Cancer and Radiation Literatures

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Abstract: 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. This is literature collection on cancer and radiation.
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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.

Literatures

Abbott, D. W., M. L. Freeman, et al. (1998). "Double-strand break repair deficiency and radiation sensitivity in BRCA2 mutant cancer cells." J Natl Cancer Inst **90**(13): 978-85.

BACKGROUND: The protein product of the BRCA2 gene mediates repair of double-strand breaks in DNA. Because a number of cancer therapies exert cytotoxic effects via the initiation of double-strand breaks, cancers comprised of cells carrying BRCA2 gene mutations may be more amenable to treatment with agents that cause such breaks. METHODS: We identified a human pancreatic adenocarcinoma cell line lacking one copy of the BRCA2 gene and containing a mutation (6174delT) in the remaining copy. In vitro and in vivo experiments were conducted with this cell line and with other carcinoma cell lines matched for similar genetic mutations, similar differentiation status, and/or similar carcinoma type to examine double-strand break repair, sensitivity to drugs that induce double-strand breaks, and radiation sensitivity. RESULTS: BRCA2-defective cells were unable to repair the double-strand DNA breaks induced by ionizing radiation. These cells were also markedly sensitive to mitoxantrone, amsacrine, and etoposide (drugs that induce double-strand breaks) (two-sided P = .002) and to ionizing radiation (two-sided P = .001). Introduction of antisense BRCA2 deoxyribonucleotides into cells possessing normal BRCA2 function led to increased sensitivity to mitoxantrone (two-sided P = .008). Tumors formed by injection of BRCA2-defective cells into nude mice were highly sensitive (>90% tumor size reduction, two-sided P = .002) to both ionizing radiation and mitoxantrone when compared with tumors exhibiting normal BRCA2 function. Histologic analysis of irradiated BRCA2-defective tumors showed a large degree of necrosis compared with that observed for control tumors possessing normal BRCA2 function. CONCLUSION: BRCA2-defective cancer cells are highly sensitive to agents that cause double-strand breaks in DNA.

Abbott, D. W., M. E. Thompson, et al. (1999). "BRCA1 expression restores radiation resistance in BRCA1-defective cancer cells through enhancement of transcription-coupled DNA repair." J Biol Chem **274**(26): 18808-12.

The breast cancer predisposition genes, BRCA1 and BRCA2, are responsible for the vast majority of hereditary breast cancer. Although BRCA2 functions to help the cell repair double-stranded DNA breaks, the function of BRCA1 remains enigmatic. Here, we develop a human genetic system to study the role of BRCA1 in oxidative DNA damage. We show that human cancer cells containing mutated BRCA1 are hypersensitive to ionizing radiation. This hypersensitivity can be reversed by the expression of forms of BRCA1 that are not growth suppressing. Reversal of hypersensitivity requires the ring finger of BRCA1, its transactivation domain, and its BRCT domain. Lastly, we show that unlike BRCA2, BRCA1 does not function in the repair of

double-stranded DNA breaks. Instead, it functions in transcription-coupled DNA repair (TCR). TCR ability correlated with radioresistance as cells containing BRCA1 showed both increased TCR and radioresistance, whereas cells without BRCA1 showed decreased TCR and radiosensitivity. These findings give physiologic significance to the interaction of BRCA1 with the basal transcription machinery.

Abela, R. A., J. Qian, et al. (2008). "Radiation improves gene delivery by a novel transferrin-lipoplex nanoparticle selectively in cancer cells." Cancer Gene Ther **15**(8): 496-507.

Selective gene transfer to tumor is critical in cancer gene therapy. We previously used ionizing radiation to improve adenovirus uptake in intrahepatic tumors but liver cytotoxicity associated with the viral administration still occurred. Here, we explore the potential of radiation for improving gene delivery by a virus-mimicking nanoparticle, transferrin (Tf)-cationic liposome-DNA complex (Tf-lipoplex). Transduction by Tf-lipoplex was highly efficient in various cell lines and further increased by radiation in a dose- and time-dependent manner. This radiation induction, which was associated with an increase in Tf-lipoplex uptake (3- to 4-folds in hepatocytes WB and lung cancer cells, LLC1), was absent when a Tf-deficient complex was used or abolished by the presence of free Tf, suggesting that Tf receptor (TfR) interaction is required for radiation induction. Radiation (10-20 Gy) markedly induced transgene (LacZ) expression in LLC1 xenografts (3.5- to 7.4-folds), correlating with increased plasmid content and TfR expression in irradiated tumors. Moreover, Tf-lipoplex-mediated gene expression was not observed in the liver or other normal tissues regardless of radiation treatment. We conclude that radiation improves Tf-lipoplex gene delivery selectively to tumor cells both in vitro and in vivo. Our findings may provide insight in developing ligand-specific lipoplex for molecularly targeted cancer gene therapy.

Adusumilli, P. S., B. M. Stiles, et al. (2005). "Radiation therapy potentiates effective oncolytic viral therapy in the treatment of lung cancer." Ann Thorac Surg **80**(2): 409-16; discussion 416-7.

BACKGROUND: Replication-competent oncolytic herpes simplex viruses with deletion of the gamma(1)34.5 gene preferentially replicate in and kill malignant cells. The gamma(1)34.5 gene codes for ICP 34.5, a protein that enhances viral replication, and is homologous to growth arrest and DNA damage protein 34 (GADD34), a radiation-inducible DNA repair gene. We hypothesized that radiation therapy may potentiate efficacy of oncolytic viral therapy by upregulating GADD34 and promoting viral replication. METHODS: The A549 and H1299 lung cancer cell lines were infected with NV1066, an oncolytic herpes simplex virus, at multiplicities of infection (number of viral particles per tumor cell) of 0.1 to 0.5 in vitro with radiation (2 to 10 Gy) or without radiation. Viral replication was determined by plaque assay, cell-to-cell spread was determined by flow cytometry, cell kill was determined by lactate dehydrogenase assay, and GADD34 induction was determined by real-time reverse transcription-polymerase chain reaction and Western blot method. Evidence of synergistic cytotoxicity dependence with GADD34 induction is further confirmed by small inhibitory RNA inhibition of GADD34 expression. RESULTS: Using both the isobologram method and combination index method of Chou and Talalay, significant synergism was demonstrated between radiation therapy and NV1066 both in vitro and in vivo. As a result of such synergism, a dose reduction for each agent (2- to 6,000-fold) can be accomplished for a wide range of therapeutic effect levels without sacrificing tumor cell kill. This effect is correlated with increased GADD34 expression and inhibited by transfection of small inhibitory RNA directed against GADD34. CONCLUSIONS: These data provide the cellular basis for the clinical investigation of combined use of radiation therapy with oncolytic herpes simplex virus therapy in the treatment of lung cancer to achieve synergistic efficacy while minimizing dosage and toxicity.

Ahmed, M. M., D. Chendil, et al. (2001). "Early growth response-1 gene: potential radiation response gene marker in prostate cancer." Am J Clin Oncol **24**(5): 500-5.

This study was undertaken to determine whether the transcription factor EGR-1 expression: (1) in the primary tumor, correlates with radiation response in terms of complete local tumor control with no evidence of disease or recurrence and no evidence of metastasis; (2) in the postirradiated biopsies correlates with residual tumor; and (3) correlates with the expression of Egr-1 target genes such as TP53, pRB, and Bax. The authors analyzed: (1) 25 pretreated surgically resected paraffin-embedded primary adenocarcinomas of the prostate for the presence of EGR-1 expression and mutation, and correlated this with clinical endpoints such as serum prostate-specific antigen levels and current clinical status; (2) 27 postirradiated biopsies of prostate for the presence of EGR-1 expression, and correlated these findings to the residual tumor status; and (3) 12 prospective prostate tumor specimens for EGR-1 expression and its target genes. EGR-1 expression was determined by immunohistochemistry and mutations were screened in two regions of the Egr-1 gene (trinucleotide AGC

repeats in transactivation domain [TD] and poly A tract in 3'UTR) by polymerase chain reaction-single strand conformational polymorphism analysis. Of 25 patients, 18 patients showed expression of EGR-1. EGR-1 overexpression correlated with treatment failure. No correlation with EGR-1 overexpression and its target genes was found, which may indirectly suggest that overexpressed EGR-1 may lack transactivation function. In summary, EGR-1 overexpression in the mutant form may provide an indication of clinical failure (local recurrence or metastasis).

Alcock, R. A., S. Dey, et al. (2002). "Farnesyltransferase inhibitor (L-744,832) restores TGF-beta type II receptor expression and enhances radiation sensitivity in K-ras mutant pancreatic cancer cell line MIA PaCa-2." Oncogene **21**(51): 7883-90.

Activated ras is known to dysregulate TGF-beta signaling by altering the expression of TGF-beta type II receptor (RII). It is well documented that tumor cells harboring mutant ras are more resistant to radiation than cells with wild-type ras. In this study, we hypothesized that the use of farnesyltransferase inhibitor (FTI, L-744,832) may directly restore TGF-beta signaling through RII expression via ras dependent or independent pathway leading to induction of radiation sensitivity. Two pancreatic cancer cell lines, BxPC-3 and MIA PaCa-2 were used in this study. FTI inhibited farnesylation of Ras protein more significantly in MIA PaCa-2 than BxPC-3 cells. In contrast, MIA PaCa-2 cells were resistant to radiation when compared to BxPC-3 cells. BxPC-3 cells were more resistant to FTI than MIA PaCa-2 cells. In combination treatment, no significant radiosensitizing effect of FTI was observed in BxPC-3 cells at 5 or 10 microM. However, in MIA PaCa-2 cells, a significant radiosensitizing effect was observed at both 5 and 10 microM concentrations (P>0.004). The TGF-beta effector gene p21(waf1/cip1) was elevated in combination treatment in MIA PaCa-2 but not in BxPC-3 cells. In MIA PaCa-2 cells, FTI induced TGF-beta responsive promoter activity as assessed by 3TP-luciferase activity. A further induction of luciferase activity was observed in MIA PaCa-2 cells treated with radiation and FTI. Induction of TGF-beta signaling by FTI was mediated through restoration of the RII expression, as demonstrated by RT-PCR analysis. In addition, re-expression of RII by FTI was associated with a decrease in DNA methyltransferase 1 (DNMT1) levels. Thus, these findings suggest that the L-744,832 treatment restores the RII expression through inhibition of DNMT1 levels causing induction of TGF-beta signaling by radiation and this forms a novel molecular mechanism of radiosensitization by FTI.

Amundson, S. A., K. T. Do, et al. (2008). "Integrating global gene expression and radiation survival parameters across the 60 cell lines of the National Cancer Institute Anticancer Drug Screen." Cancer Res **68**(2): 415-24.

The 60 cell lines of the National Cancer Institute Anticancer Drug Screen (NCI-60) constitute the most extensively characterized in vitro cancer cell model. They have been tested for sensitivity to more than 100,000 potential chemotherapy agents and have been profiled extensively at the DNA, RNA, protein, functional, and pharmacologic levels. We have used the NCI-60 cell lines and three additional lines to develop a database of responses of cancer cells to ionizing radiation. We compared clonogenic survival, apoptosis, and gene expression response by microarray. Although several studies have profiled relative basal gene expression in the NCI-60, this is the first comparison of large-scale gene expression changes in response to genotoxic stress. Twenty-two genes were differentially regulated in cells with low survival after 2-Gy gamma-rays; 14 genes identified lines more sensitive to 8 Gy. Unlike reported basal gene expression patterns, changes in expression in response to radiation showed little tissue-of-origin effect, except for differentiating the lymphoblastoid cell lines from other cell types. Basal expression patterns, however, discriminated well between radiosensitive and more resistant lines, possibly being more informative than radiation response signatures. The most striking patterns in the radiation data were a set of genes up-regulated preferentially in the p53 wild-type lines and a set of cell cycle regulatory genes down-regulated across the entire NCI-60 panel. The response of those genes to gamma-rays seems to be unaffected by the myriad of genetic differences across this diverse cell set; it represents the most penetrant gene expression response to ionizing radiation yet observed.

Ananthaswamy, H. N. (1986). "Relationship between expression of tumor-specific transplantation antigens and neoplastic transformation in an ultraviolet radiation-induced murine skin cancer." Cancer Res **46**(12 Pt 1): 6322-6.

Ultraviolet radiation-induced murine skin cancers often express highly immunogenic tumor-specific transplantation antigens (TSTA). The relationship between expression of TSTA and neoplastic transformation is not clear. I have used DNA transfection techniques to determine whether expression of TSTA and the transformed phenotype are associated at the genetic level. C3H mouse embryo

fibroblast 10T1/2 clone 8 cells were transfected with high-molecular-weight genomic DNA from a highly antigenic ultraviolet radiation-induced 2240 tumor cell line. A cotransfection protocol using pSV2-neo DNA, which confers resistance to the antibiotic G418, was used to select cells that had taken up foreign DNA. Morphologically transformed, G418-resistant colonies were isolated and tested for expression of 2240 tumor-specific antigens by means of a cytotoxic T-lymphocyte assay. None of the 12 morphologically transformed colonies tested expressed 2240 tumor-specific antigens on their cell surface as revealed by their inability to be killed by 2240 tumor-specific cytotoxic T-lymphocytes. In addition, the morphologically transformed cells did not inhibit the killing of 51Cr-labeled 2240 cells by 2240 tumor-specific cytotoxic T-lymphocytes in a cold-target inhibition assay. Cell surface expression of Class I major histocompatibility antigens was not significantly altered in 2240 DNA transformants. These results demonstrate that, in ultraviolet radiation-induced murine skin tumors, there is not coordinate expression of TSTA and the transformed phenotype, even though most ultraviolet radiation-induced skin tumors exhibit both characteristics. This finding suggests that the two phenotypes are controlled by separate genes.

Arnold, S. F., E. Tims, et al. (1999). "Regulation of transforming growth factor beta1 by radiation in cells of two human breast cancer cell lines." Radiat Res **152**(5): 487-92.

We have investigated the mechanisms by which radiation inhibits proliferation of human breast cancer cells in culture. Radiation, within the dose range used for treatment of humans, decreased the rate of proliferation of estrogen-independent MDA-MB-231 cells more effectively than it did that of estrogen-dependent MCF-7 cells. The rate of proliferation of MDA-MB-231 cells was also inhibited to a greater extent than that of MCF-7 cells by purified TGFB1. Using an ELISA specific for activated TGFB1, we found that conditioned medium from irradiated MDA-MB-231 or MCF-7 cells contained twofold more TGFB1 than that from nonirradiated cells. Conditioned medium from irradiated breast cancer cells, but not from nonirradiated cells, inhibited the growth of untreated MDA-MB-231 cells. The inhibitory activity was blocked by an anti-TGFB1 neutralizing antibody. An approximately twofold increase in the TGFB1 mRNA in irradiated cells compared to controls was found using semiquantitative reverse-transcriptase PCR. Last, the mRNA for insulin-like growth factor binding protein 3, a reported target of the cell inhibitory activity of TGFB1, was increased threefold upon irradiation. Our results demonstrate that the TGFB1 is increased after irradiation and that the activation of the TGFB1 signaling pathway may sensitize cells to the effects of radiation.

Balcer-Kubiczek, E. K., S. J. Meltzer, et al. (1997). "Csa-19, a radiation-responsive human gene, identified by an unbiased two-gel cDNA library screening method in human cancer cells." Oncogene **14**(25): 3051-7.

A novel polymerase chain reaction (PCR)-based method was used to identify candidate genes whose expression is altered in cancer cells by ionizing radiation. Transcriptional induction of randomly selected genes in control versus irradiated human HL60 cells was compared. Among several complementary DNA (cDNA) clones recovered by this approach, one cDNA clone (CL68-5) was downregulated in X-irradiated HL60 cells but unaffected by 12-O-tetradecanoyl phorbol-13-acetate, forskolin, or cyclosporin-A. DNA sequencing of the CL68-5 cDNA revealed 100% nucleotide sequence homology to the reported human Csa-19 gene. Northern blot analysis of RNA from control and irradiated cells revealed the expression of a single 0.7-kilobase (kb) messenger RNA (mRNA) transcript. This 0.7-kb Csa-19 mRNA transcript was also expressed in a variety of human adult and corresponding fetal normal tissues. Moreover, when the effect of X- or fission neutron-irradiation on Csa-19 mRNA was compared in cultured human cells differing in p53 gene status (p53-/- versus p53+/+), downregulation of Csa-19 by X-rays or fission neutrons was similar in p53-wild type and p53-null cell lines. Our results provide the first known example of a radiation-responsive gene in human cancer cells whose expression is not associated with p53, adenylate cyclase or protein kinase C.

Barcellos-Hoff, M. H. (2008). "Cancer as an emergent phenomenon in systems radiation biology." Radiat Environ Biophys **47**(1): 33-8.

Radiation-induced DNA damage elicits dramatic cell signaling transitions, some of which are directed towards deciding the fate of that particular cell, while others lead to signaling to other cells. Each irradiated cell type and tissue has a characteristic pattern of radiation-induced gene expression, distinct from that of the unirradiated tissue and different from that of other irradiated tissues. It is the sum of such events, highly modulated by genotype that sometimes leads to cancer. The challenge is to determine as to which of these phenomena have persistent effect that should be incorporated into models of how radiation increases the risk of developing cancer. The application of systems biology to radiation effects

may help to identify which biological responses are significant players in radiation carcinogenesis. In contrast to the radiation biology paradigm that focuses on genomic changes, systems biology seeks to integrate responses at multiple scales (e.g. molecular, cellular, organ, and organism). A key property of a system is that some phenomenon emerges as a property of the system rather than of the parts. Here, the idea that cancer in an organism can be considered as an emergent phenomenon of a perturbed system is discussed. Given the current research goal to determine the consequences of high and low radiation exposures, broadening the scope of radiation studies to include systems biology concepts should benefit risk modeling of radiation carcinogenesis.

Bar-Sela, G., K. M. Jacobs, et al. (2007). "Histone deacetylase inhibitor and demethylating agent chromatin compaction and the radiation response by cancer cells." Cancer J **13**(1): 65-9.

It now appears that epigenetics plays a central role in transformation, both in vitro and in vivo. The expression and regulation of DNA methylation and the subsequent chromatin structure are significantly altered in tumor cells, suggesting a direct role in the process of in vivo cellular transformation. If epigenetics and posttranslational modifications of histones play a role in transformation, then it seems logical that the genes regulating chromatin compaction may also be molecular targets and markers in profiling tumor cell resistance. Local remodeling of chromatin is a key step in the regulation of gene expression, and altering the expression of these genes might also favorably alter how tumor cells respond to anticancer agents. Several new agents that alter chromatin compaction, either methyltransferase or histone deacetylases inhibitors, are progressing through clinical trials and have shown promising preclinical interactions when combined with radiation. In this review, we discuss the potential for histone deacetylases inhibitors as radiosensitizing agents.

Bennett, L. M. (1999). "Breast cancer: genetic predisposition and exposure to radiation." Mol Carcinog **26**(3): 143-9.

The identification of breast cancer susceptibility genes, such as BRCA1, BRCA2, ATM, and p53, has been accompanied by the examination of the effects of radiation in combination with genetic mutations at these loci. Women at high risk for developing breast cancer may respond differently than the general population to low- and high-dose radiation exposures associated with screening and treatment. Epidemiologic studies are being performed to investigate the effects of radiation on subsequent breast cancer development in genetically predisposed individuals. Mouse strains with specific genetic modifications are being created to study the consequence of both inherited mutations and radiation on mammary gland carcinogenesis. Finally, studies investigating DNA damage-response pathways after radiation exposure are being performed. Recent work on the effects of several known or suspected breast cancer susceptibility genes, alone or in combination with radiation, is presented here, and directions for future research are considered.

Bhatti, P., J. P. Struewing, et al. (2008). "Polymorphisms in DNA repair genes, ionizing radiation exposure and risk of breast cancer in U.S. Radiologic technologists." Int J Cancer **122**(1): 177-82.

High-dose ionizing radiation exposure to the breast and rare autosomal dominant genes have been linked with increased breast cancer risk, but the role of low-to-moderate doses from protracted radiation exposure in breast cancer risk and its potential modification by polymorphisms in DNA repair genes has not been previously investigated among large numbers of radiation-exposed women with detailed exposure data. Using carefully reconstructed estimates of cumulative breast doses from occupational and personal diagnostic ionizing radiation, we investigated the potential modification of radiation-related breast cancer risk by 55 candidate single nucleotide polymorphisms in 17 genes involved in base excision or DNA double-strand break repair among 859 cases and 1083 controls from the United States Radiologic Technologists (USRT) cohort. In multivariable analyses, WRN V114I (rs2230009) significantly modified the association between cumulative occupational breast dose and risk of breast cancer (adjusted for personal diagnostic exposure) ($p = 0.04$) and BRCA1 D652N (rs4986850), PRKDC IVS15 + 6C > T (rs1231202), PRKDC IVS34 + 39T > C (rs8178097) and PRKDC IVS31 - 634C > A (rs10109984) significantly altered the personal diagnostic radiation exposure-response relationship (adjusted for occupational dose) ($p < or = 0.05$). None of the remaining 50 SNPs significantly modified breast cancer radiation dose-response relationships. The USRT genetic study provided a unique opportunity to examine the joint effects of common genetic variation and ionizing radiation exposure on breast cancer risk using detailed occupational and personal diagnostic exposure data. The suggestive evidence found for modification of radiation-related breast cancer risk for 5 of the 55 SNPs evaluated requires confirmation in larger studies of women with quantified radiation breast doses in the low-to-moderate range.

Billecke, C. A., M. E. Ljungman, et al. (2002). "Lack of functional pRb results in attenuated recovery of mRNA synthesis and increased apoptosis following UV radiation in human breast cancer cells." Oncogene **21**(29): 4481-9.

Lack of functional pRb results in attenuated recovery of mRNA synthesis and increased apoptosis following UV radiation in human breast cancer cells. We have previously demonstrated that a human breast cancer cell line, MDA-MB-468, which lacks the retinoblastoma protein (pRb), is particularly sensitive to low doses of ultraviolet (UV) radiation. These cells are 15-20-fold more sensitive to UV radiation than cells with wild-type pRb. In order to understand the mechanisms of the high apoptotic response of MDA-MB-468 cells to UV radiation, we examined the effects of UV on these cells with regards to both membrane-mediated events and DNA damage. We found that MDA-MB-468 cells were resistant to all ligand-induced death receptor signaling. In addition, although UV activated caspase 8 in MDA-MB-468 cells, a peptide inhibitor of caspase 8 failed to inhibit UV-induced apoptosis. We then tested the possibility that nuclear events mediated the enhanced sensitivity to UV-induced apoptosis in these cells. Unlike UV-resistant cells, MDA-MB-468 cells were unable to recover mRNA synthesis after 5 J/m2 UVC. We also found that the pRb-null DU-145 cells similarly had attenuated recovery of mRNA synthesis after UV radiation. In UV-resistant cells with wild-type pRb, the inactivation of pRb with HPV-16 E7 resulted in significant inhibition in their ability to recover mRNA synthesis and increased levels of apoptosis following UV radiation. Furthermore, pRb-null cells were deficient in repair of UV radiation-induced DNA damage. These data suggest that the sensitivity of MDA-MB-468 cells to UV radiation is due to defects in repair of DNA damage and recovery of mRNA synthesis rather than to membrane death receptor pathways. Inactivation of pRb may contribute to an increased sensitivity to UV radiation by attenuating repair of DNA lesions and recovery of mRNA synthesis following UV radiation.

Birrell, G. W. and J. R. Ramsay (1995). "Induction of p53 protein by gamma radiation in lymphocyte lines from breast cancer and ataxia telangiectasia patients." Br J Cancer **72**(5): 1096-101.

Exposure of human cells to gamma-radiation causes levels of the tumour-suppressor nuclear protein p53 to increase in temporal association with the decrease in replicative DNA synthesis. Cells from patients with the radiosensitive and cancer-prone disease ataxia telangiectasia (AT) exhibit radioresistant DNA synthesis and show a reduced or delayed gamma-radiation-induced increase in p53 protein levels. We have used Western immunoblotting with semiquantitative densitometry to examine the gamma-radiation-induced levels of p53 protein in 57 lymphoblastoid cell lines (LCLs) derived from patients with AT, carriers of the AT gene, breast cancer patients and normal donors. We confirm the previously reported reduced induction in AT homozygote LCLs (n = 8) compared with normal donor LCLs (n = 17, P = 0.01). We report that AT heterozygote LCLs (n = 5) also have a significantly reduced p53 induction when compared with LCLs from normal donors (n = 17, P = 0.02). The response of breast cancer patient cells was not significantly different from normal donor cells but 18% (5/27) had a p53 response in the AT heterozygote range (95% confidence interval) compared with only 6% (1/17) of the normal donor cells. We found no significant correlation between p53 induction and cellular radiosensitivity in LCLs from breast cancer patients. These methods may be useful in identifying individuals at greater risk of the DNA-damaging effects of ionising radiation.

Broeks, A., L. M. Braaf, et al. (2007). "Identification of women with an increased risk of developing radiation-induced breast cancer: a case only study." Breast Cancer Res **9**(2): R26.

INTRODUCTION: Radiation exposure at a young age is one of the strongest risk factors for breast cancer. Germline mutations in genes involved in the DNA-damage repair pathway (DDRP) may render women more susceptible to radiation-induced breast cancer. METHODS: We evaluated the contribution of germline mutations in the DDRP genes BRCA1, BRCA2, CHEK2 and ATM to the risk of radiation-induced contralateral breast cancer (CBC). The germline mutation frequency was assessed, in a case-only study, in women who developed a CBC after they had a first breast cancer diagnosed before the age of 50 years, and who were (n = 169) or were not (n = 78) treated with radiotherapy for their first breast tumour. RESULTS: We identified 27 BRCA1, 5 BRCA2, 15 CHEK2 and 4 truncating ATM germline mutation carriers among all CBC patients tested (21%). The mutation frequency was 24.3% among CBC patients with a history of radiotherapy, and 12.8% among patients not irradiated for the first breast tumour (odds ratio 2.18 (95% confidence interval 1.03 to 4.62); p = 0.043). The association between DDRP germline mutation carriers and risk of radiation-induced CBC seemed to be strongest in women who developed their second primary breast tumour at least 5 years after radiotherapy. Those patients had an odds ratio of 2.51 (95% confidence interval 1.03 to 6.10; p = 0.049) of developing radiation-induced breast

cancer, in comparison with non-carriers. CONCLUSION: This study shows that carriers of germline mutations in a DDRP gene have an increased risk of developing (contralateral) breast cancer after radiotherapy; that is, over and above the risk associated with their carrier status. The increased risk indicates that knowledge of germline status of these DDRP genes at the time of breast cancer diagnosis may have important implications for the choice of treatment.

Brown, E. T. and J. T. Holt (2009). "Rad51 overexpression rescues radiation resistance in BRCA2-defective cancer cells." Mol Carcinog **48**(2): 105-9.

Breast cancers with BRCA2 mutations exhibit DNA repair defects and are particularly sensitive to radiation. BRCA2 interacts with Rad51 in a complex manner involving internal BRC and C-terminal TR2 domains which play a key role in homologous recombination. BRCA2 expression also modulates Rad51 protein levels such that Rad51 protein is relatively decreased in BRCA2-defective cancer cells. This is mediated in part through BRCA2's capacity to protect Rad51 from caspase-3 proteolytic degradation. In order to distinguish between functional and expression related roles for BRCA2 we studied the results of Rad51 overexpression in mouse and human cells with inactivating BRCA2 mutations. The results show that overexpression of wild-type Rad51 partially rescues BRCA2 deficiency but that overexpression of a caspase-3 resistant Rad51 completely complements the BRCA2 defect in radiation responsiveness. These results indicate that Rad51 can compensate for some aspects of a BRCA2 gene defect and suggest that Rad51 expression levels may be an important modifier of the BRCA2 defective genotype.

Buchholz, T. A. and X. Wu (2001). "Radiation-induced chromatid breaks as a predictor of breast cancer risk." Int J Radiat Oncol Biol Phys **49**(2): 533-7.

PURPOSE: In in vivo models, radiation-induced genomic instability correlates with the risk of breast cancer development. In addition, homozygous mutations in tumor suppressor genes associated with breast cancer development adversely affects the processing and repair of radiation-induced DNA damage. We performed a case-control study to determine whether an assay measuring radiation-induced chromatid breaks correlated with the risk of having bilateral breast cancer. METHODS AND MATERIALS: Patients were prospectively studied on an institutional review board-approved protocol. We included only women with bilateral breast cancer as cases to obtain patients with a presumed genetic susceptibility for breast cancer. Controls were healthy women without a previous cancer history. A mutagen sensitivity assay using gamma-radiation was performed on lymphocytes obtained from 26 cases and 18 controls. One milliliter of whole blood was cultured with 9 mL of blood medium for 91 h and then treated with 125 cGy using a Cs-137 irradiator. Following an additional 4 h in culture, cells were treated with Colcemid for 1 h to arrest cells in metaphase. The number of chromatid breaks per cell was counted using a minimum of 50 metaphase spreads for each sample. RESULTS: Cases had a statistically higher number of gamma-radiation-induced chromatid breaks per cell than controls, with mean values of 0.61 +/- 0.24 vs. 0.45 +/- 0.14, respectively ($p = 0.034$, Wilcoxon rank sum test). Using the 75th percentile value in the control group as a definition of radiation sensitivity, the radiation-sensitive individuals had a 2.83-fold increased odds ratio for breast cancer development compared with individuals who were not radiation sensitive (95% confidence intervals of 0.83 and 9.67). CONCLUSIONS: These preliminary data suggest that sensitivity to radiation-induced chromatid breaks in lymphocytes correlates with the risk of bilateral breast cancer. Although the differences between cases and controls were statistically significant, the small sample size necessitates that this finding be validated in a larger study. More data are also needed to determine whether this sensitivity is limited to breast cancer patients with a genetic susceptibility for the disease or also applies to the general breast cancer population.

Cao, J., Y. Liu, et al. (2002). "Chromosomal aberrations, DNA strand breaks and gene mutations in nasopharyngeal cancer patients undergoing radiation therapy." Mutat Res **504**(1-2): 85-90.

Nasopharyngeal cancer (NPC) is a common disease in the south part of China, and its incidence is increasing in the southwest of China in recent years. Radiation therapy is the main therapeutic method for NPC in China. In this study, genetic changes were assessed in randomly selected nine NPC patients receiving radiation therapy by different genotoxical screening methods, the cytokinesis-block micronucleus test (CB-MNT), the buccal mucosa cell micronucleus test (BMC-MNT), the undivided lymphocyte micronucleus test (UL-MNT), chromosomal aberration (CA) test, the comet assay and the hprt gene mutation test (HPRT). Patients were used as self-control before receiving radiation therapy. Apart from the UL-MNT, all the methods detected genetic damages in NPC patients, though with different sensitivities. CB-MNT is the best biological indicator for evaluating genetic damage induced by

radiation therapy in NPC patients; followed by CA and HPRT, while the BMC-MNT is simplest method as a potential biological indicator.

Cardis, E., J. Hall, et al. (2007). "Identification of women with an increased risk of developing radiation-induced breast cancer." Breast Cancer Res **9**(3): 106.

In the previous issue of Breast Cancer Research, Broeks and collaborators present the results of a study suggesting that germline mutations in BRCA1, BRCA2, ATM or CHEK2 may double the risk of radiation-induced contralateral breast cancer following radiotherapy for a first breast cancer. The assocation appeared to be strongest among women who were below the age of 40 at the time of their first breast cancer and among women who developed their second cancer 5 years or more after the first. While there were a number of methodological issues that might limit the conclusions drawn from this paper, this is one of several recent studies suggesting that carriers of pathogenic alleles in DNA repair and damage recognition genes may have an increased risk of breast cancer following exposure to ionising radiation, even at low doses. This finding has important implications for the protection of breast cancer patients and their close relatives. If confirmed, mutation carriers may wish to consider alternatives to X-ray for diagnostic purposes. The need for tailored cancer treatment strategies in carriers should also be evaluated carefully.

Chen, X., C. A. Arciero, et al. (2006). "BRCA1-associated complexes: new targets to overcome breast cancer radiation resistance." Expert Rev Anticancer Ther **6**(2): 187-96.

Since BRCA1 was cloned a decade ago, significant progress has been made in defining its biochemical and biological functions, as well as its role in breast and ovarian cancers. BRCA1 has been implicated in many cellular processes, including DNA repair, cell cycle checkpoint control, protein ubiquitination and chromatin remodeling. This review examines the role(s) of BRCA1 in mediating these cellular processes, and discusses its potential involvement in the resistance of breast cancer to radiation-based therapies. Finally, the possibility that BRCA1-associated proteins may serve as new targets for breast cancer radiation therapy is explored. The activation or inactivation of these BRCA1-associated proteins may modify both the risk of developing cancers in BRCA1 mutation carriers and the efficacy of breast cancer therapy, including radiation.

Chendil, D., A. Das, et al. (2002). "Par-4, a pro-apoptotic gene, inhibits radiation-induced NF kappa B activity and Bcl-2 expression leading to induction of radiosensitivity in human prostate cancer cells PC-3." Cancer Biol Ther **1**(2): 152-60.

Ionizing radiation caused induction NF kappa B activity and Bcl-2 protein expression in the radioresistant p53 null human prostate cancer cell line, PC-3. Exposure of PC-3 cells to Ad5-I kappa B super-repressor inhibited radiation-induced Bcl-2 expression indicating that radiation-induced NF kappa B activity is required for the induction of Bcl-2 protein. PAR-4, a novel pro-apoptotic protein is a potent down-modulator of NF kappa B activity and bcl-2 protein expression. This study was undertaken to investigate the impact of PAR-4 expression on radiation-induced NF kappa B activity and Bcl-2 expression and its resultant radiation response in PC-3 cells. Western blot analysis indicated that enforced expression of PAR-4 in PC-3 cells down regulated radiation-induced bcl-2 protein, whereas in vector transfected cells radiation caused an induction of bcl-2 protein. In both transfectant cell lines, the bax protein levels remained unaltered after radiation. When compared to PC-3/Vector cells, PC-3/PAR-4 cells showed significant sensitivity to radiation-induced clonogenic inhibition and apoptosis. Thus, the down-regulation of bcl-2 protein by ectopic PAR-4 expression altered bcl-2: bax ratio in PC-3/PAR-4 cells and this led enhanced radiosensitivity. PAR-4 was found to inhibit the radiation-induced NF kappa B activity and NF kappa B transcriptional activity is essential for bcl-2 upregulation. In PC-3/Vector cells, radiation caused an increase in NF kappa B activity leading to upregulation of bcl-2 protein. However, in PC-3/PAR-4 cells, the radiation-induced NF kappa B activity was inhibited resulting in the transrepression of bcl-2 promoter and down-modulation of bcl-2 protein. In addition, PAR-4 was found to directly inhibit the phosphorylation and degradation of I kappa B alpha, which led to the loss of NF kappa B activity causing repression of endogenous and radiation-induced Bcl-2 protein. Together, these mechanisms suggest that PAR-4 is functionally required to cause radiation-induced apoptosis by abrogating the survival and anti-apoptotic effects of NF kappa B activity and bcl-2 function respectively.

Cheo, D. L., L. B. Meira, et al. (2000). "Ultraviolet B radiation-induced skin cancer in mice defective in the Xpc, Trp53, and Apex (HAP1) genes: genotype-specific effects on cancer predisposition and pathology of tumors." Cancer Res **60**(6): 1580-4.

Mutations in nucleotide excision repair (NER) genes in humans result in the UV-induced skin cancer-prone disease xeroderma pigmentosum (XP). Mouse models that mimic XP have provided an informative experimental system with which to study DNA repair, as well as the molecular pathology of UV

radiation-induced skin cancer. We reported previously that mice defective in the Xpc gene (Xpc-/-) are highly predisposed to UVB radiation-induced skin cancer and that the appearance of skin cancer is more rapid in Xpc Trp53 double mutants. Extended studies now demonstrate an increased predisposition to UVB radiation-induced skin cancers in Xpc heterozygous mice compared with normal mice. We also show that Xpc Trp53 double heterozygous mutants are more predisposed to skin cancer than Trp53 single heterozygous mice. No mutations were detected in the cDNA of the remaining Xpc allele, suggesting that haploinsufficiency of the Xpc gene may be operating and is a risk factor for UVB radiation-induced skin cancer in mice. Skin tumors from Xpc-/- mice were exclusively well or moderately well-differentiated squamous cell carcinomas. In Xpc+/+ and Xpc+/- mice, many of the squamous cell carcinomas were less well differentiated. We also documented previously increased predisposition to UV radiation-induced skin cancers in Xpc-/- Apex+/- mice. Here we show the absence of mutations in the cDNA of the remaining Apex allele, a further suggestive indication of haploinsufficiency and its resulting predisposition to skin cancer. The Trp53 and Apex heterozygous conditions altered the skin tumor spectrum to more poorly differentiated forms in all Xpc genotypes.

Cheo, D. L., L. B. Meira, et al. (1996). "Synergistic interactions between XPC and p53 mutations in double-mutant mice: neural tube abnormalities and accelerated UV radiation-induced skin cancer." Curr Biol **6**(12): 1691-4.

The significance of DNA repair to human health has been well documented by studies on xeroderma pigmentosum (XP) patients, who suffer a dramatically increased risk of cancer in sun-exposed areas of their skin [1,2]. This autosomal recessive disorder has been directly associated with a defect in nucleotide excision-repair (NER) [1,2]. Like human XP individuals, mice carrying homozygous mutations in XP genes manifest a predisposition to skin carcinogenesis following exposure to ultraviolet (UV) radiation [3-5]. Recent studies have suggested that, in addition to roles in apoptosis [6] and cell-cycle checkpoint control [7] in response to DNA damage, p53 protein may modulate NER [8]. Mutations in the p53 gene have been observed in 50% of all human tumors [9] and have been implicated in both the early [10] and late [11] stages of skin cancer. To examine the consequences of a combined deficiency of the XPC and the p53 proteins in mice, we generated double-mutant animals. We document a spectrum of neural tube defects in XPC p53 mutant embryos. Additionally, we show that, following exposure to UV-B radiation, XPC p53 mutant mice have more severe solar keratosis and suffer accelerated skin cancer compared with XPC mutant mice that are wild-type with respect to p53.

Chetty, C., P. Bhoopathi, et al. (2009). "Inhibition of matrix metalloproteinase-2 enhances radiosensitivity by abrogating radiation-induced FoxM1-mediated G2/M arrest in A549 lung cancer cells." Int J Cancer **124**(10): 2468-77.

Matrix metalloproteinase-2 (MMP-2), is known to degrade the collagen IV, plays a role in radiation-induced lung injury. We therefore investigated the antitumor effects of combining MMP-2 inhibition using an adenovirus expressing siRNA against MMP-2 (Ad-MMP-2-Si) with radiation therapy (IR) on A549 lung cancer cells in vitro and in vivo. IR increased MMP-2 mRNA, protein and activity in lung cancer cells. MMP-2 inhibition along with IR enhanced radiosensitivity as determined by clonogenic assay, flow cytometry and TUNEL assay. We show that MMP-2 inhibition prior to irradiation reduced p53 phosphorylation, with a corresponding reduction in the expression of the p53 downstream target gene p21(Cip1/Waf1). Irradiated tumor cells induced the FoxM1-mediated DNA repair gene, XRCC1 and Checkpoint kinases 2/1, which were abrogated with combined treatment of Ad-MMP-2-Si and IR. Further, the combination of Ad-MMP-2-Si with radiotherapy significantly increased antitumor efficacy in vivo compared to either agent alone. Indeed, histological analysis of tumor sections collected from the combination group revealed more apoptotic cells. These studies suggest that MMP-2 inhibition in combination with radiotherapy abrogates G2 cell cycle arrest leading to apoptosis and provide evidence of the antitumor efficacy of combining MMP-2 inhibition with irradiation as a new therapeutic strategy for the effective treatment of NSCLC patients.

Chung, Y. L., M. Y. Lee, et al. (2009). "Epigenetic therapy using the histone deacetylase inhibitor for increasing therapeutic gain in oral cancer: prevention of radiation-induced oral mucositis and inhibition of chemical-induced oral carcinogenesis." Carcinogenesis **30**(8): 1387-97.

In addition to genetic changes, epigenetic aberrations also play important roles in radiation- and chemical-induced disorders and carcinogenesis. The present study investigated whether epigenetic therapy with a histone deacetylase (HDAC) inhibitor has dual benefits for radiation-induced oral mucositis and chemical-induced oral carcinogenesis, which should be treated at the same time. The HDAC inhibitor phenylbutyrate was first tested to determine if it influences DNA damage repair and survival in

irradiated normal cells in vitro by investigating the patterns and dynamics of phospho-gammaH2AX foci, Rad51 foci and phospho-gammaH2AX/Rad51 colocalization and using the comet and clonogenic assays. Oral mucositis or carcinogenesis was induced in hamsters using radiation or 7,12-dimethylbenz[a]anthracene (DMBA) irritation to the cheek pouch. The ability of phenylbutyrate formed in proper carriers to prevent radiation-induced oral mucositis and inhibit chemical-induced oral carcinogenesis was assessed. The treated or untreated irradiated or DMBA-irritated oral tissues or mucosal epithelia were subjected to the studies of histology, immunohistochemistry, gene expression, comet assay, HDAC activity or oxidative stress. We found that phenylbutyrate promoted DNA repair and survival in normal cells after radiation. Compared with blank or vehicle-treated hamsters, the irradiated mucosa treated with phenylbutyrate had significantly lower oxidative stress and tumor necrosis factor-alpha expression and less severe oral mucositis of a shorter duration. A reduction of the oral tumor incidence, burden and progression by phenylbutyrate correlated with the suppression of oncomiRs and Rad51 overexpression, the upregulation of differentiation markers and the decrease of intracellular HDAC activity and oxidative stress during DMBA-induced oral carcinogenesis. Thus, epigenetic therapy using the HDAC inhibitor as an adjuvant to radiotherapy for chemical-induced oral cancer may provide a promising strategy combining the prevention of radiation-induced oral mucositis and the inhibition of oral carcinogenesis.

Chung, Y. M., B. G. Kim, et al. (2005). "Increased expression of ICAM-3 is associated with radiation resistance in cervical cancer." Int J Cancer **117**(2): 194-201.

To search for a marker that predicts the efficacy of radiation therapy in human cervical cancer, gene expression profiles between parental SiHa cervical cancer cells and radiation-resistant SiHa/R cells have been compared by the microarray technique. Microarray and Northern blot analyses demonstrated that the ICAM-3 expression was upregulated in SiHa/R cells. This increased expression of ICAM-3 in SiHa cells enhanced cell survival by about 34.3% after a 2 Gy dosage of radiation. In addition, SiHa/ICAM-3 cells showed a 2.45-fold higher level of FAK phosphorylation than that of the control cells. In tumor specimens, ICAM-3 staining was restricted to tumor stromal endothelial cells and lymphocytes. The overexpression of ICAM-3 was significantly more frequent in radiation-resistant cervical cancer specimens when compared with radiation-sensitive specimens (83.3% vs. 35.3%; $p = 0.015$). With these observations, we can suggest that an increased expression of ICAM-3 is associated with radiation resistance in cervical cancer cells and the expression of ICAM-3 can be used as a valuable biomarker to predict the radiation resistance in cervical cancer that occurs during radiotherapy.

Collis, S. J., M. J. Swartz, et al. (2003). "Enhanced radiation and chemotherapy-mediated cell killing of human cancer cells by small inhibitory RNA silencing of DNA repair factors." Cancer Res **63**(7): 1550-4.

Recent developments in the use of small inhibitory RNA molecules (siRNAs) to inhibit specific protein expression have highlighted the potential use of siRNA as a therapeutic agent. The double-strand break signaling/repair proteins ATM, ATR, and DNA-dependent protein kinase catalytic subunit (DNA-PK(cs)) are attractive targets to confer enhanced radio and chemosensitivity to tumor cells. We have designed and exogenously delivered plasmids encoding siRNAs targeting these critical kinases to human cancer cells to assess the feasibility of this concept as a clinically translatable experimental therapeutic. siRNA led to a approximately 90% reduction in target protein expression. siRNAs targeting ATM and DNA-PK(cs) gave rise to a dose-reduction factor of approximately 1.4 compared with untransfected and control vector-transfected cells at the clinically relevant radiation doses. This was greater than the radiosensitivity achieved using the phosphatidylinositol 3'-kinase inhibitor Wortmannin or DNA-PK(cs) competitive inhibitor LY294002. A similar increased sensitivity to the alkylating agent methyl methanesulfonate (MMS) was also observed for siRNA-mediated ATR silencing. Together, these data provide strong evidence for the potential use of siRNA as a novel radiation/chemotherapy-sensitizing agent.

De Benedetti, V. M., L. B. Travis, et al. (1996). "p53 mutations in lung cancer following radiation therapy for Hodgkin's disease." Cancer Epidemiol Biomarkers Prev **5**(2): 93-8.

High risks of lung cancer occur after successful treatment of Hodgkin's disease. In addition to tobacco smoking, other risk factors include radiotherapy, chemotherapy, and immunosuppression, although the relative contributions of each are unknown. We conducted p53 mutational spectrum analysis in second lung cancers after radiation therapy for Hodgkin's disease in the Netherlands and in Ontario, Canada. Lung cancer tissues from 11 patients were analyzed by p53 immunohistochemistry and DNA sequence analysis. All were male cigarette smokers, all received radiation therapy, and six also received chemotherapy. The lung cancers occurred 9.8 years (mean) after treatment. Radiation doses to lung

lobes that developed the tumors averaged 5.7 Gy (range, 3.7-11.7 Gy). Sequence analysis showed four missense and two silent p53 point mutations in five patients. There were four G:C-->A:T transitions; three of four mutated deoxyguanines occurred on the coding strand, and one was a CpG site. There were two transversions: one G:C-->C:G and one A:T-->C:G. Despite moderate or heavy smoking histories in all patients, the mutational spectrum appears to differ from usual smoking-related lung cancers in which G:C-->T:A transversions predominate. The absence of G:C-->T:A mutations and the prominence of G:C-->A:T transitions, which are characteristic of radiation and oxidative damage, suggest that radiotherapy might have caused some of the p53 mutations. These data illustrate the potential of mutation analysis to determine causes of human cancer. If confirmed in a larger series, these results imply that some radiation-induced cancers can be distinguished from those caused by other factors.

de Gruijl, F. R. (1999). "Skin cancer and solar UV radiation." Eur J Cancer **35**(14): 2003-9.

Ultraviolet (UV) radiation in sunlight is the most prominent and ubiquitous physical carcinogen in our natural environment. It is highly genotoxic but does not penetrate the body any deeper than the skin. Like all organisms regularly exposed to sunlight, the human skin is extremely well adapted to continuous UV stress. Well-pigmented skin is clearly better protected than white Caucasian skin. The sun-seeking habits of white Caucasians in developed countries are likely to have contributed strongly to the increase in skin cancer observed over the last century. Skin cancer is by far the most common type of cancer in the U.S.A. and Australia, which appears to be the result of an 'unnatural displacement' of people with sun-sensitive skin to sub-tropical regions. Although campaigns have been successful in informing people about the risks of sun exposure, general attitudes and behaviour do not yet appear to have changed to the extent that trends in skin cancer morbidity and the corresponding burden on public healthcare will be reversed. The relationship between skin cancer and regular sun exposure was suspected by physicians in the late 19th century, and subsequently substantiated in animal experiments in the early part of the 20th century. UV radiation was found to be highly genotoxic, and DNA repair proved to be crucial in fending off detrimental effects such as mutagenesis and cell death. In fact, around 1940 it was shown that the wavelength dependence of mutagenicity paralleled the UV absorption by DNA. In the 1970s research on UV carcinogenesis received a new impetus from the arising concern about a possible future depletion of the stratospheric ozone layer: the resulting increases in ambient UV loads were expected to raise skin cancer incidences. Epidemiological studies in the last decades of the 20th century have greatly refined our knowledge on the aetiology of skin cancers. Analyses of gene mutations in skin carcinomas have identified UV radiation as the cause. The relationship between the most fatal skin cancer, i.e. malignant melanoma and solar UV exposure is, however, still unclear and needs to be clarified to optimise preventive measures and minimise mortality from skin cancers.

DelloRusso, C., P. L. Welcsh, et al. (2007). "Functional characterization of a novel BRCA1-null ovarian cancer cell line in response to ionizing radiation." Mol Cancer Res **5**(1): 35-45.

The breast and ovarian cancer susceptibility gene BRCA1 plays a major role in the DNA damage response pathway. The lack of well-characterized human BRCA1-null cell lines has limited the investigation of BRCA1 function, particularly with regard to its role in ovarian cancer. We propagated a novel BRCA1-null human ovarian cancer cell line UWB1.289 from a tumor of papillary serous histology, the most common form of ovarian carcinoma. UWB1.289 carries a germline BRCA1 mutation within exon 11 and has a deletion of the wild-type allele. UWB1.289 is estrogen and progesterone receptor negative and has an acquired somatic mutation in p53, similar to the commonly used BRCA1-null breast cancer cell line HCC1937. We used ionizing radiation to induce DNA damage in both UWB1.289 and in a stable UWB1.289 line in which wild-type BRCA1 was restored. We examined several responses to DNA damage in these cell lines, including sensitivity to radiation, cell cycle checkpoint function, and changes in gene expression using microarray analysis. We observed that UWB1.289 is sensitive to ionizing radiation and lacks cell cycle checkpoint functions that are a normal part of the DNA damage response. Restoration of wild-type BRCA1 function in these cells partially restores DNA damage responses. Expression array analysis not only supports this partial functional correction but also reveals interesting new information regarding BRCA1-positive regulation of the expression of claudin 6 and other metastasis-associated genes and negative regulation of multiple IFN-inducible genes.

Du, X. L., T. Jiang, et al. (2009). "Differential expression profiling of gene response to ionizing radiation in two endometrial cancer cell lines with distinct radiosensitivities." Oncol Rep **21**(3): 625-34.

Although radiotherapy is routinely administered to high-risk endometrial carcinoma and offer a significant disease-free survival advantage, the therapeutic effect is sometimes limited by the

occurrence of radioresistance. To determine the patterns of gene expression responsible for the radioresistance and to search for potential target genes for radiotherapy, we selected two cell lines with distinct radiosensitivities using colony-formation assay from four endometrial cancer cell lines. The cell cycle distribution showed higher fractions of G2/M phase cells in the radiosensitive cell line KLE after radiation compared with the radioresistant cell line ISK. Apoptosis assessment also showed significant elevation in the percentage of early apoptosis cells in KLE cells. Subsequently, gene expression changes after X-ray exposure were analyzed by using oligonucleotide microarrays. We identified, respectively, in ISK and KLE, 227 and 354 genes that exhibited > or =2-fold difference. However, only 53 genes showing differences more than double the median expression value between the two groups were defined as radiosensitivity (or radioresistance)-related genes. Among these, genes associated with DNA-repair, apoptosis, growth factor, signal transduction, cell cycle and cell adhesion were predominant. The validity of the expression level of 10 randomly selected genes was confirmed by real-time PCR and/or Western blotting. In conclusion, the differential gene expression changes that occur after radiation in the two cell lines will provide insight into molecular mechanisms of radioresistance in endometrial carcinoma, and also the means to find potential targets to achieve further gains in therapeutic benefit.

Fallis, L. H., E. Richards, et al. (1999). "The biological response of MCF7 breast cancer cells to proteosome inhibition or gamma-radiation is unrelated to the level of p53 induction." Apoptosis **4**(2): 99-107.

The p53 tumour suppressor is stabilised following exposure to genotoxic agents, such as gamma-radiation. Cell responses to p53 stabilisation include induction of apoptosis and/or cell cycle arrest. Several studies have suggested that gamma-radiation stabilises p53 by blocking ubiquitin mediated proteolysis. Here we have compared the biological activities of p53 stabilized following exposure to gamma-radiation or treatment with the proteosome inhibitor N-acetyl-leucinyl-leucinyl-norleucinal (ALLN) in MCF7 cells with wild type p53. Stabilisation of p53 by ALLN was reversible and was not blocked by caffeine. Although ALLN was a more effective p53 stabilising agent than gamma-radiation, ALLN was not as effective at inducing cell cycle arrest/apoptosis as gamma-radiation. Although p53 stabilised by ALLN and gamma-radiation were both able to bind DNA and activate transcription, ALLN did not increase expression of BAX, which is involved in p53-induced apoptosis. Therefore, p53 stabilised by different agents is not always biologically active to the same extent and additional alterations triggered by gamma-radiation may enable p53 to activate a subset of critical target genes, such as BAX, which are required for p53 responses.

Fan, S., N. F. Twu, et al. (1998). "Down-regulation of BRCA1 and BRCA2 in human ovarian cancer cells exposed to adriamycin and ultraviolet radiation." Int J Cancer **77**(4): 600-9.

Germ-line mutations of the BRCA1 and BRCA2 genes predispose women to develop cancers of the breast and ovary, but the biologic functions of these genes remains unclear. We have investigated the responses of the BRCA1 and BRCA2 gene products to cytotoxic agents in 3 human ovarian cancer cell lines: SK-OV-3 (which contains a p53 deletion mutation), CAOV-3 (which over-expresses a mutant p53) and PA-1 (which expresses wild-type p53). In screening studies, we determined the effects of 7 different agents on BRCA1 and BRCA2 expression. We found that Adriamycin (ADR) and ultraviolet (UV)radiation significantly down-regulated BRCA1 and BRCA2 mRNA expression in SK-OV-3 cells. On the other hand, camptothecin, nitrogen mustard, taxol, vincristine and etoposide had no effect on BRCA1 or BRCA2 mRNA levels at doses that yielded degrees of cytotoxicity similar to or greater than ADR. The down-regulation of BRCA1 and BRCA2 mRNAs was dose and time dependent; significant down-regulation was first observed at 8-16 hr after exposure to ADR. BRCA1 protein levels were also down-regulated following treatment of SK-OV-3 cells with ADR. Similar results were observed in CAOV-3 and PA-1 cells treated with ADR, and this finding could not be directly attributed to ADR-induced changes in the cell cycle distribution. The ADR doses required for significant decreases of BRCA1 and BRCA2 were about 10-15, 5-10 and 2 microM, respectively, for SK-OV-3, CAOV-3 and PA-1; the IC50 doses for loss of cell viability (determined by Trypan blue dye exclusion) were 23, 14 and 0.4 microM, respectively. Thus, at equitoxic doses of ADR, PA-1 cells were more resistant to down-regulation of BRCA1 and BRCA2 than SK-OV-3 or CAOV-3. Our findings suggest that 1) BRCA1 and BRCA2 expression in human ovarian cancer cell lines is selectively down-regulated by 2 DNA-damaging agents (ADR and UV radiation); 2) these responses are not due to non-specific cytotoxicity; and 3) the BRCA1 and BRCA2 responses may be dependent, in part, on the p53 functional status of the cells. We speculate that the down-regulation of BRCA1 and BRCA2 may be part of a cellular survival response activated by certain forms of DNA damage.

Fan, Z., P. Chakravarty, et al. (2000). "Adenovirus-mediated antisense ATM gene transfer sensitizes prostate cancer cells to radiation." Cancer Gene Ther **7**(10): 1307-14.

Treatment failure after radiation therapy of prostate cancer (PC) could be a significant problem. Our objective is to design genetic radiosensitizing strategies for the treatment of PC. Cells from individuals with the genetic disorder ataxia telangiectasia (AT) are hypersensitive to ionizing radiation. Therefore, we examined whether attenuation of the AT gene product, AT mutated (ATM), in PC cells could result in an increased intrinsic radiosensitivity. A p53-mutant PC cell line, PC-3 was infected with adenoviral vectors, expressing antisense ATM RNA to various domains of the ATM gene. Immunoblot analyses of cellular extracts from antisense ATM-transfected PC-3 cells showed attenuated expression of the ATM protein within 2 days of viral infection. Compared with cells infected with an adeno-beta-galactosidase vector, antisense ATM-transfected PC-3 cells showed aberrant control of S-phase cell-cycle checkpoints after exposure to ionizing radiation. Under these conditions, the intrinsic radiosensitivity of the PC-3 cells was enhanced. Consequently antisense ATM gene therapy could serve as a paradigm for strategies that target the cellular survival mechanisms of an irradiated tumor cell and may provide therapeutic benefit to patients undergoing radiation therapy for PC.

Fogelfeld, L., T. K. Bauer, et al. (1996). "p53 gene mutations in radiation-induced thyroid cancer." J Clin Endocrinol Metab **81**(8): 3039-44.

Little is known about the role of specific oncogenes and tumor suppressor genes in radiation-induced thyroid cancer (RITC). In thyroid cancer, mutations in the p53 tumor suppressor gene have been largely confined to the more aggressive anaplastic forms. We studied point mutations in the p53 gene in 22 patients exposed in childhood to radiation in the head and neck area who later developed papillary thyroid cancers (RITC). Eighteen thyroid cancer patients without exposure to radiation, selected to match by gender and age the RITC group, were used as the control group. After histological identification, DNA was extracted from paraffin-embedded specimens. Exons 5-8 of p53 were PCR amplified and screened for mutations by single strand conformation polymorphism analysis and cycle sequencing. Four of 22 RITC patients (18%) showed missense point mutations. No missense mutations were found in the cancer control group. The missense mutations in the RITC group occurred at codon 208 in 2 patients, codon 177 in 1, and codon 217 in 1. The mutations were transitions from G to A and C to T. All patients with missense mutations were male and had lymph node involvement. Three of the 4 patients with p53 missense mutations had invasion of the cancer beyond the thyroid capsule compared to 2 of the 17 remaining RITC patients. None of the patients with p53 mutations had distant metastases or recurrence of the tumor. These results suggest that p53 gene point mutations may play a pathogenetic role in some radiation-induced, well differentiated thyroid cancers and in their local spread.

Freytag, S. O., H. Stricker, et al. (2003). "Phase I study of replication-competent adenovirus-mediated double-suicide gene therapy in combination with conventional-dose three-dimensional conformal radiation therapy for the treatment of newly diagnosed, intermediate- to high-risk prostate cancer." Cancer Res **63**(21): 7497-506.

The primary study objective was to determine the safety of intraprostatic administration of a replication-competent, oncolytic adenovirus containing a cytosine deaminase (CD)/herpes simplex virus thymidine kinase (HSV-1 TK) fusion gene concomitant with increasing durations of 5-fluorocytosine and valganciclovir prodrug therapy and conventional-dose three-dimensional conformal radiation therapy (3D-CRT) in patients with newly diagnosed, intermediate- to high-risk prostate cancer. Secondary objectives were to determine the persistence of therapeutic transgene expression in the prostate and to examine early posttreatment response. Fifteen patients in five cohorts received a single intraprostatic injection of 10(12) viral particles of the replication-competent Ad5-CD/TKrep adenovirus on day 1. Two days later, patients were administered 5-fluorocytosine and valganciclovir prodrug therapy for 1 (cohorts 1-3), 2 (cohort 4), or 3 (cohort 5) weeks along with 70-74 Gy 3D-CRT. Sextant needle biopsy of the prostate was obtained at 2 (cohort 1), 3 (cohort 2), and 4 (cohort 3) weeks for determination of the persistence of transgene expression. There were no dose-limiting toxicities and no significant treatment-related adverse events. Ninety-four percent of the adverse events observed were mild to moderate and self-limiting. Acute urinary and gastrointestinal toxicities were similar to those expected for conventional-dose 3D-CRT. Therapeutic transgene expression was found to persist in the prostate for up to 3 weeks after the adenovirus injection. As expected for patients receiving definitive radiation therapy, all patients experienced significant declines in prostate-specific antigen (PSA). The mean PSA half-life in patients administered more than 1 week of prodrug therapy was significantly shorter than that of patients receiving prodrugs for only 1 week (0.6 versus 2.0 months; $P < 0.02$) and markedly shorter than that

reported previously for patients treated with conventional-dose 3D-CRT alone (2.4 months). With a median follow-up of only 9 months, 5 of 10 (50%) patients not treated with androgen-deprivation therapy achieved a serum PSA < or = 0.5 ng/ml. The results demonstrate that replication-competent adenovirus-mediated double-suicide gene therapy can be combined safely with conventional-dose 3D-CRT in patients with intermediate- to high-risk prostate cancer. The shorter than expected PSA half-life in patients receiving more than 1 week of prodrug therapy may suggest a possible interaction between the oncolytic adenovirus and/or double-suicide gene therapies and radiation therapy.

Furre, T., E. I. Furre, et al. (2003). "Lack of inverse dose-rate effect and binding of the retinoblastoma gene product in the nucleus of human cancer T-47D cells arrested in G2 by ionizing radiation." Int J Radiat Biol **79**(6): 413-22.

PURPOSE: To investigate the radiosensitivity of human breast cancer cells, T-47D, irradiated with low dose-rates and to study activation of the retinoblastoma gene product in the G1 and G2 phases during irradiation. MATERIALS AND METHODS: Cells were irradiated with (60)Co gamma-rays with dose-rates of 0.37 and 0.94 Gy h(-1). Cell survival was measured as the ability of cells to form colonies. Cells were extracted, fixed and stained for simultaneous measurements of nuclear-bound pRB content and DNA content. Cell nuclei were stained with monoclonal antibody PMG3-245 and Hoechst 33258 was used for additional staining of DNA. Two-parametric flow cytometry measurements of pRB and DNA content were performed using a FACSTAR(PLUS) flow cytometer. RESULTS: It was observed that irradiated cells were arrested in G2. No increase in radiation sensitivity was observed when the cells accumulated in G2. Irradiation of cells at both 0.37 and 0.94 Gy h(-1) resulted in exponential dose-survival curves with nearly equal alpha values, i.e. the same radiosensitivity. However, the retinoblastoma gene product was bound in the nucleus, i.e. hypophosphorylated, in about 15% of the cells arrested in G2. CONCLUSIONS: T47-D cells accumulate in G2 during low dose irradiation, but no inverse dose-rate effect, i.e. a more efficient inactivation of cells at lower than at higher dose-rates, was observed. A population of arrested G2 cells has pRB protein bound in the nucleus, and pRB therefore could play a role in protecting cells against radiation-induced cell death in G2.

Goss, P. E. and S. Sierra (1998). "Current perspectives on radiation-induced breast cancer." J Clin Oncol **16**(1): 338-47.

PURPOSE: An approach to screening and detection of radiation-induced breast cancer is offered. Primary and secondary prevention strategies are suggested and the need for prospective clinical trials is emphasized. METHODS: Data are reviewed from published evidence of radiation-induced breast cancer secondary to atomic bomb radiation, occupational, and therapeutic exposure, especially that incurred during successful treatment of Hodgkin's disease (HD). Preclinical studies are reviewed to explore potential risk factors. RESULTS: Risk factors evident in the link between radiation and breast cancer include the differentiation of breast tissue as mediated by age and hormonal influence. Evidence is presented exploring the link between genetics and breast cancer, including specific genes such as the BRCA1 and BRCA2 genes, the p53 gene, the ataxia telangiectasia (AT) gene, and other nonspecific alterations in DNA repair proficiency. In light of these findings, steps toward primary prevention are discussed, including avoiding radiation exposure, genetic screening, and manipulation of the hormonal milieu. Secondary prevention may also be possible with the use of tamoxifen, low-fat diets, and/or the consumption of flaxseed. CONCLUSION: Our current recommendations for patients irradiated before 30 years of age for Hodgkin's disease include breast self-examination (BSE) monthly, yearly mammography 8 years postirradiation, and regular physical examinations every 6 months. Given the clear link between radiation exposure and breast cancer, we strongly recommend a prospective trial randomize patients to different levels of intensity of surveillance to monitor the efficacy of such screening efforts.

Gridley, D. S. and J. M. Slater (2004). "Combining gene therapy and radiation against cancer." Curr Gene Ther **4**(3): 231-48.

Radiation has been a well-established modality in cancer treatment for several decades. Significant improvements have been achieved in radiotherapy over the years due to technological advances and development of facilities for delivery of charged particles such as protons. Nonetheless, the potential for tumor control with radiotherapy must always be carefully balanced with the risk for normal tissue damage. In addition, tumor cells outside the immediate field of radiation exposure or that have metastasized to distant sites are not destroyed. Gene therapy offers many exciting possibilities by which the overall efficacy of radiotherapy may be improved, while minimizing unwanted side effects. This review highlights several of the most promising gene transfer approaches that are currently being evaluated in combination with radiation in the treatment of cancer. Results from studies utilizing genes encoding

molecules that function in apoptosis, radiosensitization, immune up-regulation, angiogenesis, DNA repair, normal tissue protection from radiation damage, and tumor targeting are discussed. The evidence indicates that many of these innovative gene-based strategies have great potential to augment radiotherapy, as well as other established forms of cancer treatment, in the near future.

Hall, E. J., P. B. Schiff, et al. (1998). "A preliminary report: frequency of A-T heterozygotes among prostate cancer patients with severe late responses to radiation therapy." Cancer J Sci Am **4**(6): 385-9.

PURPOSE: To investigate whether a significant proportion of prostate cancer patients who have late sequelae after high-dose external-beam conformal radiation therapy are radio-sensitive because they are carriers of ataxia-telangiectasia, that is, are heterozygous for mutations in the ATM gene. PATIENTS AND METHODS: A group of prostate cancer patients were selected who experienced severe late sequelae, specifically proctitis or cystitis, after high-dose external-beam conformal radiation therapy, together with a control group of patients treated in the same way but who did not have severe late effects. Blood samples were taken from these patients, genomic DNA extracted, and mutations sought in the ATM gene. RESULTS: Of 17 late-effect patients in whom most or all of the ATM gene has been examined, significant mutations (17.6%) were identified in three. No significant mutations were found in the control group. The incidence of ataxia-telangiectasia heterozygotes in the United States population is 1% to 2%. DISCUSSION: These preliminary data suggest that a disproportionate number, but by no means all, of prostate cancer radiotherapy patients who experience severe late effects are ataxia-telangiectasia heterozygotes. If this conclusion is confirmed, these individuals could be identified prospectively and, with dose de-escalation, spared a great deal of discomfort and suffering. As a corollary, if most of the small late-effects population were prospectively identifiable, the dose to the remaining population could potentially be escalated. Present methods of identifying mutations in a large gene, such as ATM, are cumbersome and expensive, but the technology is evolving rapidly, so that rapid screening of the ATM gene is imminent.

Hall, J. and S. Angele (1999). "Radiation, DNA damage and cancer." Mol Med Today **5**(4): 157-64.

The characterization of the rare, radiation-sensitive and cancer-prone syndromes, ataxia telangiectasia and Nijmegen breakage syndrome, has demonstrated that genetic predisposition increases the risk of developing cancer after exposure to ionizing radiation (IR). Molecular analyses of these disorders provide valuable insights into the normal function of these two gene products in the cellular response to IR-induced DNA damage. Their contribution to a cellular radiosensitive phenotype and their role in sporadic cancers can now be fully assessed. For example, the gene ataxia telangiectasia mutated (ATM) has recently been shown to be a tumour suppressor gene in T-cell prolymphocytic leukaemia, and there is increasing evidence that individuals with one mutated ATM or Nijmegen breakage syndrome (NBS1) allele have an increased predisposition to cancer.

Hamilton, J. P., F. Sato, et al. (2006). "Promoter methylation and response to chemotherapy and radiation in esophageal cancer." Clin Gastroenterol Hepatol **4**(6): 701-8.

BACKGROUND & AIMS: Multiple studies have shown that promoter methylation of tumor suppressor genes underlies esophageal carcinogenesis. Hypothetically, methylation resulting in tumor suppressor gene inactivation might result in tumors that are unresponsive to chemotherapy and radiation. Accordingly, our aim was to find methylation markers that could be used to predict response to chemoradiation. METHODS: Tumor specimens were obtained before treatment from 35 patients enrolled in a uniform chemoradiation treatment protocol. Methylation-specific quantitative polymerase chain reaction was performed on all samples. Pathology reports from esophagectomy specimens were used to define response to treatment. RESULTS: Thirteen (37%) of 35 patients were responders, and 22 (63%) of 35 patients were nonresponders. The number of methylated genes per patient was significantly lower in responders than in nonresponders (1.4 vs 2.4 genes per patient; Student t test, P = .026). The combined mean level of promoter methylation of p16, Reprimo, p57, p73, RUNX-3, CHFR, MGMT, TIMP-3, and HPP1 was also lower in responders than in nonresponders (Student t test, P = .003; Mann-Whitney test, P = .001). The frequency (15% of responders vs 64% of nonresponders; Fisher exact test, P = .01) and level (0.078 in responders vs 0.313 in nonresponders; Mann-Whitney test, P = .037) of Reprimo methylation was significantly lower in responders than in nonresponders. CONCLUSIONS: Reprimo methylation occurred at significantly lower levels and less frequently in chemoradioresponsive than in nonresponsive esophageal cancer patients, suggesting potential clinical application of this single-gene biomarker in defining prognosis and management. In addition, increased methylation of a 9-gene panel correlated significantly with poor responsiveness to chemoradiation.

Han, Z., H. Wang, et al. (2006). "Radiation-guided gene therapy of cancer." Technol Cancer Res Treat **5**(4): 437-44.

Gene therapy has been proposed as a means to combat cancer. However, systemic toxicity observed in preclinical trials suggested the importance of selectively targeted delivery and inducible gene expression in tumor tissues. Discovery of radiation-inducible promoter sequences provides one way to minimize inadvertent toxicity from gene therapy in normal tissues. Radiation is administered to selectively induce cytotoxic gene expression in the targeted tumor tissues. With promising results from phase II clinical trials using TNF-expressing adenovirus, it is possible to have radiation-guided gene therapy regimes once the tumor-targeted delivery has been achieved. Tumor endothelium is an attractive biological target for gene therapy, because it has the advantage of stability, accessibility, and bioavailability for therapeutic agents. Technological development of DNA microarray, proteomic profiling, and phage-displayed libraries accelerates the identification of tumor-specific endothelial biomarkers and discovery of its relevant affinity reagents for targeted delivery. The application of radiation-guided gene delivery, its amplification, as well as expression of gene therapy presents great opportunities to be employed as an alternative cancer treatment.

Harada, K., Y. Obiya, et al. (1997). "Cancer risk in space due to radiation assessed by determining cell lethality and mutation frequencies of prokaryotes and a plasmid during the Second International Microgravity Laboratory (IML-2) Space Shuttle experiment." Oncol Rep **4**(4): 691-5.

We participated in a space experiment conducted during the 2nd International Microgravity Laboratory Mission (IML-2) project. The aim of our study was to investigate the effects of space radiation, i.e., high-LET (linear energy transfer) cosmic radiation, on living organisms in the 'Realtime Radiation Monitoring Device (RRMD)'. The biological samples, dried E. coli DNA repair-deficient mutant cells and shuttle vector plasmid pZl89 DNA, were prepared and placed in a biospecimen box sandwiched between 'Harzlas' plastic radiation detectors. This box was then loaded into the RRMD sensor unit in the Space Shuttle 'Columbia' and an identical box was left in the NASA John F. Kennedy Space Center (KSC) as a control. 'Columbia' (flight No. STS-65) was launched from KSC in Florida, USA on July 8, 1994. The mission duration was 14.75 days and after 'Columbia' returned to earth, we studied (i) the lethal and mutagenic effects of high-LET cosmic radiation on E. coli mutants and (ii) the relationship between high-LET cosmic radiation and the mutation frequency of pZ189 DNA. There were virtually no differences between the cell viabilities of the space and control samples of Escherichia coli KMBL3835 (wild-type), KY383 (lexA-), KY385 (recA-) and KY386 (uvrA-), nor between the mutation frequency ratios of the space and control E. coli mutant samples. Furthermore, the survival and mutation frequency of the supF gene of pZ189 DNA space samples did not differ from those of the control samples. We concluded there was no cancer risk during this Space Shuttle flight.

Helland, A., H. Johnsen, et al. (2006). "Radiation-induced effects on gene expression: an in vivo study on breast cancer." Radiother Oncol **80**(2): 230-5.

BACKGROUND AND PURPOSE: Breast cancer is diagnosed worldwide in approximately one million women annually and radiation therapy is an integral part of treatment. The purpose of this study was to investigate the molecular basis underlying response to radiotherapy in breast cancer tissue. MATERIAL AND METHODS: Tumour biopsies were sampled before radiation and after 10 treatments (of 2 Gray (Gy) each) from 19 patients with breast cancer receiving radiation therapy. Gene expression microarray analyses were performed to identify in vivo radiation-responsive genes in tumours from patients diagnosed with breast cancer. The mutation status of the TP53 gene was determined by using direct sequencing. RESULTS AND CONCLUSION: Several genes involved in cell cycle regulation and DNA repair were found to be significantly induced by radiation treatment. Mutations were found in the TP53 gene in 39% of the tumours and the gene expression profiles observed seemed to be influenced by the TP53 mutation status.

Hellman, B., D. Brodin, et al. (2005). "Radiation-induced DNA-damage and gene expression profiles in human lung cancer cells with different radiosensitivity." Exp Oncol **27**(2): 102-7.

BACKGROUND: Measurements of DNA double strand breaks and their subsequent repair after in vitro irradiation has been suggested to be an alternative way of monitoring radiotherapeutic response. METHODS: In the present study, the DNA repair kinetics (using a neutral version of the Comet assay) up to 45 min after a single dose of 2 Gy was studied as well as the gene expression profiles, before and 45 min after the irradiation, in two human lung cancer cell lines with different radiosensitivity (U-1285 and U-1810). RESULTS: Immediately after the irradiation, both cell lines responded with increased levels of DNA damage. However, the induced damage was slightly higher in U-1810 (known to be radioresistant) than in U-1285 (known to be

radiosensitive), and the latter cell line also seemed to have a slightly more efficient DNA-repair. The two different lung cancer cell lines were highly heterogeneous in gene expression, both before and after the irradiation, and there was no obvious relationship between the Comet data and the microarray data. CONCLUSION: Given the fact that U-1810 has been classified as radioresistant and U-1285 as radiosensitive in clonogenic assays, the results of the present study indicate that radiation-induced DNA double strand breaks and DNA-repair efficiency are poor indicators of the intrinsic radiosensitivity of human lung cancer cells irradiated with a single dose in vitro.

Henness, S., M. W. Davey, et al. (2002). "Fractionated irradiation of H69 small-cell lung cancer cells causes stable radiation and drug resistance with increased MRP1, MRP2, and topoisomerase IIalpha expression." Int J Radiat Oncol Biol Phys **54**(3): 895-902.

PURPOSE: After standard treatment with chemotherapy and radiotherapy, small-cell lung cancer (SCLC) often develops resistance to both treatments. Our aims were to establish if fractionated radiation treatment alone would induce radiation and drug resistance in the H69 SCLC cell line, and to determine the mechanisms of resistance. METHODS AND MATERIALS: H69 SCLC cells were treated with fractionated X-rays to an accumulated dose of 37.5 Gy over 8 months to produce the H69/R38 subline. Drug and radiation resistance was determined using the MTT (3,-4,5 dimethylthiazol-2,5 diphenyltetrazolium bromide) cell viability assay. Protein expression was analyzed by Western blot. RESULTS: The H69/R38 subline was resistant to radiation (2.0 +/- 0.2-fold, $p < 0.0001$), cisplatin (14 +/- 7-fold, $p < 0.001$), daunorubicin (6 +/- 3-fold, $p < 0.05$), and navelbine (1.7 +/- 0.15-fold, $p < 0.02$). This was associated with increased expression of the multidrug resistance-associated proteins, MRP1 and MRP2, and topoisomerase IIalpha and decreased expression of glutathione-S-transferase pi (GSTpi) and bcl-2 and decreased cisplatin accumulation. Treatment with 4 Gy of X-rays produced a 66% decrease in MRP2 in the H69 cells with no change in the H69/R38 cells. This treatment also caused a 5-fold increase in topoisomerase IIalpha in the H69/R38 cells compared with a 1.5-fold increase in the H69 cells. CONCLUSIONS: Fractionated radiation alone can lead to the development of stable radiation and drug resistance and an altered response to radiation in SCLC cells.

Hill, J. W., K. Tansavatdi, et al. (2009). "Validation of the cell cycle G(2) delay assay in assessing ionizing radiation sensitivity and breast cancer risk." Cancer Manag Res **1**: 39-48.

Genetic variations in cell cycle checkpoints and DNA repair genes are associated with prolonged cell cycle G(2) delay following ionizing radiation (IR) treatment and breast cancer risk. However, different studies reported conflicting results examining the association between post-IR cell cycle delay and breast cancer risk utilizing four different parameters: cell cycle G(2) delay index, %G(2)-M, G(2)/G(0)-G(1), and (G(2)/G(0)-G(1))/S. Therefore, we evaluated whether different parameters may influence study results using a data set from 118 breast cancer cases and 225 controls as well as lymphoblastoid and breast cancer cell lines with different genetic defects. Our results suggest that cell cycle G(2) delay index may serve as the best parameter in assessing breast cancer risk, genetic regulation of IR-sensitivity, and mutations of ataxia telangiectasia mutated (ATM) and TP53. Cell cycle delay in 21 lymphoblastoid cell lines derived from BRCA1 mutation carriers was not different from that in controls. We also showed that IR-induced DNA-damage signaling, as measured by phosphorylation of H2AX on serine 139 (gamma-H2AX) was inversely associated with cell cycle G(2) delay index. In summary, the cellular responses to IR are extremely complex; mutations or genetic variations in DNA damage signaling, cell cycle checkpoints, and DNA repair contribute to cell cycle G(2) delay and breast cancer risk. The cell cycle G(2) delay assay characterized in this study may help identify subpopulations with elevated risk of breast cancer or susceptibility to adverse effects in normal tissue following radiotherapy.

Hino, O., A. J. Klein-Szanto, et al. (1993). "Spontaneous and radiation-induced renal tumors in the Eker rat model of dominantly inherited cancer." Proc Natl Acad Sci U S A **90**(1): 327-31.

Hereditary renal carcinoma (RC) in the rat, originally reported by R. Eker in 1954, is an example of a Mendelian dominant predisposition to a specific cancer in an experimental animal. At the histologic level, RCs develop through multiple stages from early preneoplastic lesions (e.g., atypical tubules) to adenomas in virtually all heterozygotes by the age of 1 year. The homozygous mutant condition is lethal at approximately 10 days of fetal life. Ionizing radiation induces additional tumors in a linear dose-response relationship, suggesting that in heterozygotes two events (one inherited, one somatic) are necessary to produce tumors, and that the predisposing gene is a tumor suppressor gene. No genetic linkage has yet been found between the Eker mutation and rat DNA sequences homologous to those in human chromosome 3p, the presumed site of the putative

tumor suppressor gene responsible for human RC. Nonrandom loss of rat chromosome 5 in RC-derived cell lines is sometimes associated with homozygous deletion of the interferon gene loci at rat chromosome bands 5q31-q33. Since this locus is not linked with the predisposing inherited gene in the Eker rat, it probably represents a second tumor suppressor gene involved in tumor progression.

Hino, S., H. Kawamata, et al. (2002). "Cytoplasmic TSC-22 (transforming growth factor-beta-stimulated clone-22) markedly enhances the radiation sensitivity of salivary gland cancer cells." Biochem Biophys Res Commun **292**(4): 957-63.

We transfected a salivary gland cancer cell line, TYS, with three different forms of TSC-22 (transforming growth factor-beta-stimulated clone-22) gene: full-length TSC-22 (TSC-22FL) containing nuclear export signal, TSC-box and leucine zipper, truncated TSC-22 (TSC-22LZ) containing only TSC-box and leucine zipper, and truncated TSC-22 with nuclear localization signal (NLS-TSC-22LZ). High expression of TSC-22FL in the cytoplasm markedly enhanced the radiation-sensitivity of TYS cells, while, moderate expression of TSC-22FL marginally affected the radiation-sensitivity. TSC-22LZ, which was expressed in the cytoplasm and the nucleus, enhanced the radiation-sensitivity of TYS cells irrespective to its expression level. NLS-TSC-22LZ, which was expressed only in the nucleus, marginally affected the radiation-sensitivity of the cells even at high expression level. Interestingly, cytoplasmic TSC-22 translocates to nucleus concomitant with radiation-induced apoptosis. These results suggest that cytoplasmic localization of TSC-22 and translocation of TSC-22 from cytoplasm to nucleus is important for regulating the cell death signal after irradiation-induced DNA damage.

Hu, J. J., T. R. Smith, et al. (2002). "Genetic regulation of ionizing radiation sensitivity and breast cancer risk." Environ Mol Mutagen **39**(2-3): 208-15.

Genetic variability in DNA repair may contribute to hypersensitivity to ionizing radiation (IR) and susceptibility to breast cancer. We used samples collected from a clinic-based breast cancer case-control study to test the working hypothesis that amino acid substitution variants of DNA repair genes may contribute to prolonged cell-cycle delay following IR and breast cancer risk. Fluorescence-activated cell sorter (FACS) analysis was used to measure cell-cycle delay. PCR-restriction fragment length polymorphism (RFLP) assays were used to determine four genotypes of three DNA repair genes: XRCC1, 194 Arg/Trp and 399 Arg/Gln; XRCC3, 241 Thr/Met; and APE1, 148 Asp/Glu. The data showed that breast cancer patients had a significantly higher delay index than that of controls ($P < 0.001$); the means +/- SD for cases and controls were 36.0 +/- 13.1 ($n = 118$) and 31.4 +/- 11.5 ($n = 225$), respectively. There was a significant dose-response relationship between delay index, categorized into quartiles, and an increasing risk of breast cancer (crude odds ratios: 1.00, 1.00, 1.27, and 2.46, respectively; P(trend) = 0.002). In controls, prolonged cell-cycle delay was significantly associated with the number of variant alleles in APE1 Asp148Glu and XRCC1 Arg399Gln genotypes (P(trend) = 0.001). Although larger studies are needed to validate the results, our data suggest that an inherited hypersensitivity to IR may contribute to human breast carcinogenesis.

Huang, A., R. Gandour-Edwards, et al. (1998). "p53 and bcl-2 immunohistochemical alterations in prostate cancer treated with radiation therapy." Urology **51**(2): 346-51.

OBJECTIVES: Radiation therapy is definitive treatment for localized prostate cancer. It causes cellular deoxyribonucleic acid (DNA) damage, which, if irreparable, results in apoptosis or programmed cell death. Overexpression of mutant p53 and/or bcl-2 proteins prolongs cell survival despite exposure to damaging agents. We examined whether abnormal expression of either gene could help to explain radiation therapy failures in prostate cancer. METHODS: Archival tissue from patients who had failed radiation therapy as treatment for prostate cancer was obtained before and after treatment. These cancer samples were examined immunohistochemically for accumulation of p53 and bcl-2 proteins. Comparison was made with specimens from patients who had no evidence of recurrent or persistent disease at least 3 years following radiation therapy. RESULTS: High rates of p53 immunopositivity were found in the prostate tissue from all groups studied. More patients who had failed radiation therapy were found to have bcl-2 immunopositive specimens than were those without evidence for recurrent disease (41% preradiation and 61% postradiation versus 8%, $P < 0.05$). More patients who failed radiation therapy had both p53 and bcl-2 immunopositive prostate tissue than did those who were treated successfully (32% preradiation and 48% postradiation versus 8%). CONCLUSIONS: bcl-2 immunopositivity, with or without concomitant detection of p53, was found in significantly more cancers of patients who failed radiation therapy. Positive staining for bcl-2 may serve as a marker for determining the radiation sensitivity of a tumor and thus may help to guide treatment options. It is also

notable that a high proportion of the prostate cancers examined were immunopositive for p53.

Hurley, P. J., D. Wilsker, et al. (2007). "Human cancer cells require ATR for cell cycle progression following exposure to ionizing radiation." Oncogene **26**(18): 2535-42.

The vast majority of cancer cells have defective checkpoints that permit the cell cycle to progress in the presence of double-strand DNA breaks (DSBs) caused by ionizing radiation (IR) and radiomimetic drugs. ATR (ataxia telangiectasia-mutated and Rad3-related) has recently been shown to be activated by DSBs, although the consequences of this activity are largely unknown. In this report, we use advanced gene targeting methods to generate biallelic hypomorphic ATR mutations in human colorectal cancer cells and demonstrate that progression of the cancer cell cycle after IR treatment requires ATR. Cells with mutant ATR accumulated at a defined point at the beginning of the S phase after IR treatment and were unable to progress beyond that point, whereas cells at later stages of the S phase during the time of irradiation progressed and completed DNA replication. The prolonged arrest of ATR mutant cancer cells did not involve the ataxia telangiectasia mutated-dependent S-phase checkpoint, but rather closely resembled a previously characterized form of cell cycle arrest termed S-phase stasis. As ATR strongly contributed to clonogenic survival after IR treatment, these data suggest that blocking ATR activity might be a useful strategy for inducing S-phase stasis and promoting the radiosensitization of checkpoint-deficient cancer cells.

Hussein, M. R. (2005). "Ultraviolet radiation and skin cancer: molecular mechanisms." J Cutan Pathol **32**(3): 191-205.

Every living organism on the surface of the earth is exposed to the ultraviolet (UV) fraction of the sunlight. This electromagnetic energy has both life-giving and life-endangering effects. UV radiation can damage DNA and thus mutagenize several genes involved in the development of the skin cancer. The presence of typical signature of UV-induced mutations on these genes indicates that the ultraviolet-B part of sunlight is responsible for the evolution of cutaneous carcinogenesis. During this process, variable alterations of the oncogenic, tumor-suppressive, and cell-cycle control signaling pathways occur. These pathways include (a) mutated PTCH (in the mitogenic Sonic Hedgehog pathway) and mutated p53 tumor-suppressor gene in basal cell carcinomas, (b) an activated mitogenic ras pathway and mutated p53 in squamous cell carcinomas, and (c) an activated ras pathway, inactive p16, and p53 tumor suppressors in melanomas. This review presents background information about the skin optics, UV radiation, and molecular events involved in photocarcinogenesis.

Iannuzzi, C. M., D. P. Atencio, et al. (2002). "ATM mutations in female breast cancer patients predict for an increase in radiation-induced late effects." Int J Radiat Oncol Biol Phys **52**(3): 606-13.

PURPOSE: Mutation of the ATM gene may be associated with enhanced radiosensitivity and increased radiation-induced morbidity. Denaturing high performance liquid chromatography (DHPLC) is a powerful new technique proven to be sensitive and accurate in the detection of missense mutations, as well as small deletions and insertions. We screened female breast cancer patients for evidence of ATM gene alterations using DHPLC. This study attempted to determine whether breast cancer patients who develop severe radiotherapy (RT)-induced effects are more likely to possess ATM mutations than patients who display normal radiation responses. METHODS AND MATERIALS: Forty-six patients with early-stage breast carcinoma underwent limited surgery and adjuvant RT. DNA was isolated from blood lymphocytes, and each coding exon of the ATM gene was amplified using polymerase chain reaction. Genetic variants were identified using DHPLC by comparing test patterns with a known wild-type pattern. All variants were subjected to DNA sequencing and compared with wild-type sequences for evidence of a mutation. A retrospective review was performed, and the Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer acute and late morbidity scoring schemes for skin and subcutaneous normal tissues were applied to quantify the radiation-induced effects. RESULTS: Nine ATM mutations were identified in 6 patients (8 novel and 1 rare). The median follow-up was 3.2 years (range 1.3-10.3). A significant correlation between ATM mutation status and the development of Grade 3-4 subcutaneous late effects was found. All 3 of the patients (100%) who manifested Grade 3-4 subcutaneous late sequelae possessed ATM mutations, whereas only 3 (7%) of the 43 patients who did not develop this form of severe toxicity harbored an ATM mutation ($p = 0.001$). One ATM mutation carrier developed Grade 4 soft tissue necrosis after RT and required hyperbaric oxygen. All 3 patients manifesting Grade 3-4 late subcutaneous responses in fact harbored 2 ATM mutations. In contrast, none of the 3 ATM carriers who had a single mutation developed a severe subcutaneous reaction. ATM mutation status did not predict for a significant increase in early effects. Of the 23 patients with Grade 2-3 moist desquamation, 4 (17%) had an ATM mutation compared with 2 (9%)

of 23 patients without desquamation (p = 0.7). CONCLUSION: Possession of an ATM mutation, particularly when 2 are present, may be predictive of an increase in subcutaneous late tissue effects after RT for breast cancer and may subsequently prove to be a relative contraindication to standard management. These patients may be better served with reduced doses of radiation. Equivalent local control remains to be tested, but this germline alteration may radiosensitize normal tissues, as well as the tumor itself. DHPLC is effective in the identification of these patients. A larger study is required to confirm these findings.

Iwakawa, M., T. Ohno, et al. (2007). "The radiation-induced cell-death signaling pathway is activated by concurrent use of cisplatin in sequential biopsy specimens from patients with cervical cancer." Cancer Biol Ther **6**(6): 905-11.

OBJECTIVE: To identify changes in gene expression related to the concurrent use of platinum compounds with radiotherapy, in the treatment of cervical cancer. PATIENTS AND METHODS: Biopsy specimens were obtained from 39 patients with squamous cell carcinoma of the uterine cervix, before and during fractionated radiotherapy. Twenty patients were treated with radiotherapy (RT) alone, while 19 received the same radiotherapy plus concomitant chemotherapy with cisplatin (CRT). Changes in gene expression induced by treatment were investigated using single-color oligo-microarrays consisting of 44K human sequences. Paraffin-embedded samples were used to examine apoptosis and the expression of protein by treatment-responsive genes. Changes in mRNA expression were assessed for these genes by real-time reverse transcriptase-polymerase chain reaction. Aberrant genomic change (detected using microarray-based comparative genomic hybridization), human papillomavirus infection, and p53 status were also evaluated. RESULTS: The expression of CDKN1A, BAX, TNFSF8, and RRM2B was consistently upregulated by CRT (9 Gy with a single administration of cisplatin). Similar expression changes were induced by RT (9 Gy) alone, although the variability between tumors was greater. Apoptotic cells were significantly increased in both groups. CRT significantly increased the numbers of cases with diffusely distributed CDKN1A-positive cells. Genetic losses at 2q33-ter and gains of 3q26-ter were detected in the samples with high frequency; 60% were positive for human papillomavirus DNA; and three tumors had deletions/mutations of the p53 gene. There was no difference in the incidence of these genomic changes between the groups, and no association was found with the changes in expression of CDKN1A, BAX, TNFSF8 or RRM2B. CONCLUSIONS: Using biopsy samples from pretreatment and midtreatment cervical tumors, we identified therapy-induced genes related to the cell death signaling pathway. CRT produced a homogenous pattern of changes in expression of known radiation-responsive genes.

Jiang, W., H. N. Ananthaswamy, et al. (1999). "p53 protects against skin cancer induction by UV-B radiation." Oncogene **18**(29): 4247-53.

To assess the role of the p53 tumor suppressor gene in skin carcinogenesis by UV radiation, mice constitutively lacking one or both copies of the functional p53 gene were compared to wild-type mice for their susceptibility to UV carcinogenesis. Heterozygous mice showed greatly increased susceptibility to skin cancer induction, and homozygous p53 knockout mice were even more susceptible. Accelerated tumor development in the heterozygotes was not associated with loss of the remaining wild-type allele of p53, as reported for tumors induced by other carcinogens, but in many cases was associated with UV-induced mutations in p53. Tumors arose on the ears and dorsal skin of mice of all three genotypes, and homozygous knockout mice also developed ocular tumors, mainly melanomas. Skin tumors in the p53 knockout mice were predominately squamous cell carcinomas and were associated with premalignant lesions resembling actinic keratoses, whereas those in the heterozygous and wild-type mice were mainly sarcomas. These results demonstrate the importance of p53 in protecting against UV-induced cancers, particularly in the eye and epidermis.

Jiao, Y., C. M. Ge, et al. (2007). "Adenovirus-mediated expression of Tob1 sensitizes breast cancer cells to ionizing radiation." Acta Pharmacol Sin **28**(10): 1628-36.

AIM: To investigate the effect of the Tob1 gene, a member of the Transducing Molecule of ErbB2/B-cell Translocation Ggene (TOB/BTG) family, by using the adenovirus-mediated expression of Tob1 on radiosensitivity in a human breast cancer cell line MDA-MB-231. METHODS: Cell survival was determined by clonogenic assay. Apoptosis was evaluated by DNA fragmentation gel electrophoresis and terminal deoxynucleotidyl transferase-mediated nick end labeling assay. Protein expression was analyzed by Western blot assay and DNA repair was measured by a host cell reactivation assay. RESULTS: We demonstrated that pre-irradiation treatment with Ad5-Tob1 significantly increased radiosensitivity, accompanying the increased induction of apoptosis and the repression of DNA damage repair. Furthermore, Ad5-Tob1-mediated radiosensitivity

correlates with the upregulation of the pro-apoptotic protein Bax and the downregulation of several DNA double strand break repair proteins, including DNA-dependent protein kinases, Ku70 and Ku80, and X-ray-sensitive complementation group 4. CONCLUSION: Tob1, as a new radiosensitizer, is a new target in the radiotherapy of breast cancer via increasing apoptosis and suppressing DNA repair.

Jongmans, W. and J. Hall (1999). "Cellular responses to radiation and risk of breast cancer." Eur J Cancer **35**(4): 540-8.

Mutations in the ataxia telangiectasia gene (ATM) result in an abnormal p53-mediated cellular response to DNA damage produced by ionising radiation. This deficiency is believed to contribute to the radiosensitivity and high cancer risk seen in ataxia telangiectasia (AT) patients and AT heterozygotes. Epidemiological studies have demonstrated that relatives of AT patients are particularly predisposed to breast cancer. This observation, together with the finding that a relatively high proportion of breast cancer patients display an abnormal severe reaction of normal tissues following radiotherapy, has led to the suggestion that AT heterozygosity plays a role in radiosensitivity and breast cancer development. The cloning of the ATM gene has allowed this possibility to be examined at the molecular level. The studies reported to date remain inconclusive, with the number of AT heterozygotes being found in radiosensitive breast cancer patients being less than would be expected based on the family studies. The potential role of several other recently identified genes which are involved in the cellular DNA damage response to ionising radiation and which could also play a role in radiosensitivity and breast cancer development are reviewed.

Josson, S., S. Y. Sung, et al. (2008). "Radiation modulation of microRNA in prostate cancer cell lines." Prostate **68**(15): 1599-606.

BACKGROUND: MicroRNAs (miRNA) are gene regulators and play an important role in response to cellular stress. METHODS: Using multiplexed quantitative real-time PCR we performed global miRNA screening of prostate cancer cells in response to radiation treatment. RESULTS: Several miRNA were significantly altered in response to radiation treatment. Significant changes were observed in miR-521 and miR-34c. To determine the role of miR-521 in radiation response we transiently overexpressed miR-521 using miR-521 mimic. The miR-521 mimic significantly sensitized prostate cancer cells to radiation treatment. Conversely, ectopic inhibition of miR-521 resulted in radiation resistance of prostate cancer cells. To determine the mechanism by which miR-521 modulates radiation sensitivity we measured the expression levels of one of its predicted target protein, Cockayne syndrome protein A (CSA). CSA is a DNA repair protein, and its levels correlated inversely with the levels of miR-521. Radiation treatment downregulated the levels of miR-521 and upregulated CSA protein. Similarly, ectopic inhibition of miR-521 resulted in increased CSA protein levels. Therefore by altering the levels of CSA protein, miR-521 sensitized prostate cancer cells to radiation treatment. CONCLUSION: miR-521 modulates the expression levels of DNA repair protein, CSA and plays an important role, in the radio-sensitivity of prostate cancer cell lines. Thus miR-521 can be a potential target for enhancing the effect of radiation treatment on prostate cancer cells.

Kasid, U., A. Pfeifer, et al. (1987). "The raf oncogene is associated with a radiation-resistant human laryngeal cancer." Science **237**(4818): 1039-41.

In order to identify the genetic factors associated with the radiation-resistant human laryngeal carcinoma cell line (SQ-20B), tumor cell DNA was transfected into NIH/3T3 cells. A high incidence (six out of six) of raf sequences was found in transfected NIH/3T3 clones and the tumorigenic potential of SQ-20B DNA could be linked to genomic fragments that represent most of the kinase domain of human c-raf-1. An apparently unaltered 3.5-kilobase pair (kb) human c-raf transcript was identified in SQ-20B cells but was not observed in the transfected NIH/3T3 cell clones. Two new transcripts (4.2 kb and 2.6 kb) were found in tumorigenic clones; the large transcript was missing in a very poorly tumorigenic clone. Cytogenetic analysis indicated that the normal autosomes of chromosome 3 were absent in SQ-20B karyotypes and had formed apparently stable marker chromosomes. Unlike the recipient NIH/3T3 cell line, 30 percent of the transformed clone-1 metaphases had minute and double-minute chromosomes representative of amplified DNA sequences. The frequency of the c-raf-1 identification by NIH/3T3 transfection of SQ-20B DNA suggests the presence of some genetic abnormality within this locus.

Kassem, H., V. Sangar, et al. (2002). "A potential role of heat shock proteins and nicotinamide N-methyl transferase in predicting response to radiation in bladder cancer." Int J Cancer **101**(5): 454-60.

The use of definitive radiotherapy for treatment of invasive bladder cancer has the advantage of preserving bladder function, but tumour regression is only achieved in approximately 40-50% of patients. Knowledge of the molecular basis of sensitivity to ionizing radiation and identification of potential molecular predictors will provide useful information

regarding patient response and thus help clinicians to individualize treatment. The recent application of cDNA expression array technology provides a useful tool to investigate hundreds or even thousands of genes in a single experiment. In our study, we have used the Atlas human stress cDNA array trade mark to investigate the expression profile of stress-related and DNA repair genes in a radioresistant bladder carcinoma cell line (MGH-U1) and its radiosensitive subclone (S40b). This provides an ideal situation to study genes related to radiation because the genotypes of both cell lines are basically similar and differential changes detected are likely to be related to the different radiosensitivity phenotype. Of 234 genes blotted on the array, 3 genes (Heat shock protein 90, Heat shock protein 27 and Nicotinamide N-methyl transferase) showed consistent downregulation in the radiosensitive clone in 2 independent experiments. These results were further confirmed for HSP27 and NNMT using Sybr Green I-based real-time QRT-PCR. The role of heat shock proteins (HSPs) in response to radiation remains to be determined; however, the results of our present work suggest a possible role of HSP27 in determining radiosensitivity. Our study also opens avenues for the investigation of genes, such as NNMT, which has not previously been linked to response to radiation.

Kaufmann, W. K., L. Filatov, et al. (2006). "Radiation clastogenesis and cell cycle checkpoint function as functional markers of breast cancer risk." Carcinogenesis **27**(12): 2519-27.

BACKGROUND: Familial breast cancer is associated with mutations in several genes (BRCA1, BRCA2, p53, ATM) whose protein products protect against radiation-induced genotoxicity. This study tested whether sporadic breast cancer was associated with constitutive radiation hypersensitivity. METHODS: Blood lymphocytes and EBV-transformed lymphoblasts from patients with newly diagnosed breast cancer and controls without cancer were evaluated for ionizing radiation (IR)-induced chromosomal aberrations and cell cycle delays. Lymphoblasts from patients with ataxia telangiectasia (AT) and heterozygous AT carriers were tested as positive controls for radiation hypersensitivity. RESULTS: Lymphoblasts from AT patients and AT carriers displayed G2-irradiation, chromosomal hypersensitivity (GICH). Irradiated G2 phase lymphocytes from breast cancer cases and controls displayed 3-fold inter-individual variation in frequencies of chromatid damage. However, the percentage of breast cancer cases with damage frequencies in excess of 2 SD of the control mean (8/102 or 8%) was not significantly elevated compared to controls (2/48 or 4%, P=0.5). Lymphoblasts sampled 24 h after 3 Gy of IR also varied in the ratios of cells with 4N and 2N DNA content (4N/2N ratio), as a measure of cell cycle checkpoint function. 4N/2N ratios in irradiated lymphoblasts were strongly correlated with the fractions of S phase cells in un-irradiated control cultures (Pearson's correlation coefficient, r=0.87). After normalization to S fraction, the radiation-induced increment in the 4N/2N ratio was significantly elevated in AT lymphoblasts but not in lymphoblasts from AT carriers. The fraction of breast cancer cases with reduced checkpoint function (2/45 or 4%) was equal to the control fraction (2/45 or 4%). For breast cancer cases and controls, GICH in primary lymphocytes was not associated with reduced cell cycle checkpoint function in lymphoblasts. CONCLUSION: Constitutive radiation hypersensitivity in blood lymphocytes and lymphoblasts was not a useful biomarker for identifying women at increased risk of breast cancer.

Kavgaci, H., F. Ozdemir, et al. (2005). "Effect of the farnesyl transferase inhibitor L-744,832 on the colon cancer cell line DLD-1 and its combined use with radiation and 5-FU." Chemotherapy **51**(6): 319-23.

BACKGROUND: Ras oncogenes are found in 25% of human tumors and they significantly affect prognosis. One of the major fields studied to improve anticancer drugs is blockade of the oncogenic ras protein function. One of the mechanisms to block the function of these proteins is to block farnesylation using a farnesyl transferase inhibitor (FTI) and thus to prevent the ras from anchoring to the cell membrane. METHODS: In this study, we investigated the effects of FTI L-744,832 either alone or in combination with 5-fluorouracil (5-FU; 1 microM/l) and radiotherapy (2, 6, and 10 Gy) on the colon cancer cell line DLD-1 with mutations in K-, N- and H-ras, c-myb, c-myc, p53, fos, sis and DNA repair genes. Drugs were added 3 h after cultivation. Radiotherapy was performed on the 3rd day of the study. On the 3rd day, medium and drugs were changed. Evaluations were performed on the 6th day. RESULTS: Administration of L-744,832, neither alone nor its combination with 5-FU and radiation, affected the number of DLD-1 cells and apoptosis rates. Regarding its effects on the cell cycle, L-744,832 was shown to lead to G(0)/G(1) and G(2)/M accumulation in a dose-dependent manner when administered alone. However, in combination with 5-FU, only a G(0)/G(1) accumulation was observed. CONCLUSION: Our study showed that FTI L-744,832 does not effect the cell number and apoptosis rate of DLD-1 cells and it cannot overcome 5-FU and radiation resistance, although it is able to modify some phases of the cell cycle.

Kim, C. Y., A. J. Giaccia, et al. (1992). "Differential expression of protein kinase C epsilon protein in lung cancer cell lines by ionising radiation." Br J Cancer **66**(5): 844-9.

The effect of ionising radiation on the regulation of gene and protein expression is complex. This study focuses on the translational regulational of the epsilon isoform of protein kinase C by ionising radiation. We found that protein kinase C epsilon is rapidly increased in the human lung adenocarcinoma cell line A549 following irradiation. Western blots showed increased accumulation of this protein at doses as low as 75 cGy after 15 min post irradiation. Maximal induction (11-fold over unirradiated cells) of PKC epsilon occurred at 150 cGy within 1 h after treatment by X-rays in A549 cells. The increased levels of PKC epsilon protein after X-rays does not require de novo protein or RNA synthesis, suggesting that this increase is post-translationally controlled. In contrast to A549 cells PKC epsilon levels in the large cell lung carcinoma cell line NCI H661 were not induced by radiation. In the small cell lung carcinoma cell line NCI N417, PKC epsilon was also not induced but a higher molecular weight PKC epsilon protein, suggestive of phosphorylation, appeared at 2 h after irradiation. The variation in induction or phosphorylation of PKC epsilon by ionising radiation in the cell lines tested in this study suggested that no clear correlation existed between intrinsic radiation sensitivity and PKC epsilon induction. To determine whether PKC epsilon does play a role in cell survival to irradiation, we used the protein kinase inhibitor staurosporin to decrease PKC activity and found that staurosporin sensitised cells to killing by ionising radiation. Pulsed field gel electrophoresis, however, indicated that DNA double-strand break repair was not decreased, suggesting that PKC epsilon is modifying the fidelity of rejoining and not the overall magnitude of repair. The regulation of PKC by ionising radiation will be discussed with respect to the biological consequences of gene induction by DNA damage agents.

Kyprianou, N., E. D. King, et al. (1997). "bcl-2 over-expression delays radiation-induced apoptosis without affecting the clonogenic survival of human prostate cancer cells." Int J Cancer **70**(3): 341-8.

In this study we evaluated the effect of over-expression of the bcl-2 gene, a potent apoptosis suppressor, on radiation-induced apoptotic cell death in 2 human prostate cancer cell lines, androgen-independent PC-3 cells and androgen-sensitive LNCaP cells. Cells were transfected with the bcl-2 gene and bcl-2 transfectant clones isolated under neomycin selection; bcl-2 gene integration and level of mRNA and protein expression in the cloned transfectants were examined by Southern, Northern and Western blot analyses, respectively. Parental, neo control and bcl-2-expressing cells were exposed to single or fractionated doses of ionizing irradiation, and the cellular response to radiation was determined at 24, 48 and 72 hr post-irradiation, on the basis of: (i) loss of cell viability, (ii) clonogenic survival and (iii) induction of apoptotic DNA fragmentation. At 24 hr post-irradiation all cell lines, i.e., parental and bcl-2 transfectants, failed to form colonies, though the majority of bcl-2-expressing cells did not exhibit apoptotic morphology; bcl-2 over-expression in both cell lines reduced apoptosis 48 hr post-irradiation from 20-25% to 5% at a dose of 2,000 cGy. By 72 hr, bcl-2 over-expression afforded a 3-fold protection from radiation-induced apoptosis. There was no significant difference, however, in the clonogenic survival of the parental and bcl-2-expressing cells. Furthermore, there was a 24 hr delay in induction of the apoptosis marker gene SGP-2/TRPM-2 in the bcl-2-expressing cells, co-incidental with the delay in apoptotic DNA fragmentation.

Kyprianou, N. and S. Rock (1998). "Radiation-induced apoptosis of human prostate cancer cells is independent of mutant p53 overexpression." Anticancer Res **18**(2A): 897-905.

BACKGROUND: Previous studies have demonstrated that androgen-independent prostate cancer cancer cells undergo apoptosis in response to ionizing irradiation. The p53 protein controls cell cycle arrest and apoptosis by acting as a checkpoint control that halts the cell cycle in G1, while DNA damage is present. In this study the effect of overexpression of mutant p53 protein, on radiation-induced apoptotic cell death of human prostate cancer cells PC-3 was investigated. MATERIALS AND METHODS: PC-3 cells were transfected with the plasmid encoding the mutant p53 sequence, and the neomycin resistance gene. Selected transfectant clones, were characterized at the molecular level (gene integration, and level of mRNA and protein expression) and cloned transfectants expressing high levels of p53 protein were treated with increasing doses of ionizing irradiation. The cellular response to radiation was determined on the basis of: a) clonogenic survival (colony forming ability of irradiated cells); b) induction of apoptosis as determined by the terminal transferase assay; c) apoptotic DNA fragmentation; and d) induction of expression of genes associated with prostate-apoptosis. RESULTS: Both mutant p53 transfectant and parental PC-3 cells underwent apoptosis in response to ionizing irradiation following similar kinetics of induction of DNA fragmentation. In addition, the magnitude of induction of expression of

prostate apoptosis associated genes, SGP-2 and TGF-beta, was similar in the mutant p53 overexpressing and parental PC-3 cells and coincidental with DNA fragmentation. CONCLUSIONS: These findings seriously challenge the involvement of p53 in radiation-induced apoptosis in human prostate cancer cells and suggest that p53 mutations provide no selective advantage in the development of radioresistance of prostate tumor cells within the context of p53 independent apoptotic pathway.

Lee, C., J. S. Kim, et al. (2004). "PTEN gene targeting reveals a radiation-induced size checkpoint in human cancer cells." Cancer Res **64**(19): 6906-14.

Following DNA damage, human cells arrest primarily in the G(1) and G(2) phases of the cell cycle. Here, we show that after irradiation, human cancer cells with targeted deletion of PTEN or naturally occurring PTEN mutations can exert G(1) and G(2) arrests but are unable to arrest in size. Pharmacological inhibition of phosphoinositol-3-kinase or mTOR in PTEN(-/-) cells restored the size arrest, whereas siRNA-mediated depletion of TSC2 in PTEN(+/+) cells attenuated the size arrest. Radiation treatment potentiated Akt activation in PTEN(-/-) but not PTEN(+/+) cells. Finally, abrogation of the size arrest via PTEN deletion conferred radiosensitivity both in vitro and in vivo. These results identify a new tumor suppressor gene-regulated, DNA damage-inducible arrest that occurs simultaneously with the G(1) and G(2) arrests but is genetically separable from them. We suggest that aberrant regulation of cell size during cell cycle arrest may be important in human cancer pathogenesis.

Leong, T., J. Whitty, et al. (2000). "Mutation analysis of BRCA1 and BRCA2 cancer predisposition genes in radiation hypersensitive cancer patients." Int J Radiat Oncol Biol Phys **48**(4): 959-65.

PURPOSE: The dose intensity of radiotherapy (RT) used in cancer treatment is limited in rare individuals who display severe normal tissue reactions after standard RT treatments. Novel predictive assays are required to identify these individuals prior to treatment. The mechanisms responsible for such reactions are unknown, but may involve dysfunction of genes involved in the sensing and response of cells to DNA damage. The breast cancer susceptibility genes BRCA1 and BRCA2 are implicated in DNA damage repair and the control of genome stability. The purpose of this study was to determine if clinical radiation hypersensitivity is related to mutations of the BRCA1 and BRCA2 genes. Such information is of potential use in the clinical management of BRCA mutation carriers and their families. METHODS AND MATERIALS: Twenty-two cancer patients who developed severe normal tissue reactions after RT were screened for mutations of BRCA1 and BRCA2, using various methods including protein truncation testing, direct DNA sequencing, and a PCR-based BRCA1 exon 13 duplication test. RESULTS: No mutations were detected in the 22 patients tested, despite screening for the majority of commonly described types of mutations of BRCA1 and BRCA2. CONCLUSION: These early results suggest that genes other than BRCA1 and BRCA2 probably account for most cases of clinical radiation hypersensitivity, and that screening for mutations of BRCA1 and BRCA2 is unlikely to be useful in predicting response to radiotherapy. However, it has not been excluded that some BRCA1 or BRCA2 heterozygotes might experience unexpected RT toxicity; further BRCA mutation screening on radiation sensitive individuals is warranted.

Li, Z., L. Xia, et al. (2001). "Effector genes altered in MCF-7 human breast cancer cells after exposure to fractionated ionizing radiation." Radiat Res **155**(4): 543-53.

Understanding the molecular mechanisms involved in the response of tumors to fractionated exposures to ionizing radiation is important for improving radiotherapy and/or radiochemotherapy. In the present study, we examined the expression of stress-related genes in an MCF-7 cell population (MCF-IR20) that has been derived through treatment with fractionated irradiation (2 Gy per fraction with a total dose of 40 Gy). MCF-IR20 cells showed a 1.6-fold increase in sensitization with dose at 10% isosurvival in a clonogenic assay, and a reduced growth delay (approximately 15 h compared to approximately 27 h), compared to the parental MCF-7 cells treated with a single dose of 5 Gy. To determine which effector genes were altered in the MCF-IR20 cells, the expression of stress-related effector genes was measured using a filter with 588 genes (Clontech) that included major elements involved in cell cycle control, DNA repair, and apoptosis. Compared to MCF-7 cells that were not exposed to fractionated radiation, 19 genes were up- regulated (2.2-5.1-fold) and 4 were down-regulated (2.7-3.4- fold) in the MCF-IR20 cells. In agreement with the array results, 6 up-regulated genes tested by RT-PCR showed elevated expression. Also, activities of the stress-related transcription factors NFKB, TP53 and AP1 showed a 1.2-4.5-fold increase after a single dose of 5 Gy in MCF-IR20 cells compared with parental MCF-7 cells. However, when the radioresistant MCF-IR20 cell were cultured for more than 12 passages after fractionated irradiation (MCF-RV), radioresistance was lost, with the radiosensitivity being the same as

the parental MCF- 7 cells. Interestingly, expression levels of CCNB1, CD9 and CDKN1A in MCF-RV cells returned to levels expressed by the parental cells, whereas the expression levels of three other genes, MSH2, MSH6 and RPA remained elevated. To determine if any of the changes in gene expression could be responsible for the induced radioresistance, CCNB1 and CDKN1A, both of which were up-regulated in MCF-IR20 cells and down-regulated in MCF-RV cells, were studied further by transfection with antisense oligonucleotides. Antisense of CCNB1 significantly reduced the clonogenic survival of MCF-IR20 cells at doses of 5 and 10 Gy, from 42% to 26% and from 5.7% to 1.0%, respectively. Antisense of CDKN1A, however, had no effect on radiation survival of MCF-IR20 cells. In summary, these results suggest that stress-related effector genes are altered in cells after treatment with fractionated irradiation, and that up-regulation of CCNB1 is responsible, at least in part, for radioresistance after fractionated irradiation.

Lindel, K., P. Burri, et al. (2005). "Human papillomavirus status in advanced cervical cancer: predictive and prognostic significance for curative radiation treatment." Int J Gynecol Cancer **15**(2): 278-84.

Human papillomavirus (HPV) infection plays a major role in oncogenesis of squamous cell carcinoma of the cervix. This study was performed to investigate if HPV status and E2 gene integrity are prognostic parameters for clinical outcome and predictive for radiation response. Forty women with locally advanced cervical cancer treated with curative radiotherapy were analyzed for HPV infection and E2 gene integrity by multiplex polymerase chain reaction. Statistical analyses were performed for overall survival, disease-free survival (DFS), local progression-free survival, and treatment response (clinical complete remission). Twenty-eight (70%) of 40 carcinomas were HPV positive. The only significant factor for a better overall survival, DFS, and local progression-free survival was HPV positivity ($P < 0.02$, $P = 0.02$, and $P < 0.05$, log-rank, respectively). HPV-positive tumors had a significantly better clinical complete remission (67% vs 33%, $P = 0.04$, Fisher's exact test). An intact E2 gene region showed a trend for a better DFS ($P = 0.1$, log-rank). This study reveals HPV as an independent prognostic parameter for outcome and radiation response. Integration of the virus genome into host cell DNA might be a molecular target to determine the treatment response of HPV-positive cancers.

Liu, S. S., A. N. Cheung, et al. (2003). "Differential gene expression in cervical cancer cell lines before and after ionizing radiation." Int J Oncol **22**(5): 1091-9.

Our previous study demonstrated a correlation between increased apoptotic index in pre-radiotherapy cervical cancer and poor patient survival. Apoptosis may thus play an important role in the response of cervical cancer to radiation. Most of cervical carcinomas are associated with human papillomavirus (HPV), and the oncoproteins E6 and E7 disrupt the functions of tumour suppressor genes, resulting in genetic alteration. To understand the multiple genetic changes related to cell radiosensitivity and the induction of apoptosis, two cervical cancer cell lines, SiHa (with HPV infection) and C-33A (contains a mutant p53 gene), were selected for present studied. The gene expression patterns in these cell lines were compared before and after radiation. When compared to normal cervical tissues, differential expressions were observed in 46 genes among the two cell lines studied. Thirty-three genes showed altered expressions after radiation induction. Three out of ten genes that showed differential responsiveness to radiation in the two cell lines were further confirmed by reverse transcriptase-polymerase chain reaction (RT-PCR). Bak and c-abl were found to be potential genes that may play important roles in signaling apoptosis in cervical cancer cells following radiation induction.

Lu, B., Y. Mu, et al. (2004). "Survivin as a therapeutic target for radiation sensitization in lung cancer." Cancer Res **64**(8): 2840-5.

Expression of survivin is elevated in most malignancies, especially in radiation-resistant cell lines. In this study, we investigated how radiation affects survivin expression in primary endothelial cells as well as in malignant cell lines. We found that 3 Gy significantly reduced survivin protein level in human umbilical vein endothelial cells (HUVECs) but not in tumor cell lines. Flow cytometry studies suggest that the down-regulation of survivin is independent of cell cycle. In addition, survivin mRNA level was also down-regulatable by irradiation. However, it was abrogated by actinomycin D-mediated inhibition of gene transcription. Luciferase reporter gene assays suggest that irradiation suppressed the survivin promoter. p53 overexpression reduced survivin expression, but overexpression of a p53 mutant failed to abolish the radiation-induced down-regulation in HUVECs. Alteration of p53 status in Val138 lung cancer cell line also failed to restore the radiation-inducible down-regulation. Overexpression of survivin in 293 cells prevented apoptosis induced by irradiation and increased cell viability after irradiation. The inhibition of survivin using antisense oligonucleotides caused a significant decrease in cell

viability of irradiated H460 lung cancer cells. These data suggest that radiation transcriptionally down-regulates survivin in HUVECs. This regulatory mechanism is defective in malignancies and is not mediated by p53. Survivin overexpression may lead to resistance to radiotherapy by inhibiting apoptosis and enhancing cell viability. The inhibition of survivin results in sensitization of H460 lung cancer cells to radiation. These studies suggest that survivin may be a target for cancer therapy.

Lu, X., C. Yang, et al. (2008). "Inactivation of gadd45a sensitizes epithelial cancer cells to ionizing radiation in vivo resulting in prolonged survival." Cancer Res **68**(10): 3579-83.

Ionizing radiation (IR) therapy is one of the most commonly used treatments for cancer patients. The responses of tumor cells to IR are often tissue specific and depend on pathway aberrations present in the tumor. Identifying molecules and mechanisms that sensitize tumor cells to IR provides new potential therapeutic strategies for cancer treatment. In this study, we used two genetically engineered mouse carcinoma models, brain choroid plexus carcinoma (CPC) and prostate, to test the effect of inactivating gadd45a, a DNA damage response p53 target gene, on tumor responses to IR. We show that gadd45a deficiency significantly increases tumor cell death after radiation. Effect on survival was assessed in the CPC model and was extended in IR-treated mice with gadd45a deficiency compared with those expressing wild-type gadd45a. These studies show a significant effect of gadd45a inactivation in sensitizing tumor cells to IR, implicating gadd45a as a potential drug target in radiotherapy management.

Lupold, S. E. and R. Rodriguez (2005). "Adenoviral gene therapy, radiation, and prostate cancer." Rev Urol **7**(4): 193-202.

Viral gene therapy has exceptional potential as a specifically tailored cancer treatment. However, enthusiasm for cancer gene therapy has varied over the years, partly owing to safety concerns after the death of a young volunteer in a clinical trial for a genetic disease. Since this singular tragedy, results from numerous clinical trials over the past 10 years have restored the excellent safety profile of adenoviral vectors. These vectors have been extensively studied in phase I and II trials as intraprostatically administered agents for patients with locally recurrent and high-risk local prostate cancer. Promising therapeutic responses have been reported in several studies with both oncolytic and suicide gene therapy strategies. The additional benefit of combining gene therapy with radiation therapy has also been realized; replicating adenoviruses inhibit DNA repair pathways, resulting in a synergistic sensitization to radiation. Other, nonreplicating suicide gene therapy strategies are also significantly enhanced with radiation. Combined radiation/gene therapy is currently being studied in phase I and II clinical trials and will likely be the first adenoviral gene therapy mechanism to become available to urologists in the clinic. Systemic gene therapy for metastatic disease is also a major goal of the field, and clinical trials are currently under way for hormone-resistant metastatic prostate cancer. Second- and third-generation "re-targeted" viral vectors, currently being developed in the laboratory, are likely to further improve these systemic trials.

Mamon, H. J., W. Dahlberg, et al. (2003). "Differing effects of breast cancer 1, early onset (BRCA1) and ataxia-telangiectasia mutated (ATM) mutations on cellular responses to ionizing radiation." Int J Radiat Biol **79**(10): 817-29.

PURPOSE: The ataxia-telangiectasia mutated (ATM) gene encodes a protein kinase, the activation of which is an early event in the cellular response to ionizing radiation. One of the many substrates of ATM is BRCA1 (breast cancer 1, early onset gene), which has been associated with susceptibility to breast and ovarian cancer, and has been implicated in DNA repair processes. Various cellular responses to radiation were analysed in cells with mutations in ATM or BRCA1 in an attempt to clarify which effects of ATM can be mediated through BRCA1. MATERIALS AND METHODS: The response to radiation of cells with mutations in ATM or BRCA1 was examined, as were BRCA1-mutant tumour cells transfected with an exogenous wild-type BRCA1 allele. Assays included cell-survival curves, studies of potentially lethal damage repair, measurement of chromosomal aberrations and of G1 arrest, and Western blot analysis of lysates of irradiated cells to determine the phosphorylation of the product of the human Mdm2 gene (HDM2). RESULTS: Both ATM and BRCA1 mutations were associated with sensitivity to ionizing radiation, deficient repair of potentially lethal damage and markedly increased chromosomal aberrations. A BRCA1-mutated tumour cell line HCC1937, like ATM mutant cells, did not exhibit a normal G1 arrest but, unlike ATM mutant cells, did exhibit phosphorylation of HDM2. Expression of wild-type BRCA1 in HCC1937 cells partially restored radioresistance, restored repair of potentially lethal damage and markedly reduced radiation-induced chromosomal aberrations. G1 arrest, however, was not restored by expression of BRCA1. CONCLUSIONS: The results are consistent with a model in which ATM phosphorylation of BRCA1 regulates DNA repair functions, particularly those involved in potentially lethal damage repair and

chromosomal integrity, but not other aspects of the cellular response to radiation such as G1 cell cycle arrest. To the authors' knowledge, this is the first demonstration of the ability of exogenously expressed BRCA1 to restore the ability to perform potentially lethal damage repair and maintain chromosomal integrity in irradiated cells.

Mendonca, M. S., D. L. Farrington, et al. (2004). "Homozygous deletions within the 11q13 cervical cancer tumor-suppressor locus in radiation-induced, neoplastically transformed human hybrid cells." Genes Chromosomes Cancer **39**(4): 277-87.

Studies on nontumorigenic and tumorigenic human cell hybrids derived from the fusion of HeLa (a cervical cancer cell line) with GM00077 (a normal skin fibroblast cell line) have demonstrated "functional" tumor-suppressor activity on chromosome 11. It has been shown that several of the neoplastically transformed radiation-induced hybrid cells called GIMs (gamma ray induced mutants), isolated from the nontumorigenic CGL1 cells, have lost one copy of the fibroblast chromosome 11. We hypothesized, therefore, that the remaining copy of the gene might be mutated in the cytogenetically intact copy of fibroblast chromosome 11. Because a cervical cancer tumor suppressor locus has been localized to chromosome band 11q13, we performed deletion-mapping analysis of eight different GIMs using a total of 32 different polymorphic and microsatellite markers on the long arm (q arm) of chromosome 11. Four irradiated, nontumorigenic hybrid cell lines, called CONs, were also analyzed. Allelic deletion was ascertained by the loss of a fibroblast allele in the hybrid cell lines. The analysis confirmed the loss of a fibroblast chromosome 11 in five of the GIMs. Further, homozygous deletion (complete loss) of chromosome band 11q13 band sequences, including that of D11S913, was observed in two of the GIMs. Detailed mapping with genomic sequences localized the homozygous deletion to a 5.7-kb interval between EST AW167735 and EST F05086. Southern blot hybridization using genomic DNA probes from the D11S913 locus confirmed the existence of homozygous deletion in the two GIM cell lines. Additionally, PCR analysis showed a reduction in signal intensity for a marker mapped 31 kb centromeric of D11S913 in four other GIMs. Finally, Northern blot hybridization with the genomic probes revealed the presence of a novel >15-kb transcript in six of the GIMs. These transcripts were not observed in the nontumorigenic hybrid cell lines. Because the chromosome 11q13 band deletions in the tumorigenic hybrid cell lines overlapped with the minimal deletion in cervical cancer, the data suggest that the same gene may be involved in the development of cervical cancer and in radiation-induced carcinogenesis. We propose that a gene localized in proximity to the homozygous deletion is the candidate tumor-suppressor gene.

Millikan, R. C., J. S. Player, et al. (2005). "Polymorphisms in DNA repair genes, medical exposure to ionizing radiation, and breast cancer risk." Cancer Epidemiol Biomarkers Prev **14**(10): 2326-34.

An epidemiologic study was conducted to determine whether polymorphisms in DNA repair genes modify the association between breast cancer risk and exposure to ionizing radiation. Self-reported exposure to ionizing radiation from medical sources was evaluated as part of a population-based, case-control study of breast cancer in African-American (894 cases and 788 controls) and White (1,417 cases and 1,234 controls) women. Genotyping was conducted for polymorphisms in four genes involved in repair of radiation-induced DNA damage, the double-strand break repair pathway: X-ray cross-complementing group 3 (XRCC3) codon 241 Thr/Met, Nijmegen breakage syndrome 1 (NBS1) codon 185 Glu/Gln, X-ray cross-complementing group 2 (XRCC2) codon 188 Arg/His, and breast cancer susceptibility gene 2 (BRCH2) codon 372 Asn/His. Allele and genotype frequencies were not significantly different in cases compared with controls for all four genetic polymorphisms, and odds ratios for breast cancer were close to the null. Combining women with two, three, and four variant genotypes, a positive association was observed between breast cancer and number of lifetime mammograms (P(trend) < 0.0001). No association was observed among women with zero or one variant genotype (P = 0.86). Odds ratios for radiation treatments to the chest and number of lifetime chest X-rays were slightly elevated but not statistically significant among women with two to four variant genotypes. The study has several limitations, including inability to distinguish between diagnostic and screening mammograms or reliably classify prediagnostic mammograms and chest X-rays in cases. Prospective studies are needed to address whether common polymorphisms in DNA repair genes modify the effects of low-dose radiation exposure from medical sources.

Mirzayans, R., L. Enns, et al. (1996). "Faulty DNA polymerase delta/epsilon-mediated excision repair in response to gamma radiation or ultraviolet light in p53-deficient fibroblast strains from affected members of a cancer-prone family with Li-Fraumeni syndrome." Carcinogenesis **17**(4): 691-8.

Dermal fibroblast strains cultured from affected members of a cancer-prone family with Li-Fraumeni syndrome (LFS) harbor a point mutation in one allele of the p53 tumor suppressor gene, resulting

in loss of normal p53 function. In this study we have examined the ability of these p53-deficient strains to carry out the long-patch mode of excision repair, mediated by DNA polymerases delta and epsilon, after exposure to 60Co gamma radiation or far ultraviolet (UV) (chiefly 254 nm) light. Repair was monitored by incubation of the irradiated cultures in the presence of aphidicolin (apc) or 1-beta-D-arabinofuranosylcytosine (araC), each a specific inhibitor of long-patch repair, followed by measurement of drug-induced DNA strand breaks (reflecting non-ligated strand incision events) by alkaline sucrose velocity sedimentation. The LFS strains displayed deficient repair capacity in response to both gamma rays and UV light. The repair anomaly in UV-irradiated LFS cultures was manifested not only in the overall genome, but also in the transcriptionally active, preferentially repaired c-myc gene. Using autoradiography we also assessed unscheduled DNA synthesis (UDS) after UV irradiation and found this conventional measure of repair replication to be deficient in LFS strains. Moreover, both apc and araC decreased the level of UV-induced UDS by approximately 75% in normal cells, but each had only a marginal effect on LFS cells. We further demonstrated that the LFS strains are impaired in the recovery of both RNA and replicative DNA syntheses after UV treatment, two molecular anomalies of the DNA repair deficiency disorders xeroderma pigmentosum and Cockayne's syndrome. Together these results imply a critical role for wild-type p53 protein in DNA polymerase delta/epsilon-mediated excision repair, both the mechanism operating on the entire genome and that acting on expressed genes.

Mitsuhashi, M., D. Peel, et al. (2009). "Enhanced Expression of Radiation-induced Leukocyte CDKN1A mRNA in Multiple Primary Breast Cancer Patients: Potential New Marker of Cancer Susceptibility." Biomark Insights **4**: 201-9.

This study was designed to discover blood biomarkers of cancer susceptibility using invasive multiple (n = 21), single primary breast cancer (n = 21), and control subjects (n = 20). Heparinized whole blood was incubated at 37 degrees C for 2 hours after 0-10 Gy of radiation, then cell cycle arrest marker CDKN1A and apoptosis marker BBC3 mRNA were quantified. This epidemiological study was practically feasible because radiation-induced mRNA was preserved for at least 1 day whenever blood was stored at 4 degrees C (r(2) = 0.901). Moreover, blood could be stored frozen after radiation treatment (r(2) = 0.797). Radiation-induced CDKN1A and BBC3 mRNA were dose dependent, and the degree of induction of CDKN1A was correlated with that of BBC3 (r(2) = 0.679). Interestingly, multiple primary cases showed higher induction of CDKN1A mRNA than single primary and control groups, whereas BBC3 did not show such differences. The results suggested that cancer susceptibility represented by the multiple primary breast cancer cases was related to over-reaction of CDKN1A mRNA, not BBC3. The study also suggests that ex vivo gene expression analysis could potentially be used as a new tool in epidemiological studies for cancer and radiation sensitivity research.

Moon, S., S. Holley, et al. (2006). "Associations between G/A1229, A/G3944, T/C30875, C/T48200 and C/T65013 genotypes and haplotypes in the vitamin D receptor gene, ultraviolet radiation and susceptibility to prostate cancer." Ann Hum Genet **70**(Pt 2): 226-36.

Ultraviolet radiation (UVR) may protect against prostate cancer via a mechanism involving vitamin D. Thus, the vitamin D receptor (VDR) gene is a susceptibility candidate, though published data are discrepant. We studied the association of prostate cancer risk with five VDR single nucleotide polymorphisms (SNPs): G/A(1229) (SNP 1), A/G(3944) (SNP 2), T/C(30875) (SNP 3), C/T(48200) (SNP 4) and C/T(65013) (SNP 5), in 430 cancer and 310 benign prostatic hypertrophy (BPH) patients. The SNP 2 GG genotype frequency was lower in cancer than BPH patients (odds ratio = 0.63, 95% CI = 0.41-0.98, p = 0.039). SNPs 1 and 2, and SNPs 4 and 5, were in linkage disequilibrium. Two copies of haplotypes comprising SNPs 1-2, G-G (odds ratio = 0.63, p = 0.039), SNPs 2-3 G-C (odds ratio = 0.45, p = 0.008) and SNPs 1-2-3 G-G-C (odds ratio = 0.44, p = 0.006), but not SNPs 1-3, G-C (odds ratio = 0.81, p = 0.34), were associated with reduced risk (reference, no copies of the haplotypes). These associations were observed after stratification of subjects by extent of UVR exposure. These data show that SNP 2 GG genotype mediates prostate cancer risk, complementing studies reporting this allele is protective in malignant melanoma pathogenesis. They further suggest that published associations of risk with SNP 1 may result from linkage disequilibrium with SNP 2.

Nakagawa, T., N. Otsuki, et al. (2008). "Additive effects of oral fluoropyrimidine derivative S-1 and radiation on human hypopharyngeal cancer xenografts." Acta Otolaryngol **128**(8): 936-40.

CONCLUSION: The results presented here provide evidence of the enhancing effect of oral fluoropyrimidine derivative S-1 in concomitant chemoradiotherapy for head and neck cancer and further insights into its biological mechanism.

OBJECTIVE: To investigate the additive effect of S-1 and radiation for human hypopharyngeal cancer. MATERIALS AND METHODS: Nude mice bearing hypopharyngeal cancer cells (H891) were used for an in vivo model. S-1 was administered at a volume of 0.01 mg/g body weight per mouse for 14 days, and tumors were irradiated with 2.0 Gy on days 1 and 8. Mice treated with either radiation or S-1 alone were used as controls. The growth of tumors in each group was measured and, after completion of the treatment, a focused DNA array was used to determine mRNA expression levels in the tumors of 132 genes related to 5-fluorouracil (5-FU), radiation or carcinogenesis. RESULTS: The additive antitumor effect of S-1 and radiation was statistically confirmed on day 14 (p=0.01). DNA array assay showed significant changes in expression of several genes, including DNA repair gene POLD, angiogenesis-related genes bFGF and TP, DNA topoisomerase TOP2A, and nucleoside transporter gene ENT1.

Nakamura, M., K. Masutomi, et al. (2005). "Efficient inhibition of human telomerase reverse transcriptase expression by RNA interference sensitizes cancer cells to ionizing radiation and chemotherapy." Hum Gene Ther **16**(7): 859-68.

Telomerase activation plays critical roles in tumor growth and progression in part through the maintenance of telomere structure. Indeed, the ubiquitous expression of telomerase in human cancers makes telomerase a promising target for cancer therapy. Genetic, pharmacologic, and antisense methods to inhibit telomerase have been described; however, in most cases, cancer cell death was observed only after many cell divisions. Here, using retroviral delivery of small interfering RNAs (siRNAs) specific for the human telomerase reverse transcriptase (hTERT), we successfully inhibited telomerase activity in cervical cancer cell lines. Cells lacking hTERT expression exhibited significantly decreased telomerase activity and showed shortened telomeres and telomeric 3' overhangs with passage. These cells entered replicative senescence after a considerable number of cell divisions. Notably, the proliferative rate of these cells was significantly impaired, compared with control cells with telomerase activity, even in low-passage cells (population doubling 5). Likewise, colony-forming ability and tumorigenicity in mice were attenuated in low-passage cells lacking hTERT. We further examined the effects of chemotherapy and ionizing radiation on cells in which hTERT expression was suppressed. Cells lacking hTERT showed a significantly increased sensitivity, compared with control cells, to ionizing radiation or chemotherapeutic agents that induce DNA double- strand breaks, such as topoisomerase inhibitors or bleomycin. These findings suggest that an siRNA-based strategy can be applied to the development of novel telomerase inhibitors, the antitumor effects of which may be enhanced in combination with ionizing radiation and chemotherapy.

Nakashima, T., M. Masuda, et al. (1999). "Induction of apoptosis in maxillary sinus cancer cells by 5-fluorouracil, vitamin A and radiation (FAR) therapy." Eur Arch Otorhinolaryngol **256 Suppl 1**: S64-9.

The triple combination of 5-fluorouracil (5-FU), vitamin A and radiation (FAR therapy) has been used since 1972 to treat malignant tumors of the head and neck at Kyushu University. Using nick end labeling of tumor specimens, cells of human maxillary sinus carcinomas were observed previously to undergo apoptosis in response to FAR therapy. The present study evaluated the in vitro effects of FAR therapy on a human maxillary sinus cancer (IMC-4) cell line. We further compared the effects of FAR therapy on this cell line with those effects seen on tissue samples taken from patients with maxillary sinus cancers. DNA electrophoresis and electron microscopic examination of the IMC-4 cells after treatment with FAR therapy revealed typical apoptotic features. The effects of 50-100 micrograms/ml 5-FU, 10(-4) M all-trans-retinoic acid and radiation to 6 Gy on IMC-4 cells were evaluated by trypan blue dye exclusion and a cell colony formation assay. 5-FU and radiation caused direct cell death, while vitamin A mainly inhibited cell growth. The combination of these treatment as FAR therapy synergistically enhanced cell death and inhibited cell growth. Flow cytometry demonstrated that FAR-treated cells were arrested in the G1 phase of the cell cycle before undergoing apoptosis. To further investigate possible biological parameters influencing a tumor's apoptotic sensitivity, we also examined the expression of p53 in human maxillary sinus cancer cells and analyzed the relationship between p53 expression and apoptosis. However, no relationship was found between these two markers at the time point studied.

Neta, R. (2000). "The promise of molecular epidemiology in defining the association between radiation and cancer." Health Phys **79**(1): 77-84.

Molecular epidemiology involves the inclusion in epidemiologic studies of biologic measurements made at a genetic and molecular level and aims to improve the current knowledge of disease etiology and risk. One of the goals of molecular epidemiology studies of cancer is to determine the role of environmental and genetic factors in initiation and progression of malignancies and to use this knowledge to develop preventive strategies. This approach

promises extraordinary opportunities for revolutionizing the practice of medicine and reducing risk. However, this will be accompanied by the need to address and resolve many challenges, such as ensuring the appropriate interpretation of molecular testing and resolving associated ethical, legal, and social issues. Traditional epidemiologic approaches determined that exposure to ionizing radiation poses significantly increased risk of leukemia and several other types of cancer. Such studies provided the basis for setting exposure standards to protect the public and the workforce from potentially adverse effects of ionizing radiation. These standards were set by using modeling approaches to extrapolate from the biological effects observed in high-dose radiation studies to predicted, but mostly unmeasurable, effects at low radiation doses. It is anticipated that the addition of the molecular parameters to the population-based studies will help identify the genes and pathways characteristic of cancers due to radiation exposure of individuals, as well as identify susceptible or resistant subpopulations. In turn, the information about the molecular mechanisms should aid to improve risk assessment. While studies on radiogenic cancers are currently limited to only a few candidate genes, the exponential growth of scientific knowledge and technology promises expansion of knowledge about identity of participating genes and pathways in the future. This article is meant to provide an introductory overview of recent advances in understanding of carcinogenesis at the molecular level, with an emphasis of the aspects that may be of use in establishing the association between radiation and cancer.

Ng, C. E., Q. Y. Liu, et al. (2007). "Transcriptomic profiling of radiation-resistant or -sensitive human colorectal cancer cells: acute effects of a single X-radiation dose." Int J Oncol **30**(6): 1369-80.

We previously isolated several clones that were closely-related genetically from a human colorectal tumor (HCT116) cell line. These clones displayed significantly different X-radiation response phenotypes. In this paper, we investigated how a single dose of X-radiation modulated the transcriptomic profiles of either the radiation-resistant (HCT116Clone2_XRR) or the radiation-sensitive (HCT116CloneK_XRS) clone when each was compared to a reference clone, HCT116Clone10_control. The latter represented a control clone that displayed a similar X-radiation response as the parental HCT116 cells. Pooled RNAs were obtained from HCT116Clone2_XRR, HCT116CloneK_XRS or HCT116Clone10_control cells either before or at 10 min, 6 or 24 h after treatment with 4-Gy X-radiation. Transcriptomic profiles were assessed by cDNA microarrays. At least three independent experiments were carried out for each time point and statistical analysis was performed by paired t-test ($p<0.05$). From 19,200 genes/ESTs examined, we identified only 120 genes/ESTs that were differentially expressed at any one of these four time points. Interestingly, different patterns of gene modulation were observed between the radiation-sensitive and radiation-resistant clones. However, the fold changes of gene modulation were generally small (2-3 fold). Surprisingly, only 12.7% of 79 genes involved in DNA damage sensor/repair and cell cycle and between 2.6 and 9.2% of 76 genes involved in apoptosis, were significantly modulated in these early time points following irradiation. By comparison, up to 10% of 40 known housekeeping genes were differentially expressed. Thus in our experimental model, we were able to detect the up-regulation or down-regulation of mostly novel genes and/or pathways in the acute period (up to 24 h) following a single dose of 4-Gy X-radiation.

Nuyts, S., L. Van Mellaert, et al. (2001). "The use of radiation-induced bacterial promoters in anaerobic conditions: a means to control gene expression in clostridium-mediated therapy for cancer." Radiat Res **155**(5): 716-23.

Nuyts, S., Van Mellaert, L., Theys, J., Landuyt, W., Lambin, P. and Anne, J. The Use of Radiation-Induced Bacterial Promoters in Anaerobic Conditions: A Means to Control Gene Expression in Clostridium-Mediated Therapy for Cancer. Radiat. Res. 155, 716-723 (2001). Apathogenic clostridia, which have been genetically engineered to express therapeutic genes, will specifically target hypoxic and necrotic regions in tumors. This specificity can be improved further if the expression of these genes is controlled by a radiation-induced promoter, leading to spatial and temporal control of gene expression. We isolated two radiation-inducible genes of the SOS repair system of Clostridium. Northern blot experiments confirmed radiation activation of the recA and recN genes at a dose of 2 Gy. The promoter region of these genes was isolated and used to regulate expression of the lacZ gene under anaerobic conditions. For the recA promoter, a significant increase of beta-galactosidase activity of 20-30% was seen after 2 Gy irradiation. The recN promoter did not show a significant induction and had a 50-100 times lower basal expression. Treatment of the recombinant clostridial cultures with the cytostatic agent mitomycin C also resulted in a significant increase of beta-galactosidase activity that was under the control of recA or recN promoter. Oxygen does not appear to be necessary in the activation of the SOS repair system by irradiation as tested with Escherichia coli

since recA-deficient and recA-containing strains showed similar survival after treatment with UV and ionizing radiation in the presence or absence of oxygen.

Ohnishi, K., H. Inaba, et al. (2004). "C-terminal peptides of p53 molecules enhance radiation-induced apoptosis in human mutant p53 cancer cells." Apoptosis **9**(5): 591-7.

We propose here a novel p53-targeting radio-cancer therapy using p53 C-terminal peptides for patients having mutated p53. Hoechst 33342 staining showed that X-ray irradiation alone efficiently induced apoptotic bodies in wild-type p53 (wt p53) human head and neck cancer cells transfected with a neo control vector (SAS/neo cells), but hardly induced apoptotic bodies in mutation-type p53 (m p53) cells transfected with a vector carrying the m p53 gene (SAS/m p53). In contrast, transfection of p53 C-terminal peptides (amino acid residues 361-382 or 353-374) via liposomes caused a remarkable increase of apoptotic bodies in X-ray-irradiated SAS/m p53 cells, but did not enhance apoptotic bodies in X-ray-irradiated SAS/neo cells. In immunocytochemical analysis, positively stained cells for active type caspase-3 were observed at high frequency after X-ray irradiation in the SAS/m p53 cells pre-treated with p53 C-terminal peptides. In SAS/neo cells, positively stained cells for active type caspase-3 were observed with X-ray irradiation alone. Furthermore, protein extracts from X-ray-irradiated SAS/m p53 cells showed higher DNA-binding activity of p53 to p53 consensus sequence when supplemented in vitro with p53 C-terminal peptides than extracts from non-irradiated SAS/m p53 cells. These results suggest that radiation treatment in the presence of p53 C-terminal peptides is more effective for inducing p53 -mediated apoptosis than radiation treatment alone or p53 C-terminal peptide treatment alone, especially in m p53 cancer cells. This novel tool for enhancement of apoptosis induction in m p53 cells might be useful for p53-targeted radio-cancer therapy.

Ohnishi, K., Z. Scuric, et al. (2006). "siRNA targeting NBS1 or XIAP increases radiation sensitivity of human cancer cells independent of TP53 status." Radiat Res **166**(3): 454-62.

NBS1 is essential for the repair of radiation-induced DNA double-strand breaks (DSBs) in yeast and higher vertebrate cells. In this study, we examined whether suppressed NBS1 expression by small interference RNA (siRNA) could enhance radiation sensitivity in cancer cells with different TP53 status. We used human non-small cell lung cancer cells differing in TP53 gene status (H1299/wtp53 cells bearing wild-type TP53 or H1299/mp53 cells bearing mutant TP53). A DNA cassette expressing siRNA targeted for the NBS1 gene was transfected into those cell lines, and radiation sensitivity was examined with a colony-forming assay. Cellular levels of NBS1 and other proteins were analyzed using Western blotting. We found that the radiation sensitivity of H1299/wtp53 and H1299/mp53 cells was enhanced by transfection of the DNA cassette. In the NBS1-siRNA-transfected cells, we observed decreased constitutive expression of NBS1 protein and decreased radiation-induced accumulation of phosphorylated NBS1 protein. In addition, radiation-induced expression of the transcription factor NF-kappaB (NFKB) and XIAP (X-chromosome-linked inhibitor of apoptosis protein) was suppressed by NBS1-siRNA. Enhanced X-ray sensitivity after NBS1-siRNA transfection was achieved in TP53 wild-type cells and sensitivity was even more pronounced in TP53 mutant cells. The transfection of siRNA targeted for XIAP also enhanced X-ray sensitivity even more for TP53 mutant cells compared to TP53 wild-type cells. Our data suggest that the sensitization to radiation results from NBS1-siRNA-mediated suppression of DNA repair and/ or X-ray-induced cell survival signaling pathways through NFKB and XIAP. siRNA targeting appears to be a novel radiation-sensitizing agent, particularly in human TP53 mutant cancer cells.

Ohnishi, T. (2005). "The role of the p53 molecule in cancer therapies with radiation and/or hyperthermia." J Cancer Res Ther **1**(3): 147-50.

In recent years, cancer-related genes have been analyzed as predictive indicators for cancer therapies. Among those genes, the gene product of a tumor suppressor gene p53 plays an important role in cancer therapy, because the p53 molecule induces cell-cycle arrest, apoptosis and depression of DNA repair after cancer therapies such as radiation, hyperthermia and anti-cancer agents. An abnormality of the p53 gene might introduce low efficiency in their cancer therapies. Mutations of p53 are observed at a high frequency in human tumors, and are recognized in about half of all malignant tumors in human. In the both systems of a human cell culture and their transplanted tumor, the sensitivities to radiation, heat and anti-cancer agents were observed in wild-type p53 cells, but not in mutated or deleted p53 cells. In this review, we discuss the p53 activation signaling pathways through the modification of p53 molecules such as phosphorylation after radiation and/or hyperthermia treatments.

Ohnishi, T., A. Takahashi, et al. (2008). "p53 Targeting can enhance cancer therapy via radiation,

heat and anti-cancer agents." Anticancer Agents Med Chem **8**(5): 564-70.

In recent years, genes associated with cancer have been studied to assess their possible use as predictive indicators for cancer therapies. Among these, the gene product of the tumor suppressor gene p53 was found to play an important role in cancer therapy. The p53 molecule induces cell-cycle arrest, apoptosis and DNA repair after cells are subjected to cancer therapies involving radiation, heat and various anti-cancer agents. Mutations in p53 are observed at a high frequency in human tumors, and are present in about half of all malignant tumors in humans. Sensitization to radiation, heat and anti-cancer agents was observed in cells containing wild type p53, but not in cells containing mutated p53. This review discusses p53 activation of signaling pathways after exposure to cancer therapies which target p53; such therapies include chemical chaperones, the p53 gene, p53-C terminal peptides, and p53-targeting agents which enhance p53-central signal transduction pathways.

Oka, A., Y. Harima, et al. (2000). "Determination of p53-mediated transactivational ability in radiation-treated cervical cancer." Cancer Detect Prev **24**(3): 275-82.

To establish a new predictor of human cervical cancer radioresponse, we investigated the transactivational ability of p53 gene in tumor tissue for use as a marker of both pretreatment and postirradiation levels of mRNA of its downstream gene, WAF1. A total of 38 wild-type p53-bearing patients with histologically proved uterine cervical cancer were treated with definitive radiotherapy. Their p53 status was investigated using a single-strand conformation polymorphism analysis, and human papilloma virus 16, 18, 33, and 58 E6 was determined by polymerase chain reaction in pretreatment biopsy specimens. WAF1 mRNA was estimated by reverse transcriptase-polymerase chain reaction in both pretreatment specimens and those obtained after the administration of 10.8 Gy. Undetectable or low pretreatment levels of WAF1 mRNA were associated with complete response in the majority of cases, whereas only a few patients with a high pretreatment WAF1 level responded to treatment (P = .03). The increase in the postirradiation level of WAF1 mRNA positively correlated with better treatment response and long survival (P = .02). Although the human papilloma virus infection did not change the radiation response directly, it decreased the inducibility of WAF1. Consequently, the lower inducibility of WAF1 resulted in a poor treatment response. This is the first clinical report showing that the transactivational ability of p53 may be a determinant of the efficacy of cervical cancer radiotherapy.

O'Malley, B. W., Jr., D. Li, et al. (2003). "Molecular disruption of the MRN(95) complex induces radiation sensitivity in head and neck cancer." Laryngoscope **113**(9): 1588-94.

OBJECTIVES/HYPOTHESIS: The goal of the project was to develop a novel treatment strategy for head and neck cancer that induces radiation sensitivity. We hypothesized that the normal cellular DNA repair response in head and neck squamous cell carcinoma after radiation therapy can be blocked by a dominant negative disruption of the functioning MRN(95) protein complex. To test this hypothesis, we have developed a novel molecular therapy that inhibits the MRN(95) complex in tumor cells. Disruption the MRN(95) complex and thus DNA repair should result in enhanced tumor killing after classic external-beam radiation therapy. STUDY DESIGN: Experiments with human head and neck squamous cell carcinoma cell lines in vitro were performed. METHODS: Recombinant adenovirus vectors carrying the genes for enhancing radiation were generated. Human head and neck squamous cell carcinoma cells were treated with recombinant adenovirus vectors carrying the mutated p95 gene (p95-300), which contains the C-terminus 300 amino acids of the Nbs1(p95) protein. Tumor cells were also treated with adenovirus vector carrying full-length p95 protein or DL312 control virus; then all cell lines were subjected to 2 Gy irradiation. Cell growth curves were determined through colorimetric tetrazolium salt assay. RESULTS: Both the Ad-p95-300 and Ad-p94-his (full-length wild-type gene) demonstrated significant antitumor effect alone and in combination with radiation therapy compared with control samples. Cell cycle analysis demonstrated a shift toward the G2/M phase of the cell cycle. Analysis of telomerase activity demonstrated a significant decrease in telomerase activity after molecular therapy alone, and a greater decrease when combined with radiation therapy. CONCLUSION: Adenovirus-mediated mutant or full-length p95 molecular therapy demonstrated efficacy for the treatment of head and neck squamous cell carcinoma in vitro. This novel molecular therapy strategy induced significant radiation sensitization, induced a relative G2/M arrest, and decreased telomerase activity, all of which enhance the benefit of radiation therapy.

Oshita, F., Y. Kato, et al. (1999). "A feasibility study of continuous etoposide infusion combined with thoracic radiation for non-small cell lung cancer." Oncol Rep **6**(2): 263-8.

We conducted a feasibility study of continuous etoposide infusion, which was expected to suppress DNA repair after radiation, combined with radiation in patients with advanced non-small cell lung cancer (NSCLC). Between July 1995 and January 1997, 10 patients with NSCLC were registered. Thirty-six mg/m2/day etoposide was infused continuously for a mean of 19 days (range 14-26). Patients tolerated a mean total dose of accelerated hyperfractionated thoracic radiotherapy (1.5 Gy twice per day) of 52.6 Gy (range 33-60). The primary tumors of 7 patients showed partial responses and distant metastasis progression occurred before primary tumor progression in all 7 responders. The hematological adverse effects of chemoradiotherapy were grade 3 or 4 leukopenia in all 10 patients, grade 3 anemia developed in 3, and 2 had grade 3 thrombocytopenia. Six patients contracted infections and one of them died of pneumonia. The major non-hematological adverse effect was esophagitis, which was grade 3 in 3 patients, one of whom died of renal dysfunction. The serum etoposide concentrations were 1.6-2.0 microgram/ml, except in one patient, who had liver dysfunction due to B-type hepatitis. DNA repair gene XRCC1 mRNA expression in peripheral blood mononuclear cells was analyzed, using the reverse transcriptase-polymerase chain reaction, in 8 patients and was suppressed during etoposide infusion in 2. No relationship was observed between serum etoposide concentration and XRCC1 expression and clinical outcome. In conclusion, continuous etoposide infusion combined with thoracic radiation induces severe toxicity and should be given only after careful consideration.

Pajonk, F., A. van Ophoven, et al. (2005). "The proteasome inhibitor MG-132 sensitizes PC-3 prostate cancer cells to ionizing radiation by a DNA-PK-independent mechanism." BMC Cancer **5**: 76.

BACKGROUND: By modulating the expression levels of specific signal transduction molecules, the 26S proteasome plays a central role in determining cell cycle progression or arrest and cell survival or death in response to stress stimuli, including ionizing radiation. Inhibition of proteasome function by specific drugs results in cell cycle arrest, apoptosis and radiosensitization of many cancer cell lines. This study investigates whether there is also a concomitant increase in cellular radiosensitivity if proteasome inhibition occurs only transiently before radiation. Further, since proteasome inhibition has been shown to activate caspase-3, which is involved in apoptosis, and caspase-3 can cleave DNA-PKcs, which is involved in DNA-double strand repair, the hypothesis was tested that caspase-3 activation was essential for both apoptosis and radiosensitization following proteasome inhibition. METHODS: Prostate carcinoma PC-3 cells were treated with the reversible proteasome inhibitor MG-132. Cell cycle distribution, apoptosis, caspase-3 activity, DNA-PKcs protein levels and DNA-PK activity were monitored. Radiosensitivity was assessed using a clonogenic assay. RESULTS: Inhibition of proteasome function caused cell cycle arrest and apoptosis but this did not involve early activation of caspase-3. Short-time inhibition of proteasome function also caused radiosensitization but this did not involve a decrease in DNA-PKcs protein levels or DNA-PK activity. CONCLUSION: We conclude that caspase-dependent cleavage of DNA-PKcs during apoptosis does not contribute to the radiosensitizing effects of MG-132.

Pestell, K. E., C. J. Medlow, et al. (1998). "Characterisation of the p53 status, BCL-2 expression and radiation and platinum drug sensitivity of a panel of human ovarian cancer cell lines." Int J Cancer **77**(6): 913-8.

The P53 gene is frequently mutated in late stage ovarian cancer and has been proposed as a determinant of radiation and chemosensitivity. We have therefore determined the p53 functional status, P53 sequence, radiation sensitivity and cytotoxicity of cisplatin and the novel platinum analogue, AMD473, in a panel of 6 human ovarian cancer cell lines. Constitutive p53 protein levels were low in A2780, CH1, LK1, LK2 and PA1 but were markedly induced following irradiation. In OV1P, constitutive p53 protein was readily detectable and levels were induced slightly following irradiation. p21WAF1/CIP1 and MDM-2 mRNA were constitutively expressed in all the cell lines and expression was induced markedly following irradiation. There was marked radiation induced G1/S arrest in A2780 but only partial arrests in CH1, LK1, LK2, PA1 and OV1P lines. No mutations were found in A2780, CH1, LK1, LK2 and PA1 cells by single-strand conformational polymorphism (SSCP) analysis but a heterozygous point mutation was found in exon 5 of OV1P. All the cell lines were radiation sensitive and also relatively sensitive to cisplatin; however, OV1P was the most resistant being consistent with its heterozygous P53 status. AMD473 was less potent than cisplatin but a similar pattern of drug sensitivity was observed with the exception of LK2, which was resistant. CH1, LK1, LK2 and PA1 all expressed BCL-2 protein but there was no expression in A2780 and OV1P. Our results suggest an overall association between wild type P53 and radiation and platinum drug sensitivity in these ovarian cancer cell lines.

Piening, B. D., P. Wang, et al. (2009). "A radiation-derived gene expression signature predicts clinical

outcome for breast cancer patients." Radiat Res **171**(2): 141-54.

Activation of the DNA damage response pathway is a hallmark for early tumorigenesis, while loss of pathway activity is associated with disease progression. Thus we hypothesized that a gene expression signature associated with the DNA damage response may serve as a prognostic signature for outcome in cancer patients. We identified ionizing radiation-responsive transcripts in human lymphoblast cells derived from 12 individuals and used this signature to screen a panel of cancer data sets for the ability to predict long-term survival of cancer patients. We demonstrate that gene sets induced or repressed by ionizing radiation can predict clinical outcome in two independent breast cancer data sets, and we compare the radiation signature to previously described gene expression-based outcome predictors. While genes repressed in response to radiation likely represent the well-characterized proliferation signature predictive of breast cancer outcome, genes induced by radiation likely encode additional information representing other deregulated biological properties of tumors such as checkpoint or apoptotic responses.

Price, F. M., R. Parshad, et al. (1991). "Radiation-induced chromatid aberrations in Cockayne syndrome and xeroderma pigmentosum group C fibroblasts in relation to cancer predisposition." Cancer Genet Cytogenet **57**(1): 1-10.

We showed previously that the persistence of chromatid breaks and gaps after G2 phase irradiation with X-rays or near-UV visible light characterizes skin fibroblasts from individuals with cancer-prone genetic diseases. This abnormal response appears to result from deficient DNA repair during G2 and to be associated with cancer proneness. We have, therefore, compared the responses of cells from two genetic disorders, Cockayne syndrome (CS) and xeroderma pigmentosum complementation group C(XP-C), both of which exhibit cellular hypersensitivity to sunlight, but only one of which, XP, manifests a high rate of sunlight-induced cancer. CS cells, in contrast to XP cells, showed a normal G2 response to irradiation with either X-rays or near-UV visible light. However, CS cells showed a deficiency in repair of DNA damage inflicted by light during S and G1 phases of the cell cycle. The present results support the concept that deficient DNA repair during G2 phase plays a role in carcinogenesis. This deficient repair in the presence of DNA damage and continuous cell cycling from activation of proto-oncogenes or loss of suppressor genes may be necessary and sufficient for cancer development.

Quarmby, S., H. Fakhoury, et al. (2003). "Association of transforming growth factor beta-1 single nucleotide polymorphisms with radiation-induced damage to normal tissues in breast cancer patients." Int J Radiat Biol **79**(2): 137-43.

PURPOSE: To investigate whether transforming growth factor beta-1 (TGFbeta1) single nucleotide polymorphisms were associated with the susceptibility of breast cancer patients to severe radiation-induced normal tissue damage. MATERIALS AND METHODS: PCR-RFLP assays were performed for TGFbeta1 gene polymorphisms on DNA obtained from 103 breast cancer patients who received radiotherapy. The G-800A, C-509T, T+869C and G+915C polymorphic sites were examined, and genotype and allele frequencies of two subgroups of patients were calculated and compared. RESULTS: The less prevalent -509T and +869C alleles were significantly associated with a subgroup of patients who developed severe radiation-induced normal tissue fibrosis (n=15) when compared with those who did not (n=88) (odds ratio=3.4, p=0.0036, and 2.37, p=0.035, respectively). Furthermore, patients with the -509TT or +869CC genotypes were between seven and 15 times more likely to develop severe fibrosis. CONCLUSIONS: These findings imply a role for the -509T and +869C alleles in the pathobiological mechanisms underlying susceptibility to radiation-induced fibrosis. Their predictive value would be limited to patients who are -509TT or +869CC, but if "fibrosis-associated" polymorphic sites in other genes could be identified, it may be possible to detect fibrosis prone individuals before radiotherapy with greater certainty.

Rajaraman, P., P. Bhatti, et al. (2008). "Nucleotide excision repair polymorphisms may modify ionizing radiation-related breast cancer risk in US radiologic technologists." Int J Cancer **123**(11): 2713-6.

Exposure to ionizing radiation has been consistently associated with increased risk of female breast cancer. Although the majority of DNA damage caused by ionizing radiation is corrected by the base-excision repair pathway, certain types of multiple-base damage can only be repaired through the nucleotide excision repair pathway. In a nested case-control study of breast cancer in US radiologic technologists exposed to low levels of ionizing radiation (858 cases, 1,083 controls), we examined whether risk of breast cancer conferred by radiation was modified by nucleotide excision gene polymorphisms ERCC2 (XPD) rs13181, ERCC4 (XPF) rs1800067 and rs1800124, ERCC5 (XPG) rs1047769 and rs17655; and ERCC6 rs2228526. Of the 6 ERCC variants examined, only ERCC5 rs17655 showed a borderline main effect association with breast cancer risk

(OR(GC) = 1.1, OR(CC) = 1.3; p-trend = 0.08), with some indication that individuals carrying the C allele variant were more susceptible to the effects of occupational radiation (EOR/Gy(GG) = 1.0, 95% CI = <0, 6.0; EOR/Gy(GC/CC) = 5.9, 95% CI = 0.9, 14.4; p(het) = 0.10). ERCC2 rs13181, although not associated with breast cancer risk overall, statistically significantly modified the effect of occupational radiation dose on risk of breast cancer (EOR/Gy(AA) = 9.1, 95% CI = 2.1-21.3; EOR/Gy(AC/CC) = 0.6, 95% CI = <0, 4.6; p(het) = 0.01). These results suggest that common variants in nucleotide excision repair genes may modify the association between occupational radiation exposure and breast cancer risk.

Rakozy, C., D. J. Grignon, et al. (1999). "p53 gene alterations in prostate cancer after radiation failure and their association with clinical outcome: a molecular and immunohistochemical analysis." Pathol Res Pract **195**(3): 129-35.

This study evaluates the prevalence of p53 gene mutations in prostate cancer in salvage prostatectomies after radiation failure using single strand conformational polymorphism (SSCP) and direct sequencing of the polymerase chain reaction (PCR) product. Findings were correlated with immunohistochemically (IHC) detectable p53 expression in residual prostate cancer. The usefulness of p53 as a marker of clinical outcome was evaluated. Thirty-three cases were available for molecular and immunohistochemical analysis. Immunohistochemical stains for p53 were performed with clone DO7. PCR-SSCP for mutations in the coding region of p53 DNA (exons 4-9) was performed on all immunopositive cases and 12 of 23 immunonegative cases. All samples with an SSCP shift were sequenced for the respective exon. Patients were evaluated for biochemical failure for 1-82 months (median 38 months) following surgery. Immunohistochemical p53 reactivity was noted in 10 of 33 (30%) patients. Among p53 immunopositive cases SSCP shifts were seen in 7 of 10 (70%) samples with 5 of the 7 (71%) showing p53 mutations. Univariate analysis revealed abnormal expression of p53 protein by immunohistochemistry to be a significant predictor of poorer outcome (p = 0.025, log rank), however this was not independent of pathologic stage, surgical margin status and Gleason score. The presence of p53 gene mutations by PCR-SSCP and direct sequencing did not predict for outcome. In our study 30% of prostate cancers at the time of salvage prostatectomy after radiation failure expressed immunohistochemically detectable p53. PCR-SSCP and sequencing shows that not all of these cases have detectable mutations in the most frequent mutation sites (exons 4-9). Clinical failure is more common in the group of prostate cancer patients with abnormal p53 immunoreactivity.

Ribeiro, J. C., A. R. Barnetson, et al. (1997). "Relationship between radiation response and p53 status in human bladder cancer cells." Int J Radiat Biol **72**(1): 11-20.

Mutations in the p53 tumour suppressor gene are found at high frequency in bladder cancer. There is strong evidence that p53 plays an important role in controlling the cell cycle after DNA damage by ionizing radiation. However, the effect of loss of p53 function on radiosensitivity is not yet clear. Radiotherapy combined with chemotherapy is the most common treatment for patients with invasive bladder cancer. Recently three bladder cancer cell lines have been established and this paper investigates the p53 status and clonogenic survival of these cell lines following irradiation. It was found that one line expresses wt p53 (UCRU-BL-13) and two lines contain a codon 72 polymorphism (UCRU-BL-17 and UCRU-BL-28). UCRU-BL-17 cells also contain a point mutation affecting codon 280. The level of p53 expression in the cell lines is clearly different, with UCRU-BL-17 expressing a higher level of p53 compared with UCRU-BL-13; UCRU-BL-28 expressed intermediate levels. The clonogenic survival of these cell lines has been determined. It was found that the line expressing a p53 mutation was more sensitive than those with wild type p53, providing support for a model in which loss of p53 function is associated with increased radiosensitivity, possibly due to reduced p53-dependent DNA repair.

Ritter, M. A., K. W. Gilchrist, et al. (2002). "The role of p53 in radiation therapy outcomes for favorable-to-intermediate-risk prostate cancer." Int J Radiat Oncol Biol Phys **53**(3): 574-80.

PURPOSE: Some prostate cancers may have molecular alterations that render them less responsive to radiation therapy; identification of these alterations before treatment might allow improved treatment optimization. This study investigated whether p53, a potential molecular determinant, could predict long-term radiation therapy outcome in a restricted group of relatively favorable-risk prostate cancer patients treated uniformly with irradiation alone. METHODS AND MATERIALS: This study included 53 patients previously treated with radiotherapy for favorable-to-intermediate-risk prostate cancer. These patients were selected for relatively low pretreatment PSAs (< or =21 ng/mL) and Gleason scores (< or =7) to decrease the likelihood of nonlocalized disease, because disease localization was necessary to examine the efficacy of localized radiation therapy. The status of p53 was

immunohistochemically assessed in paraffin-embedded pretreatment biopsy specimens, along with appropriate controls. This marker was selected based upon a usable mutation prevalence in early-stage prostate cancer and its potential linkage with radiation response via cell cycle, DNA repair, and cell death pathways. Correlation between p53 mutation and clinical outcome was analyzed in univariate and multivariate fashion and included conventional prognosticators, such as stage, grade, and PSA. Freedom from biochemical failure was determined using American Society for Therapeutic Radiology and Oncology criteria. Limitations of prior studies were potentially avoided by requiring adequate posttreatment follow-up (median follow-up in nonfailing patients of 5.1 years), as well as pretreatment PSA and Gleason scores that suggested localized disease, and uniformity of treatment. RESULTS: The total group of 53 favorable-to-intermediate-risk patients demonstrated an actuarial biochemical failure rate of 35% at 5 years. Forty percent of all specimens had a greater than 10% labeling index for p53 mutation, and actuarial biochemical control was found to strongly and independently correlate with p53 status. Patients with higher p53 labeling indices demonstrated significantly higher PSA failure rates ($p < 0.001$). In contrast, p53 status did not correlate with pretreatment PSA, grade, or tumor stage. Similarly, pretreatment PSA (log-rank 0.22), Gleason score (log-rank 0.93), and T stage (log-rank 0.15) were not prognostic for outcome in this group of patients selected for their relatively favorable clinical characteristics. CONCLUSIONS: (1) p53 status in pretreatment biopsies strongly predicted for long-term biochemical control after radiation therapy in favorable-to-intermediate-risk prostate cancer patients. (2) If validated in other independent clinical data sets, p53 status should be considered as a stratification factor in future clinical trials and could be useful in guiding treatment. Abnormal p53 status might favor surgical management, aggressive dose escalation, or p53-targeted therapy.

Rothkamm, K. and M. Lobrich (2002). "Misrepair of radiation-induced DNA double-strand breaks and its relevance for tumorigenesis and cancer treatment (review)." Int J Oncol **21**(2): 433-40.

The faithful repair of DNA double-strand breaks (DSBs) is probably one of the most critical tasks for a cell in order to maintain its genomic integrity since these lesions may lead to chromosome breaks or rearrangements, mutations, cell death or cancer. DSBs can arise spontaneously during normal cellular DNA metabolism or may be induced by exogenous agents such as ionizing radiation. To overcome the danger that emanates from these lesions, eukaryotic cells have evolved specific pathways for processing DSBs by either homology-dependent or non-homologous repair mechanisms. This review focuses on the formation of genomic rearrangements that arise by joining incorrect break ends and on the factors that influence repair fidelity. Recent studies indicate that the probability for a break to be incorrectly rejoined is fairly low when DSBs are spatially separated but increases drastically when multiple breaks coincide. The formation of genomic rearrangements in situations of multiple breaks is mediated by non-homologous end-joining, the predominant DSB repair pathway in mammalian cells. Interestingly, the same pathway is required for efficiently preserving chromosomal integrity in situations of separated breaks. Furthermore, the probability for a DSB to be faithfully repaired depends on its genomic location and on the cell cycle position. Methods for assaying DSB repair are discussed, again with emphasis on experimental systems that allow to determine whether a DSB is correctly or incorrectly rejoined.

Rukin, N. J., C. Luscombe, et al. (2007). "Prostate cancer susceptibility is mediated by interactions between exposure to ultraviolet radiation and polymorphisms in the 5' haplotype block of the vitamin D receptor gene." Cancer Lett **247**(2): 328-35.

Vitamin D receptor (VDR) polymorphisms are prostate cancer risk candidates. We determined if SNPs in haplotype block sub-regions C2 (SNPs C2-1, G/C(3436), C2-2, A/G(3944)) or C1 (C1-1, C/T(20965), C1-2, C/T(30056)) are associated with risk in an ultraviolet radiation (UVR)-dependent manner. In men with very low exposure, SNPs in both sub-regions were associated with risk. Various haplotypes in haplotype block C including G(3436)-A(3944)-C(20965)-C(30056), (G or C)-A-C-C and G-A-(C or T)-C were significantly associated with increased risk (odds ratios between 1.95 and 2.37). These findings suggest various block C SNPs are associated with prostate cancer risk via a mechanism involving exposure to sunlight.

Saga, Y., M. Suzuki, et al. (2002). "Enhanced expression of thymidylate synthase mediates resistance of uterine cervical cancer cells to radiation." Oncology **63**(2): 185-91.

It has been shown that there is an inverse relationship between the level of thymidylate synthase (TS) and therapeutic outcomes in patients with malignancies including cervical cancer. To clarify the mechanism of the poor prognosis of cervical cancer with high TS expression, we introduced TS cDNA to the human uterine cervical cancer cell line SKG-II and evaluated the effect of TS expression on its

radiosensitivity. After selection, stable transformants of SKG-II cells expressing high level of TS (SKG-II/TS) and control cells (SKG-II/luciferase) were obtained. The level of TS measured by the FdUMP-TS binding assay was significantly higher ($p < 0.05$) in SKG-II/TS than in control (2.0 +/- 0.1 and 1.3 +/- 0.1 pmol/mg, respectively). No difference was observed in in vitro cell growth or in vivo tumor growth. On evaluation of in vitro radiosensitivity, the 50% growth inhibitory dose (ID(50)) was 3.1 +/- 0.1 Gy in SKG-II/TS and was significantly higher ($p < 0.01$) than that in control (2.2 +/- 0.1 Gy). From these results, it is suggested that one of the reasons of poor outcome of cervical cancer to radiation is high TS expression.

Sakakura, C., E. A. Sweeney, et al. (1997). "Overexpression of bax enhances the radiation sensitivity in human breast cancer cells." Surg Today **27**(1): 90-3.

Bax-alpha, a splice variant of bax which promotes apoptosis, is expressed in many kinds of untransformed cell lines and breast tissue, whereas only weak or no expression could be detected in breast cancer cell lines and malignant breast tissue. Human breast cancer MCF-7 cells, which have a weak bax gene expression, were stably transfected with pCX2neo bax, encoding human bax and neomycin-resistant genes, and two unique clones (MCF-7/bax-1 and MCF-7/bax-2) were thus generated which expressed different levels of bax-alpha. Sensitivity to ionizing radiation (IR) was examined and each was more sensitive to IR than the parental MCF-7 cells. The degree of enhancement in radiosensitivity was dependent on the expression level of bax, and IR was found to induce intranucleosomal DNA fragmentation in stable transfectant but not in parent cells, thus suggesting that this sensitization is due to apoptosis. We suggest that exogenous bax-alpha expression might therefore be one of the factors determining cellular radiosensitivity in MCF-7 breast cancer cells and may potentially have a therapeutic application by enhancing radiation sensitivity in breast cancer cells.

Sankaranarayanan, K. and R. Chakraborty (1995). "Cancer predisposition, radiosensitivity and the risk of radiation-induced cancers. I. Background." Radiat Res **143**(2): 121-43.

This paper presents an overview of current knowledge on genetic predisposition to cancer and on enhanced sensitivity of cancer-predisposed genotypes to cancers induced by ionizing radiation. It is intended to provide a background and set the stage for the next papers in this series in which we will assess how such heterogeneity (with respect to predisposition to cancer and presence of radiosensitivity genotypes) in a population may affect estimates of the risk of radiation-induced cancers. The main findings and/or conclusions of the present paper are the following: (1) "Cancer-predisposing genes" (i.e. those at which germinal mutations predispose to cancer) are present in the human genome; these genes are responsible not only for the rare familial cancer syndromes but also for a proportion of the common cancers. At least 21 such genes have now been cloned (including 9 tumor suppressor genes, 11 DNA repair genes and 1 proto-oncogene); further, at least 8 putative tumor suppressor genes and a gene involved in ataxia telangiectasia have been localized to specific chromosomes. (2) These genes play crucial roles in the control of cellular proliferation, programmed cell death (apoptosis) and/or one or another DNA repair pathway. Consequently, mutations in these genes are likely to "liberate" the cells from the normal constraints imposed by them, resulting in unconstrained growth characteristic of cancer. (3) At present, the evidence for increased sensitivity of cancer-predisposed genotypes to radiation-induced cancers is limited. However, current knowledge of the known functions of the cancer-predisposing genes and of the consequences of mutations in these provide (a) sufficient grounds for assuming that the genotypes of those predisposed to cancer may be at an increased risk for radiation-induced cancers and (b) the rationale for attempts to estimate quantitatively the impact of genotype-dependent differences in cancer predisposition and radiosensitivity on cancer risks in an irradiated population.

Scott, B. R., S. A. Belinsky, et al. (2009). "Radiation-stimulated epigenetic reprogramming of adaptive-response genes in the lung: an evolutionary gift for mounting adaptive protection against lung cancer." Dose Response **7**(2): 104-31.

Humans are continuously exposed to low-level ionizing radiation from natural sources. However, harsher radiation environments persisted during our planet's early years and mammals survived via an evolutionary gift--a system of radiation-induced natural protective measures (adaptive protection). This system includes antioxidants, DNA repair, apoptosis of severely damaged cells, epigenetically regulated apoptosis (epiapoptosis) pathways that selectively remove precancerous and other aberrant cells, and immunity against cancer. We propose a novel model in which the protective system is regulated at least in part via radiation-stress-stimulated epigenetic reprogramming (epireprogramming) of adaptive-response genes. High-dose radiation can promote epigenetically silencing of adaptive-response genes (episilencing), for example via promoter-associated DNA and/or histone methylation and/or histone

deacetylation. Evidence is provided for low linear-energy-transfer (LET) radiation-activated natural protection (ANP) against high-LET alpha-radiation-induced lung cancer in plutonium-239 exposed rats and radon-progeny-exposed humans. Using a revised hormetic relative risk model for cancer induction that accounts for both epigenetic activation (epiactivation) and episilencing of genes, we demonstrate that, on average, >80% of alpha-radiation-induced rat lung cancers were prevented by chronic, low-rate gamma-ray ANP. Interestingly, lifetime exposure to residential radon at the Environmental Protection Agency's action level of 4 pCi L(-1) appears to be associated with on average a > 60% reduction in lung cancer cases, rather than an increase. We have used underlined italics to indicate newly introduced terminology.

Scott, S. D., M. C. Joiner, et al. (2002). "Optimizing radiation-responsive gene promoters for radiogenetic cancer therapy." Gene Ther **9**(20): 1396-402.

We have been developing synthetic gene promoters responsive to clinical doses of ionizing radiation (IR) for use in suicide gene therapy vectors. The crucial DNA sequences utilized are units with the consensus motif CC(A/T)(6)GG, known as CArG elements, derived from the IR-responsive Egr1 gene. In this study we have investigated the parameters needed to enhance promoter activation to radiation. A series of plasmid vectors containing different enhancer/promoters were constructed, transiently transfected into tumor cells (MCF-7 breast adenocarcinoma and U-373MG glioblastoma) and expression of a downstream reporter assayed. Results revealed that increasing the number of CArG elements, up to a certain level, increased promoter radiation-response; from a fold-induction of 1.95 +/- 0.17 for four elements to 2.74 +/- 0.17 for nine CArGs of the same sequence (for MCF-7 cells). Specific alteration of the core A/T sequences caused an even greater positive response, with fold-inductions of 1.71 +/- 0.23 for six elements of prototype sequence compared with 2.96 +/- 0.52 for one of the new sequences following irradiation. Alteration of spacing (from six to 18 nucleotides) between elements had little effect, as did the addition of an adjacent Sp1 binding site. Combining the optimum number and sequence of CArG elements in an additional enhancer was found to produce the best IR induction levels. Furthermore, the improved enhancers also performed better than the previously reported prototype when used in in vitro and in vivo experimental GDEPT. We envisage such enhancers will be used to drive suicide gene expression from vectors delivered to a tumor within an irradiated field. The modest, but tight expression described in the present study could be amplified using a molecular 'switch' system as previously described using Cre/LoxP. In combination with targeted delivery, this strategy has great potential for significantly improving the efficacy of cancer treatment in the large number of cases where radiotherapy is currently employed.

Scott, S. L., J. D. Earle, et al. (2003). "Functional p53 increases prostate cancer cell survival after exposure to fractionated doses of ionizing radiation." Cancer Res **63**(21): 7190-6.

External beam radiation therapy is an effective therapy for localized prostate cancer, although failures occur at high rates. One variable that may affect the radiosensitivity of prostate tumor cells is their p53 status because this gene controls radiation-induced cell cycle arrest, apoptosis, and the repair of DNA damage. Using a system in which p53 function was conditionally restored to p53-null PC3 prostate cancer cells by stable transfection with a human temperature-sensitive p53 mutant allele, we tested the hypothesis that functional p53 increases cell cycle arrest and contributes to increased clonogenic survival after ionizing radiation (IR) of prostate carcinoma cells. Cell cycle arrest and clonogenic survival in response to single and multiple daily exposures to clinically relevant 2-Gy doses of IR were examined. Whereas the temperature-sensitive p53 protein was activated by phosphorylation after IR exposure at both the restrictive and permissive temperatures, Cdkn1/p21 was only induced by functional p53 (at the permissive temperature). In the presence of functional p53, the maintenance of G(2) arrest was significantly longer ($P < 0.01$), and a small increase in cell survival measured by clonogenic assay was seen after exposure to a single 2-Gy dose of IR. However, functional p53 significantly increased clonogenic survival ($P < 0.01$) after exposure to daily doses of 2 Gy of IR and contributed to a more sustained G(2) arrest and increased G(1) arrest in response to the multifraction regimen. These studies implicate the presence of wild-type p53 with increased survival of prostate carcinoma cells after fractionated exposure to radiation. Additionally, the data provide evidence that wild-type p53 in prostate tumor cells may reduce the effectiveness of radiation therapy.

Servomaa, K., A. Kiuru, et al. (1996). "p53 mutations associated with increased sensitivity to ionizing radiation in human head and neck cancer cell lines." Cell Prolif **29**(5): 219-30.

The p53 tumour suppressor gene is activated following cellular exposure to DNA-damaging agents. The functions of wild-type p53 protein include transient blocking of cell cycle progression, direct or indirect stimulation of DNA repair machinery and

triggering of apoptosis if DNA repair fails. Therefore, the status of p53 protein may be critically associated with tumour cell radiosensitivity. In the present study we examine the intrinsic radiosensitivity of 20 human carcinoma cell lines derived from 15 patients with different types of head and neck tumour. Radiosensitivities were measured in a 96-well plate clonogenic assay in terms of the mean inactivation dose, surviving fraction at 2 Gy, and constants alpha and beta in the linear quadratic survival curve. The p53 allele status was determined by amplifying exons 4-10 by the polymerase chain reaction (PCR), screening for mutations using single-strand conformation polymorphism (SSCP) analysis and determining the exact type and location of a mutation by direct sequencing. The results showed that prevalence of p53 mutations in squamous cell carcinoma (SCC) cell lines is high (80%), and that deletion of one or both wild-type alleles is common (75%). Intrinsic radiosensitivity of the cell lines varied greatly in terms of mean inactivation dose, from 1.4 +/- 0.1 to 2.6 +/- 0.2 Gy. Radiosensitivity correlated well with the p53 allele status so that cell lines carrying a wild-type p53 allele were significantly ($P < 0.01$) more radioresistant (mean inactivation dose 2.23 +/- 0.15 Gy) than cell lines which lacked a wild-type gene (1.82 +/- 0.24 Gy). Evaluation of our own results and those published in the literature lead us to conclude that absence of the wild-type p53 allele in human head and neck cancer cell lines is associated with increased radiosensitivity. However, the sensitivity is also strongly dependent on the exact type and location of the p53 mutation.

Shafman, T. D., S. Levitz, et al. (2000). "Prevalence of germline truncating mutations in ATM in women with a second breast cancer after radiation therapy for a contralateral tumor." Genes Chromosomes Cancer **27**(2): 124-9.

Patients treated with conservative surgery and radiation therapy for early-stage breast cancer develop a contralateral breast cancer at a rate of approximately 0.75% per year. Ataxia-telangiectasia (AT) is an autosomal recessive disease that is characterized by increased sensitivity to ionizing radiation (IR) and cancer susceptibility. Epidemiologic studies have suggested that AT carriers, who comprise 1% of the population, may be at an increased risk for developing breast cancer, particularly after exposure to IR. To test this hypothesis, we analyzed blood samples from 57 patients who developed a contralateral breast cancer at least 6 months after completion of radiation therapy for an initial breast tumor. A cDNA-based truncation assay in yeast was used to test for heterozygous mutations in the ATM gene, which is responsible for AT. No mutations were detected. Our findings fail to support the hypothesis that AT carriers account for a significant fraction of breast cancer cases arising in women after exposure to radiation. Genes Chromosomes Cancer 27:124-129, 2000.

Shimada, K., M. Nakamura, et al. (2003). "Androgen and the blocking of radiation-induced sensitization to Fas-mediated apoptosis through c-jun induction in prostate cancer cells." Int J Radiat Biol **79**(6): 451-62.

PURPOSE: To clarify the key mechanism by which androgen makes prostate cancer cells highly resistant to Fas-mediated apoptosis. MATERIALS AND METHODS: The role of c-jun induction by 10 nM dihydrotestosterone (DHT) in 5 Gy radiation-induced up-regulation of Fas and sensitization to the apoptosis was studied by using the human prostate cancer cell line LNCaP. RESULTS: On exposure to 5 Gy radiation, LNCaP cells demonstrated high sensitization to Fas-mediated apoptosis through increased Fas expression, stabilized p53 expression and binding to p53 response elements within the promoter and first intronic region of the Fas gene. Following treatment with DHT, in vivo binding of p53 to its response elements was strongly inhibited. In addition, DHT significantly up-regulated c-jun expression through extracellular stress-regulated kinase (ERK) activation, and transfection of an antisense oligonucleotide for c-jun or ERK inhibition by PD98059 cancelled DHT-mediated suppression of radiation-induced transactivation of Fas gene and sensitization to Fas-mediated apoptosis. CONCLUSIONS: Radiation-induced Fas sensitization in prostate cancer cell was mediated through p53-dependent transactivation of the Fas gene, which can be blocked by androgen stimulation mainly through induction of c-jun.

Shin, S., H. J. Cha, et al. (2009). "Alteration of miRNA profiles by ionizing radiation in A549 human non-small cell lung cancer cells." Int J Oncol **35**(1): 81-6.

Ionizing radiation (IR) is widely used in cancer treatment and in biological studies. It disrupts cellular homeostasis through multiple mechanisms including changes of the expression profile of genes. Although microRNAs (miRNAs) have recently been recognized as important post-transcriptional regulators and are involved in various biological processes, whether miRNAs play any roles in the cellular response to IR, is not well examined. We investigated the profile of miRNA expression following IR in the human lung carcinoma cell line A549, and the expression profiles of IR-responsive miRNAs were confirmed by qRT-PCR. The target mRNAs of IR-responsive miRNAs were predicted with a target

prediction tool. Microarray analysis identified 12 and 18 miRNAs in 20- and 40 Gy-exposed A549 cells, respectively, that exhibited more than 2-fold changes in their expression levels. Of these, four were changed in only 20-Gy-treated cells, ten only in 40-Gy-treated cells, and eight miRNAs were found to change after both treatments. qRT-PCR analysis of a subset of the miRNAs showed patterns of regulation as the microarray data, although the magnitude of the changes differed in the two data sets. Target prediction for IR-responsive miRNAs suggests that they target genes related to apoptosis, regulation of cell cycle, and DNA damage and repair. Taken together, these data suggest that miRNA expression is affected by radiation, and they may be involved in the regulation of radiation responses.

Siden, T. S., W. Golembieski, et al. (1994). "Physical map of small cell lung cancer deletion region on short arm of human chromosome 3 (3p13-22) based on radiation fusion hybrid analysis." Somat Cell Mol Genet **20**(2): 121-32.

Deletion of DNA sequences from various regions of the short arm of human chromosome 3 (3p13-14, 3p21, and 3p25) has been observed during the development of a variety of solid tumors, including lung and renal cell carcinomas. In this study we have used a set of radiation fusion hybrids to generate a physical map of chromosome 3p to orient the search for putative tumor suppressor genes. Eighty-six human-hamster radiation fusion hybrids were screened on Southern blots for the retention of 55 human chromosome 3p DNA markers. The high marker density enabled us to identify a set of successively overlapping chromosome fragments in the 3p13-22 area guided by eight markers with previously known order. Twenty-four map intervals were suggested using breakpoints determined by partial fragment overlaps. The final order between the markers derived is consistent with previous information about localizations for 26 of the markers to three larger cytogenetic intervals.

Sigurdson, A. J., P. Bhatti, et al. (2007). "Polymorphisms in apoptosis- and proliferation-related genes, ionizing radiation exposure, and risk of breast cancer among U.S. Radiologic Technologists." Cancer Epidemiol Biomarkers Prev **16**(10): 2000-7.

BACKGROUND: Although genes involved in apoptosis pathways and DNA repair pathways are both essential for maintaining genomic integrity, genetic variants in DNA repair have been thought to increase susceptibility to radiation carcinogenesis, but similar hypotheses have not generally been raised about apoptosis genes. For this reason, potential modification of the relationship between ionizing radiation exposure and breast cancer risk by polymorphic apoptosis gene variants have not been investigated among radiation-exposed women. METHODS: In a case-control study of 859 cases and 1,083 controls within the U.S. Radiologic Technologists cohort, we assessed breast cancer risk with respect to 16 candidate variants in eight genes involved in apoptosis, inflammation, and proliferation. Using carefully reconstructed cumulative breast dose estimates from occupational and personal diagnostic ionizing radiation, we also investigated the joint effects of these polymorphisms on the risk of breast cancer. RESULTS: In multivariate analyses, we observed a significantly decreased risk of breast cancer associated with the homozygous minor allele of CASP8 D302H [rs1045485, odds ratio (OR), 0.3; 95% confidence interval (95% CI), 0.1-0.8]. We found a significantly increased breast cancer risk with increasing minor alleles for IL1A A114S (rs17561); heterozygote OR 1.2 (95% CI, 1.0-1.4) and homozygote OR 1.5 (95% CI, 1.1-2.0), P(trend) = 0.008. Assuming a dominant genetic model, IL1A A114S significantly modified the dose-response relationship between cumulative personal diagnostic radiation and breast cancer risk, adjusted for occupational dose (P(interaction) = 0.004). CONCLUSION: The U.S. Radiologic Technologists breast cancer study provided a unique opportunity to examine the joint effects of common genetic variation and ionizing radiation exposure to the breast using detailed occupational and personal diagnostic dose data. We found evidence of effect modification of the radiation and breast cancer dose-response relationship that should be confirmed in studies with more cases and controls and quantified radiation breast doses in the low-to-moderate range.

Singh-Gupta, V., H. Zhang, et al. (2009). "Radiation-induced HIF-1alpha cell survival pathway is inhibited by soy isoflavones in prostate cancer cells." Int J Cancer **124**(7): 1675-84.

We previously showed that treatment of prostate cancer cells with soy isoflavones and radiation resulted in greater cell killing in vitro, and caused downregulation of NF-kappaB and APE1/Ref-1. APE1/Ref-1 functions as a redox activator of transcription factors, including NF-kappaB and HIF-1alpha. These molecules are upregulated by radiation and implicated in radioresistance of cancer cells. We extended our studies to investigate the role of HIF-1alpha survival pathway and its upstream Src and STAT3 molecules in isoflavones and radiation interaction. Radiation induced phosphorylation of Src and STAT3 leading to induction of HIF-1alpha. Genistein, daidzein or a mixture of soy isoflavones did not activate this pathway. These data were observed

both in PC-3 (AR-) and C4-2B (AR+) androgen-independent cell lines. Pretreatment with isoflavones inhibited Src/STAT3/HIF-1alpha activation by radiation and nuclear translocation of HIF-1alpha. These findings correlated with decreased expression of APE1/Ref-1 and DNA binding activity of HIF-1alpha and NF-kappaB. In APE1/Ref-1 cDNA transfected cells, radiation caused a greater increase in HIF-1alpha and NF-kappaB activities but this effect was inhibited by pretreatment with soy prior to radiation. Transfection experiments indicate that APE1/Ref-1 inhibition by isoflavones impairs the radiation-induced transcription activity of NF-kappaB and HIF-1alpha. This mechanism could result in the inhibition of genes essential for tumor growth and angiogenesis, as demonstrated by inhibition of VEGF production and HUVECs tube formation. Our novel findings suggest that the increased responsiveness to radiation mediated by soy isoflavones could be due to pleiotropic effects of isoflavones blocking cell survival pathways induced by radiation including Src/STAT3/HIF-1alpha, APE1/Ref-1 and NF-kappaB.

Smith, D. J., M. Jaggi, et al. (2006). "Metallothioneins and resistance to cisplatin and radiation in prostate cancer." Urology **67**(6): 1341-7.

OBJECTIVES: The metallothioneins (MTs) are a family of small molecular weight trace metal and free radical scavenging proteins well established to play a role in the resistance to chemotherapy and radiotherapy in human cancers. MT gene expression is upregulated in response to the presence of metal ions such as zinc. Because prostatic tissue has the greatest concentration of zinc in the human body, in this study we analyzed the effect of MT induction by zinc in prostate cancer (PCa). METHODS: The activation of MT gene expression in response to zinc treatment in LNCaP and C4-2 PCa cells was shown by Western blotting and DNA microarray analysis. Chemotherapy and radiation sensitivity assays of cells after treatment with cisplatin or radiation were performed in the presence, or absence, of 150 microM ZnSO4, and cell viability was measured after 72 hours by MTS viability and clonogenic and flow cytometry assays. The experiments were repeated three times and the data analyzed. RESULTS: Increasing concentrations of ZnSO4 upregulated MT expression in a dose-dependent manner. Microarray analysis demonstrated a specific increase in MT expression. Cells treated with zinc demonstrated a significantly decreased sensitivity to cisplatin and radiotherapy compared with controls ($P < 0.05$). CONCLUSIONS: Our data have confirmed that treatment of PCa with zinc causes an increase in MT expression, which is significantly associated with resistance to cisplatin chemotherapy and radiotherapy in PCa. Therapeutic targeting of MT may therefore provide a means to overcome resistance to radiotherapy and cisplatin chemotherapy in PCa.

Soehnge, H., A. Ouhtit, et al. (1997). "Mechanisms of induction of skin cancer by UV radiation." Front Biosci **2**: d538-51.

Ultraviolet (UV) radiation is the carcinogenic factor in sunlight; damage to skin cells from repeated exposure can lead to the development of cancer. UV radiation has been mainly implicated as the cause of non-melanoma skin cancer, although some role for UV in malignant melanoma has been suggested. The induction of skin cancer is mainly caused by the accumulation of mutations caused by UV damage. Cellular mechanisms exist to repair the DNA damage, or to induce apoptosis to remove severely damaged cells; however, the additive effects of mutations in genes involved in these mechanisms, or in control of the cell cycle, can lead to abnormal cell proliferation and tumor development. The molecular events in the induction of skin cancer are being actively investigated, and recent research has added to the understanding of the roles of tumor suppressor and oncogenes in skin cancer. UV radiation has been shown to induce the expression of the p53 tumor suppressor gene, and is known to produce "signature" mutations in p53 in human and mouse skin cancers and in the tumor suppressor gene patched in human basal cell carcinoma. The role of UV radiation in suppression of immune surveillance in the skin, which is an important protection against skin tumor development, is also being investigated. The knowledge gained will help to better understand the ways in which skin cancer arises from UV exposure, which will in turn allow development of better methods of treatment and prevention.

Sohda, M., H. Ishikawa, et al. (2004). "Pretreatment evaluation of combined HIF-1alpha, p53 and p21 expression is a useful and sensitive indicator of response to radiation and chemotherapy in esophageal cancer." Int J Cancer **110**(6): 838-44.

Tumor hypoxia has been known to be associated with resistance to radiation and chemotherapy (CRT). Hypoxia-inducible factor-1alpha (HIF-1alpha), a transcription factor induced by hypoxic condition, plays a major role in the pleiotropic response observed under hypoxic conditions. It encodes proteins that play key roles in critical development and physiologic processes, including angiogenesis, glucose transport and erythropoiesis. On the other hand, cell cycle- and apoptosis-control genes p53 and p21 may play major roles in the tumor response to cytotoxic agents such as radiation and chemotherapy. Previous reports have suggested that the regulation of p53 and p21 is HIF-1-

dependent. Our aim was to evaluate the expression of the HIF-1alpha, p53 and p21 proteins by immunohistochemistry in biopsy specimens of esophageal squamous cell carcinoma, which were obtained endoscopically from 65 patients before CRT, and then determine whether the levels of expression of these proteins predicted the clinical effectiveness of CRT in individual cancers. Also, to assess the relationship between expression of these proteins and cell death and cellular proliferation activity, we evaluated Ki67 expression and the apoptosis index (TUNEL). HIF-1alpha expression in esophageal cancer was significantly and negatively related to the response to CRT, independently of p53 and p21 expression. Interestingly, 44.4% (12/27) of the HIF-1alpha-negative group showed a complete response to therapy. There was no significant correlation between the expression of HIF-1alpha, p53 and p21 and proliferation and apoptosis. HIF-1alpha overexpression may predict resistance to CRT and may be a helpful guide in choosing between therapeutic strategies, such as intensive combined modality therapy vs. palliative therapy. Combined immunohistochemical evaluation of HIF-1alpha, p53 and p21 protein expression at the pretreatment biopsy is a very useful and powerful indicator of sensitivity to CRT in human esophageal cancer. Our data also indicate the importance of having a clear grasp of the degree of hypoxia (HIF-1alpha) of a tumor, rather than its cellular character (proliferation and apoptosis), to indicate the likely impact of CRT.

Sreekumar, A., M. K. Nyati, et al. (2001). "Profiling of cancer cells using protein microarrays: discovery of novel radiation-regulated proteins." Cancer Res **61**(20): 7585-93.

The advent of DNA microarray technology will likely have a major impact on the molecular classification and understanding of human cancer. Obtaining a global perspective of proteins expressed in cancer cells is considerably more challenging. Here we describe a microarray-based platform that can be used to measure protein levels and activities in a complex biological milieu such as a cellular lysate. Using a protein microarray made up of 1920 elements (146 distinct antibodies) we were able to monitor alterations of protein levels in LoVo colon carcinoma cells treated with ionizing radiation. The protein microarray approach revealed radiation-induced up-regulation of apoptotic regulators including p53, DNA fragmentation factor 40/caspase activated DNase, DNA fragmentation factor 45/inhibitor of caspase activated DNase, tumor necrosis factor-related apoptosis-inducing ligand, death receptor 5, decoy receptor 2, FLICE-like inhibitory protein, signal transducers and activators of transcription 1alpha, and uncoupling protein 2, among others. Consistent with this observation, an increased percentage of apoptosis was observed in irradiated LoVo cells. Interestingly, we also observed radiation-induced down-regulation of carcinoembryonic antigen, a prototypic cancer biomarker. Selected proteins assessed by microarray were validated by traditional immunoblotting. Taken together, our work suggests that protein/antibody microarrays will facilitate high-throughput proteomic studies of human cancer and carcinogenesis.

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.

Stuschke, M., A. Sak, et al. (2002). "Radiation-induced apoptosis in human non-small-cell lung cancer cell lines is secondary to cell-cycle progression

beyond the G2-phase checkpoint." Int J Radiat Biol **78**(9): 807-19.

PURPOSE: To characterize the relationship between cell-cycle progression and radiation-induced apoptosis in NSCLC cell lines with different p53 status. MATERIALS AND METHODS: Cell lines with functional (H460, A549) and non-functional p53 (H661 and H520) were irradiated with 20 Gy. Multiparameter flow-cytometry was used to follow the progression of synchronized cells through the cell cycle after irradiation. RESULTS: Delayed apoptosis was observed after cell-cycle progression beyond the G2 block, either in the late G2/M-phase of the same cell cycle being irradiated (H661, H520) or in the G1-phase of the subsequent cell cycle (H460, A549). The apoptotic fraction in H661 and H520 was 60-80% at 144h after irradiation, higher than in A549 and H460 (5 and 35%, respectively). As an alternative to apoptosis in cells cycling beyond the G2 restriction point, hyperploid cells were generated by all cell lines. Inhibition of cell-cycle progression through the G2/M-phase efficiently reduced the induction of late apoptosis. After irradiation in S-phase, 50-60% of cells with functional p53 remained arrested at the G2 restriction point until 144 h post-irradiation, while only 20% of the H661 or H520 did so. CONCLUSIONS: These data characterize radiation-induced apoptosis in NSCLC cell lines as a removal pathway of clonogenically inactivated cells secondary to cell-cycle progression beyond G2/M, and is unlikely to be a critical factor for cellular radiation sensitivity.

Sutton, D., S. Kim, et al. (2006). "Efficient suppression of secretory clusterin levels by polymer-siRNA nanocomplexes enhances ionizing radiation lethality in human MCF-7 breast cancer cells in vitro." Int J Nanomedicine **1**(2): 155-62.

Small interfering RNA molecules (siRNA) hold great promise to specifically target cytoprotective factors to enhance cancer therapy. Like antisense RNA strategies, however, the use of siRNA is limited because of in vivo instability. As a first step to overcome delivery issues, a series of graft copolymers of polyethylene glycol and polyethylenimine (PEI-g-PEG) were synthesized and investigated as nontoxic carriers for delivery of siRNA targeting the signaling peptide of secretory clusterin (sCLU), a prosurvival factor that protects cells from ionizing radiation (IR) injury, as well as chemotherapeutic agents. Three copolymers with different PEG grafting densities were tested for their abilities to bind and form nanocomplexes with siRNA. A copolymer composed of 10 PEG grafts (2 kDa each) per PEI polymer (2k10 copolymer) gave the highest binding affinity to siRNA by ethidium bromide exclusion assays, and had the smallest nanocomplex size (115 +/- 13 nm diameter). In human breast cancer MCF-7 cells, 2k10-siRNA-sCLU nanocomplexes suppressed both basal as well as IR-induced sCLU protein expression, which led to an over 3-fold increase in IR-induced lethality over 2k10-siRNA scrambled controls. In summary, this study demonstrates the proof-of-principle in using nanoparticle-mediated delivery of specific siRNAs to enhance the lethality of IR exposure in vitro, opening the door for siRNA-mediated knockdown of specific cytoprotective factors, such as DNA repair, anti-apoptotic, free radical scavenging, and many other proteins.

Sylvain, V., S. Lafarge, et al. (2001). "Molecular pathways involved in response to ionizing radiation of ID-8 mouse ovarian cancer cells expressing exogenous full-length Brca1 or truncated Brca1 mutant." Int J Oncol **19**(3): 599-607.

BRCA1 germline mutations have been linked to the development of hereditary breast and ovarian cancers. Recent studies suggest that BRCA1 may function in the regulation of basic cellular processes, including gene transcription, and sensing and/or repair of DNA damage. To further delineate the BRCA1 upstream and downstream steps involved in its role in the cellular response to ionizing radiation, we compared the effects of expression of an exogenous full-length Brca1 with those of a truncated Brca1 mutant in the ID-8 mouse ovarian cancer cell line after irradiation. We found that expression of both full-length and truncated Brca1 increased resistance to ionizing radiation. Expression of truncated, but not full-length, Brca1 then allowed us to identify new potential downstream targets of mutated BRCA1 like MAPK/ERK pathway members and also key genes involved in mutated BRCA1 signaling pathway response to ionizing radiation such as p53 and p21WAF1/CIP1. We therefore established an in vitro mouse model for studying the molecular effects of human BRCA1 germline mutations.

Tao, Y., P. Zhang, et al. (2007). "Enhancement of radiation response by inhibition of Aurora-A kinase using siRNA or a selective Aurora kinase inhibitor PHA680632 in p53-deficient cancer cells." Br J Cancer **97**(12): 1664-72.

Overexpression of Aurora-A kinase has been correlated with cancer susceptibility and poor prognosis in several human cancers. In this study, we evaluated the effect of inhibition of Aurora-A kinase on cell cycle progression and tumour cell survival after exposure to ionising radiation (IR). Combined IR and Aurora-A inhibition by short interfering RNA (siRNA) or by PHA680632 (a selective Aurora kinase inhibitor with submicromolar activity against Aurora-

A) prior to IR led to an enhancement of radiation-induced annexin V positive cells, micronuclei formation, and Brca1 foci formation only in cells with deficient p53. However, the drug brought about additive to sub-additive interaction with radiation with regard to in vitro clonogenic survival. Cell cycle analysis revealed a high >4N DNA content 24 h after PHA680632 exposure. DNA content >4N was reduced dramatically when cells were irradiated combined with PHA680632 simultaneously. In vivo xenografts (p53-/- HCT116) of a mice study showed enhanced tumour growth delay (TGD) after the PHA680632-IR combinatorial treatment compared with IR alone. These results demonstrate that PHA680632 in association with radiation leads to an additive effect in cancer cells, especially in the p53-deficient cells, but does not act as a radiosensitiser in vitro or in vivo.

Terleth, C., T. van Laar, et al. (1997). "A lack of radiation-induced ornithine decarboxylase activity prevents enhanced reactivation of herpes simplex virus and is linked to non-cancer proneness in xeroderma pigmentosum patients." Cancer Res **57**(19): 4384-92.

Patients with xeroderma pigmentosum (XP), a DNA repair disorder, run a large risk of developing skin cancer in sun-exposed areas. Cancer proneness in these patients correlates with a mammalian SOS-like response, "enhanced reactivation (ER) of viruses." Here, we report that radiation-induced activation of the ornithine decarboxylase (ODC) gene, a putative proto-oncogene, is required for this response. Various diploid fibroblast strains derived from a non-cancer-prone subclass of XP patients, which lack the ER response, were irradiated with 2 J/m2 and assessed for gene induction. In these fibroblasts, an absence of induction of ODC by UV-C was observed at the levels of mRNA, protein, and enzyme activity. This lack of induction is quite specific because the genes for fos and collagenase were induced as they were in normal XP cells. The apparent linkage between non-cancer proneness and a lack of ER and ODC induction was confirmed in a fibroblast strain derived from a patient with another DNA repair disorder, trichothiodystrophy, which does not lead to cancer proneness: in these cells, no induction of the ER response nor of ODC occurs after UV-C irradiation. Repair deficiency, however, is not essential because the simultaneous lack of ODC and ER induction after 10 J/m2 UV-C was found in at least one repair-proficient fibroblast. Next, a specific inhibitor of ODC, difluoromethylornithine, at a dose of 10 mM, completely blocked the ER response in cultured normal skin fibroblasts, suggesting that the ODC enzyme is in fact essential for the ER response. Difluoromethylornithine, although it did not affect other processes such as DNA repair, leads to a block in the cell division cycle at the G1-S transition. Interestingly, other blockers of this transition, wortmannin (500 nM) and mimosine (100 mM), also decreased the ER response. Finally, the ER and ODC responses also seem to be linked after treatment with X-irradiation (3 Gy), suggesting that both are part of a general response to DNA damage, at least in human skin fibroblasts. Apart from the abnormal ER and ODC responses, fibroblasts from non-tumor-prone XP patients react in the same way to radiation as do fibroblasts from tumor-prone XP patients with respect to other parameters. Thus, the lack of ODC induction after radiation may help to protect XP patients against skin carcinogenesis.

Tevz, G., S. Kranjc, et al. (2009). "Controlled systemic release of interleukin-12 after gene electrotransfer to muscle for cancer gene therapy alone or in combination with ionizing radiation in murine sarcomas." J Gene Med **11**(12): 1125-37.

BACKGROUND: The present study aimed to evaluate the antitumor effectiveness of systemic interleukin (IL)-12 gene therapy in murine sarcoma models, and to evaluate its interaction with the irradiation of tumors and metastases. To avoid toxic side-effects of IL-12 gene therapy, the objective was to achieve the controlled release of IL-12 after intramuscular gene electrotransfer. METHODS: Gene electrotransfer of the plasmid pORF-mIL12 was performed into the tibialis cranialis in A/J and C57BL/6 mice. Systemic release of the IL-12 was monitored in the serum of mice after carrying out two sets of intramuscular IL-12 gene electrotransfer of two different doses of plasmid DNA. The antitumor effectiveness of IL-12 gene electrotransfer alone or in combination with local tumor or lung irradiation with X-rays, was evaluated on subcutaneous SA-1 and LPB tumors, as well as on lung metastases. RESULTS: A synergistic antitumor effect of intramuscular gene electrotransfer combined with local tumor irradiation was observed as a result of the systemic distribution of IL-12. The gene electrotransfer resulted in up to 28% of complete responses of tumors. In combination with local tumor irradiation, the curability was increased by up to 100%. The same effect was observed for lung metastases, where a potentiating factor of 1.3-fold was determined. The amount of circulating IL-12 was controlled by the number of repeats of gene electrotransfer and by the amount of the injected plasmid. CONCLUSIONS: The present study demonstrates the feasibility of treatment by IL-12 gene electrotransfer combined with local tumor or lung metastases irradiation on sarcoma tumors for translation into the clinical setting.

Tillmanns, T. D., S. A. Kamelle, et al. (2005). "Sensitization of cervical cancer cell lines to low-dose radiation by retinoic acid does not require functional p53." Gynecol Oncol **97**(1): 142-50.

OBJECTIVE: Current therapy for cervical cancer includes radiation therapy. Retinoic acid (RA) can increase the sensitivity of cervical cancer cell lines to radiation. The mechanism of this sensitization may not involve the p53 protein because the human papillomavirus (HPV) E6 protein, which is present in the majority of cervical cancers, promotes p53 degradation. The objective of this study was to determine if p53 is involved in the mechanism of RA radiosensitization. METHOD: The effects of radiation on cervical (SiHa, CC-1, and C33a) and vulvar (SW962) cancer cell lines under various experimental conditions were evaluated using clonogenic, Coulter Counter, electrophoretic mobility shift (EMSA) and a multi-probe RNase protection assay of p53-inducible genes. RESULTS: RA (5 microM 9-cis-RA) radiosensitized the SiHa and CC-1 cell lines that contain HPV-degraded p53, but did not radiosensitize the SW962 cell line, which is HPV negative and contains wild-type p53, nor the C33a cell line, which contains mutant p53 (R273C). Expression of mutant p53 (R273H) in SiHa cells increased the growth rate, but did not prevent RA-induced differentiation or radiosensitization at clinically relevant doses. Inhibition of p53 transactivation with pifithirin alpha did not prevent RA radiosensitization of SiHa at 5 Gy. RA repressed c-fos mRNA expression in control and irradiated SiHa cultures, but did not repress bcl-x(L), p53, GADD45, p21, bax, bcl-2, or mcl-1 mRNA expression. CONCLUSIONS: The mechanism of RA radiosensitization does not require functional p53 and may involve c-fos in cervical cancer cell lines.

Tjebbes, G. W., P. A. Kreijveld, et al. (2002). "P53 tumor suppressor gene mutations in laryngeal cancer and in recurrent disease following radiation therapy." Oral Oncol **38**(3): 296-300.

In this study we performed p53 sequencing based mutation analysis in laryngeal cancers and matched recurrent disease following irradiation. The question is if irradiation affects the DNA and introduces or deletes mutations so that p53 cannot be used as a clonal marker anymore. P53 mutations were identified in fresh-frozen laryngectomy specimens with either primary laryngeal cancers, treated by surgery and irradiation post-operative with local failure during follow-up, or with recurrent laryngeal cancers following primary irradiation. In 21 tumors the p53 status was analyzed by direct sequencing full-length mRNA through RT-PCR. DNA sequencing analysis of exons 2 through 11 was performed when RNA isolation could not be performed. The marker mutation identified in this way was detected by DNA sequencing of the corresponding exon in formalin-fixed deparaffinized tumor biopsy samples in respectively matched recurrent disease following surgery and irradiation or primary tumor before irradiation. DNA sequencing analysis of the corresponding exon of peripheral blood leukocytes excluded the presence of germline mutations or polymorphisms. In 16 out of 21 tumors (71%), a mutation was identified. Fifteen of these marker mutations were detected in the matched tumor biopsy sample (94%). The only case lacking the marker mutation probably was a second primary tumor. We conclude that we find no direct evidence for induction or loss of p53 mutations following irradiation. Consequently, p53 may be used as a diagnostic tool when histological examination fails, for example in discriminating between the presence of a second primary tumor in the same area versus recurrent disease.

Truman, J. P., N. Gueven, et al. (2005). "Down-regulation of ATM protein sensitizes human prostate cancer cells to radiation-induced apoptosis." J Biol Chem **280**(24): 23262-72.

Treatment with the protein kinase C activator 12-O-tetradecanoylphorbol 12-acetate (TPA) enables radiation-resistant LNCaP human prostate cancer cells to undergo radiation-induced apoptosis, mediated via activation of the enzyme ceramide synthase (CS) and de novo synthesis of the sphingolipid ceramide (Garzotto, M., Haimovitz-Friedman, A., Liao, W. C., White-Jones, M., Huryk, R., Heston, D. W. W., Cardon-Cardo, C., Kolesnick, R., and Fuks, Z. (1999) Cancer Res. 59, 5194-5201). Here, we show that TPA functions to decrease the cellular level of the ATM (ataxia telangiectasia mutated) protein, known to repress CS activation (Liao, W.-C., Haimovitz-Friedman, A., Persaud, R., McLoughlin, M., Ehleiter, D., Zhang, N., Gatei, M., Lavin, M., Kolesnick, R., and Fuks, Z. (1999) J. Biol. Chem. 274, 17908-17917). Gel shift analysis in LNCaP and CWR22-Rv1 cells demonstrated a significant reduction in DNA binding of the Sp1 transcription factor to the ATM promoter, and quantitative reverse transcription-PCR showed a 50% reduction of ATM mRNA between 8 and 16 h of TPA treatment, indicating that TPA inhibits ATM transcription. Furthermore, treatment of LNCaP, CWR22-Rv1, PC-3, and DU-145 human prostate cells with antisense-ATM oligonucleotides, which markedly reduced cellular ATM levels, significantly enhanced radiation-induced CS activation and apoptosis, leading to apoptosis at doses as a low as 1 gray. These data suggest that the CS pathway initiates a generic mode of radiation-induced

apoptosis in human prostate cancer cells, regulated by a suppressive function of ATM, and that ATM might represent a potential target for pharmacologic inactivation with potential clinical applications in human prostate cancer.

Tsai, K. K., J. Stuart, et al. (2009). "Low-dose radiation-induced senescent stromal fibroblasts render nearby breast cancer cells radioresistant." Radiat Res **172**(3): 306-13.

In addition to cell cycle arrest, DNA repair or/and apoptosis, ionizing radiation can also induce premature senescence, which could lead to very different biological consequences depending on the cell type. We show in this report that low-dose radiation-induced senescent stromal fibroblasts stimulate proliferation of cocultured breast carcinoma cells. Such effects of senescent fibroblasts appear to result from their ability to induce the expression in carcinoma cells of mitotic genes and subsequent mitotic division. The elevated proliferation of breast carcinoma cells correlates with resistance to radiation as well as to adriamycin. Of interest is the observation that exposure to lower doses (<20 cGy) augments the ability of senescent fibroblasts to promote the survival of cocultured breast carcinoma cells. The resistance appears to be mediated partially by the Akt pathway, because expression of a dominant negative Akt mutant in breast carcinoma cells results in a partial reversal of the radioresistance. The ability of fibroblasts to modulate the radiosensitivity of nearby carcinoma cells implicates the importance of targeting the stroma during therapy.

Turner, B. C., E. Harrold, et al. (1999). "BRCA1/BRCA2 germline mutations in locally recurrent breast cancer patients after lumpectomy and radiation therapy: implications for breast-conserving management in patients with BRCA1/BRCA2 mutations." J Clin Oncol **17**(10): 3017-24.

PURPOSE: Breast cancer patients treated conservatively with lumpectomy and radiation therapy (LRT) have an estimated lifetime risk of local relapse (ipsilateral breast tumor recurrence [IBTR]) of 10% to 15%. For breast cancer patients carrying BRCA1 or BRCA2 (BRCA1/2) mutations, the outcome of treatment with LRT with respect to IBTR has not been determined. In this study, we estimate the frequency of BRCA1/2 mutations in a study of breast cancer patients with IBTR treated with LRT. PATIENTS AND METHODS: Between 1973 and 1994, there were 52 breast cancer patients treated with LRT who developed an IBTR within the prior irradiated breast and who were willing to participate in the current study. From our database, we also identified 52 control breast cancer patients treated with LRT without IBTR. The control patients were individually matched to the index cases with respect to multiple clinical and pathologic parameters. Lymphocyte DNA specimens from all 52 locally recurrent patients and 15 of the matched control patients under age 40 were used as templates for polymerase chain reaction amplification and dye-primer sequencing of exons 2 to 24 of BRCA1, exons 2 to 27 of BRCA2, and flanking intron sequences. RESULTS: After LRT, eight (15%) of 52 breast cancer patients had IBTR with deleterious BRCA1/2 mutations. By age, there were six (40%) of 15 patients with IBTR under age 40 with BRCA1/2 mutations, one (9.0%) of 11 between ages 40 and 49, and one (3.8%) of 26 older than age 49. In comparison to the six (40%) of 15 of patients under age 40 with IBTR found to have BRCA1/2 mutations, only one (6.6%) of 15 matched control patients without IBTR and had a BRCA1/2 mutation (P =.03). The median time to IBTR for patients with BRCA1/2 mutations was 7.8 years compared with 4.7 years for patients without BRCA1/2 mutations (P =.03). By clinical and histologic criteria, these relapses represented second primary tumors developing in the conservatively treated breast. All patients with BRCA1/2 mutations and IBTR underwent successful surgical salvage mastectomy at the time of IBTR and remain alive without evidence of local or systemic progression of disease. CONCLUSION: In this study, we found an elevated frequency of deleterious BRCA1/2 mutations in breast cancer patients treated with LRT who developed late IBTR. The relatively long time to IBTR, as well as the histologic and clinical criteria, suggests that these recurrent cancers actually represent new primary breast cancers. Early onset breast cancer patients experiencing IBTR have a disproportionately high frequency of deleterious BRCA1/2 mutations. This information may be helpful in guiding management in BRCA1 or BRCA2 patients considering breast-conserving therapy.

Valdagni, R., T. Rancati, et al. (2009). "To bleed or not to bleed. A prediction based on individual gene profiling combined with dose-volume histogram shapes in prostate cancer patients undergoing three-dimensional conformal radiation therapy." Int J Radiat Oncol Biol Phys **74**(5): 1431-40.

PURPOSE: The main purpose of this work was to try to elucidate why, despite excellent rectal dose-volume histograms (DVHs), some patients treated for prostate cancer exhibit late rectal bleeding (LRB) and others with poor DVHs do not. Thirty-five genes involved in DNA repair/radiation response were analyzed in patients accrued in the AIROPROS 0101 trial, which investigated the correlation between LRB and dosimetric parameters. METHODS AND MATERIALS: Thirty patients undergoing conformal

radiotherapy with prescription doses higher than 70 Gy (minimum follow-up, 48 months) were selected: 10 patients in the low-risk group (rectal DVH with the percent volume of rectum receiving more than 70 Gy [V70Gy] < 20% and the percent volume of rectum receiving more than 50 Gy [V50Gy] < 55%) with Grade 2 or Grade 3 (G2-G3) LRB, 10 patients in the high-risk group (V70Gy > 25% and V50Gy > 60%) with G2-G3 LRB, and 10 patients in the high-risk group with no toxicity. Quantitative reverse-transcriptase polymerase chain reaction was performed on RNA from lymphoblastoid cell lines obtained from Epstein-Barr virus-immortalized peripheral-blood mononucleated cells and on peripheral blood mononucleated cells. Interexpression levels were compared by using the Kruskal-Wallis test. RESULTS: Intergroup comparison showed many constitutive differences: nine genes were significantly down-regulated in the low-risk bleeder group vs. the high-risk bleeder and high-risk nonbleeder groups: AKR1B1 (p = 0.019), BAZ1B (p = 0.042), LSM7 (p = 0.0016), MRPL23 (p = 0.015), NUDT1 (p = 0.0031), PSMB4 (p = 0.079), PSMD1 (p = 0.062), SEC22L1 (p = 0.040), and UBB (p = 0.018). Four genes were significantly upregulated in the high-risk nonbleeder group than in the other groups: DDX17 (p = 0.048), DRAP1 (p = 0.0025), RAD23 (p = 0.015), and SRF (p = 0.024). For most of these genes, it was possible to establish a cut-off value that correctly classified most patients. CONCLUSIONS: The predictive value of sensitivity and resistance to LRB of the genes identified by the study is promising and should be tested in a larger data set.

van der Horst, G. T., L. Meira, et al. (2002). "UVB radiation-induced cancer predisposition in Cockayne syndrome group A (Csa) mutant mice." DNA Repair (Amst) **1**(2): 143-57.

Cockayne syndrome (CS) is an inherited photosensitive neurodevelopmental disorder caused by a specific defect in the transcription-coupled repair (TCR) sub-pathway of NER. Remarkably, despite their DNA repair deficiency, CS patients do not develop skin cancer. Here, we present a mouse model for CS complementation group A. Like cells from CS-A patients, Csa-/- mouse embryonic fibroblasts (MEFs): (i) are ultraviolet (UV)-sensitive; (ii) show normal unscheduled DNA synthesis (indicating that the global genome repair sub-pathway is unaffected); (iii) fail to resume RNA synthesis after UV-exposure and (iv) are unable to remove cyclobutane pyrimidine dimers (CPD) photolesions from the transcribed strand of active genes. CS-A mice exhibit UV-sensitivity and pronounced age-dependent loss of retinal photoreceptor cells but otherwise fail to show the severe developmental and neurological abnormalities of the human syndrome. In contrast to human CS, Csa-/- animals develop skin tumors after chronic exposure to UV light, indicating that TCR in mice protects from UV-induced skin cancer development. Strikingly, inactivation of one Xpc allele (encoding a component of the damage recognition complex involved in the global genome repair sub-pathway) in Csa-/- mice resulted in a strongly enhanced UV-mediated skin cancer sensitivity, indicating that in a TC repair defective background, the Xpc gene product may be a rate-limiting factor in the removal of UV-induced DNA lesions.

Vares, G., K. Ory, et al. (2004). "Progesterone prevents radiation-induced apoptosis in breast cancer cells." Oncogene **23**(26): 4603-13.

Sex steroid hormones play an essential role in the control of homeostasis in the mammary gland. Although the involvement of progesterone in cellular proliferation and differentiation is well established, its exact role in the control of cell death still remains unclear. As dysregulation of the apoptotic process plays an important role in the pathogenesis of breast cancer, we investigated the regulation of apoptosis by progesterone in various breast cancer cell lines. Our results show that progesterone treatment protects against radiation-induced apoptosis. This prevention appears to be mediated by the progesterone receptor and is unrelated to p53 status. There is also no correlation with the intrinsic hormonal effect on cell proliferation, as the presence of cells in a particular phase of the cell cycle. Surprisingly, progesterone partly allows bypassing of the irradiation-induced growth arrest in G(2)/M in PgR+ cells, leading to an increase in cell proliferation after irradiation. One consequence of this effect is a higher rate of chromosome damage in these proliferating progesterone-treated cells compared to what is observed in untreated irradiated cells. We propose that progesterone, by inhibiting apoptosis and promoting the proliferation of cells with DNA damage, potentially facilitates the emergence of genetic mutations that may play a role in malignant transformation.

Varghese, S., R. K. Schmidt-Ullrich, et al. (1999). "Enhanced radiation late effects and cellular radiation sensitivity in an ATM heterozygous breast cancer patient." Radiat Oncol Investig **7**(4): 231-7.

We observed severe late effects in a patient treated with radiation therapy for breast cancer. Radiation survival studies of patient fibroblasts show an enhanced cellular radiation sensitivity (Do = 0.95 Gy). Genetic analysis reveals that the patient is heterozygous for a mutated ATM gene. Protein truncation test (PTT) and sequence analysis identified

a truncation within the leucine zipper domain, corresponding to a fragment previously reported to exhibit dominant negative function. These findings demonstrate that ATM heterozygosity may be associated with enhanced clinical radiation sensitivity and suggest a clinical relevance to this truncation that results in a dominant negative-acting protein.

Vorobtsova, I. E. (1989). "Increased cancer risk as a genetic effect of ionizing radiation." IARC Sci Publ(96): 389-401.

The well known genetic effects of ionizing radiation include severe developmental disorders in the progeny of irradiated parents resulting in embryonic death, stillbirth and early postnatal mortality, congenital abnormalities, malformations and fertility disturbances in live-born organisms. These effects are considered to be due to gross mutations (genomic, chromosomal and those of essential genes). Physiological inferiority and an increased cancer risk in phenotypically normal offspring of irradiated parents appear to be two further types of genetic effect of radiation. The genetic background of these effects is suggested to be induced recessive polygene mutations and regulatory DNA alterations, which may lead to instability of the hereditary apparatus of cells, activation of protooncogenes and other inducible processes. A comparison of somatic and genetic effects of radiation shows certain similarities, not only in phenomenology, but probably also in pathogenetic mechanisms.

Wakatsuki, M., T. Ohno, et al. (2008). "p73 protein expression correlates with radiation-induced apoptosis in the lack of p53 response to radiation therapy for cervical cancer." Int J Radiat Oncol Biol Phys **70**(4): 1189-94.

PURPOSE: p73 belongs to the p53 tumor suppressor family of genes and can inhibit cell growth in a p53-like manner by inducing apoptosis or cell cycle arrest. Here, we investigated whether p73 could compensate for impaired p53 function in apoptosis induced by radiation therapy (RT) for cervical cancer. METHODS AND MATERIALS: Sixty-eight patients with squamous cell carcinoma of the cervix who received definitive RT combined with (n=37) or without (n=31) cisplatin were investigated. Biopsy specimens were excised from the cervical tumor before RT and after 9 Gy. RESULTS: Mean apoptosis index (AI) was 0.93% before RT and 1.97% after 9 Gy with a significant increase ($p<0.001$). For all patients, there was a significant correlation between p73 expression positivity after 9 Gy and AI ratio (AI after 9 Gy/AI before RT) ($p=0.021$). Forty-one patients were regarded as the p53-responding group according to the expression of p53 after 9 Gy, whereas the remaining 27 patients were regarded as the p53-nonresponding group. A significant correlation between p73 expression after 9 Gy and AI ratio was observed in the p53-non-responding group ($p<0.001$) but not in the p53-responding group ($p=0.940$). CONCLUSION: Our results suggest that p73 plays an important role in compensating for the lack of p53 function in radiation-induced apoptosis of cervical cancer.

Wang, J. A., S. Fan, et al. (1999). "Ultraviolet radiation down-regulates expression of the cell-cycle inhibitor p21WAF1/CIP1 in human cancer cells independently of p53." Int J Radiat Biol **75**(3): 301-16.

PURPOSE: To investigate the regulation of G1 cyclin-dependent kinase inhibitor p21WAF1/CIP1 by ultraviolet (UV) radiation in human carcinoma cells. MATERIALS AND METHODS: Human cancer cell lines were irradiated with UV-C (254 nm) radiation, and their responses were characterized by Western blotting, Northern blotting, semi-quantitative RT-PCR analysis, trypan blue staining and flow cytometric cell cycle analysis. RESULTS: At 24 h after UV irradiation, p21 expression was down-regulated in various cancer cell types (breast, prostrate, cervix, colon, glioma, squamous cancers), independently of their p53 genetic and functional status. UV-mediated down-regulation of p21 was dose- and time-dependent, was observed at the protein and mRNA levels, and did not correlate with cytotoxicity. Reduction of p21 protein levels required about 4 and 1 h, respectively, in MCF-7 and MDA-MB-231 breast cancer cells; some of the UV-induced decreases in p21 levels in these cell lines was due to enhanced proteasomal degradation. Despite decreased p21 levels, UV-irradiated breast cancer cells with wild-type p53 (MCF-7) retained the capacity for G1 cell-cycle arrest, whereas UV-treated cells with mutant p53 (MDA-MB-231) accumulated in S phase, suggesting a p53-dependent G1 checkpoint in MCF-7. UV treatment caused other alterations in cell-cycle regulatory, DNA repair and tumour suppressor genes, as described in this report. CONCLUSIONS: In contrast to X-rays, UV causes down-regulation of the cell-cycle inhibitor p21 in tumour cells. It is postulated that this may be an adaptation to promote the growth and survival of transformed cells.

Weichselbaum, R. R., H. Ishwaran, et al. (2008). "An interferon-related gene signature for DNA damage resistance is a predictive marker for chemotherapy and radiation for breast cancer." Proc Natl Acad Sci U S A **105**(47): 18490-5.

Individualization of cancer management requires prognostic markers and therapy-predictive

markers. Prognostic markers assess risk of disease progression independent of therapy, whereas therapy-predictive markers identify patients whose disease is sensitive or resistant to treatment. We show that an experimentally derived IFN-related DNA damage resistance signature (IRDS) is associated with resistance to chemotherapy and/or radiation across different cancer cell lines. The IRDS genes STAT1, ISG15, and IFIT1 all mediate experimental resistance. Clinical analyses reveal that IRDS(+) and IRDS(-) states exist among common human cancers. In breast cancer, a seven-gene-pair classifier predicts for efficacy of adjuvant chemotherapy and for local-regional control after radiation. By providing information on treatment sensitivity or resistance, the IRDS improves outcome prediction when combined with standard markers, risk groups, or other genomic classifiers.

Whitfield, J. F. (2006). "Parathyroid hormone: a novel tool for treating bone marrow depletion in cancer patients caused by chemotherapeutic drugs and ionizing radiation." Cancer Lett **244**(1): 8-15.

Between 1958 and the late 1970s it was learned that PTH (the parathyroid hormone) could directly stimulate the initiation of DNA replication by murine CFU-S (colony-forming unit-spleen) cells via cyclic AMP, stimulate the proliferation of normal and X-irradiated murine and rat bone marrow cells, control hematopoiesis, and increase the survival of X-irradiated mice and rats when injected any time between 18h before and 3h after X-irradiation. Since then, it has been shown that the hematopoietic stem cell niche consists of PTH receptor-bearing, osteoblastic trabecular bone-lining cells that maintain the stem cells' (HSCs') proliferatively quiescent 'stemness' by various gene up-regulating and down-regulating signals caused by the tight adhesion of the HSCs to the osteoblastic niche-lining cells. Stimulating the osteoblastic lining cells with recombinant human PTH-(1-34) (Forteo) causes a cyclic AMP-mediated enlargement of the HSC pool and promotes bone marrow transplant engraftment and growth and the survival of lethally irradiated mice. But this is only the beginning of the exploitation of the PTHs for marrow engraftment. It must now be determined whether the marrow engraftment-enhancing action of this potent bone growth-stimulating PTH can be extended from mice to rats and monkeys. It must be determined whether two other PTH peptides, rhPTH-(1-84) [Preos]and [Leu(27)]cyclo(Glu(22)-Lys(26))hPTH-(1-31)NH(2) [Ostabolin-C]) are as effective as or better than rhPTH-(1-34)(Forteo). Since, all three peptides are on the market, or nearing the market, for safely and strongly stimulating bone growth and treating osteoporosis one or all of them may become valuable tools for safely promoting the engraftment of peripherally harvested HSCs in cancer patients whose bone marrows have been 'emptied' by chemotherapeutic drugs or ionizing radiation.

Wu, X., J. Gu, et al. (2006). "Genetic variations in radiation and chemotherapy drug action pathways predict clinical outcomes in esophageal cancer." J Clin Oncol **24**(23): 3789-98.

PURPOSE: Understanding how specific genetic variants modify drug action pathways may provide informative blueprints for individualized chemotherapy. METHODS: We applied a pathway-based approach to examine the impact of a comprehensive panel of genetic polymorphisms on clinical outcomes in 210 esophageal cancer patients. RESULTS: In the Cox proportional hazards model, MTHFR Glu429Ala variant genotypes were associated with significantly improved survival (hazard ratio [HR] = 0.56; 95% CI, 0.35 to 0.89) in patients treated with fluorouracil (FU). The 3-year survival rates for patients with the variant genotypes and the wild genotypes were 65.26% and 46.43%, respectively. Joint analysis of five polymorphisms in three FU pathway genes showed a significant trend for reduced recurrence risk and longer recurrence-free survival as the number of adverse alleles decreased (P = .004). For patients receiving platinum drugs, the MDR1 C3435T variant allele was associated with significantly reduced recurrence risk (HR = 0.25; 95% CI, 0.10 to 0.64) and improved survival (HR = 0.44; 95% CI, 0.23 to 0.85). In nucleotide excision repair genes, there was a significant trend for a decreasing risk of death with a decreasing number of high-risk alleles (P for trend = .0008). In base excision repair genes, the variant alleles of XRCC1 Arg399Gln were significantly associated with the absence of pathologic complete response (odds ratio = 2.75; 95% CI, 1.14 to 6.12) and poor survival (HR = 1.92; 95% CI, 1.00 to 3.72). CONCLUSION: Several biologically plausible associations between individual single nucleotide polymorphisms and clinical outcomes were found. Our data also strongly suggest that combined pathway-based analysis may provide valuable prognostic markers of clinical outcomes.

Xia, L., A. Paik, et al. (2004). "p53 activation in chronic radiation-treated breast cancer cells: regulation of MDM2/p14ARF." Cancer Res **64**(1): 221-8.

Mammalian cells chronically exposed to ionizing radiation (IR) induce stress response with a tolerance to the subsequent cytotoxicity of IR. Although p53 is well documented in IR response, the signaling network causing p53 activation in chronic

IR remains to be identified. Using breast carcinoma MCF+FIR cells that showed a transient radioresistance after exposure chronically to fractionated IR (FIR), the present study shows that the basal DNA binding and transcriptional activity of p53 was elevated by FIR. p53-controlled luciferase activity was strikingly induced (approximately 7.9-fold) with little enhancement of p53/DNA binding activity (approximately 1.3-fold). The phosphorylated p53 (Thr 55) was increased in the cytoplasm and nucleus of MCF+FIR but not in the sham-FIR control cells. On the contrary, the sham-FIR control MCF-7 cells showed a low p53 luciferase transcription (approximately 3-fold) but a striking enhancement of p53/DNA binding (12-fold) after 5 Gy of IR. To determine the signaling elements regulating p53 activity, DNA microarray of MCF+FIR using sham-FIR MCF-7 cells as a reference demonstrated that the mRNA of p21, MDM2, and p14ARF was up-regulated. Time course Western blot analysis, however, showed no difference in p21 induction. In contrast, MDM2 that was absent in control cells and was predominantly induced by IR was not induced in MCF+FIR cells. In agreement with MDM2 inhibition, MDM2-inhibitory protein p14ARF was increased in MCF+FIR cells. In summary, these results demonstrate that up-regulation of p14ARF paralleled with MDM2 inhibition contributes to p53 accumulation in the nucleus and causes a high responsiveness of p53 in chronic IR-treated breast cancer cells.

Xu, L., K. F. Pirollo, et al. (1999). "Transferrin-liposome-mediated systemic p53 gene therapy in combination with radiation results in regression of human head and neck cancer xenografts." Hum Gene Ther **10**(18): 2941-52.

The use of cationic liposomes as nonviral vehicles for the delivery of therapeutic molecules is becoming increasingly prevalent in the field of gene therapy. We have previously demonstrated that the use of the transferrin ligand (Tf) to target a cationic liposome delivery system resulted in a significant increase in the transfection efficiency of the complex [Xu, L., Pirollo, K.F., and Chang, E.H. (1997). Hum. Gene Ther. 8, 467-475]. Delivery of wild-type (wt) p53 to a radiation-resistant squamous cell carcinoma of the head and neck (SCCHN) cell line via this ligand-targeted, liposome complex was also able to revert the radiation resistant phenotype of these cells in vitro. Here we optimized the Tf/liposome/DNA ratio of the complex (LipT) for maximum tumor cell targeting, even in the presence of serum. The efficient reestablishment of wtp53 function in these SCCHN tumor cells in vitro, via the LipT complex, restored the apoptotic pathway, resulting in a significant increase in radiation-induced apoptosis that was directly proportional to the level of exogenous wtp53 in the tumor cells. More significantly, intravenous administration of LipT-p53 markedly sensitized established SCCHN nude mouse xenograft tumors to radiotherapy. The combination of systemic LipT-p53 gene therapy and radiation resulted in complete tumor regression and inhibition of their recurrence even 6 months after the end of all treatment. These results indicate that this tumor-specific, ligand-liposome delivery system for p53 gene therapy, when used in concert with conventional radiotherapy, can provide a new and more effective means of cancer treatment.

Xu, Q. Y., Y. Gao, et al. (2008). "Identification of differential gene expression profiles of radioresistant lung cancer cell line established by fractionated ionizing radiation in vitro." Chin Med J (Engl) **121**(18): 1830-7.

BACKGROUND: Radiotherapy plays a critical role in the management of non-small cell lung cancer (NSCLC). This study was conducted to identify gene expression profiles of acquired radioresistant NSCLC cell line established by fractionated ionizing radiation (FIR) by cDNA microarray. METHODS: The human lung adenocarcinoma cell line Anip973 was treated with high energy X-ray to receive 60 Gy in 4 Gy fractions. The radiosensitivity of Anip973R and its parental line were measured by clonogenic assay. Gene expression profiles of Anip973R and its parental line were analyzed using cDNA microarray consisting of 21 522 human genes. Identified partly different expressive genes were validated by quantitative reverse transcription-polymerase chain reaction (Q-RT-PCR). RESULTS: Fifty-nine upregulated and 43 downregulated genes were identified to radio-resistant Anip973R. Up-regulated genes were associated with DNA damage repair (DDB2), extracellular matrix (LOX), cell adhesion (CDH2), and apoptosis (CRYAB). Down-regulated genes were associated with angiogenesis (GBP-1), immune response (CD83), and calcium signaling pathway (TNNC1). Subsequent validation of selected eleven genes (CD24, DDB2, IGFBP3, LOX, CDH2, CRYAB, PROCR, ANXA1 DCN, GBP-1 and CD83) by Q-RT-PCR was consistent with microarray analysis. CONCLUSIONS: Fractionated ionizing radiation can lead to the development of radiation resistance. Altered gene profiles of radioresistant cell line may provide new insights into mechanisms underlying clinical radioresistance for NSCLC.

Xu, Y., F. Fang, et al. (2008). "SN52, a novel nuclear factor-kappaB inhibitor, blocks nuclear import of

RelB:p52 dimer and sensitizes prostate cancer cells to ionizing radiation." Mol Cancer Ther **7**(8): 2367-76.

The activation of nuclear factor-kappaB (NF-kappaB) is thought to protect cancer cells against therapy-induced cytotoxicity. RelB, a member of the NF-kappaB family in the alternative pathway, is uniquely expressed at a high level in prostate cancer with high Gleason scores. Here, we show that ionizing radiation (IR) enhances nuclear import of RelB, leading to up-regulation of its target gene, manganese superoxide dismutase (MnSOD), and renders prostate cancer cells resistant to IR. To selectively block RelB nuclear import, we designed a cell-permeable SN52 peptide, a variant of the SN50 peptide that has been shown to block nuclear import of NF-kappaB family members in the classic pathway. Inhibition of IR-induced NF-kappaB activation by SN50 and SN52 was achieved by selectively interrupting the association of p50 and p52 with nuclear import factors importin-alpha1 and importin-beta1. Importantly, SN52 seems to be more efficient for radiosensitization of prostate cancer cells at clinically relevant radiation doses and has less cytotoxicity to normal prostate epithelial cells compared with the toxicity observed with SN50. These results suggest that targeting the alternative pathway is a promising approach to selectively radiosensitize prostate cancers and that SN52 may serve as a prototype biological agent for sensitizing prostate cancers to clinically relevant doses of IR.

Yoshikawa, R., H. Yanagi, et al. (2002). "Prognostic values of radiation-induced p53 in adjacent normal mucosa and p21WAF1/CIP1 expression in rectal cancer patients." Int J Oncol **21**(6): 1223-8.

DNA damage induces p53-mediated cell cycle arrest in which p21WAF1/CIP1, a cyclin-dependent kinase inhibitor, may play a critical role by being regulated via wild-type p53. Although adjuvant preoperative radiotherapy in rectal carcinoma is generally believed to improve the prognosis, it remains unclear which factors control the response. We investigated the interactions between the underlying mechanisms of cell cycle perturbation in response to radiotherapy, and local recurrence and distant metastasis in patients undergoing radical surgery for rectal carcinoma. A retrospective review was carried out in which 63 cases of Dukes' B or C, well or moderately differentiated rectal carcinomas in the lower two-thirds of the rectum, with or without preoperative radiotherapy, were immunohistochemically analyzed using antibodies to p53 and p21WAF1/CIP1. Induced p53 expression in adjacent normal mucosa, as seen in seven of 35 cases with radiotherapy, and mutually exclusive p21WAF1/CIP1 immunoreactivity, was strongly associated with local recurrence (P=0.0001). Furthermore, high p21WAF1/CIP1 expression was associated with a lack of distant metastasis (P=0.032). Our data suggest that there are some cases in which p53 overexpression in adjacent normal mucosa induced by radiotherapeutic treatment might heighten the risk of local recurrence, and that p21WAF1/CIP1 induction independent of the status of the p53 gene showing radiosensitivity might lead to a less distant metastasis.

Zellweger, T., K. Chi, et al. (2002). "Enhanced radiation sensitivity in prostate cancer by inhibition of the cell survival protein clusterin." Clin Cancer Res **8**(10): 3276-84.

PURPOSE: The purpose of this study is to evaluate the role of the cell survival gene clusterin in radiation-induced cell death in human LNCaP and PC-3 prostate cancer models. Experimental Design: Radiation sensitivities were compared in parental and clusterin-overexpressing LNCaP cells and in PC-3 cells and tumors treated with antisense or mismatch clusterin oligonucleotides. RESULTS: Clusterin-overexpressing LNCaP cells were less sensitive to irradiation with significantly lower cell death rates (23% after 8 Gy) compared with parental LNCaP cells (50% after 8 Gy) 3 days after irradiation. Clusterin expression in PC-3 cells after radiation was found to be up-regulated in a dose-dependent manner in vitro by 70% up to 12 Gy and in vivo by 84% up to 30 Gy. Inhibition of clusterin expression in PC-3 cells using antisense oligonucleotides (ASOs) occurred in a sequence- and dose-dependent manner and significantly enhanced radiation-induced apoptosis and decreased PC-3 cell growth rate and plating efficiency. Compared with mismatch control oligonucleotide treatment, clusterin ASO treatment enhanced radiation therapy and significantly reduced PC-3 tumor volume in vivo by 50% at 9 weeks. In addition, TUNEL staining revealed increased number of apoptotic cells in clusterin ASO-treated and irradiated PC-3 tumors, compared with treatment with mismatch control oligonucleotides plus radiation. CONCLUSIONS: These findings support the hypothesis that clusterin acts as a cell survival protein that mediates radioresistance through the inhibition of apoptosis. In vivo results further suggest that inactivation of clusterin using ASO technology might offer a novel strategy to improve results of radiation therapy for prostate cancer patients.

Zhang, M., S. Li, et al. (2003). "Ionizing radiation increases adenovirus uptake and improves transgene expression in intrahepatic colon cancer xenografts." Mol Ther **8**(1): 21-8.

Specific targeting and transgene expression in tumors are critical in adenovirus gene therapy for intrahepatic colon carcinoma metastases. In this study, we investigated if ionizing radiation could increase adenoviral uptake by cells. Various human cell lines and rat hepatocytes were irradiated prior to exposure to a cytomegalovirus (CMV) promoter-driven green fluorescent protein (GFP) marker gene adenoviral vector. We found that gamma-radiation increased the number of GFP-positive cells in a dose- and time-dependent manner for most cells, ranging from 4.6- to 27.1-fold after a 4-Gy treatment. No induction occurred for lentiviral vector, lipofection, or naked plasmid exposure. Preincubation of cells with adenovirus failed to show an increase, suggesting that radiation might mediate adenoviral infection by inducing viral uptake into cells. We demonstrated that radiation induced internalization of a fluorescence-labeled adenovirus (Cy3-Ad) and an increase in intracellular viral DNA content. Rats bearing intrahepatic colon carcinoma xenografts were irradiated in the tumor region followed by portal venous administration of an adenoviral vector containing a CMV-beta-galactosidase (beta-gal) gene. Radiation increased beta-gal activity in tumors as much as 5.4-fold after a 25-Gy treatment. These data suggest that a combination of regional radiation treatment with adenovirus gene therapy is a rational strategy for improving adenoviral targeting and transgene expression in tumors.

Zhou, C., P. Huang, et al. (2005). "The carboxyl-terminal of BRCA1 is required for subnuclear assembly of RAD51 after treatment with cisplatin but not ionizing radiation in human breast and ovarian cancer cells." Biochem Biophys Res Commun **336**(3): 952-60.

BRCA1 plays an important role in maintaining genomic stability through its involvement in DNA repair. Although it is known that BRCA1 and RAD51 form distinct DNA repair subnuclear complexes, or foci, following environmental insults to the DNA, the role of BRCA1 in this process remains to be characterized. The purpose of the study was therefore to determine the role of BRCA1 in the formation of RAD51 foci following treatment with cisplatin and ionizing radiation. We found that although a functional BRCA1 is required for the subnuclear assembly of BRCA1 foci following treatment with either ionizing radiation or cisplatin, a functional BRCA1 is required for RAD51 foci to form following treatment with cisplatin but not with ionizing radiation. Similar results were obtained in SKOV-3 cells when the level of BRCA1 expression was knocked down by stable expression of a retrovirus-mediated small-interfering RNA against BRCA1. We also found that the carboxyl-terminal of BRCA1 contains uncharacterized phosphorylation sites that are responsive to cisplatin. The functional BRCA1 is also required for breast and ovarian cancer cells to mount resistance to cisplatin. These results suggest that the carboxyl-terminal of BRCA1 is required for the cisplatin-induced recruitment of RAD51 to the DNA-damage site, which may contribute to cisplatin resistance.

Zhou, C., J. L. Smith, et al. (2003). "Role of BRCA1 in cellular resistance to paclitaxel and ionizing radiation in an ovarian cancer cell line carrying a defective BRCA1." Oncogene **22**(16): 2396-404.

BRCA1, the gene responsible for approximately half of all cases of hereditary breast cancer and almost all cases of combined hereditary breast and ovarian cancer, has been implicated in the maintenance of genomic stability through DNA repair. This function is mediated, at least in part, through two tandem BRCA1 C-terminal (BRCT) repeats. The role of BRCA1 in the development of ovarian cancer is poorly understood, partially owing to the lack of ovarian cancer cell lines with defective BRCA1. The purpose of this study was to further characterize an endometrioid ovarian cancer cell line, SNU-251, which was previously reported to carry a nonsense mutation (from G to A) at amino acid 1815 of BRCA1. In addition, we examined the role of BRCA1 in the cell cycle and in the responses to the chemotherapy drug paclitaxel and ionizing radiation. Loss of the C-terminal 49 amino acids due to this point mutation did not affect the expression of the truncated BRCA1 protein, but caused a loss of transcriptional activation of the endogenous p21(WAF1/CIP1) gene, and could not sustain arrest in the G(2)/M phase of the cell cycle. The BRCA1 mutation in SNU-251 cells inhibited BRCA1 subnuclear assembly for DNA-damage repair and increased cellular sensitivity to ionizing radiation and paclitaxel. This sensitivity was reversed by reintroduction of ectopic wild-type BRCA1. Our results suggest that the deletion of the C-terminal 49 amino acids of BRCA1 results in a loss of BRCA1 function in the SNU-251 cell line. BRCA1 helps to mediate the resistance to both radiation and paclitaxel. Therefore, SNU-251 may be a useful model for studying the molecular mechanism of BRCA1 in the resistance of ovarian cancer to ionizing radiation and chemotherapy treatment and in the development of hereditary human ovarian cancer.

References

1. Abbott, D. W., M. E. Thompson, et al. (1999). "BRCA1 expression restores radiation resistance in BRCA1-defective cancer cells through enhancement of transcription-coupled DNA repair." J Biol Chem **274**(26): 18808-12.

2. Abbott, D. W., M. L. Freeman, et al. (1998). "Double-strand break repair deficiency and radiation sensitivity in BRCA2 mutant cancer cells." J Natl Cancer Inst **90**(13): 978-85.
3. Abela, R. A., J. Qian, et al. (2008). "Radiation improves gene delivery by a novel transferrin-lipoplex nanoparticle selectively in cancer cells." Cancer Gene Ther **15**(8): 496-507.
4. Adusumilli, P. S., B. M. Stiles, et al. (2005). "Radiation therapy potentiates effective oncolytic viral therapy in the treatment of lung cancer." Ann Thorac Surg **80**(2): 409-16; discussion 416-7.
5. Ahmed, M. M., D. Chendil, et al. (2001). "Early growth response-1 gene: potential radiation response gene marker in prostate cancer." Am J Clin Oncol **24**(5): 500-5.
6. Alcock, R. A., S. Dey, et al. (2002). "Farnesyltransferase inhibitor (L-744,832) restores TGF-beta type II receptor expression and enhances radiation sensitivity in K-ras mutant pancreatic cancer cell line MIA PaCa-2." Oncogene **21**(51): 7883-90.
7. Amundson, S. A., K. T. Do, et al. (2008). "Integrating global gene expression and radiation survival parameters across the 60 cell lines of the National Cancer Institute Anticancer Drug Screen." Cancer Res **68**(2): 415-24.
8. Ananthaswamy, H. N. (1986). "Relationship between expression of tumor-specific transplantation antigens and neoplastic transformation in an ultraviolet radiation-induced murine skin cancer." Cancer Res **46**(12 Pt 1): 6322-6.
9. Arnold, S. F., E. Tims, et al. (1999). "Regulation of transforming growth factor beta1 by radiation in cells of two human breast cancer cell lines." Radiat Res **152**(5): 487-92.
10. Balcer-Kubiczek, E. K., S. J. Meltzer, et al. (1997). "Csa-19, a radiation-responsive human gene, identified by an unbiased two-gel cDNA library screening method in human cancer cells." Oncogene **14**(25): 3051-7.
11. Barcellos-Hoff, M. H. (2008). "Cancer as an emergent phenomenon in systems radiation biology." Radiat Environ Biophys **47**(1): 33-8.
12. Bar-Sela, G., K. M. Jacobs, et al. (2007). "Histone deacetylase inhibitor and demethylating agent chromatin compaction and the radiation response by cancer cells." Cancer J **13**(1): 65-9.
13. Bennett, L. M. (1999). "Breast cancer: genetic predisposition and exposure to radiation." Mol Carcinog **26**(3): 143-9.
14. Bhatti, P., J. P. Struewing, et al. (2008). "Polymorphisms in DNA repair genes, ionizing radiation exposure and risk of breast cancer in U.S. Radiologic technologists." Int J Cancer **122**(1): 177-82.
15. Billecke, C. A., M. E. Ljungman, et al. (2002). "Lack of functional pRb results in attenuated recovery of mRNA synthesis and increased apoptosis following UV radiation in human breast cancer cells." Oncogene **21**(29): 4481-9.
16. Birrell, G. W. and J. R. Ramsay (1995). "Induction of p53 protein by gamma radiation in lymphocyte lines from breast cancer and ataxia telangiectasia patients." Br J Cancer **72**(5): 1096-101.
17. Broeks, A., L. M. Braaf, et al. (2007). "Identification of women with an increased risk of developing radiation-induced breast cancer: a case only study." Breast Cancer Res **9**(2): R26.
18. Brown, E. T. and J. T. Holt (2009). "Rad51 overexpression rescues radiation resistance in BRCA2-defective cancer cells." Mol Carcinog **48**(2): 105-9.
19. Buchholz, T. A. and X. Wu (2001). "Radiation-induced chromatid breaks as a predictor of breast cancer risk." Int J Radiat Oncol Biol Phys **49**(2): 533-7.
20. Cao, J., Y. Liu, et al. (2002). "Chromosomal aberrations, DNA strand breaks and gene mutations in nasopharyngeal cancer patients undergoing radiation therapy." Mutat Res **504**(1-2): 85-90.
21. Cardis, E., J. Hall, et al. (2007). "Identification of women with an increased risk of developing radiation-induced breast cancer." Breast Cancer Res **9**(3): 106.
22. Chen, X., C. A. Arciero, et al. (2006). "BRCA1-associated complexes: new targets to overcome breast cancer radiation resistance." Expert Rev Anticancer Ther **6**(2): 187-96.
23. Chendil, D., A. Das, et al. (2002). "Par-4, a pro-apoptotic gene, inhibits radiation-induced NF kappa B activity and Bcl-2 expression leading to induction of radiosensitivity in human prostate cancer cells PC-3." Cancer Biol Ther **1**(2): 152-60.
24. Cheo, D. L., L. B. Meira, et al. (1996). "Synergistic interactions between XPC and p53 mutations in double-mutant mice: neural tube abnormalities and accelerated UV radiation-induced skin cancer." Curr Biol **6**(12): 1691-4.
25. Cheo, D. L., L. B. Meira, et al. (2000). "Ultraviolet B radiation-induced skin cancer in mice defective in the Xpc, Trp53, and Apex (HAP1) genes: genotype-specific effects on cancer predisposition and pathology of tumors." Cancer Res **60**(6): 1580-4.
26. Chetty, C., P. Bhoopathi, et al. (2009). "Inhibition of matrix metalloproteinase-2 enhances radiosensitivity by abrogating radiation-induced FoxM1-mediated G2/M arrest in A549 lung cancer cells." Int J Cancer **124**(10): 2468-77.
27. Chung, Y. L., M. Y. Lee, et al. (2009). "Epigenetic therapy using the histone deacetylase inhibitor for increasing therapeutic gain in oral cancer: prevention of radiation-induced oral mucositis and inhibition of chemical-induced oral carcinogenesis." Carcinogenesis **30**(8): 1387-97.
28. Chung, Y. M., B. G. Kim, et al. (2005). "Increased expression of ICAM-3 is associated with radiation resistance in cervical cancer." Int J Cancer **117**(2): 194-201.
29. Collis, S. J., M. J. Swartz, et al. (2003). "Enhanced radiation and chemotherapy-mediated cell killing of human cancer cells by small inhibitory RNA silencing of DNA repair factors." Cancer Res **63**(7): 1550-4.
30. De Benedetti, V. M., L. B. Travis, et al. (1996). "p53 mutations in lung cancer following radiation therapy for Hodgkin's disease." Cancer Epidemiol Biomarkers Prev **5**(2): 93-8.
31. de Gruijl, F. R. (1999). "Skin cancer and solar UV radiation." Eur J Cancer **35**(14): 2003-9.
32. DelloRusso, C., P. L. Welcsh, et al. (2007). "Functional characterization of a novel BRCA1-null ovarian cancer cell line in response to ionizing radiation." Mol Cancer Res **5**(1): 35-45.
33. Du, X. L., T. Jiang, et al. (2009). "Differential expression profiling of gene response to ionizing radiation in two endometrial cancer cell lines with distinct radiosensitivities." Oncol Rep **21**(3): 625-34.
34. Fallis, L. H., E. Richards, et al. (1999). "The biological response of MCF7 breast cancer cells to proteosome inhibition or gamma-radiation is unrelated to the level of p53 induction." Apoptosis **4**(2): 99-107.
35. Fan, S., N. F. Twu, et al. (1998). "Down-regulation of BRCA1 and BRCA2 in human ovarian cancer cells exposed to adriamycin and ultraviolet radiation." Int J Cancer **77**(4): 600-9.
36. Fan, Z., P. Chakravarty, et al. (2000). "Adenovirus-mediated antisense ATM gene transfer sensitizes prostate cancer cells to radiation." Cancer Gene Ther **7**(10): 1307-14.
37. Fogelfeld, L., T. K. Bauer, et al. (1996). "p53 gene mutations in radiation-induced thyroid cancer." J Clin Endocrinol Metab **81**(8): 3039-44.
38. Freytag, S. O., H. Stricker, et al. (2003). "Phase I study of replication-competent adenovirus-mediated double-suicide gene therapy in combination with conventional-dose three-dimensional conformal radiation therapy for the treatment of newly diagnosed, intermediate- to high-risk prostate cancer." Cancer Res **63**(21): 7497-506.
39. Furre, T., E. I. Furre, et al. (2003). "Lack of inverse dose-rate effect and binding of the retinoblastoma gene product in the nucleus of human cancer T-47D cells arrested in G2 by ionizing radiation." Int J Radiat Biol **79**(6): 413-22.
40. Goss, P. E. and S. Sierra (1998). "Current perspectives on radiation-induced breast cancer." J Clin Oncol **16**(1): 338-47.
41. Gridley, D. S. and J. M. Slater (2004). "Combining gene therapy and radiation against cancer." Curr Gene Ther **4**(3): 231-48.
42. Hall, E. J., P. B. Schiff, et al. (1998). "A preliminary report: frequency of A-T heterozygotes among prostate cancer patients with severe late responses to radiation therapy." Cancer J Sci Am **4**(6): 385-9.
43. Hall, J. and S. Angele (1999). "Radiation, DNA damage and cancer." Mol Med Today **5**(4): 157-64.
44. Hamilton, J. P., F. Sato, et al. (2006). "Promoter methylation and response to chemotherapy and radiation in esophageal cancer." Clin Gastroenterol Hepatol **4**(6): 701-8.
45. Han, Z., H. Wang, et al. (2006). "Radiation-guided gene therapy of cancer." Technol Cancer Res Treat **5**(4): 437-44.
46. Harada, K., Y. Obiya, et al. (1997). "Cancer risk in space due to radiation assessed by determining cell lethality and mutation

frequencies of prokaryotes and a plasmid during the Second International Microgravity Laboratory (IML-2) Space Shuttle experiment." Oncol Rep **4**(4): 691-5.
47. Helland, A., H. Johnsen, et al. (2006). "Radiation-induced effects on gene expression: an in vivo study on breast cancer." Radiother Oncol **80**(2): 230-5.
48. Hellman, B., D. Brodin, et al. (2005). "Radiation-induced DNA-damage and gene expression profiles in human lung cancer cells with different radiosensitivity." Exp Oncol **27**(2): 102-7.
49. Henness, S., M. W. Davey, et al. (2002). "Fractionated irradiation of H69 small-cell lung cancer cells causes stable radiation and drug resistance with increased MRP1, MRP2, and topoisomerase IIalpha expression." Int J Radiat Oncol Biol Phys **54**(3): 895-902.
50. Hill, J. W., K. Tansavatdi, et al. (2009). "Validation of the cell cycle G(2) delay assay in assessing ionizing radiation sensitivity and breast cancer risk." Cancer Manag Res **1**: 39-48.
51. Hino, O., A. J. Klein-Szanto, et al. (1993). "Spontaneous and radiation-induced renal tumors in the Eker rat model of dominantly inherited cancer." Proc Natl Acad Sci U S A **90**(1): 327-31.
52. Hino, S., H. Kawamata, et al. (2002). "Cytoplasmic TSC-22 (transforming growth factor-beta-stimulated clone-22) markedly enhances the radiation sensitivity of salivary gland cancer cells." Biochem Biophys Res Commun **292**(4): 957-63.
53. Hu, J. J., T. R. Smith, et al. (2002). "Genetic regulation of ionizing radiation sensitivity and breast cancer risk." Environ Mol Mutagen **39**(2-3): 208-15.
54. Huang, A., R. Gandour-Edwards, et al. (1998). "p53 and bcl-2 immunohistochemical alterations in prostate cancer treated with radiation therapy." Urology **51**(2): 346-51.
55. Hurley, P. J., D. Wilsker, et al. (2007). "Human cancer cells require ATR for cell cycle progression following exposure to ionizing radiation." Oncogene **26**(18): 2535-42.
56. Hussein, M. R. (2005). "Ultraviolet radiation and skin cancer: molecular mechanisms." J Cutan Pathol **32**(3): 191-205.
57. Iannuzzi, C. M., D. P. Atencio, et al. (2002). "ATM mutations in female breast cancer patients predict for an increase in radiation-induced late effects." Int J Radiat Oncol Biol Phys **52**(3): 606-13.
58. Iwakawa, M., T. Ohno, et al. (2007). "The radiation-induced cell-death signaling pathway is activated by concurrent use of cisplatin in sequential biopsy specimens from patients with cervical cancer." Cancer Biol Ther **6**(6): 905-11.
59. Jiang, W., H. N. Ananthaswamy, et al. (1999). "p53 protects against skin cancer induction by UV-B radiation." Oncogene **18**(29): 4247-53.
60. Jiao, Y., C. M. Ge, et al. (2007). "Adenovirus-mediated expression of Tob1 sensitizes breast cancer cells to ionizing radiation." Acta Pharmacol Sin **28**(10): 1628-36.
61. Jongmans, W. and J. Hall (1999). "Cellular responses to radiation and risk of breast cancer." Eur J Cancer **35**(4): 540-8.
62. Josson, S., S. Y. Sung, et al. (2008). "Radiation modulation of microRNA in prostate cancer cell lines." Prostate **68**(15): 1599-606.
63. Kasid, U., A. Pfeifer, et al. (1987). "The raf oncogene is associated with a radiation-resistant human laryngeal cancer." Science **237**(4818): 1039-41.
64. Kassem, H., V. Sangar, et al. (2002). "A potential role of heat shock proteins and nicotinamide N-methyl transferase in predicting response to radiation in bladder cancer." Int J Cancer **101**(5): 454-60.
65. Kaufmann, W. K., L. Filatov, et al. (2006). "Radiation clastogenesis and cell cycle checkpoint function as functional markers of breast cancer risk." Carcinogenesis **27**(12): 2519-27.
66. Kavgaci, H., F. Ozdemir, et al. (2005). "Effect of the farnesyl transferase inhibitor L-744,832 on the colon cancer cell line DLD-1 and its combined use with radiation and 5-FU." Chemotherapy **51**(6): 319-23.
67. Kim, C. Y., A. J. Giaccia, et al. (1992). "Differential expression of protein kinase C epsilon protein in lung cancer cell lines by ionising radiation." Br J Cancer **66**(5): 844-9.
68. Kyprianou, N. and S. Rock (1998). "Radiation-induced apoptosis of human prostate cancer cells is independent of mutant p53 overexpression." Anticancer Res **18**(2A): 897-905.
69. Kyprianou, N., E. D. King, et al. (1997). "bcl-2 over-expression delays radiation-induced apoptosis without affecting the clonogenic survival of human prostate cancer cells." Int J Cancer **70**(3): 341-8.
70. Lee, C., J. S. Kim, et al. (2004). "PTEN gene targeting reveals a radiation-induced size checkpoint in human cancer cells." Cancer Res **64**(19): 6906-14.
71. Leong, T., J. Whitty, et al. (2000). "Mutation analysis of BRCA1 and BRCA2 cancer predisposition genes in radiation hypersensitive cancer patients." Int J Radiat Oncol Biol Phys **48**(4): 959-65.
72. Li, Z., L. Xia, et al. (2001). "Effector genes altered in MCF-7 human breast cancer cells after exposure to fractionated ionizing radiation." Radiat Res **155**(4): 543-53.
73. Lindel, K., P. Burri, et al. (2005). "Human papillomavirus status in advanced cervical cancer: predictive and prognostic significance for curative radiation treatment." Int J Gynecol Cancer **15**(2): 278-84.
74. Liu, S. S., A. N. Cheung, et al. (2003). "Differential gene expression in cervical cancer cell lines before and after ionizing radiation." Int J Oncol **22**(5): 1091-9.
75. Lu, B., Y. Mu, et al. (2004). "Survivin as a therapeutic target for radiation sensitization in lung cancer." Cancer Res **64**(8): 2840-5.
76. Lu, X., C. Yang, et al. (2008). "Inactivation of gadd45a sensitizes epithelial cancer cells to ionizing radiation in vivo resulting in prolonged survival." Cancer Res **68**(10): 3579-83.
77. Lupold, S. E. and R. Rodriguez (2005). "Adenoviral gene therapy, radiation, and prostate cancer." Rev Urol **7**(4): 193-202.
78. Mamon, H. J., W. Dahlberg, et al. (2003). "Differing effects of breast cancer 1, early onset (BRCA1) and ataxia-telangiectasia mutated (ATM) mutations on cellular responses to ionizing radiation." Int J Radiat Biol **79**(10): 817-29.
79. Mendonca, M. S., D. L. Farrington, et al. (2004). "Homozygous deletions within the 11q13 cervical cancer tumor-suppressor locus in radiation-induced, neoplastically transformed human hybrid cells." Genes Chromosomes Cancer **39**(4): 277-87.
80. Millikan, R. C., J. S. Player, et al. (2005). "Polymorphisms in DNA repair genes, medical exposure to ionizing radiation, and breast cancer risk." Cancer Epidemiol Biomarkers Prev **14**(10): 2326-34.
81. Mirzayans, R., L. Enns, et al. (1996). "Faulty DNA polymerase delta/epsilon-mediated excision repair in response to gamma radiation or ultraviolet light in p53-deficient fibroblast strains from affected members of a cancer-prone family with Li-Fraumeni syndrome." Carcinogenesis **17**(4): 691-8.
82. Mitsuhashi, M., D. Peel, et al. (2009). "Enhanced Expression of Radiation-induced Leukocyte CDKN1A mRNA in Multiple Primary Breast Cancer Patients: Potential New Marker of Cancer Susceptibility." Biomark Insights **4**: 201-9.
83. Moon, S., S. Holley, et al. (2006). "Associations between G/A1229, A/G3944, T/C30875, C/T48200 and C/T65013 genotypes and haplotypes in the vitamin D receptor gene, ultraviolet radiation and susceptibility to prostate cancer." Ann Hum Genet **70**(Pt 2): 226-36.
84. Nakagawa, T., N. Otsuki, et al. (2008). "Additive effects of oral fluoropyrimidine derivative S-1 and radiation on human hypopharyngeal cancer xenografts." Acta Otolaryngol **128**(8): 936-40.
85. Nakamura, M., K. Masutomi, et al. (2005). "Efficient inhibition of human telomerase reverse transcriptase expression by RNA interference sensitizes cancer cells to ionizing radiation and chemotherapy." Hum Gene Ther **16**(7): 859-68.
86. Nakashima, T., M. Masuda, et al. (1999). "Induction of apoptosis in maxillary sinus cancer cells by 5-fluorouracil, vitamin A and radiation (FAR) therapy." Eur Arch Otorhinolaryngol **256 Suppl 1**: S64-9.
87. Neta, R. (2000). "The promise of molecular epidemiology in defining the association between radiation and cancer." Health Phys **79**(1): 77-84.
88. Ng, C. E., Q. Y. Liu, et al. (2007). "Transcriptomic profiling of radiation-resistant or -sensitive human colorectal cancer cells: acute effects of a single X-radiation dose." Int J Oncol **30**(6): 1369-80.
89. Nuyts, S., L. Van Mellaert, et al. (2001). "The use of radiation-induced bacterial promoters in anaerobic conditions: a means to control gene expression in clostridium-mediated therapy for cancer." Radiat Res **155**(5): 716-23.

90. Ohnishi, K., H. Inaba, et al. (2004). "C-terminal peptides of p53 molecules enhance radiation-induced apoptosis in human mutant p53 cancer cells." Apoptosis **9**(5): 591-7.
91. Ohnishi, K., Z. Scuric, et al. (2006). "siRNA targeting NBS1 or XIAP increases radiation sensitivity of human cancer cells independent of TP53 status." Radiat Res **166**(3): 454-62.
92. Ohnishi, T. (2005). "The role of the p53 molecule in cancer therapies with radiation and/or hyperthermia." J Cancer Res Ther **1**(3): 147-50.
93. Ohnishi, T., A. Takahashi, et al. (2008). "p53 Targeting can enhance cancer therapy via radiation, heat and anti-cancer agents." Anticancer Agents Med Chem **8**(5): 564-70.
94. Oka, A., Y. Harima, et al. (2000). "Determination of p53-mediated transactivational ability in radiation-treated cervical cancer." Cancer Detect Prev **24**(3): 275-82.
95. O'Malley, B. W., Jr., D. Li, et al. (2003). "Molecular disruption of the MRN(95) complex induces radiation sensitivity in head and neck cancer." Laryngoscope **113**(9): 1588-94.
96. Oshita, F., Y. Kato, et al. (1999). "A feasibility study of continuous etoposide infusion combined with thoracic radiation for non-small cell lung cancer." Oncol Rep **6**(2): 263-8.
97. Pajonk, F., A. van Ophoven, et al. (2005). "The proteasome inhibitor MG-132 sensitizes PC-3 prostate cancer cells to ionizing radiation by a DNA-PK-independent mechanism." BMC Cancer **5**: 76.
98. Pestell, K. E., C. J. Medlow, et al. (1998). "Characterisation of the p53 status, BCL-2 expression and radiation and platinum drug sensitivity of a panel of human ovarian cancer cell lines." Int J Cancer **77**(6): 913-8.
99. Piening, B. D., P. Wang, et al. (2009). "A radiation-derived gene expression signature predicts clinical outcome for breast cancer patients." Radiat Res **171**(2): 141-54.
100. Price, F. M., R. Parshad, et al. (1991). "Radiation-induced chromatid aberrations in Cockayne syndrome and xeroderma pigmentosum group C fibroblasts in relation to cancer predisposition." Cancer Genet Cytogenet **57**(1): 1-10.
101. Quarmby, S., H. Fakhoury, et al. (2003). "Association of transforming growth factor beta-1 single nucleotide polymorphisms with radiation-induced damage to normal tissues in breast cancer patients." Int J Radiat Biol **79**(2): 137-43.
102. Rajaraman, P., P. Bhatti, et al. (2008). "Nucleotide excision repair polymorphisms may modify ionizing radiation-related breast cancer risk in US radiologic technologists." Int J Cancer **123**(11): 2713-6.
103. Rakozy, C., D. J. Grignon, et al. (1999). "p53 gene alterations in prostate cancer after radiation failure and their association with clinical outcome: a molecular and immunohistochemical analysis." Pathol Res Pract **195**(3): 129-35.
104. Ribeiro, J. C., A. R. Barnetson, et al. (1997). "Relationship between radiation response and p53 status in human bladder cancer cells." Int J Radiat Biol **72**(1): 11-20.
105. Ritter, M. A., K. W. Gilchrist, et al. (2002). "The role of p53 in radiation therapy outcomes for favorable-to-intermediate-risk prostate cancer." Int J Radiat Oncol Biol Phys **53**(3): 574-80.
106. Rothkamm, K. and M. Lobrich (2002). "Misrepair of radiation-induced DNA double-strand breaks and its relevance for tumorigenesis and cancer treatment (review)." Int J Oncol **21**(2): 433-40.
107. Rukin, N. J., C. Luscombe, et al. (2007). "Prostate cancer susceptibility is mediated by interactions between exposure to ultraviolet radiation and polymorphisms in the 5' haplotype block of the vitamin D receptor gene." Cancer Lett **247**(2): 328-35.
108. Saga, Y., M. Suzuki, et al. (2002). "Enhanced expression of thymidylate synthase mediates resistance of uterine cervical cancer cells to radiation." Oncology **63**(2): 185-91.
109. Sakakura, C., E. A. Sweeney, et al. (1997). "Overexpression of bax enhances the radiation sensitivity in human breast cancer cells." Surg Today **27**(1): 90-3.
110. Sankaranarayanan, K. and R. Chakraborty (1995). "Cancer predisposition, radiosensitivity and the risk of radiation-induced cancers. I. Background." Radiat Res **143**(2): 121-43.
111. Scott, B. R., S. A. Belinsky, et al. (2009). "Radiation-stimulated epigenetic reprogramming of adaptive-response genes in the lung: an evolutionary gift for mounting adaptive protection against lung cancer." Dose Response **7**(2): 104-31.
112. Scott, S. D., M. C. Joiner, et al. (2002). "Optimizing radiation-responsive gene promoters for radiogenetic cancer therapy." Gene Ther **9**(20): 1396-402.
113. Scott, S. L., J. D. Earle, et al. (2003). "Functional p53 increases prostate cancer cell survival after exposure to fractionated doses of ionizing radiation." Cancer Res **63**(21): 7190-6.
114. Servomaa, K., A. Kiuru, et al. (1996). "p53 mutations associated with increased sensitivity to ionizing radiation in human head and neck cancer cell lines." Cell Prolif **29**(5): 219-30.
115. Shafman, T. D., S. Levitz, et al. (2000). "Prevalence of germline truncating mutations in ATM in women with a second breast cancer after radiation therapy for a contralateral tumor." Genes Chromosomes Cancer **27**(2): 124-9.
116. Shimada, K., M. Nakamura, et al. (2003). "Androgen and the blocking of radiation-induced sensitization to Fas-mediated apoptosis through c-jun induction in prostate cancer cells." Int J Radiat Biol **79**(6): 451-62.
117. Shin, S., H. J. Cha, et al. (2009). "Alteration of miRNA profiles by ionizing radiation in A549 human non-small cell lung cancer cells." Int J Oncol **35**(1): 81-6.
118. Siden, T. S., W. Golembieski, et al. (1994). "Physical map of small cell lung cancer deletion region on short arm of human chromosome 3 (3p13-22) based on radiation fusion hybrid analysis." Somat Cell Mol Genet **20**(2): 121-32.
119. Sigurdson, A. J., P. Bhatti, et al. (2007). "Polymorphisms in apoptosis- and proliferation-related genes, ionizing radiation exposure, and risk of breast cancer among U.S. Radiologic Technologists." Cancer Epidemiol Biomarkers Prev **16**(10): 2000-7.
120. Singh-Gupta, V., H. Zhang, et al. (2009). "Radiation-induced HIF-1alpha cell survival pathway is inhibited by soy isoflavones in prostate cancer cells." Int J Cancer **124**(7): 1675-84.
121. Smith, D. J., M. Jaggi, et al. (2006). "Metallothioneins and resistance to cisplatin and radiation in prostate cancer." Urology **67**(6): 1341-7.
122. Soehnge, H., A. Ouhtit, et al. (1997). "Mechanisms of induction of skin cancer by UV radiation." Front Biosci **2**: d538-51.
123. Sohda, M., H. Ishikawa, et al. (2004). "Pretreatment evaluation of combined HIF-1alpha, p53 and p21 expression is a useful and sensitive indicator of response to radiation and chemotherapy in esophageal cancer." Int J Cancer **110**(6): 838-44.
124. Sreekumar, A., M. K. Nyati, et al. (2001). "Profiling of cancer cells using protein microarrays: discovery of novel radiation-regulated proteins." Cancer Res **61**(20): 7585-93.
125. 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.
126. Stuschke, M., A. Sak, et al. (2002). "Radiation-induced apoptosis in human non-small-cell lung cancer cell lines is secondary to cell-cycle progression beyond the G2-phase checkpoint." Int J Radiat Biol **78**(9): 807-19.
127. Sutton, D., S. Kim, et al. (2006). "Efficient suppression of secretory clusterin levels by polymer-siRNA nanocomplexes enhances ionizing radiation lethality in human MCF-7 breast cancer cells in vitro." Int J Nanomedicine **1**(2): 155-62.
128. Sylvain, V., S. Lafarge, et al. (2001). "Molecular pathways involved in response to ionizing radiation of ID-8 mouse ovarian cancer cells expressing exogenous full-length Brca1 or truncated Brca1 mutant." Int J Oncol **19**(3): 599-607.
129. Tao, Y., P. Zhang, et al. (2007). "Enhancement of radiation response by inhibition of Aurora-A kinase using siRNA or a selective Aurora kinase inhibitor PHA680632 in p53-deficient cancer cells." Br J Cancer **97**(12): 1664-72.
130. Terleth, C., T. van Laar, et al. (1997). "A lack of radiation-induced ornithine decarboxylase activity prevents enhanced reactivation of herpes simplex virus and is linked to non-cancer proneness in xeroderma pigmentosum patients." Cancer Res **57**(19): 4384-92.
131. Tevz, G., S. Kranjc, et al. (2009). "Controlled systemic release of interleukin-12 after gene electrotransfer to muscle for cancer gene therapy alone or in combination with ionizing radiation in murine sarcomas." J Gene Med **11**(12): 1125-37.
132. Tillmanns, T. D., S. A. Kamelle, et al. (2005). "Sensitization of cervical cancer cell lines to low-dose radiation by retinoic acid does not require functional p53." Gynecol Oncol **97**(1): 142-50.

133. Tjebbes, G. W., P. A. Kreijveld, et al. (2002). "P53 tumor suppressor gene mutations in laryngeal cancer and in recurrent disease following radiation therapy." Oral Oncol **38**(3): 296-300.
134. Truman, J. P., N. Gueven, et al. (2005). "Down-regulation of ATM protein sensitizes human prostate cancer cells to radiation-induced apoptosis." J Biol Chem **280**(24): 23262-72.
135. Tsai, K. K., J. Stuart, et al. (2009). "Low-dose radiation-induced senescent stromal fibroblasts render nearby breast cancer cells radioresistant." Radiat Res **172**(3): 306-13.
136. Turner, B. C., E. Harrold, et al. (1999). "BRCA1/BRCA2 germline mutations in locally recurrent breast cancer patients after lumpectomy and radiation therapy: implications for breast-conserving management in patients with BRCA1/BRCA2 mutations." J Clin Oncol **17**(10): 3017-24.
137. Valdagni, R., T. Rancati, et al. (2009). "To bleed or not to bleed. A prediction based on individual gene profiling combined with dose-volume histogram shapes in prostate cancer patients undergoing three-dimensional conformal radiation therapy." Int J Radiat Oncol Biol Phys **74**(5): 1431-40.
138. van der Horst, G. T., L. Meira, et al. (2002). "UVB radiation-induced cancer predisposition in Cockayne syndrome group A (Csa) mutant mice." DNA Repair (Amst) **1**(2): 143-57.
139. Vares, G., K. Ory, et al. (2004). "Progesterone prevents radiation-induced apoptosis in breast cancer cells." Oncogene **23**(26): 4603-13.
140. Varghese, S., R. K. Schmidt-Ullrich, et al. (1999). "Enhanced radiation late effects and cellular radiation sensitivity in an ATM heterozygous breast cancer patient." Radiat Oncol Investig **7**(4): 231-7.
141. Vorobtsova, I. E. (1989). "Increased cancer risk as a genetic effect of ionizing radiation." IARC Sci Publ(96): 389-401.
142. Wakatsuki, M., T. Ohno, et al. (2008). "p73 protein expression correlates with radiation-induced apoptosis in the lack of p53 response to radiation therapy for cervical cancer." Int J Radiat Oncol Biol Phys **70**(4): 1189-94.
143. Wang, J. A., S. Fan, et al. (1999). "Ultraviolet radiation down-regulates expression of the cell-cycle inhibitor p21WAF1/CIP1 in human cancer cells independently of p53." Int J Radiat Biol **75**(3): 301-16.
144. Weichselbaum, R. R., H. Ishwaran, et al. (2008). "An interferon-related gene signature for DNA damage resistance is a predictive marker for chemotherapy and radiation for breast cancer." Proc Natl Acad Sci U S A **105**(47): 18490-5.
145. Whitfield, J. F. (2006). "Parathyroid hormone: a novel tool for treating bone marrow depletion in cancer patients caused by chemotherapeutic drugs and ionizing radiation." Cancer Lett **244**(1): 8-15.
146. Wu, X., J. Gu, et al. (2006). "Genetic variations in radiation and chemotherapy drug action pathways predict clinical outcomes in esophageal cancer." J Clin Oncol **24**(23): 3789-98.
147. Xia, L., A. Paik, et al. (2004). "p53 activation in chronic radiation-treated breast cancer cells: regulation of MDM2/p14ARF." Cancer Res **64**(1): 221-8.
148. Xu, L., K. F. Pirollo, et al. (1999). "Transferrin-liposome-mediated systemic p53 gene therapy in combination with radiation results in regression of human head and neck cancer xenografts." Hum Gene Ther **10**(18): 2941-52.
149. Xu, Q. Y., Y. Gao, et al. (2008). "Identification of differential gene expression profiles of radioresistant lung cancer cell line established by fractionated ionizing radiation in vitro." Chin Med J (Engl) **121**(18): 1830-7.
150. Xu, Y., F. Fang, et al. (2008). "SN52, a novel nuclear factor-kappaB inhibitor, blocks nuclear import of RelB:p52 dimer and sensitizes prostate cancer cells to ionizing radiation." Mol Cancer Ther **7**(8): 2367-76.
151. Yoshikawa, R., H. Yanagi, et al. (2002). "Prognostic values of radiation-induced p53 in adjacent normal mucosa and p21WAF1/CIP1 expression in rectal cancer patients." Int J Oncol **21**(6): 1223-8.
152. Zellweger, T., K. Chi, et al. (2002). "Enhanced radiation sensitivity in prostate cancer by inhibition of the cell survival protein clusterin." Clin Cancer Res **8**(10): 3276-84.
153. Zhang, M., S. Li, et al. (2003). "Ionizing radiation increases adenovirus uptake and improves transgene expression in intrahepatic colon cancer xenografts." Mol Ther **8**(1): 21-8.
154. Zhou, C., J. L. Smith, et al. (2003). "Role of BRCA1 in cellular resistance to paclitaxel and ionizing radiation in an ovarian cancer cell line carrying a defective BRCA1." Oncogene **22**(16): 2396-404.
155. Zhou, C., P. Huang, et al. (2005). "The carboxyl-terminal of BRCA1 is required for subnuclear assembly of RAD51 after treatment with cisplatin but not ionizing radiation in human breast and ovarian cancer cells." Biochem Biophys Res Commun **336**(3): 952-60.
156. PubMed (2011). http://www.ncbi.nlm.nih.gov/pubmed.
157. Cancer. Wikipedia. (2011) http://en.wikipedia.org/wiki/Cancer.

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