superior to 18F-FDG PET/computed tomography. For patients with nonoperable metastatic disease, novel chemotherapeutic agents, such as vandetanib, targeting rearranged during transfection, vascular endothelial growth factor receptor and epidermal growth factor receptor, are showing promise. Such agents are currently in phase II trials. SUMMARY: There have been several recent advances in the diagnosis, molecular biology, imaging, and treatment options of MTC. By potentially downstaging of

disease, and treating metastatic disease more effectively, overall survival and outcomes of patients may improve.

Romei, C., R. Ciampi, et al. (2008). "BRAFV600E mutation, but not RET/PTC rearrangements, is correlated with a lower expression of both thyroperoxidase and sodium iodide symporter genes in papillary thyroid cancer." Endocr Relat Cancer **15**(2): 511-20.

A low sodium iodide symporter (NIS) expression has been shown in papillary thyroid carcinomas (PTCs) harboring the BRAFV600E mutation. In the present study, we analyzed the mRNA expression of thyroid differentiation genes, glucose transporter (GLUT)-1 and GLUT-3, in 78 PTCs according to the presence of BRAFV600E or RET/PTC rearrangements. We found BRAFV600E and RET/PTC rearrangements in 35.8 and 19.4% of PTCs respectively. The mRNA expression of NIS and thyroperoxidase (TPO) genes were significantly lower ($P<0.0001$ and $P=0.004$ respectively) in BRAFV600E-positive PTC with respect to non-mutated samples. In support of this result, immunohistochemistry showed that the percentage of NIS-positive cells was significantly lower ($P=0.005$) in BRAFV600E-mutated PTC (mean 53.5%) than in negative cases (mean 72.6%). In contrast, no difference either in NIS or in any other thyroid differentiation genes' mRNA expression was found in PTC with or without RET/PTC rearrangements. When GLUT-1 and GLUT-3 mRNA expression was considered, no correlation was found either in BRAFV600E- nor in RET/PTC-mutated cases. In conclusion, this study confirmed the presence of a genetic alteration of BRAF and/or RET oncogenes in 64% of PTC cases and revealed a significant correlation of BRAFV600E mutation with a lower expression of both NIS and TPO. This latter finding could indicate that an early dedifferentiation process is present at the molecular level in BRAFV600E-mutated PTC, thus suggesting that the previously demonstrated poor prognostic significance of BRAFV600E mutation could be related to the dedifferentiation process more than to a more advanced stage at diagnosis.

Saez, C., M. A. Martinez-Brocca, et al. (2006). "Prognostic significance of human pituitary tumor-transforming gene immunohistochemical expression in differentiated thyroid cancer." J Clin Endocrinol Metab **91**(4): 1404-9.

CONTEXT: Human securin pituitary tumor-transforming gene (hPTTG) is overexpressed in a variety of primary neoplasias, including differentiated thyroid cancer (DTC). OBJECTIVE: The objective of this study was to examine the immunohistochemical expression of hPTTG in DTC and its association with known prognostic factors. DESIGN: hPTTG expression was analyzed by immunostaining on paraffin-embedded tissues. Clinical data were used to determine any associations between the expression of hPTTG and prognostic variables of DTC. A median follow-up of 43 months allowed us to analyze the persistence of disease and the response to radioiodine therapy. SETTING: The study was conducted at a tertiary university hospital. PATIENTS: Ninety-five patients undergoing surgical resection for DTC ($n = 60$) or benign nodular thyroid disease ($n = 35$) were studied. MAIN OUTCOME MEASURE: The main outcome measure was the association between hPTTG expression and prognostic factors in DTC. RESULTS: Among DTC cases, 21 (35%) had low and 39 (65%) had high hPTTG immunostaining. Adjacent nonneoplastic thyroid tissue was largely unstained. Among benign nodular thyroid disease cases, immunostaining was detected focally in eight (22.8%). A significant association was found between hPTTG expression and the presence of nodal ($P < 0.01$) or distant metastases ($P < 0.05$). A significant association with TNM was also found, because 83.3% of advanced TNM stages showed elevated hPTTG ($P < 0.05$). The association between hPTTG overexpression and decreased radioiodine uptake during follow-up was also significant ($P < 0.05$). The expression levels of hPTTG were confirmed as an independent prognostic factor for persistent disease (relative risk, 3.0; 95% confidence interval, 1.1-8.7; $P < 0.05$). CONCLUSIONS: Immunohistochemical analysis of hPTTG is of potential value in the determination of tumor aggressiveness in DTC.

Sala, E., L. Mologni, et al. (2006). "A rapid method for the purification of wild-type and V804M mutant ret catalytic domain: A tool to study thyroid cancer." Int J Biol Macromol **39**(1-3): 60-5.

RET (rearranged during transfection) is a transmembrane tyrosine kinase and acts as co-receptor of glial-derived neurotrophic factor (GDNF) family neurothrofic factors in complex with GFRalpha family proteins; RET is important for development of enteric nervous system and renal organogenesis during embryonal life. Alterations in Ret gene are related to several neoplasias: point mutations are identified in medullary thyroid carcinoma (MTC) and multiple endocrine neoplasias 2A and B (MEN2A and B), while translocations and chromosomal inversions cause papillary thyroid carcinoma (PTC). We expressed recombinant RET kinase domain (rRET) containing the active site, the ATP binding pocket, and the activation loop with regulatory activity, with the Baculovirus expression system. RET was purified

by a two-step procedure consisting of an anion exchange chromatography followed by nickel affinity chromatography. Moreover a biochemical characterization of the recombinant product was performed in order to verify its activity (by ELISA) and physical state (dynamic light scattering). We used rRET to validate an ELISA-based kinase assay, by testing inhibitors reported in literature such as PP1 and PP2. This method represents an easy system to screen potential inhibitors found by computational methods. We also produced V804M mutants to identify inhibitors that can overcome resistance to PP1 and ZD6474. The catalytic domain of RET can be used also for X-ray diffraction to obtain information about the three-dimensional structure, necessary for a rational design of selective inhibitors: it represents an important tool to understand the molecular mechanisms causing thyroid cancer and to care it.

Santarpia, L., A. K. El-Naggar, et al. (2008). "Phosphatidylinositol 3-kinase/akt and ras/raf-mitogen-activated protein kinase pathway mutations in anaplastic thyroid cancer." J Clin Endocrinol Metab **93**(1): 278-84.

CONTEXT: Anaplastic thyroid carcinoma (ATC) can occur in the setting of differentiated thyroid carcinoma (DTC), which suggests a continuum in malignant progression from DTC to ATC. The Ras/Raf-MAPK and the phosphatidylinositol 3-kinase/Akt signaling pathways play critical roles in DTC tumorigenesis, but their roles in the pathogenesis of ATC are poorly defined. OBJECTIVE: Our objective was to explore the potential contributions of these two pathways in ATC pathogenesis. DESIGN, SETTING, AND SUBJECTS: The mutational status of BRAF, PIK3CA, PTEN, and RAS genes was analyzed in genomic DNA from microdissected tumor specimens of 36 cases of ATC, and in 16 samples of paired-matched lymph node metastases. PIK3CA copy number gain was assessed by real-time quantitative PCR. We performed immunohistochemistry for phospho-ERK and phospho-AKT in 26 cases of ATC. RESULTS: DTC was present in half of the cases. BRAF V600E mutation was identified in nine of 36 (25%) ATCs; seven cases had identical mutations in both the ATC and DTC components. PIK3CA kinase domain mutations were found in five (14%) ATCs, one of which had mutations in both differentiated and anaplastic areas. RAS and PTEN mutations were each found in two (6%) ATCs. PIK3CA gain copy number was found notably increased in 14 (39%) ATCs. CONCLUSIONS: BRAF mutations appear to play a role in the tumorigenesis of a subset of ATCs, and the majority of lymph node metastases. PIK3CA alterations occur preferentially in the later stages of ATC and were the most relevant events during thyroid cancer progression. The activation of both pathways suggests an important role in ATC dedifferentiation.

Sbrana, I., F. Veroni, et al. (2006). "Chromosomal fragile sites FRA3B and FRA16D show correlated expression and association with failure of apoptosis in lymphocytes from patients with thyroid cancer." Genes Chromosomes Cancer **45**(5): 429-36.

It has been suggested that common fragile sites (cFSs) are related to cancer development. This appears to be the case for FRA3B and FRA16D, localized in two tumor-suppressor genes (FHIT and WWOX, respectively) that are altered by deletions or loss of heterozygosity (LOH) in many cancers. The features responsible for fragility have not yet been identified. Attenuation of checkpoint control and apoptosis resistance seem to be the cell phenotypes associated with unusual chromosome fragility. We propose that breakage at specific cFS could derive from early epigenetic events at loci involved in radiation carcinogenesis. This article contains supplementary Material available at http://www.interscience.wiley.com/jpages/1045-2257/suppmat.

Schagdarsurengin, U., A. M. Richter, et al. (2009). "Frequent epigenetic inactivation of RASSF10 in thyroid cancer." Epigenetics **4**(8): 571-6.

The Ras association domain family (RASSF) encodes for distinct tumor suppressors and several members are frequently silenced in human cancer. In our study, we analyzed the role of a novel RASSF member termed RASSF10 in thyroid carcinogenesis. The RASSF10 CpG island promoter was intensively methylated in nine thyroid cancer cell lines and in 66% of primary thyroid carcinomas. RASSF10 methylation was significantly increased in primary thyroid carcinoma compared to normal thyroid and follicular adenoma (0 and 10%, respectively; $p < 0.004$). Patients with cancerous lymph nodes were significantly hypermethylated for RASSF10 in primary thyroid tumors compared to those with non-affected lymph nodes (79 vs. 36%; $p = 0.047$). RASSF10 promoter hypermethylation correlated with a reduced expression and treatment with a DNA methylation inhibitor reactivated RASSF10 transcription. In summary, our data show frequent epigenetic inactivation of RASSF10 in thyroid cancer. These results suggest that RASSF10 may encode a novel epigenetically inactivated candidate tumor suppressor gene in thyroid carcinogenesis.

Schlumberger, M., L. Lacroix, et al. (2007). "Defects in iodide metabolism in thyroid cancer and implications for the follow-up and treatment of

patients." Nat Clin Pract Endocrinol Metab **3**(3): 260-9.

The two major steps of iodine metabolism--uptake and organification--are altered in thyroid cancer tissues. Organification defects result in a rapid discharge of radioiodine from thyroid cells, a short effective half-life of iodine, and a low rate of thyroid hormone synthesis. These defects are mainly due to decreased expression of functional genes encoding the sodium-iodide symporter and thyroid peroxidase and could result in a low radiation dose to thyroid cancer cells. TSH stimulation that is achieved with injections of recombinant human TSH, or long-term withdrawal of thyroid hormone treatment increases iodine-131 uptake in two-thirds of patients with metastatic disease and increases thyroglobulin production in all patients with metastases, even in the absence of detectable uptake. Serum thyroglobulin determination obtained following TSH stimulation and neck ultrasonography is the most sensitive combination for the detection of small tumor foci. Radioiodine treatment is effective when a high radiation dose can be delivered (in patients with high uptake and retention of radioiodine) and when tumor foci are sensitive to the effects of radiation therapy (younger patients, with a well-differentiated tumor and/or with small metastases). The other patients rarely respond to radioiodine treatment, and when progression occurs, other treatment modalities should be considered. Novel strategies are currently being explored to restore iodine uptake in cancer cells that are unable to concentrate radioiodine.

Schott, M. and W. A. Scherbaum (2004). "Immunotherapy and gene therapy of thyroid cancer." Minerva Endocrinol **29**(4): 175-87.

Most forms of thyroid cancer have a good prognosis. Some tumours, however, dedifferentiate and may finally develop into highly malignant anaplastic thyroid carcinomas with a low survival time. Due to their dedifferentiation these tumours are inaccessible to classical therapeutic options as radioiodide treatment or thyrotropin-suppression. Radical surgical revision of the tumour masses is the therapy of choice of patients with limited disease stages including patients with medullary thyroid carcinomas. Despite progress in radiation and chemotherapy regimes, many metastatic forms remain, however, incurable by conventional therapies. During the past few years new developments in immunology have revealed increasing information about the molecular basis of tumour-host interactions. The multitude of information resulting from basic science in cellular immunology, together with the availability of biologic reagents in pharmacological amounts, has opened new venues for the development of immunotherapy approaches for patients with different kind of cancers including thyroid malignancies. This review describes some most important developments in cellular immunotherapies e.g. dendritic cells-based protocols and gene therapy. It also provides a brief overview on the role of cytokines and antibodies in the treatment of advanced thyroid malignancies.

Schweppe, R. E., A. A. Kerege, et al. (2009). "Distinct genetic alterations in the mitogen-activated protein kinase pathway dictate sensitivity of thyroid cancer cells to mitogen-activated protein kinase kinase 1/2 inhibition." Thyroid **19**(8): 825-35.

BACKGROUND: The mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway plays an important role in papillary and anaplastic thyroid cancer (PTC and ATC) due to activating mutations in BRAF, RAS, or rearrangements in RET/PTC1. The objective of this study was to thoroughly test whether the BRAF V600E mutation predicts response to mitogen-activated protein kinase kinase 1/2 (MKK1/2) inhibition, as shown in other tumor types, using an authenticated panel of thyroid cancer cell lines. METHODS: PTC and ATC cells harboring distinct mutations in the MAPK pathway were treated with two different inhibitors selective for MKK1/2 (CI-1040 or U0126). The consequences of MKK1/2 inhibition on cell growth, survival, invasion, and MAPK signaling was determined. RESULTS: Inhibition of MKK1/2 using CI-1040 or U0126 differentially inhibits the growth of a panel of PTC and ATC cell lines in two-dimensional culture, with those harboring the BRAF V600E mutation (SW1736) or BRAF-V600E/PI3K-E542K mutations (K1) being the most sensitive, the RET/PTC1 rearrangement (TPC1) and BRAF V600E mutant (BCPAP), intermediate, and the HRAS-G13R mutant (C643), the least sensitive. Growth of these cells is more sensitive to MKK1/2 inhibition when grown in 2% versus 10% serum. Baseline levels of phospho-ERK1/2 were similar in all of the cell lines, and inhibition phospho-ERK1/2 did not predict sensitivity to MKK1/2 inhibition. When cells are grown in three-dimensional culture, MKK1/2 inhibition of growth correlates with mutational status (BRAF > RET/PTC1 > RAS). Finally, PTC and ATC invasiveness is differentially inhibited by CI-1040, which is independent of tumor type or mutation present. CONCLUSIONS: Different mutations in the MAPK pathway play distinct roles in the growth and invasion of thyroid cancer cells. These results indicate that MKK1/2 inhibitors have the potential to inhibit thyroid cancer growth and invasion, but that responses differ based on mutation status and growth conditions.

Schweppe, R. E., J. P. Klopper, et al. (2008). "Deoxyribonucleic acid profiling analysis of 40 human thyroid cancer cell lines reveals cross-contamination resulting in cell line redundancy and misidentification." J Clin Endocrinol Metab **93**(11): 4331-41.

CONTEXT: Cell lines derived from human cancers provide critical tools to study disease mechanisms and develop novel therapies. Recent reports indicate that up to 36% of cell lines are cross-contaminated. OBJECTIVE: We evaluated 40 reported thyroid cancer-derived cell lines using short tandem repeat and single nucleotide polymorphism array analysis. RESULTS: Only 23 of 40 cell lines tested have unique genetic profiles. The following groups of cell lines are likely derivatives of the same cell line: BHP5-16, BHP17-10, BHP14-9, and NPA87; BHP2-7, BHP10-3, BHP7-13, and TPC1; KAT5, KAT10, KAT4, KAT7, KAT50, KAK1, ARO81-1, and MRO87-1; and K1 and K2. The unique cell lines include BCPAP, KTC1, TT2609-C02, FTC133, ML1, WRO82-1, 8505C, SW1736, Cal-62, T235, T238, Uhth-104, ACT-1, HTh74, KAT18, TTA1, FRO81-2, HTh7, C643, BHT101, and KTC-2. The misidentified cell lines included the DRO90-1, which matched the melanoma-derived cell line, A-375. The ARO81-1 and its derivatives matched the HT-29 colon cancer cell line, and the NPA87 and its derivatives matched the M14/MDA-MB-435S melanoma cell line. TTF-1 and Pax-8 mRNA levels were determined in the unique cell lines. CONCLUSIONS: Many of these human cell lines have been widely used in the thyroid cancer field for the past 20 yr and are not only redundant, but not of thyroid origin. These results emphasize the importance of cell line integrity, and provide the short tandem repeat profiles for a panel of thyroid cancer cell lines that can be used as a reference for comparison of cell lines from other laboratories.

Scouten, W. T., A. Patel, et al. (2004). "Cytoplasmic localization of the paired box gene, Pax-8, is found in pediatric thyroid cancer and may be associated with a greater risk of recurrence." Thyroid **14**(12): 1037-46.

The paired box-8 protein (Pax-8) has been observed in the nucleus of normal adult thyroids, follicular adenomas, follicular thyroid cancers, and papillary thyroid cancers (PTC) but not undifferentiated thyroid cancers. To our knowledge, Pax-8 has not been studied in pediatric thyroid cancer. Because of the more favorable prognosis for PTC in children compared to young patients, we hypothesized that Pax-8 expression might be different in pediatric thyroid cancers. To test this, we stained 47 thyroid lesions from children and young patients for Pax-8. Pax-8 was located in the cytoplasm (cPAX) or nucleus (nPAX) in the majority of samples. There was no significant difference in nPAX between benign and malignant lesions. However, cPAX was more commonly seen in PTC than autoimmune diseases ($p = 0.01$) and the intensity of cPAX staining correlated with tumor size ($p = 0.041$), metastasis, age, completeness of resection, local invasion, and tumor size (MACIS) scores ($p = 0.045$), and the presence of invasion, metastasis, recurrence, or persistence ($p = 0.012$). Disease-free survival was significantly reduced for cancers with intense cPAX staining ($p = 0.0003$). These data show that cPAX is common in PTC, and although limited by small sample size, suggest an association with higher MACIS scores, an aggressive clinical course, and an increased risk of clinically evident recurrence for children and young patients.

Shen, W. T., T. S. Wong, et al. (2005). "Valproic acid inhibits growth, induces apoptosis, and modulates apoptosis-regulatory and differentiation gene expression in human thyroid cancer cells." Surgery **138**(6): 979-84; discussion 984-5.

BACKGROUND: Among the most promising new therapies for thyroid cancer are the histone deacetylase inhibitors. Valproic acid (VA) is an anticonvulsant that inhibits histone deacetylase activity at nontoxic concentrations. We hypothesized that VA would have antineoplastic effects on human thyroid cancer cells. METHODS: We treated 1 papillary and 3 follicular thyroid cancer cell lines with VA (0.5-2 mmol/L) for 24 to 72 hours. Cell proliferation was measured with a cell proliferation assay kit. Annexin V-fluorescein isothiocyanate was used to quantitate cells that were undergoing apoptosis. Quantitative polymerase chain reaction was used to measure expression of apoptosis-regulatory and differentiation genes. RESULTS: VA inhibited growth in all cell lines by 26% to 59% at 48 hours and up to 77% at 72 hours. Nineteen percent to 30% of VA-treated cells underwent apoptosis, compared with 4% to 8% of the control cells. Expression of pro survival genes bcl-2 and bcl-xl was down-regulated by 10% to 60%; expression of the proapoptosis gene bax was up-regulated by 23% to 85%. Sodium-iodide symporter and thyroglobulin messenger RNA expression were up-regulated by 93% to 370% in follicular cell lines but remained unchanged in the papillary cell line. CONCLUSION: VA inhibits growth, induces apoptosis, and modulates apoptosis-regulatory and differentiation gene expression in thyroid cancer cells. These findings suggest that VA may be useful clinically for patients with thyroid cancers of follicular cell origin.

Shibru, D., K. W. Chung, et al. (2008). "Recent developments in the clinical application of thyroid cancer biomarkers." Curr Opin Oncol **20**(1): 13-8.

PURPOSE OF REVIEW: The aim of this article is to provide an update on the status of the clinical application of thyroid cancer biomarkers. RECENT FINDINGS: Our understanding of the tumor cell biology of thyroid cancer of follicular cell origin has improved and modern genomic technological tools are providing new data that may have clinical ramifications. The common somatic genetic changes in thyroid cancer of follicular cell origin (RET/PTC, NTRK, RAS, BRAF, PAX8-PPARgamma) are generally mutually exclusive, with distinct genotype-histologic subtype of thyroid cancer and genotype-phenotype associations observed. Mutation analysis in thyroid nodule fine needle aspiration biopsy has been applied to improve the diagnostic accuracy of fine needle aspiration biopsy and cytologic examination. Gene expression profiling studies have identified numerous diagnostic biomarkers of thyroid cancer that are beginning to be applied in fine needle aspiration biopsy samples to improve diagnosis. The BRAF mutation has recently been shown to be associated with disease aggressiveness, and as an independent prognostic biomarker. SUMMARY: There has been significant progress toward identifying biomarkers that could improve the accuracy of fine needle aspiration biopsy in the evaluation of patients with thyroid nodule and predicting disease aggressiveness. Future clinical trials evaluating the accuracy and cost-effectiveness of applying these biomarkers in the management of thyroid neoplasm should be considered.

Silva, S. N., O. M. Gil, et al. (2005). "Association of polymorphisms in ERCC2 gene with non-familial thyroid cancer risk." Cancer Epidemiol Biomarkers Prev **14**(10): 2407-12.

The ERCC2 protein is an evolutionary conserved ATP-dependent helicase that is associated with a TFIIH transcription factor complex and plays an important role in nucleotide excision repair. Mutations in this gene are responsible for xeroderma pigmentosum and also for Cocayne syndrome and trichothiodystrophy. Several single nucleotide polymorphisms have been identified in the ERCC2 locus. Among them, a G23591A polymorphism in the codon 312 results in an Asp --> Asn substitution in a conserved region and a A35931C polymorphism in the codon 751 results in a Lys --> Gln substitution. Because these polymorphisms have been associated with an increased risk for several types of cancers, we carried out an hospital based case-control study in a Caucasian Portuguese population to evaluate the potential role of these polymorphisms on the individual susceptibility to thyroid cancer. The results obtained did not reveal a significant association between each individual polymorphism studied (G23591A and A35931C) and an increased thyroid cancer risk, but individuals homozygous for non-wild-type variants are overrepresented in patients group. The evaluation of the different haplotypes generated by these polymorphisms showed that individuals simultaneously homozygous for rare variants of both polymorphisms have an increased risk for thyroid cancer [adjusted odds ratio (OR) 3.084; 95% confidence interval (95% CI), 1.347-7.061; P = 0.008] and for papillary thyroid-type tumors (adjusted OR, 2.997; 95% CI, 1.235-7.272; P = 0.015) but not for follicular thyroid-type tumors. These results suggest that genetic polymorphisms in this gene might be associated with individual susceptibility towards thyroid cancer, mainly papillary-type tumors, but larger studies are required to confirm these results.

Siraj, A. K., M. Al-Rasheed, et al. (2008). "RAD52 polymorphisms contribute to the development of papillary thyroid cancer susceptibility in Middle Eastern population." J Endocrinol Invest **31**(10): 893-9.

Genetic polymorphisms of DNA repair genes seem to determine the DNA repair capacity. We hypothesized that polymorphisms of genes responsible for DNA repair may be associated with risk of thyroid cancer. To evaluate the role of genetic polymorphisms of DNA repair genes in thyroid cancer, we conducted a hospital-based case-control study in Saudi population. Two hundred and twenty-three incident papillary thyroid cancer cases and 229 controls recruited from Saudi Arabian population were analyzed for 21 loci in 8 selected DNA repair genes by PCR-restriction fragment length polymorphism including non-homologous end joining pathway genes LIGIV (LIGlV ASP62HIS, PRO231SER, TRP46TER), XRCC4 Splice 33243301G>A and XRCC7 ILE3434THR; homologous recombination pathway genes XRCC3 ARG94HIS and THR241MET, RAD51 UTR 15452658T>C, 15455419A>G, RAD52 2259 and GLN221GLU, conserved DNA damage response gene Tp53 PRO47SER, PRO72ARG, Tp53 UTR 7178189A>C and base excision repair gene XRCC1 ARG194TRP, ARG280HIS, ARG399GLN, ARG559GLN. RAD52 GLN221GLU genotypes CG and variants carrying G allele showed statistical significance and very high risk of developing thyroid cancer compared to wild type [CG vs CC; p<0.001, odds ratio (OR)=15.57, 95% confidence interval (CI)=6.56-36.98, CG+GG vs CC; p<0.001, OR=17.58, 95% CI=7.44-41.58]. Similarly, RAD52 2259 genotypes CT and variant allele T showed a

significant difference in terms of risk estimation (CT vs CC; p<0.05, OR=1.53, 95% CI=1.03-2.28, CT+TT vs CC; p<0.001, OR=1.922, 95% CI=1.31-2.82). Remaining loci demonstrated no significance with risk. Of the 21 loci screened, RAD52 2259 and RAD52 GLN221GLU may be of importance to disease process and may be associated with papillary thyroid cancer risk in Saudi Arabian population.

Siraj, A. K., P. Bavi, et al. (2007). "Genome-wide expression analysis of Middle Eastern papillary thyroid cancer reveals c-MET as a novel target for cancer therapy." J Pathol **213**(2): 190-9.

In an attempt to find genes that may be of importance in malignant progression of papillary thyroid carcinoma (PTC) in the Middle East, which therefore can be targeted in cancer therapy, we screened and validated the global gene expression in PTC using cDNA expression arrays and immunohistochemistry (IHC) on tumour tissue microarrays. Twenty-nine PTC tissue specimens were compared with seven non-cancerous thyroid specimens by use of cDNA microarray. Results for selected genes were confirmed by quantitative real-time PCR. Protein expression of selected genes was further studied using a tissue microarray consisting of 536 PTCs and compared with histologically non-cancerous tissue samples. One hundred and ninety-six genes were overexpressed in PTC tissues relative to non-cancerous thyroid tissues. The genes that were up-regulated in PTC were involved in cell cycle regulation, cell signaling, and oncogenesis. Among these genes, c-MET was identified by immunohistochemical methods as a protein that is overexpressed in 37% of PTCs and was significantly associated with more aggressive behaviour, eg higher stage, nodal involvement, and tall cell variant (p value = 0.01, 0.01 and 0.04, respectively). In this study, 55% of the PTC cases expressed activated AKT (P-AKT), which suggests that activated AKT may play an important role in PTC tumourigenesis. The fact that most of the PTC cases that had activated AKT showed overexpression of c-MET (p = 0.027) leads us to hypothesize that c-MET may be an alternative mechanism of AKT activation in Middle Eastern PTCs. Finally, our data suggest that c-MET dysregulation is associated with aggressive behaviour and may serve as a molecular biomarker and potential therapeutic target in this disease.

Siraj, A. K., M. Ibrahim, et al. (2008). "Polymorphisms of selected xenobiotic genes contribute to the development of papillary thyroid cancer susceptibility in Middle Eastern population." BMC Med Genet **9**: 61.

BACKGROUND: The xenobiotic enzyme system that enables us to detoxify carcinogens exhibits identifiable genetic polymorphisms that are highly race specific. We hypothesized that polymorphisms of these genes may be associated with risk of thyroid cancer. To evaluate the role of genetic polymorphisms of xenobiotic genes in thyroid cancer, we conducted a hospital-based case-control study in Saudi population. METHODS: 223 incident papillary thyroid cancer cases and 513 controls recruited from Saudi Arabian population were analyzed for the association between polymorphisms in genes encoding folic acid metabolizing enzymes MTHFR and six xenobiotics-metabolizing enzymes including CYP1A1 T3801C, C4887A, GSTP1 A1578G, C2293T, GSTM1, GSTT1, NAT2 G590A, NQO*1 C609T, using PCR-RELP. RESULTS: Among selected genes, CYP1A1 C4887A genotypes CA, AA and variant allele A demonstrated significant differences and greater risk of developing thyroid cancer comparing to wild type genotype CC (CA vs. CC; p < 0.0001, OR = 1.91, 95% CI = 1.36-2.70, AA vs. CC; p < 0.001, OR = 3.48, 95% CI = 1.74-6.96 and CA+AA vs. CC; p < 0.0001, OR = 2.07, 95% CI = 1.49-2.88). GSTT1 null showed 3.48 times higher risk of developing thyroid cancer (p < 0.0001, 95% CI = 2.48-4.88) while GSTM1 null showed protective effect (p < 0.05, OR = 0.72, 95% CI = 0.52-0.99). Remaining loci demonstrated no significance with risk. CONCLUSION: Of the 9 polymorphisms screened, we identified GST, GSTM1 and CYP1A1 C4887A, may be of importance to disease process and may be associated with papillary thyroid cancer risk in Saudi Arabian population.

Smallridge, R. C., L. A. Marlow, et al. (2009). "Anaplastic thyroid cancer: molecular pathogenesis and emerging therapies." Endocr Relat Cancer **16**(1): 17-44.

Anaplastic thyroid cancer (ATC) is a rare malignancy. While external beam radiation therapy has improved locoregional control, the median survival of approximately 4 months has not changed in more than half a century due to uncontrolled systemic metastases. The objective of this study was to review the literature in order to identify potential new strategies for treating this highly lethal cancer. PubMed searches were the principal source of articles reviewed. The molecular pathogenesis of ATC includes mutations in BRAF, RAS, catenin (cadherin-associated protein), beta 1, PIK3CA, TP53, AXIN1, PTEN, and APC genes, and chromosomal abnormalities are common. Several microarray studies have identified genes and pathways preferentially affected, and dysregulated microRNA profiles differ from differentiated thyroid cancers. Numerous

proteins involving transcription factors, signaling pathways, mitosis, proliferation, cell cycle, apoptosis, adhesion, migration, epigenetics, and protein degradation are affected. A variety of agents have been successful in controlling ATC cell growth both in vitro and in nude mice xenografts. While many of these new compounds are in cancer clinical trials, there are few studies being conducted in ATC. With the recent increased knowledge of the many critical genes and proteins affected in ATC, and the extensive array of targeted therapies being developed for cancer patients, there are new opportunities to design clinical trials based upon tumor molecular profiling and preclinical studies of potentially synergistic combinatorial novel therapies.

Smith, V. E., M. L. Read, et al. (2009). "A novel mechanism of sodium iodide symporter repression in differentiated thyroid cancer." J Cell Sci **122**(Pt 18): 3393-402.

Differentiated thyroid cancers and their metastases frequently exhibit reduced iodide uptake, impacting on the efficacy of radioiodine ablation therapy. PTTG binding factor (PBF) is a proto-oncogene implicated in the pathogenesis of thyroid cancer. We recently reported that PBF inhibits iodide uptake, and have now elucidated a mechanism by which PBF directly modulates sodium iodide symporter (NIS) activity in vitro. In subcellular localisation studies, PBF overexpression resulted in the redistribution of NIS from the plasma membrane into intracellular vesicles, where it colocalised with the tetraspanin CD63. Cell-surface biotinylation assays confirmed a reduction in plasma membrane NIS expression following PBF transfection compared with vector-only treatment. Coimmunoprecipitation and GST-pull-down experiments demonstrated a direct interaction between NIS and PBF, the functional consequence of which was assessed using iodide-uptake studies in rat thyroid FRTL-5 cells. PBF repressed iodide uptake, whereas three deletion mutants, which did not localise within intracellular vesicles, lost the ability to inhibit NIS activity. In summary, we present an entirely novel mechanism by which the proto-oncogene PBF binds NIS and alters its subcellular localisation, thereby regulating its ability to uptake iodide. Given that PBF is overexpressed in thyroid cancer, these findings have profound implications for thyroid cancer ablation using radioiodine.

Solini, A., S. Cuccato, et al. (2008). "Increased P2X7 receptor expression and function in thyroid papillary cancer: a new potential marker of the disease?" Endocrinology **149**(1): 389-96.

Nucleotides are increasingly recognized as nonredundant extracellular signals for chemotaxis, cell growth, and cytokine release. Effects of extracellular nucleotides are mediated by P2 receptors, among which the P2X(7) subtype is attracting increasing attention for its involvement in apoptosis, cell growth, and cytokine release. Recent studies showed that P2X(7) is overexpressed in chronic lymphocytic leukemia and breast and prostate cancer. The aim of the present study was to better understand the clinical significance of P2X(7) receptor expression in normal and cancer human thyroid tissues. P2X(7) receptor message and protein expression and functional activity were tested in two cell lines (FB1 and FB2) established from either anaplastic or papillary primary thyroid cancer and in several histological samples of human papillary cancer. Finally, the thyroid carcinoma cell lines had at least a 3-fold higher intracellular ATP concentration and maintained at least a 3-fold higher extracellular ATP level, compared with control cells. These data suggest that an enhanced P2X(7)R function might be a feature of human thyroid cancer.

Spitzweg, C. (2009). "Gene therapy in thyroid cancer." Horm Metab Res **41**(6): 500-9.

A variety of promising gene therapy approaches have been examined for treatment of follicular cell-derived and medullary thyroid cancer, including corrective gene therapy, cytoreductive gene therapy as well as immunomodulatory gene therapy. In addition, cloning of the NIS gene has provided us with a powerful cytoreductive gene therapy strategy based on targeted NIS gene transfer followed by radionculide ((131)I, (188)Re, (211)At) therapy. The data summarized in this article clearly demonstrate the high potential of currently available gene therapy approaches for future therapy of advanced dedifferentiated and medullary thyroid cancer, in particular as part of a multimodality approach. One of the major hurdles on the way to clinical application of gene therapy approaches in metastasized thyroid cancer is optimal tumor-specific targeting in the presence of low toxicity. Replication-selective viral vectors and novel biodegradable polymers as highly efficient nonviral vectors seem to be most promising candidates for the development of efficient and safe systemic gene therapy strategies. The bystander effect that is associated with some of the above listed gene therapy strategies provides a powerful means to compensate for the limited tumor spread of viral and nonviral vectors. Based on its dual function as therapy and reporter gene allowing noninvasive imaging by (123)I-scintigraphy and (124)I-PET imaging, NIS gene therapy offers the advantage of detailed characterization of in vivo vector biodistribution as

well as localization, level, and duration of transgene expression - an essential prerequisite for exact planning and monitoring of clinical gene therapy trials with the aim of individualized therapy.

Spitzweg, C., C. H. Baker, et al. (2007). "Image-guided radioiodide therapy of medullary thyroid cancer after carcinoembryonic antigen promoter-targeted sodium iodide symporter gene expression." Hum Gene Ther **18**(10): 916-24.

In contrast to follicular cell-derived thyroid cancer, medullary thyroid cancer (MTC) remains difficult to treat because of its unresponsiveness to radioiodine therapy, or to conventional chemo- and radiotherapy. We therefore examined the feasibility of radioiodine therapy of MTC after human sodium iodide symporter (hNIS) gene transfer, using the tumor-specific carcinoembryonic antigen (CEA) promoter for transcriptional targeting. NIS gene transfer was performed in vivo in human MTC cell (TT) xenografts, using adenoviral vectors carrying the NIS gene linked to the cytomegalovirus promoter (Ad5-CMV-NIS) or a CEA promoter fragment (Ad5-CEA-NIS). Functional NIS expression was confirmed by immunostaining as well as in vivo (123)I gamma-camera imaging followed by application of a therapeutic (131)I dose. TT cell xenografts in nude mice injected intratumorally with Ad5-CEA-NIS accumulated 7.5 +/- 1.2% ID/g (percentage injected dose per gram tumor tissue; 5 x 10(8) PFU) and 12 +/- 2.95% ID/g (1 x 10(9) PFU) with an average biological half-life of 6.1 +/- 0.8 and 23.6 +/- 3.7 hr, respectively, as compared with accumulation of 8.4 +/- 0.9% ID/g with a biological half-life of 12 +/- 8 hr after application of Ad5-CMV-NIS (5 x 10(8) PFU). After Ad5-CEA-NIS-mediated NIS gene transfer in TT cell xenografts administration of a therapeutic dose of 111 MBq (3 mCi) of (131)I resulted in a significant reduction of tumor growth associated with significantly lower calcitonin serum levels in treated mice as well as improved survival. We conclude that a therapeutic effect of (131)I was demonstrated in vivo in MTC cell xenografts after adenovirus-mediated induction of tumor-specific iodide accumulation by CEA promoter-directed hNIS expression.

Spitzweg, C. and J. C. Morris (2004). "Gene therapy for thyroid cancer: current status and future prospects." Thyroid **14**(6): 424-34.

Despite multimodality treatment for thyroid cancer, including surgical resection, radioiodine therapy, thyrotropin (TSH)-suppressive thyroxine treatment, and chemotherapy/radiotherapy, survival rates have not improved over the last decades. Therefore, development and evaluation of novel treatment strategies, including gene therapy, are urgently needed. NIS gene delivery into medullary and follicular cell-derived thyroid cancer cells has been shown to be capable of establishing or restoring radioiodine accumulation and might therefore represent an effective therapy for medullary and dedifferentiated thyroid tumors that lack iodide accumulating activity. The data summarized in this review article clearly demonstrate that the currently available strategies represent potentially curative novel therapeutic approaches for future gene therapy of thyroid cancer. The combination of different therapeutic genes has been demonstrated to be very useful to enhance therapeutic efficacy and seems to have a promising role at least as part of a multimodality approach for advanced thyroid cancer.

Stankov, K., S. Landi, et al. (2006). "GSTT1 and M1 polymorphisms in Hurthle thyroid cancer patients." Cancer Lett **240**(1): 76-82.

Glutathione S-transferases (GST) are an important part of cell defense against numerous genotoxic compounds and ROS. In order to test the possibility of association between the GSTT1 and M1 null allele variant, and the risk of TCO (thyroid carcinoma with cell oxyphilia), a case-control study was carried out. The rationale for our study was that according to the important roles of GST enzymes in cells and association of GST null genotypes with many types of tumors, inactivating polymorphisms may be genetic susceptibility factors in the etiology of oxyphilic thyroid tumors characterized by mitochondrial dysfunction, increased ROS production and resistance to chemio- and radio-therapy. We found the frequency of GSTT1 null genotype of 19.2% in cases and 15.7% in controls, with an adjusted odds ratio (OR) of 1.4 (95% confidence interval (CI), 0.70-2.81), and a frequency of GSTM1 null genotype of 59% in cases with oxyphilic tumors and of 55.6% in controls (OR 1.24; 95% CI, 0.62-2.48), indicating that the GSTT1 and M1 null genotypes do not increase the risk of development of oxyphilic tumors.

Straight, A. M., K. Oakley, et al. (2006). "Aplidin reduces growth of anaplastic thyroid cancer xenografts and the expression of several angiogenic genes." Cancer Chemother Pharmacol **57**(1): 7-14.

BACKGROUND: Anaplastic thyroid cancer (ATC) is one of the most aggressive and highly lethal human cancers. Median survival after diagnosis is 4-6 months despite available radiotherapy and chemotherapy. Additional treatments are needed for ATC. Vascular endothelial growth factor (VEGF) is a potent angiogenic stimulus, which is expressed by ATC. Previously, anti-VEGF antibody was used to block VEGF-dependent angiogenesis in ATC

xenografts. This treatment induced partial (56%) but not complete tumor regression. Aplidin (APLD) is a marine derived antitumor agent currently in phase II clinical studies. Multiple activities of this compound have been described which likely contribute to its antiproliferative effect. Notably, APLD has been shown to have antiangiogenic properties which include: inhibition of VEGF secretion, reduction in the synthesis of the VEGF receptor (FLT-1), and blockade of matrix metalloproteinase production by endothelial cells. We hypothesized that Aplidin, with its broad spectrum of action and antiangiogenic properties, would be a potentially effective drug against ATC. APLD significantly reduced ATC xenograft growth (low dose, 20% reduction, P = 0.01; high dose, 40% reduction, P < 0.001). This was associated with increased levels of apoptosis related proteins polyadenosylribose polymerase 85 (PARP-85, 75% increase, P = 0.024) and caspase 8 (greater than fivefold increase, P = 0.03). APLD treatment was further associated with lost or reduced expression of several genes that support angiogenesis to include: VEGF, hypoxia inducible factor 1(HIF-1), transforming growth factor-beta (TGFbeta), TGFbeta receptor 2 (TGFbetaR2), melanoma growth stimulating factor 1 (GRO1), cadherin, and vasostatin. This data supports the hypothesis that APLD may be an effective adjunctive therapy against ATC. The demonstrated molecular impact against angiogenic related genes specifically supports future strategies combining APLD with VEGF interacting agents.

Strock, C. J., J. I. Park, et al. (2006). "Activity of irinotecan and the tyrosine kinase inhibitor CEP-751 in medullary thyroid cancer." J Clin Endocrinol Metab **91**(1): 79-84.

CONTEXT: Medullary thyroid cancer (MTC) is a cancer of the parafollicular C cells that commonly presents with an inherited or acquired RET gene mutation. There is currently no effective systemic treatment for MTC. OBJECTIVE: The objective of this study was to investigate a systemic therapeutic approach to treat MTC. We studied the sensitivity of an MTC cell line and xenograft to irinotecan, alone and in combination with the tyrosine kinase inhibitor, CEP-751. RESULTS: In TT cell culture and xenografts, irinotecan treatment was highly effective. This effect was augmented by treatment with CEP-751. Treatment of TT cell xenografts resulted in durable complete remission in 100% of the mice, with median time to recurrence of 70 d for irinotecan alone and more than 130 d for irinotecan plus CEP-751. Although irinotecan induced an S phase checkpoint arrest in TT cells, CEP-751 in combination with irinotecan resulted in a loss of this arrest. CEP-751 induced a loss in the induction of the DNA repair program marked by phospho-H2AX and the checkpoint pathway marked by the activated Chk1 pathway. CONCLUSIONS: Irinotecan treatment was highly effective in a preclinical model of human MTC, resulting in complete remission in 100% of the xenografts treated. The duration of remission was further enhanced by combination with the kinase inhibitor, CEP-751. These results suggest that irinotecan, alone or in combination, may be useful for the treatment of MTC.

Stulp, R. P., J. C. Herkert, et al. (2008). "Thyroid cancer in a patient with a germline MSH2 mutation. Case report and review of the Lynch syndrome expanding tumour spectrum." Hered Cancer Clin Pract **6**(1): 15-21.

Lynch syndrome (HNPCC) is a dominantly inherited disorder characterized by germline defects in DNA mismatch repair (MMR) genes and the development of a variety of cancers, predominantly colorectal and endometrial. Although the risks for some tumour types, including breast cancer, soft tissue sarcoma and prostate cancer, are not significantly increased in Lynch syndrome, MMR deficiency in the presence of a corresponding germline defect has been demonstrated in incidental cases of a growing range of tumour types, which is reviewed in this paper. Interestingly, the MSH2-associated tumour spectrum appears to be wider than that of MLH1 and generally the risk for most extra-colonic cancers appears to be higher for MSH2 than for MLH1 mutation carriers. Together with a previously reported case, our findings show that anaplastic thyroid carcinoma can develop in the setting of Lynch syndrome. Uncommon Lynch syndrome-associated tumour types might be useful in the genetic analysis of a Lynch syndrome suspected family if samples from typical Lynch syndrome tumours are unavailable.

Sturgeon, C. and O. H. Clark (2005). "Familial nonmedullary thyroid cancer." Thyroid **15**(6): 588-93.

Familial nonmedullary thyroid cancer (FNMTC) is a syndrome of familial clustering of thyroid cancers of follicular cell origin. It is characterized by multifocality, early onset, more recurrences, and a higher degree of aggressiveness than nonfamilial thyroid cancers of follicular cell origin. An autosomal dominant inheritance pattern with reduced penetrance appears likely in most pedigrees. Although several candidate genes responsible for isolated clinical variants of FNMTC have been identified in single families, the gene(s) responsible for the vast majority of FNMTC cases has yet to be identified. Members of FNMTC cohorts should be followed longitudinally with physical

examination and ultrasonography, and aggressively treated when cancer is diagnosed. When cancer is diagnosed, total thyroidectomy should be performed, and most patients should have a prophylactic central neck dissection and a therapeutic lateral functional neck dissection, postoperative radioiodine ablation and thyroid-stimulating hormone (TSH) suppressive therapy. Close follow-up with stimulated thyroglobulin levels, neck ultrasounds, and radioiodine scans are also central to the management strategy.

Sturgis, E. M., C. Zhao, et al. (2005). "Radiation response genotype and risk of differentiated thyroid cancer: a case-control analysis." Laryngoscope **115**(6): 938-45.

BACKGROUND: Radiation is the only clear etiologic agent for differentiated thyroid cancer (DTC). Understanding the factors affecting sensitivity to gamma radiation and susceptibility to DTC will be critical to early detection and prevention of DTC. HYPOTHESIS: Germline variants of double-strand break repair genes are markers of DTC risk. OBJECTIVE: Determine the frequency of common single nucleotide polymorphisms of genes of the double-strand break repair pathway in patients with DTC and cancer-free controls. STUDY DESIGN: Case-control study. METHODS: This study included 134 patients with DTC, 79 patients with benign thyroid lesions, and 166 cancer-free control subjects. To avoid ethnic confounding, all subjects were non-Hispanic whites. Genotype analyses were performed on DNA isolated from peripheral blood lymphocytes. Multivariate logistic regression analyses were performed to estimate the risk of DTC associated with each variant genotype. RESULTS: The XRCC3 18067T polymorphic allele was found significantly more commonly among the DTC cases than for the control subjects (P=.006). After multivariate adjustment, having the XRCC3 18067T allele was associated with an increased risk of DTC (adjusted odds ratio [OR] = 2.1; 95% confidence interval [CI] = 1.3 to 3.4; P = .004). In addition, there was a suggestion that the XRCC3 18067T polymorphic allele was more common among the patients with benign thyroid disease (P = .054), and the homozygous polymorphic genotype was associated with risk for benign thyroid disease (adjusted OR = 2.1; 95% CI = 0.9-4.9; P = .078). CONCLUSIONS: In this case-control analysis, the XRCC3 18067T polymorphism is associated with DTC risk. However, such work needs confirmation in larger studies.

Subramanian, M., T. Pilli, et al. (2009). "Knockdown of IG20 gene expression renders thyroid cancer cells susceptible to apoptosis." J Clin Endocrinol Metab **94**(4): 1467-71.

AIM: The aim of the study was to investigate the expression and function of the IG20 gene in thyroid cancer cell survival, proliferation, and apoptosis. The IG20 gene expression levels were higher in benign and malignant thyroid tumors and in WRO and FRO cells relative to normal tissues. Predominantly, MADD and DENN-SV isoforms of IG20 gene were expressed. IG20 knockdown resulted in increased spontaneous, TRAIL-, and TNFalpha-induced apoptosis in WRO, but not FRO, cells. FRO cell resistance to apoptosis is likely due to caspase-8 deficiency. CONCLUSION: IG20 knockdown renders WRO cells more susceptible to spontaneous, TRAIL-, and TNFalpha-induced apoptosis and thus demonstrates the prosurvival function of the IG20 gene in thyroid cancer. These observations, combined with overexpression of IG20 noted in thyroid tumor tissues, may suggest a potential role in thyroid cancer survival and growth and indicate that IG20 may be targeted either alone or in conjunction with TRAIL or TNFalpha treatment in certain thyroid cancers.

Suh, I., S. Filetti, et al. (2009). "Distinct loci on chromosome 1q21 and 6q22 predispose to familial nonmedullary thyroid cancer: a SNP array-based linkage analysis of 38 families." Surgery **146**(6): 1073-80.

BACKGROUND: Familial nonmedullary thyroid cancer (FNMTC) is associated with earlier onset and more aggressive behavior than its sporadic counterpart. Although candidate chromosomal loci have been proposed for isolated families with variants of FNMTC, the etiology of most cases is unknown. We aimed to identify loci linked to FNMTC susceptibility using single-nucleotide polymorphism (SNP) array-based linkage analysis in a broad sampling of affected families. METHODS: We enrolled and pedigreed 38 FNMTC families. Genomic DNA was extracted from the peripheral blood of 110 relatives, and hybridized to Affymetrix SNP arrays. We performed genotyping and linkage analysis, calculating exponential logarithm-of-the-odds (LOD) scores to identify chromosomal loci with a significant likelihood of linkage. RESULTS: Forty-nine affected and 61 unaffected members of FNMTC families were genotyped. In pooled linkage analysis of all families, 2 distinct loci with significant linkage were detected at 6q22 and 1q21 (LOD=3.3 and 3.04, respectively). CONCLUSION: We have identified 2 loci on chromosomes 1 and 6 that demonstrate linkage in a broad sampling of FNMTC families. Our findings suggest the presence of germline mutations in heretofore-undiscovered genes at these loci, which may potentially lead to accurate genetic tests. Future

studies will consist of technical validation and subset analyses of higher-risk pedigrees.

Sunde, M., K. C. McGrath, et al. (2004). "TC-1 is a novel tumorigenic and natively disordered protein associated with thyroid cancer." Cancer Res **64**(8): 2766-73.

A novel gene, thyroid cancer 1 (TC-1), was found recently to be overexpressed in thyroid cancer. TC-1 shows no homology to any of the known thyroid cancer-associated genes. We have produced stable transformants of normal thyroid cells that express the TC-1 gene, and these cells show increased proliferation rates and anchorage-independent growth in soft agar. Apoptosis rates also are decreased in the transformed cells. We also have expressed recombinant TC-1 protein and have undertaken a structural and functional characterization of the protein. The protein is monomeric and predominantly unstructured under conditions of physiologic salt and pH. This places it in the category of natively disordered proteins, a rapidly expanding group of proteins, many members of which play critical roles in cell regulation processes. We show that the protein can be phosphorylated by cyclic AMP-dependent protein kinase and protein kinase C, and the activity of both of these kinases is up-regulated when cells are stably transfected with TC-1. These results suggest that overexpression of TC-1 may be important in thyroid carcinogenesis.

Takahashi, Y., J. Hamada, et al. (2004). "Expression profiles of 39 HOX genes in normal human adult organs and anaplastic thyroid cancer cell lines by quantitative real-time RT-PCR system." Exp Cell Res **293**(1): 144-53.

HOX genes are well known as master control genes in embryonic morphogenesis. We hypothesized that HOX genes give cells spatial information to maintain tissue- or organ-specificity in adult body and that the deregulated expression of HOX genes results in tumor development. We established a comprehensive analysis system to quantify expression of 39 human HOX genes based on the real-time reverse transcription PCR (RT-PCR) method. Analysis of 39 HOX genes of 20 normal adult organs by this system revealed that 5' HOX genes were expressed in organs in the caudal parts of the body, and that the more caudal regions the more numbers of HOX genes were expressed. It was also found that the expression patterns of HOX genes were more similar in the adjacent genes on the same cluster rather than in those belonging to the same paralogs. Compared with normal thyroid tissues, thyroid cancer cell lines showed the altered expression of some HOX genes, especially Abd-B homeobox family genes. Our results showed that HOX genes were organ-specifically expressed in adult body and that the deregulated expressions of Abd-B family genes were implicated in thyroid tumor development.

Takakura, S., N. Mitsutake, et al. (2008). "Oncogenic role of miR-17-92 cluster in anaplastic thyroid cancer cells." Cancer Sci **99**(6): 1147-54.

Micro RNAs (miRNAs) are non-coding small RNAs and constitute a novel class of negative gene regulators that are found in both plants and animals. Several miRNAs play crucial roles in cancer cell growth. To identify miRNAs specifically deregulated in anaplastic thyroid cancer (ATC) cells, we performed a comprehensive analysis of miRNA expressions in ARO cells and primary thyrocytes using miRNA microarrays. MiRNAs in a miR-17-92 cluster were overexpressed in ARO cells. We confirmed the overexpression of those miRNAs by Northern blot analysis in ARO and FRO cells. In 3 of 6 clinical ATC samples, miR-17-3p and miR-17-5p were robustly overexpressed in cancer lesions compared to adjacent normal tissue. To investigate the functional role of these miRNAs in ATC cells, ARO and FRO cells were transfected with miRNA inhibitors, antisense oligonucleotides containing locked nucleic acids. Suppression of miR-17-3p caused complete growth arrest, presumably due to caspase activation resulting in apoptosis. MiR-17-5p or miR-19a inhibitor also induced strong growth reduction, but only miR-17-5p inhibitor led to cellular senescence. On the other hand, miR-18a inhibitor only moderately attenuated the cell growth. Thus, we have clarified functional differences among the members of the cluster in ATC cells. In conclusion, these findings suggest that the miR-17-92 cluster plays an important role in certain types of ATCs and could be a novel target for ATC treatment.

Temmim, L., A. K. Ebraheem, et al. (2006). "Cyclin D1 protein expression in human thyroid gland and thyroid cancer." Anat Histol Embryol **35**(2): 125-9.

Cell cycle progression is facilitated by cyclin dependent kinases (CDKs) that are activated by cyclins, including Cyclin D1 and inhibited by CDK inhibitors. Evidence of the involvement of cyclin gene alterations and over expression of various cyclins in human cancer is growing. The role of Cyclin D1 in malignant progression of papillary carcinomas of the thyroid has yet to be established. We therefore studied the expression of Cyclin D1 protein in thyroid carcinomas of young Kuwaiti patients (36 cases of conventional papillary thyroid carcinoma, 12 cases of its follicular variant, one case of tall cell thyroid carcinoma and one case of medullary carcinoma) using immunohistochemistry. In 23 patients (46%)

circumscribed areas of cells were detected that showed a distinct to strong nuclear staining for immunoreactive Cyclin D1 whereas the remaining bulk of the carcinoma cells were negative or only showed a slight cytoplasmic staining. None of the tested clinical or path histological parameters showed a statistically significant correlation with the focal immunostaining. This does not rule out that the detected foci with positive nuclear Cyclin D1 immunostaining are areas where a progressive transformation to a more malignant phenotype occurs which eventually leading to lymph node and distant metastases.

Tomoda, C., A. Miyauchi, et al. (2004). "Cribriform-morular variant of papillary thyroid carcinoma: clue to early detection of familial adenomatous polyposis-associated colon cancer." World J Surg **28**(9): 886-9.

The cribriform-morular variant (CMV) of papillary thyroid carcinoma (PTC) is a rare histologic subtype of PTC that shows a combination of growth patterns including cribriform and spindle cell areas. The thyroid cancer with this unique histology was originally reported in patients with familial adenomatous polyposis (FAP), although it was later found in patients without polyposis as well. Because of its rarity, its clinical features are not clear. We reviewed seven patients with CMV-PTC who were found among 4194 patients with PTC in our pathology files between June 1991 and March 2003. The prevalence of CMV was 0.16% among all PTCs. We invited these patients to our hospital so we could obtain a detailed family history and recommend colonoscopic examination and germline APC gene analysis. Two patients without subjective symptoms had polyposis of the colon and colon cancers. Germline APC gene mutations were found in both patients. The father of a patient who refused the invitation was revealed to have undergone surgery for colon polyposis. In the remaining four patients, neither polyposis nor APC gene mutation was found. Common clinical features included a young age (mean 25 years), predominance of females, circumscribed tumors, negative node metastasis, and no recurrence of the thyroid cancer after surgery. Two of the three patients with colon polyposis had bilateral multiple thyroid tumors, whereas the remaining four (without polyposis) had a solitary tumor. The histopathology of CMV in patients with PTC should arouse a suspicion of FAP, especially if there are multiple tumors. This finding can lead to early detection of colon cancer.

Traugott, A. and J. F. Moley (2005). "Medullary thyroid cancer: medical management and follow-up." Curr Treat Options Oncol **6**(4): 339-46.

Medullary thyroid carcinoma (MTC) is a neuroendocrine malignancy that occurs in hereditary (25%) and sporadic (75%) clinical settings. MTC is present in all patients with the multiple endocrine neoplasia type 2 (MEN 2) syndromes. MTCs produce calcitonin, measurement of which indicates the presence of tumor in at-risk individuals and the effectiveness of therapy in treated patients. Surgery is the mainstay of therapy for primary and recurrent disease. Routine serial postoperative measurement of calcitonin levels should be done. Patients with elevated calcitonin levels should have imaging by computed tomography scan, magnetic resonance imaging, and/or fluorodeoxyglucose positron emission tomography to identify sites of recurrence and metastasis. The role of radiation therapy is not well defined. There is no effective systemic therapy for MTC at present. Activating mutations in a tyrosine kinase receptor gene are present in the majority of MTCs, and experience with tyrosine kinase inhibitors and other agents in the setting of clinical trials is critical for the identification of effective systemic treatment.

Tsai, J. H., C. H. Tsai, et al. (2005). "Association of viral factors with non-familial breast cancer in Taiwan by comparison with non-cancerous, fibroadenoma, and thyroid tumor tissues." J Med Virol **75**(2): 276-81.

To study the etiologic factors of non-familial breast cancer, the polymerase chain reaction (PCR) and Southern hybridization were used to detect six viruses including human papillomavirus (HPV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex virus (HSV)-1, HSV-2, and human herpesvirus (HHV)-8 DNA in 69 patients with breast cancer and 60 specimens from non-cancerous or other individuals with thyroid tumors or fibroadenoma (non-breast cancer controls). Two specimens from patients with a familial history of breast cancer and five breast cancer specimens with negative results for beta-globin, which was used as internal control, were excluded from this study. Eight (12.9%) HSV-1, 28 (45.2%) EBV, 47 (75.8%) CMV, 8 (12.9%) HPV, and 28 (45.2%) HHV-8 positive samples out of the 62 breast cancer specimens were detected; no HSV-2 DNA was detected in any group. Among the viral gene-positive breast cancer samples, 12 (23.1%) were positive for 1 virus, 16 (30.8%) were positive for 2 viruses, 21 (40.4%) were positive for 3 viruses, and 3 (5.8%) were positive for 4 viruses. Among the viral gene-positive specimens of the control groups, only one virus, CMV, was found in the non-cancerous and thyroid tumor specimens, while multiple viruses were found in the fibroadenoma specimens. The viruses associated with breast cancer were HHV-8 > EBV (P

<0.01). The viruses associated with fibroadenoma were HSV-1 and HHV-8 > EBV (P <0.01). The presence of more than one virus was found predominantly in breast cancer and exclusively found in fibroadenoma. CMV was the only virus associated with thyroid tumors.

Ulisse, S., E. Baldini, et al. (2007). "Transforming acidic coiled-coil 3 and Aurora-A interact in human thyrocytes and their expression is deregulated in thyroid cancer tissues." Endocr Relat Cancer **14**(3): 827-37.

Aurora-A kinase has recently been shown to be deregulated in thyroid cancer cells and tissues. Among the Aurora-A substrates identified, transforming acidic coiled-coil (TACC3), a member of the TACC family, plays an important role in cell cycle progression and alterations of its expression occur in different cancer tissues. In this study, we demonstrated the expression of the TACC3 gene in normal human thyroid cells (HTU5), and its modulation at both mRNA and protein levels during cell cycle. Its expression was found, with respect to HTU5 cells, unchanged in cells derived from a benign thyroid follicular tumor (HTU42), and significantly reduced in cell lines derived from follicular (FTC-133), papillary (B-CPAP), and anaplastic thyroid carcinomas (CAL-62 and 8305C). Moreover, in 16 differentiated thyroid cancer tissues, TACC3 mRNA levels were found, with respect to normal matched tissues, reduced by twofold in 56% of cases and increased by twofold in 44% of cases. In the same tissues, a correlation between the expression of the TACC3 and Aurora-A mRNAs was observed. TACC3 and Aurora-A interact in vivo in thyroid cells and both proteins localized onto the mitotic structure of thyroid cells. Finally, TACC3 localization on spindle microtubule was no more observed following the inhibition of Aurora kinase activity by VX-680. We propose that Aurora-A and TACC3 interaction is important to control the mitotic spindle organization required for proper chromosome segregation.

Varkondi, E., F. Gyory, et al. (2005). "Oncogene amplification and overexpression of oncoproteins in thyroid papillary cancer." In Vivo **19**(2): 465-70.

BACKGROUND: Several oncogene aberrations have been found in papillary thyroid cancer, the incidence of which has increased after the accident in Chernoby. The occurrence and prognostic significance of these aberrations may have importance in therapeutic strategies. MATERIALS AND METHODS: Tumour tissues from 24 patients were investigated by Dot-blot DNA hybridisation for c-myc, Ha-ras amplification and p53 deletion, and by immunohistochemical method for cyclin D1, p53 and p21 overexpression. RESULTS: Overexpression of p53 protein was detected in 66.6%, with p21 expression (25%) without any influence on tumour phenotype. Cyclin D1 overexpression was found in 50% to be associated with p21, in inverse relation to lymphocytic infiltration. Overexpression of estrogen receptor was shown in 4 cyclin D1-positive samples (17%). CONCLUSION: Our results suggest that cyclin D1 overexpression is associated with poor prognosis. The co-expression of cyclin D1 and p21 causes a CDK-independent, estrogen receptor-mediated effect of the cyclin D1 also described in breast cancer.

Vasko, V., S. Hu, et al. (2005). "High prevalence and possible de novo formation of BRAF mutation in metastasized papillary thyroid cancer in lymph nodes." J Clin Endocrinol Metab **90**(9): 5265-9.

CONTEXT: The role of the T1799A BRAF mutation in lymph node metastasis of papillary thyroid cancer (PTC) is not clear. OBJECTIVE: Our objective was to explore the relationship between BRAF mutation and lymph node metastasis of PTC by examining the mutation in both the primary tumors and their paired lymph node metastases. DESIGN: We isolated genomic DNA from primary thyroid tumors and paired lymph node metastases and performed direct sequencing of exon 15 of the BRAF gene mutation that carries the T1799A mutation. RESULTS: In a series of 33 cases, 21 harbored the T1799A mutation in the primary tumors, and 17 (81%) of them harbored the same mutation also in the paired lymph node metastases. Twelve cases did not harbor the T1799A mutation in the primary tumors, among which nine cases also did not harbor BRAF mutation in the lymph node-metastasized tumors, whereas the other three did harbor the T1799A mutation in lymph node-metastasized tumor tissues. A novel tandem TG1799-1800AA mutation within one allele was found in a lymph node-metastasized tumor but not in the primary tumor. This mutation results in the change of codon 600 (GTG) of the gene to GAA with the consequent amino acid change (V600E) in the B-type Raf (BRAF) protein, same as that caused by the T1799A mutation alone. CONCLUSION: The high prevalence of BRAF mutation in lymph node-metastasized PTC tissues from BRAF mutation-positive primary tumors and the possible de novo formation of BRAF mutation in lymph node-metastasized PTC were consistent with a role of BRAF mutation in facilitating the metastasis and progression of PTC in lymph nodes.

Vella, V., R. Mineo, et al. (2004). "Interleukin-4 stimulates papillary thyroid cancer cell survival: implications in patients with thyroid cancer and

concomitant Graves' disease." J Clin Endocrinol Metab **89**(6): 2880-9.

IL-4, a pleiotropic cytokine mainly produced by activated helper T lymphocytes type 2 (Th2), is known to protect thyroid cells from autoimmune damage. Acting via its receptors (IL-4Ralpha), IL-4 has antiproliferative and apoptotic effects in many malignancies. Its effect in thyroid cancer is unknown. We found that surgical specimens of thyroid carcinomas express both IL-4Ralpha and IL-4 in the majority of cases. Thyroid glands affected by Graves' disease also express IL-4. We also studied a panel of eight thyroid cancer cell lines from different histotypes and found that thyroid cancer cells express high levels of IL-4Ralpha although they do not express IL-4. We then compared the biological effects of IL-4 in TPC-1, a thyroid cancer cell line, and in MCF-7 breast cancer cells. IL-4 very weakly stimulated thyroid cancer cell proliferation, but it was very effective in protecting thyroid cancer cells from apoptosis induced by staurosporin. The protective effect of IL-4 was similar in magnitude to that of IGF-I and was associated with up-regulation of the antiapoptotic molecule Bcl-2 and weak down-regulation of the proapoptotic molecule Bax. Moreover, IL-4 slightly potentiated the survival effect of IGF-I. In contrast, IL-4 reduced growth and induced apoptosis in MCF-7 cells. Taken together, these findings suggest that thyroid cancer cells receive significant protection from apoptosis by IL-4 produced in the thyroid gland by activated T lymphocytes when concomitant Graves' disease is present.

Vella, V., C. Puppin, et al. (2009). "DeltaNp73alpha inhibits PTEN expression in thyroid cancer cells." Int J Cancer **124**(11): 2539-48.

DeltaNp73 is a N-terminally truncated p53 family member with a dominant negative function, which is upregulated in cancer. PTEN is a lipid phosphatase, which is involved in the attenuation of tyrosine kinase signaling. PTEN expression is increased by p53, and its function is blunted in several malignancies. Because in most of the thyroid carcinomas, DeltaNp73alpha is upregulated, whereas PTEN expression down regulated, we investigated whether DeltaNp73alpha may influence PTEN expression in this cell model. We found that DeltaNp73alpha overexpression in thyroid cancer cells reduces PTEN expression, whereas DeltaNp73alpha down-regulation by siRNA increases PTEN expression. Real-time PCR indicated that overexpression of DeltaNp73alpha is able to reduce PTEN mRNA levels. Moreover, chromatin immunoprecipitation (ChIP) and luciferase assays indicated that DeltaNp73alpha binds to -1031-779 region of the PTEN promoter, which is a different site than that for p53, thereby inhibiting promoter activity. Interestingly, also the transcriptionally active p73 isoforms (TAp73alpha and TAp73beta) bound to this DNA sequence and, at variance with DeltaNp73alpha, stimulated PTEN promoter activity to an extent similar to that of p53. In accordance with its effect on PTEN protein levels, DeltaNp73alpha increased phospho-Akt protein content and, as a consequence, Mdm2-mediated p53 degradation. This effect of DeltaNp73alpha resulted in increased thyroid cancer cell proliferation and reduced apoptosis and was reverted by the PI3-kinase inhibitor LY294002, indicating the role of Akt pathway in this effect. Taken together, these results indicate a novel p73 regulated mechanism for PTEN expression in thyroid cancer cells, and that, also through this mechanism, DeltaNp73alpha exerts its protumorigenic effect.

Vivacqua, A., D. Bonofiglio, et al. (2006). "17beta-estradiol, genistein, and 4-hydroxytamoxifen induce the proliferation of thyroid cancer cells through the g protein-coupled receptor GPR30." Mol Pharmacol **70**(4): 1414-23.

The higher incidence of thyroid carcinoma (TC) in women during reproductive years compared with men and the increased risk associated with the therapeutic use of estrogens have suggested a pathogenetic role exerted by these steroids in the development of TC. In the present study, we evaluated the potential of 17beta-estradiol (E2), genistein (G), and 4-hydroxyta-moxifen (OHT) to regulate the expression of diverse estrogen target genes and the proliferation of human WRO, FRO, and ARO thyroid carcinoma cells, which were used as a model system. We have ascertained that ARO cells are devoid of estrogen receptors (ERs), whereas both WRO and FRO cells express a single variant of ERalpha that was neither transactivated, modulated, nor translocated into the nucleus upon treatment with ligands. However, E2, G, and OHT were able either to induce the transcriptional activity of c-fos promoter constructs, including those lacking the estrogen-responsive elements, or to increase c-fos, cyclin A, and D1 expression. It is noteworthy that we have demonstrated that the G protein-coupled receptor 30 (GPR30) and the mitogen-activated protein kinase (MAPK) pathway mediate both the up-regulation of c-fos and the growth response to E2, G, and OHT in TC cells studied, because these stimulatory effects were prevented by silencing GPR30 and using the MEK inhibitor 2'-amino-3'-methoxyflavone (PD 98059). Our findings provide new insight into the molecular mechanisms through which estrogens may induce the progression of TC.

Vriens, M. R., J. M. Schreinemakers, et al. (2009). "Diagnostic markers and prognostic factors in thyroid cancer." Future Oncol **5**(8): 1283-93.

There has been considerable progress identifying biomarkers in thyroid tumors that improve the accuracy of fine-needle aspiration biopsy and also help predict tumor aggressiveness or behavior. In this review we address both the clinical potential of molecular biomarkers and their usefulness, based on the most recent literature. We describe the current best clinical staging systems and the common somatic mutations in thyroid cancer. The BRAF mutation is the most common mutation in papillary thyroid cancer and has recently been reported to be associated with disease aggressiveness; it is also an independent predictor of tumor behavior. Combined testing of RET/PTC, NTRK, RAS and PAX8-PPARgamma, which are mutually exclusive mutations, helps improve the accuracy of fine-needle aspiration biopsy. Gene-expression profiling studies have identified a variety of potential molecular markers to help distinguish benign from malignant thyroid neoplasms. Expression analysis of differentially expressed microRNAs also appears to be a promising diagnostic approach for distinguishing benign from malignant thyroid neoplasm. It is especially useful for indeterminate nodules by fine-needle aspiration biopsy.

Vriens, M. R., I. Suh, et al. (2009). "Clinical features and genetic predisposition to hereditary nonmedullary thyroid cancer." Thyroid **19**(12): 1343-9.

BACKGROUND: Approximately 5% of the nonmedullary thyroid cancers are hereditary. Hereditary nonmedullary thyroid cancer may occur as a minor component of familial cancer syndromes (familial adenomatous polyposis, Gardner's syndrome, Cowden's disease, Carney's complex type 1, Werner's syndrome, and papillary renal neoplasia) or as a primary feature (familial nonmedullary thyroid cancer [FNMTC]). The goal of this article was to review our current knowledge on the hereditary nonmedullary thyroid cancer. SUMMARY: Epidemiologic and clinical kindred studies have demonstrated that FNMTC is a unique clinical entity. Most studies suggest that FNMTC is associated with more aggressive disease than sporadic cases, with higher rates of multicentric tumors, lymph node metastasis, extrathyroidal invasion, and shorter disease-free survival. A hereditary predisposition to nonmedullary thyroid cancer is well established, but the susceptibility genes for isolated FNMTC have not been identified. However, additional susceptibility loci for FNMTC have been recently identified in classic isolated cases of FNMTC (1q21, 6q22, 8p23.1-p22, and 8q24). CONCLUSIONS: More studies are needed to validate chromosomal susceptibility loci and identify the susceptibility genes for FNMTC. The discovery of the predisposing genes may allow for screening and early diagnosis, which could lead to improved outcomes for patients and their families.

Wang, H. Q., Z. X. Du, et al. (2007). "Different induction of GRP78 and CHOP as a predictor of sensitivity to proteasome inhibitors in thyroid cancer cells." Endocrinology **148**(7): 3258-70.

Proteasome inhibitors represent a novel class of antitumor agents with preclinical and clinical evidence of activity against hematological malignancies and solid tumors. Emerging lines of evidence suggest that the unfolded protein response is implicated in proteasome inhibitors-induced apoptosis. Glucose-regulated protein 78 kDa (GRP78) and CCAAT/enhancer-binding protein homologous protein (CHOP) as part of the unfolded protein response play critical roles in cell survival or death. Here we demonstrate that induction of GRP78 and CHOP are differently regulated upon proteasome inhibition in different thyroid cancer cell lines, and GRP78 levels as well as preferential induction of GRP78 or CHOP appears to be involved in the responsiveness. Insensitive ARO, 8305C, and 8505C cell lines inherently express relatively high levels of GRP78 compared with sensitive cell lines, and its levels are further up-regulated upon treatment with proteasome inhibitors. CHOP levels are dramatically induced in sensitive cell lines until 24 h after proteasome inhibition. On the other hand, only a slight increase is observed at 4 h in insensitive cell lines, and this increase is unable to be detected after 8 h. Insensitive cells are sensitized to proteasome inhibition by suppression of GRP78. Furthermore, suppression of CHOP induction or overexpression of GRP78 partially prevents proteasome inhibition-mediated cell death. Our study indicates a molecular mechanism by which the sensitivity of thyroid cancer cells is regulated by the level of GRP78 as well as preferential induction of GRP78 or CHOP upon treatment with proteasome inhibitors. Our experiments therefore suggest a novel approach toward sensitization of thyroid cancer cells to proteasome inhibitors.

Wang, M. H., W. Lee, et al. (2007). "Altered expression of the RON receptor tyrosine kinase in various epithelial cancers and its contribution to tumourigenic phenotypes in thyroid cancer cells." J Pathol **213**(4): 402-11.

Aberrant expression of the RON receptor tyrosine kinase has been implicated in the pathogenesis of epithelial tumours. The aim of this study was to determine RON expression in various

normal epithelial cells and their corresponding tumours by immunohistochemistry. The role of RON in regulating tumourigenic phenotypes was also studied using thyroid cancer cells as a model. RON was almost exclusively expressed at variable levels in normal epithelial cells from the digestive track, lung, kidney, pancreas, liver, breast, bladder, skin, and others. Among 15 types of cancer studied, RON was overexpressed in significant numbers in cancers derived from breast (56%), colon (51%), lung (48), thyroid (42%), skin (37%), bladder (36%), and pancreas (33%). In contrast, limited RON overexpression was observed in cancers from stomach, kidney, brain, liver, ovary, and prostate. Detailed analysis of thyroid tissues showed that RON was hardly detected in normal thyroid cells, moderately expressed in adenoma samples, but overexpressed in about half of papillary and follicular cancer specimens. Overexpression correlated with advanced clinical stage and was associated with lymph node metastasis. In cultured thyroid cancer cells, RON was highly expressed, with constitutive phosphorylation. Activation of RON increased cell growth and migration via the MAP kinase and AKT pathways. Silencing RON expression significantly prevented cell growth and increased cell apoptotic death. These findings show that RON overexpression occurs in a particular group of epithelial cancers. The requirement for RON in sustaining tumourigenic phenotypes suggests that it is a potential target for therapeutic intervention.

Ward, L. S., E. C. Morari, et al. (2007). "Identifying a risk profile for thyroid cancer." Arq Bras Endocrinol Metabol **51**(5): 713-22.

The large use of simple and effective diagnostic tools has significantly contributed to the increase in diagnosis of thyroid cancer over the past years. However, there is compelling evidence that most micropapillary carcinomas have an indolent behavior and may never evolve into clinical cancers. Therefore, there is an urgent need for new tools able to predict which thyroid cancers will remain silent, and which thyroid cancers will present an aggressive behavior. There are a number of well-established clinical predictors of malignancy and recent studies have suggested that some of the patients laboratory data and image methods may be useful. Molecular markers have also been increasingly tested and some of them appear to be very promising, such as BRAF, a few GST genes and p53 polymorphisms. In addition, modern tools, such as immunocytochemical markers, and the measure of the fractal nature of chromatin organization may increase the specificity of the pathological diagnosis of malignancy and help ascertain the prognosis. Guidelines designed to select nodules for further evaluation, as well as new methods aimed at distinguishing carcinomas of higher aggressiveness among the usually indolent thyroid tumors are an utmost necessity.

Watanabe, R., Y. Hayashi, et al. (2009). "Possible involvement of BRAFV600E in altered gene expression in papillary thyroid cancer." Endocr J **56**(3): 407-14.

Somatic mutations in BRAF, especially BRAFV600E, are frequently identified in papillary thyroid cancer (PTC) tumors. It has been established that expression levels of numbers of genes are characteristically altered in PTC, however, the link between BRAF mutation and gene expression patterns are still elusive. In the present study, we analyzed relative expression levels of the wild type BRAF and BRAFV600E mRNA by using quantitative PCR (qPCR) and cDNAPCR- RFLP in 19 PTC specimens and adjacent normal thyroid tissues. BRAFV600E mRNA was detected in 17 out of 19 PTC specimens, and the expression levels were valuable among the specimens, suggesting alternative expression of BRAFV600E in each cell and/or alternative population of BRAFV600E-positive clones in the tumor. We then analyzed expression levels of 20 genes by qPCR, and analyzed for possible correlation with expression levels of BRAFV600E mRNA. Expression levels of fibronectin, vimentin and CITED1 (Cbp/p300 interacting protein with glutamic acid and aspartic acid rich carboxyl terminal domain) were positively correlated with those of BRAFV600E, suggesting pathophysiological links between activated BRAF and overexpression of these genes. Among these genes expression of vimentin was decreased by inhibiting BRAF expression in NPA cells that express BRAFV600E by means of siRNA, suggesting activated BRAF positively regulate expression of vimentin. Collectively, our analyses illustrated the possibilities that variable expression of BRAFV600E may modify characters of PTC through its effects on gene expression.

Weber, F. and C. Eng (2005). "Gene-expression profiling in differentiated thyroid cancer--a viable strategy for the practice of genomic medicine?" Future Oncol **1**(4): 497-510.

Thyroid neoplasias have been largely ignored as an active field of investigation due to the overall favorable prognosis of differentiated nonmedullary thyroid cancers. However, differentiated thyroid cancers have the highest estimated annual percentage increase in incidence amongst all cancer sites. Furthermore, no significant progress has been made to improve survival, especially for advanced disease. Compounding the problem, there remains a lack of

highly accurate preoperative markers or molecular-based predictive models to differentiate benign from malignant follicular neoplasias, thus we continue to rely upon surgery for diagnostic purposes in this subset of patients. Therefore, new approaches are necessary to identify potential novel diagnostic, prognostic and therapeutic algorithms, which would not only allow accurate early diagnosis but also personalized patient management, with clinical management and surveillance tailored according to the genetic signature of the patient. The advent of modern genomic technologies, such as global gene-expression profiling, may begin to provide the data required for the evidence-based practice of genomic medicine as it relates to thyroid neoplasia. However, it is already clear that genomic technology alone is insufficient to fully achieve this vision.

Weier, H. U., J. Kwan, et al. (2009). "Kinase expression and chromosomal rearrangements in papillary thyroid cancer tissues: investigations at the molecular and microscopic levels." J Physiol Pharmacol **60 Suppl 4**: 47-55.

Structural chromosome aberrations are known hallmarks of many solid tumors. In the papillary form of thyroid cancer (PTC), for example, activation of the receptor tyrosine kinase (RTK) genes, ret or the neurotrophic tyrosine kinase receptor type I (NTRK1) by intra- or interchromosomal rearrangements have been suggested as a cause of the disease. The 1986 accident at the nuclear power plant in Chernobyl, Ukraine, led to the uncontrolled release of high levels of radioisotopes. Ten years later, the incidence of childhood papillary thyroid cancer (chPTC) near Chernobyl had risen by two orders of magnitude. Tumors removed from some of these patients showed aberrant expression of the ret RTK gene due to a ret/PTC1 or ret/PTC3 rearrangement involving chromosome 10. However, many cultured chPTC cells show a normal G-banded karyotype and no ret rearrangement. We hypothesize that the "ret-negative" tumors inappropriately express a different oncogene or have lost function of a tumor suppressor as a result of chromosomal rearrangements, and decided to apply molecular and cytogenetic methods to search for potentially oncogenic chromosomal rearrangements in Chernobyl chPTC cases. Knowledge of the kind of genetic alterations may facilitate the early detection and staging of chPTC as well as provide guidance for therapeutic intervention.

Weier, H. U., T. B. Tuton, et al. (2006). "Molecular cytogenetic characterization of chromosome 9-derived material in a human thyroid cancer cell line." Cytogenet Genome Res **114**(3-4): 284-91.

The incidence of papillary thyroid carcinoma (PTC) increases significantly after exposure of the head and neck region to ionizing radiation, yet we know neither the steps involved in malignant transformation of thyroid epithelium nor the specific carcinogenic mode of action of radiation. Such increased tumor frequency became most evident in children after the 1986 nuclear accident in Chernobyl, Ukraine. In the eight years following the accident, the average incidence of childhood PTCs (chPTC) increased 70-fold in Belarus, 200-fold in Gomel, 10-fold in the Ukraine and 50-fold in Tschnigov, Kiev, Rovno, Shitomyr and Tscherkassy compared to the rate of about 1 tumor incidence per 106 children per year prior to 1986 (Likhtarev et al., 1995; Sobolev et al., 1997; Jacob et al., 1998). To study the etiology of radiation-induced thyroid cancer, we formed an international consortium to investigate chromosomal changes and altered gene expression in cases of post-Chernobyl chPTC. Our approach is based on karyotyping of primary cultures established from chPTC specimens, establishment of cell lines and studies of genotype-phenotype relationships through high resolution chromosome analysis, DNA/cDNA micro-array studies, and mouse xenografts that test for tumorigenicity. Here, we report the application of fluorescence in situ hybridization (FISH)-based techniques for the molecular cytogenetic characterization of a highly tumorigenic chPTC cell line, S48TK, and its subclones. Using chromosome 9 rearrangements as an example, we describe a new approach termed 'BAC-FISH' to rapidly delineate chromosomal breakpoints, an important step towards a better understanding of the formation of translocations and their functional consequences.

Wells, S. A., Jr. and M. Santoro (2009). "Targeting the RET pathway in thyroid cancer." Clin Cancer Res **15**(23): 7119-23.

The RET (rearranged during transfection) protooncogene encodes a single pass transmembrane receptor that is expressed in cells derived from the neural crest and the urogenital tract. As part of a cell-surface complex, RET binds glial derived neurotrophic factor (GDNF) ligands in conjunction with GDNF-family alpha co-receptors (GFRalpha). Ligand-induced activation induces dimerization and tyrosine phosphorylation of the RET receptor with downstream activation of several signal transduction pathways. Activating germline RET mutations play a central role in the development of the multiple endocrine neoplasia (MEN) syndromes MEN2A, MEN2B, and familial medullary thyroid carcinoma (FMTC) and also in the development of the congenital abnormality Hirschsprung's disease. Approximately 50% of patients with sporadic MTC have somatic

RET mutations, and a significant portion of papillary thyroid carcinomas result from chromosomal inversions or translocations, which activate RET (RET/PTC oncogenes). The RET protooncogene has a significant place in cancer prevention and treatment. Timely thyroidectomy in kindred members who have inherited a mutated RET allele, characteristic of MEN2A, MEN2B, or FMTC, can prevent MTC, the most common cause of death in these syndromes. Also, recently developed molecular therapeutics that target the RET pathway have shown activity in clinical trials of patients with advanced MTC, a disease for which there has been no effective therapy.

Wreesmann, V. B., E. M. Sieczka, et al. (2004). "Genome-wide profiling of papillary thyroid cancer identifies MUC1 as an independent prognostic marker." Cancer Res **64**(11): 3780-9.

Clinicopathological variables used at present for prognostication and treatment selection for papillary thyroid carcinomas (PTCs) do not uniformly predict tumor behavior, necessitating identification of novel prognostic markers. Complicating the assessment is the long natural history of PTC and our rudimentary knowledge of its genetic composition. In this study we took advantage of differences in clinical behavior of two distinct variants of PTC, the aggressive tall-cell variant (TCV) and indolent conventional PTC (cPTC), to identify molecular prognosticators of outcome using complementary genome wide analyses. Comparative genome hybridization (CGH) and cDNA microarray (17,840 genes) analyses were used to detect changes in DNA copy number and gene expression in pathological cPTC and TCV. The findings from CGH and cDNA microarray analyses were correlated and validated by real-time PCR and immunohistochemical analyses on a series of 100 cases of cPTC and TCV. Genes identified by this approach were evaluated as prognostic markers in cPTC by immunohistochemistry on tissue arrays. CGH identified significant differences in the presence (76 versus 27%; P = 0.001) and type of DNA copy number aberrations in TCV compared with cPTC. Recurrent gains of 1p34-36, 1q21, 6p21-22, 9q34, 11q13, 17q25, 19, and 22 and losses of 2q21-31, 4, 5p14-q21, 6q11-22, 8q11-22, 9q11-32, and 13q21-31 were unique to TCV. Hierarchical clustering of gene expression profiles revealed significant overlap between TCV and cPTC, but further analysis identified 82 dysregulated genes differentially expressed among the PTC variants. Of these, MUC1 was of particular interest because amplification of 1q by CGH correlated with MUC1 amplification by real-time PCR analysis and protein overexpression by immunohistochemistry in TCV (P = 0.005). Multivariate analysis revealed a significant association between MUC1 overexpression and treatment outcome, independent of histopathological categorization (P = 0.03). Analysis of a validation series containing a matched group of aggressive and indolent cPTCs confirmed the association between MUC1 overexpression and survival (relative risk, 2.3; 95% confidence interval, 1.1-5.5; P = 0.03). Our data suggest that MUC1 dysregulation is associated with aggressive behavior of PTC and may serve as a prognostic marker and potential therapeutic target in this disease.

Xu, J., M. Capezzone, et al. (2005). "Activation of nicotinamide N-methyltransferase gene promoter by hepatocyte nuclear factor-1beta in human papillary thyroid cancer cells." Mol Endocrinol **19**(2): 527-39.

We previously demonstrated that the human nicotinamide N-methytransferase (NNMT) gene was highly expressed in many papillary thyroid cancers and cell lines. The expression in other papillary and follicular cancers or cell lines and normal thyroid cells was low or undetectable. To gain an understanding of the molecular mechanism of this cell-specific expression, the NNMT promoter was cloned and studied by luciferase reporter gene assay. The promoter construct was expressed highly in papillary cancer cell lines, including those with higher (e.g. BHP 2-7) and lower (e.g. BHP 14-9) NNMT gene expression, and expressed weakly in follicular thyroid cancer cell lines. Further study with 5'-deletion promoter construct suggested that the NNMT promoter was regulated differently in BHP 2-7 and BHP 14-9 cells. In BHP 2-7 cells, promoter activity was dependent on an upstream sequence. In BHP 14-9 cells, sequence in the basal promoter region contributed notably to the overall promoter activity. RT-PCR or Western blot analysis indicated that hepatocyte nuclear factor-1beta (HNF-1beta) was expressed in only papillary cancer cell lines with high NNMT gene expression. HNF-1beta was not expressed or expressed very weakly in other papillary, follicular, and Hurthle cancer cell lines and primary cultures of normal thyroid cells and benign thyroid conditions. A HNF-1 binding site was identified in the NNMT basal promoter region. Mutations in this site decreased NNMT promoter activity in the HNF-1beta-positive BHP 2-7 cells, but not in the HNF-1beta-negative BHP 14-9 cells. HNF-1beta bound to the HNF-1 site specifically as a homodimer as determined by gel retardation assays with HNF-1beta-specific antibody. Cotransfection of a HNF-1beta expression plasmid increased NNMT promoter activity significantly in both HNF-1beta-positive and -negative thyroid cancer cell lines and Hep G2 liver cancer cells. Furthermore, transient expression of

HNF-1beta in BHP 14-9 cells increased endogenous NNMT protein levels. In summary, HNF-1beta functions as a transcription activator for NNMT gene expression in some papillary thyroid cancer cells.

Xu, J., S. Filetti, et al. (2008). "Expression of hepatocyte nuclear factor-1alpha mRNA in human anaplastic thyroid cancer cell lines and tumors." Thyroid **18**(5): 533-9.

BACKGROUND: Hepatocyte nuclear factor (HNF)-1alpha and HNF-1beta are related transcription factors that are mainly expressed in liver cells. Our previous study showed that HNF-1beta was highly expressed in papillary thyroid cancer cell lines and tumors. HNF-1alpha mRNA, however, was not detected in differentiated thyroid cancer cell lines. The objective of this study was to determine whether HNF-1alpha is expressed in dedifferentiated anaplastic thyroid cancer cells. METHODS: Total RNA isolated from six anaplastic thyroid cancer cell lines and 38 surgical samples was analyzed for HNF-1alpha mRNA by conventional reverse-transcription polymerase chain reaction (RT-PCR) or real-time RT-PCR. HNF-1alpha DNA binding activity was measured by gel retardation assay and HNF-1alpha protein was identified by Western blotting. RESULTS: HNF-1alpha mRNA was expressed in four of the six anaplastic cell lines. The presence of HNF-1alpha protein and DNA binding activity was detected in three lines with higher HNF-1alpha mRNA level. Three cell lines also expressed HNF-1beta. HNF-1alpha transcripts were also detected in five out of six anaplastic tumors, but not in the papillary tumors except one with weak PCR signal. CONCLUSION: HNF-1alpha mRNA was detected in high frequency in anaplastic thyroid cancer cell lines and tumors. HNF-1alpha might play a role in the pathogenesis of anaplastic thyroid cancer.

Xu, J. and J. M. Hershman (2006). "Histone deacetylase inhibitor depsipeptide represses nicotinamide N-methyltransferase and hepatocyte nuclear factor-1beta gene expression in human papillary thyroid cancer cells." Thyroid **16**(2): 151-60.

Nicotinamide N-methyltransferase (NNMT) catalyzes N-methylation of nicotinamide and other structural analogues. NNMT gene expression is enhanced in many papillary thyroid cancer cells and activated by hepatocyte nuclear factor (HNF)-1beta. In this work, we studied the effects of depsipeptide, a histone deacetylase inhibitor, on NNMT gene expression in BHP 18-21 papillary thyroid cancer cells. Depsipeptide reduced NNMT mRNA level in a dose-dependent and time-dependent manner as determined by semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR). In contrast, expression of the sodium iodide symporter (NIS), a gene with differentiated function, waas enhanced in the treated cells. NNMT protein level determined by Western blot analysis and NNMT catalytic activity was also reduced significantly in the depsipeptide-treated cells. To study the mechanism of NNMT gene repression by depsipeptide, effects of depsipeptide on NNMT promoter activity were determined by luciferase reporter gene assay. NNMT promoter activity was significantly reduced in the HNF-1beta-positive BHP 18-21 cells but not in the HNF-1beta-negative BHP 14-9 papillary cancer cells. A mutant reporter construct with mutations in a HNF-1 site in the NNMT basal promoter region did not respond to depsipeptide in both HNF-1beta protein levels, and abolished activity of DNA binding to the HNF-1 site in the NNMT promoter region. Protein synthesis inhibitor cycloheximide and proteasome inhibitor MG-132 enhanced HNF-1beta stability in the depsipeptide-treated cells. In summary, depsipeptide represses NNMT and HNF-1beta gene expression in some papillary thyroid cancer cells. the repression of NNMT by depsipeptide is at the transcription level through downregulation of transcription activator HNF-1beta.

Xu, X., G. Rao, et al. (2006). "Clinicopathological significance of major histocompatibility complex class I-related chain a and B expression in thyroid cancer." J Clin Endocrinol Metab **91**(7): 2704-12.

CONTEXT: Major histocompatibility complex class I-related chains A and B (MICA/B) are two stress-inducible ligands for the immunoreceptor NKG2D that is expressed on cytotoxic T cells and natural killer (NK) cells. It is not known whether MICA/B expression is up-regulated in thyroid cancer as a result of oncogene activation. OBJECTIVE: The objective of the investigation was to study MICA/B expression and regulation in thyroid cancer and its role in mediating the cytotoxicity of NK cells. METHODS: MICA/B expression in thyroid cancer was analyzed by immunohistochemical staining. Cell surface MICA/B levels in thyroid tumor cell lines and fresh tumor cells were analyzed by flow cytometric analysis. The susceptibility of thyroid tumor cells to NK cell killing was tested by using (51)Cr release assay. RESULTS: MICA/B was expressed at moderate or high levels in 18 of 39 papillary thyroid carcinomas and four of eight anaplastic thyroid carcinomas. MICA/B expression was confirmed by flow cytometric analysis in three fresh thyroid neoplasms. MICA/B expression was detected in eight of 10 thyroid tumor cell lines and correlated with their sensitivity to killing by the NKG2/D-positive NK-92 cells. Blocking of NKG2D and MICA/B interaction by specific antibodies partially led to the inhibition of

NK-92 cell-mediated cytotoxicity. The MAPK inhibitors were able to block MICA/B expression in MRO87 and HeLa cells. Transient transfection of mutant BRAF and RAS oncogenes led to increased MICA/B expression in 293 cells and WRO82 cells. CONCLUSION: MICA/B expression is up-regulated in thyroid cancer, probably due to the activation of the MAPK pathway. MICA/B in thyroid cancer plays an important role in NK-92 cell-mediated cytotoxicity.

Yamanaka, K., Y. Ito, et al. (2007). "Immunohistochemical study of glypican 3 in thyroid cancer." Oncology **73**(5-6): 389-94.

In 123 patients with thyroid cancer, expression of glypican 3 (GPC3) was immunohistochemically investigated in tissue samples and the biological significance of GPC3 in thyroid cancer was examined. GPC3 was scarcely expressed in the normal thyroid gland, but was dramatically enhanced in certain types of cancers: 100% in follicular carcinoma (20/20 cases) and 70% in papillary carcinoma (48/69 cases). Expression of GPC3 in follicular carcinoma was significantly higher than that of follicular adenoma ($p < 0.0019$). In contrast, GPC 3 was not expressed in 17 cases of anaplastic carcinoma. A high expression of GPC3 mRNA was confirmed in cancer lesions, which were strongly positive for immunohistochemical staining. In 69 cases of papillary carcinoma, GPC3 was expressed at an early stage, suggesting that GPC3 expression in thyroid cancer is an early event in developing papillary carcinoma. Further studies are required to determine biological functions and molecular mechanisms underlying the upregulation of GPC3 in thyroid cancer.

Yano, Y., H. Kamma, et al. (2007). "Growth suppression of thyroid cancer cells by adenylcyclase activator." Oncol Rep **18**(2): 441-5.

Thyroid stimulating hormone (TSH) is known to increase intracytoplasmic cyclic adenosine monophosphate (cAMP) and to regulate the growth of normal follicular cells. The aim of this study was to explore the role of the cAMP-mediated signaling pathway stimulated by TSH as a cell growth modulator in human thyroid cancer cells. One papillary thyroid cancer cell line, K1 cells and two anaplastic thyroid cancer cell lines, TTA1 and TTA2 cells were treated with forskolin, which directly activates adenyl cyclase to raise the level of intracellular cAMP. Forskolin suppressed thyroid cancer cell proliferations, especially in K1 cells, in a dose-dependent manner and induced growth arrest at the G0/G1 phase of the cell cycle. We also examined the expression of mitogen activated protein kinase (MAPK) after the forskolin treatment. Forskolin reduced the activation of growth factor induced MAPK activity. In conclusion, we demonstrated that forskolin was involved in G1 arrest and MAPK activation in K1 thyroid cancer cells. Our study suggests that the TSH signal mediated by cAMP acts as a negative regulator in thyroid cancer cells, unlike that in normal follicular cells.

Yates, C. M., A. Patel, et al. (2006). "Erythropoietin in thyroid cancer." J Endocrinol Invest **29**(4): 320-9.

Erythropoietin (Epo) and the epo-receptor (EpoR) have been implicated in tumor growth, invasion and metastasis. We previously demonstrated Epo and EpoR expression in a small group of archived papillary thyroid cancers (PTC), but were unable to examine functional integrity using formalin-fixed tissues. In the present study, we examined the in vitro expression, induction and function of Epo and EpoR in papillary (NPA), follicular (WRO) and anaplastic (ARO-81) thyroid cancer cells. We found that all three cell lines expressed Epo and EpoR mRNA and that the hypoxia-mimetic cobalt induced Epo expression in all cell lines. None of the growth factors we examined (thyrotropin, vascular endothelial growth factor, IGF-I, or human Epo) altered Epo or EpoR gene expression. Importantly, however, administration of Epo to NPA but not WRO cells resulted in significant alterations in the expression of several mitogenic genes including cyclooxygenase-2 (COX-2), beta-casein (CSN2), wild type p53-induced gene-1 (WIG1) and cathepsin D (CTSD). Epo treated ARO-81 cells only had an increase in CSN2 expression. We conclude that Epo and EpoR are expressed by thyroid cancers and that stimulation of the Epo/EpoR signal pathway results in changes that could impact on the clinical behavior of thyroid cancers.

Yaylim-Eraltan, I., N. Bozkurt, et al. (2008). "L-myc gene polymorphism and risk of thyroid cancer." Exp Oncol **30**(2): 117-20.

L-myc gene polymorphism is a representative genetic trait responsible for an individual's susceptibility to several cancers. However, there have been no reports concerning the association between thyroid cancer and L-myc gene polymorphism. AIM: To analyze the distribution of L-myc gene polymorphism in Turkish patients with thyroid disorders and thyroid cancers. METHODS: We used a molecular genotyping method, polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP). We studied 138 patients of whom 47 had multinodular goiter, 13 had follicular cancer and 69 had papillar cancer, in comparison with control group of 109 healthy individuals. RESULTS: No significant difference in the distribution of

genotypes was observed between thyroid patients and controls. Carrying SS or LS genotype revealed a 1.96-fold (95% CI 0.573-6.706) risk for the occurrence of follicular cancer when compared with controls, and 3.11-fold (95% CI 0.952-10.216), when compared with multinodular goiter patients (p=0.04). CONCLUSION: We suggest that L-myc genotype profiling together with other susceptibility factors, may be useful in the screening for thyroid nodular malignancy.

Ying, H., M. C. Willingham, et al. (2008). "The steroid receptor coactivator-3 is a tumor promoter in a mouse model of thyroid cancer." Oncogene **27**(6): 823-30.

The molecular genetic events underlying thyroid carcinogenesis are not well understood. Mice harboring a dominant-negative mutant thyroid hormone receptor-beta (TRbeta(PV/PV) mice) spontaneously develop follicular thyroid carcinoma similar to human cancer. The present study aimed to elucidate the role of the steroid receptor coactivator-3 (SRC-3) in thyroid carcinogenesis in vivo by using the offspring from the cross of TRbeta(PV/PV) and SRC-3(-/-) mice. TRbeta(PV/PV) mice deficient in SRC-3 (TRbeta(PV/PV)SRC-3(-/-) mice) had significantly increased survival, decreased thyroid tumor growth, delayed tumor progression and lower incidence of distant metastasis as compared with TRbeta(PV/PV) mice with SRC-3 (TRbeta(PV/PV)SRC-3(+/+) mice). Further, in vivo and in vitro analyses of multiple signaling pathways indicated that SRC-3 deficiency could lead to (1) inhibition of cell cycle progression at the G(1)/S transition via controlling the expression of cell cycle regulators, such as E2F1; (2) induction of apoptosis by controlling the expression of the Bcl-2 and caspase-3 genes and (3) suppression of neovascularization and metastasis, at least in part, through modulating the vascular endothelial growth factor gene expression. Taken together, SRC-3 could play important roles through regulating multiple target genes and signaling pathways during thyroid carcinogenesis, understanding of which should direct future therapeutic options for thyroid cancer.

Yu, W., I. Imoto, et al. (2007). "A novel amplification target, DUSP26, promotes anaplastic thyroid cancer cell growth by inhibiting p38 MAPK activity." Oncogene **26**(8): 1178-87.

Anaplastic thyroid cancer (ATC) is one of the most lethal of all human tumors, but cytogenetic information concerning ATC is extremely limited. Using our in-house array-based comparative genomic hybridization and 14 ATC cell lines with further fluorescence in situ hybridization analysis, we demonstrated amplification of the DUSP26 gene, known by another report as MAP kinase phosphatase-8. DUSP26 was overexpressed in ATC cell lines and primary ATC tumor samples. When overexpressed, either exogenously or endogenously, DUSP26 promoted growth of the ATC cells. DUSP26 encodes a protein containing a dual-specificity phosphatase domain that can dephosphorylate itself. DUSP26 effectively dephosphorylates p38 and has a little effect on extracellular signal-regulated kinase in ATC cells. DUSP26 protein formed a physical complex with p38, and promoted survival of ATC cells by inhibiting p38-mediated apoptosis. Our findings suggest that DUSP26 may act as an oncogene in ATC, and might be a useful diagnostic marker and therapeutic target of this disease.

Zhang, H. Y., H. Q. Wang, et al. (2008). "Regulation of tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis by DJ-1 in thyroid cancer cells." Endocr Relat Cancer **15**(2): 535-44.

DJ-1, a cancer-associated protein protects cells from multiple toxic stresses. The expression of DJ-1 and its influence on thyroid cancer cell death has not been investigated so far. We analyzed DJ-1 expression in human thyroid carcinoma cell lines and the effect of DJ-1 on tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis. DJ-1 was expressed in human thyroid carcinoma cell lines; small interfering RNA-mediated downregulation of its levels significantly sensitized thyroid carcinoma cells to TRAIL-induced apoptosis, whereas the forced exogenous expression of DJ-1 significantly suppressed cell death induced by TRAIL. We also report here that TRAIL-induced thyroid cancer cell apoptosis is mediated by oxidative stress and that DJ-1, a potent nutritional antioxidant, protects cancer cells from apoptosis at least in part by impeding the elevation of reactive oxygen species levels induced by TRAIL and impairing caspase-8 activation. Subsequently, we investigated DJ-1 expression in 52 normal and 74 primary thyroid carcinomas from patients of China Medical University. The protein was not detectable in the 52 specimens of normal thyroid, while 70 out of 74 analyzed carcinomas (33 out of 33 follicular, 17 out of 19 papillary, 12 out of 13 medullar, and 8 out of 9 anaplastic) were clearly positive for DJ-1 expression. Our data demonstrated that DJ-1 is specifically expressed in thyroid carcinomas and not in the normal thyroid tissue. In addition, the protein modulates the response to TRAIL-mediated apoptosis in human neoplastic thyroid cells, at least partially through its antioxidant property.

Zhang, Y., G. L. Guo, et al. (2008). "Do Polybrominated Diphenyl Ethers (PBDEs) Increase

the Risk of Thyroid Cancer?" Biosci Hypotheses **1**(4): 195-199.

An increased incidence of thyroid cancer has been reported in many parts of the world including the United States during the past several decades. Recently emerging evidence has demonstrated that polyhalogenated aromatic hydrocarbons (PHAHs), particularly polybrominated diphenyl ethers (PBDEs), alter thyroid hormone homeostasis and cause thyroid dysfunction. However, few studies have been conducted to test whether exposure to PBDEs and other PHAHs increases the risk of thyroid cancer. Here, we hypothesize that elevated exposure to PHAHs, particularly PBDEs, increases the risk of thyroid cancer and may explain part of the increase in incidence of thyroid cancer during the past several decades. In addition, genetic and epigenetic variations in metabolic pathway genes may alter the expression and function of metabolic enzymes which are involved in the metabolism of endogenous thyroid hormones and the detoxification of PBDEs and other PHAHs. Such variation may result in different individual susceptibilities to PBDEs and other PHAHs and the subsequent development of thyroid cancer. The investigation of this hypothesis will lead to an improved understanding of the role of PBDEs and other PHAHs in thyroid tumorigenesis and may provide a real means to prevent this deadly disease.

Zhao, H., J. Zhang, et al. (2008). "Reduced expression of N-Myc downstream-regulated gene 2 in human thyroid cancer." BMC Cancer **8**: 303.

BACKGROUND: NDRG2 (N-Myc downstream-regulated gene 2) was initially cloned in our laboratory. Previous results have shown that NDRG2 expressed differentially in normal and cancer tissues. Specifically, NDRG2 mRNA was down-regulated or undetectable in several human cancers, and over-expression of NDRG2 inhibited the proliferation of cancer cells. NDRG2 also exerts important functions in cell differentiation and tumor suppression. However, it remains unclear whether NDRG2 participates in carcinogenesis of the thyroid. METHODS: In this study, we investigated the expression profile of human NDRG2 in thyroid adenomas and carcinomas, by examining tissues from individuals with thyroid adenomas (n = 40) and carcinomas (n = 35), along with corresponding normal tissues. Immunohistochemistry, quantitative RT-PCR and western blot methods were utilized to determine both the protein and mRNA expression status of Ndrg2 and c-Myc. RESULTS: The immunostaining analysis revealed a decrease of Ndrg2 expression in thyroid carcinomas. When comparing adenomas or carcinomas with adjacent normal tissue from the same individual, the mRNA expression level of NDRG2 was significantly decreased in thyroid carcinoma tissues, while there was little difference in adenoma tissues. This differential expression was confirmed at the protein level by western blotting. However, there were no significant correlations of NDRG2 expression with gender, age, different histotypes of thyroid cancers or distant metastases. CONCLUSION: Our data indicates that NDRG2 may participate in thyroid carcinogenesis. This finding provides novel insight into the important role of NDRG2 in the development of thyroid carcinomas. Future studies are needed to address whether the down-regulation of NDRG2 is a cause or a consequence of the progression from a normal thyroid to a carcinoma.

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