

Hereditary Cancer Syndromes

PR-1 Plasma IGF-I is inversely associated with colorectal adenoma recurrence. E. T. Jacobs,¹ M. Elena Martinez,¹ D. S. Alberts,¹ E. L. Ashbeck,¹ S. M. Gapstur,² P. Lance,¹ P. A. Thompson¹. ¹Arizona Cancer Center, Tucson, AZ, ²Northwestern University, Chicago, IL.

The IGF-I axis has been proposed to be a significant factor in the development of certain cancers, including colorectal. However, results from epidemiological studies suggest modest effects on colorectal cancer risk. Using cross-sectional and prospective study designs within the same cohort, we investigated whether plasma IGF-I, IGFBP-1, and IGFBP-3 were associated with colorectal adenoma characteristics at baseline, and whether their levels were related to odds for adenoma recurrence. Plasma levels of each marker were measured at baseline in 299 male participants in the Wheat Bran Fiber Trial who were followed for recurrence of their adenomatous lesions. In cross-sectional analyses, plasma IGF-I was significantly positively associated with the presence of adenomas with any villous features ($p=0.04$). In contrast, IGF-I levels were inversely associated with odds of colorectal adenoma recurrence, with ORs (95% CIs) of 0.72 (0.37-1.38) and 0.52 (0.27-1.00) for the second and third tertiles of IGF-I, respectively, as compared to the first tertile (p -trend=0.05). The inverse association was stronger for advanced adenoma recurrence (p -trend=0.04) than for non-advanced recurrence (p -trend=0.16). These results suggest that while IGF-I may have adverse effects in existing premalignant lesions of the colorectum, individuals with elevated IGF-I levels are protected from the formation of new lesions in the colorectum.

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PR-2 FGFR2 is a breast cancer susceptibility gene in Israeli populations. L. Raskin,¹ G. Rennert,² S. B. Gruber¹. ¹Departments of Internal Medicine, Epidemiology, and Human Genetics, University of Michigan Medical School and School of Public Health, Ann Arbor, MI, ²Department of Community Medicine and Epidemiology, Carmel Medical Center and Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology and Clalit Health Services national Cancer Control Center, Haifa, Israel.

We validated genome-wide association scan (GWAS) findings of FGFR2 as a risk factor for breast cancer and estimated the contribution of FGFR2 polymorphisms to breast cancer risk in diverse ethnic groups within the Jewish and other Middle Eastern populations. Taking advantage of a large ongoing, population-based case-control study of the molecular and environmental etiology of breast cancer in Israel, we genotyped four FGFR2 SNPs (rs11200014, rs2981579, rs1219648, rs2420946) in intron 2 and tested for association of these SNP and their haplotypes with breast cancer risk in the series of 1529 women with breast cancer and 1528 controls.

We found evidence of significant associations between risk of breast cancer and all four studied SNPs in FGFR2 (p for trend for all SNPs are <0.0001). The analysis in different ethnic groups showed significant association of all four studied SNPs with breast cancer risk in Ashkenazi and Sephardi Jews (p for trend for all SNPs are <0.01), with a similar, but not significant trend in Arabs. There was no significant effect modification of the association between FGFR2 SNPs and risk of breast cancer by age at diagnosis, first degree family history of breast cancer, or ethnicity.

Haplotype analysis identified four common haplotypes (five in Sephardi Jews and Arabs) with frequencies >0.01 . The common AAGT haplotype, previously demonstrated as a risk haplotype in white non-Hispanic women was significantly associated with breast cancer risk in Ashkenazi (OR=1.22, 95%CI=1.04-1.42, $p=0.0125$) and Sephardi Jews (OR=1.42, 95%CI=1.14-1.76, $p=0.015$) compared to the reference GGAC haplotype. A second low frequency haplotype (AAAC) was significantly associated with breast cancer risk in Sephardi Jews only (OR=1.98, 95%CI=1.16-3.36, $p=0.0121$). This haplotype (AAAC) was about 4 times more frequent in Sephardi Jews and Arabs than in Ashkenazi Jews. The reference GGAC haplotype was the most frequent in Ashkenazi Jews and Arabs, but not in Sephardi Jews. None of the haplotypes showed a significant association with breast

cancer risk in Arabs, although power is limited in this group and the direction of the associations was consistent with those observed in other populations. The GAGT haplotype, equally frequent among Sephardi Jews and Arabs (0.016 and 0.019 respectively), but less frequent in Ashkenazi Jews (0.008), was also significantly associated with risk of breast cancer. The present study shows that FGFR2 is a breast cancer susceptibility gene in Ashkenazi and Sephardi Jews and appears to confer modest risk in Arab populations as well. The population attributable risk for rs1219648 is 16% for Ashkenazi and 22% for Sephardi Jews, and could be as high as 12% in Arabs. Our findings may help to identify FGFR2 causal variants for breast cancer.

[This abstract also presented in Poster Session B on board B74.]

Bioactive Food Components and Cancer Prevention

PR-3 Consumption of raw, but not cooked, cruciferous vegetables and reduction of bladder cancer risk. L. Tang, G. R. Zirpoli, K. Guru, K. B. Moysich, Y. Zhang, C. B. Ambrosone, S. E. McCann. Roswell Park Cancer Institute, Buffalo, NY.

Cruciferous vegetables contain isothiocyanates (ITCs), which show potent chemopreventive activity against bladder cancer in both *in vitro* and *in vivo* studies. However, previous epidemiological studies investigating cruciferous vegetable intake and bladder cancer risk have been inconsistent. Cooking can substantially reduce or destroy ITCs, and could account for study inconsistencies. In this hospital-based case-control study involving 275 individuals with incident, primary bladder cancer and 825 individuals without cancer, we examined usual pre-diagnostic intake of raw and cooked cruciferous vegetables in relation to bladder cancer risk. Odds ratios (OR) and 95% confidence intervals (CI) were estimated with unconditional logistic regression, adjusting for smoking and other bladder cancer risk factors. We observed a strong and statistically significant inverse association between bladder cancer risk and raw cruciferous vegetables (adjusted OR for highest vs. lowest category= 0.57, 95% CI = 0.38-0.84), with a clear dose-response trend ($P = 0.004$); there were no significant associations for fruit, total vegetables, or total cruciferous vegetables. The reduced risks observed for total raw crucifers were also observed for individual raw crucifer. The inverse associations were stronger in current smokers and heavy smokers. Risk reduction was greatest among non-smokers with three or more servings per month of raw cruciferous vegetables (adjusted OR = 0.27, 95% CI = 0.13-0.56). These data indicate that cruciferous vegetables, when consumed raw, may reduce risk of bladder cancer, a protective effect consistent with a role of dietary ITCs as chemopreventive agents against bladder cancer.

[This abstract also presented in Poster Session B on board B47.]

PR-4 Inhibition of colorectal tumorigenesis in azoxymethane (AOM)-treated rats by green tea polyphenols. H. Xiao, X. Hao, B. Simi, J. Ju, M. Lee, G. Lu, B. S. Reddy, C. S. Yang. Rutgers, the State University of New Jersey, Piscataway, NJ.

The cancer preventive activity of tea and its constituents has been demonstrated in different animal models. However, the results on colon cancer prevention by tea polyphenols in rats have not been consistent. Previously, our results showed that dietary administration of Polyphenon E (PPE, a standardized green tea polyphenol preparation) inhibited the formation of aberrant crypt foci in AOM-treated rats. Herein, we conducted a study with PPE as the preventive agent in the AOM-induced rat colon cancer model using the colorectal tumor as an endpoint. F344 rats were given two weekly injections of AOM (15 mg/kg), and then fed a 20% high fat diet with or without 0.24% PPE for 34 weeks. In the positive control group, 96% of rats developed colorectal tumors with multiplicity of 3.64 ± 0.31 , and tumor tissues showed highly elevated levels of Akt and phospho-Akt, and decreased levels of caspase-3 activation, in comparison to the normal colorectal mucosa. Dietary PPE treatment did not change liver or kidney weight, but decreased body weight by 5% in the treated group compared to the control. The decrease in body weight may be due to

inhibition of lipid absorption by tea polyphenols (as suggested by our study in a high fat diet-induced obesity mouse model). Dietary PPE treatment significantly decreased tumor multiplicity and tumor size by 55% ($p < 0.01$) and 45% ($p < 0.05$), respectively. Moreover, treatment with 0.24% PPE also significantly decreased the incidence of adenocarcinoma (from 61% to 27%, $p < 0.05$), the multiplicity of adenoma (by 45%, $p < 0.05$), and the multiplicity of adenocarcinoma (by 80%, $p < 0.01$). Dietary PPE treatment significantly increased the levels of major tea polyphenols, i.e., EGC, EC, EGCG and ECG, in both plasma and colorectal mucosa of treated rats. Our results also showed that PPE treatment decreased levels of PGE₂, LTB₄ and 8-isoprostane in plasma of treated rats. Taken together, we demonstrated convincingly the inhibition of rat colon carcinogenesis by dietary treatment with green tea polyphenols. (Supported by NIH grant CA88961).

[This abstract also presented in Poster Session A on board A134.]

Novel Targets for Prevention of Breast Cancer

PR-5 Tamoxifen modulates tumor suppressor gene methylation in breast cells and growth factors in plasma in women at increased risk for breast cancer. C. M. Lewis,¹ D. Bu,¹ R. Ashfaq,¹ X. Xie,¹ A. Miller,² W. Dooley,³ J. O'Shaughnessy,⁴ B. Arun,⁵ K. Hunt,⁵ D. M. Euhus¹. ¹UT Southwestern Medical Center, Dallas, TX, ²Cancer Therapy and Research Center, San Antonio, TX, ³The University of Oklahoma Health Sciences Center, Oklahoma City, OK, ⁴Baylor Sammons Cancer Center, Texas Oncology, PA, US Oncology, Dallas, TX, ⁵UT MD Anderson Cancer Center, Houston, TX.

Background: Tamoxifen reduces the incidence of invasive and *in situ* breast cancer by 50% in high risk women, but its effects on benign breast tissue biology have not been systematically studied. This randomized, prospective placebo-controlled double-blind Phase IIa trial was designed to identify biomarkers that are modulated by tamoxifen locally in benign breast tissue as well as systemically in plasma.

Methods: Between May 2002 and March 2007, 31 premenopausal and 42 postmenopausal women defined as high risk by the Gail model or with a personal history of LCIS were randomized to tamoxifen (20 mg/day) or placebo for 3 months. Blood and breast tissue were collected at baseline and post-treatment. Endpoints included growth hormones and binding proteins in plasma, cytological classifications and tumor suppressor gene methylation in FNA, and proliferation (Ki67) and apoptosis (TUNEL) in tissue cores.

Results: Tamoxifen treatment was associated with an increase in SHBG and total estradiol but a reduction in plasma albumin resulting in an increase in free estradiol in the plasma ($P=0.003$). Levels of IGF1 decreased while IGF2, IGFBP1 and IGFBP3 levels increased. The net effect was a significant decrease in IGF1/IGFBP1 and IGF1/IGFBP3 ratios ($P=0.013$ and $P=0.001$). Prolactin levels were not altered during treatment. The three month tamoxifen treatment did not reduce FNA cellularity or the prevalence of cytological atypia. However, promoter methylation of RASSF1A and APC decreased in the tamoxifen group compared to the placebo group ($P=0.04$). Methylation of cyclin D2, H-cadherin, CST6, HIN-1, and RAR 2 was not affected. There was no reduction in the proliferative index and no increase in apoptosis.

Conclusion: Tamoxifen increased total and free estradiol and beneficially modulated IGF/IGFBP ratios in the plasma. Tamoxifen also decreased APC and RASSF1A methylation in benign breast epithelial cells and this effect was independent of IGF modulation. This work was supported by the National Cancer Institute grant: N01-CN-95139.

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PR-6 Combination chemoprevention of HER2/neu-induced breast cancer using a COX-2 inhibitor and an RXR-selective retinoid.

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The inducible prostaglandin synthase isoform cyclooxygenase-2 (COX-2) is overexpressed in ~40% of human breast carcinomas, and in precancerous breast lesions, particularly in association with overexpression of human epidermal growth factor receptor 2 (HER2/neu). Experimental breast cancer can be suppressed by genetic or pharmacological ablation of Cox-2, suggesting potential clinical utility of COX-2 inhibitors with respect to breast cancer. Importantly, several clinical trials have found reduced colorectal adenoma formation in individuals administered selective COX-2 inhibitors. However, such trials have also identified increased cardiovascular risk associated with COX-2 inhibitor use. The goal of this research was to test whether improved chemopreventive efficacy could be achieved by combining submaximal doses of a selective COX-2 inhibitor and a retinoid X receptor-selective retinoid (rexinoid). The rate of HER2/neu-induced mammary tumor formation was substantially delayed by coadministration of the COX-2 inhibitor celecoxib (500 ppm in diet) and the rexinoid LGD1069 (10 mg/kg body weight; oral gavage) to MMTV/neu mice. Median time to tumor formation was increased from 304 days in control animals to >600 days in the celecoxib/LGD1069 group ($P<0.0001$). The combination was substantially more effective than either drug individually. Tumor multiplicity was also significantly reduced in the combination group relative to the control cohort (44% of control; $P=0.027$). Similarly, increased suppression of aromatase activity was observed in mammary tissues from the combination cohort relative to that achieved with either agent singly (44% of control; $P<0.00001$). Regulation of aromatase expression and activity by COX-derived prostaglandins is well established. Interestingly however, single agent LGD1069 significantly reduced mammary aromatase activity (71% of control; $P<0.0001$) without modulating eicosanoid levels. Our data demonstrate that together celecoxib and LGD1069 have potent anticancer efficacy, potentially worthy of clinical evaluation.

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