Cancer and Carcinogens Research 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 paper is the collections of literatures on carcinogen research.
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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

Al-Saleh, I., J. Arif, et al. (2008). "Carcinogen DNA adducts and the risk of colon cancer: case-control study." Biomarkers **13**(2): 201-16.

Colorectal cancer represents 8.5% of all tumours at the King Faisal Specialist Hospital & Research Centre. Environmental and dietary carcinogens such as polycyclic aromatic hydrocarbons (PAHs) and heterocyclic amines (HCAs) have long been suspected to play a prominent role in colon cancer aetiology. We designed a case-control study to test the hypothesis of whether or not the presence of DNA adducts can play a role in the aetiology of colon cancer. DNA adducts were measured in 24 cancerous and 20 non-cancerous tissue samples of newly diagnosed colon cancer patients by (32)P-post-labelling technique. Normal tissue from 19 hospital patients served as controls. The mean levels of adducts per 10(10) nucleotides in cancerous and non-cancerous tissue were 151.75+/-217.27 and 114.81+/-186.10, respectively; however, only adducts in cancerous tissue were significantly higher than controls (32.78+/-57.51 per 10(10) nucleotides) with p-values of 0.017. No BPDE-DNA adducts were found. No relationship was found between urinary cotinine as a marker of tobacco smoke and 1-hydroxypyrene as an indicator of an individual's internal dose of PAHs and DNA adducts. In a logistic regression model, only adducts in cancerous tissue were associated with the subsequent risk of colon cancer, with an odds ratio of 3.587 (95% confidence interval 0.833-15.448) after adjustment for age and the duration of living in the current region, but of a borderline significance (p=0.086). Although it is difficult to arrive at a definite conclusion from a small dataset, our preliminary results suggest the potential role of DNA adducts in the colon carcinogenesis process. Additional studies with larger sample sizes are needed to confirm our preliminary finding. It is also important to identify the structural characterization of these unknown DNA adducts in order to have a better understanding of whether or not environmental carcinogens play a role in the aetiology of colon cancer.

Aranganathan, S., J. P. Selvam, et al. (2008). "Effect of hesperetin, a citrus flavonoid, on bacterial enzymes and carcinogen-induced aberrant crypt foci in colon cancer rats: a dose-dependent study." J Pharm Pharmacol **60**(10): 1385-92.

Hesperetin, an important bioactive compound in Chinese traditional medicine, has antioxidant and anticarcinogenic properties. Hesperetin is found in abundance in orange and grape juices (200-590 mg L(-1)) consumed in the daily diet. We have investigated the effect of different doses of hesperetin on faecal and colonic mucosal bacterial enzymes and aberrant crypt foci (ACF) in 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis in male Wistar rats. The rats were divided into six groups and were fed a modified pellet diet for 16 weeks. Group 1 served as control and group 2 received the modified pellet diet along with hesperetin (30 mg kg(-1)). The

rats in groups 3-6 rats were given a weekly subcutaneous injection of DMH (20 mg kg(-1)) for the first four weeks. Hesperetin was supplemented orally at different doses (10, 20 or 30 mg kg(-1)) for a total of 16 weeks. At the end of the experimental period all rats were killed. In DMH-treated rats, the activity of faecal and colonic mucosal bacterial enzymes, such as beta-glucuronidase, beta-galactosidase, beta-glucosidase, nitroreductase, sulfatase and mucinase, were significantly elevated, but in rats supplemented hesperetin along with DMH the activity was significantly lowered ($P < 0.05$). The total number of aberrant crypts was significantly increased in unsupplemented DMH-treated rats, while hesperetin supplementation to DMH-treated rats significantly reduced the total number of crypts. The results demonstrated that hesperetin supplementation at a dose of 20 mg kg(-1) played a potent role in suppressing the formation of aberrant crypt foci and reducing the activity of bacterial enzymes in colon cancer.

Baumeister, P., S. Schwenk-Zieger, et al. (2009). "Transforming Growth Factor-alpha reduces carcinogen-induced DNA damage in mini-organ cultures from head-and-neck cancer patients." Mutat Res **677**(1-2): 42-5.

Epidermal Growth Factor Receptor (EGFR) signalling is particularly important in the biology of the vast majority of solid human malignancies. EGFR is over-expressed in up to 90-100% of head-and-neck squamous cell carcinomas (HNSCC), and increased expression of EGFR and its ligand Transforming Growth Factor-alpha (TGF-alpha) is not limited to malignant cells, but also detected in histologically normal mucosa of HNSCC patients, supporting the hypothesis of field carcinogenesis. Permanent EGFR activation via an autocrine stimulatory pathway is thought to be a major factor forcing pre-neoplastic tissue towards malignancy. Our study evaluates the impact of stimulation by TGF-alpha on carcinogen-induced and oxidative DNA damage in mucosa tissue cultures of macroscopically normal biopsies from tumour patients and controls. Effects of TGF-alpha on DNA-repair capacity were investigated. To assess DNA fragmentation, alkaline single-cell gel electrophoresis (comet assay) was used. Stimulation of cultures during 24 h with TGF-alpha decreased benzo(a)pyrene diolepoxide (BPDE)-induced DNA damage by 36% in the tumour group ($p < 0.001$) and by 7% in controls ($n = 30$). No statistically significant impact on oxidatively induced DNA fragmentation in both groups ($n = 15$ and 20, respectively), or DNA repair could be shown n ($n = 6$). The exact mechanism by which TGF-alpha stimulation reduces BPDE-induced DNA fragmentation remains unclear. It was shown in clinical studies, that EGFR targeting has synergistic effects with chemotherapy in HNSCC and reverses chemo-resistance of epithelial tumours, which was shown to be the consequence of altered expression of multidrug resistance (mdr) efflux pumps. EGFR downstream signalling regulates the transcription of MDR1 (p-glycoproteine) and glutathione homeostasis. BPDE is a substrate of mdr1 and is detoxified by glutathione conjugation. However, our results show a strong DNA-stabilizing effect of stimulation by TGF-alpha in mucosa tissue cultures of tumour patients and may therefore be seen as a physiological response to continued carcinogenic impact on the epithelium of the upper aerodigestive tract.

Blanco-Aparicio, C., L. Perez-Gallego, et al. (2007). "Mice expressing myrAKT1 in the mammary gland develop carcinogen-induced ER-positive mammary tumors that mimic human breast cancer." Carcinogenesis **28**(3): 584-94.

AKT1/PKB is a serine/threonine protein kinase that regulates biological processes such as proliferation, apoptosis and growth in a variety of cell types. To assess the oncogenic capability of an activated form of AKT in vivo we have generated several transgenic mouse lines that overexpress in the mammary epithelium the murine Akt1 gene modified with a myristoylation signal, which renders active this protein by localizing it to the plasma membrane. We demonstrate that expression of myristoylated AKT in the mammary glands increases the susceptibility of these mice to the induction of mammary tumors of epithelial origin by the carcinogen 9,10-dimethyl-1,2 benzanthracene (DMBA). We have found that while carcinogen-treated wild-type mice show mostly mammary tumors of sarcomatous origin, AKT transgenic mice treated with DMBA developed mainly adenocarcinoma or adenosquamous tumors, all of them displaying activated AKT. We analyzed other possible molecular alterations cooperating with AKT and found that neither Ras nor beta-catenin/Wnt pathways seemed altered nor p53 mutated. We have found that 100% of mammary DMBA-induced tumors and benign lesions in myrAKT mice are estrogen receptor (ERalpha)-positive and are more frequent than in wild-type littermates. These data show that AKT activation cooperates with deregulation of the estrogen receptor in the DMBA-induced mammary tumorigenesis model and recapitulate two characteristics of some human breast tumors. Thus, our model might provide a preclinical relevant model system to study the role of AKT and ERalpha in breast tumorigenesis and the response of mammary gland tumors to chemotherapeutics.

Bockman, D. E. (2008). "Transition to pancreatic cancer in response to carcinogen." Langenbecks Arch Surg **393**(4): 557-60.

It has become obvious that the traditional assumptions about the transition from normal pancreas to pancreatic cancer are incomplete. Experimental studies reveal that the earliest changes during transition to pancreatic adenocarcinoma involve premalignant lesions that are derived from acinar, islet, and ductal cells. OBSERVATIONS: Changes are rapid, occurring in days. As part of redifferentiation and transformation to adenocarcinoma, cells regain the characteristics of developing pancreas. Elements significant in identifying precursor cell types include Pdx1, hedgehog signaling, notch signaling, and nestin, an intermediate filament expressed by precursor cell types. Thus pancreatic carcinogenesis is not simply a matter of transition of ductal cells to cancer cells months after insult by the carcinogen; ductal cells are not the sole source transitioning to cancer, and PanINs are not the sole route to adenocarcinoma. Tubular complexes, derived from multiple cell sources, are included in routes to pancreatic cancer. Markers characteristic of developing pancreas are consistent with this transition. Cells previously thought to be terminally differentiated become, in effect, stem cells.

Butler, L. M., Y. Duguay, et al. (2005). "Joint effects between UDP-glucuronosyltransferase 1A7 genotype and dietary carcinogen exposure on risk of colon cancer." Cancer Epidemiol Biomarkers Prev **14**(7): 1626-32.

The UDP-glucuronosyltransferase 1A7 (UGT1A7) gene is polymorphic and encodes an enzyme involved in the detoxification of heterocyclic amines (HCA) and polycyclic aromatic hydrocarbons (PAH). Consumption of pan-fried and well-done meat are surrogates for HCA and PAH exposure and are possibly associated with colon cancer. We have evaluated whether UGT1A7 allelic variations are associated with colon cancer and whether UGT1A7 genotype modified associations among meat intake, exposure to HCAs and PAHs, and colon cancer in a population-based case-control study of African Americans (197 cases and 202 controls) and whites (203 cases and 210 controls). As part of a 150-item food frequency questionnaire, meat intake was assessed by cooking method and doneness and used to estimate individual HCA and PAH exposure. UGT1A7 alleles (UGT1A7*1, UGT1A7*2, UGT1A7*3, and UGT1A7*4) were measured and genotypes were categorized into predicted activity groups (high: *1/*1, *1/*2, *2/*2; intermediate: *1/*3, *1/*4, *2/*3; low: *3/*3, *3/*4, *4/*4). There was no association with UGT1A7 low versus high/intermediate genotype [odds ratio (OR), 1.1; 95% confidence interval (95% CI), 0.7-1.8], regardless of race. Greater than additive joint effects were observed for UGT1A7 low genotype and HCA-related factors. For example, equal to or greater than the median daily intake of the HCA, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (DiMeIQx) and having UGT1A7 low genotype was positively associated with colon cancer (OR, 2.4; 95% CI, 1.2-4.8), compared with less than the median daily intake and UGT1A7 high/intermediate genotypes. These data suggest that the associations among cooked meat-derived compound exposure, and colon cancer are modified by the UGT1A7 genotype.

Chen, G. F., F. L. Chan, et al. (2004). "Mitochondrial DNA mutations in chemical carcinogen-induced rat bladder and human bladder cancer." Oncol Rep **12**(2): 463-72.

Mitochondrial (mt) DNA mutations have been described recently in different tumors, whereas similar studies focusing on bladder cancer are scarce. In an effort to understand the significance of mtDNA mutations in bladder cancer, we investigated the mtDNA alterations in both clinical human bladder cancer and in a carcinogen-induced rat bladder cancer model. Human bladder cancer tissues were obtained by radical cystectomy and transurethral resection of bladder tumors. Rat bladder tumors were induced in SD rats by treatment with N-butyl-N-(4-hydroxybutyl) nitrosamine in drinking water for 24-28 weeks. Genomic DNA was extracted from tumor specimens and microdissected normal bladder mucosae. Mitochondrial genes and D-loop region were amplified by PCR. The amplified PCR fragments were either cloned into plasmid vector or used for direct DNA sequencing. The results of DNA sequence revealed numerous point mutations in the non-coding D-loop region and different mtDNA genes in both human and rat bladder cancers. In addition, we also detected deletions of variable lengths in mononucleotide repeats in the D-loop region, ND2, ATPase 8 and COIII genes in human bladder cancer samples. Our results show that mtDNA exhibits a high rate of mutations in both human and rat bladder cancers. We also demonstrate that the repetitive sequences of mononucleotides within the mt genome are unstable and subjected to deletions. The high incidence of mtDNA mutations in bladder cancer suggests that mtDNA and mitochondria could play an important role in the process of carcinogenesis and also mtDNA could be valuable as a marker for early bladder cancer diagnosis.

Church, T. R., K. E. Anderson, et al. (2009). "A prospectively measured serum biomarker for a tobacco-specific carcinogen and lung cancer in

smokers." Cancer Epidemiol Biomarkers Prev **18**(1): 260-6.

No prior studies have related a tobacco-specific carcinogen to the risk of lung cancer in smokers. Of the over 60 known carcinogens in cigarette smoke, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is specific to tobacco and causes lung cancer in laboratory animals. Its metabolites, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides (total NNAL), have been studied as biomarkers of exposure to NNK. We studied the relation of prospectively measured NNK biomarkers to lung cancer risk. In a case-control study nested in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, we randomly selected 100 lung cancer cases and 100 controls who smoked at baseline and analyzed their baseline serum for total NNAL, cotinine, and r-1,t-2,3,c-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT), a biomarker of polycyclic aromatic hydrocarbon exposure and metabolic activation. To examine the association of the biomarkers with all lung cancers and for histologic subtypes, we computed odds ratios for total NNAL, PheT, and cotinine using logistic regression to adjust for potential confounders. Findings: Individual associations of age, smoking duration, and total NNAL with lung cancer risk were statistically significant. After adjustment, total NNAL was the only biomarker significantly associated with risk (odds ratio, 1.57 per unit SD increase; 95% confidence interval, 1.08-2.28). A similar statistically significant result was obtained for adenocarcinoma risk, but not for nonadenocarcinoma. This first reporting of the effect of the prospectively measured tobacco-specific biomarker total NNAL, on risk of lung cancer in smokers provides insight into the etiology of smoking-related lung cancer and reinforces targeting NNK for cancer prevention.

Ciolino, H. P., S. E. Bass, et al. (2008). "Sulindac and its metabolites induce carcinogen metabolizing enzymes in human colon cancer cells." Int J Cancer **122**(5): 990-8.

Sulindac is a nonsteroidal antiinflammatory drug that has been demonstrated to be a potent chemopreventive agent against colorectal cancer in both human and animal models. In vivo, sulindac may be reversibly reduced to the active antiinflammatory compound, sulindac sulfide, or irreversibly oxidized to sulindac sulfone. Sulindac has also been shown to inhibit polycyclic aromatic hydrocarbon (PAH)-induced cancer, but the molecular mechanisms of its antitumor effect remain unclear. In this study, we investigated the effects of sulindac and its metabolites on the expression of enzymes that metabolize and detoxify PAHs in 2 human colon cancer cell lines, LS180 and Caco-2. Sulindac and sulindac sulfide induced a sustained, concentration-dependent increase in CYP enzyme activity as well as an increase in the mRNA levels of CYP1A1, CYP1A2 and CYP1B1. Sulindac and sulindac sulfide induced the transcription of the CYP1A1 gene, as measured by the level of heterogeneous nuclear CYP1A1 RNA and verified by the use of actinomycin D as a transcription inhibitor. Chromatin immunoprecipitation assays demonstrated that sulindac and sulindac sulfide also increased the nuclear level of activated aryl hydrocarbon receptor, the transcription factor which mediates CYP expression. Additionally, sulindac and both metabolites increased the activity and mRNA expression of the carcinogen detoxification enzyme NAD(P)H:quinone oxidoreductase, as well as the expression of UDP-glucuronosyltransferase mRNA. These results show an overall upregulation of carcinogen metabolizing enzymes in colon cancer cells treated with sulindac, sulindac sulfide and sulindac sulfone that may contribute to the established chemoprotective effects of these compounds.

Cotterchio, M., B. A. Boucher, et al. (2008). "Red meat intake, doneness, polymorphisms in genes that encode carcinogen-metabolizing enzymes, and colorectal cancer risk." Cancer Epidemiol Biomarkers Prev **17**(11): 3098-107.

Colorectal cancer literature regarding the interaction between polymorphisms in carcinogen-metabolizing enzymes and red meat intake/doneness is inconsistent. A case-control study was conducted to evaluate the interaction between red meat consumption, doneness, and polymorphisms in carcinogen-metabolizing enzymes. Colorectal cancer cases diagnosed 1997 to 2000, ages 20 to 74 years, were identified through the population-based Ontario Cancer Registry and recruited by the Ontario Family Colorectal Cancer Registry. Controls were sex-matched and age group-matched random sample of Ontario population. Epidemiologic and food questionnaires were completed by 1,095 cases and 1,890 controls; blood was provided by 842 and 1,251, respectively. Multivariate logistic regression was used to obtain adjusted odds ratio (OR) estimates. Increased red meat intake was associated with increased colorectal cancer risk [OR (> 5 versus < or = 2 servings/wk), 1.67 (1.36-2.05)]. Colorectal cancer risk also increased significantly with well-done meat intake [OR (> 2 servings/wk well-done versus < or = 2 servings/wk rare-regular), 1.57 (1.27-1.93)]. We evaluated interactions between genetic variants in 15 enzymes involved in the metabolism of carcinogens in overcooked meat (cytochrome P450, glutathione S-transferase, UDP-glucuronosyltransferases, SULT, NAT, mEH, and AHR). CYP2C9 and NAT2 variants

were associated with colorectal cancer risk. Red meat intake was associated with increased colorectal cancer risk regardless of genotypes; however, CYP1B1 combined variant and SULT1A1-638G>A variant significantly modified the association between red meat doneness intake and colorectal cancer risk. In conclusion, well-done red meat intake was associated with an increased risk of colorectal cancer regardless of carcinogen-metabolizing genotype, although our data suggest that persons with CYP1B1 and SULT1A1 variants had the highest colorectal cancer risk.

Derby, K. S., K. Cuthrell, et al. (2009). "Exposure to the carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in smokers from 3 populations with different risks of lung cancer." Int J Cancer **125**(10): 2418-24.

Native Hawaiian smokers are at higher risk and Japanese-American smokers at lower risk of lung cancer (LC), compared with white smokers, even after accounting for smoking history. Because variation in carcinogen exposure/metabolism may occur separately of smoking amount, we compared urinary biomarkers of uptake and detoxification of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-a potent lung carcinogen-among 578 smokers in these ethnic/racial groups in Hawaii. We measured the NNK metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronide (NNAL-Gluc) and examined total NNAL (NNAL + NNAL-Gluc) and the NNAL detoxification ratio (NNAL-Gluc:NNAL). Native Hawaiians and Japanese-Americans had lower age- and sex-adjusted mean total NNAL, compared with whites. When further adjusting for urinary nicotine equivalents (the sum of nicotine, cotinine, trans-3'-hydroxycotinine and their respective glucuronides), only the difference between Japanese-Americans and whites was eliminated. Therefore, consistent with their lower LC risk, a lower cigarette smoke exposure explains the lower NNK dose of Japanese-Americans, but it does not explain that of Native Hawaiians. The mean detoxification ratio was also lower in Native Hawaiians and Japanese-Americans, compared with whites, even after adjusting for nicotine equivalents ($p < 0.0001$). Lower NNAL glucuronidation in Native Hawaiians might contribute to their increased LC risk; however, this is inconsistent with the low glucuronidation ratio similarly observed in the low-risk Japanese-American group and because Native Hawaiians had lower total NNAL levels. Thus, exposure and detoxification of NNK are unlikely to explain, by themselves, the differences in LC risk among the 3 populations studied.

Dumstorf, C. A., S. Mukhopadhyay, et al. (2009). "REV1 is implicated in the development of carcinogen-induced lung cancer." Mol Cancer Res **7**(2): 247-54.

The somatic mutation hypothesis of cancer predicts that reducing the frequency of mutations induced by carcinogens will reduce the incidence of cancer. To examine this, we developed an antimutator strategy based on the manipulation of the level of a protein required for mutagenic bypass of DNA damage induced by the ubiquitous carcinogen benzo[a]pyrene. The expression of this protein, REV1, was reduced in mouse cells using a vector encoding a gene-specific targeting ribozyme. In the latter cells, mutagenesis induced by the activated form of benzo[a]pyrene was reduced by >90%. To examine if REV1 transcripts could be lowered in vivo, the plasmid was complexed with polyethyleneimine, a nonviral cationic polymer, and delivered to the lung via aerosol. The endogenous REV1 transcript in the bronchial epithelium as determined by quantitative real-time PCR in laser capture microdissected cells was reduced by 60%. There was a significant decrease in the multiplicity of carcinogen-induced lung tumors from 6.4 to 3.7 tumors per mouse. Additionally, REV1 inhibition completely abolished tumor formation in 27% of the carcinogen-exposed mice. These data support the central role of the translesion synthesis pathway in the development of lung cancer. Further, the selective modulation of members of this pathway presents novel potential targets for cancer prevention. The somatic mutation hypothesis of cancer predicts that the frequency of cancers will also be reduced.

Guo, J. Y., X. Li, et al. (2004). "Dietary soy isoflavones and estrone protect ovariectomized ERalphaKO and wild-type mice from carcinogen-induced colon cancer." J Nutr **134**(1): 179-82.

Consumption of soy foods has been weakly associated with reduced colon cancer risk. Colon cancer risk is influenced by estrogen exposure, although the mechanism through which this occurs is not defined. Conversion of estradiol (E2) to estrone (E1) may be protective in the colon. We hypothesized that dietary phytoestrogens, or E1, would reduce colon tumorigenesis via an estrogen receptor (ER)-dependent mechanism. Ovariectomized ERalphaKO or wild-type (WT) female mice were fed diets containing casein (Casein), soy protein without isoflavones (Soy-IF), soy protein + genistein (Soy+Gen), soy protein + NovaSoy (Soy+NSoy) or soy protein + estrone (Soy+E1) from weaning. Colon tumors were induced with azoxymethane. Tumor incidence was affected by diet but not genotype. Colon tumor incidence was lower in ERalphaKO and WT mice fed the Soy+E1 diet compared with those

fed the casein or Soy-IF diets. Mice fed Soy+NSoy had a lower tumor incidence than mice fed casein, but not Soy-IF. Genistein did not affect tumor incidence. Soy protein, independently of phytoestrogens or E1, significantly reduced relative colon weight, tumor burden and multiplicity. Relative colon weight was lower (P=0.008) in mice fed Soy+E1 than in the other soy-fed groups. Tumor incidence in this group was lower than in the casein and soy-IF-fed groups and tended to be lower than in the others (P=0.020). Hence, soy protein and NSoy protect mice from colon cancer, and E1 further reduces colon tumorigenesis in mice, independently of ERalpha.

Guo, S., S. Yang, et al. (2005). "Green tea polyphenol epigallocatechin-3 gallate (EGCG) affects gene expression of breast cancer cells transformed by the carcinogen 7,12-dimethylbenz[a]anthracene." J Nutr **135**(12 Suppl): 2978S-2986S.

Since the 1980s, the incidence of late-onset breast cancer has been increasing in the United States. Known risk factors, such as genetic modifications, have been estimated to account for approximately 5 to 10% of breast cancer cases, and these tend to be early onset. Thus, exposure to and bioaccumulation of ubiquitous environmental chemicals, such as polycyclic aromatic hydrocarbons (PAHs), have been proposed to play a role in this increased incidence. Treatment of female Sprague-Dawley rats with a single dose of the PAH 7,12-dimethylbenz[a]anthracene (DMBA) induces mammary tumors in approximately 90 to 95% of test animals. We showed previously that female rats treated with DMBA and given green tea as drinking fluid displayed significantly decreased mammary tumor burden and invasiveness and a significantly increased latency to first tumor. Here we used cDNA microarray analysis to elucidate the effects of the green tea polyphenol epigallocatechin-3 gallate (EGCG) on the gene expression profile in a DMBA-transformed breast cancer cell line. RNA was isolated, in quadruplicate, from D3-1 cells treated with 60 mug/mL EGCG for 2, 7, or 24 h and subjected to analysis. Semiquantitative RT-PCR and Northern blot analyses confirmed the changes in the expression of 12 representative genes seen in the microarray experiments. Overall, our results documented EGCG-altered expression of genes involved in nuclear and cytoplasmic transport, transformation, redox signaling, response to hypoxia, and PAHs.

Hein, D. W. (2006). "N-acetyltransferase 2 genetic polymorphism: effects of carcinogen and haplotype on urinary bladder cancer risk." Oncogene **25**(11): 1649-58.

A role for the N-acetyltransferase 2 (NAT2) genetic polymorphism in cancer risk has been the subject of numerous studies. Although comprehensive reviews of the NAT2 acetylation polymorphism have been published elsewhere, the objective of this paper is to briefly highlight some important features of the NAT2 acetylation polymorphism that are not universally accepted to better understand the role of NAT2 polymorphism in carcinogenic risk assessment. NAT2 slow acetylator phenotype(s) infer a consistent and robust increase in urinary bladder cancer risk following exposures to aromatic amine carcinogens. However, identification of specific carcinogens is important as the effect of NAT2 polymorphism on urinary bladder cancer differs dramatically between monoarylamines and diarylamines. Misclassifications of carcinogen exposure and NAT2 genotype/phenotype confound evidence for a real biological effect. Functional understanding of the effects of NAT2 genetic polymorphisms on metabolism and genotoxicity, tissue-specific expression and the elucidation of the molecular mechanisms responsible are critical for the interpretation of previous and future human molecular epidemiology investigations into the role of NAT2 polymorphism on cancer risk. Although associations have been reported for various cancers, this paper focuses on urinary bladder cancer, a cancer in which a role for NAT2 polymorphism was first proposed and for which evidence is accumulating that the effect is biologically significant with important public health implications.

Huang, J. K., C. J. Huang, et al. (2005). "Independent [Ca2+]i increases and cell proliferation induced by the carcinogen safrole in human oral cancer cells." Naunyn Schmiedebergs Arch Pharmacol **372**(1): 88-94.

The effect of the carcinogen safrole on intracellular Ca2+ movement and cell proliferation has not been explored previously. The present study examined whether safrole could alter Ca2+ handling and growth in human oral cancer OC2 cells. Cytosolic free Ca2+ levels ([Ca2+]i) in populations of cells were measured using fura-2 as a fluorescent Ca2+ probe. Safrole at a concentration of 325 microM started to increase [Ca2+]i in a concentration-dependent manner. The Ca2+ signal was reduced by 40% by removing extracellular Ca2+, and was decreased by 39% by nifedipine but not by verapamil or diltiazem. In Ca2+-free medium, after pretreatment with 650 microM safrole, 1 microM thapsigargin (an endoplasmic reticulum Ca2+ pump inhibitor) barely induced a [Ca2+]i rise; in contrast, addition of safrole after thapsigargin treatment induced a small [Ca2+]i rise. Neither inhibition of phospholipase C with 2

microM U73122 nor modulation of protein kinase C activity affected safrole-induced Ca2+ release. Overnight incubation with 1 microM safrole did not alter cell proliferation, but incubation with 10-1000 microM safrole increased cell proliferation by 60+/-10%. This increase was not reversed by pre-chelating Ca2+ with 10 microM of the Ca2+ chelator BAPTA. Collectively, the data suggest that in human oral cancer cells, safrole induced a [Ca2+]i rise by causing release of stored Ca2+ from the endoplasmic reticulum in a phospholipase C- and protein kinase C-independent fashion and by inducing Ca2+ influx via nifedipine-sensitive Ca2+ entry. Furthermore, safrole can enhance cell growth in a Ca2+-independent manner.

Hussain-Hakimjee, E. A., X. Peng, et al. (2006). "Growth inhibition of carcinogen-transformed MCF-12F breast epithelial cells and hormone-sensitive BT-474 breast cancer cells by 1alpha-hydroxyvitamin D5." Carcinogenesis **27**(3): 551-9.

Several studies have established the active form of vitamin D(3) as an effective tumor-suppressing agent; however, its antitumor activity is achieved at doses that are hypercalcemic in vivo. Therefore, less calcemic vitamin D(3) analog, 1alpha-hydroxy-24-ethyl-cholecalciferol (1alpha[OH]D5), was evaluated for its potential use in breast cancer chemoprevention. Previously, 1alpha(OH)D5 showed anticarcinogenic activity in several in vivo and in vitro models. However, its effects on growth of normal tissue were not known. The present study was conducted to determine the effects of 1alpha(OH)D5 on the growth of normal mouse mammary gland and normal-like human breast epithelial MCF-12F cells and to compare these effects with carcinogen-transformed MCF-12F and breast cancer cells. No significant difference was observed in the growth or morphology of cultured mouse mammary gland and MCF-12F cells in the presence of 1alpha(OH)D5. However, the transformed MCF-12F cells underwent growth inhibition (40-60%, $P < 0.05$) upon 1alpha(OH)D5 treatment as determined by cell viability assays. Cell cycle analysis showed marked increase (50%) in G-1 phase for cells treated with 1alpha(OH)D5 compared with the controls. Moreover, the percentage of cells in the synthesis (S) phase of cell cycle was decreased by 70% in transformed MCF-12F, BT-474 and MCF-7 cells. The growth arrest was preceded by an increase in expression of cell cycle regulatory proteins p21(Waf-1) and p27(Kip-1). In addition, differential expression studies of parent and transformed MCF-12F cell lines using microarrays showed that prohibitin mRNA was increased 4-fold in the transformed cells. These results indicate that the growth inhibitory effect of 1alpha(OH)D5 was achieved in both carcinogen-transformed MCF-12F and breast cancer cells at a dose that was non-inhibitory in normal-like breast epithelial cells.

Iqbal, M., Y. Okazaki, et al. (2009). "Curcumin attenuates oxidative damage in animals treated with a renal carcinogen, ferric nitrilotriacetate (Fe-NTA): implications for cancer prevention." Mol Cell Biochem **324**(1-2): 157-64.

Curcumin (diferuloylmethane), a biologically active ingredient derived from rhizome of the plant Curcuma longa, has potent anticancer properties as demonstrated in a plethora of human cancer cell lines/animal carcinogenesis model and also acts as a biological response modifier in various disorders. We have reported previously that dietary supplementation of curcumin suppresses renal ornithine decarboxylase (Okazaki et al. Biochim Biophys Acta 1740:357-366, 2005) and enhances activities of antioxidant and phase II metabolizing enzymes in mice (Iqbal et al. Pharmacol Toxicol 92:33-38, 2003) and also inhibits Fe-NTA-induced oxidative injury of lipids and DNA in vitro (Iqbal et al. Teratog Carcinog Mutagen 1:151-160, 2003). This study was designed to examine whether curcumin possess the potential to suppress the oxidative damage caused by kidney-specific carcinogen, Fe-NTA, in animals. In accord with previous report, at 1 h after Fe-NTA treatment (9.0 mg Fe/kg body weight intraperitoneally), a substantial increased formation of 4-hydroxy-2-nonenal (HNE)-modified protein adducts in renal proximal tubules of animals was observed. Likewise, the levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) and protein reactive carbonyl, an indicator of protein oxidation, were also increased at 1 h after Fe-NTA treatment in the kidneys of animals. The prophylactic feeding of animals with 1.0% curcumin in diet for 4 weeks completely abolished the formation of (i) HNE-modified protein adducts, (ii) 8-OHdG, and (iii) protein reactive carbonyl in the kidneys of Fe-NTA-treated animals. Taken together, our results suggest that curcumin may afford substantial protection against oxidative damage caused by Fe-NTA, and these protective effects may be mediated via its antioxidant properties. These properties of curcumin strongly suggest that it could be used as a cancer chemopreventive agent.

Kelsey, K. T., T. Hirao, et al. (2005). "TP53 alterations and patterns of carcinogen exposure in a U.S. population-based study of bladder cancer." Int J Cancer **117**(3): 370-5.

The molecular pathology of bladder cancer has been the subject of considerable interest, and current efforts are targeted toward elucidating the interrelationships between individual somatic gene

loss and both etiologic and prognostic factors. Mutation of the TP53 gene has been associated with more invasive bladder cancer, and evidence suggests that TP53 mutation, independent of stage, may be predictive of outcome in this disease. However, there is no consensus in the literature that bladder carcinogen exposure is associated with inactivation of the TP53 gene. Work to date has been primarily hospital based and, as such, subject to possible bias associated with selection of more advanced cases for study. We examined exposure relationships with both TP53 gene mutation and TP53 protein alterations in a population-based study of 330 bladder cancer cases in New Hampshire. Tobacco smoking was not associated with TP53 alterations. We found a higher prevalence of TP53 inactivation (i.e., mutation and nuclear accumulation) among hair dye users (odd ratio [OR] = 4.1; 95% confidence interval [CI] 1.2-14.7), and the majority of these mutations were transversions. Men who had "at risk" occupations were more likely to have mutated TP53 tumors (OR = 2.9; 95% CI 1.1-7.6). There also was a relative absence of TP53 mutation (OR = 0.4; 95% CI 0.0-2.9) and TP53 protein alterations (OR = 0.6; 95% CI 0.3-1.4) in bladder cancers from individuals with higher arsenic exposure. Our data suggest that there is exposure-specific heterogeneity in inactivation of the TP53 pathway in bladder cancers and that integration of the spectrum of pathway alterations in population-based approaches (capturing the full range of exposures to bladder carcinogens) may provide important insights into bladder tumorigenesis.

Kim, H., P. Hall, et al. (2004). "Chemoprevention by grape seed extract and genistein in carcinogen-induced mammary cancer in rats is diet dependent." J Nutr **134**(12 Suppl): 3445S-3452S.

Many popular dietary supplements are enriched in polyphenols such as the soy isoflavones, tea catechins, and resveratrol (from grape skins), each of which has been shown to have chemopreventive activity in cellular models of cancer. The proanthocyanidins, which are oligomers of the catechins, are enriched in grape seeds and form the basis of the dietary supplement grape seed extract (GSE). Evidence suggests that the proanthocyanidins may be metabolized to the monomeric catechins. This study was carried out to determine whether GSE added to rodent diets protected against carcinogen-induced mammary tumorigenesis in rats and whether this was affected by the composition of the whole diet. Female rats were begun on 5%, 1.25%, or 0% (control) GSE-supplemented diets at age 35 d. At age 50 d they were administered 7,12-dimethylbenz[a]anthracene (DMBA) in sesame oil at 80 mg/kg body weight. They were weighed and monitored weekly for tumor development until 120 d after DMBA administration. Administration of GSE in AIN-76A diet did not show any protective activity of GSE against DMBA-induced breast cancer. However, administration of GSE in a laboratory dry food diet (Teklad 4% rodent diet) resulted in a 50% reduction in tumor multiplicity. In similar experiments, genistein administered in AIN-76A diet also failed to show chemopreventive activity against the carcinogen N-methyl-N-nitrosourea; however, when administered at the same dose in the Teklad 4% rodent diet, genistein exhibited significant chemopreventive activity (44-61%). These results demonstrate that GSE is chemopreventive in an animal model of breast cancer; moreover, the diet dependency of the chemopreventive activity for both GSE and genistein suggests that whether or not a compound is chemopreventive may depend on the diet in which the agent is administered.

Kirman, C. R., L. M. Sweeney, et al. (2004). "Addressing nonlinearity in the exposure-response relationship for a genotoxic carcinogen: cancer potency estimates for ethylene oxide." Risk Anal **24**(5): 1165-83.

Ethylene oxide (EO) has been identified as a carcinogen in laboratory animals. Although the precise mechanism of action is not known, tumors in animals exposed to EO are presumed to result from its genotoxicity. The overall weight of evidence for carcinogenicity from a large body of epidemiological data in the published literature remains limited. There is some evidence for an association between EO exposure and lympho/hematopoietic cancer mortality. Of these cancers, the evidence provided by two large cohorts with the longest follow-up is most consistent for leukemia. Together with what is known about human leukemia and EO at the molecular level, there is a body of evidence that supports a plausible mode of action for EO as a potential leukemogen. Based on a consideration of the mode of action, the events leading from EO exposure to the development of leukemia (and therefore risk) are expected to be proportional to the square of the dose. In support of this hypothesis, a quadratic dose-response model provided the best overall fit to the epidemiology data in the range of observation. Cancer dose-response assessments based on human and animal data are presented using three different assumptions for extrapolating to low doses: (1) risk is linearly proportionate to dose; (2) there is no appreciable risk at low doses (margin-of-exposure or reference dose approach); and (3) risk below the point of departure continues to be proportionate to the square of the dose. The weight of evidence for EO supports the use of a nonlinear assessment. Therefore, exposures to

concentrations below 37 microg/m3 are not likely to pose an appreciable risk of leukemia in human populations. However, if quantitative estimates of risk at low doses are desired and the mode of action for EO is considered, these risks are best quantified using the quadratic estimates of cancer potency, which are approximately 3.2- to 32-fold lower, using alternative points of departure, than the linear estimates of cancer potency for EO. An approach is described for linking the selection of an appropriate point of departure to the confidence in the proposed mode of action. Despite high confidence in the proposed mode of action, a small linear component for the dose-response relationship at low concentrations cannot be ruled out conclusively. Accordingly, a unit risk value of 4.5 x 10(-8) (microg/m3)(-1) was derived for EO, with a range of unit risk values of 1.4 x 10(-8) to 1.4 x 10(-7) (microg/m3)(-1) reflecting the uncertainty associated with a theoretical linear term at low concentrations.

Kraunz, K. S., H. H. Nelson, et al. (2006). "Homozygous deletion of p16INK4a and tobacco carcinogen exposure in nonsmall cell lung cancer." Int J Cancer **118**(6): 1364-9.

Inactivation of p16(INK4a) in the Rb pathway is among the most common somatic alterations observed in nonsmall cell lung cancers (NSCLCs). While epigenetic inactivation of the p16(INK4a) gene promoter has been shown to be associated with increased tobacco carcinogen exposure, little investigation of any similar association of homozygous deletion or mutation of p16(INK4a) and tobacco use has been completed. In 177 consecutive NSCLCs, we examined the determinants of p16(INK4a) homozygous deletion and mutation, including the pattern of tobacco smoking and asbestos exposure. We observed that p16(INK4a) homozygous deletion occurred at a higher frequency in never smokers as compared to former and current smokers ($p = 0.01$). This observation suggested that tumors from these patients might be more prone to DNA deletion events; consistent with this, epigenetic silencing of the DNA double-strand break repair genes FancF and BRCA1 was also associated with homozygous deletion of p16(INK4a)($p = 0.002$ and $p = 0.06$, respectively). Finally, mutation of p16(INK4a) was rare and only occurred in patients who were smokers. Hence, the character of somatic alteration in the Rb pathway (deletion, mutation or methylation silencing) in NSCLC is associated with the pattern of tobacco exposure, suggesting that susceptibility to lung cancer is, at least in part, mediated by the biological mechanism that selects for the character of the induced somatic lesion.

Kuang, S. Q., L. Liao, et al. (2005). "Mice lacking the amplified in breast cancer 1/steroid receptor coactivator-3 are resistant to chemical carcinogen-induced mammary tumorigenesis." Cancer Res **65**(17): 7993-8002.

Amplified in breast cancer 1 (AIB1; steroid receptor coactivator-3, p/CIP, RAC3, ACTR, TRAM-1, or NCoA-3) is a transcriptional coactivator for nuclear receptors and certain other transcription factors and is a newly defined oncogene overexpressed in human breast cancer. Although the role and molecular mechanism of AIB1 in normal physiology and in breast cancer are currently under intensive investigation, the role of AIB1 in determination of the susceptibility of mammary gland to chemical carcinogens remains uncharacterized. In this study, we used back-crossed FVB wild-type (WT) and AIB1 mutant mice to assess the role of AIB1 in mammary gland development and in carcinogen-induced tumorigenesis. We show that mammary ductal growth was delayed in AIB1-/- mice with FVB strain background, and mammary ductal outgrowths emanating from the AIB1-/- mammary epithelial transplants in WT mice also were attenuated, indicating that the role of AIB1 in mammary ductal growth is a mammary epithelial autonomous function. In mice treated with the chemical carcinogen 7,12-dimethylbenz[a]anthracene (DMBA), AIB1 deficiency protected the mammary gland, but not the skin, from tumorigenesis. AIB1 deficiency suppressed the up-regulation of the insulin receptor substrate (IRS)-1 and IRS-2 and thereby inhibited the activation of Akt, expression of cyclin D1, and cell proliferation. The suppression of these components for insulin-like growth factor-I signaling might be partially responsible for the decreased DMBA-induced mammary tumor initiation and progression in AIB1-/- mice. Our results suggest that AIB1 may serve as a potential target for prevention of carcinogen-induced breast cancer initiation and for treatment of breast cancer progression.

Lacko, M., M. B. Oude Ophuis, et al. (2009). "Genetic polymorphisms of smoking-related carcinogen detoxifying enzymes and head and neck cancer susceptibility." Anticancer Res **29**(2): 753-61.

Smoking and the consumption of alcohol are the main risk factors for head and neck cancer. However, interindividual variation in the activity of enzymes involved in the detoxification of tobacco smoke (pro)carcinogens, such as microsomal epoxide hydrolase (mEH), glutathione-S-transferases (GSTs) and uridine 5'-diphosphate (UDP)-glucuronosyltransferase (UGTs), may influence the process of carcinogenesis. Genetic polymorphisms of these enzymes may alter their activity and may thus

modulate the risk for squamous cell carcinomas of the head and neck (SCCHN). A literature review on the role of mEH, GSTs and UGTs polymorphisms in relation to SCCHN was performed and the results summarized. For mEH polymorphisms, some of the studies revealed a relationship between genetic polymorphisms of these enzymes and an altered risk for SCCHN, whereas others did not. The presence of null polymorphisms in GSTM1 or GSTT1 were associated with an increased risk for SCCHN. For the UGTs, only variants in UGT1A7 and UGT1A10 have been studied, both of which were associated with an altered risk for SCCHN.

Larsen, R. I. (2003). "An air quality data analysis system for interrelating effects, standards, and needed source reductions: Part 13--Applying the EPA Proposed Guidelines for Carcinogen Risk Assessment to a set of asbestos lung cancer mortality data." J Air Waste Manag Assoc **53**(11): 1326-39.

The Clean Air Act Amendments of 1990 (CAAA-90) list 189 hazardous air pollutants (HAPs) for which "safe" ambient concentrations are to be determined. The primary purpose of this paper is to develop two mathematical models, lognormal and logarithmic, that effectively express excess lung cancer mortality as a function of asbestos concentration for an example set of data and also to suggest using these two models for additional HAPs. The secondary purpose of this paper is to calculate a "safe" asbestos concentration by first assuming a default linear extrapolation (to one excess death per million people, as specified for carcinogenic HAPs). The resulting "safe" concentration is an impossible-to-achieve 1/1000 of present background asbestos concentrations. A letter to the editor and a response in this Journal issue use additional asbestos data that suggest that the "safe" concentration should be about 730 times higher than first calculated here and that a default nonlinear extrapolation should be used instead, with the "safe" concentration proportional to the desired mortality level raised to the 0.39 power. These results suggest that the most important problem in setting a "safe" concentration for each carcinogenic HAP is to determine the correct nonlinear extrapolation to use for each HAP.

Lauber, S. N. and N. J. Gooderham (2007). "The cooked meat derived genotoxic carcinogen 2-amino-3-methylimidazo[4,5-b]pyridine has potent hormone-like activity: mechanistic support for a role in breast cancer." Cancer Res **67**(19): 9597-602.

The cooked meat-derived heterocyclic amine 2-amino-3-methylimidazo[4,5-b]pyridine (PhIP) is activated by CYP1A2 to the N-hydroxy metabolite, then esterified by acetyl transferase and sulfur transferase into unstable DNA-reactive products that can lead to mutation. The genotoxicity of PhIP has been implicated in its carcinogenicity. Yet, CYP1A2-null mice are still prone to PhIP-mediated cancer, inferring that alternative mechanisms must be operative in tumor induction. PhIP induces tumors of the breast, prostate, and colon in rats and lymphoma in mice. This profile of carcinogenicity is indicative of hormonal involvement. We recently reported that PhIP has potent estrogenic activity inducing transcription of estrogen (E2)-regulated genes, proliferation of E(2)-dependent cells, up-regulation of progesterone receptor, and stimulation of mitogen-activated protein kinase signaling. In this report, we show for the first time that PhIP at doses as low as of 10(-11) mol/L has direct effects on a rat pituitary lactotroph model (GH3 cells) and is able to induce cell proliferation and the synthesis and secretion of prolactin. This PhIP-induced pituitary cell proliferation and synthesis and secretion of prolactin can be attenuated by an estrogen receptor (ER) inhibitor, implying that PhIP effects on lactotroph responses are ERalpha mediated. In view of the strong association between estrogen, progesterone, prolactin, and breast cancer, the PhIP repertoire of hormone-like activities provides further mechanistic support for the tissue-specific carcinogenicity of the chemical. Furthermore, the recent epidemiology studies that report an association between consumption of cooked red meat and premenopausal and postmenopausal human breast cancer are consonant with these observations.

Lillie, M. A., C. M. Ambrus, et al. (2004). "Breast cancer in intraductal carcinogen-treated non-human primates." J Med **35**(1-6): 271-5.

Eight female Macaca arctoides monkeys were given dimethylbenzanthracene (DMBA) directly into the milk ducts. During a 4-year observation period, ending with euthanasia and autopsy, no mammary cancers were noticed. However, one animal developed a superficial localized squamous cell carcinoma. DMBA is highly carcinogenic in rodents, e.g. producing a high incidence of breast cancer in C3H mice. It was concluded that carcinogenicity testing should be extended beyond testing in rodents to non-human primates in order to distinguish "primary rodent carcinogens" from those highly active in primates as well. Studies are in progress to study carcinogens in human cell lines transplanted into nu/nu mice.

Lindsey, H. (2003). "EPA assesses cancer risk due to childhood carcinogen exposure." Lancet Oncol **4**(4): 201.

Marsit, C. J., M. R. Karagas, et al. (2006). "Carcinogen exposure and gene promoter hypermethylation in bladder cancer." Carcinogenesis **27**(1): 112-6.

Tobacco smoking, certain occupational exposures, and exposure to inorganic arsenic in drinking water have been associated with the occurrence of bladder cancer. However, in these tumors the exposure-associated pattern of somatic alterations in genes in the causal pathway for disease has been poorly characterized. In particular, the mechanism by which arsenic induces bladder cancer and the effects of lower environmental levels of exposure remain uncertain. Animal and in-vitro studies have suggested that arsenic and other exposures may act through epigenetic mechanisms. We, therefore, examined, in a population-based study of human bladder cancer, the relationship between epigenetic silencing of three tumor suppressor genes, p16(INK4A), RASSF1A and PRSS3, and exposure to both tobacco and arsenic in bladder cancer. Promoter methylation of each of these genes occurred in approximately 30% of bladder cancers, and both RASSF1A and PRSS3 promoter methylation were associated with advanced tumor stage (P<0.001 and P<0.04, respectively). Arsenic exposure, measured as toenail arsenic, was associated with RASSF1A (P<0.02) and PRSS3 (P<0.1) but not p16INK4A promoter methylation, in models adjusted for stage and other factors. Cigarette smoking was associated with a >2-fold increased risk of promoter methylation of the p16INK4A gene only, with greater risk seen in patients with exposures more recent to disease diagnosis. These results, from human bladder tumors, add to the body of animal and in vitro evidence that suggests a role in epigenetic alterations for bladder carcinogens.

Marsit, C. J., M. R. Karagas, et al. (2006). "Carcinogen exposure and epigenetic silencing in bladder cancer." Ann N Y Acad Sci **1076**: 810-21.

Tobacco smoking, certain occupational exposures, and exposure to inorganic arsenic in drinking water have been associated with the occurrence of bladder cancer. However, in these tumors the exposure-associated pattern of somatic alterations in genes in the causal pathway for disease has been poorly characterized. Animal and in vitro studies have suggested that arsenic, tobacco carcinogens, and other exposures may act through epigenetic mechanisms. We, therefore, examined, in a population-based study of human bladder cancer (n = 351), the relationship between epigenetic silencing of the tumor-suppressor genes, p16(INK4A), RASSF1A, PRSS3, and the four SFRP genes and exposure to both tobacco and arsenic in bladder cancer. Promotor methylation silencing of each of these genes occurred in approximately 30-50% of bladder cancers. Epigenetic silencing of RASSF1A and PRSS3 and any of the SFRP genes were each significantly associated with advanced tumor stage ($P < 0.001$, $P < 0.04$, and $P < 0.005$, respectively). Arsenic exposure, measured as toenail arsenic, was associated with RASSF1A ($P < 0.02$) and PRSS3 ($P < 0.1$) but not p16(INK4A) or SFRP promotor methylation, in models adjusted for stage and other risk factors. Cigarette smoking was associated with a greater than twofold increased risk of promotor methylation of the p16(INK4A) gene, with greater risk seen in patients with exposures more recent to disease diagnosis, and smoking was also significantly associated with any SFRP gene methylation ($P < 0.01$). These results from human bladder tumors, add to the body of animal and in vitro evidence that suggests bladder carcinogens play a crucial role in the induction of important epigenetic alterations.

Mennuni, C., S. Ugel, et al. (2008). "Preventive vaccination with telomerase controls tumor growth in genetically engineered and carcinogen-induced mouse models of cancer." Cancer Res **68**(23): 9865-74.

The telomerase reverse transcriptase, TERT, is an attractive target for human cancer vaccination because its expression is reactivated in a conspicuous fraction of human tumors. Genetic vaccination with murine telomerase (mTERT) could break immune tolerance in different mouse strains and resulted in the induction of both CD4+ and CD8+ telomerase-specific T cells. The mTERT-derived immunodominant epitopes recognized by CD8+ T cells were further defined in these mouse strains and used to track immune responses. Antitumor efficacy of telomerase-based vaccination was investigated in two cancer models closely resembling human diseases: the TRAMP transgenic mice for prostate cancer and a carcinogen-induced model for colon cancer. TERT overexpression in tumor lesions was shown in both models by immunohistochemistry, thus reinforcing the similarity of these tumors to their human counterparts. Repeated immunizations with mTERT-encoding DNA resulted in a significant delay of tumor formation and progression in both the prostate cancer and the colon cancer models. Moreover, evaluation of the intratumoral infiltrate revealed the presence of telomerase-specific T cells in vaccinated mice. The safety of vaccination was confirmed by the absence of histomorphologic changes on postnecropsy analysis of several organs and lack of adverse effects on blood cell counts. These results indicate that TERT vaccination can elicit antigen-specific immunosurveillance and imply this

antigen as a potential candidate for preventive cancer vaccines.

Nagini, S., P. V. Letchoumy, et al. (2009). "Of humans and hamsters: a comparative evaluation of carcinogen activation, DNA damage, cell proliferation, apoptosis, invasion, and angiogenesis in oral cancer patients and hamster buccal pouch carcinomas." Oral Oncol **45**(6): e31-7.

The hamster buccal pouch (HBP) carcinogenesis model is one of the most well characterized animal systems for analyzing the development of oral squamous cell carcinoma (OSCC), a common malignancy worldwide. HBP carcinomas that closely mimic human OSCC are useful in understanding the molecular mechanisms of neoplastic transformation. The present study is a comparative evaluation of markers of carcinogen activation, oxidative stress, cell proliferation, apoptosis, invasion, and angiogenesis in human and hamster OSCCs. Enhanced expression of CYP1A1 and CYP1B1 isoforms in both human and hamster oral tumours was associated with significantly increased expression of 8-hydroxy 2-deoxyguanosine (8-OHdG) indicating oxidative DNA damage. Analysis of markers of cell survival and proliferation revealed increased expression of PCNA, GST-P, and NF-kappaB with downregulation of p21, p53 and IkappaB in both human and hamster OSCCs. In addition, both human and hamster oral carcinomas displayed invasive, and angiogenic properties as revealed by dysregulated cytokeratin expression, downregulation of RECK, and increased expression of uPA, MMP-2 and-9, HIF-1alpha, and VEGF. The results reveal aberrant expression of multiple molecules in key signaling pathways in both human OSCCs and HBP carcinomas rendering the HBP model as an important tool for monitoring oral oncogenesis.

Nakagama, H., M. Nakanishi, et al. (2005). "Modeling human colon cancer in rodents using a food-borne carcinogen, PhIP." Cancer Sci **96**(10): 627-36.

Animal models provide researchers with powerful tools to elucidate multistage mechanisms for cancer development and to gain further insights into the biological roles of various cancer-related genes in in vivo situations. As for colon cancer models in rodents, Apc-disrupted mice, including ApcMin, have been one of the most widely utilized animal models to dissect the molecular events implicated in the development of intestinal tumors. In rats, several models have been established using chemical carcinogens, including azoxymethane and 2-amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine (PhIP). The former is a representative colon carcinogenic alkylating agent, and the latter a heterocyclic amine produced while cooking meat and fish, which people are exposed to in ordinary life. It is of great importance to note that PhIP preferentially targets the colon and prostate gland in male rats, and the mammary glands in female rats. Cancers in these three organs are common in Western countries and are currently increasing in Japan, where modern dietary habits are rapidly becoming more like those of the West. In the present article, the history of PhIP-induced colon cancer models in rodents, activation/detoxification mechanisms of PhIP with regard to the formation of PhIP-DNA adducts, mechanistic approaches to dissect the molecular events involved in the development of colon cancer by PhIP, and epidemiological evidence of human exposure to PhIP are overviewed. The induction of Paneth cell maturation/differentiation in PhIP-induced colon cancers, genetic traits affecting susceptibility to colon carcinogenesis, and the biological relevance of colon cancer models in rodents to studying human colon carcinogenesis are also discussed.

Nakajima, T., S. Enomoto, et al. (2008). "DNA methylation: a marker for carcinogen exposure and cancer risk." Environ Health Prev Med **13**(1): 8-15.

Cancers arise as a consequence of multiple genetic and epigenetic alterations. Many genes aberrantly methylated in cancers have been identified in recent years, and their use in cancer diagnosis and therapy is currently under investigation. During our genome-wide screening for a novel tumor-suppressor gene in gastric cancers, we found that only a small amount of aberrant methylation was present, even in non-cancerous gastric mucosae. A subsequent large-scale analysis of the gastric mucosae of healthy individuals and gastric cancer patients using quantitative methylation-specific PCR (qMSP) revealed that Helicobacter pylori infection potently induced aberrant DNA methylation in non-cancerous gastric mucosae and that these high methylation levels can decrease following cessation of the H. pylori infection. Helicobacter pylori infection induced the methylation of specific genes among 48 genes that can be methylated in gastric cancer cell lines. Most importantly, the methylation levels in the gastric mucosae of individuals without H. pylori infection correlated with their risk of gastric cancer. These findings show that a field for cancerization is formed by H. pylori infection and that this field can be measured using DNA methylation as a marker. The concept of an "epigenetic field for cancerization" has been also demonstrated for colon and breast cancers, and it is possibly present for other cancers and other diseases. Applied knowledge of epigenetic changes in human diseases has now started to make an impact on

the prevention, diagnostics, and therapeutics of these diseases.

Oguri, T., S. V. Singh, et al. (2003). "The carcinogen (7R,8S)-dihydroxy-(9S,10R)-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene induces Cdc25B expression in human bronchial and lung cancer cells." Cancer Res **63**(4): 771-5.

Cdc25B regulates cell cycle progression and genetic stability. Here, we report that exposure to the environmental carcinogen (7R,8S)-dihydroxy-(9S,10R)-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (anti-BPDE) causes a marked increase in the expression of Cdc25B mRNA and protein levels in terminal squamous differentiated human bronchial epithelial cells and in lung cancer cells, but not in undifferentiated bronchial cells. In addition, the growth rate of lung cancer cells was increased significantly in comparison with untreated cells after chronic exposure to 0.1 micro M anti-BPDE. Furthermore, increased Cdc25B expression and decreased Cdk1 phosphorylation were observed in anti-BPDE-treated cells. We postulate that the induction of Cdc25B expression in lung cancer cells by the ultimate carcinogen anti-BPDE accelerates the further development of lung carcinogenesis.

Ozawa, S., T. Katoh, et al. (2002). "Association of genotypes of carcinogen-activating enzymes, phenol sulfotransferase SULT1A1 (ST1A3) and arylamine N-acetyltransferase NAT2, with urothelial cancer in a Japanese population." Int J Cancer **102**(4): 418-21.

Carcinogenic aromatic amines such as 4-aminobiphenyl, which is contained in tobacco smoke, are one of the causal factors of urothelial epithelial cancers. 4-Aminobiphenyl has been shown to be bioactivated through N-hydroxylation by hepatic cytochrome (CYP) 1A2 and subsequently through O-sulfation and O-acetylation by phenol sulfating sulfotransferase, ST1A3 (SULT1A1), and arylamine N-acetyltransferase, NAT2, respectively. In a case-control study for urothelial epithelial cancers, low activity alleles of NAT2 are overall high-risk alleles (OR 2.11; 95% CI 1.08-4.26). Wild-type ST1A3*1 ((213)Arg) alleles were slightly overrepresented in nonsmoking urothelial cancer patients (82.6% vs. 69.7%) and in smoking cancer patients (76.7% and 74.3%) compared to a variant ST1A3*2 ((213)His) allele. In combination of ST1A3 and NAT2 genotypes for analyses of urothelial cancer risk, the highest OR of 2.45 (95% CI 1.04-5.98) was obtained with ST1A3*1 and NAT2 slow genotype among the 4 combinations. Recombinant ST1A3*1 enzyme showed a tendency of catalyzing higher in vitro 3'-phosphoadenosine 5'-phosphosulfate-dependent DNA adduct formation than ST1A3*2 (2.84 +/- 0.49 and 2.22 +/- 0.11 adducts/10(8) nucleotides). Combined analyses of different alleles of carcinogenic aromatic amine-activating phase II enzymes were applied to urothelial cancer risk for the first time and showed the highest risk combination of ST1A3 and NAT2 alleles.

Pacini, S., T. Punzi, et al. (2009). "A paradox of cadmium: a carcinogen that impairs the capability of human breast cancer cells to induce angiogenesis." J Environ Pathol Toxicol Oncol **28**(1): 85-8.

Cadmium, a highly persistent heavy metal, has been categorized as a human carcinogen. Even though it is known that cadmium acts as estrogens in breast cancer cells, several studies failed to demonstrate whether cadmium is a causal factor for breast cancer. The lack of a strong association between cadmium and breast cancer could be found in the antiangiogenic properties of this heavy metal, which might counteract its carcinogenic properties in the progression of breast cancer. In this study, we exposed estrogen-responsive breast cancer cells to subtoxic levels of cadmium, and we evaluated their angiogenic potential using the chick embryo chorioallantoic membrane assay. Exposure of breast cancer cells to subtoxic levels of cadmium significantly inhibited the angiogenic potential of the breast cancer cell line, suggesting the possibility that cadmium might negatively regulate the production of proangiogenic factors in breast cancer cells. Our results suggest that cadmium might exert a paradoxical effect in breast cancer: on the one hand, it could promote carcinogenesis, and, on the other hand, it could delay the onset of tumors by inhibiting breast cancer cell-induced angiogenesis.

Paolini, M., S. Z. Abdel-Rahman, et al. (2003). "Beta-carotene: a cancer chemopreventive agent or a co-carcinogen?" Mutat Res **543**(3): 195-200.

Evidence from both epidemiological and experimental observations have fueled the belief that the high consumption of fruits and vegetables rich in carotenoids may help prevent cancer and heart disease in humans. Because of its well-documented antioxidant and antigenotoxic properties, the carotenoid beta-carotene (betaCT) gained most of the attention in the early 1980s and became one of the most extensively studied cancer chemopreventive agents in population-based trials supported by the National Cancer Institute. However, the results of three randomized lung cancer chemoprevention trials on betaCT supplementation unexpectedly contradicted the large body of epidemiological evidence relating to the potential benefits of dietary carotenoids. Not only did betaCT show no benefit, it was associated with significant increases in lung cancer incidence, cardiovascular diseases, and total mortality. These

findings aroused widespread scientific debate that is still ongoing. It also raised the suspicion that betaCT may even possess co-carcinogenic properties. In this review, we summarize the current data on the co-carcinogenic properties of betaCT that is attributed to its role in the induction of carcinogen metabolizing enzymes and the over-generation of oxidative stress. The data presented provide convincing evidence of the harmful properties of this compound if given alone to smokers, or to individuals exposed to environmental carcinogens, as a micronutrient supplement. This has now been directly verified in a medium-term cancer transformation bioassay. In the context of public health policies, while the benefits of a diet rich in a variety of fruits and vegetables should continue to be emphasized, the data presented here point to the need for consideration of the possible detrimental effects of certain isolated dietary supplements, before mass cancer chemoprevention clinical trials are conducted on human subjects. This is especially important for genetically predisposed individuals who are environmentally or occupationally exposed to mutagens and carcinogens, such as those found in tobacco smoke and in industrial settings.

Pavek, P., G. Merino, et al. (2005). "Human breast cancer resistance protein: interactions with steroid drugs, hormones, the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine, and transport of cimetidine." J Pharmacol Exp Ther **312**(1): 144-52.

The breast cancer resistance protein (BCRP/ABCG2) is an ATP-binding cassette drug efflux transporter that extrudes xenotoxins from cells, mediating drug resistance and affecting the pharmacological behavior of many compounds. To study the interaction of human wild-type BCRP with steroid drugs, hormones, and the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP), we expressed human BCRP in the murine MEF3.8 fibroblast cell line, which lacks Mdr1a/1b P-glycoprotein and Mrp1, and in the polarized epithelial MDCKII cell line. We show that PhIP was efficiently transported by human BCRP in MDCKII-BCRP cells, as was found previously for murine Bcrp1. Furthermore, we show that six out of nine glucocorticoid drugs, corticosterone, and digoxin increased the accumulation of mitoxantrone in the MEF3.8-BCRP cell line, indicating inhibition of BCRP. In contrast, aldosterone and ursodeoxycholic acid had no significant effect on BCRP. The four most efficiently reversing glucocorticoid drugs (beclomethasone, 6alpha-methylprednisolone, dexamethasone, and triamcinolone) and 17beta-estradiol showed a significantly reduced BCRP-mediated transepithelial transport of PhIP by MDCKII-BCRP cells, with the highest reduction of PhIP transport ratio for beclomethasone (from 25.0 +/- 1.1 to 2.7 +/- 0.0). None of the tested endogenous steroids or synthetic glucocorticoids or digoxin, however, were transported substrates of BCRP. We also identified the H(2)-receptor antagonist drug cimetidine as a novel efficiently transported substrate for human BCRP and mouse Bcrp1. The generated BCRP-expressing cell lines thus provide valuable tools to study pharmacological and toxicological interactions mediated by BCRP and to identify new BCRP substrates.

Perera, F. P., L. A. Mooney, et al. (2002). "Associations between carcinogen-DNA damage, glutathione S-transferase genotypes, and risk of lung cancer in the prospective Physicians' Health Cohort Study." Carcinogenesis **23**(10): 1641-6.

DNA damage from polycyclic aromatic hydrocarbons (PAH) and other aromatic/hydrophobic compounds has been implicated in case-control studies as a risk factor for lung cancer, as have common polymorphisms in the glutathione S-transferase (GST) genes involved in carcinogen detoxification. However, their joint effects have not been evaluated in prospective studies, leaving open questions about predictive value of these biomarkers. In this matched case-control study nested within the prospective Physicians' Health Study, we evaluated whether biomarkers measured in white blood cells (WBC) significantly predicted risk, alone and in combination, after controlling for level of smoking. The biomarkers reported here are aromatic/hydrophobic-DNA adducts and polymorphisms in genes coding for the GSTM1 and GSTP1 enzymes. Our study population was composed of 89 cases of primary lung cancer and 173 controls, matched in a 1:2 ratio on smoking, age and duration of follow up. Adducts were measured in WBC DNA by the nuclease P1-enhanced (32)P-post-labeling method. Genotypes (GSTM1 null versus non-null and GSTP1 Val versus GSTP1 Ile) were determined by genomic amplification and restriction fragment length polymorphism analysis. Among current smokers, adducts were significant predictors of lung cancer risk (after adjusting for GST genotypes, OR = 3.10, 95% CI 1.07, 9.01). The combined GSTM1 null/GSTP1 Val genotype was associated with lung cancer overall and especially among former smokers, before and after adjusting for adducts (OR for former smokers = 4.21, CI 1.08, 16.41; adjusted OR = 4.68, CI 1.17, 18.71). Among cases only, adducts were significantly higher among current or former smokers with the GSTM1 non-null/GSTP1 Ile genotype. The two risk factors (adducts and genotypes) appear to be independent predictors of risk. The findings underscore the complex and important role of

biological susceptibility as a determinant of risk from carcinogens found in tobacco smoke and other environmental compounds.

Rundle, A. (2006). "Carcinogen-DNA adducts as a biomarker for cancer risk." Mutat Res **600**(1-2): 23-36.

Carcinogen-DNA adducts are thought to be a useful biomarker in epidemiologic studies seeking to show that environmental exposures to xenobiotics cause cancer. This paper reviews the literature in this field from an epidemiologic perspective and identifies several common problems in the epidemiologic design and analysis of these studies. Carcinogen-DNA adducts have been used in studies attempting to link xenobiotic exposures to hepatocellular carcinoma, smoking related cancers and breast cancer. Adduct measurements have been useful in further implicating aflatoxin exposure in the etiology of hepatocellular carcinoma. For smoking related cancers, associations with carcinogen-DNA adducts are commonly seen in current smokers but less so in ex- or non-smokers. In breast cancer the associations have been inconsistent and weak and there is little evidence that carcinogen-DNA adducts implicate xenobiotic exposures in the etiology of breast cancer. Methodological issues common to these studies are the use of target versus surrogate tissues and how this choice impacts control selection, disease effects on adduct levels, the time period reflected by adduct levels, the use of inappropriate statistical analyses and small sample sizes. It is unclear whether the lack of association between carcinogen-DNA adducts and cancer reflects a lack of association between the xenobiotic exposure of interest and cancer or the effects of these methodological issues. A greater focus needs to be placed on designs that allow measurements of adduct levels in tissues collected years prior to cancer diagnosis, there is little need for further hospital based case-control studies in which adducts are measured at the time of or after diagnosis. New designs that address these issues are suggested in the paper.

Saarinen, N. M., A. Warri, et al. (2008). "Dietary lariciresinol attenuates mammary tumor growth and reduces blood vessel density in human MCF-7 breast cancer xenografts and carcinogen-induced mammary tumors in rats." Int J Cancer **123**(5): 1196-204.

Lariciresinol is a dietary lignan that accounts for a significant portion of the total phytoestrogen intake from Western foods. Recent epidemiological studies suggest that high dietary intake of lignans and lariciresinol is associated with reduced breast cancer risk. However, no causal relationship between lariciresinol intake and breast cancer development has been established. In this study, we investigated for the first time the effects and possible mechanisms of action of lariciresinol on hormone responsive mammary cancer in vivo in dimethylbenz[a]anthracene induced mammary cancer in rats, and in human MCF-7 breast cancer xenografts in athymic mice. For tumor bearing rats, lariciresinol (3 or 15 mg/kg of body weight) or vehicle was administered p.o. daily for 9 weeks. For E2-maintained ovariectomized athymic mice bearing orthotopic MCF-7 tumors, control diet (AIN-93G) or lariciresinol containing diet (AIN-93G supplemented with 20 or 100 mg of lariciresinol/kg of diet) was administered for 5 weeks. In both models, lariciresinol administration inhibited the tumor growth and tumor angiogenesis. In MCF-7 cells, enterolactone significantly inhibited the E2-stimulated VEGF secretion. Moreover, in MCF-7 xenografts, lariciresinol administration enhanced tumor cell apoptosis and increased estrogen receptor beta expression. Lariciresinol and its further metabolites secoisolariciresinol, enterodiol and enterolactone were found in serum of both rats and athymic mice confirming a similar lignan metabolism pattern as in humans. These findings indicate conceivable importance of dietary lignan lariciresinol in inhibition of breast cancer development.

Simoes, M. L., S. L. Hockley, et al. (2008). "Gene expression profiles modulated by the human carcinogen aristolochic acid I in human cancer cells and their dependence on TP53." Toxicol Appl Pharmacol **232**(1): 86-98.

Aristolochic acid (AA) is the causative agent of urothelial tumours associated with aristolochic acid nephropathy. These tumours contain TP53 mutations and over-express TP53. We compared transcriptional and translational responses of two isogenic HCT116 cell lines, one expressing TP53 (p53-WT) and the other with this gene knocked out (p53-null), to treatment with aristolochic acid I (AAI) (50-100 microM) for 6-48 h. Modulation of 118 genes was observed in p53-WT cells and 123 genes in p53-null cells. Some genes, including INSIG1, EGR1, CAV1, LCN2 and CCNG1, were differentially expressed in the two cell lines. CDKN1A was selectively up-regulated in p53-WT cells, leading to accumulation of TP53 and CDKN1A. Apoptotic signalling, measured by caspase-3 and -7 activity, was TP53-dependent. Both cell types accumulated in S phase, suggesting that AAI-DNA adducts interfere with DNA replication, independently of TP53 status. The oncogene MYC, frequently over-expressed in urothelial tumours, was up-regulated by AAI, whereas FOS was down-regulated. Observed modulation of genes involved in endocytosis, e.g. RAB5A, may be relevant to the known inhibition of receptor-mediated

endocytosis, an early sign of AA-mediated proximal tubule injury. AAI-DNA adduct formation was significantly greater in p53-WT cells than in p53-null cells. Collectively, phenotypic anchoring of the AAI-induced expression profiles to DNA adduct formation, cell-cycle parameters, TP53 expression and apoptosis identified several genes linked to these biological outcomes, some of which are TP53-dependent. These results strengthen the importance of TP53 in AA-induced cancer, and indicate that other alterations, e.g. to MYC oncogenic pathways, may also contribute.

Spink, B. C., J. A. Bennett, et al. (2009). "Long-term estrogen exposure promotes carcinogen bioactivation, induces persistent changes in gene expression, and enhances the tumorigenicity of MCF-7 human breast cancer cells." Toxicol Appl Pharmacol **240**(3): 355-66.

The cumulative exposure to estrogens is an important determinant in the risk of breast cancer, yet the full range of mechanisms involving estrogens in the genesis and progression of breast cancer remains a subject of debate. Interactions of estrogens and environmental toxicants have received attention as putative factors contributing to carcinogenesis. Mechanistic studies have demonstrated interactions between estrogen receptor alpha (ERalpha) and the aryl hydrocarbon receptor (AhR), with consequences on the genes that they regulate. Many studies of ERalpha and AhR-mediated effects and crosstalk between them have focused on the initial molecular events. In this study, we investigated ERalpha- and AhR-mediated effects in long-term estrogen exposed (LTEE) MCF-7 human breast cancer cells, which were obtained by continuous culturing for at least 12 weeks in medium supplemented with 1 nM of 17beta-estradiol (E(2)). With these LTEE cells and with parallel control cells cultured without E(2) supplementation, we performed an extensive study of cytochrome P450 (CYP) induction, carcinogen bioactivation, global gene expression, and tumorigenicity in immunocompromised mice. We found that LTEE cells, in comparison with control cells, had higher levels of AhR mRNA and protein, greater responsiveness for AhR-regulated CYP1A1 and CYP1B1 induction, a 6-fold higher initial level of benzo(a)pyrene-DNA adducts as determined by liquid chromatography tandem mass spectrometry, marked differences in the expression of numerous genes, and a higher rate of E(2)-dependent tumor growth as xenografts. These studies indicate that LTEE causes adaptive responses in MCF-7 cells, which may reflect processes that contribute to the overall carcinogenic effect of E(2).

Swanson, S. M. and K. Christov (2003). "Estradiol and progesterone can prevent rat mammary cancer when administered concomitantly with carcinogen but do not modify surviving tumor histology, estrogen receptor alpha status or Ha-ras mutation frequency." Anticancer Res **23**(4): 3207-13.

An early full-term pregnancy is protective against mammary cancer in both humans and rodents. Treating rats with two hormones of pregnancy, estradiol and progesterone, for 5 weeks renders the rat mammary glands refractory to carcinogenesis. Our objectives was to determine if a shortened regimen (3 weeks) would be as effective as the 5-week regimen and to determine if the mammary gland was vulnerable to carcinogenic insult during the hormone treatments. We also examined cancers that survived the chemopreventive regimen to see if those tumors were particularly aggressive compared to control tumors (i.e., less differentiated, estrogen receptor alpha (ER alpha)-negative or harbored mutations in Ha-ras). In the first experiment, Lewis rats were injected with N-methyl-N-nitrosourea (MNU, 50 mg/kg) at 50 days of age. At 60 days of age, the rats were either mated and allowed to nurse their young for 3 weeks, treated with hormone vehicle for 5 weeks, or 17 beta-estradiol (E, 20 micrograms) and progesterone (P, 4 mg) 5 times per week for 3 or 5 weeks. All the rats exposed to MNU but not estradiol and progesterone developed multiple mammary cancers. Pregnancy reduced multiplicity to 0.40 cancers/rat. Treatments of estradiol and progesterone for 3 or 5 weeks reduced cancer multiplicity and increased latency to a similar degree as pregnancy. Mammary cancers from each group displayed a similar spectra of histologic class, estrogen receptor alpha (ER alpha) content and Ha-ras mutation status. In the second experiment, 50-day-old rats were treated for five weeks with either estradiol and progesterone or vehicle as above beginning at 60 days of age and treated with MNU at 50, 64, 78 or 92 days of age. In each case, estradiol and progesterone treatments resulted in significantly reduced mammary tumor frequency. These results demonstrate that a three-week regimen of estradiol and progesterone can protect the mammary gland from chemically-induced carcinogenesis even when carcinogen exposure occurs concomitant with estradiol and progesterone stimulation.

Trosko, J. E. and B. L. Upham (2005). "The emperor wears no clothes in the field of carcinogen risk assessment: ignored concepts in cancer risk assessment." Mutagenesis **20**(2): 81-92.

The following is a position paper challenging the paradigm that 'carcinogen = mutagen', and that the current rodent bioassay to predict risks to human

cancers is relevant and useful. Specifically, we review current observations concerning carcinogenesis that might lead to another approach for assessing the identification of human carcinogenic hazards and the risk assessment that chemicals might pose. We give a brief review of the multistage and multimechanism process of cancer in a tissue that involves not only genotoxic but also epigenetic events, and the importance of stem and progenitor cells in the development of cancer. We focus on the often ignored 'epigenetic' effects of carcinogens and the role of cell communication systems in epigenetically altering gene expression that leads to an imbalance of cell proliferation, differentiation and apoptosis in a tissue that can contribute to the cancer process. To draw attention to the fact that the current paradigm and policy to test toxic chemicals is often misleading and incorrect, we discuss how oxidative stress, in spite of the DNA damaging data, most probably contributes to cancer at the epigenetic level. Additionally, we briefly review how this mutagenic concept has greatly diverted attention away from doing research on the lower molecular weight, non-genotoxic, polycyclic aromatic hydrocarbons (PAHs), and how these low molecular weight PAHs are etiologically more relevant to the disease potential of environmental mixtures such as cigarette smoke.

Vainio, H. (2003). "Acrylamide in heat-processed foods--a carcinogen looking for human cancer?" Eur J Epidemiol **18**(12): 1105-6.

van der Hel, O. L., H. B. Bueno-de-Mesquita, et al. (2005). "Cumulative genetic defects in carcinogen metabolism may increase breast cancer risk (The Netherlands)." Cancer Causes Control **16**(6): 675-81.

Variants in the metabolic genes NAT1, NAT2, GSTM1 or GSTT1, may cause differences in individual detoxifying capacity of possible carcinogens. We examined the cumulative effect of putative at risk genotypes on breast cancer risk and we examined the extent to which these polymorphisms modify the association between smoking and breast cancer. A case cohort study was conducted in the DOM cohort with 676 breast cancer cases and a random sample of 669 individuals. No effect of the NAT1, NAT2 or GSTM1 genotypes on breast cancer risk was observed. However, women with GSTT1 null genotype had a 30% increased breast cancer risk compared to women with GSTT1 present (RR = 1.30 (95% confidence interval (CI) 1.04-1.64)). Smoking did not influence breast cancer risk nor did genetic variations in NAT1, NAT2 or GSTM1 in combination with smoking. Compared to women who never smoked with GSTT1 present, women with GSTT1 null genotype and who formerly smoked showed an increased breast cancer risk (RR = 2.55 (95% CI 1.10-5.90)), but current smokers who smoked 20 cigarettes or more per day did not (RR = 1.06 (95% CI 0.51-2.18)). Increasing numbers of putative at risk genotypes increased breast cancer risk in a dose dependent manner (p for trend 0.01). The risk was more than doubled in women with all four risk genotypes, RR = 2.45 (95% CI 1.24-4.86), compared to women with zero putative at risk genotypes. In conclusion, the results of this study suggest that presence of three or more putative at risk genotypes increases breast cancer risk.

van Herwaarden, A. E., J. W. Jonker, et al. (2003). "The breast cancer resistance protein (Bcrp1/Abcg2) restricts exposure to the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine." Cancer Res **63**(19): 6447-52.

The food carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) is the most abundant heterocyclic amine found in various protein containing foods. PhIP is mutagenic and carcinogenic in rodents, inducing lymphomas in mice and colon, mammary and prostate carcinomas in rats. It has also been implicated in human breast carcinogenesis. Humans on a normal Western diet are exposed to PhIP on a daily basis. The breast cancer resistance protein (BCRP/ABCG2) transports various anticancer drugs from cells, causing multidrug resistance. By its presence in the apical membrane of the intestine and bile canalicular membrane, it also protects the body from substrate drugs and toxins. We investigated whether Bcrp1 could affect PhIP exposure of the body because this could implicate BCRP activity in the cancer risk due to PhIP. Using polarized cell lines, we found that PhIP is efficiently transported by murine Bcrp1. In vivo pharmacokinetic studies showed that at a dose of 1 mg/kg [(14)C]PhIP the area under the curve for oral administration was 2.9-fold higher in Bcrp1(-/-) compared with wild-type mice (306 +/- 39 versus 107 +/- 15 h.ng/ml) and, for i.v. administration, 2.2 fold higher (386 +/- 36 versus 178 +/- 8.9 h.ng/ml). Wild-type mice cleared [(14)C]PhIP mainly by fecal excretion, but this shifted to primarily urinary excretion in Bcrp1(-/-) mice. In mice with a cannulated gall bladder, both hepatobiliary and direct intestinal excretion of [(14)C]PhIP were greatly impaired in Bcrp1(-/-) compared with wild-type mice (9.0 +/- 4.4 versus 36.5 +/- 9.4% and 1.5 +/- 0.8 versus 4.2 +/- 1.5%, respectively). We conclude that Bcrp1 effectively restricts the exposure of mice to ingested PhIP by decreasing its uptake from the gut lumen and by mediating hepatobiliary and intestinal elimination. Intra- or interindividual differences in BCRP activity in humans may thus also affect the

exposure to PhIP and related food carcinogens, with possible implications for cancer susceptibility.

Xiao, H. and S. V. Singh (2007). "p53 regulates cellular responses to environmental carcinogen benzo[a]pyrene-7,8-diol-9,10-epoxide in human lung cancer cells." Cell Cycle **6**(14): 1753-61.

The p53 tumor suppressor is a mutational target of environmental carcinogen anti-benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE). We now demonstrate that p53 plays an important role in regulation of cellular responses to BPDE. Exposure of p53-null H1299 human lung cancer cells to BPDE resulted in S and G(2) phase cell cycle arrest, but not mitotic block, which correlated with induction of cyclin B1 protein expression, down-modulation of cell division cycle 25C (Cdc25C) and Cdc25B protein levels, and hyperphosphorylation of Cdc25C (S216), cyclin-dependent kinase 1 (Cdk1; Y15), checkpoint kinase 1 (Chk1; S317 and S345) and Chk2 (T68). The BPDE-induced S phase block, but not the G(2)/M phase arrest, was significantly attenuated by knockdown of Chk1 protein level. The BPDE-mediated accumulation of sub-diploid fraction (apoptotic cells) was significantly decreased in H1299 cells transiently transfected with both Chk1 and Chk2 specific siRNAs. The H460 human lung cancer cell line (wild-type p53) was relatively more sensitive to BPDE-mediated growth inhibition and enrichment of sub-diploid apoptotic fraction compared with H1299 cells. The BPDE exposure failed to activate either S or G(2) phase checkpoint in H460 cells. Instead, the BPDE-treated H460 cells exhibited a nearly 8-fold increase in sub-diploid apoptotic cells that was accompanied by phosphorylation of p53 at multiple sites. Knockdown of p53 protein level in H460 cells attenuated BPDE-induced apoptosis but enforced activation of S and G(2) phase checkpoints. In conclusion, the present study points towards an important role of p53 in regulation of cellular responses to BPDE in human lung cancer cells.

Young, M. R. (2008). "Use of carcinogen-induced premalignant oral lesions in a dendritic cell-based vaccine to stimulate immune reactivity against both premalignant oral lesions and oral cancer." J Immunother **31**(2): 148-56.

Select groups of premalignant oral lesions carry a high risk of development of secondary premalignant lesions and oral squamous cell carcinoma (OSCC). The goal of the present study was to determine the feasibility of using premalignant lesion-pulsed dendritic cells as a treatment option to prevent development of secondary lesions and development of OSCC. Mice that were treated with the carcinogen 4-nitroquinoline-1-oxide (4NQO) developed premalignant oral lesions and, subsequently, OSCC. Immunohistochemical analyses showed that these 4NQO-induced lesions and OSCC both overexpressed the tumor antigens epidermal growth factor receptor, RAGE and, to a lesser extent, MUC1. Because there was shared overexpression of tumor antigens on premalignant oral lesions and OSCC, dendritic cells pulsed with lysates of 4NQO-induced premalignant lesion cells were tested in vitro and in vivo for their capacity to stimulate T-cell reactivity to premalignant lesion cells and to OSCC. Spleen cells that were sensitized during coculture or in vivo with premalignant lesion-pulsed dendritic cells were cytolytic toward both premalignant lesion cells and OSCC, and secreted increased levels of interferon -gamma in response to challenge with premalignant lesion cells or OSCC as compared with spleen cells that were sensitized with keratinocyte-pulsed dendritic cells. Levels of CD8+ Tcells and interferon-gamma release were also increased in lesions of mice that were vaccinated with premalignant lesion-pulsed dendritic cells. The mice that were vaccinated against premalignant lesions were also more resistant to OSCC challenge. These studies show the feasibility of using premalignant oral lesions to stimulate immune reactivity against both premalignant oral lesions and

Yu, S. and A. N. Kong (2007). "Targeting carcinogen metabolism by dietary cancer preventive compounds." Curr Cancer Drug Targets **7**(5): 416-24.

Prevention is one of the most important and promising strategies to control cancer. Many dietary bioactive compounds, mostly phytochemicals, have been found to decrease the risk of carcinogenesis. Modulating the metabolism and disposition pathways of carcinogens represents one of the major mechanisms by which dietary compounds prevent carcinogenesis. In the present review, the specific molecular targets of dietary compounds within carcinogen metabolism, including various enzymes and transporters and their regulatory signaling pathways, are briefly reviewed. The expression of phase I enzymes, which presumably mostly activate carcinogens, is mainly regulated by xenobiotics sensing nuclear receptors such as AhR, CAR, PXR, and RXR. On the other hand, phase II enzymes catalyze the conjugations of carcinogens and generally are transcriptionally controlled by the Nrf2/ARE signaling pathways. The Nrf2/ARE signaling pathway, which regulates the expression of many detoxifying enzymes, is a major target of dietary compounds. The final excretion of carcinogens and their metabolites is mediated by phase III transporters, which share many regulatory mechanisms with phase I/II enzymes. Indeed, the expression of metabolizing enzymes and transporters is often coordinately

regulated. Besides transcriptional regulation, the activities of phase I/II enzymes and phase III transporters could be directly activated or inhibited by dietary compounds. Furthermore, genetic polymorphisms have profound effects on the individual response to dietary compounds. Finally, the effects of cancer prevention and the risk of carcinogenesis are determined by the network composed of known/unknown molecular targets and signaling pathways and its interaction with various xenobiotics, including carcinogens, drugs, and diet. With the rapid advances in the post genomic sciences, it could be possible to decipher this network and better predict the clinical outcomes of cancer prevention by dietary bioactive compounds.

Zhang, C., E. Naftalis, et al. (2006). "Carcinogen-induced DNA double strand break repair in sporadic breast cancer." J Surg Res **135**(1): 120-8.

Induction of DNA double strand breaks and alterations in the repair of these breaks is implicated in breast carcinogenesis. Prior studies have demonstrated that peripheral blood mononuclear cells (PBMC) from breast cancer patients exhibit increased numbers of DNA strand breaks after exposure to ionizing radiation, but these studies did not specifically measure DNA double strand breaks and it is not known whether chemical carcinogens produce similar effects. PBMC from 32 women undergoing breast surgery were genotyped at nine loci of seven DNA repair genes. DNA double strand break repair was measured using the neutral comet assay after exposure to ionizing radiation (0.5 Gy) or bioactivated benzo[a]pyrene (B[a]P, 5 microM. RESULTS: PBMC from breast cancer patients showed higher levels of residual DNA double strand breaks 30 min after exposure to radiation than PBMC from patients with benign breast disease (1.40 times baseline [95% confidence intervals [CI] 1.29-1.51] versus 1.24 times baseline [95% CI 1.15-1.33], respectively, P = 0.04). The response to B[a]P trended in the same direction, but did not reach statistical significance. The MGMT K178R variant genotype was associated with improved DNA double strand break repair in PBMC exposed to B[a]P. Reduced repair of radiation-induced DNA double strand breaks in PBMC is a robust biomarker of breast cancer risk. Reduced DNA repair capacity may have a genetic component even in sporadic breast cancer.

Zhou, G. D., N. Popovic, et al. (2005). "Tissue-specific attenuation of endogenous DNA I-compounds in rats by carcinogen azoxymethane: possible role of dietary fish oil in colon cancer prevention." Cancer Epidemiol Biomarkers Prev **14**(5): 1230-5.

I-compounds are bulky covalent DNA modifications that are derived from metabolic intermediates of nutrients. Some I-compounds may play protective roles against cancer, aging, and degenerative diseases. Many carcinogens and tumor promoters significantly reduce I-compound levels gradually during carcinogenesis. Colon cancer is the second leading cause of cancer death in the United States, whereas cancer of the small intestine is relatively rare. Here we have studied levels of I-compounds in DNA of colon and duodenum of male Sprague-Dawley rats treated with azoxymethane. The effects of dietary lipids (fish oil or corn oil) on colon and duodenal DNA I-compounds were also investigated. Rats fed a diet containing fish oil or corn oil were treated with 15 mg/kg azoxymethane. Animals were terminated 0, 6, 9, 12, or 24 hours after injection. I-compound levels were analyzed by the nuclease P1-enhanced (32)P-postlabeling assay. Rats treated with azoxymethane displayed lower levels of I-compounds in colon DNA compared with control groups (0 hour). However, I-compound levels in duodenal DNA were not diminished after azoxymethane treatment. Animals fed a fish oil diet showed higher levels of I-compounds in colonic DNA compared with corn oil groups (mean adduct levels for fish and corn oil groups were 13.35 and 10.69 in 10(9) nucleotides, respectively, P = 0.034). Taken together, these results support claims that fish oil, which contains a high level of omega-3 polyunsaturated fatty acids, may have potent chemopreventive effects on carcinogen-induced colon cancer. The fact that duodenal I-compounds were not diminished by azoxymethane treatment may have been due to the existence of tissue-specific factors protecting against carcinogenesis. In conclusion, our observations show that endogenous DNA adducts may serve not only as sensitive biomarkers in carcinogenesis and cancer prevention studies, but are also helpful to further our understanding of the chemopreventive properties of omega-3 fatty acids and mechanisms of carcinogenesis.

Zhu, F., C. X. Jin, et al. (2003). "Response of human REV3 gene to gastric cancer inducing carcinogen N-methyl-N'-nitro-N-nitrosoguanidine and its role in mutagenesis." World J Gastroenterol **9**(5): 888-93.

To understand the response of human REV3 gene to gastric cancer inducing carcinogen N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and its role in human mutagenesis. The response of the human REV3 gene to MNNG was measured in human 293 cells and FL cells by RT-PCR. By using antisense technology, mutation analysis at HPRT locus (on which lesion-targeted mutation usually occurs) was conducted in human transgenic cell line FL-REV3(-)

by 8-azaguanine screening, and mutation occurred on undamaged DNA template was detected by using a shuttle plasmid pZ189 as the probe in human transgenic cell lines 293-REV3(-) and FL-REV3(-). The blockage effect of REV3 was measured by combination of reverse transcription-polymerase chain reaction to detect the expression of antisense REV3 RNA and Western blotting to detect the REV3 protein level. RESULTS: The human REV3 gene was significantly activated by MNNG treatment, as indicated by the upregulation of REV3 gene expression at the transcriptional level in MNNG-treated human cells, with significant increase of REV3 expression level by 0.38 fold, 0.33 fold and 0.27 fold respectively at 6 h, 12 h and 24 h in MNNG-treated 293 cells ($P<0.05$); and to 0.77 fold and 0.65 fold at 12 h and 24 h respectively in MNNG-treated FL cells ($P<0.05$). In transgenic cell line (in which REV3 was blocked by antisense REV3 RNA), high level of antisense REV3 RNA was detected, with a decreased level of REV3 protein. MNNG treatment significantly increased the mutation frequencies on undamaged DNA template (untargeted mutation), and also at HPRT locus (lesion-targeted mutation). However, when REV3 gene was blocked by antisense REV3 RNA, the MNNG-induced mutation frequency on undamaged DNA templates was significantly decreased by 3.8 fold ($P<0.05$) and 5.8 fold ($P<0.01$) respectively both in MNNG-pretreated transgenic 293 cells and FL cells in which REV3 was blocked by antisense RNA, and almost recovered to their spontaneous mutation levels. The spontaneous HPRT mutation was disappeared in REV3-disrupted cells, and induced mutation frequency at HPRT locus significantly decreased from 8.66 x 10(-6) in FL cells to 0.14 x 10(-6) in transgenic cells as well ($P<0.01$). CONCLUSION: The expression of the human REV3 can be upregulated at the transcriptional level in response to MNNG. The human REV3 gene plays a role not only in lesion-targeted DNA mutagenesis, but also in mutagenesis on undamaged DNA templates that is called untargeted mutation.

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