

## Angiogenesis and Invasion

## Cell, Molecular, and Tumor Biology

## Angiogenesis and Invasion

**A1 The zinc-finger E-box-binding transcriptional repressor Snail promotes tumor progression and angiogenesis in non-small cell lung cancer.** Jane Yanagawa,<sup>1</sup> Tonya C. Walser,<sup>1</sup> Li X. Zhu,<sup>2</sup> Jie Luo,<sup>1</sup> Long-Shen Hong,<sup>1</sup> Michael C. Fishbein,<sup>1</sup> Lee Goodglick,<sup>1</sup> Robert M. Strieter,<sup>3</sup> Sherven Sharma,<sup>2</sup> Steven M. Dubinett<sup>1</sup>. <sup>1</sup>UCLA, Los Angeles, CA; <sup>2</sup>VA Greater Los Angeles Health Care Center, Los Angeles, CA; <sup>3</sup>University of Virginia, Charlottesville, VA.

The zinc-finger E-box-binding transcriptional repressor Snail has been implicated in tumor progression of several malignancies. Best known as a transcriptional repressor of the adherens junction component E-cadherin, Snail has predominantly been associated with the epithelial-mesenchymal transition (EMT), invasion, and metastasis. However, the role of Snail in non-small cell lung cancer (NSCLC) is not yet defined. Immunohistochemistry of human lung adenocarcinoma and squamous cell carcinoma sections revealed specific nuclear staining of tumor cells in all patient samples. A human lung adenocarcinoma cell line H441 was stably transduced with a Snail expressing retroviral vector. Western analysis verified up-regulation of Snail and down-regulation of E-cadherin in the Snail over-expressing cells (H441-Snail) as compared to vector control cells (H441-V). In three-dimensional spheroid culture, H441V cells grew into large, tight spheroids while H441-Snail cells were markedly dis cohesive, reflecting a Snail-mediated phenotypic change consistent with EMT. To examine the effects of Snail over-expression *in vivo*, severe combined immunodeficiency (SCID) mice were injected subcutaneously with either H441-Snail cells or with H441-V cells. Mice were sacrificed 6 weeks later. The primary tumor burden in mice bearing H441-Snail tumors was five fold greater than the primary tumor burden of mice bearing H441-V tumors ( $p < 0.005$ ). To evaluate the incidence of metastases, these cell lines were tested in an orthotopic model. H441-V and H441-Snail cells were transthoracically injected into the left lung of SCID mice. Organs were then harvested and analyzed by flow cytometry gated on the human marker, CD49b. The incidence of metastases to the right lung, liver, bone marrow, and adrenal glands were significantly increased in the mice bearing H441-Snail tumors ( $p < 0.05$ ). Because H441-Snail tumors appeared grossly more hemorrhagic as compared to H441-V tumors at the time of harvest, primary tumors were homogenized and analyzed by ELISA for levels of two angiogenic factors known to play a role in NSCLC tumor angiogenesis: CXCL8 and CXCL5. H441-Snail tumors were associated with increased levels of CXCL8 ( $p < 0.05$ ) and CXCL5 ( $p < 0.05$ ) as compared to H441-V tumors. Based on these results, SCID mice injected with H441-Snail cells were treated with a CXCR2 (CXCL8 and CXCL5 receptor) blocking antibody. These mice exhibited reduced tumor burden and metastases as compared to mice bearing H441-Snail tumors that were treated with a control antibody. In summary, Snail contributes to tumor progression in NSCLC by inducing tumor angiogenesis, as evidenced by elevated levels of angiogenic factors (CXCL8 and CXCL5) and the reversal of increased tumor burden and metastases with CXCR2 blockade.

**A2 Dauricine inhibits IGF-I-induced HIF-1 $\alpha$  protein accumulation and VEGF expression.** Xudong Tang, Gang Li, Keyuan Zhou. Guangdong Medical College, Zhanjiang 524023, China.

Dauricine (Dau), a bisbenzylisoquinoline alkaloid derivative isolated from the rhizome of *Menispermum dauricum* DC, has been found to have anticancer activities. However, the effect of Dau on tumor angiogenesis is still unclear. A growing body of evidence has demonstrated that hypoxia-inducible factor (HIF)-1 $\alpha$  and vascular endothelial growth factor (VEGF) play an important role in tumor angiogenesis. In this study, we investigated the effect of Dau on HIF-1 $\alpha$  and VEGF expression induced by insulin-like growth factor-I (IGF-I) in human breast cancer cells (MCF-7). Our results

showed that Dau significantly inhibited IGF-I-induced HIF-1 $\alpha$  protein accumulation, but had no effect on HIF-1 $\alpha$  mRNA expression. Meanwhile, Dau also remarkably suppressed VEGF expression at both protein and mRNA levels in response to IGF-I. Mechanistically, we found that Dau suppressed IGF-I-induced HIF-1 $\alpha$  and VEGF protein expression mainly *via* blocking the activation of PI-3K/AKT/mTOR signaling pathway. Functionally, we found that Dau dramatically abrogated IGF-I-stimulated formation of capillary tube-like structures *in vitro*. Taken together, our results suggested that Dau may be a promising antiangiogenic agent for human breast cancer.

Supported by National Natural Science Foundation of China (No. 30872944) and Guangdong Administration of Traditional Chinese Medicine (No.2008166).

**A3 Bcl-w-induced invasive pathways in gastric cancer cells.** Hong Duck Um, In Hwa Bae, Sung Hwan Yoon. KIRAMS, Seoul, Republic of Korea.

We have reported previously that Bcl-w, a pro-survival member of the Bcl-2 family, is expressed in gastric cancer cells, particularly in those with an infiltrative morphology. Functional studies utilizing Bcl-w-overexpressing cells have consistently demonstrated that Bcl-w enhances not only the survivability of the cancer cells, but also their migratory and invasive potentials. The Bcl-w-induced invasive pathway was determined to involve a sequential activation of phosphoinositide 3-kinase (PI3K), Akt, and Sp1, which subsequently results in the expression of metalloproteinase-2 (MMP-2). In this study, we have demonstrated that Bcl-w also induces the urokinase-type plasminogen activator (uPA), which was prevented by treatment with pharmacological inhibitors of PI3K, Akt, or Sp1. These inhibitors also attenuated the ability of Bcl-w to promote cell migration. Similar results were obtained when the actions of MMP-2 and uPA were blocked using their inhibitors or small interfering RNAs (siRNAs), thereby suggesting that these enzymes stimulate migratory signals in this system. Bcl-w overexpression was shown to activate focal adhesion kinase (FAK) in a manner dependent on PI3K, Akt, Sp1, MMP-2, and uPA, and the prevention of FAK action using its siRNAs or dominant negative mutants abolished Bcl-w-induced migration. Overall, it appears that Bcl-w induces both MMP-2 and uPA via the PI3K-Akt-Sp1 pathway, which then promotes cell invasion not only via the degradation of the extracellular matrix, but also via the stimulation of the FAK-dependent migratory pathway.

**A4 Antagonistic effects of sulphated polysaccharides from *Turbinaria conoides* (J. Agardh) on tumor cell migration and angiogenesis.** Caroline R. Delma,<sup>1</sup> Karthikeyan Ramalingam,<sup>2</sup>

Vijayabaskar Pandian,<sup>1</sup> Albert Baskar,<sup>3</sup> Ignacimuthu Savarimuthu,<sup>3</sup> Balasubramanian Thangavelu,<sup>1</sup> Somasundaram T. Somasundaram<sup>1</sup>. <sup>1</sup>Annamalai University, Parangipettai, India; <sup>2</sup>University of Texas Health Science Centre, San Antonio, TX; <sup>3</sup>Entomology Research Institute, Loyola College, Chennai, India.

Seaweeds are considered to be a rich source of biologically active substances like antioxidants, anti-viral, anti-inflammatory and anticoagulant agents. There are some evidences that seaweeds contain compounds with a relatively high antioxidant and antiproliferative activity. Seaweeds are low in fats but contain vitamins and bioactive compounds, like terpenoids and sulfated polysaccharides. Sulphated polysaccharides from brown algae are found to exhibit diverse biological activities like anticoagulant, antihyperlipidemic, antiviral, antioxidant, antitumor, antimetastatic and antiangiogenic activities. In this study, the antiproliferative, antiinvasive and antiangiogenic properties of sulphated polysaccharides from brown seaweed, *Turbinaria conoides* against colon carcinoma cell line COLO 320DM were investigated. Crude sulphated polysaccharide was extracted from *T. conoides*, collected from Mandapam coast, South east coast of India. The antiproliferative activity of the seaweed polysaccharides on COLO 320DM was evaluated by MTT assay and DNA fragmentation studies. The inhibitory potential on the migration

of this invasive cell line was assessed by wound healing assay. The effect of sulphated polysaccharides against angiogenesis was investigated by chorio allantoic membrane (CAM) assay in chick embryo. It was observed that sulphated polysaccharides from *T. conoides* inhibited the proliferation of COLO 320DM cells at a concentration of 1mg/ml by 40% at 24 hours. DNA fragmentation as evidenced by ladder formation in agarose gel indicates that the antiproliferative activity of seaweed polysaccharides is due to the induction of apoptosis. The migration of the carcinoma cell line was effectively inhibited by the seaweed extract. The antiinvasive property increased with increasing concentrations. However, the activity was more or less similar at 24 and 48 hours. In the CAM assay, it was found that sulphated polysaccharides from the brown seaweed inhibited vasculogenesis in a developing chick embryo. These results clearly indicate that sulphated polysaccharides from brown algae are potential molecular leads for antimetastatic drug development.

#### A5 Inhibition of tumor cell migration and angiogenesis by sulphated polysaccharides from *Sargassum wightii* (Greville).

Caroline R. Delma,<sup>1</sup> Karthikeyan Ramalingam,<sup>2</sup> Vijayabaskar Pandian,<sup>1</sup> Albert Baskar,<sup>3</sup> Ignacimuthu Savarimuthu,<sup>3</sup> Balasubramanian Thangavelu,<sup>1</sup> Somasundaram T. Somasundaram<sup>1</sup>. <sup>1</sup>Annalai University, Parangipettai, India; <sup>2</sup>University of Texas Health Science Centre at San Antonio, San Antonio, TX; <sup>3</sup>Entomology Research Institute, Loyola College, Chennai, India.

Seaweeds are potential renewable resources of the marine environment. They are low in fats but contain vitamins and bioactive compounds like terpenoids and sulphated polysaccharides. Sulphated polysaccharides from brown algae are found to exhibit diverse biological activities like anticoagulant, antihyperlipidemic, antiviral, antioxidant, antitumor, antimetastatic and antiangiogenic activities. In this study, the antiproliferative, antiinvasive and antiangiogenic properties of sulphated polysaccharides from brown seaweed, *Sargassum wightii* (Greville) against colon carcinoma cell line COLO 320DM were investigated. Crude sulphated polysaccharide was extracted from *S. wightii* collected from Mandapam coast, South east coast of India. The antiproliferative activity of the seaweed polysaccharides on COLO 320DM was evaluated by MTT assay and DNA fragmentation studies. The inhibitory potential on the migration of this invasive cell line was assessed by wound healing assay. The effect of sulphated polysaccharides against angiogenesis was investigated by chorio allantoic membrane (CAM) assay in chick embryo. Sulphated polysaccharides from *S. wightii* exhibited moderate growth inhibitory activity against COLO 320DM at a concentration of 1mg/ml at 24 hours. DNA fragmentation studies revealed characteristic ladder formation which indicates that seaweed polysaccharides have effected the induction of apoptosis. The seaweed extract was found to inhibit the migration of the colon carcinoma cell line. The antiinvasive property increased with increasing concentrations. However, the activity was more or less similar at 24 and 48 hours. The CAM assay showed that sulphated polysaccharides from the brown seaweed exhibited promising activities against neoangiogenesis. These results clearly indicate that sulphated polysaccharides from brown algae are potential molecular leads for antimetastatic drug development.

### Cancer Genetics/Gene Expression

**A6 Stat5a and pregnancy protection in the CERM mouse model.** Anne M. Miermont, Priscilla A. Furth. Lombardi Comp. Cancer Center at Georgetown University, Washington, DC.

**Introduction:** Estrogen Receptor  $\alpha$  (ER $\alpha$ ) is a key regulator in normal mammary gland development and acts as a growth factor in breast cancer development. We have developed the CERM mouse model with targeted ER $\alpha$  overexpression and deregulation specifically to the mammary gland.

By 4 months of age, the CERM mice develop ER $\alpha$ -initiated mammary ductal hyperplasia and ductal carcinoma in-situ which mimic human disease (Frech et al., 2005). In women, there is evidence that early full-term pregnancy decreases lifetime ER $\alpha$ -positive breast cancer risk. Paradoxically, there is also evidence that breast cancer risk is transiently increased within ten years of early full-term pregnancy (Schedin, 2006). One potential mechanism for the lifetime protective effect is believed to occur via differentiation (Russo et al., 2006). In addition, it is well established that STAT5a, a latent cytoplasmic transcription factor, is highly activated during pregnancy and lactation, and is involved in alveolar proliferation and terminal differentiation (Liu et al, 1996 & 1997). Therefore, we hypothesized that Stat5a contributes to the protective effects of pregnancy. The CERM mice develop hyperplastic alveolar nodules (HANs) by 12 months of age, therefore we postulated that pregnancy would protect against HANs in the CERM mice, which have Stat5a, and that loss of Stat5a alleles would reduce the protective effects of pregnancy.

**Methods:** The CERM mouse model, which has targeted ER $\alpha$  transgene expression to mammary epithelial cells, was bred with the Stat5a knockout mouse model to generate CERM, CERM/Stat5a<sup>+/+</sup> and CERM/Stat5a<sup>-/-</sup> mice. The mice went through several pregnancies and lactation cycles before being necropsied at 12-13 months of age.

**Results:** At 12 months of age, CERM mice showed an increased incidence of mammary hyperplastic alveolar nodules (HANs) (p= 0.0254) over wild type (WT) mice. That increase is reduced with loss of one Stat5a alleles (CERM/Stat5a<sup>+/-</sup>) and further reduced to zero (p=0.0022) with loss of 2 Stat5a alleles (CERM/Stat5a<sup>-/-</sup>). We observed an unexpected finding with parity in the CERM and CERM/Stat5a<sup>+/-</sup> mice. At 12 months of age, the incidence of HANs was not reduced in multiparous CERM versus nulliparous CERM mice as well as in multiparous CERM/Stat5a<sup>+/-</sup> versus nulliparous CERM/Stat5a<sup>+/-</sup> mice. Since the incidence of HANs was zero in the nulliparous CERM/Stat5a<sup>-/-</sup> mice, the protective effects of pregnancy were not studied with loss of two Stat5a alleles. However, we had generated one 12 month old parous CERM/Stat5a<sup>-/-</sup> mice, which interestingly developed HANs as opposed to nulliparous CERM/Stat5a<sup>-/-</sup> mice, which do not develop HANs. Interestingly, additional preliminary data demonstrated that parity in the CERM and CERM/Stat5a<sup>+/-</sup> mice can lead to the development of different neoplastic lesions that were variably ER $\alpha$  positive or negative.

**Conclusion:** Pregnancy in our CERM model did not confer protection against HANs development at 12 months of age. This lack of pregnancy protection seemed to occur via a Stat5a-independent ER $\alpha$ -dependent growth pathway. Since Stat5a loss leads to lack of HAN development in nulliparous mice, Stat5a loss seemed to have a different impact in multiparous mice which showed development of HANs. Further studies will determine which signaling pathways are involved in lack of pregnancy protection in our models.

**A7 Identification of novel genetic alterations in GI tumors using whole-genome profiling.** Murali Bashyam,<sup>1</sup> Ratheesh Raman,<sup>1</sup> Kotapalli Viswakalyan,<sup>1</sup> Pandila Ramaswamy,<sup>1</sup> Ajay Chaudhary,<sup>1</sup> Chandrakant K. Reddy,<sup>1</sup> Swarnalata Gowrishankar,<sup>1</sup> Mukta Srinivasulu,<sup>2</sup> R. A. Sastry,<sup>3</sup> Sujith Pattnaik,<sup>4</sup> Mohana Vamsy<sup>4</sup>. <sup>1</sup>Centre for DNA Fingerprinting and Diagnostics, Hyderabad, India; <sup>2</sup>MNJ Institute of Oncology and Regional Cancer Centre, Hyderabad, India; <sup>3</sup>Nizam's Institute of Medical Sciences, Hyderabad, India; <sup>4</sup>Indo-American Cancer Institute and Research Centre, Hyderabad, India.

In our previous studies, we identified novel pancreatic cancer genes using a combination of array-based comparative genomic hybridization (aCGH) and gene expression microarrays (Neoplasia, 2007 and Kwei and PLOS Genet, 2008). In the current study, we have used a multipronged approach to address two important GI cancers prevalent in India. Colorectal cancer (CRC) is a major health problem worldwide and is usually an age-related disease. A small proportion of patients present at an

early age due to familial cancer syndromes. Surprisingly, there appears to be a high proportion of patients in India who present at an early age without a family background and succumb to aggressive metastatic tumors. Using immunohistochemistry, mutation screening and microsatellite instability analysis of more than 150 samples, we show that Wnt signaling, a hallmark of CRC in the West, is not a major feature in a majority of young patients in India unlike older patients. There does not appear to be a significant difference between the two categories of CRC patients in other parameters including gender, lifestyle, diet, tumor location, frequency of microsatellite instability, and Loss of Heterozygosity at the BRCA1 locus. In addition, we have identified several novel mutations in the APC gene in Wnt positive tumors. Determination of genome-wide copy number alterations and transcript profiles is expected to delineate the genetic aberrations that may lead to CRC in the young in India.

Squamous cell carcinoma of the esophagus (ESCC) is common in India and Asia but rare in the West, as against esophagus adenocarcinoma which is common in the West but not in India. ESCC is a poorly studied highly aggressive cancer with survival rates below 15% in most populations. We have carried out molecular analysis of about 75 ESCC samples and 26 adenocarcinoma samples using archived as well as fresh resected tumors. Results indicate that unlike the ESCC samples, a significant proportion of adenocarcinoma samples exhibited an activated Wnt signaling pathway. Inactivation of p53 however appeared to be a common event in both Esophagus cancer subtypes. Analysis of copy number alterations using array-based CGH has revealed a recurrent amplification at 10q21 in ESCC samples. The amplification harbored genes that exhibited comparably altered transcript levels, as determined by array-based transcript profiling. We are also carrying out molecular analyses of mixed adeno-squamous tumors of the esophagus; results are expected to reveal whether the squamous and adeno components have an independent origin or vice versa.

**A8 Deregulated microRNAs associated with cervical cancer metastasis.** Y.F. Wong, T.H. Cheung, S.F. Yim, M.K.N. Man, T.S. Lau, M.K.L. Chan, T.K.H. Chung. The Chinese University of Hong Kong, Shatin, NT, Hong Kong.

Cervical cancer is one of the most common gynecologic malignancy in Hong Kong. The identification of pretreatment markers with predictive significance for the presence of lymph node metastasis in low stage of cervical cancer is clinically important. MicroRNAs (miRNAs) are noncoding RNA molecules of 21 to 24 nt that regulate the expression of target genes in a post-transcriptional manner. Recent evidence indicates that miRNAs play essential roles in tumorigenesis and cancer metastasis. This study was to evaluate the deregulated miRNAs associated with cervical cancer metastasis. A total of 20 cervical squamous cell carcinomas, in which 10 had pathologically confirmed lymph node metastasis and 10 no metastasis, were included in the study. RNAs were extracted from microdissected tumor tissue specimens. Real-time reverse transcription PCR technology-based miRNA array enabling quantitation of 365 human mature miRNAs, was used to profile global miRNA expression in cervical cancer. dChip software was used for biostatistical analysis of data. Global miRNA expression data obtained from all 20 arrays were normalized, and then the corresponding model-based expression index was calculated. Unsupervised hierarchical clustering analysis of data showed a distinct separation between cancers in the presence and absence of lymph node metastasis. Supervised hierarchical clustering analysis revealed that of 13 significantly differentially expressed miRNAs, 10 miRNAs including miR-137 (4.76-fold), miR-203 (3.82-fold), miR-594 (3.58-fold), miR-149 (3.05-fold), miR-365 (2.61-fold), miR-556 (2.43-fold), miR-33 (2.32-fold), miR-627 (2.12-fold), miR-20a (2.05-fold) and miR-653 (2.04-fold) were up-regulated and 3 miRNAs including miR-187 (-4.31), miR-4095p (-2.65-fold) and miR-615 (-2.1-fold) were down-regulated in the cervical squamous cell carcinomas with lymph node metastasis compared to that without metastasis. The

results obtained from this preliminary study indicate that the deregulation of miRNAs appears to be involved in the cervical cancer progression, and miRNA expression signature can be of potential importance as predictive biomarker in cervical cancer metastasis. Further validation of such microRNA pattern by the testing set and an independent cohort of patients with cervical cancer as well as the functional study of these deregulated miRNAs are ongoing.

**A9 Expression of insulin-like growth factor II and of the type 1 receptor gene in esophageal adenocarcinoma.** Ronghua Zhao, Ron Geyer, John Decoteau, Alan Casson. College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

**Aim:** To explore potential biological mechanisms underlying the association between obesity and esophageal malignancy, we studied the expression of insulin-like growth factor-II (IGF-II) and of the type 1 receptor (IGF-IR) gene in a well characterized series of patients with primary esophageal adenocarcinoma (EADC) and matched histologically normal esophageal epithelia (NE).

**Patients and Methods:** Banked esophageal tissues (EADC and matched NE, obtained with informed consent) were available from a consecutive series of 52 patients who underwent esophageal resection without preoperative chemoradiation therapy. Following extraction of RNA, expression of IGF-II and IGF-IR mRNA was determined using reverse transcription and real time polymerase chain reaction (RT-PCR). The  $2^{-\Delta\Delta CT}$  (Livak) method was used to quantitate tissue expression of IGF-II and IGF-IR mRNA, which was correlated with measured body mass index (BMI), clinicopathological findings, and outcome.

**Results:** As expected, significant correlations between tumor stage, differentiation and survival were found for the 49 male and 3 female patients in this series, who ranged in age from 36 to 85 years (median: 60.5 years). A significant association was seen between BMI and IGF-II mRNA expression in NE tissues ( $r_s=0.26$ ,  $p=0.075$ ). Significantly higher IGF-II mRNA expression was seen in EADC tumors (median 6.45, 95%CI 7.00-13.46) compared to matched NE tissues (median 1.32, 95%CI 1.37-4.31,  $p<0.01$ ), particularly in poorly differentiated tumors. On multivariate analysis, IGF-II expression was an independent predictor of disease-free survival ( $p=0.02$ ) and overall survival ( $p=0.03$ ). Expression levels of IGF-II mRNA correlated significantly with IGF-IR in both EADC ( $r_s=0.47$ ,  $p<0.01$ ) and NE ( $r_s=0.46$ ,  $p<0.01$ ). Furthermore, IGF-IR mRNA expression was found to be significantly higher in EADC (median 1.57; 95%CI 1.47-3.86) compared with matched NE (median 0.57; 95%CI 0.50-0.85) ( $p<0.01$ ), but only in patients with the A variant of the common IGF-IR G3174A polymorphism, which has recently been shown to modulate the obesity risk for EADC.

**Conclusions:** These studies further implicate perturbations of the IGF axis in the molecular pathogenesis of human EADC, and suggest a plausible mechanistic basis to explain the association between obesity and risk for malignancy. The significant association between IGF-II expression and survival suggests that IGF-II may be a clinically relevant biomarker for prognosis, or molecular target for novel anti-cancer therapies.

**A10 Gene expression analysis of malignancy associated changes in normal bronchial epithelium.** Kelsie L. Thu, Jennifer Campbell, Cathie Garnis, Bradley P. Coe, Wan L. Lam, Calum MacAulay, Stephen Lam. British Columbia Cancer Research Centre, Vancouver, British Columbia, Canada.

**Background:** Lung cancer is the number one cancer-related cause of death in the world. The poor prognosis for lung cancer patients is largely due to the lack of an effective non-invasive method to detect the disease in its early stage. Using quantitative nuclear morphometry of sputum cells, we have previously demonstrated that in the presence of tumor, cytologically normal appearing diploid cells may exhibit malignancy-associated changes (MACs) and that these changes may be used as surrogate markers for early detection. The molecular basis for MACs is not known.

**Objective:** Our objective is to identify malignancy-associated gene expression changes in normal bronchial epithelial cells obtained by bronchial brushing.

**Methods:** We compared the pre- and post-surgical resection gene expression profiles of bronchial epithelial cells obtained by brushing of airways in the lung opposite to the one containing the lung cancer. Agilent whole genome expression arrays were used to generate mRNA transcript profiles for seven paired normal bronchial brushings taken before and 3 to 6 months after surgical resection of the tumor. *Ingenuity Pathway Analysis* software was used to identify downstream expression targets of the soluble signaling factors VEGF, TGF- $\beta$ , TNF- $\alpha$ , and IL-8, which have been reported to be secreted by lung tumor cells. The target genes identified were analyzed for malignancy induced differential gene expression between normal bronchial epithelial cells from cancerous and non-cancerous environments.

**Results:** We detected differential expression of multiple target genes in the bronchial brush cells. Genes that were found to be differentially expressed included downstream targets of the signaling molecules EGF, TGF- $\beta$ , and VEGF.

**Conclusion:** Soluble signaling molecules secreted by lung malignancies induce MACs, as evident by altered gene expression after removal of the tumor. Although further investigation is necessary, our results are consistent with previous observation that lung tumors can induce MACs in normal appearing bronchial cells in the vicinity of the tumor. MACs may be a potential marker for early detection of lung cancer. This data also provides evidence that MACs can be measured quantitatively in bronchial brush cells using gene expression analysis.

**A11 Mutation at 235th nucleotide in mutated Myc gene down regulates overexpression and cancer growth: A computational genomic approach.** Samarendra Kumar Ray, Latchumi N., Yamini A., Meera K., Naresh S., Chandrasekhar R. R., Gayathri S., Soumyarupa De, Gayatri Ankem. Birla Institute of Technology & Science, Pilani, Goa Campus, Zuarinagar, India.

The work refers to development of computational methodology for characterization of coding sequence of the proto-oncogene Myc present in chromosome 8 of human genome. The mutation at 288th codon where AGC is replaced by AAC results in the substitution of serine to asparagine. This causes over expression of the gene to become an oncogene which ultimately results in uncontrolled tumor formation. A computational methodology has been designed based on Shannon's information theory which mimics the functional weights of the nucleotides. Three parameters, TMIC (Total Modular Information Content), BAI (Binding Affinity Index) and IPCIC (Integrated Per Class Information Content), have been developed to calculate the functional values of the nucleotides. In the process of calculating the parameters, the chemical attribute considered is the partial charge on heteroatoms of A, T, G and C constituting the molecules. The partial charges were calculated using Gaussian software. Two concepts have been utilized in the computational methodology-degeneracy of codons and sensitivity operations of different orders. The IPCIC values of the nucleotides A, T, G and C are 3.84076, 2.8133, 3.2800 and 2.7968 respectively. Using these functional weights of the nucleotides and following 4th order sensitivity operations, the characteristic value of the normal Myc gene was calculated to be 207.1965 and that of the mutated gene was 818.2066. An approach has been initiated by mutating codons following degeneracy principle so that the protein product of the gene is unchanged. The mutation is done at places other than 288th position. Correction of mutated codon at 288th position is not energetically feasible as internal energy of mutated gene is less than that of the normal gene. The mutated Myc gene is more stable than that of the normal gene. The results show that, replacing GGA by GGG at 235th position of mutated Myc gene sequence brings the characteristic value of mutated Myc gene closer to that of normal gene. This gives a strong physical implication that

the virulence of the cancerous gene can be reduced to that of the normal gene, thus reducing its over expression. Such an approach can be employed in cancer research for treatment of cancer for addressing malignant growth. Degeneracy of codons indicates that resolution of mutational effect of gene is not unique. Characteristic value of mutated gene would come to normal /near normal by mono as well as multi point mutational implant. Scope of the work is multivariate. Further studies are in progress.

**A12 Increased expression of STEAP1 gene as potential poor prognostic marker of prostate cancer.** Shadia M. Ihlaseh, Greicy H. R. Gambarini, Jose Carlos S. Trindade Fo., Maria A. Domingues, Silvia R. Rogatto, Joao Lauro V. de Camargo. UNESP- Sao Paulo State University, Botucatu, Brazil.

Prostate cancer is a public health problem due to its high incidence and mortality rates. *STEAP1* and *STEAP2* genes (six-transmembrane epithelial antigen of prostate 1 e 2) are mapped at 7q21.13 region, where we have previously described genomic gains by array comparative genomic hybridization (aCGH) of primary prostate adenocarcinomas (PCa). The involvement of these genes in PCa development and progression remains to be clarified. They express tumor antigens and are putative molecular markers. The objective of this study was to determine *STEAP1* and *STEAP2* genes expressions by real time quantitative PCR (qPCR) and *STEAP1* protein expression by immunohistochemistry in samples of PCa, in surrounding non-neoplastic tissues, and in prostate nodular hyperplasia. Patients were classified in three groups of recurrence risk depending on serum PSA levels, Gleason scores, and tumor stages for qPCR analysis. Increased *STEAP1* and *STEAP2* genes expressions were registered in 16% and 21% of the 55 samples, respectively; both genes presented simultaneous increased expression in three high-risk cases. Similar pattern of expression of these genes were detected in the majority of PCa and in surrounding non-neoplastic tissues. Compared to surrounding non-neoplastic tissues, there was significant increase of *STEAP1* expression in tumor samples with Gleason scores  $\geq 7(4+3)$  and in cases with vascular invasion. There was a trend for increased *STEAP1* expression in high-risk cases when compared to the moderate-risk group. In accordance with gene expression, higher levels of *STEAP1* protein were expressed in PCa compared to surrounding non-neoplastic tissues. Our data suggest that increased *STEAP1* expression is related to clinical and pathological parameters of PCa patients and its use as a poor prognostic marker should be considered.

## Cell Cycle

**A13 Chemoprevention agent SHetA2 induces G1 arrest through modulation of a biological system driven by Cyclin D1.** Chioniso P. Masamha, Doris Mangiaracina Benbrook. University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Flexible heteroarotinoid (Flex-Het) compounds induce apoptosis through direct targeting of mitochondria and Bcl-2 proteins, inhibit angiogenesis through regulation of cytokine expression and induce differentiation through increased E-Cadherin expression. In addition, the lead Flex-Het, SHetA2, induces G1 cell cycle arrest through induction of Cyclin D1 protein phosphorylation, ubiquitination and degradation. Overexpression of Cyclin D1 abrogated the G1 arrest. SHetA2 counteraction of carcinogen-induced Cyclin D1 expression was associated with SHetA2 chemoprevention of transformation in an organotypic model. Cyclin D1 is a tightly controlled protein that drives G1 cell cycle progression. Overexpression of Cyclin D1 is frequently observed in ovarian cancer and is believed to be an early event in ovarian tumorigenesis. The hypothesis of this study was that SHetA2 induces G1 arrest through modulation of a biological system driven by Cyclin D1.

## Cell Cycle

Cyclin D1 degradation was associated with several expected downstream events in 2 ovarian cancer cell lines. Co-IP assays demonstrated that Cyclin D1 loss was associated with relocation of p21 from the Cyclin D1 complex to the Cyclin E2 complex. Consistent with the stimulatory effect of p21 on the CyclinD1-Cdk4/6 complex and the inhibitory effect on the CyclinE2-Cdk2 kinase activity, Western blot analysis demonstrated that phosphorylation of Rb on serine 780, known to be induced by CyclinD1-Cdk4/6-p21, was decreased. This was followed by decreased Rb phosphorylation on serine 612, which is known to be induced by CyclinE2-Cdk2. SHetA2 inhibition of Cyclin A protein expression, confirmed that the repression of Rb phosphorylation prevented the release of E2F from Rb, thus preventing expression of S Phase genes. An rtPCR array demonstrated that 7 genes involved in cell cycle regulation were significantly altered above background in both ovarian cancer cell lines. Ingenuity analysis demonstrated that several of these genes are involved in an interacting biological system driving the cell cycle. A Cdk4 inhibitor, p16, and the down-stream gene Bax were up-regulated, while several Cyclin D1 and E2F interacting genes were also regulated.

Study of upstream events driving Cyclin D1 degradation demonstrated that the GSK3 kinase, known to induce Cyclin D1 threonine 286 phosphorylation, was phosphorylated/inhibited by SHetA2 and not involved in the cell cycle arrest. This phosphorylation event could be due to the SHetA2 induced phosphorylation of Akt serine 473, which has been shown to lead to increased GSK3 phosphorylation. Experiments with libraries of chemical and siRNA kinase inhibitors demonstrated that inhibition of kinases, such as EGF-R, mTOR, PKC, DNA-PK, contributed to SHetA2 growth inhibition, depending on their expression and activity patterns in the specific cell lines evaluated.

In conclusion, SHetA2 inhibition of Cyclin D1 induced a number of changes in down-stream and associated targets consistent with G1 arrest. Experiments are ongoing to identify the specific kinase responsible for SHetA2 induction of Cyclin D1 phosphorylation. Knowledge of SHetA2 modulation of the biological system regulating cell cycle progression provides important information, such as response biomarkers, that can be used in translational research associated with planned chemoprevention trials.

Supported by CA106713

## Cell Death

**A14 The role of Livin in oncogenesis reveals a novel mode of gene regulation.** Lazar Itay, Riki Perlman, Luna Kadouri, Dina Ben-Yehuda. Hadassha, Jerusalem, Israel.

Acquired resistance to apoptosis is a hallmark of all types of cancer. The Inhibitor of Apoptosis Protein (IAP) family members is able to inhibit apoptosis induced by a variety of stimuli mainly by binding to and inhibiting caspases.

We and others previously identified the IAP Livin. We further showed that Livin functions as a regulator of apoptosis that can undergo cleavage by effector caspases to produce a truncated form with paradoxical pro-apoptotic activity.

In this study we describe the role of Single Nucleotide Polymorphism (SNP) in the regulation of Livin in normal and malignant cells and demonstrate how the 528 (C/T) SNP regulates Livin protein expression. We show that Livin is expressed in a non-random monoallelic manner as only the 528T allele is expressed in normal blood and in early-stage melanoma. In advanced melanoma, by mechanism of DNA overrepresentation, both 528T and 528C mRNA alleles of Livin are expressed, resulting in expression of the Livin protein.

The variable expression of the Livin protein plays a major role in the survival of melanoma patients: We show that high Livin expression is an independent adverse prognostic marker. Importantly, we show that low-intermediate expression of Livin is associated with favorable prognosis in comparison to no Livin expression.

**PR-3 A novel function of poly(ADP-ribose) polymerase-1 in modulation of autophagy and necrosis under oxidative stress.** Qing Huang, You-Tong Wu, Hui-Ling Tan, Choon-Nam Ong, Han-Ming Shen. National University of Singapore, Singapore, Singapore.

This abstract is being presented as a short talk in Concurrent Session 5. A full abstract is printed in the Proffered Papers: Oral Presentation Abstracts section of the conference proceedings. (Presented on board number A15)

**A16 External Qi of Yan Xin Qigong induces apoptosis in estrogen-independent breast cancer cells through inhibition of the Akt/NF- $\kappa$ B pathway.** Xin Yan,<sup>1</sup> Hua Shen,<sup>2</sup> Hongjian Jiang,<sup>3</sup> Dan Hu,<sup>4</sup> Chengsheng Zhang,<sup>5</sup> Jun Wang,<sup>2</sup> Xinqi Wu.<sup>4</sup> <sup>1</sup>Chongqing Institute of Traditional Chinese Medicine, Chongqing, China; <sup>2</sup>New Medical Science Research Institute, New York, NY; <sup>3</sup>Brigham and Women's Hospital, Harvard Medical School, Boston, MA; <sup>4</sup>Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA; <sup>5</sup>McMaster University, Hamilton, Ontario, Canada.

The concept External Qi of Qigong refers to the technology and ability of the "Qi deployment" therapy and health preservation in traditional Chinese medicine (TCM). External Qi therapy of TCM has long been one of the medical practices and under the management of the health authorities in China. Studies have shown substantial beneficial effects of Yan Xin Qigong (YXQ) that originated from TCM on disease management, weight control, and optimizing caloric intake and diet habit. The external Qi of Yan Xin Qigong (YXQ-EQ) has previously been shown to have potent antioxidative properties. In the present study we have investigated the effect of YXQ-EQ on the Akt/NF- $\kappa$ B signaling pathway that is crucial for tumor growth and progression in estrogen-independent breast cancer MDA-MB-231 cells. YXQ-EQ significantly inhibited constitutive and EGF-stimulated Akt phosphorylation. Furthermore, YXQ-EQ substantially inhibited constitutive NF- $\kappa$ B activity and downregulated the expression of NF- $\kappa$ B target genes Bcl-2, Bcl-xL, XIAP and survivin. YXQ-EQ treatment caused a dramatic reduction in the viability of MDA-MB-231 cells, accompanied by DNA fragmentation, cleavage of PARP, and activation of caspase-3 and caspase-9. These findings suggest that YXQ-EQ might induce apoptosis in MDA-MB-231 cells through inactivation of Akt/NF- $\kappa$ B signaling. YXQ-EQ could potentially be a novel approach for breast cancer prevention and therapy.

**DNA Methylation/Epigenetics and Chromatin Regulation**

**A18 CpG island microarray analysis of minute bronchial epithelial cell specimens.** Ian M. Wilson, Stephen L. Lam, Wan L. Lam. British Columbia Cancer Research Centre, Vancouver, British Columbia, Canada.

**Background:** DNA methylation abnormalities are both very early events in cancer development and very widespread events in advanced neoplastic disease. Given their early occurrence in neoplasia, DNA methylation changes have many potential applications as biomarkers in serum, sputum, and exfoliated bronchial cells. To date, analysis of epigenetic events has been limited by technique throughput and by DNA requirement, limiting this approach to larger specimens, or only a few genes at a time. The ability to determine methylation status simultaneously at many loci using the limited amounts of starting tissue material recoverable from the small airways of the lung or sputum would greatly facilitate the use of DNA methylation as both a discovery tool and as a biological marker.

**Objective:** To improve on current methylation profiling technologies and adapt them to the investigation of minute amounts of DNA from exfoliated bronchial epithelial cells collected by bronchial brushing.

**Materials and Methods:** Exfoliated bronchial cells were collected during bronchoscopy from peripheral airways from patients with and without non-small cell lung cancer (NSCLC). DNA was extracted by standard biochemical means. Using only 250 ng of genomic DNA, which is available from a single bronchial brush, methylation profiles were generated for the epithelial cells using a 244k oligonucleotide microarray from Agilent that covers 27,800 CpG islands across the entire genome. Methylated segments of the genome were differentiated from unmethylated segments by adapting the methylated DNA immunoprecipitation (MeDIP) protocol which relies on the specificity of anti-5'-methylcytosine for immunoprecipitation. The methylated fragments and input DNA (without immunoprecipitation) from the same sample were differentially labeled with Cyanine-3 and Cyanine-5 respectively and competitively co-hybridized to the CpG island array. Dye ratios at individual spots on the array were mapped to their genomic location and are used to infer methylation status at that locus. This approach generates a DNA methylation map spanning nearly every CpG island in the genome.

**Results:** Importantly, we have reduced the material requirement for oligonucleotide microarray profiling of DNA methylation levels to only 250 ng of genomic DNA, without requiring subsequent sample amplification (thus eliminating this source of potential bias). Using this new technique, we showed that CpG island DNA methylation profiles can be produced reliably from airway epithelial cells using very limited amounts of DNA with high correlations between replicate experiments. We also demonstrated DNA methylation alterations that are specific to the airways of patients with NSCLC, highlighting the potential role of this technique as a new tool in screening and early detection of lung cancers.

**Conclusion:** We have demonstrated that CpG island profiling of airway epithelial cells using a modified MeDIP procedure is a powerful tool for the analysis of DNA methylation profiles. This technique can be used for large-scale genome-wide assessment of methylation changes occurring in the peripheral airways and provide a method to study lung cancer development in former smokers as well as for developing early detection biomarkers and targets for chemoprevention.

**A19 Identification of epigenetic hits can predict breast cancer risk.** John A. Fischer, Stefano Rossetti, Richard T. Cheney, Nicoletta Sacchi. Roswell Park Cancer Institute, Buffalo, NY.

RARB2 is a master tumor suppressor that mediates the growth-inhibitory action of retinoic acid (RA). Homozygosity for epigenetically silent *RARB2* alleles, which results into loss of RARB2 tumor suppressor activity, leads to RA-resistance, and apparently precedes the acquisition of morphological transformation of breast epithelial cells (Bistulfi et al., Cancer Research, 2006). *RARB2* epigenetic silencing is marked by chromatin repressive changes, including DNA methylation of the *RARB2* CpG island (Sirchia et al., Oncogene, 2000). In a mechanistic study, we demonstrated that methylation arises at a specific epicenter in the *RARB2* CpG island, which we call the *RARB2* methylation epicenter (RME) (Ren et al., MCB, 2005). Here we show that RME methylation is detectable in both benign and ductal carcinoma *in situ* within the same patient tissue sample. This finding implies sequential epigenetic silencing of *RARB2* alleles. Timely identification of the first epigenetic hit in breast tissue of women at risk of breast cancer could prevent progression to RME methylation homozygosity, the consequent loss of RARB2-mediated tumor suppressor function, RA-resistance, and morphological epithelial cell transformation.

This work was supported by the Roswell Park Alliance Foundation Award (NS), the Breast Cancer Coalition of Rochester (NS), the Susan Komen Foundation (SR).

**A20 Deacetylation of histone H3 at lysine 9 with ethanol in human colonic epithelial cells.** Hyeran Jang,<sup>1</sup> Mary P. Moyer,<sup>2</sup> Sang-Woon Choi<sup>1</sup>. <sup>1</sup>JM USDA Human Nutrition Research Center at Tufts University, Boston, MA; <sup>2</sup>INCELL Corporation LLC, San Antonio, TX.

Epidemiologic observations have implicated chronic excess ethanol consumption as a risk factor for various cancers including colon cancer. Recent epigenetic studies demonstrated that ethanol causes selective acetylation of histone H3 at lysine 9 (H3K9) in the liver, lung, spleen and testes, indicating that ethanol may inhibit histone deacetylase activity, or enhance histone acetyltransferase activity in those tissues. We therefore investigated the effect of ethanol on histone H3 modifications in colonic epithelial cells. NCM460, human colonic epithelial cells were incubated with 100mM of ethanol for 24, 48, or 72 hr and then the acetylation and methylation states of histone H3K9 were measured by immunoblot analysis using site-specific antibodies. We found that ethanol decreased acetylation of histone H3K9 in a time-dependent manner (71, 21, 17%, respectively,  $P < 0.0001$ ), while the histone deacetylase inhibitor, trichostatin A, which was used as a positive control, increased H3K9 acetylation. However, both ethanol and trichostatin A had little effect on histone H3K9 methylation. This is the first report of the colon-specific post-translational deacetylation of histone H3 with ethanol.

## Gene Regulation and Transcriptional Control

**A21 Identification of the role of Snail and Slug in radio resistance of ovarian cancer.** Sharmila A. Bapat,<sup>1</sup> Nawneet Kurrey,<sup>1</sup> Swati Jalgaonkar<sup>2</sup>. <sup>1</sup>National Centre for Cell Science, Pune, India; <sup>2</sup>Institute for Biotechnology and Bioinformatics, Pune, India.

The transcription repressors *Snail* and *Slug* play an important role in mediation of epithelial-mesenchymal transition (EMT), that is essential for cell migration during development and in epithelial cancer progression. EMT is effected at the transcriptional level through interactions of these repressors with the DNA via their zinc finger domains, and specific binding to consensus sites containing the core sequence CAGGTG in the promoters of their target genes. A large majority of currently identified targets include cell junction components that include E-Cadherin, Cytokeratin 18, Occludin, Claudin, etc. Disintegration of cell junctions brought about by these factors thus mediates cell migration. However, it is not known whether the two transcriptional repressors Snail and Slug play distinct or redundant roles in carcinogenesis; it seems likely that the two factors may regulate targets differentially. More recently, additional roles for these molecules in mediating cell survival under conditions of stress have been suggested, although the specific mechanisms underlying the same are not well elucidated. Identification of the complete repertoire of gene targets of these factors in the context of cancer thus, could be critical in understanding their contribution to cellular transformation.

Here, we describe a strategy employed by us towards such an identification of transcription factor targets. It was observed that *Snail* and *Slug* are significantly upregulated in response to  $\gamma$ -radiation in ovarian cancer cells. To explore whether this correlated with a role for the molecules in mediating cell survival, we developed a comprehensive cell system comprising of an ovarian epithelial cell line A4, Snail and Slug overexpressing A4 cell clones (termed as SNA and SLA respectively) and radioresistant AR cells. Further, using a ChIP-PCR strategy, we identified that overexpression of either Snail or Slug leads to repression of several genes including those involved in cell cycle/damage checkpoints, chromatin modifiers, cell signaling and apoptosis. A similar set of genes was also found to be repressed in the AR cells that constitutively up regulated the two transcription factors during their acquisition of the radioresistant phenotype. Integrating these results with a bioinformatics strategy, we identified the entire repertoire of putative *Snail* and *Slug* targets on a genome-wide scale. Further validation of a subset of novel targets of these factors within this database was carried out in A4, SNA, SLA and AR cells at steady-state as well as on exposure to a LD50 dose of  $\gamma$ -radiation. This further revealed the changing profiles and correlations between the repressed genes and a functional annotation of the expression patterns. In conclusion, we report that the two transcriptional repressors Snail and Slug have common as well as specific targets and, targeting of these subsets of genes seems to be important in mediating radiation resistance in ovarian cancer. Thereby, our integrative approach of bioinformatics and use of functional screens identified distinct and unique roles for these two factors in ovarian cancer progression and acquisition of a recalcitrant phenotype of the disease.

**A22 HIF-1 mediates up-regulation of urokinase-type activator receptor (uPAR) in cervical cancer cells.** Hirotaka Nishi, Keiichi Isaka. Tokyo Medical University, Tokyo, Japan.

Hypoxia occurs during development of cervical cancer and is considered to correlate with its invasion. Hypoxia induces cancer cells to have more invasive property through the urokinase plasminogen activator receptor (uPAR) expression. We sought to determine the regulator(s) of uPAR expression during hypoxia. According to the invasion assays, cervical cancer cell lines, CaSki and CA, under hypoxic condition (1% O<sub>2</sub>) showed more invasive property than those under normoxia. By western blot analysis, hypoxia enhanced the endogenous hypoxia inducible factor (HIF)-1 $\alpha$  and uPAR protein expression. uPAR mRNA level was also up-regulated by hypoxia using realtime RT-PCR. Overexpression of HIF-1 $\alpha$  which is induced by hypoxia activated the transcriptional activity of the uPAR promoter by luciferase assays. HIF-1 protein bound the putative HIF-1 response element on the uPAR promoter using the NoShift Transcription Factor Assay kit, and additional luciferase assays show that this is essential for uPAR transactivation by HIF-1. HIF-1 overexpression enhanced the endogenous uPAR expression and introduction of siRNA for HIF-1 $\alpha$  diminishes uPAR expression during hypoxia. These results indicate the up-regulation of uPAR by hypoxia in cervical cancer cells is mediated through HIF-1. Using cervical cancer cell tissues, we also demonstrated that the level of uPAR mRNA in normal cervix is significantly lower than cervical cancer. Our results provide evidence that regulation of uPAR expression by HIF-1 represents a mechanism for cervical cancer invasion during hypoxia.

**A23 Expression profile of genes altered by ester-protected hydroxybenzyl phosphates (EHBP) in T-leukemia cells.** Niharika Nath,<sup>1</sup> Ravinder Kodela,<sup>2</sup> Daniel Boring,<sup>3</sup> James A. Crowell,<sup>3</sup> Khosrow Kashfi<sup>2</sup>. <sup>1</sup>City University of New York Medical School and New York Institute of Technology, New York, NY; <sup>2</sup>City University of New York Medical School, New York, NY; <sup>3</sup>Division of Cancer Prevention, National Cancer Institute, Bethesda, MD.

**Introduction:** Acute lymphoblastic leukemia (ALL) is the primary cause of cancer-related mortality in children. Novel compounds and different therapeutic approaches are required for more effective treatments. Nitric oxide-releasing aspirin (NO-ASA), comprised of an ASA molecule covalently bound to an NO liberating moiety through an aromatic spacer was reported to inhibit Jurkat T cell growth and other cancer cell lines of colon, pancreatic and breast origin, including experimentally-induced tumors in various animal models. Recently, we and others showed that the mechanism of action of NO-ASA did not involve released NO or aspirin but that the spacer molecule joining these two entities was the biologically active component. The role of the NO-releasing moiety was simply that of a leaving group in the production of a reactive quinone methide (QM) intermediate. The ASA component apparently had a very limited or no biological contribution. On this basis, we synthesized two compounds as models in which the NO-releasing group was replaced with a substituted phosphate (Agent 1: No NO, NO-ASA); and one in which the aspirin component was replaced with an acetyl group and the -ONO<sub>2</sub> leaving group was replaced by a substituted phosphate group (Agent 2: No NO, No-aspirin, NO-ASA). In this study we investigated whether these three compounds may alter the gene expression of biological signaling pathways that are potential targets in carcinogenesis, and to elucidate the role of the NO-releasing moiety and the aspirin component of NO-ASA in the differential expression of these genes.

**Methods:** Agents: NO-ASA (2-(acetyloxy)-4-[(nitrooxy)methyl] phenyl ester, Agent 2: (2-(acetyloxy)-4-[(diethylphosphate)methyl] phenyl ester, Agent 3: (4-acetyloxybenzyl diethylphosphate) were synthesized, purified and NMR verified by us. Cell line: Jurkat T-acute lymphoblastic leukemia; the IC<sub>50</sub> for cell growth inhibition for the three compounds was determined by an MTT assay. Following treatment of the Jurkat cells with

NO-ASA, agent 2 or agent 3, at their respective IC50s, mRNA was isolated and labeled according to standard protocols and kits. The oligo arrays set from SuperArray were used. The blots were hybridized and the ratios of the expression levels quantified and calculated. Potential candidate genes that were differentially expressed were validated by real time RT-PCR.

**Results:** All three drugs were potent growth inhibitors of human leukemia Jurkat T cells. The genes that were prominently induced by all three drugs included FMO4, HSPA1A, HSPA6, HSPH1, FOS, CYP24 A1, DDIT3; this induction occurred in a concentration dependent manner. These may provide mechanistic insights into the growth inhibition observed with these agents. Other moderately altered genes included HYOU1, SOD1, SOD2. Interestingly, all 3 agents had the same effect in terms of fold-changes in gene expression for many of these candidate genes. This strongly suggests that the NO-releasing group and the aspirin component of NO-ASA have a negligible role in modulating these gene expressions. We propose that the growth inhibitory properties of all three agents are associated with the spacer component of the molecules which generates a quinone methide intermediate. The significance of the genes induced or repressed and the relationship of their gene expression with the cell growth inhibition will be discussed.

**A24 Endothelial cell aging and possible role in angioprevention: Increased expression of TGF $\beta$  and other antiangiogenic factors.** Adriana Albini,<sup>1</sup> Luca Generoso,<sup>1</sup> Valentina Mirisola,<sup>2</sup> Francesco Romeo,<sup>2</sup> Ulrich Pfeffer<sup>2</sup>. <sup>1</sup>MultiMedica, Milan, Italy; <sup>2</sup>National Institute for Cancer Research, Genoa, Italy.

Alterations of endothelium are associated with increased risk in cardiovascular diseases; they are also involved in tumor angiogenesis, one of the main "rate limiting step" in tumor progression and spreading. For this reason in the last years several agents have been patented for the cancer therapy thanks to their ability to inhibit tumor angiogenesis and now angiogenesis is considered also target prevention.

Since senescence is considered an "anti-cancer" phenomenon we are evaluating the molecular changes that occur during aging in endothelial cells. We conducted this study on endothelial cells derived from Human Umbilical Vein (HUV $\beta$ Cs) of early and late passages, from both masculin and feminin origin and we have analyzed the expression profile at different stages of in culture aging.

Several genes are regulated during this process; among these there is a significant reduction of the expression of genes such as *E-selectin* and *CCL2* from cells of both male or female origin. This reduction could correlate with a decrease of the ability of endothelial cells to recruit inflammatory cells, and may have implications for metastatic spread of cancer. *Angiopoietin 2 (ANG2)* also seem to decrease during the endothelial cell senescence; this could be related to a potential impairment of the capacity to undergo remodeling in "aged" vessels. Interestingly, all three genes show a 2-fold higher expression in females as compared to males and the rate of decrease is different although the trend is the same. These data could suggest that there may be important gender-specific differences in gene expression of endothelial cells, and that these may have an effect on the senescence process.

Finally we have evidence that *TGF $\beta$ 1*, a molecule related to *TGF $\beta$*  induction, is increased with passages in culture and is very high in aged endothelial cells. Probably it could clarify the pro-apoptotic and antiangiogenic activity of *TGF $\beta$* .

Taken together, these data confirm that physiological endothelial cell senescence could be associated with a reduction in anti-angiogenic potential; this property suggests that some tumors could be less angiogenic in the elderly.

## Inflammation and Cancer Initiation and Promotion

**PR-11 Snail-induced and EMT-mediated early lung cancer development: Promotion of invasion and expansion of stem cell populations.** Tonya C. Walsler,<sup>1</sup> Jane Yanagawa,<sup>1</sup> Jie Luo,<sup>1</sup> Ming Liu,<sup>1</sup> Lee Goodglick,<sup>1</sup> Long-Sheng Hong,<sup>1</sup> Michael C. Fishbein,<sup>1</sup> Jerry W. Shay,<sup>2</sup> John D. Minna,<sup>2</sup> Steven M. Dubinett<sup>1</sup>. <sup>1</sup>David Geffen School of Medicine at UCLA, Los Angeles, CA; <sup>2</sup>University of Texas Southwestern Medical Center, Dallas, TX.

This abstract is being presented as a short talk in Concurrent Session 12. A full abstract is printed in the Proffered Papers: Oral Presentation Abstracts section of the conference proceedings. (Presented on board number A25)

**A26 IL-1 $\beta$  dependent epithelial-mesenchymal transition in non-small cell lung cancer.** Eileen L. Heinrich, Burt Charuworn, Miriam Dohadwala, Steven M. Dubinett. UCLA, Los Angeles, CA.

Pulmonary diseases associated with a heightened risk of lung cancer, such as emphysema and pulmonary fibrosis, show both increased and deregulated inflammation. Tobacco smoke exposure is associated with chronic airway inflammation and is a strong risk factor for the development of lung cancer. Inflammatory mediators and inflammatory cells are over expressed in the pulmonary microenvironment of both smokers and patients with non-small cell lung cancer (NSCLC). Expression of interleukin 1 $\beta$  (IL-1 $\beta$ ) is increased in a number of cancers, including lung cancer and is associated with tumor aggressiveness and poor patient outcomes. A chronic increase in inflammatory mediators in the lung tumor microenvironment can lead to increased tumor promotion, invasion, angiogenesis and metastasis; however the mechanism by which this occurs has yet to be elucidated.

We have found that IL-1 $\beta$  exposure leads to the down regulation of E-cadherin in NSCLC cell lines. Loss of E-cadherin is a hallmark of epithelial-mesenchymal transition (EMT) in which epithelial cells lose cell-cell junctions, undergo cytoskeletal reorganization and basement membrane degradation and switch from cadherin to integrin mediated adhesion. This transformation results in cells with a mesenchymal phenotype. An important feature of mesenchymal cells is their ability to migrate independently and invade locally - prerequisites for metastasis. Following IL-1 $\beta$  exposure we see, along with the loss of E-cadherin, changes in cellular morphology, down regulation of cytokeratin 18 and an up regulation of mesenchymal markers, particularly N-cadherin, vimentin and SNAI2 (Slug) - a transcriptional repressor of E-cadherin. Slug expression is increased at both the mRNA and protein levels and is found via chromatin immunoprecipitation to be bound to the E-cadherin promoter following IL-1 $\beta$  exposure. Further, in NSCLC cell lines which do not express E-cadherin, Slug expression is elevated. Blocking Slug expression using siRNA inhibits IL-1 $\beta$  mediated down regulation of E-cadherin.

IL-1 $\beta$  has many well-characterized effects on signaling pathways and protein expression, including the up regulation of COX-2 which has been shown previously to decrease E-cadherin expression. However, the IL-1 $\beta$  mediated effects on E-cadherin expression are COX-2 independent. Blocking COX-2 activity with celecoxib does not abrogate the E-cadherin down regulation seen here. Additionally, others have found that loss of E-cadherin is associated with resistance to epidermal growth factor receptor tyrosine kinase inhibitors which are currently in use as treatment and have been suggested as prevention agents for NSCLC. Thus, the presence of inflammatory mediators in the pulmonary microenvironment may play a specific role in promoting resistance to targeted prevention and therapy.

## Oncogenes/Tumor Suppressor Genes

## Oncogenes/Tumor Suppressor Genes

**A27 Contribution of DNA methylation in development of lung cancer in former smokers.** Emily A. Vucic, Ian M. Wilson, Bradley P. Coe, Raj Chari, Stephen Lam, Wan L. Lam. British Columbia Cancer Research Centre, Vancouver, British Columbia, Canada.

**Background:** Lung cancer is the most common cause of cancer death worldwide with more than 1.2 million people dying of the disease each year. Half of newly diagnosed lung cancer patients are former smokers. Understanding why former smokers develop lung cancer is clearly important to the development of early detection, prevention and treatment strategies for these people. The effects of cigarette smoke on the epigenome are widespread as both global DNA methylation and local DNA methylation have been identified, associated with genomic instability and tumor suppressor gene (TSG) silencing, respectively. In a cancer-specific context, upregulation of oncogenes and silencing of tumor suppressor genes both occur as a result of tobacco smoke exposure. Therefore, molecular studies examining tumors at genomic and epigenomic levels will likely identify causal genetic events involved in cancer development in former smokers.

**Objective:** The objective of this study is to determine the contribution of DNA methylation as a mechanism to lung cancer development in former smokers.

**Hypothesis:** As smoking induces methylation changes in bronchioepithelial cells, we hypothesize that this constitutes the first hit to TSG inactivation. In order to inactivate both alleles, these methylation changes would be maintained in tumors where a second hit would be found at the same gene loci.

**Materials and Methods:** Epithelial cells from former smokers (those with >10 years of smoking cessation) were collected from peripheral airways during routine bronchoscopy. Half of the cells were fixed in Cytolyt and the other half in RNA later for DNA and RNA extraction, respectively. Copy number profiling was performed by array comparative genomic hybridization (aCGH) using whole-genome tiling path SMRT v2 BAC array. Methylation analysis was performed by coupling affinity based enrichment of methylated sequences with hybridization to the same aCGH platform described above. Expression status of genes was determined by gene expression microarray analysis using Agilent 44K expression arrays. **RESULTS:** Preliminary MeDIP aCGH of bronchial brush cells from eight former smokers who had previous surgical removal of Stage I NSCLC, and eight former smokers without NSCLC, of similar age (68±7 versus 62±6), revealed distinct differences in frequency of DNA methylation between cancer and non-cancer groups. For example, at 11p13 the cancer group shows hypermethylation at the *WT1* locus, a known TSG. Analysis in 62 NSCLC tumors for gene dosage, showed a high frequency of loss at the *WT1* locus. To examine downstream effects of these events in tumors, expression of *WT1* was assessed and found to be significantly underexpressed in the majority of NSCLC tumors compared to a normal lung reference. Collection of more samples and further integrative analysis is currently underway.

**Conclusion:** Differences in methylation between these two groups may explain why some former smokers develop cancer while others remain cancer free despite similar lifestyle changes. As methylation is a reversible DNA modification, this knowledge would prompt the development and application of DNA demethylation chemopreventative agents and unique therapeutic strategies.

**A28 Loss of inhibitory IRS-1 phosphorylation is an early event in mTOR-dependent endometrial hyperplasia and carcinoma.**

Adrienne S. McCampbell,<sup>1</sup> Heather A. Harris,<sup>2</sup> Judy S. Crabtree,<sup>2</sup> Richard C. Winneker,<sup>2</sup> Cheryl L. Walker,<sup>3</sup> Russell R. Broadus<sup>1</sup>. <sup>1</sup>University of Texas M.D. Anderson Cancer Center, Department of Pathology, Houston, TX; <sup>2</sup>Wyeth Research, Endocrinology and Reproductive Division, Women's Health and Musculoskeletal Biology, Collegeville, PA; <sup>3</sup>University of Texas M.D. Anderson Cancer Center, Department of Carcinogenesis, Science Park Research Division, Smithville, TX.

Activation of IGF-I receptor signaling contributes to the development of endometrioid-type endometrial carcinoma in humans and in rodent models. This signaling pathway is under both positive and negative regulation, including S6K phosphorylation of IRS-1 at S636/639, which occurs downstream of mTOR activation to inhibit this adapter protein. We observed activation of mTOR signaling with a high frequency in human endometrial hyperplasia and carcinoma, but an absence of IRS1 phosphorylation at S636/639, despite the high levels of activated S6K in these tumors. To explore when during disease progression mTOR activation and loss of inhibitory IRS-1 phosphorylation occurred, we utilized the Eker rat (*Tsc2<sup>EK/+</sup>*) model for this disease, where endometrial hyperplasia develops as a result of loss of *Tsc2*, the "gatekeeper" for mTOR signaling. We observed mTOR activation early in progression in hyperplasias and in some histologically normal appearing epithelial cells, suggesting that event(s) in addition to loss of *Tsc2* were required for progression to hyperplasia. In contrast, IRS-1 phosphorylation at S636/639 was observed in normal appearing epithelium, but was absent from all hyperplasias, indicating loss of IRS-1 inhibition by S6K occurred during progression to hyperplasia. Furthermore, treatment of Eker rats with an mTOR inhibitor resulted in a decreased incidence of endometrial hyperplasia [54/79 hyperplasias/uterine cross section in vehicle controls to 6/90 in treated rats ( $p < 0.001$ )] and decreased the proliferative index of hyperplastic endometrial epithelial cells by >95% ( $p < 0.05$ ). Since progression from normal epithelium to endometrial carcinoma proceeds via the intermediate step of endometrial hyperplasia, these data suggest a progression sequence where activation of mTOR is followed by loss of negative feedback to IRS-1 during the initial stages of development of this disease.

**A30 MicroRNA-27a functions as an oncogene in gastric adenocarcinoma by targeting prohibitin.** Hua Tang, Tao Liu, Yuanyuan Lang, Min Liu, Yixuan Li, Xin Li. Tianjin Medical University, Tianjin, China.

MicroRNAs (miRNAs) may function as oncogenes or tumor suppressors. Here, we show that miR-27a is up-regulated in human gastric adenocarcinoma. Suppression of miR-27a inhibits gastric cancer cell growth. Subsequently, prohibitin is identified as a potential miR-27a target, combining bioinformatics and microarray analysis. EGFP report experiment also confirms that the 3' untranslated region (3' UTR) of prohibitin carries the directly binding site of miR-27a. After knockdown of miR-27a in gastric cancer cells, mRNA level and protein level of prohibitin are both elevated. Down-regulation of prohibitin by miR-27a may explain why suppression of miR-27a can inhibit gastric cancer cell growth, further supporting that miR-27a functions as an oncogene.

**Chemoprevention and Biological Therapies 1****Mechanisms of Chemoprevention**

**A31 Intestinal resistance to celecoxib in Min/+ mice involves ECM changes and an expanded population of TGF $\beta$ -expressing myofibroblasts.** Jennifer S. Davids, Adelaide Carothers, Beatrice Damas, Monica M. Bertagnolli. Brigham and Women's Hospital, Boston, MA.

The COX-2 inhibitor, celecoxib, inhibits prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) production and intestinal adenoma formation in mice and in humans. Previously, we showed that treatment of Min/+ mice with dietary celecoxib for 3 weeks reduced PGE<sub>2</sub> and induced tumor regression, whereas treatment for 5 months resulted in increased PGE<sub>2</sub> and tumor growth (Carothers et al. *Cancer Res.* 2006; 66: 6432-8). In the small intestine, cross-talk between epithelial and stromal cells occurs across the extracellular matrix (ECM). Heparan sulfate proteoglycans (HSPGs) within the ECM sequester and modulate the activities of growth factors and latent TGF $\beta$  ligands. TGF $\beta$  stimulates differentiation of fibroblasts into PGE<sub>2</sub>-producing myofibroblasts. These stromal cells play important roles in intestinal tumorigenesis and in the chemopreventive effect of nonsteroidal anti-inflammatory drugs. We tested the hypothesis that celecoxib resistance in the intestine of Min/+ mice involves ECM regulation of HSPGs and TGF $\beta$  signaling in epithelial and stromal cells. Using immunohistochemistry (IHC), we compared the non-tumor mucosa and adenomas of untreated Min/+ controls with Min/+ mice treated with celecoxib for 3 weeks and for 5 months. We performed IHC for the HSPG membrane protein syndecan-1, cytokines TGF $\beta$ 1, 2, 3 (TGF $\beta$ ), and the nuclear downstream TGF $\beta$  effector, Smad4. IHC was also performed to characterize subepithelial stromal cells using F4/80, a macrophage-specific marker, as well as  $\alpha$ -smooth muscle actin (SMA) and vimentin, myofibroblast-specific markers. In comparison to untreated Min/+ small intestine, celecoxib treatment for 3 weeks increased membrane-localized syndecan-1, TGF $\beta$ , and nuclear Smad4 in crypt and villus enterocytes. Under these conditions, few SMA+ and vimentin+ myofibroblasts or F4/80+ macrophages were present in the pericryptal submucosa and lamina propria. Thus, brief treatment may sequester latent TGF $\beta$  and growth factors in the ECM, blocking their downstream signaling. A reciprocal effect was produced by 5 month treatment, in which there was markedly reduced expression of membrane-localized syndecan-1, TGF $\beta$ , and nuclear Smad4 in the crypt and villus enterocytes. There was also a dramatic increase in the number of myofibroblasts in the lamina propria and pericryptal submucosa ( $P < .01$ ). Thus, celecoxib resistance was associated with TGF $\beta$ -induced differentiation of fibroblasts into PGE<sub>2</sub>-producing myofibroblasts. These results suggest that the duration of celecoxib treatment affects the cross-talk between epithelial and stromal cells through the ECM, providing a novel mechanism for celecoxib resistance. Moreover, elevated numbers of myofibroblasts in the human colon may be predictive of chemoprevention failure.

**A32 Proteomic signatures of deoxycholic acid and ursodeoxycholic acid treated human colon cancer cells.** Masako Nakanishi, Antoine Menoret, Anthony T. Vella, Daniel W. Rosenberg. University of Connecticut Health Center, Farmington, CT.

Dietary factors play an important role in the pathogenesis of colorectal cancer (CRC), due in part to the influence of the bile acids. While the secondary bile acid, deoxycholic acid (DCA), has been associated with tumor promotion, one tertiary bile acid in particular, ursodeoxycholic acid (UDCA), has been demonstrated to exert chemopreventive properties in the colon. Although its efficacy against high-grade adenomas has already been demonstrated in phase III clinical trials, mechanisms by which UDCA suppresses colon tumorigenesis remain unclear. In order to gain further insight into the tumor modifying properties of the bile acids, we compared the effects of DCA and UDCA on human colon tumor cells (CaCo-2) using

proteomic analyses. In the following study, CaCo-2 cells grown at 70% confluence were treated with either 10  $\mu$ M deoxycholic acid (DCA) or 1 mM ursodeoxycholic acid (UDCA) for 72 hours. For the proteomic analysis, protein was first fractionated by PF2D isochromatofocusing on a Beckman Coulter ProteomeLab PF2D platform. Fractions corresponding to a linear gradient between pH 8.0 and 4.0 were collected and processed through an automated autoloader for further separation by HPRP-PF2D reverse phase chromatography. Two dimensional protein expression maps displaying isoelectric point (pI) versus hydrophobicity were generated by the ProteoView/DeltaVue software package. Pairwise analysis of these complex protein chromatograms demonstrated common peak profiles (signatures) as well as unique peaks (fingerprints) characteristic of UDCA or DCA treatment. One of these protein peaks that exhibited a differential expression pattern between the two drug treatments was identified by MALDI and tandem mass spectrometry sequencing as heat-shock protein 60 (Hsp60). Furthermore, this protein was found to be less oxidized upon treatment with UDCA, suggesting the attenuation of oxidative stress present in cancer cells. Further analyses on the role of Hsp60 as well as resultant diminished oxidative stress will enable the discovery of potential mechanisms by which DCA and UDCA control cellular pathways.

**A33 Superior efficacy of dietary 3,3'-diindolylmethane (DIM) amongst several indoles to inhibit prostate cancer growth via induction of the p75NTR tumor suppressor protein.** Fatima Khwaja,<sup>1</sup> Isadora Posey,<sup>2</sup> Xueqing Song,<sup>2</sup> Shehla Wynne,<sup>1</sup> Daniel Djakiew<sup>1</sup>.

<sup>1</sup>Georgetown University School of Medicine, Washington, DC; <sup>2</sup>University of the District of Columbia, Washington, DC.

A comparison of the indoles etodolac, indomethacin, 5-methylindole-3-acetic acid, indole-3-carbinol, 3,3'-diindolylmethane (DIM) and the related ketorolac molecule showed superior efficacy of DIM, a dietary indole, to induce expression of the p75NTR tumor suppressor and inhibit survival of the PC-3 prostate cancer cell line. Each indole that induced p75NTR expression exhibited the same rank-order for inhibition of prostate cell survival. Comparison of the activity of DIM, the most potent of the indoles, between several cell lines, showed that DIM induced p75NTR at low concentrations in prostate cells (PC-3, DU-145), followed by bladder (T24), with breast (MCF-7) and fibroblast cells (3T3) being the least responsive to DIM. DIM induction of p75NTR levels in the various cell lines occurred in the same rank-order as inhibition of cell survival. Since the consumption of cruciferous vegetables has been reported to reduce the risk of prostate cancer through the release of indole-3-carbinol (I3C) which is subsequently metabolized in the acidic environment of the stomach to DIM, the superior activity of DIM to induce expression of the p75NTR tumor suppressor protein and inhibit cell survival suggests a mechanism of action for the anticancer activity of DIM in the prevention of prostate cancer. In order to demonstrate a cause and effect between DIM and p75NTR inhibition of survival, we used a dominant negative of p75NTR to rescue DIM induced loss of cell survival. In order to investigate the mechanism of action by which p75NTR transduces a biochemical signaling cascade leading to cell death of prostate cancer cells we utilized knockdown with p38 MAPK siRNA to rescue DIM induction of p75NTR thereby implicating p38 MAPK in DIM induction of cell death. Moreover, DIM treatment induced phosphorylation of p38 MAPK within one minute, suggesting the target of DIM is proximal to p38 MAPK. Hence, these results suggest that the anti-cancer activity of cruciferous vegetables may be mediated in part by DIM and that the mechanism of action occurs, in part, by activation of the p38 MAPK pathway leading to expression of the p75NTR death receptor with subsequent apoptosis of prostate cancer cells.

## Mechanisms of Chemoprevention

**A34 Curcumin downregulates IL-8 in ER negative DCIS breast cancer model: Correlation between HER-2-induced inflammation and tumorigenicity.** Tilda Barliya, Kori Gall-Troselj, Carl Crawford, Meena Katdare. Carcinogenesis and Chemoprevention Lab, Department of Surgery Weill Cornell Medical College, New York, NY.

**Introduction:** Overexpression of HER-2 oncogene is reported in more than 60% of ductal carcinoma *in situ* (DCIS) breast cancer, including 30% estrogen receptor negative chemotherapy resistant high-grade tumors with metastatic phenotype. Recent studies show high correlation between MRI characteristics and lower ER score, higher grade, much higher CD68 count (inflammatory marker) and extensive comedo necrosis of DCIS. The inflammatory cytokine IL-8 is found to be overexpressed in most ER- breast cancers and is recently suggested as a marker of tumor invasion and metastasis. Curcumin (CUR) the active component of the spice turmeric, known for its immunomodulatory, antimutagenic and antitumorigenic properties and was found to inhibit cell proliferation and mitogenic signal transduction pathways in ER negative, HER-2 positive DCIS breast cancer model.

**Material and Methods:** Normal 184-B5 breast epithelial cells and transformed 184-B5/HER cells in log phase were exposed to 0, 5, 10 and 20 M CUR for 24 and 48 hours and analyzed for dose response, cell viability, cell cycle distribution, gene expression and HER-2 signal transduction protein markers.

**Results:** 184-B5/HER cells exhibit hyper-proliferation, shorter population doubling time, higher saturation density, reduced rate of apoptosis, higher anchorage independent growth and tumorigenicity compared to the non-tumorigenic ER-, PR-, HER2-, EGFR+ and p53+ parental immortalized 184-B5 cells. Cell cycle analysis of 184-B5/HER cells exposed to 20 M CUR showed cytostatic arrest of growth, 85% inhibition of colony formation, decreased  $G_0/G_1$ :  $S+G_2/M$  ratio and increased apoptosis. Microarray data showed 557 probes to be modulated in 20 M CUR (24 hrs) treated 184-B5/HER cells (117 upregulated, 380 downregulated). These include reduced expression of cell cycle regulatory genes such as cyclin A (-2.79), cyclin B2 (-3.89), cyclin D1 (-1.50), cdk2 (-1.69) and Cdc25c (-3.80). Decreased levels of Cyclin B2, cdk2 and Cdc25c cell cycle proteins in 184-B5/HER cells, reflect CUR induced cell cycle arrest in S and G2/M phases. Treatment with CUR decreased NF $\kappa$ B (-1.86) in 184-B5/HER cells suggesting modulation of downstream signaling pathways including the inflammatory cascade. We observed a 10 fold higher levels of interleukin-8 (IL-8) gene expression in 184-B5/HER cells compared to 184-B5 cells, while treatment with CUR dramatically reduced the IL-8 gene expression as revealed by both microarray and real-time PCR.

**Conclusion:** Treatment of breast epithelial 184-B5/HER cells with CUR show preventive efficacy in this ER negative DCIS model via cytostatic arrest in S and G2/M phases of the cell cycle, by downregulation of ERK pathway and induction of apoptosis, verified at both the gene and protein levels. Moreover, CUR down-regulates key regulatory proteins such as NF $\kappa$ B and IL-8 expression thus preventing the inflammatory cascade and possible progression of DCIS into invasive ductal carcinoma. Reduced expression of metastatic molecular marker IL-8 by CUR in 184-B5/HER cells suggests its preventive/therapeutic potential.

Support: NIH-NCI CA122394.

**A35 JS-K, a nitric oxide-donating prodrug, inhibits the growth of leukemic jurkat cells and modulates  $\beta$ -catenin/TCF signaling.**

Niharika Nath,<sup>1</sup> Mitali Chattopadhyay,<sup>2</sup> Liliya Pospishil,<sup>2</sup> Lucyna Z. Cieciora,<sup>2</sup> Joseph E. Saavedra,<sup>3</sup> Larry K. Keefer,<sup>4</sup> Khosrow Kashfi.<sup>2</sup> <sup>1</sup>City University of New York Medical School and New York Institute of Technology, New York, NY; <sup>2</sup>City University of New York Medical School, New York, NY; <sup>3</sup>Basic Research Program National Cancer Institute at Frederick, SAIC-Frederick, MD; <sup>4</sup>Laboratory of Comparative Carcinogenesis, National Cancer Institute at Frederick, Frederick, MD.

**Introduction:** Leukemia is the most common form of cancer in children and acute lymphoblastic leukemia (ALL) is the primary cause of cancer-

related mortality. The deregulation of the Wnt signaling cascade and its components has been implicated in T-cell ALL as well as in B-cell chronic lymphocytic leukemia (CLL). The protein  $\beta$ -catenin is a central player of the Wnt signaling pathway that regulates cell-cell adhesion and may promote leukemia cell proliferation. The stabilization and accumulation of  $\beta$ -catenin have a powerful regulatory role in proliferation and differentiation.  $\beta$ -catenin is expressed in T-ALL cells, tumor lines of hematopoietic origin and primary leukemia cells but is undetectable in normal peripheral blood T cells. Among the leukemic cell lines,  $\beta$ -catenin is expressed in high levels in Jurkat T-cells. Currently, the role of  $\beta$ -catenin and its regulation in non-adherent cells are not very clear. JS-K [O2-(2,4-dinitrophenyl)1-[(4-ethoxycarbonyl)piperazin-1-yl]diazene-1-ium-1,2-diolate] is a prodrug of the diazeniumdiolate class that releases NO through a reaction catalyzed by GST utilizing glutathione. This agent has been shown to inhibit the growth of various cancer cell lines, and in HL-60 cells (leukemia cell line) it induced apoptosis, and oxidative stress through the induction of the intrinsic pathway. However, the effect of this agent on the Wnt/  $\beta$ -catenin pathway is essentially unknown. This report highlights the effects of JSK on the growth properties of human Jurkat T-acute lymphoblastic leukemia cells and on the  $\beta$ -catenin signaling pathway.

**Methods:** JS-K: synthesized by us. Cell cycle phase distribution: flow cytometry; apoptosis: subdiploid (sub-G0/G1) peak in DNA content histograms. Transient transfection: 400 ng of luciferase reporter constructs TOPflash or FOPflash and 200 ng of pSV-  $\beta$ gal vector as internal control. Luciferase activity: per manufacturer's instructions;  $\beta$ -galactosidase activity: standard protocols.  $\beta$ -catenin protein levels: immunoblotting.

**Results:** JS-K inhibited the growth of Jurkat cells, IC50s of  $14 \pm 2 \mu$ M and  $9 \pm 1 \mu$ M at 24 h and 48 hr, respectively. This effect was due, in part, to a dose-dependent induction of apoptosis as observed by flow cytometry of annexin V stained JS-K treated cells. Jurkat cells constitutively express high levels of  $\beta$ -catenin, which translocates to the nucleus, interacting with TCF-4, modulating transcription of specific genes. In the cell line SW480, which also expresses high levels of  $\beta$ -catenin and is an adherent cell line of colorectal cancer type that transfects with higher efficiency than Jurkat cells, we found that JS-K dose-dependently inhibited  $\beta$ -catenin/TCF-4 signaling by reporter assays. In Jurkat cells, JS-K reduced  $\beta$ -catenin levels in the nucleus and cytoplasm in a dose-dependent manner. JS-K, dose-dependently altered the distribution of the cells in the cell cycle, causing an arrest in G0/G1.

**Conclusions:** These findings establish a strong inhibitory effect of JS-K in human Jurkat T-acute lymphoblastic leukemia cells, an effect that is modulated through inhibition of Wnt/  $\beta$ -catenin/TCF-4 signaling and through induction of apoptosis. Our findings underscore the potential therapeutic application of JS-K to leukemias.

**A36 Combinatorial attack on radiation-induced neoplastic transformation by phenylbutyrate: Involvement of Hsp90 molecular chaperone and histone deacetylase.** Alexandra C. Miller. Uniformed Services University/Armed Forces Radiobiology Research Institute, Bethesda, MD.

Phenyl fatty acids like phenylacetate (PA) and phenylbutyrate (PB) have shown anti-cancer efficacy in clinical trials and in both *in vitro* and *in vivo* models. The mechanism of action of these agents is not fully understood however. *In vitro* studies have demonstrated that PB treatment induces apoptosis and growth rate effects in a time- and dose- dependent manner in the p21ras-expressing hematopoietic cells, Ras-DC and the human chronic myeloid leukemia blast crisis cells, (CML-BC) K562 cells. Both leukemia cell types are characterized by deregulated activity of the oncoprotein tyrosine kinase known as Bcr-Abl frequently observed in radiation-associated leukemias. Molecular studies are underway to characterize the effect of PB on Bcr-Abl expression in these leukemia cells since Bcr-Abl is a well known client protein for Hsp90. Furthermore, gene expression profile analysis of PB treatment of leukemia cells and human

osteoblast cells (HOS) demonstrated that PB down-regulated Hsp90 suggesting that PB may affect molecular chaperoning. The anti-leukemic activity of PB was investigated using the cobalt radiation-induced neoplastic transformation model, HOS cells. HOS cells were exposed to a total of 2 Gy ( $^{60}\text{Co}$ , 50 cGy/exposure) and transformed foci were formed within 35 days. Cellular incubation with PB either pre- or post-radiation significantly suppressed the radiation-induced transformation. Molecular studies also showed that PB induced acetylation of histones H3 and H4 and apoptosis in this cell model. A radiation leukemia rodent model is currently being employed to evaluate PB's anti-leukemia efficacy. Although preliminary, these data suggest that PB involvement in both Hsp90 molecular chaperoning and inhibition of histone deacetylase is mechanistically related to its anti-leukemia efficacy.

**A37 Modulation of *GSTP1* and *NQO1* by plant-derived chemopreventive agents in human lung normal, immortalized, and malignant bronchial cells.** Xiang-Lin Tan<sup>1,2</sup> Shengli Xiong<sup>1,2</sup> Miao Shi<sup>1</sup>, Weiguo Han<sup>1,2</sup> Simon D. Spivack<sup>1,2,3,4,5</sup> <sup>1</sup>Division of Pulmonary Medicine, Albert Einstein College of Medicine, Bronx, NY; <sup>2</sup>Laboratory of Human Toxicology and Molecular Epidemiology, Wadsworth Center, New York State Department of Health, Albany, NY; <sup>3</sup>Environmental Health Sciences, School of Public Health, University at Albany, Albany, NY; <sup>4</sup>Department of Epidemiology, Albert Einstein College of Medicine, Bronx, NY; <sup>5</sup>Department of Genetics, Albert Einstein College of Medicine, Bronx, NY.

Many phytochemicals possess putative cancer-preventive properties. However, none have yet demonstrated clear benefit for lung cancer prevention in clinical trials. In this study, we applied an *in vitro* system of assay to systematically investigate the influence of putative anti-mutagen, phase II metabolism inducing naturally-occurring mixtures and single compounds, including green tea extracts (GTE), broccoli sprout ITC extracts (ITCs), epigallocatechin gallate (EGCG), sulforaphane (SFN), phenethyl isothiocyanate (PEITC) and benzyl isothiocyanate (BITC), for phase II enzymes *GSTP1* and *NQO1* in the mRNA and protein level. Primary normal human bronchial epithelial cells (NHBE), immortalized human bronchial epithelial cells (HBEC), and overtly malignant lung adenocarcinoma cells (A549) were incubated with 0.5, 1.0, 5.0  $\mu\text{g/ml}$  mixture or 0.5, 1.0, 2.0  $\mu\text{M}$  single index compounds for 24h, 48h and 7 days, respectively. The proliferation of normal and malignant cells determined by MTT assay was significantly reduced by all studied agents, with relative activities of BITC > PEITC > ITCs > SFN > EGCG > GTE. We then determined the mRNA expression by RNA-specific quantitative RT-PCR previously developed in the laboratory, and the protein expression by conventional Western blot. Overall, the mixtures GTE and ITC increased *GSTP1* and *NQO1* mRNA levels up to two-fold (1 $\mu\text{g/ml}$ ) to four-fold (5  $\mu\text{g/ml}$ ) increased. However, these mRNA levels were decreased by single compounds PEITC and BITC, in a time and dose dependent manner. EGCG and SFN did not show a consistent effect on the mRNA expression of both *GSTP1* and *NQO1* genes, although SFN showed two- to four-fold induction activity for *NQO1* mRNA expression at 0.5-1.0  $\mu\text{M}$ , uniquely for NHBE. The results were largely confirmed at the protein level, although there was some mRNA-protein discordance. Our results suggest that further *in vitro* and *in vivo* screening studies are needed to discover new, more potent phase II-inducing chemopreventive agents for lung cancer, along with mechanistic studies of gene regulation for these key upstream anti-carcinogenesis genes. Such studies are in process in the laboratory.

**A38 Structure-activity relationship of closely-related flavonoids with possible chemopreventive qualities.** Petra A. Tsuji,<sup>1</sup> Katherine K. Stephenson,<sup>2</sup> Kristina L. Wade,<sup>2</sup> Hua Liu,<sup>2</sup> Jed W. Fahey.<sup>1</sup> <sup>1</sup>National Cancer Institute, Rockville, MD; <sup>2</sup>Johns Hopkins University, Baltimore, MD.

Dietary flavonoids are now widely presumed to have human health benefits, and increased intake is epidemiologically correlated with decreased risk of cardiovascular disease and cancer. The general

mechanisms thought to be involved in chemoprevention through dietary compounds include the inhibition of the carcinogen-bioactivating phase I enzymes and/or induction of carcinogen-bioinactivating phase II enzymes. Methoxylated flavonoids have been shown to have chemopreventive activity in a number of *in vitro* assays, and we have selected 27 of them as the objects of this work. Furthermore, these flavonoids were analyzed to investigate possible structure-activity relationships for effective, non-toxic agents with chemopreventive potential.

The flavonoids are a structurally diverse class of low molecular weight polyphenolic benzo-c-pyrone derivatives. The basic structure is composed of two benzene rings (A and B) separated by an oxygen-containing heterocyclic ring. Substituent groups can attach on each of the molecule's rings, with hydroxyl, methoxy groups or sugar moieties being present most frequently. Neither hydroxylation nor methoxylation solely on the B-ring had a strong inducing effect on the phase II enzyme NAD(P)H:quinone-reductase (*NQO1*) in murine hepatoma cells. However, flavonoids with a methoxy-group substitution at the 5-position of the A-ring were the most potent inducers of *NQO1*. Other flavonoids were equally potent inducers, but exhibited much higher toxicity in at least one of the cell lines tested. Certain methoxylated flavonoids that are found in dietary fruits and vegetables have significant potential for prevention of chemically-induced carcinogenesis and should be investigated further in animal models of cancer prevention.

**A39 Dosage-dependent regulation of caspase 8 activation in LNCaP and DU-145 human prostate cancer cell lines by the Chinese medicinal herb *Scutellaria barbata*.** Dinh L. Q. Nguyen, Rachel R. Devadhason, Brian Y. Y. Wong. Pacific Union College, Angwin, CA.

*Scutellaria barbata* (SB) (known in traditional Chinese medicine as Ban-Zhi-Lian) is a perennial herb. It has been used to treat appendicitis, hepatitis, pulmonary abscess, and several cancers. LNCaP cells are derived from a human prostate cancer cell line with mutated androgen receptors (AR) while DU-145 is a classical human prostate cell line without androgen receptors. Our previous data indicated that SB activated apoptosis in LNCaP cells via caspases 3, 8, and 9. Histopathological data revealed that the herb had the ability to delay tumor progression and development in TRAMP mice. Recent Western blot data from LNCaP cells undergoing treatment with SB showed increased expression of Akt protein while decreased phosphorylation of Akt in the cells. In this study, we compared the effect of AR-dependence on the herb's apoptosis activation through caspase 8 on these two different cell lines. The two cell lines were incubated with distilled water as a control group and 100  $\mu\text{L}$  and 200 $\mu\text{L}$  of SB as experimental groups for 2 and 8 hours separately. A specific carboxyfluorescein (FAM) labeled peptide fluoromethyl ketone (FMK) caspase inhibitor (FAM-Peptide-FMK) was used to label caspase 8-activated cells. Caspase positive (+) cells appeared green whereas necrotic cells were stained red with propidium iodide and observed under a fluorescent microscope. Pictures were taken and the activation/necrosis ratio (A/N ratio) was analyzed using ImageJ software (developed by the National Institutes of Health). Significant induction of caspase 8 by SB was observed in androgen receptor positive LNCaP cells. In both 2 and 8 hour incubation, SB activated more caspase 8 in LNCaP cells than in DU-145 cells treated at 100  $\mu\text{L}$  and 200  $\mu\text{L}$  concentrations (A/N<sub>LNCaP</sub> > A/N<sub>DU-145</sub>). In addition, DU-145 exhibited significant dosage-dependent induction of caspase 8 by SB, while dosage-dependence was not observed in LNCaP cells (A/N<sub>100 $\mu\text{L}$</sub>  = A/N<sub>200 $\mu\text{L}$</sub> ). These data indicate that SB might activate apoptosis through an up-stream androgen receptor pathway, thus suggesting a plausible mechanism for this herb's activation of the apoptotic pathway in prostate cancer cells.

**A40 The enhancement of p53 stability via the inhibition of JNK-modulated ubiquitination with the activation of redox factor 1 (Ref-1) in response to non-genotoxic antioxidant selenomethionine (semet).** Hwa Jin Jung,<sup>1</sup> Ju Han Lee,<sup>1</sup> Seok Won Jeong,<sup>1</sup> Jee Young Kwon,<sup>1</sup> Jee Yeon Park,<sup>1</sup> Hye Lim Kim,<sup>1</sup> Young Ju Lee,<sup>2</sup> Sung-Goo Chang,<sup>3</sup> Young Rok Seo<sup>1</sup>. <sup>1</sup>Department of Pharmacology, Institute for Basic Medical Science (IBMS), School of Medicine, Kyung Hee University, Seoul, Republic of Korea; <sup>2</sup>Department of Pharmacology, School of Medicine, Kyung Hee University, Seoul, Republic of Korea; <sup>3</sup>Department of Urology, School of Medicine, Kyung Hee University, Seoul, Republic of Korea.

p53 has been known to play an important role as a tumor suppressor gene for the maintaining of genome stability. In the field of cancer prevention study, p53 has been issued to clarify the mechanism of activation under the non-genotoxic cancer preventive agents containing antioxidant selenium. In previous our study, p53 has been reported to be the function of transcriptional activation activated without the genotoxicity through the redox modulation in response to selenomethionine (SeMet). In this study, we investigated that the mechanism of p53 protein stabilization enhanced by SeMet. Our data showed that the ubiquitinated p53 was decreased in the cells exhibiting the downregulation of JNK which is one of E3-ubiquitin ligases compared with in mock-treated cells suggesting that the JNK-modulated p53 ubiquitination might be inhibited by SeMet. To define the mechanism of the enhanced p53 stability in the presence of SeMet, redox factor 1 (Ref-1) which has been documented as one of the factors modulating the redox status in response to antioxidants was downregulated using siRNA. Our data showed the first time that the interaction of JNK and p53 was increased in Ref-1 siRNA-treated cells in the presence of SeMet implicating that the Ref-1 activated by SeMet might be involved in the inhibition of JNK-mediated p53 ubiquitination. Our study suggested that the enhancement of p53 stabilization in response to SeMet might provide an important clue for the cancer prevention.

## Natural Product-based Agents

**A42 The dietary agents indole-3-carbinol and myo-inositol prevent lung cancer induced by vinyl carbamate in A/J mice.** Fekadu Kassie, Ilze Matise, Stephen Kalscheuer, Pramod Upadhyaya, Stephen S. Hecht. University of Minnesota, Minneapolis, MN.

Because of their expected safety, food-derived products could be highly interesting for development as chemopreventive agents. Among promising cancer preventive dietary agents are indole-3-carbinol (I3C) and *myo*-inositol (MI). I3C is derived from the enzymatic hydrolysis of indolyl glucosinolates contained in cruciferous vegetables. MI is found in a variety of animal and plant foods in its free form, as an inositol-containing phospholipid, and inositol hexaphosphate (IP6, phytic acid.). In earlier studies, we found that both I3C and MI prevent lung adenoma induced by tobacco smoke carcinogens in A/J mice. In the present study, we examined the efficacy of I3C and MI to prevent lung adenocarcinoma. Female A/J mice were injected i.p. with two doses of vinyl carbamate (VC, 0.32 mg per mouse) dissolved in physiological saline, 1 week apart, and then randomized into the different treatment groups. After waiting 1 week following the second injection of the carcinogen, mice were maintained either on untreated control diet, or diets supplemented with I3C (70  $\mu$ mol/g diet) or MI (56  $\mu$ mol/g diet) for 15 weeks. At necropsy, lungs were removed and grossly visible lesions on the surface of all lobes of the lung were counted and the size of the tumors determined using a dissecting microscope. Then, the left lobe of the lung from each mouse was preserved in buffered formalin for histopathological analysis. Mice treated with VC and fed conventional diet had  $42.6 \pm 11.9$  lung tumors/mouse. Compared to mice treated with VC alone, carcinogen-treated mice given I3C or MI-supplemented diet had a significantly lower tumor multiplicity,  $31.6 \pm 4.8$  and  $33.9 \pm 6.1$  lung tumors, corresponding to a reduction by 26% and 20% respectively ( $p = 0.0003$  for VC + I3C group and  $p = 0.0005$  for VC + MI group compared using Wilcoxon non-parametric test). Classification of the tumors into three different size groups (<1 mm, 1-2 mm, and > 2 mm) showed that the multiplicity of only the larger tumors was reduced by the chemopreventive agents ( $25.4 \pm 6.8$ ,  $16.4 \pm 8.3$  and  $0.69 \pm 1.3$  tumors/mouse for < 1 mm, 1-2 mm, and > 2 mm size groups, respectively, in mice treated with VC alone versus  $28.8 \pm 4.9$ ,  $2.8 \pm 2.0$ , and  $0.05 \pm 0.22$  tumors/mouse in the group treated with VC + I3C and  $26.8 \pm 5.1$ ,  $6.7 \pm 2.3$ , and  $0.40 \pm 0.6$  tumors/mouse in VC + MI-treated mice). Furthermore, we determined the effect of the chemopreventive agents on the incidence and multiplicity of the different microscopic lesions (hyperplasia, adenoma, adenoma with cellular pleomorphism and adenocarcinoma). Preliminary results showed that I3C reduced the incidence (71% in the VC group versus 36% in the VC + I3C group) and multiplicity (1.1 /mouse in the VC group versus 0.4 /mouse in the VC + I3C group) of adenocarcinoma. I3C also reduced the multiplicity (3.6/mouse in the VC group versus 2.4 /mouse in the VC + I3C group) of adenoma with cellular pleomorphism lesions. In mice maintained on MI-supplemented diet, there was no change either in the incidence or multiplicity of adenocarcinoma but the multiplicity of adenoma with cellular pleomorphism lesions was markedly reduced (3.6/mouse in the VC group versus 1.5/mouse in the VC + MI group). The chemopreventive agents did not affect the incidence or multiplicity of hyperplastic or adenoma lesions. In conclusion, our results indicate the potential lung cancer chemopreventive effects of I3C and MI in former smokers.

**A43 Hops (*humulus lupulus*) induce detoxification enzymes *in vivo*.** Birgit M. Dietz, Ghenet K. Hagos, Jian Guo, Matthew Main, Daniel D. Lantvit, Norman R. Farnsworth, Guido F. Pauli, Richard B. van Breemen, Judy L. Bolton. UIC, Dept. of Medicinal Chemistry & Pharmacognosy, UIC/NIH Center for Botanical Dietary Supplement Research, Chicago, IL.

Extracts of hop strobiles, the female parts of *Humulus lupulus* L., are popular botanical dietary supplements as alternative treatment for menopausal symptoms (1). Cancer preventive properties of hops, which are in part mediated through the induction of detoxification enzymes, have frequently been reported (2). This effect has been mainly attributed to one of its major constituents, xanthohumol (XN) (3). However, the chemopreventive effects have so far only been demonstrated *in vitro* and have not been confirmed *in vivo*. Therefore, we conducted an *in vivo* study in adult female rats and analyzed the effect of oral administration of a standardized hop extract (7.5 g extract/kg BW per day, containing 2% XN) or s.c. injection of XN (20 mg/day) on detoxification enzymes, such as NAD(P)H:quinone oxidoreductase 1 (NQO1) and glutathione-S-transferase (GST). Analysis of various tissues revealed that while pure XN and oral hops significantly induced NQO1 activity in liver and colon tissue, only oral hops and not pure xanthohumol induced NQO1 activity in the uterus. Interestingly, hops but not xanthohumol significantly induced GST activity in the liver and mammary gland suggesting that other hop constituents and/or an additional mechanism might be responsible for these effects. Transcription of many detoxification enzymes is regulated through the antioxidant response element (ARE) and its transcription factor Nrf2, which is repressed under basal conditions by Keap1 (4). It has been shown that XN alkylates Keap1 and therefore enhances the concentration of free Nrf2 in the nucleus which results in ARE activation (5). Another pathway leading to ARE activation is mediated by protein kinase C (PKC) that phosphorylates Nrf2 leading to accumulation of Nrf2 in the nucleus (6). To investigate whether this pathway is involved in the observations described above, additional experiments with HepG2-ARE-luciferase C8 cells were performed. The cells were treated with hop extract or XN with or without the selective PKC inhibitor, Ro-32-0432, and the ARE-luciferase induction was determined. The results revealed that hops- but not XN-regulated ARE induction is in part mediated by PKC. Possible active compounds are under investigation. These results suggest that hop dietary supplements have the potential as preventive agents against cancer through induction of detoxifying enzymes.

#### References:

- Overk, C. R., Guo, J., Chadwick, L. R., Lantvit, D. D., Minassi, A., Appendino, G., Chen, S. N., Lankin, D. C., Farnsworth, N. R., Pauli, G. F., van Breemen, R. B. and Bolton, J. L. (2008) *Chem. Biol. Interact.* June 20<sup>th</sup>.
- Gerhäuser, C., Alt, A., Heiss, E., Gamal-Eldeen, A., Klimo, K., Knauft, J., Neumann, I., Scherf, H. R., Frank, N., Bartsch, H. and Becker, H. (2002) *Mol. Cancer Ther.* 1, 959-969.
- Dietz, B. M., Kang, Y. H., Liu, G., Eggler, A. L., Yao, P., Chadwick, L. R., Pauli, G. F., Farnsworth, N. R., Mesecar, A. D., van Breemen, R. B. and Bolton, J. L. (2005) *Chem. Res. Toxicol.* 18, 1296-1305.
- Itoh, K., Wakabayashi, N., Katoh, Y., Ishii, T., Igarashi, K., Engel, J. D. and Yamamoto, M. (1999) *Genes Dev.* 13, 76-86.
- Liu, G., Eggler, A. L., Dietz, B. M., Mesecar, A. D., Bolton, J. L., Pezzuto, J. M. and van Breemen, R. B. (2005) *Anal. Chem.* 77, 6407-6414.
- Huang, H. C., Nguyen, T. and Pickett, C. B. (2002) *J. Biol. Chem.* 277, 42769-42774.

**A44 Carnosol, a dietary diterpene functions as a dual AR and ER- $\alpha$  antagonist in prostate cancer.** Jeremy J. Johnson, Deeba N. Syed, Chenelle R. Heren, Imtiaz A. Siddiqui, Hasan Mukhtar. University of Wisconsin, Madison, WI.

Emerging evidence suggest that androgens may not be the only hormone responsible for the etiology of prostate cancer (PCa). Recent data has suggested that estrogens may be another important piece to the PCa puzzle. For example the use of the Selective Estrogen Receptor Modulator (SERM) toremifene in HG-PIN patients for one year resulted in a 21% reduction in the conversion of HG-PIN to PCa after one year of treatment. It is well established that race correlates with the levels of androgens with African Americans having the highest, followed by Caucasians and lowest in Japanese males. A similar trend has been observed in regard to serum estrogen levels and it is noteworthy that with age estrogen levels remain constant while androgens levels decrease. Given that experimental animal models suggest that both estrogens and androgens are necessary for PCa development it may be a reasonable approach to simultaneously disrupt both androgen and estrogen signaling in PCa. Several SERMs that include tamoxifen, toremifene, and fulvestrant have been evaluated for their ability to also target the AR and function as a dual AR and ER antagonist, however, experiments have suggested these effects are saturable and in some instances SERMs exert AR agonist properties. In our quest to find a dual AR and ER antagonist present in dietary substances that humans consume we found that the dietary diterpene Carnosol displays growth inhibitory properties in the hormone sensitive prostate (LNCaP and CWR22Rv1) and breast (MCF7 and AU565) cancer cell lines. Given the structural similarity between Carnosol to dihydrotestosterone and estradiol we hypothesized that it may interact with both the androgen and estrogen receptor alpha (ER- $\alpha$ ). Using a TR-FRET cell free biochemical assay we found that Carnosol can interact with both AR and ER- $\alpha$  with similar potencies. Next, we evaluated the antagonist and agonist properties of Carnosol using a functional assay where the AR and ER- $\alpha$  ligand binding domains are expressed as a fusion protein in HEK293 cells. We observed that Carnosol exhibits antagonist properties that are dose responsive and even more interestingly, regardless of dose, did not exert agonist properties. In LNCaP, CWR22Rv1, and MCF7 cells Carnosol was found to disrupt both androgen and estrogen signaling in a dose and time dependent manner. Our finding that Carnosol displays the ability to disrupt both androgen and estrogen signaling simultaneously suggesting that Carnosol may be developed or chemically modified through more rigorous structure activity relationship studies of other such chemical entities for a new class of investigational agents - a dual AR/ER modulator.

**A45 Hydroxylated polymethoxyflavones: A novel class of agents for colon cancer prevention.** Hang Xiao,<sup>1</sup> Shiming Li,<sup>2</sup> Huanyu Jin,<sup>3</sup> Chung S. Yang,<sup>3</sup> Chi-Tang Ho<sup>3</sup>. <sup>1</sup>University of Massachusetts, Amherst, MA; <sup>2</sup>WellGen, Inc., North Brunswick, NJ; <sup>3</sup>Rutgers, The State University of New Jersey, New Brunswick, NJ.

Orange peels have been used as flavoring and traditional Chinese medicine for many centuries. Polymethoxyflavones (PMFs) are a unique class of flavonoids, and almost exclusively exist in the citrus genus, particularly in the peels of sweet oranges (*Citrus senensis*) and mandarin oranges (*Citrus reticulata*). Major PMFs are permethoxylated PMFs (Me-PMFs) such as nobiletin, 3,5,6,7,8,3',4'-heptamethoxyflavone (HMF), and tangeretin, which have been shown to inhibit cancer cell growth *in vitro* and *in vivo*. Recently, we have isolated a class of novel PMFs, namely hydroxylated PMFs (OH-PMFs), from sweet orange peel. These OH-PMFs can be formed from their corresponding Me-PMFs counterparts by hydrolysis naturally. Herein, we studied the effects of three major OH-PMFs, namely 5-hydroxy-6,7,8,3',4'-pentamethoxyflavone (5HMPMF), 5-hydroxy-3,6,7,8,3',4'-hexamethoxyflavone (5HHMF), and 5-hydroxy-6,7,8,4'-tetramethoxyflavone (5HTMF), on colon cancer cells, and compared their effects with their corresponding Me-PMFs counterparts,

## Natural Product-based Agents

namely nobiletin, HMF, and tangeretin, respectively. Our results showed that OH-PMFs significantly inhibited colon cancer cell (HCT116 and HT29) growth in a dose dependent fashion, and these effects were much stronger than those produced by their corresponding Me-PMFs counterparts. Cell cycle analysis by flow cytometry demonstrated that at 24 h, 5HPMF caused cell cycle arrest at G2/M phase, while 5HHMF resulted in arrest at G0/G1 phase. In contrast, at much lower concentration, 5HTMF significantly increased the sub-G0/G1 cell population, indicating possible DNA degradation due to cell death. Annexin V/PI co-staining assay was used to detect possible apoptosis after treatments with OH-PMFs for 48 h. It was found that all three OH-PMFs increased the apoptotic cell population, but 5HTMF showed a much stronger pro-apoptotic effect at much lower concentration in comparison to 5HPMF and 5HHMF. The distinct effects of these three OH-PMFs on cell cycle and apoptosis of colon cancer cells suggest that each OH-PMFs may have different molecular targets in the cancer cells. Moreover, three OH-PMFs profoundly modulated proteins related to cell proliferation and apoptosis. OH-PMFs, namely 5HPMF and 5HHMF, produced profound tumor-suppressive changes on these proteins, while their corresponding Me-PMFs, namely nobiletin or HMF did not cause any noticeable change on the same proteins tested. On the other hand, tangeretin caused various tumor-suppressive changes on the proteins tested, however, its effects were either similar or to less extent than those produced by 5HTMF at much lower concentrations. Overall, at 48 h, three OH-PMFs decreased the level of K-RAS and phosphorylation of AKT, increased the level of p16, and activated caspase cascade, while 5HTMF also increased the level of p16 and decreased the level of Mcl-1. Our results demonstrated that OH-PMFs are promising novel agents for colon cancer prevention.

**A46 A black raspberry extract inhibits the proliferation of cervical cancer cells partially through proapoptotic effects.** Zhaoxia Zhang, Leigh G. Seamon, Thomas J. Knobloch, Gary D. Stoner, David E. Cohn, David M. O'Malley, Electra D. Paskett, Jeffrey M. Fowler, Christopher M. Weghorst. The Ohio State University, Columbus, OH.

Cervical carcinoma, the second most common female cancer worldwide, is the seventh leading cause of cancer death in women. Although pap smears to screen abnormal cervical cytology have helped reduce mortality rates, it remains a challenge to manage preinvasive and invasive cervical lesions. The long latency of cervical cancer allows preventive and therapeutic interventions before progressing onto invasive disease. Thus, phytochemical-based cancer chemoprevention and the role of bioactive food components have attracted significant interest in current cancer-fighting interventions. We have previously shown that lyophilized black raspberries (LBR, *Rubus occidentalis*) inhibit the cell proliferation of human oral squamous cell carcinoma cells *in vitro* as well as oral tumorigenesis *in vivo*. Given the similar epidemiologic basis of oral and cervical cancers, we evaluated whether an LBR extract had anti-proliferative effects in cervical cancer cells *in vitro* and began to elucidate the possible regulatory molecular mechanisms. We found that LBR inhibited the proliferation of three human cervical cancer cell lines, HeLa (HPV16-/HPV18+) SiHa (HPV16+/HPV18-) and C-33A (HPV18-/HPV16-), in a dose-dependent manner (at 25, 50, 100 or 200µg/ml for 1, 3 and 5 days, respectively) to a maximum of 44%, 53% and 45% respectively ( $P < 0.005$ ). While exogenous hydrogen peroxide ( $H_2O_2$ , 100µM), a common reactive oxygen species (ROS) intermediate and inhibitor of cell growth, inhibited C-33A proliferation, HeLa and SiHa cells were not significantly affected. The anti-proliferative effects of either LBR or LBR+catalase were similar in all cell lines as shown by WST1 (tetrazolium salt cleavage) and crystal violet assays, which supporting the hypothesis that decreased proliferation is specific to LBR bioactive components and independent *in vitro* peroxide generation. Flow cytometric analysis of propidium iodide and Annexin V staining showed that LBR induced apoptotic markers in all three cell lines, and did not change cell cycle progression. LBR treatment induced

hypercondensation and volume contracted nuclei in HeLa cells as demonstrated by fluorescence microscopy analysis of Hoechst 33342 staining. In conclusion, an extract of lyophilized black raspberries demonstrated significant anti-proliferative and pro-apoptotic activities in human cervical cancer cells *in vitro*. Therefore, black raspberries and their bioactive components may represent promising candidates for the phytochemical-based chemoprevention of cervical cancer and warrant further investigation.

**A47 Drinking water with red beetroot food dye antagonizes esophageal carcinogenesis in NMBA-treated rats.** John F. Lechner,<sup>1</sup> Li-Shu Wang,<sup>2</sup> Steven J. Schwartz,<sup>2</sup> Gary D. Stoner.<sup>2</sup> <sup>1</sup>University So. Maine, Portland, ME; <sup>2</sup>Ohio State University, Columbus, OH.

This study was undertaken to determine if the oral consumption of red beetroot dye would result in an inhibition of *N*-nitrosomethylbenzylamine (NMBA)-induced tumors in the rat esophagus. Rats were treated with NMBA and given either regular water *ad libitum* or water containing 78 µg/ml of commercial red beetroot dye, E162. The number of NMBA-induced esophageal papillomas was reduced by 45% ( $p < 0.001$ ) in animals that received the dye when compared to controls. Dye treatment also resulted in reduced rates of cell proliferation in both precancerous esophageal lesions and in papillomas of NMBA-treated rats, as measured by immunohistochemical staining of Ki-67 in esophageal tissue specimens. The effects of beetroot dye on angiogenesis (microvessel density by CD34 immunostaining), inflammation (by CD45 immunostaining), and apoptosis (by TUNEL staining) in esophageal tissue specimens were also determined. When compared to rats treated with NMBA only, the levels of angiogenesis and inflammation in the beetroot dye-consuming animals was reduced and the apoptotic rate was increased. Thus, the mechanism(s) of chemoprevention by the active constituents of red beetroot dye include reducing cell proliferation, angiogenesis and inflammation, and stimulating apoptosis. Importantly, consumption of the dye in the drinking water for a period of 35 weeks did not appear to induce any overt toxicity. Based on the fact that red beetroot dye contains betacyanins, which have strong anti-oxidant activity, it is postulated that these effects are mediated through inhibition of oxygen radical-induced signal transduction.

**A50 Soy isoflavones and vitamin D in breast cancer prevention.** Radharani Marik,<sup>1</sup> Saraswati Sukumar,<sup>2</sup> Martha A. Zeiger,<sup>1</sup> Vered Stearns,<sup>2</sup> Christopher B. Umbricht.<sup>1</sup> <sup>1</sup>Johns Hopkins School Of Medicine, Baltimore, MD; <sup>2</sup>Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD.

**Background:** Breast cancer incidence is higher in Western women compared to Asian women. Since Asian women have a 10-25-fold higher soya isoflavone intake, soya products may be useful in breast cancer chemoprevention. The dietary soya isoflavones daidzein and genistein possess structures similar to estrogen, and may mimic or antagonize estrogen effects in different tissues. Daidzein and genistein may be also be involved in gene regulation by modulating epigenetic events such as DNA methylation, directly or through an estrogen receptor dependent process. We also hypothesized that cellular differentiating vitamin D3 (VTD) may improve the isoflavone sensitivity to the breast cancer cell line.

**Methods:** We treated three breast cancer cell lines (MCF7, 21PT and T47D) with daidzein and genistein alone and in combination with VTD for 96 hours. Effects were then compared to those seen with the known methyltransferase inhibitor 5'-deoxy-azacytidine (AZA).

**Results:** Daidzein and genistein inhibited the growth of all three the breast cancer cell lines in a dose dependent manner. The combination of VTD with isoflavones showed an additive effect. Isoflavone treatment reversed methylation of several breast cancer candidate genes, including Hin-1, RAR beta and Rass-F1, associated with an induction of their expression levels. Combinations of VTD with daidzein or genistein showed more demethylation and mRNA re-expression than isoflavones alone.

Furthermore, daidzein or genistein treatment resulted in a significant decrease of estrogen receptor alpha (ERA) level as well as an increase in ER beta (ERB) levels. These results imply that isoflavones may also protect from breast cancer by lowering ERA levels while inducing ERB levels. Indeed, recent studies indicate that high ERB levels are associated with a reduced breast cancer risk and that ERB is an important modulator of proliferation and invasion of breast cancer cells.

ERB has several splice variants: ERB1, ERB2 (ERBcx) and ERB5. Among these variants, ERB1 forms a functionally active heterodimer with ERA, while ERB2 and ERB5 form non-functional heterodimers. High ratios of ERB2 to ERB1 or ERB5 to ERB1 block ERA downstream signaling. In our study, we observed induction of ERB2 > ERB1 levels isoflavone treatments, and ERA downstream signaling was decreased compared to the untreated control cells. Combination therapy of these isoflavones with VTD also resulted in improved induction of erb2 > ERB1 levels than isoflavone treatments alone.

**Conclusion:** Isoflavones can inhibit the growth of breast cancer cell lines MCF7, 21PT and T47D in a dose dependent manner. These isoflavones can both demethylate and re-express critical differentiating genes, and combination treatment with VTD shows more pronounced effects. Inhibition of ERA, ERA downstream genes and activation of ERB along with an increase of the ERB2 to ERB1 ratio by isoflavones in breast cancer cell lines imply the use of isoflavones may be an effective chemopreventive agent that will be well tolerated and may have similar benefits to more toxic agents such as AZA.

**A51 Chemopreventive effects of Brazilian herb oral feeding on UVB induced photocarcinogenesis.** Harukuni Tokuda,<sup>1</sup> Masafumi Kaneko,<sup>2</sup> Mitsuaki Yamashita,<sup>3</sup> Akira Iiida<sup>4</sup>. <sup>1</sup>Kyoto Prefectural University of Medicine, Kyoto, Japan; <sup>2</sup>Takasaki University of Welfare and Health, Takasaki, Japan; <sup>3</sup>Takasaki University of Welfare and Health, Takasaki, Japan; <sup>4</sup>Kinki University, Nara, Japan.

Ultraviolet (UV) light is the most common cause of skin cancer in humans and it is very acute problem in our life style during living. Several effects of UVB (290-320 nm) are thought to contribute to skin carcinogenesis and generation of free radicals and related oxidants produced by UVB exposure. *Tabebuia avellanedae* (Bignoniaceae)(TA), which is native in South America from Brazil to northern Argentina, is well known in traditional folk medicine used for the treatment of various disease during five hundred years. The inner bark of this plant produced in Brazil is distributed in Asia and Japan as a healthy purpose herb tea. Previously, we reported that extract essence of TA(TA ess.) and including naphthoquinones type compounds, NQ801, inhibited TPA-induced *in vitro* assay for chemopreventive potency and also inhibited TPA-induced *in vivo* assay on two-stage mouse skin test. We have now extended these investigations to a new tumorigenesis model in which we initiated the tumors with UVB irradiation and promoted with 1.7 nmol of TPA in SENCAR mice. These tumor -free mice, which had a high risk of developing skin tumors, using these experimental systems, were then treated drinking with TA ess. Oral feeding of 0.0025 % of TA ess., two weeks before and after tumor initiation resulted in a significant reduction in tumor incidence (40 %) accompanied by an extension(> 20 %) of the tumor latency and also decrease papilloma multiplicity. These results provide a basis for further development of TA ess. against sunlight-induced skin cancer in humans.

**A52 Vitamin K1 enhances sorafenib effects on HCC and induces apoptosis: A nontoxic prevention strategy.** Brian I. Carr, Gang Wei, Meifung Wang. Thomas Jefferson University, Philadelphia, PA.

HCC is usually multifocal and non surgical at presentation in the US and arises on a cirrhotic liver which tolerates chemotherapy poorly. A defect in vitamin K metabolism in HCC results in secretion of the useful tumor marker DCP, or des gamma carboxy prothrombin. We previously showed that natural vitamin K, which has no known toxicities in adult humans, is a weak HCC growth inhibitor. The FDA recently approved the multikinase inhibitor sorafenib for HCC treatment. We now show that at doses of each agent (vitamin K1 12.5 g/ml and sorafenib 2.5 g/ml) that do not independently alter HCC growth, the combination inhibits growth of Hep3B, PLC/PRF/5 and JM1 HCC cell lines at 24-72 hr and induces apoptosis, as supported by FACS analysis (without G1 or S phase arrest), TUNEL stain, cleavage of caspase 3 and PARP and reduced expression of survivin, Bcl-2 and Mcl-1 at 6-24 hr. At later times, a profound decrease in phospho-ERK and cyclin D1 was found (36-48 hr). The results show that the non-toxic vitamin K1 can decrease the concentrations at which sorafenib works and provides a possible approach to cancer prevention in patients with cirrhosis, who are at risk for HCC development.

**A53 Apoptosis initiated in colon cancer cells when treated with muscadine grape extract.** Lane E. Hannah,<sup>1</sup> Vondina R. Brown,<sup>1</sup> Damayanthi Ranwala,<sup>2</sup> Michael J. Wargovich<sup>1</sup>. <sup>1</sup>Medical University of South Carolina, Charleston, SC; <sup>2</sup>Clemson University, Clemson, SC.

Research suggests strong correlations between the inflammation process and colon cancer progression, making it an attractive target for anti-inflammatory drugs and compounds. Muscadines (*Vitis rotundifolia*), a grape species native to the Southeastern United States, are rich in polyphenols containing many biologically important flavonoids that may have potential anti-inflammatory and anti-oxidant properties. Current research has shown that the muscadine grape has a higher total phenolic and flavonoid content than commercially available red grapes. A hallmark of progressive colon cancers is disruption of different signaling pathways leading to increased proliferation and escape from apoptotic mechanisms. We hypothesize that extracts containing high levels of biologically important polyphenols from the muscadine pomace would induce cell cycle arrest and apoptosis in HT-29 and HT-15 colon cancer cell lines. Polyphenolic compounds were extracted from muscadine pomace using 80% methanol in 6N hydrochloric acid at 60°C. Whole concentrated extract (WCE) was fractionized using an Oasis HLB column to yield three different muscadine fractions (MF). We compared the cytotoxic effects of both cell lines when treated with WCE versus three different muscadine fractions (MF-1, MF-2, and MF-3). Cell proliferation assays showed a decrease in viable cell proliferation in both cell lines treated with MF-3. Apoptosis was confirmed through the use of flow cytometry and we found evidence of G1 arrest in MF-3 treated cells. Immunoblots for apoptosis-related proteins suggest apoptotic activation of the caspase-3 pathway in treated cells compared to non-treated cells. We conclude that extracts of muscadine grape contain biologically active polyphenols that inhibit the growth of HT-29 and HT-15 colon cancer cells via an apoptotic pathway.

## Natural Product-based Agents

**A54 Epigenetic changes in colon cancer cell lines treated with the tea polyphenolic, EGCG.** Vondina R. Brown, Hannah E. Lane, Michael J. Wargovich. Medical University of South Carolina, Charleston, SC.

Retinoids are known for their role in regulating cell growth and proliferation and for activating tumor suppressor genes. We have previously shown that Retinoid X Receptor alpha (RXR alpha) is silenced in tumors of the colon cancer AOM-APCmin+ mouse model. Upon treatment with green tea, RXR alpha expression was restored and intestinal tumorigenesis was inhibited. We hypothesize that tea polyphenols, especially epigallocatechin gallate (EGCG), induce modification of gene expression through effects of DNA methyltransferases (DNMTs). We first determined the optimal range of EGCG concentration with which to treat the cells by performing cytotoxicity assays. We treated HT-29 and HCT116 colon cancer cells with EGCG concentrations of 0, 50, 100 or 150µM for 24 and 48 hours. The cell lines were chosen based on their methylation status. HCT116 cells are sensitive to methylation while HT-29 cells are not. Using nuclear fractions of the cell lysates, we probed for presence of the most common DNMTs using western blotting. We found that expression of DNMT1, DNMT3a and DNMT3b was inhibited in a dose dependent manner following treatment with EGCG at both 24 and 48 hour time points. We also used the DNMT inhibitor 5-aza-2dC as a positive control to recover DNMT expression in the cells treated with EGCG. We can conclude that the silencing of RXR alpha may be due in part to by repressing effects of EGCG on DNMT activity.

**A55 A screening method for identifying potential angiogenesis inhibitors.** Kelli Caldwell, Shirley Charlton, Diane C. Krill. Point Park University, Pittsburgh, PA.

**Objective:** Plants produce chemical compounds to prevent encroachment by competitors. These turf wars that generate defense products in plants have potential medicinal value for humans. For example, compounds with anti-angiogenic properties are useful in combating cancer by preventing new blood vessel formation to support the tumor. The search for new angiogenesis inhibitors is ongoing, and utilizes a variety of time-intensive cell culture models. In this report we introduce a rapid, inexpensive method for screening potential anti-angiogenic compounds in a model system that stimulates the production of secondary defense chemicals in plants, thus increasing the potential yield of effective compounds.

Plant chemicals under investigation for anti-angiogenic activity include flavonoids, a broad group of compounds that are present in most photosynthesizing cells of plants. Flavonoids share a common phenyl-chromanone structure (C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub>), and include thousands of compounds, subdivided into 6 major categories; flavones, flavonols, isoflavones, flavanols, flavanones, and anthocyanins. Humans do not synthesize flavonoids, but derive an average of 1 gram per day in their diet. Their reported pharmacological properties include antioxidative, anti-inflammatory and antiproliferative properties.

**Methods:** In order to induce defense chemical production, we chose thyme and mint plants to combine because thyme was observed to inhibit the spread of mint, a highly invasive vascular plant. The thyme plants grown alone, as well as the mint alone, were analyzed as controls. The plant leaves and stems were extracted and the flavonoid products were analyzed by reverse phase HPLC. The flavonoids that appear in the thyme in the presence of mint, that are not present in the thyme plants grown alone, were considered induced by the presence of mint. The induced compounds were collected and tested in a vertical plate assay that measures root length as a quantitative assay for drug sensitivity. The compounds were applied to the vascular root tissue of mint seedlings 5-7 days after germination.

**Results:** Of eight *Thymus vulgaris* compounds that were induced by the presence of mint, 5 were tested by bioassay, and one was found to have an inhibitory effect. Known angiogenesis inhibitors, VEGF receptor tyrosine kinase and ameliotide hydrochloride, significantly reduced root growth compared to the control. An HPLC-isolated compound b, which eluted at 15.2 minutes, reduced the growth of root vascular tissue compared to the control and vehicle control, and 50% as well as known angiogenesis inhibitors.

**Conclusion:** This report demonstrates that induced secondary defense chemicals produced by thyme plants include flavonoid compounds. Useful inhibitors may be identified with this rapid, sensitive, and inexpensive technique.

**A56 Alterations in female reproductive organs of cyclic rats treated with aqueous extract of moringa oleifera lam: Indication of possible role in epithelial ovarian cancer.** Chinmoy K. Bose. Netaji Subhas Chandra Bose Cancer Research Institute, Kolkata, India.

**Background:** A hormonal etiology of epithelial ovarian cancer has long been suspected seeing its incidence menopausal age, and now the role of FSHR has also been demonstrated. Many ovarian cancer cell line express FSHR in them. In studies of the anticancer potential of plants used in folk medicine of Bengal, extracts of plants such as *Oroxylum indicum*, *Moringa oleifera lam*, *Aegles marmelos* could be considered as potential sources of anticancer compounds. Amongst them only *Moringa oleifera lam* has unique anticancer as well as hormonal property, which may or may not be attributable to isothiocyanate or glucosinolate that it contains. An animal experiment was planned to see effect of *Moringa* root extract in female reproductive system of mice.

**Methods:** 5 adult female mice of swiss strain of 30 gm each 2-control, 3-treated kept on stock diet, pellet, having nutritional value of 7 days. An aqueous extract of the root was prepared according to a traditional method. In brief, 500 g of the root were placed in a container with 750 ml of water and boiled for 30 min. The preparation was left standing to cool and was then filtered. The filtrate, containing 66.7 mg root in 1 mL, was placed in small vials and kept in 4 degree C refrigerator until use. 1 ml of extract was used orally daily for 45 days.

**Results:** Attenuation/shrinkage of ovary and uterus was seen while mice tolerated the herb extract well. There was reversal to pre estrus phase of adult mice as was revealed by PAP smear from vagina. In histology there was absence of follicles in comparison to control ovary. There was lesser amount of fibrosis in treated ovary.

**Conclusion:** Isothiocyanate of *Moringa* may inhibit proliferation of ovarian granulosa and other cells as it induces apoptosis via caspase-9 and -3 pathways, a family of calcium-dependent cysteine proteases. It may also act by inhibiting ERK1/2 and Akt survival signaling while simultaneously activating pro-apoptotic p38 and JNK1/2. There are reports to show that *Moringa* induced decrease in cerebral dopamine and norepinephrine which may influence and lower NGF and FSHR through central mechanisms. There is strong possibility of using this agent in epithelial ovarian cancer and, as such, a cell line experiment is urgently necessary.

**A57 Lipid extract from the ostrich fern *Matteucia struthiopteris* displays potent anti-cancer effects in microtumors from human malignant cell lines via disruption of actin cytoskeleton.** Sarah Crawford, Rafaella Penarreta, Deanna Diamond. Southern Connecticut State University, New Haven, CT.

Previous studies from the Cancer Biology Research Laboratory identified primitive plants of moss, fern and lichen species with anti-cancer activities. Current research involves the extraction of a lipid-containing fraction from the ostrich fern, *Matteucia struthiopteris*, with 1,000-fold greater anti-cancer activity than whole plant aqueous homogenates in pre-clinical assays of microtumors prepared from several solid tumor malignancies, including drug-resistant glioblastoma multiforme. Confocal laser microscopy of phalloidin-stained tumor cells demonstrated that a primary effect of the plant extracts is the disruption of cortical actin cytoskeleton in the cell membrane. This represents a previously unrecognized therapeutic target for anti-cancer agents.

The targeted actin-cytoskeleton may also comprise a novel chemopreventive target. A novel biophysical model, proposed by Crawford, suggests that the active lipid fraction may affect cytoskeletal integrity as a consequence of the interaction of plant isoprenoids and dolichols on tumor membrane fluidity. Increased membrane fluidity may interfere with the dynamic assembly of actin cytoskeletal components essential to cell motility and focal adhesions essential for tumor viability and metastasis.

\*Patent pending 2008

## Clinical Prevention Trials

### Breast Cancer

**A58 Breast cancer risk estimates from the CARE and GAIL models among black women: Howard University Cancer Center experience.** Lucile L. Adams-Campbell,<sup>1</sup> Kephher Makambi,<sup>2</sup> Wayne Al Frederick,<sup>2</sup> Wortia McCaskill-Stevens<sup>3</sup>. <sup>1</sup>Lombardi Comprehensive Cancer Center at Georgetown University, Washington, DC; <sup>2</sup>Howard University Cancer Center, Washington, DC; <sup>3</sup>National Cancer Institute, Bethesda, MD.

The Gail model has been used to predict invasive breast cancer risk in women using the following risk factors: age, age at menarche, age at first live birth, number of first degree relatives with breast cancer, and number of previous benign breast biopsy examinations. However, this model underestimates breast cancer risk in black women. The Contraceptive and Reproductive Experience (CARE) model has been developed to replace the Gail model in predicting breast cancer risk in black women. Using a sample of 883 black women who were screened for the STAR chemoprevention trial, we compared breast cancer risk estimates from the Gail and CARE models. The mean 5-year breast cancer risk was 0.88% for the Gail model and 1.29% for the CARE model. Using the usual cut-point of 1.67% or above for elevated risk, there is significant difference in the proportion of women with elevated breast cancer risk between the Gail and the CARE models (McNemar's test,  $p < 0.0001$ ). Among those with a family history of breast cancer, the mean risk was 1.57% for the Gail model and 1.88% for the CARE model. For both models, there was a significant mean risk difference between those with and without a family history of breast cancer (Wilcoxon rank-sum test,  $p < 0.0001$ ).

**Conclusion:** The CARE model is an improvement of the Gail model for estimating the risk of invasive breast cancer among black women and will improve counseling, risk assessment, prescribing of chemoprevention agents and eligibility for chemoprevention trials

**A59 A randomized, 3-arm placebo-controlled phase II presurgical trial of celecoxib or exemestane in postmenopausal breast cancer patients: Preliminary results.** Bernardo Bonanni,<sup>1</sup> Sara Gandini,<sup>1</sup> Serena Mora,<sup>1</sup> Paolo Veronesi,<sup>1</sup> Francesco Valenti,<sup>1</sup> Alberto Luini,<sup>1</sup> Matteo Lazzeroni,<sup>1</sup> Davide Serrano,<sup>1</sup> Harriet Johansson,<sup>1</sup> Pietro Caldarella,<sup>1</sup> Marco Colleoni,<sup>1</sup> Giuseppe Pelosi,<sup>1</sup> Andrea Decensi<sup>2</sup>. <sup>1</sup>European Institute of Oncology, Milan, Italy; <sup>2</sup>E.O. Ospedali Galliera, Genoa, Italy.

The presurgical model is a useful tool to screen and evaluate drug activity and select new chemopreventive agents. Cancer cell proliferation measured by ki-67 is considered to be a reliable surrogate biomarker in assessing the efficacy of chemopreventive agents. Exemestane (Exe), a highly specific steroidal aromatase inactivator, has shown clinical efficacy in the treatment of postmenopausal breast cancer (BC) patients. Epidemiologic evidence suggests that anti-inflammatory drugs reduce the risk of BC. Celecoxib (Cel) is a selective inhibitor of the enzyme COX-2 (Cyclooxygenase-2), which is overexpressed in human breast tumors, BC cell lines, and rodent mammary tumors, and it has shown significant antiproliferative and proapoptotic properties. No clinical data are known so far on the antiproliferative activity of Cel in breast cancer cells.

The primary endpoint of the present study is to assess the antiproliferative activity (as measured by ki-67 percentage change) of Exe 25mg/d or Cel 800 mg/d relative to placebo in postmenopausal ER positive BC patients treated for 6 weeks before surgery. Estimating a 20% increase in the placebo arm and a 25 % decrease in the treatment arms with 80% power and two-tailed significance at 5%, a total of 90 subjects (18 in the placebo arm and 36 in each treatment arm) was required.

Secondary endpoints were changes in ER and PgR expression, apoptosis, gene expression profile focusing on nuclear receptor coregulators (AIB1, SRC1, TIF-2/GRIP1, N-CoR and SMRT) on frozen tissue, circulating biomarkers (sex hormones, markers of inflammation and hemostasis, insulin-like growth factors, bone markers) and genomic DNA analyses of genetic polymorphisms related to BC, hormone metabolism, response to DNA damage, and cardiovascular events.

One hundred and one patients were randomized to either Exe 25 mg/d ( $n=41$ ), or Cel 800 mg/d ( $n=40$ ) or placebo ( $n=20$ ).

Baseline median age and BMI were 62, 65, 61 years, 26, 25, 27 in the Exe, Cel and placebo group, respectively.

At baseline median ki67% was 21, 18, 18 in the Exe, Cel and placebo arm, respectively.

After adjustment for baseline values, Ls means of changes of ki67 were -11.5 (-14.1, -8.9), 1.3 (-1.4, 3.9), 3.2 (-0.5, 6.8) ( $P$  among arms  $< .001$ ;  $P$  among active treatments  $< .001$ ).

After adjustment for baseline values, Ls means of changes of PgR were -26.2 (-34.1, -18.4), -6.3 (-14.3, 1.7), 2.1 (-8.8, 13.1) ( $P$  among arms  $< .001$ ;  $P$  among active treatments  $< .001$ ).

After adjustment for baseline values, Ls means of changes of ER were -2.6 (-7.8, 2.7), 0.8 (-4.5, 6.1), -0.7 (-8.0, 6.7) ( $P$  among arms 0.671;  $P$  among active treatments 0.374).

Compliance was higher than 75% and no severe AEs were observed.

In this postmenopausal population, Exe has shown a significant reduction of cell proliferation and PgR expression. Bone biomarkers are currently being measured as long as the other secondary endpoints and final results will be helpful to plan further prevention studies with Exe in high-risk subjects. Conversely, only a modest non significant modulation of PgR expression was observed in the Cel arm despite the relatively high dose of the drug. Evaluation of secondary endpoints, including expression of Cox-2 activity, will help to better elucidate the activity of both agents.

## Breast Cancer

**A60 A randomized, 3-arm phase II presurgical trial of weekly tamoxifen or raloxifene versus placebo in premenopausal breast cancer patients: Preliminary results.** Guerrieri-Gonzaga Aliana,<sup>1</sup> Sara Gandini,<sup>1</sup> Bernardo Bonanni,<sup>1</sup> Serena Mora,<sup>1</sup> Alberto Luini,<sup>1</sup> Fabio Bassi,<sup>1</sup> Viviana Galimberti,<sup>1</sup> Mattia Intra,<sup>1</sup> Matteo Lazzeroni,<sup>1</sup> Davide Serrano,<sup>1</sup> Debora Macis,<sup>1</sup> Giuseppe Renne,<sup>1</sup> Nicole Rotmensz,<sup>1</sup> Andrea Decensi<sup>2</sup>.  
<sup>1</sup>European Institute of Oncology, Milan, Italy; <sup>2</sup>E.O. Ospedali Galliera, Genoa, Italy.

The presurgical model is a useful tool to screen and evaluate drug activity and select new chemopreventive agents. Cancer cell proliferation measured by Ki-67 is considered to be a reliable surrogate biomarker in assessing the efficacy of chemopreventive agents. We previously demonstrated that tamoxifen (Tam) given in breast cancer (BC) patients at lower daily doses (1 and 5 mg/d) before surgery for 4 weeks reduced the tissue expression of Ki-67 to the same extent of the standard 20 mg/d dose. Given the long half-life of Tam and its metabolites a weekly dose (10mg/w) may be worth testing to assess the minimal active dose. Raloxifene (Ral), another SERM, has been shown to reduce Ki-67 in a preoperative setting and has been shown to be an effective BC preventive agent only in postmenopausal women so far.

The primary endpoint of the present study was to assess the activity of Tam 10mg/w or Ral 60 mg/d relative to placebo as measured by Ki-67 percentage change in premenopausal ER positive BC patients treated for 6 weeks before surgery. Estimating a 20% relative increase of Ki-67 in the placebo arm and a 25% decrease in both treatment arms with a 80% power and a two-tailed test 5% significance, a total of 90 subjects (18 in the placebo arm and 36 in each treatment arm) was required.

Secondary endpoints were changes in ER and PgR expression, apoptosis, gene expression profile focusing on nuclear receptor coregulators (AIB1, SRC1, TIF-2/GRIP1, N-CoR and SMRT) on frozen tissue, circulating biomarkers (hormones, markers of inflammation and hemostasis, growth factors, bone markers) and genomic DNA analyses of genetic polymorphisms related to breast cancer, hormone metabolism, response to DNA damage, and cardiovascular events.

Ninety-four patients were allocated to either Tam 10 mg/w (n=37), or Ral 60 mg/d (n=38) or placebo (n=19).

Baseline median age and BMI were 44, 46, 44 years, and 22, 23, 23 in the Tam, Ral and placebo group respectively. Baseline median ki-67 was 22, 22, 15 in the Tam, Ral and placebo arm, respectively.

After adjustment for baseline values, Ls means of changes of ki67 were -0.3 (-3.7, 3.1), 0.3 (-3, 3.6), -2.4 (-7.3, 2.5), Ls means of changes of PgR were -1.2 (-9.4, 7), -6.4 (-14.4, 1.6), 6.7 (-5, 18.4) and Ls means of changes of ER were -0.5 (-5.6, 4.6), 0.4 (-4.7, 5.4), 0.6 (-6.7, 7.9) in the Tam, Ral and Placebo arm, respectively. No statistically significant differences among arms nor between treatments and placebo have been observed.

Overall compliance measured with pill count was above 80%. No severe AEs occurred.

Contrary to previous observation with daily administration of low dose Tam, a weekly dose of 10 mg did not show a significant antiproliferative effect in premenopausal women. Likewise, Ral had a similar null effect on ki-67. ER and PgR were not significantly modulated by both drugs. Our results indicate that administration of weekly Tam and Ral at the standard dose and schedule did not change the proliferative index of breast cancer in premenopausal women with early BC. Evaluation of hormonal metabolism, growth factors (e.g., IGFs), ER/PgR coregulators, is ongoing and will help in elucidating these biological effects.

## Colon and Other Gastrointestinal Cancers

**A61 Pilot study of difluoromethylornithine (DFMO) in patients with Barrett's esophagus and dysplasia.** Frank A. Sinicrope,<sup>1</sup> Russell Broaddus,<sup>2</sup> Nina Joshi,<sup>3</sup> Bruce Morlan,<sup>1</sup> Ilan R. Kirsch,<sup>3</sup> Eugene Gerner,<sup>4</sup> Waun Ki Hong<sup>2</sup>. <sup>1</sup>Mayo Clinic, Rochester, MN; <sup>2</sup>MD Anderson Cancer Center, Houston, TX; <sup>3</sup>National Cancer Institute, Bethesda, MD; <sup>4</sup>Arizona Cancer Center, Tucson, AZ.

**Background:** Patients with Barrett's esophagus (BE) and dysplasia are ideal candidates for chemopreventive strategies to reduce cancer risk. Difluoromethylornithine (DFMO) is a promising chemopreventive agent that inhibits polyamine synthesis involved in the regulation of cell growth and differentiation. We conducted a pilot study to examine the effects of DFMO on esophageal histopathology, mucosal polyamine content, and gene expression.

**Materials and Methods:** Patients (n=10) with histologically confirmed BE and low grade dysplasia were enrolled in a single-arm study of DFMO (0.5 g/m<sup>2</sup>/d) given continuously for 6 months. Esophagoscopy with biopsies (4 quadrants every 1 cm) was performed at baseline, 3 and 6 months, and at 12 months (where available). Grading of dysplasia was performed by a GI pathologist blinded to clinical/biomarker data. Audiology was also performed. Mucosal polyamine content was measured by high-performance liquid chromatography, and analyzed using paired t-tests. Gene expression profiling was performed using a cDNA two-color chip, and was compared between baseline and 6 months. Expression data was normalized using the two-channel fastlo routine and differential expression was computed using a Wilcoxon signed-rank test.

**Results:** After 6 months of DFMO treatment, histopathological response data demonstrate regression of dysplasia (n= 1), stable disease (n= 8), and progression of dysplasia (n= 1). Within the stable category, 2 patients with extensive low grade dysplasia showed only focal dysplasia based upon 4 or more biopsies at 6 months. Histopathological improvements seen at 6 months were maintained at 12 months. Overall, DFMO was well tolerated. One patient had subclinical, unilateral ototoxicity. Modulation of polyamines (spermine, spermidine, putrescine, histamine) was shown only for putrescine which decreased with treatment (6 mo., p=0.07) and showed recovery to baseline at 12 months (p=0.01). Gene expression profiling revealed modulated genes of potential biological importance (*KLF5*, *KAL1*, *RFC5*, *TIMM8a*, *TC10*) and of these, Krüppel-like factor 5 (*KLF5*) was downregulated whereas the others were upregulated.

**Conclusion:** Further study of the chemopreventive effect of DFMO upon BE with dysplasia is suggested. DFMO treatment suppressed mucosal putrescine levels and downregulated *KLF5*, an important mediator of cell proliferation.

**A62 Magnesium, calcium, and colorectal adenoma recurrence: Results from a randomized trial.** Qi Dai,<sup>1</sup> John Baron<sup>2</sup>. <sup>1</sup>Vanderbilt University, Nashville, TN; <sup>2</sup>Dartmouth Medical School, Lebanon, NH.

**Background:** Mean magnesium intake in the US population is similar to that in East Asian populations with traditionally low risks of colorectal cancer and other chronic diseases, while the ratio of calcium to magnesium is much higher in the US population. It was recently reported that intakes of calcium or magnesium were associated with a reduced risk of adenoma or hyperplastic polyps only when dietary ratios of calcium to magnesium intake was low.

**Objective:** To test whether calcium supplementation reduces the risk of colorectal adenoma recurrence only when calcium/magnesium intake ratio is low. Design: The Calcium Polyp Prevention Study was a double-blind, placebo-controlled, randomized trial of 4 years of calcium supplementation (1000 mg) for the prevention of colorectal adenoma recurrence among 930 subjects. Participants underwent two follow-up colonoscopies, 1 year and 4 years after the qualifying exam. A validated semi-quantitative food frequency questionnaire was given at study entry and year 4 to obtain

subjects' usual diet over the previous year. The primary outcome in this analysis was the recurrence of adenomas during the main risk period (i.e. adenomas detected after the year 1 colonoscopy through the year 4 exam). This end point allowed for a latent period of calcium effect and minimized the number of adenomas overlooked at the qualifying colonoscopy.

**Results:** We found that dietary ratios of calcium to magnesium intake modified the effect of calcium treatment on adenoma recurrence (p for interaction: 0.075 for all adenoma and 0.046 for tubular adenoma during the main risk period). Calcium supplementation reduced the risk of adenoma recurrence only if the dietary ratio of calcium to magnesium intake was low before treatment and remained low during the treatment period. The RR (95%CI) was 0.68 (0.52-0.90) among those with the baseline ratio below the median in comparison to 0.98 (95%CI=0.75-1.28) for those above. Findings were similar when the intake ratio at year 4 was considered and when we repeated the analyses using adenomas detected during the full study period (e.g. including adenomas identified at year 1) and found very similar results. The sample size, however, became small when advanced adenomas were used as outcome; none of the associations were significant in the stratified analyses by calcium: magnesium ratio and the calcium effect did not differ substantially by ratio stratum (p for interaction, 0.575). We also found that high dietary intake of magnesium was related to a reduced risk of adenoma recurrence among subjects in the calcium treatment arm with a low ratio of calcium to magnesium intake; the RRs (95%CI) for any adenoma were 0.66 (0.39-1.13) and 0.58 (0.32-1.06) for the intermediate and the highest intake tertiles vs. the lowest intake tertile of magnesium (p for trend, 0.054). There was no association observed in the placebo arm.

**Conclusions:** We found that calcium supplementation reduced the risk of adenomas only among subjects with a low calcium:magnesium intake ratio. These findings, if confirmed, may provide a new avenue for the personalized prevention of colorectal cancer. *The abstract is on behalf of the Polyp Prevention Study Group.*

**PR-14 Regression of rectal polyps in familial adenomatous polyposis patients with freeze-dried black raspberries.** Gary D. Stoner,<sup>1</sup> Henrietta Hasson,<sup>2</sup> Christine L. Sardo,<sup>1</sup> Li-Shu Wang,<sup>1</sup> Dennis K. Pearl,<sup>1</sup> Anthony J. Buchta,<sup>3</sup> Carol A. Burke.<sup>2</sup> <sup>1</sup>The Ohio State University, Columbus, OH; <sup>2</sup>The Cleveland Clinic Foundation, Cleveland, OH; <sup>3</sup>Central Ohio Compounding Pharmacy, Columbus, OH.

This abstract is being presented as a short talk in Concurrent Session 15. A full abstract is printed in the Proffered Papers: Oral Presentation Abstracts section of the conference proceedings. (Presented on board number A63)

**A64 Ester-protected hydroxybenzyl phosphates (EHBP) inhibit the growth of various cultured human cancer cells: Evidence of a tissue type-independent effect.** Ravinder Kodela,<sup>1</sup> Mitali Chattopadhyay,<sup>1</sup> Niharika Nath,<sup>2</sup> Lucyna Z. Cieciora,<sup>2</sup> Liliya Pospishill,<sup>2</sup> Daniel Boring,<sup>3</sup> James A. Crowell,<sup>3</sup> Khosrow Kashfi.<sup>1</sup> <sup>1</sup>City University of New York Medical School, New York, NY; <sup>2</sup>City University of New York Medical School and New York Institute of Technology, New York, NY; <sup>3</sup>Division of Cancer Prevention, National Cancer Institute, Bethesda, MD.

**Introduction:** Nitric oxide-releasing nonsteroidal anti-inflammatory drugs (NO-NSAIDs) consisting of a conventional NSAID to which an NO releasing moiety -ONO<sub>2</sub> is covalently attached through a spacer have emerged as a new class of pharmaceutical agents. For several years we have been exploring the mechanism of action of NO-NSAIDs and in particular NO-aspirin (NO-ASA), with emphasis on their application to cancer. Our structure-activity studies with NO-ASA indicated that NO was pivotal for biological activity. However, careful re-examination regarding the contribution to the overall biological effect of each of the three structural components of NO-ASA, led to the surprising conclusion that the NO-releasing moiety was not required for the observed biological effects.

Rather, it was the spacer that was responsible for the biological actions of NO-ASA. The NO-releasing moiety was acting as a leaving group leading to the generation of a reactive quinone methide (QM) intermediate which behaved as a powerful electrophile. The ASA component made no biological contribution (BBRC 2007:358, 1096-1101). We therefore synthesized a series of ortho, para, and meta EHBPs, in which the -ONO<sub>2</sub> leaving group was replaced by a substituted phosphate as the leaving group and the ASA was replaced by an acetate. We also incorporated electron donating/withdrawing groups about the aromatic spacer in order to evaluate the effect of substitution on QM formation/stability and biological activity.

**Methods:** EHBPs as potential drugs: para-, ortho-, and meta-acetyloxybenzyl diethyl phosphate (Agents 1-3 respectively), p-acetyloxy-3-methylbenzyl diethyl phosphate (Agent 4), and p-acetyloxy-2-chlorobenzyl diethyl phosphate (Agent 5) were synthesized and purified at our lab with <sup>1</sup>H-NMR verifying the structures. In some experiments we also used NO-ASA for comparison. Cell lines: Human colon, breast, leukemia, and pancreatic cancers. Growth inhibition: colorimetric MTT assay kit. Cell cycle phase distribution: flow cytometry. Apoptosis: subdiploid (sub-G<sub>0</sub>/G<sub>1</sub>) peak in DNA content histograms.

**Results:** Agent 1 was very effective in inhibiting the growth of all cell lines with IC<sub>50</sub>s of 0.6-0.8 μM, in contrast, p-NO-ASA had significantly higher IC<sub>50</sub>s, 10-20 μM. Agent 2 was less potent than agent 1 in all cell lines with IC<sub>50</sub>s of 63-155 μM, presumably because of steric interference between the diethyl phosphate and the acetyloxy groups making it difficult for the QM to form. Agent 3 was the least effective, with IC<sub>50</sub>s of 162-451 μM, this agent is comparable in potency to m-NO-ASA, both of which do not form QMs. Agent 4 containing an electron donating group, was extremely potent, IC<sub>50</sub>s being 0.3-0.5 μM. Agent 5 containing an electron withdrawing group had slightly higher IC<sub>50</sub>s, 0.5-1.0 μM. Agent 1 induced dose-dependent apoptosis, which was significantly higher compared to p-NO-ASA. Agent 1 dose-dependently altered the distribution of the cells in the cell cycle, causing an arrest in G<sub>0</sub>/G<sub>1</sub>.

**Conclusions:** EHBPs inhibited the growth of various human cancer cells, indicating a tissue type independent effect. They exercise pleiotropic effects involving cell death as well as cell cycle phase transitions. These results raise the possibility that EHBPs possess chemotherapeutic activity against a wide variety of human cancers.

**A65 Identification of inflammation-associated plasma markers as response indicators in human colorectal cancer patients consuming freeze-dried black raspberries.** Roycelynn A. Mentor-Marcel,<sup>1</sup> Gerd Bobe,<sup>1</sup> Christine Sardo,<sup>2</sup> Li-Shu Wang,<sup>2</sup> Paul S. Albert,<sup>3</sup> Gary D. Stoner,<sup>2</sup> Nancy H. Colburn.<sup>1</sup> <sup>1</sup>NCI-Frederick, Frederick, MD; <sup>2</sup>Ohio State University Comprehensive Cancer Center, Columbus, OH; <sup>3</sup>National Cancer Institute, Bethesda, MD.

**Background:** Inflammatory cytokines have been reported as biomarkers of colorectal adenoma risk in humans, however, little is known of biomarkers of *response* to interventions that may be useful as preventive or therapeutic agents for colorectal cancer. Freeze-dried black raspberries given to colorectal cancer patients were previously shown to be efficacious in altering cellular parameters associated with colorectal tumor development (i.e. apoptosis, cell proliferation, angiogenesis). We hypothesized that berry-induced changes in these cellular responses would be associated with changes in plasma levels of inflammatory cytokines that may serve as measures of efficacy of a 2-4 week dietary intervention of berries in colorectal cancer patients.

**Methods:** 26 patients who had not received prior therapy consumed a slurry of black raspberries (20g in 100 ml drinking water) 3 times a day for 2-4 weeks. Plasma was collected before and after berry treatment and was analyzed for 9 inflammation-associated proteins.

**Results:** 2 of 9 inflammation-associated plasma proteins were increased (i.e. IL-1β, GM-CSF) with berry treatment. Whether these

## Colon and Other Gastrointestinal Cancers

diet-induced plasma changes correlate with changes in apoptosis, cell proliferation, and angiogenesis is being determined.

**Conclusion:** In summary, IL-1 $\beta$  and GM-CSF emerged as plasma proteins that were increased in colorectal cancer patients upon berry treatment. Changes in a subset of inflammation-associated plasma proteins may serve as response indicators to berry treatment under efficacious conditions and thus may be useful as a short term assay to monitor response to berry-based interventions for treatment of colorectal cancer.

## Head and Neck Cancers

**A66 Age-related differences in black raspberry modulated NF $\kappa$ B expression in oral squamous cell carcinoma patients.** Jeanette M. Ferguson, Thomas J. Knobloch, Christine L. Sardo, Lana K. Uhrig, Bruce C. Casto, Blake M. Warner, Kun Huang, Gulcin Ozer, David E. Schuller, Enver Ozer, Amit Agrawal, Christopher M. Weghorst. The Ohio State University, Columbus, OH.

**Background:** Black raspberries represent a food-based chemopreventive agent rich in polyphenolics compounds, including anthocyanins, that have been shown to reduce inflammation. Numerous studies have demonstrated that inflammation plays a prominent role in tumor growth, progression, and metastasis. Common risk factors for oral squamous cell carcinoma (OSCC), such as tobacco use, also contribute to the inflammatory process.

Cell culture and animal studies have shown altered gene expression in tumor and diseased tissues following exposure to lyophilized black raspberries (LBR) or their extracts. Consistently, these chemopreventive studies show the ability of LBR to modulate the expression of genes prominently associated with inflammation, including members of the NF $\kappa$ B family. In OSCC cells exposed to LBR extract in culture, we have shown a down-regulation of inflammatory biomarkers, including NF $\kappa$ B1D, NF $\kappa$ B1 and NF $\kappa$ B2. To transition these in vitro findings into an in vivo setting, we have used the hamster cheek pouch (HCP) model of OSCC to demonstrate a striking suppression of oral tumor lesion incidence and multiplicity (Anticancer Res 22, 2002) following dietary LBR treatment.

Ultimately, the role of these essential pre-clinical studies is to translate into a human patient-based clinical investigation. Consequently, the aim of our ongoing Phase 1 Clinical Trial is to evaluate the molecular changes in oral cavity tissues following short-term, locoregional, oral exposure to LBR troches. According to protocols approved by the IRB of The Ohio State University, biopsy-confirmed OSCC patients were administered three LBR troches 4x/day (4.3g cumulative dose) between pre-surgical enrollment and their normally scheduled surgery. Tissue biopsies were obtained from tumor and distant normal tissues at enrollment and during surgical resection, after an exposure range of 2.5-34 days. Using a partial interim cohort of patients, NF $\kappa$ B1 was used as a surrogate endpoint biomarker for inflammation in order to assess LBR-dependent gene expression changes.

**Methods:** TaqMan real-time PCR assays were used to examine NF $\kappa$ B1 gene expression in a partial interim cohort of 24 patients. Experimental gene expression levels were normalized to GAPDH, RPS18, B2M, HPRT, and ACTB "housekeeping" gene expression levels. The interim patient cohort was stratified into "young" ( $\leq$  45 years of age) and "older" ( $>$  45 years of age) groups for the purpose of this analysis.

**Results:** When patients were grouped into young ( $n = 7$ ) and older ( $n = 17$ ) populations, statistically significant differences were seen between LBR-modulated NF $\kappa$ B1 expression in tumor tissue compared to pre-treated, patient matched tumor tissue, with regard to age. Patients in the older population showed a significant decrease in NF $\kappa$ B1 expression in tumor tissue following LBR exposure compared to the younger population ( $p = 0.003$ ).

**Conclusions:** These results suggest that inflammation can play a prominent role in the development of OSCC in older patients, and that LBR

treatment may help alleviate the pathological effects of the inflammatory process in this population of patients. The lack of significant NF $\kappa$ B1 LBR-dependent modulation in the young OSCC population may signify that NF $\kappa$ B-independent or other functional pathways play an important role in tumorigenesis.

Support: NIH/NIDCR R21DE016361, ACS RSGT-06-126-01-CNE

## Pediatric Malignancies

**A68 Molecular response of pediatric chronic myeloid leukemia (CML) with imatinib mesylate therapy.** Jayasri Basak, Soma Mukhopadhyay, Sukanta Konar, Ashis Mukhopadhyay, Netaji Subhas Chandra Bose Cancer Research Institute, Kolkata, India.

**Background:** Childhood cases ( $<$ 18yrs of age) represent approximately 2% of all patients who develop chronic myeloid leukemia (CML). The disease in children is similar in behavior to that of adults, however the outcome of treatment with stem cell transplant is better in young individuals. The aim of our study was to see the molecular response of CML with imatinib mesylate therapy.

**Materials and Methods:** During the period from January 2003 to December 2007 we selected 56 cases of pediatric chronic myeloid leukemia who could not afford bone marrow transplant treatment in the pediatric Haemato-Oncology Department of Netaji Subhas Chandra Bose Cancer Research Institute, a tertiary cancer center from eastern India. All cases were Philadelphia chromosome and bcr/abl molecular transcript positive. The distribution of patients in the age group 1-5, 6-12 & 12-18 were 4 (7.14%), 15 (26.79%) and 37 (66.07%) respectively. The dose of imatinib mesylate (Veenat in Natco Pharma) given was 100 mg in 1-5 yrs, 200 mg in 6-12 yrs and 400 mg in older children. The maximum duration given was 5 years. bcr/abl molecular testing was done by RT-PCR method. In all patients it was done before diagnosis at 6 months, 1 year and then yearly up to 5 years.

**Results:** With median follow up of 25 months (range 4 months - 48 months), Twenty-five children (44.64%) showed complete molecular response. Sixteen patients (28.57%) showed complete hematological, partial cytogenetic and partial molecular response. Eleven patients (19.64%) showed partial haematological response only. Four patients (7.14%) died during treatment because of blast crisis.

**Conclusion:** Imatinib is a good curative drug in chronic myeloid leukemia in pediatric patients.

## Prostate and Other Genitourinary Tract Cancers

**A69 mRNA and microRNA profiling of the microenvironment in prostate biopsies: Valuable tool for risk assessment and prevention trials.** Larisa Nonn, Avani Vaishnav, Vijayalakshmi Ananthanarayanan, Lindsay Gallagher, Peter H. Gann. University of Illinois at Chicago, Chicago, IL.

Due to the widespread use of PSA testing, diagnosis of PCa typically relies on needle biopsies, which provide sparse, random sampling of the prostate. About 70-80% of these biopsies are negative for cancer, and the non-malignant tissue samples, which may contain valuable risk information, are routinely archived in paraffin after formalin fixation. Although RNA is degraded in formalin-fixed paraffin-embedded (FFPE) tissue, recent studies have shown that, with adequate attention to methodological detail, meaningful gene and microRNA (miRNA) expression data can be obtained from these specimens. We have optimized and validated several methods to overcome the challenges of working with prostate biopsies, which requires the ability to work with extremely limited amounts of material as well as the ability to isolate relatively homogeneous cell populations. miRNA signatures are rapidly being

identified for many cancers including prostate cancer. Compared to mRNAs, miRNAs are highly stable in FFPE tissue due their small size, and thus may be ideal biomarkers in prostate biopsies. Epithelial and stromal tissues were collected from FFPE prostate biopsies by laser capture microdissection (LCM) from three patients. Tissue was LCM-collected in a similar manner from paired frozen tissue from the same patients. Total RNA was isolated and the expression of mRNA and miRNAs analyzed by qRT-PCR (mention PreAmp). Expression of 7 epithelial or stromal-specific genes (K18, PSA, NKX3.1, AMACR, IGF1, TIMP3, Desmin) showed agreement between the FFPE-biopsies and the frozen tissue in all three patients. Normal and PCa tissue was available for two patients. Expression of three PCa-related genes (PCA3, AMACR and NKX3.1) also showed agreement between the FFPE-biopsies and frozen tissue. As predicted, PCA3 and AMACR were elevated and NKX3.1 decreased in PCa relative to normal areas. Analysis of three miRNAs reported to be down-regulated in PCa (miR-125b, miR-22, miR-16) showed that these miRNAs have higher expression in the stromal compartment. However, normal epithelium and PCa showed similar levels of miRNA expression. The difference between our methodology and the previously published reports for these miRNAs and PCa is that we collected the tissue by LCM. This suggests that previous findings showing decreased expression of these miRNAs in PCa may be an artifact resulting from decreased stromal tissue in the PCa lesions. This is the first study to directly compare RNA expression from FFPE and frozen tissues from the same patient and also the first study to examine miRNA expression in LCM-collected tissue from prostate biopsies. We show that with this methodology, RNA profiling of mRNA and miRNA from FFPE-biopsy material is quite feasible. More importantly, in the small set of samples, RNA expression in the FFPE-biopsy was reflective of the whole prostate and the relative expression was similar to frozen material. Our goal is to apply this methodology to archival prostate biopsy banks in order to identify RNA markers predictive of PCa. These methods will also facilitate measurement of RNA markers in prostate cancer prevention clinical trials in which pre and post-biopsy material has been collected.

### Other Organ Sites

**A71 Molecular trial for the action of a metal-based drug in HPV positive cervical cancer cells.** Priya Srinivas,<sup>1</sup> Rakesh Satheesh Nair,<sup>1</sup> Maliyeckal Ramakrishna Panicker Prathapachandra Kurup,<sup>2</sup> Mini Kuriakose<sup>2</sup>. <sup>1</sup>Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, India; <sup>2</sup>Cochin University of Science and Technology, Kochi, India.

Cervical cancer is one of the most prevalent female cancers in world. The treatment for cervical cancer includes chemotherapy. Most potential standard chemotherapeutic agents have side effects. A need-based approach for developing new anti cancer agents from metal compounds with fewer side effects will have enormous implications. Opportunities exist to exploit metal-based drugs in the discovery and development of pharmaceuticals against cancers. Vanadium is a transition metal widely distributed in the environment, which is also a dietary micronutrient. The antidiabetic and anticancer activities of the oxovanadium complexes are recently known. In this study, human cervical cancer cell line SiHa, which is HPV16 - positive has been employed to determine the anticarcinogenic property of two oxovanadium complexes, OVK 49 and OVK 89. Among these OVK 49 proved to be more effective than OVK 89. Both complexes inhibited the growth of SiHa cells in a concentration and time dependent manner. Treatment of cells with oxovanadium complexes caused loss of mitochondrial membrane potential ( $\Delta\Psi_m$ ) and morphological changes characteristic of apoptosis, such as the nuclear condensation. Moreover oxovanadium complex induced apoptosis, which involved release of mitochondrial cytochrome c and activation of caspase 8 and 9. The molecular mechanism behind the apoptotic induction due to these oxovanadium complexes is well established in this study. The E6

oncoprotein of human papillomaviruses has the potential to functionally antagonize p53. Here it has also been investigated whether this reflects the regulation of p53 expression in HPV positive cervical cells. It has also been found that p21 was efficiently induced and HPV E6, E7 p53 and GADD45 were decreased by OVK 49 and 89 in SiHa cells. These results suggests that inspite of the presence of E6 protein HPV 16 positive cervical cancer cell lines are capable of responding efficiently to DNA damage provoked by metal based drug treatment through a p53 dependent pathway.

**A72 HESA-A: Bright prospect for prevention and treatment of malignancies.** Amrollah Ahmadi. Cancer Research Center, Isfahan, Iran, Islamic Republic of Iran.

**Background:** Cancer is one of the leading causes of death in the world, particularly in developing countries that represents a tremendous burden on patients, families and societies. More than 70% of all cancer deaths occur in low and middle income countries, where resources available for prevention, diagnosis and treatment of cancer are limited or nonexistent. The effect of different types of drugs on quality of life and survival of end-stage cancer patients has been investigated, however many side effects of these drugs limited their usage. HESA-A is a compound of natural origin, consisting of rare elements and organic materials, which in several animal and cellular studies have shown powerful anticancer effects and less toxicity on normal cells. The aim of the present study was to investigate the efficacy and safety of HESA-A in the prevention and treatment of Osteosarcoma as a lethal malignancy entire the world.

**Methods:** In an experimental study, 40 rabbits were randomly allocated to one of two groups of administered HESA-A (study group) and placebo (control group) for 10 days. After this time, carcinogen agent was inserted under the periost for Osteosarcoma formation and bone radiography and magnetic resonance imaging were performed after three months. Also, cases were followed for 6 months.

**Results:** In study group, among 20 rabbits, pathological bone changes were not detected in 16 cases and after 6 months following-up, all cases were alive and no changes in their appetite and weight were observed. Besides, in placebo group, all cases suffered from Osteosarcoma. In the second stage, control cases were divided into two groups (10 rabbits were treated with HESA-A and 10 cases as non-treated group) and followed-up for 6 months. All non-treated cases were died, whereas treated cases were survived and their appetite and weight were improved.

**Conclusion:** This study proved the preventive and curative effects of HESA-A on Osteosarcoma and create a bright prospect for the future in the treatment of cancers.

## Familial and Genetic Epidemiology

## Epidemiology/Lifestyle Factors 1

## Familial and Genetic Epidemiology

**A74 Association between *FGFR2* SNPs, breast cancer risk factors, and clinical and pathological features of breast tumors in the Western New York Exposures and Breast Cancer (WEB) study.** Catalin Marian,<sup>1</sup> Jing Nie,<sup>2</sup> Amy Millen,<sup>2</sup> M. Trevisan,<sup>3</sup> M. Russel,<sup>3</sup> T. Nochajski,<sup>3</sup> Christine B. Ambrosone,<sup>3</sup> A. Hutson,<sup>3</sup> Stephen Edge,<sup>3</sup> Dominica Vito,<sup>2</sup> Peter G. Shields,<sup>1</sup> Jo L. Freudenheim.<sup>2</sup> <sup>1</sup>Georgetown University Medical Center, Washington, DC; <sup>2</sup>State University of New York at Buffalo, Buffalo, NY; <sup>3</sup>Roswell Park Cancer Institute, Buffalo, NY.

Previous observations indicate that *FGFR2* is involved in estrogen-related breast carcinogenesis and is amplified and overexpressed in breast cancer, the levels of expression being higher in ER-positive than ER-negative cell lines and tumors.

In two recent genome-wide association studies (GWAS), SNPs in intron 2 of *FGFR2* were associated with breast cancer risk with ORs of about 1.6. To date, 2 studies have found stronger associations of *FGFR2* SNPs with ER+, PR+, lower grade, node positive breast tumors and one study found a positive correlation with family history of breast cancer.

Here we investigated 4 SNPs (rs11200014, rs1219648, rs2420946, rs2981579) in intron 2 of *FGFR2* in order to replicate the GWAS findings and assess the association with breast cancer risk factors, and clinical and pathological features of breast tumors in a population-based case-control study, the Western New York Exposures and Breast Cancer study. Genomic DNA and SNP genotyping was performed by allelic discrimination real time PCR for 991 cases and 1698 controls. Demographics, reproductive history, lifetime alcohol consumption and smoking were queried by interview. Clinical and pathological characteristics of 803 tumors were abstracted from hospital records. Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI).

We confirmed the overall association of variant alleles of *FGFR2* intron 2 SNPs with breast cancer risk (OR 1.52, CI 1.20-1.94) as well as previously reported associations with ER+ (1.36, 1.11-1.67), PR+ (1.47, 1.19-1.83), lower grade tumors (1.57, 1.10-2.25) and family history of breast cancer (2.00, 1.50-2.67). We also found an association with negative node tumors in our sample set (1.29, 1.05-1.58). Additionally, we observed associations with Ki67- (1.33, 1.16-1.52), lower stage (1.42, 1.21-1.66) and smaller size (1.39, 1.21-1.59) tumors. Also, all four SNPs were associated with older age (1.96, 1.47-2.61), higher education (1.51, 1.17-1.94), higher postmenopausal BMI (1.53, 1.15-2.05), younger age at menarche (1.48, 1.05-2.08), lower number of births (2.55, 1.77-3.68), and previous benign breast disease (2.28, 1.76-2.95). Marginal association with alcohol drinking (OR 1.46, CI 0.92-2.32) and significant association with smoking status (OR 1.94, 1.37-2.75) were found.

This is the first study that comprehensively investigates interactions of *FGFR2* SNPs with breast cancer risk factors, and clinical and pathological features of breast tumors in relation to breast cancer risk. Our results support the hypothesis that *FGFR2* is involved in estrogen-related breast carcinogenesis, suggested by the association with ER+ tumors and reproductive history. Moreover, lifestyle exposures like alcohol and smoking seem to modulate the association of *FGFR2* SNPs and breast cancer, perhaps through estrogen related mechanisms as well, although further studies are needed to elucidate these mechanisms.

Supported by the following grants: DAMD-17-03-1-0446, DAMD-17-00-1-0417 and NCI-RO1CA92040

Marian C. was supported by an AVON-AACR International Scholar in Breast Cancer Research Award

**A75 DNA repair genotypes associated with benign breast disease in women at high breast cancer risk.** Timothy J. Jorgensen,<sup>1</sup> Kathy J. Helzlsouer,<sup>2</sup> Sandra C. Hoffman,<sup>3</sup> Judith Hoffman-Bolton,<sup>3</sup> Rosa M. Crum,<sup>3</sup> Kala Visvanathan.<sup>3</sup> <sup>1</sup>Georgetown University School of Medicine, Washington, DC; <sup>2</sup>Mercy Medical Center, Baltimore, MD; <sup>3</sup>Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

**Background:** Benign breast disease (BBD) is a risk factor for breast cancer and may have a heritable component. Little is known about genetic factors associated with BBD risk. Deficient DNA repair has been implicated in the etiology of breast cancer. Since DNA repair occurs early in the breast carcinogenesis pathway, it is logical that polymorphic forms of DNA repair genes may alter BBD risk. No prior studies have reported on the association between DNA repair variants and BBD.

**Methods:** We examined the association between a panel of DNA repair genotypes and BBD in a sub-cohort of women within CLUE II, a population based cohort study in Washington County, Maryland. The women were followed from 1989 to 2003 with biennial questionnaires from 1996 onwards eliciting information on many health outcomes, including physician diagnosis of BBD and breast biopsies. 3,212 women reporting no prior diagnosis of BBD at baseline were genotyped for non-synonymous SNPs in 8 DNA repair genes (ERCC2, ERCC4, ERCC5, RAD23B, XPC, XRCC1, XRCC2, and XRCC4) that had previously been implicated in cancer risk. BBD-free survival time was fitted to age-adjusted Cox proportional hazards model and analyzed by DNA repair genotype.

**Results:** Carriers of the variant alleles of XRCC1 codon 194 and ERCC4 codon 415 were significantly more likely to be diagnosed with BBD than non-carriers — hazard ratios (HR) = 1.36, 95%CI 1.06-1.74 and 1.39, 95%CI 1.09-1.76, respectively. The association between the ERCC4 variant and BBD was even greater among women with a positive family history of breast cancer. In this subgroup, the variant ERCC4 allele was associated with a 2.7 fold increase in breast cancer risk (HR = 2.63, 95% CI 1.52-4.66, p interaction = 0.02).

**Conclusion:** Variant ERCC4 and XRCC1 genotypes are significantly associated with a diagnosis of BBD in this population, particularly in women with a family history of breast cancer. These same genes have also been implicated in the risk of breast cancer in other studies. Taken together, these studies support the notion that these two DNA repair genes may have a common role in the etiology of both BBD and breast cancer.

**A76 Nuclear androgen receptor expression is linked to two SNPs in prostate tumor tissue.** Gregory L. Judson,<sup>1</sup> Jennifer A. Sinnott,<sup>1</sup> Jennifer R. Stark,<sup>1</sup> Kathryn Penny,<sup>1</sup> Meir J. Stampfer,<sup>1</sup> David J. Hunter,<sup>1</sup> Massimo Loda,<sup>2</sup> Michelangelo Fiorentino,<sup>2</sup> Stephen Finn,<sup>2</sup> Fredrick Schumacher,<sup>3</sup> Edward L. Giovannucci,<sup>1</sup> Lorelei A. Mucci<sup>1</sup>. <sup>1</sup>Harvard School of Public Health and Channing Laboratory, Boston, MA; <sup>2</sup>Dana-Farber Cancer Institute, Boston, MA; <sup>3</sup>Harvard School of Public Health and University of Southern California, Boston, MA.

**Introduction:** The androgen receptor (AR) plays a critical role in the pathogenesis of prostate cancer (PCa), and down-regulated expression in prostatic epithelium is linked to decreased differentiation and increased proliferation of tumors cells. Genetic variation in the AR gene, located on the X-chromosome, has previously been linked with PCa risk. Whether the effect of AR variants on cancer risk is explained through regulation of prostate AR expression is not well understood. This study examines whether six SNPs located in AR, as well as the CAG repeat, are related to expression of cytoplasmic and nuclear AR in prostate tumor tissue.

**Methods:** We genotyped 6 SNPs with minor allele frequencies greater than 5% located in a 310 kb region overlapping the AR gene and the CAG repeat in 270 PCa cases diagnosed among participants in the Health Professionals Follow-up Study and the Physicians Health Study. AR expression was assessed by immunohistochemistry in tumor tissue obtained from archival prostatectomy blocks. To determine if expression levels varied by genotype, we conducted an ANOVA analysis with AR expression modeled as the dependent variable.

**Results:** We found two SNPs that were significantly associated with AR nuclear expression. Men who carried the rs1337082 variant allele (18.2% of men) had reduced AR nuclear expression ( $p=0.03$ ) compared to wild-type in the non-adjusted analysis. In addition, men with the rs6152 variant allele (13.6% of men) also had decreased AR expression ( $p=0.04$ ) when adjusted for age at diagnosis. When adjusting for age, the association between rs1337082 and AR expression also became stronger ( $p=0.02$ ). The linkage disequilibrium  $R^2$  value was 0.63. Approximately 2% of the variation in AR expression can be explained by these two SNPs. The four other SNPs examined were weakly but not significantly associated with AR expression. CAG repeat length was not correlated with expression of AR.

**Discussion:** rs1337082 (approximately 40 kb downstream of AR) is an A/G polymorphism while rs6152 (exon 1) is a silent G/A polymorphism. The link between nuclear AR expression and these two non-coding SNPs provides evidence of novel genetic factors that may influence AR expression.

**A78 Common single nucleotide polymorphisms in immunoregulatory genes and multiple myeloma risk among women in Connecticut.** Kyoung-Mu Lee,<sup>1</sup> Baris Dalsu,<sup>1</sup> Yawei Zhang,<sup>2</sup> H. Dean Hosgood,<sup>2</sup> Meredith Yeager,<sup>1</sup> Shelia H. Zahm,<sup>1</sup> Sophia S. Wang,<sup>1</sup> Mark P. Purdue,<sup>1</sup> Stephen Chanock,<sup>1</sup> Tongzhang Zheng,<sup>2</sup> Lan Qing<sup>1</sup>. <sup>1</sup>National Cancer Inst, Bethesda, MD; <sup>2</sup>Yale School of Medicine, New Haven, CT.

**Background:** Increased risk of multiple myeloma has been observed among those who have a family history, suggesting a genetic component in multiple myeloma development. Also, a number of studies have shown that changes in immunoregulatory role of T cells may contribute to the development of multiple myeloma.

**Objective:** In light of the relationship between immune system dysregulation and multiple myeloma risk, we investigated whether genetic variation in 153 immune function genes are associated with multiple myeloma susceptibility in a population-based case-control study conducted among White Connecticut women.

**Methods:** Tagging single-nucleotide polymorphisms (SNPs;  $N=876$ ) were selected using a pairwise linkage-disequilibrium based algorithm. Odds ratios (ORs) and 95% confidence intervals (CIs) for SNP genotypes were estimated using unconditional logistic regression. Tests of association for gene regions were conducted using the minP test. We applied the false discovery rate (FDR) method to the minP test results as a means of

controlling for multiple comparisons. Haplotype analyses among subjects were performed using the haplo.stat statistical package in the R program. Haplotype frequencies were estimated from genotype data using the expectation-maximization algorithm while at the same time excluding those with frequencies less than 1%, and were evaluated by the global score test.

**Results:** Six out of 75 gene regions were significantly ( $P<0.05$ ) associated with multiple myeloma using the MinP test. However, only the *PTMS-LAG3-CD4-GPR162-LEPREL* gene region remained noteworthy after adjustment for multiple tests using the FDR method (min $P=0.0018$ ; noteworthy at a level of FDR control of 0.14). In this region, a total of six SNPs in two genes (*CD4* and *LAG3*) were significantly associated with risk ( $P_{\text{trend}}<0.05$ ), with the strongest association observed for rs11064392 (OR<sub>AG/GG</sub>=2.53, 95% CI=1.59-4.02;  $P_{\text{trend}}=0.0001$ ). Haplotype analyses also supported an association with rs11064392.

**Conclusion:** Our findings suggest that genetic variation in *CD4* may influence susceptibility to multiple myeloma.

## General Epidemiology and Biostatistics

**A79 Hormonal factors and breast cancer risk in nulliparous women.** Sara J. Schonfeld,<sup>1</sup> Ruth M. Pfeiffer,<sup>1</sup> James V. Lacey Jr.,<sup>1</sup> Patricia Hartge,<sup>1</sup> Michele M. Doody,<sup>1</sup> Robert T. Greenlee,<sup>2</sup> Yikyung Park,<sup>1</sup> Catherine Schairer,<sup>1</sup> Arthur Schatzkin,<sup>1</sup> Alice Sigurdson,<sup>1</sup> Kala Visvanathan<sup>3</sup>. <sup>1</sup>Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD; <sup>2</sup>Epidemiology Research Center, Marshfield Clinic Research Foundation, Marshfield, WI; <sup>3</sup>Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

**Background:** Nulliparous women are a growing population in the U.S. that is known to be at elevated risk for breast cancer. A term pregnancy is believed to exert a protective effect on the ductal structures in the breast through terminal differentiation. It is biologically plausible that limited ductal maturation in nulliparous women renders the breast more susceptible to the effects of hormonal factors.

**Methods:** We assessed the association between hormonal and other factors and breast cancer risk by parity among postmenopausal women. Data were combined from four prospective cohort studies [The Breast Cancer Detection Demonstration Project Follow-up Study, the NIH-AARP Diet and Health Study, The Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, and The United States Radiologic Technologist Study]. Each dataset was analyzed using multivariable Cox Proportional Hazards models to estimate hazard ratios (HR) and 95% confidence intervals (CI) separately for nulliparous and parous women for known risk factors. Study-specific results were combined using a meta-analytic random effects model.

**Results:** A total of 1,611 breast cancers were reported among 32,636 nulliparous women and 8,284 breast cancers among 207,763 parous women. The meta-analytic HRs for the one of the strongest risk factors, current use of menopausal hormone therapy for  $\geq 5$  years, were 1.46 (95% CI: 1.10-1.82) and 1.50 (95% CI: 1.42-1.59) for nulliparous and parous women, respectively. Patterns of risk were similar for nulliparous and parous women for other factors including ages at menarche and last menstrual period, body mass index, family history of breast cancer, and benign breast disease. Some study-specific variability was detected.

**Conclusions:** Similar associations between hormonal risk factors and breast cancer were observed for nulliparous and parous women. However, given their elevated baseline risk, these risk factors are likely to contribute to a greater absolute risk of breast cancer among nulliparous women.

**A80 Detection bias, statin drug use, and advanced prostate cancer risk.** Alison M. Mondul, Brian Caffo, Elizabeth A. Platz. Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

**Background:** Some previous prospective studies support that statin drugs (HMG Co-A reductase inhibitors) may protect against advanced prostate cancer. However, there is concern that these results may be due to detection bias. Men are likely to be screened for both elevated PSA and cholesterol by their internists, and thus are more likely to undergo both diagnostic workup for prostate cancer and be prescribed a statin. Therefore, men who take a statin may be less likely to be diagnosed with advanced prostate cancer, irrespective of a causal relation. Ideally, the possibility of this bias would be eliminated by studying the association between statin drugs and prostate cancer in an unscreened population, but because statins and PSA screening both became available at the same time (late 1980s), it is impossible to study this association in the pre-PSA era in the US. Thus, we performed a simulation to determine whether this source of detection bias is explanatory.

**Methods:** 3,000 datasets with 100,000 men without a prostate cancer diagnosis were simulated for two populations, one with a high (65%) and one with a low (15%) prevalence of PSA screening. For both populations we investigated three scenarios for the true association between statin use and advanced prostate cancer:  $RR=1.0$ ,  $0.75$ , and  $0.5$ ; in each we set the association for statin use and local disease to  $RR=1.0$ . We set the prevalence of statin use to 10% and varied the percentage of statins users who underwent PSA screening from 0 - 100%. We assumed the following: an annual prostate cancer incidence rate of 1%, a weighted average of the screened and unscreened men; a risk of prostate cancer diagnosis in screened men twice that in unscreened men; and an advanced stage at diagnosis in 20% and 40% of cases in screened and unscreened men, respectively.

**Results:** The observed association ( $RR_{obs}$ ) between statin use and local and total prostate cancer varied with the correlation between PSA screening and statin use. As PSA screening and statin use became more coincident, the  $RR_{obs}$  between statin use and both local and total prostate cancer was biased upward from the true null association. This bias was stronger in the population with a low prevalence of PSA screening: e.g., assuming that 70% of men using statins had PSA screening, the  $RR_{obs}$  for total and local prostate cancer, respectively, were 1.03 and 1.04 in the population with 65%, and 1.56 and 1.88 in the population with 15% prevalence of PSA screening. However, in all the simulated scenarios, there was very little bias in the  $RR_{obs}$  for statin use and advanced prostate cancer: e.g., assuming that 70% of men using statins had PSA screening and that the true  $RR$  for advanced prostate cancer was 1.0, the  $RR_{obs}$  for advanced prostate cancer was 0.98 in the population with a 65% prevalence of PSA screening, and 0.98 in the population with a 15% prevalence of PSA screening.

**Discussion:** Under the scenarios and assumptions we used, this simulation suggests that detection bias due to the correlation between PSA screening and statin use is unlikely to explain the inverse association observed between statin use and advanced prostate cancer in some US and European cohorts, but could account for the positive association for total prostate cancer that has been reported in some studies.

**A81 Cutaneous lymphoma incidence patterns in the United States: A population-based study of 3,884 cases.** Porcia T. Bradford, Susan S. Devesa, Jorge R. Toro. National Institutes of Health, Rockville, MD.

**Background:** Primary cutaneous lymphomas (CL) represent 27 % of all non-Hodgkin lymphoma (NHL) and are a diverse group of lymphoid neoplasms manifesting heterogeneous clinical, histologic, immunophenotypic, cytogenetic, and molecular features. Given their rarity and heterogeneity, cutaneous lymphomas present substantial diagnostic and therapeutic challenges. There have been no prior large population-based descriptive studies focusing on CL in the United States.

**Methods:** Using the Surveillance, Epidemiology and End Results (SEER) program, we analyzed CL incidence rates (IR) and patient relative survival rates according to race, gender, and histologic type using the 2005 criteria of the World Health Organization/European Organization for Research and Treatment of Cancer (WHO/EORTC) classification. Age-adjusted (2000 US standard) IR were calculated using the SEER\*Stat software public use program version 6.4.4 (Surveillance Research Program); IR were expressed as new cases per 1,000,000 person-years and were analyzed by age, gender, race, ethnicity, and year of diagnosis.

**Results:** 3,884 CL were diagnosed in 16 SEER registries during 2001 to 2005. Cutaneous T-cell lymphomas (CTCLs) were the most common group of cutaneous lymphomas (72%), while cutaneous B-cell lymphomas (CBCLs) accounted for 28% of all cutaneous lymphomas. IR for both cell types of CL and their histologic subtypes were all higher among males than females. Overall age-adjusted CL IR were highest among Non-Hispanic Whites and Blacks (11.5/1,000,000 person-years), intermediate in Hispanic Whites (7.9), and lowest among Asian/Pacific Islanders (7.2). CTCL IR were highest among Blacks, whereas the IR of CBCL overall and subtypes were highest among Non-Hispanic Whites and lowest among Blacks. The 5-year relative survival rates for patients with CTCL and CBCL were 87% and 89%, respectively. Patients with primary cutaneous diffuse large B-cell lymphoma-leg type had the poorest survival rates (50%).

**Conclusion:** CL incidence rates vary markedly by race, gender, and histological type, supporting the notion that the histologic variants of CL represent distinct clinical entities. Further investigations using large populations and molecular tools are warranted to elucidate the etiology of the diverse spectrum of cutaneous lymphomas.

**A82 Squamous cell carcinomas versus adenocarcinomas: Trends, mechanisms, and prevention.** Julia Kravchenko,<sup>1</sup> Igor Akushevich<sup>2</sup>.

<sup>1</sup>Duke Comprehensive Cancer Center, Durham, NC; <sup>2</sup>Duke University, Durham, NC.

Changing trends over recent decades in squamous cell carcinomas (SCCs) compared to adenocarcinomas (ACs) at various organs/sites have been reported in the U.S. and many other countries. The phenomenon has no clear explanation. This dynamic, however, is important for developing future cancer prevention strategies. Our aim is to study aspects important for general cancer prevention strategies: incidence trends of SCCs and ACs (for each cancer histotype, regardless of tumor site), and parameters characterizing differences in the mechanisms of ACs compared to SCCs.

**Data and Methods:** The most prevalent ACs and SCCs for 12 cancer sites were selected, and their time trends for age-adjusted incidence rates analyzed using SEER data for a 31-year period (1973-2003). Their age-specific incidence rates were analyzed using biologically motivated models of carcinogenesis, such as the Armitage-Doll model, two-stage clonal expansion model, and models with hidden frailty. In total, each of 186 sex-, race-, time period-, and histology-specific age patterns of incidence rates were fit to 10 models. The model providing the best fit for most age-patterns (i.e., frailty model with Weibull baseline and gamma-distributed frailty) was generalized to include parameters characterizing carcinogenesis (e.g., latency, number of stages of carcinogenesis, and others).

**Results:** The incidence of all SCCs analyzed (lung, esophageal and cervical) decreased. In contrast, the incidence of seven out of twelve ACs (lung, esophageal, hepatic, renal, breast, prostate, cervical) increased (e.g., ACs of lung and esophagus are now predominant over SCCs - incidence shifted about 15 years ago). Only two out of twelve ACs (uterine and gastric) decreased.

SCCs and ACs with corresponding time trends for cancer histotypes where checked for histotype-related similarity in underlying carcinogenesis mechanisms - expected differences were detected. The number of carcinogenesis stages did not differ for most ACs, regardless of cancer site, such as lung, stomach, esophagus, colon, rectum, pancreas, kidney, corpus uteri, and breast duct carcinomas. SCCs of lung, esophagus, and cervix

uteri did not differ from each other by number of carcinogenesis stages but differed substantially from ACs (SCCs had more stages than ACs).

**Conclusions:** Over the past decades, SCCs incidence continued to decline, while incidence of ACs, and their proportion compared to SCCs, increased. That may reflect changes associated with histotype-specific risk factors (e.g., smoking and HPV - for SCCs) occurring over the past several decades, including the effects of preventive strategies (e.g., anti-smoking strategies, screening effectiveness to detect precursor lesions). SCCs and ACs may differ not only by their time trends and associated risk factors, but by mechanisms these factors influencing tumorigenesis. Cancer risk factors have been clearly established for SCCs, but remain unclear for most ACs (e.g., obesity, diet, sex hormones, physical activity), making it more difficult to develop preventive strategies for ACs. Analyses of SCCs and ACs not only per site/organ, but also by histotype in general from various perspectives could be important to understand mechanisms of tumor development and thus planning of future preventive measures (e.g., risk factor intervention, screening, chemoprevention).

**A83 Organophosphate pesticides and sex steroid hormones in NHANES III.** Carol H. Christensen,<sup>1</sup> Sabine Rohrmann,<sup>2</sup> Nader Rifai,<sup>3</sup> Elizabeth A. Platz<sup>1</sup>. <sup>1</sup>Bloomberg School of Public Health, Baltimore, MD; <sup>2</sup>German Cancer Research Center, Heidelberg, Germany; <sup>3</sup>Harvard Medical School and Children's Hospital Boston, Boston, MA.

**Background:** Organophosphate pesticides (OP) may perturb hormone balance, and thus may affect men's health, e.g., prostate cancer. We evaluated whether men from the general population with higher urinary OP metabolites had higher or lower concentrations of sex steroid hormones than men with lower levels.

**Methods:** Concentrations of 3,5,6-trichloro-2-pyridinol (TCPY), a chlorpyrifos metabolite, and 4-nitrophenol (4-NP), a methyl parathion metabolite, previously were measured in spot urine for 1,338 men ages 20-59 who participated in the Third National Health and Nutrition Examination Survey (NHANES III). We measured serum concentrations of testosterone, estradiol, and sex hormone binding globulin (SHBG) for 1,637 men  $\geq$  12 years old who participated in the morning session of Phase I of NHANES III. 189 men participated in both sub-studies, forming the study population. Linear regression was used to estimate geometric mean hormone concentration by binary category of creatinine-corrected urinary OP concentration adjusting for age, US region, smoking, race, education, body mass index, and physical activity. We tested for trend across quartiles of TCPY and across 3 categories ( $<$  limit of detection (LOD), 2 categories  $\geq$  LOD) of 4-NP by entering a single ordinal term in the model with values at the category medians, the coefficient for which was evaluated by the Wald test.

**Results:** Mean testosterone (95% confidence interval) was significantly higher in men with higher TCPY ( $\geq$  median: 5.55 (4.95, 6.22)) compared with lower ( $<$ median: (4.93 (4.43, 5.48)). Although not statistically significant, mean estradiol and SHBG concentrations were also higher comparing higher to lower TCPY. There was no statistically significant difference in mean testosterone ( $<$ LOD: 5.44 (4.85, 6.10);  $\geq$ LOD: 5.14 (4.54, 5.83)) or estradiol ( $<$ LOD: 37.83 (34.88, 41.03);  $\geq$ LOD: 35.94 (32.94, 39.21)) between men with 4-NP concentration  $\geq$ LOD versus  $<$ LOD, although men with higher 4-NP tended to have lower hormone levels. SHBG did not differ by 4-NP category. None of the trends was significant ( $p>0.10$ ).

**Conclusion:** This cross-sectional study, the first in a general population of US adult men, suggests positive associations of chlorpyrifos with testosterone, estradiol, SHBG, and inverse associations of methyl parathion with testosterone and estradiol. These findings differ somewhat from those in occupationally-exposed groups. Given that a) our findings are compatible with the hypothesis that OPs influence hormones even at low exposure, b) OPs were available to consumers and commonly used, and c) a prior study observed a higher prostate cancer risk in commercial applicators who used an OP and had a family history of prostate cancer

(Am J Epidemiol 2003;157:800-14), future studies are warranted on OPs and men's health, including prostate cancer.

Funding: Maryland Cigarette Restitution Fund at Johns Hopkins

**A84 Grade- and stage-specific age incidence patterns of serous ovarian epithelial cancer among different racial and ethnic groups in the United States.** Rayna K. Matsuno,<sup>1</sup> Raluca Popovici,<sup>2</sup> Philip Grimley,<sup>3</sup> William F. Anderson,<sup>4</sup> Kala Viswanathan<sup>1</sup>. <sup>1</sup>Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; <sup>2</sup>George Washington University, Washington, DC; <sup>3</sup>Uniformed Services University of the Health Sciences, Bethesda, MD; <sup>4</sup>National Cancer Institute, Rockville, MD.

**Background:** Molecular and epidemiologic studies suggest that low- and high-grade serous ovarian epithelial cancers (sOEC) have distinct etiological pathways. However little is known about patterns in tumor grade and stage among different racial and ethnic groups in the US.

**Methods:** Data on sOEC (ICD-O-3 codes 8441, 8460-1) were obtained from the Surveillance, Epidemiology, and End Results 17-registry database (SEER17) for non-Hispanic Whites (NHW), Hispanic Whites (HW), African Americans (AA), and Asian Pacific Islanders (API), ages 20-84 years, for the period 2000-2005. Tumor stage was based on the American Joint Committee on Cancer Classification (AJCC) 6th edition, and was categorized as early (Stage I-II) and late (Stage III-IV). Tumor grade was categorized as low (well or moderately differentiated) and high (poorly differentiated or undifferentiated). Overall age-adjusted and age-specific rates of sOEC were calculated and stratified by race/ethnicity, stage, and/or grade using Poisson regression. Likelihood ratio tests were used to assess age interactions; age-specific incidence rate curves were smoothed using regression splines.

**Results:** There were a total of  $n=11,569$  cases of sOEC; of which 79.8% were NHW, 9.2% HW, 5.4% AA, and 5.3% API. The age-adjusted rate for high-grade sOEC was nearly three times the rate for low-grade sOEC (IRR=2.85). Within low-grade sOEC, the IRR=2.62 for late- to early-stage; within high grade sOEC, the IRR=6.67 for late- to early-stage. Age-specific rates were similar for all groups except HW. There was significant age interaction with tumor grade ( $p<0.001$  for both early and late stage), though within early-stage, the main effect of grade was non-significant ( $p=0.078$ ). When age-specific rates were stratified by stage and grade, there was a highly significant age interaction in WH compared to WHN (reference group) in the late-stage/high-grade stratum ( $p=2.37 \times 10^{-05}$ ).

**Conclusion:** Grade appears to play an important role in late-stage sOEC, particularly among WH. Though age incidence patterns by grade were generally similar among race/ethnicity groups in late-stage sOEC, the age incidence pattern for high-grade sOEC within late-stage was distinct for WH.

**A85 Incidence rates of exocrine and endocrine pancreatic cancers in the United States.** Jing Zhou, Lindsey Enewold, Kangmin Zhu. United States Military Cancer Institute, Washington, DC.

Pancreatic cancer is the most fatal of all major cancers with an incidence rate that approximates mortality. However, studies describing pancreatic cancer incidence, which may provide insight into its etiology, have been sparse particularly in terms of histology and tumor stage. The purpose of this study was to examine the incidence rates of exocrine and endocrine pancreatic cancers by age at diagnosis, gender, race/ethnicity and stage of the disease using data from the Surveillance, Epidemiology, and End Results (SEER) program of the National Cancer Institute from 1973 to 2005. Based on the data from nine SEER registries, the age-adjusted incidence rates, expressed per 100,000, of exocrine and endocrine pancreatic cancer were higher among males (8.18 and 0.24) than females (6.02 and 0.17), and the rates tend to be higher among Blacks (10.12 and 0.23) than Whites (6.78 and 0.20) and other races (6.01 and 0.16). The incidence rate for exocrine pancreatic cancer was generally stable over the 33 year period, whereas the incidence rate for endocrine pancreatic cancer

increased over time. This difference in trend by histology existed across age, gender and racial groups. The increase in endocrine tumors was observed for all stages of disease: localized, regional and distant. However, it was most prominent for localized tumors. Additional analysis showed similar trends for exocrine and endocrine tumors across four racial/ethnic groups (Non-Hispanic White, Hispanic White, Non-Hispanic Black, Asian and Pacific Islander) using data from 1992 to 2005 for thirteen SEER registries. While improvement in diagnosis may have contributed to the rising incidence of endocrine tumors, other factors may also be related.

## Obesity, Metabolism, and Cancer

**A86 Body fatness at young ages and risk of breast cancer throughout life.** Heather J. Baer, Shelley S. Tworoger, Susan E. Hankinson, Walter C. Willett. Brigham & Women's Hospital and Harvard Medical School, Boston, MA.

Obesity and weight gain during adulthood are associated with breast cancer risk. Recent studies suggest that body fatness at younger ages may be related to risk of breast cancer independently of adult adiposity, but this needs to be examined in further detail. We conducted a prospective analysis of body fatness during childhood and adolescence and risk of breast cancer in two established cohort studies, the Nurses' Health Study (NHS) and the Nurses' Health Study II (NHS II). Participants were 188,860 women who recalled their body fatness at ages 5, 10, and 20 using a validated 9-level pictogram, where level 1 represents the most lean and level 9 represents the most overweight. A total of 7582 cases of breast cancer were documented among these women during the follow-up period (1988-2004 for the NHS and 1989-2005 for the NHS II). Cox proportional hazards models were used to estimate relative risks (RRs) and 95% confidence intervals (CIs) for body fatness at each age and for average body fatness during childhood (ages 5-10) and adolescence (ages 10-20), adjusting for breast cancer risk factors. Initial analyses were conducted separately in the NHS and the NHS II; however, because none of the associations for body fatness at young ages differed significantly by cohort ( $P > 0.10$  for all tests of heterogeneity), the data were combined. Body fatness at each age was inversely associated with risk of both premenopausal and postmenopausal breast cancer, and there were no significant interactions with menopausal status ( $P > 0.10$ ). The multivariate RR for average adolescent body fatness  $\geq$  level 5.5 compared to level 1 was 0.54 (95% CI: 0.37-0.78,  $P$  trend  $< 0.0001$ ) for premenopausal breast cancer, and the comparable RR for postmenopausal breast cancer was 0.66 (95% CI: 0.53-0.82,  $P$  trend  $< 0.0001$ ). When current body mass index (BMI) was included in the multivariate models, the associations for premenopausal breast cancer were slightly attenuated (RR for average adolescent body fatness  $\geq$  level 5.5 compared to level 1 = 0.59, 95% CI: 0.41-0.86), whereas the associations for postmenopausal breast cancer became stronger (comparable RR = 0.60, 95% CI: 0.48-0.74). Among all women, adjusting for menopausal status as well as other risk factors, the multivariate RR for adolescent body fatness  $\geq$  level 6.5 compared to level 1 was 0.57 (95% CI: 0.37-0.87,  $P$  trend  $< 0.0001$ ). The inverse association for average adolescent body fatness was stronger for women who weighed less than 8.5 pounds at birth (multivariate RR for  $\geq$  level 4.5 compared to level 1 = 0.64, 95% CI: 0.56-0.73,  $P$  trend  $< 0.0001$ ) than for those who weighed 8.5 pounds or more (comparable RR = 0.89, 95% CI: 0.63-1.26,  $P$  trend = 0.05), and this interaction was statistically significant ( $P = 0.04$ ). In addition, the inverse association for average adolescent body fatness was somewhat stronger for estrogen receptor negative (ER-) than for ER positive (ER+) tumors; the multivariate RR for  $\geq$  level 4.5 compared to level 1 was 0.56 (95% CI: 0.42-0.76) for ER- and 0.72 (95% CI: 0.63-0.83) for ER+ tumors ( $P$  for heterogeneity = 0.03), although both trends were highly significant ( $P < 0.0001$ ). These findings confirm previous studies indicating that body fatness at young ages has a strong inverse association

with risk of breast cancer throughout life, and they suggest that body fatness at young ages acts through a different biologic pathway than adult BMI.

**A88 Body mass index, height, and risk of lymphatic malignancies: A prospective cohort study.** Leo J. Schouten,<sup>1</sup> Romana D. Pylpchuk,<sup>1</sup> R. Alexandra Goldbohm,<sup>2</sup> Harry C. Schouten,<sup>1</sup> Piet A. van den Brandt<sup>1</sup>.  
<sup>1</sup>Maastricht University, Maastricht, Netherlands; <sup>2</sup>TNO-Quality of Life, Leiden, Netherlands.

**Introduction:** Several studies have observed that body mass index (BMI) is associated with an increased risk of lymphatic malignancies (LM) or with one of its subgroups. Other studies have not observed an association, and the results are therefore inconclusive. Very few studies have investigated whether BMI around age 20 and height are associated with LM risk. We investigated therefore the association between BMI, BMI at age 20 and height and the risk of LM in a prospective cohort study.

**Methods:** In 1986, the Netherlands Cohort Study on Diet and Cancer (NLCS) was initiated. A self-administered questionnaire on diet and other risk factors for cancer was completed by 120,852 men and women, aged 55-69 at baseline. Data were processed and analyzed using the case-cohort approach, enumerating the cases for the entire cohort, and estimating the person years at risk in the cohort using a subcohort. This subcohort was randomly sampled from the entire cohort immediately after the baseline measurement and is being followed up for vital status. Cohort members who reported cancer at baseline were excluded from analysis. Follow-up for cancer was established by annual record linkages with the Netherlands Cancer Registry and the nationwide pathology registry. After 13.3 years of follow-up, data regarding from 1,042 LM cases and from 4,588 subcohort members were available for analysis.

Using the histology codes provided by the cancer registries and the abstracts provided by the pathology registry, the LM cases were subdivided into categories based on the third edition of the WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues. There were 232 cases of diffuse large-cell lymphoma (DLCL), 79 cases of follicular lymphoma (FL), 71 cases of Waldenström macroglobulinemia/ immunocytoma (WMI), 171 cases of chronic lymphatic leukemia (CLL), and 291 cases of multiple myeloma (MM). Other subgroups were too small ( $N < 70$ ) for analysis.

Rate ratios (RRs) were estimated using the Cox proportional hazards model, with multivariate adjustment for confounders.

**Results:** BMI at baseline was not associated with LM risk, the RR per increment of 4 kg/m<sup>2</sup> was 1.03 (95% confidence interval (CI): 0.94-1.13). RRs were not different between males and females. BMI at baseline was not associated with increased risk of any of the LM subtypes.

BMI around age 20 was associated with LM risk overall (RR per 4 kg/m<sup>2</sup> increment 1.13; 95% CI: 1.01-1.25), with DLCL risk (RR 1.20; 95% CI: 0.98-1.47) and WMI risk (RR 1.41; 95% CI: 1.01-1.95). The association between BMI around age 20 and LM risk was higher in males (RR per increment of 4 kg/m<sup>2</sup> increment: 1.22; 95% CI: 1.03-1.44) than in females (RR: 1.05; 95% CI: 0.91-1.21).

Height was positively associated with LM risk: the RR for 5 cm increment was 1.08 (95% CI: 1.02-1.15) for LM overall. The association between height and LM risk was higher in females (RR per increment of 5 cm: 1.14; 95% CI: 1.04-1.24) than in males (RR: 1.05; 95% CI: 0.89-1.13). For the DLCL subtype, the RR was 1.19 (95% CI: 1.07, 1.33).

**Conclusion:** The positive associations between both BMI at age 20, height and the risk of LM suggest that exposures during early life play a role in the etiology of LM.

**A89 Association of genes involved in lipid metabolism-peroxidation and risk of renal cancer in the Central European Renal Cancer Case-Control Study.** Lee E. Moore,<sup>1</sup> Paul Brennan,<sup>2</sup> Allison Meisner,<sup>1</sup> Rayjean Hung,<sup>2</sup> Sara Karami,<sup>1</sup> Philip S. Rosenberg,<sup>1</sup> Meredith Yeager,<sup>1</sup> Chanock Stephen,<sup>1</sup> David Zaridze,<sup>3</sup> Vsevolod Matveev,<sup>3</sup> Vladimir Janout,<sup>4</sup> Helena Kollarova,<sup>4</sup> Vladimir Bencko,<sup>5</sup> M. Navritalova,<sup>6</sup> Neonilia Szeszenia-Dabrowska,<sup>7</sup> Dana Mates,<sup>8</sup> Ivana Holcatova,<sup>5</sup> Paolo Boffetta,<sup>2</sup> Chow Wong-Ho,<sup>1</sup> Nathaniel Rothman<sup>1</sup>. <sup>1</sup>National Cancer Institute, Bethesda, MD; <sup>2</sup>IARC, Lyon, France; <sup>3</sup>CRC, Moscow, Russian Federation; <sup>4</sup>Faculty of Medicine, Olomouc, Czech Republic; <sup>5</sup>Faculty of Medicine, Prague, Czech Republic; <sup>6</sup>Faculty of Medicine, Brno, Czech Republic; <sup>7</sup>Faculty of Medicine, Lodz, Poland; <sup>8</sup>Faculty of Medicine, Bucharest, Romania.

Recent descriptive studies have reported increases in kidney cancer incidence since the 1970s in the United States and globally. Lipid peroxidation has been suggested as a unifying mechanistic pathway by which several known risk factors including obesity, hypertension, and chemicals directly damage cells of the proximal renal tubules and induce renal carcinogenesis. This study investigated the association between renal cell cancer (RCC) risk and variation in genes that modify the effects of lipid peroxidation, inflammation, and oxidative stress. We conducted a case-control study of RCC (987 cases and 1298 controls) from Central and Eastern Europe and analyzed genomic DNA for 635 single nucleotide polymorphisms (SNPs) thirty-eight candidate genes using an Illumina Oligo Pool-All (OPA). First, the minimum p-value permutation test (MINP) was used to identify genes that remained significant with an FDR<5%. Subsequently, a haplotype-based sliding window analysis of three consecutive SNPs was used to identify chromosome regions of interest that remained significant at a FDR<5%. Six genes were selected for in-depth analysis after multiple testing correction of the single marker associations: *APOE*, *GPX4*, *NOS2A* and *PTGS2*. The overall gene-level p-values for these genes were 0.017, 0.020, 0.055 and 0.069 using the MINP test, respectively. The minimum FDR-adjusted p-values in a sliding window haplotype analysis were 0.0005, 0.0007, 0.0002 and 1.09x10<sup>-5</sup>, respectively. For these regions, age-, sex- and center-adjusted haplotype relative risks were computed using the HaploStats package in R. After adjustment, a strong signal centered around the promoter region of *APOE* gene (rs405509) remained significantly associated with decreased risk of RCC compared to persons homozygous for the referent haplotype (OR=0.73 95% CI:0.59-0.91); global p=0.001). A second haplotype window spanning from IVS7+11 to IVS12-52 of the *NOS2A* gene that was significantly associated with increased risk (OR=1.36 (95% CI:1.05-1.78); p-global p=0.006). Additional haplotype windows spanning the transmembrane and tyrosine kinase domains of *INSR* (rs28601) and intron 2 of *LEPR* (rs970467) genes also were investigated further (FDR-adjusted p <0.05). After adjustment in Haplostats, two additional high risk haplotypes were identified that were significantly associated with risk. To our knowledge this is the first and largest study of RCC conducted to evaluate these genes in relation to RCC. Although replication and fine mapping studies will be required to confirm these findings, this study supports the hypothesis variation in genes influencing lipid metabolism/peroxidation may increase susceptibility to sporadic kidney cancer.

**A90 Anthropometry, cigarette smoking, alcohol consumption, and the risk of thyroid cancer in the U.S. Radiologic Technologists Study.** Cari Meinhold,<sup>1</sup> Elaine Ron,<sup>1</sup> Sara Schonfeld,<sup>1</sup> Bruce Alexander,<sup>2</sup> D. Michal Freedman,<sup>1</sup> Martha Linet,<sup>1</sup> Amy Berrington de Gonzalez<sup>1</sup>. <sup>1</sup>National Cancer Institute, Rockville, MD; <sup>2</sup>University of Minnesota School of Public Health, Minneapolis, MN.

Several case-control studies suggest that BMI may increase and cigarette smoking may reduce the risk of thyroid cancer, but results from prospective studies are limited. We investigated the associations between anthropometric factors, cigarette smoking, alcohol consumption and

thyroid cancer risk by following a cohort of 67,055 female and 20,557 male radiologic technologists in the United States from 1983 through 2006. Hazards ratios (HRs) and 95% confidence intervals (CIs) were calculated using proportional hazards models adjusted for age, sex, smoking status, and benign thyroid conditions, and where appropriate, height or BMI. After a mean follow-up of 15.8 years, 268 (229 female and 39 male) incident thyroid cancer cases were ascertained. Obesity (BMI  $\geq 30.0$  kg/m<sup>2</sup>) was associated with an increased risk of thyroid cancer (HR for women= 1.74, 95% CI 1.22, 2.49; HR for men= 2.27, 95% CI 1.00, 5.13) compared to BMI in the normal range (18.5-24.9 kg/m<sup>2</sup>). Compared to never smokers, current but not former smokers had a reduced risk of thyroid cancer (HR for women= 0.52, 95% CI 0.34, 0.79; HR for men= 0.28, 95% CI 0.08, 0.94). Alcohol consumption and smoking duration and intensity were not associated with thyroid cancer after adjustment for current smoking. The results were similar after restricting to confirmed (N=223, 83%) or papillary (N=191, 86%) thyroid cancers. We estimate that obesity may account for 9% of thyroid cancers in this cohort and 22% of incident thyroid cancers in the United States currently. In this prospective study, obesity increases and current cigarette smoking decreases the risk of thyroid cancer in both women and men.

**A91 Obesity and the *TMPRSS2:ERG* translocation in prostate cancer.** Lorelei A. Mucci,<sup>1</sup> Jing Ma,<sup>1</sup> Sven Perner,<sup>1</sup> Meir J. Stampfer,<sup>1</sup> Tobias Kurth,<sup>1</sup> Mark Rubin,<sup>2</sup> Philip W. Kantoff<sup>3</sup>. <sup>1</sup>Brigham and Women's Hospital, Boston, MA; <sup>2</sup>Weill Medical College of Cornell, New York, NY; <sup>3</sup>Dana Farber Cancer Institute, Boston, MA.

**Background:** The recently identified *TMPRSS2:ERG* translocation is an early and common somatic event in prostate cancer pathogenesis involving the *TMPRSS2* promoter, which is androgen regulated, and *ERG*, a member of the ETS family of transcriptional factors. Regulation of *TMPRSS2* by androgens is intriguing and suggests both a potential mechanism whereby a man's hormonal milieu could drive the progression of prostate tumor cells and also a mechanism by which androgen deprivation therapy may work. We explored the relationship of obesity, with its known relation with lower circulating testosterone and higher estradiol, on prostate cancer risk according to *TMPRSS2:ERG* fusion status.

**Methods:** The study was nested among 29,067 men in the prospective Physicians' Health Study (PHS) during 1982 to 2008. Information on obesity was collected through structured questionnaires at baseline and follow-up, and included total obesity (weight and body mass index, BMI) and central obesity (waist circumference) measures. We characterized the *TMPRSS2:ERG* fusion by fluorescence in situ hybridization on archival tumor tissue from 305 men diagnosed with incident prostate cancer during 25 years of follow-up. We applied logistic regression models to compare the association of obesity on relative risk of fusion positive and fusion negative prostate cancer.

**Results:** Almost half the men in PHS were overweight (BMI 25-26.9 kg/m<sup>2</sup>) or very overweight/obese (BMI 27 kg/m<sup>2</sup> or greater) at the start of follow-up. Prevalence of the *TMPRSS2:ERG* translocation among the men with prostate cancer was 40.0%, in line with other studies. The association of obesity on prostate cancer risk differed in relation to the translocation. Men with a higher BMI at baseline had a reduced risk of developing fusion positive prostate cancer (7% lower risk per increase in BMI, p for trend ~ 0.0021). Compared to healthy weight men, those who were very overweight/obese had a 60% lower risk of fusion positive prostate cancer (RR=0.43, 95% CI 0.22-0.83). Moreover, men with a larger waist circumference were at lower risk of developing fusion positive cancer (p for trend ~0.0017). In contrast, there was no association between total or central adiposity measures at baseline and risk of fusion-negative prostate cancer.

## Obesity, Metabolism, and Cancer

**Discussion:** Obesity appears to reduce the risk of prostate tumors that carry the TMPRSS2:ERG fusion, but is unrelated to risk of prostate cancers that occur via fusion-independent mechanisms. These preliminary data provide insight into the link between the hormonal milieu and the heterogeneity of prostate cancer.

**A92 Oxidative stress, obesity, and breast cancer risk: Results from the Shanghai women health study (SWHS).** . Qi Dai,<sup>1</sup> Yu-Tang Gao,<sup>2</sup> Xiao-Ou Shu,<sup>1</sup> Gong Yang,<sup>1</sup> Ginger Milne,<sup>1</sup> Qiuyin Cai,<sup>1</sup> Wangqing Wen,<sup>1</sup> Nathaniel Rothman,<sup>3</sup> Hui Cai,<sup>1</sup> Honglan Li,<sup>2</sup> Yongbing Xiang,<sup>2</sup> Wong-Ho Chow,<sup>3</sup> Wei Zheng<sup>1</sup>. <sup>1</sup>Vanderbilt University, Nashville, TN; <sup>2</sup>Shanghai Cancer Institute, Shanghai, China; <sup>3</sup>Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD.

**Background:** Increased reactive oxygen species may exhaust the antioxidant capability of human defense systems, leading to oxidative stress and cancer development. Urinary F<sub>2</sub>-isoprostanes, secondary end products of lipid peroxidation, are more accurate markers of oxidative stress than other available biomarkers. No prospective study has investigated whether levels of 15-F<sub>2t</sub>-Isop and its metabolite (15-F<sub>2t</sub>-IsopM) are related to breast cancer risk.

**Methods:** We conducted a nested case-control study within the Shanghai Women's Health Study, a population-based cohort study of 74,942 Chinese women between 40 and 70 years of age. Prediagnostic urinary 15-F<sub>2t</sub>-Isop and 15-F<sub>2t</sub>-IsopM were measured by gas chromatography-mass spectrometry for 436 breast cancer cases and 852 individually matched controls.

**Results:** Urinary excretion of isoprostanes was not significantly different between cases and controls. However, among overweight women, levels of isoprostanes were positively associated with breast cancer risk, which became stronger with increasing BMI. Among women with a BMI<sub>≥</sub>29, the odds ratio (OR) increased to 10.27 (2.41-43.80) for the highest compared to the lowest tertile of 15-F<sub>2t</sub>-IsopM (p for trend, 0.003; p for interaction, 0.0004). In contrast, 15-F<sub>2t</sub>-Isop and 15-F<sub>2t</sub>-IsopM were inversely associated with breast cancer risk among non-overweight women. Among women with a BMI<sub>≤</sub>23, breast cancer risk was reduced with increasing 15-F<sub>2t</sub>-Isop levels in a dose-response manner (p for trend, 0.006), with an OR of 0.46 (95%CI: 0.26-0.80) for the highest tertile versus the lowest (p for interaction, 0.006).

**Conclusion:** Our results suggest that the role of oxidative stress in breast cancer development may depend on adiposity and menopausal status.

**A93 Ethnic and anthropometric correlates of IGF axis in the United States.** Jessica M. Faupel-Badger,<sup>1</sup> David Berrigan,<sup>2</sup> Rachel Ballard-Barbash,<sup>2</sup> Nancy Potischman<sup>2</sup>. <sup>1</sup>National Cancer Institute, Cancer Prevention Fellowship Program, Bethesda, MD; <sup>2</sup>National Cancer Institute, Division of Cancer Control and Population Sciences, Bethesda, MD.

Insulin-like growth factor-1 (IGF-1) levels are positively related to some cancers and negatively related to cardiovascular disease. These conditions are also related to insulin resistance and high body weight leading to the hypothesis that IGF-1 levels may, in part, mediate the association of high body weight with these health outcomes. Using the National Health and Nutrition Examination Survey (NHANES) III population, we examined the associations between IGF-1, IGFBP-3, and the IGF-1/IGFBP-3 molar ratio with anthropometric measures in a large, United States population-based study where these associations could also be stratified by race/ethnicity and gender. The study population consisted of 3168 women and 2635 men who participated in NHANES III and provided a fasting morning serum sample. The study population was 44% non-Hispanic white, 28.2% non-Hispanic black, and 27.7% Mexican-American. On average, the female participants in the study were approximately 45 years old with a body mass index (BMI) of 26.3 while the male participants were approximately 43 years old with a BMI of 26.9. Linear regression models were used to

determine the associations of IGF-1, IGFBP-3 and IGF-1/IGFBP-3 molar ratio with anthropometric variables across race/ethnicity and gender. The anthropometric measures included height, weight, waist-to-hip ratio, waist circumference, sum of skinfolds, and percent body fat and were obtained by trained personnel in the NHANES mobile examination center. IGF-1 and IGFBP-3 were measured by staff at Diagnostic System Laboratories (DSL, Inc., Webster, TX). BMI, waist-to-hip ratio, and waist circumference were inversely associated with IGF-1 levels across all race/ethnicity and gender subgroups except for non-Hispanic black men. The magnitude of the regression coefficients differed across racial/ethnic groups. In contrast, very few anthropometric measures were significantly associated with IGFBP-3 levels. The exception to this is non-Hispanic black men, where all anthropometric measures except height were positively associated with IGFBP-3 levels. The IGF-1/IGFBP-3 molar ratio was inversely associated with all anthropometric measures, except height, in all subgroups of the population. In addition, when examining mean levels of biomarkers across tertile of BMI, Mexican-American men and women had the lowest levels of IGF-1 and non-Hispanic white men and women had the highest levels of IGFBP-3. For the IGF-1/IGFBP-3 molar ratio, non-Hispanic black men and women had the highest ratios across all tertiles of BMI. The racial and ethnic differences in the levels of IGF-1, IGFBP-3, and the IGF-1/IGFBP-3 molar ratio and longitudinal evaluation of their associations with anthropometric measures merit further investigation since IGF-1 levels have been hypothesized to be a contributor to many health outcomes. Our data are not consistent with the hypothesis that the association of high IGF-1 levels with health outcomes such as increased cancer risk is mediated through high BMI.

**A94 Racial/ethnic differences in the association between body composition/fat distribution and sex steroid hormones in men in NHANES III.** David S. Lopez. Johns Hopkins School of Public Health, Baltimore, MD.

**Background:** Body composition and fat distribution vary by race/ethnicity and these differences are thought, in part, to explain racial/ethnic differences in common chronic disease risk. We previously observed that obesity is associated with serum hormone concentrations adjusting for race/ethnicity. We also previously saw that serum testosterone was highest in Mexican-American (MA) compared with non-Hispanic black (NHB) and white (NHW) men, and estradiol concentration was highest in NHB compared with NHW and MA men. The present aim was to evaluate whether the association of body composition/fat distribution with sex steroid hormones varies by race/ethnicity in adult men.

**Methods:** We conducted a cross-sectional study in the Third National Health and Nutrition Examination Survey. We measured serum hormone concentrations in stored samples by immunoassay for 1,256 men aged 20+ years who participated in the morning examination session of Phase I. Weight, height, and waist circumference (WC) were measured during the physical examination. Percent body fat (%BF) was calculated from bioelectrical impedance, height, weight, and age. Body composition/fat distribution were categorized using combinations of overall (%BF >25%) and central (WC >102 cm) obesity: 1) obese (>25%)/obese (>102 cm), 2) obese (>25%)/non-obese (≤102 cm), 3) non-obese (≤25%)/obese (>102 cm), and 4) non-obese (≤25%)/non-obese (≤102 cm). Geometric mean hormone concentrations in the four categories were compared by race/ethnicity. We used linear regression to adjust for age, smoking, alcohol, physical activity, WC, %BF, and mutually for the hormones.

**Results:** In all racial/ethnic groups, men who were obese/obese, obese/non-obese, or non-obese/obese had lower testosterone concentrations than men who were non-obese/non-obese. However, lower testosterone was primarily associated with central obesity in MA men (see bolded values for patterns: ob/ob: 4.23, ob/n-ob: 5.62, n-ob/ob: 3.28, n-ob/n-ob: 6.19 ng/mL); with both central and overall obesity in NHB men

(ob/ob: 3.89, ob/n-ob: 4.64, n-ob/ob: 4.62, n-ob/n-ob: 5.70 ng/mL); and with central obesity in NHW men, which was more pronounced in men who had both overall and central obesity (ob/ob: 4.07, ob/n-ob: 5.16, n-ob/ob: 4.44, n-ob/n-ob: 5.82 ng/mL). Patterns were the same for free as for total testosterone: MA (ob/ob: 0.091, ob/n-ob: 0.116, n-ob/ob: 0.064, n-ob/n-ob: 0.117 ng/mL), NHB (ob/ob: 0.083, ob/n-ob: 0.094, n-ob/ob: 0.100, n-ob/n-ob: 0.110 ng/mL), and NHW men (ob/ob: 0.086, ob/n-ob: 0.104, n-ob/ob: 0.090, n-ob/n-ob: 0.110 ng/mL).

**Conclusion:** In this nationally representative study, the association of adiposity with sex steroid hormones varied by race/ethnicity in adult men. Specifically, WC influenced testosterone to a greater extent than %BF in MA men, whereas both % BF and WC influenced testosterone in NHB men, and WC had the greater influence especially in the presence of high %BF in NHW men. These findings have import in the analysis of adiposity and risk of cancer and other diseases by race/ethnicity.

**A95 Genetic variability in energy metabolism and pancreatic cancer risk: A population-based case-control study in Minnesota.** Jianjun Zhang,<sup>1</sup> Ishwori Dhakal,<sup>1</sup> Myron Gross,<sup>2</sup> Nicholas Lang,<sup>1</sup> Fred Kadlubar,<sup>1</sup> Susan Kadlubar,<sup>1</sup> Kristin Anderson<sup>2</sup>. <sup>1</sup>University of Arkansas for Medical Sciences, Little Rock, AR; <sup>2</sup>University of Minnesota, Minneapolis, MN.

Pancreatic cancer is one of the leading causes of cancer death in developed countries. Although the etiology of pancreatic cancer remains elusive, based on epidemiologic studies, obesity and type-2 diabetes appear to increase risk of this fatal disease. Therefore, it is possible that polymorphisms in genes involved in energy metabolism modulate pancreatic cancer risk. We investigated this hypothesis using a case-control study conducted during 1994-1998 in Minnesota. Cases (n=189), aged 20 years or older, were ascertained from all hospitals in the metropolitan area of the Twin Cities and the Mayo Clinic; from the later, only cases residing in the Upper Midwest of the US were recruited. Controls (n=486) were randomly selected from the general population and frequency matched to cases by age (within 5 years) and sex. Polymorphisms in four genes were genotyped: leptin (LEP, -2548G>A, rs7799039), leptin receptor (LEPR, Gln223Arg, rs1137101), and neuropeptide Y (NPY, Leu7Pro, rs16139) (regulating energy intake), and  $\beta$ 2-adrenoceptor (ADRB2, Gly16Arg, rs1042713) (regulating energy expenditure). Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated using unconditional logistic regression. After adjustment for age, sex, race, education, cigarette smoking, and alcohol use, a statistically significantly reduced risk of pancreatic cancer was observed in subjects who were heterozygous or homozygous for the NPY7Pro allele as compared with those who were homozygous for the NPY7Leu allele [OR (95%CI): 0.39 (0.15-0.89)]. The variant allele (16Arg) of ADRB2 was associated with a borderline significantly increased risk [OR (95%CI): 1.47 (0.99-2.21)]. No appreciable effects were detected for the LEP and LEPR sequence variants examined. The genetic associations described above were not markedly modified by physical activity or dietary intake of energy, fat, or fiber. This study offers novel data suggesting that genetic variability in the regulation of energy intake and expenditure influences pancreatic cancer risk.

**A96 Association of leptin with sex steroid hormones, insulin, and CRP among adult men in NHANES III.** Gabriel Y. Lai,<sup>1</sup> Sabine Rohrmann,<sup>2</sup> Nader Rifai,<sup>3</sup> Elizabeth A. Platz<sup>1</sup>. <sup>1</sup>Johns Hopkins Bloomberg Sch. of Public Health, Baltimore, MD; <sup>2</sup>German Cancer Research Center, Heidelberg, Germany; <sup>3</sup>Children's Hospital and Harvard Medical School, Boston, MA.

**Objective:** We previously observed an inverse association between leptin concentration and risk of prostate cancer, which was contrary to our hypothesis (AACR 2008). It is possible that the association between leptin - a hormone secreted by adipocytes - and prostate cancer or other cancers may be explained by confounding by other hormone and metabolic

biomarkers. To address this issue, we evaluated the association of leptin with sex steroid hormones, insulin, and C-reactive protein (CRP) among a nationally representative sample of US men in the Third National Health and Nutrition Survey (NHANES III).

**Methods:** 1149 men aged 20+ years and who attended the morning examination session of Phase I of NHANES III (1988-1991) were included. We measured serum concentrations of testosterone, androstenediol glucuronide (AAG) (a metabolite of dihydrotestosterone), estradiol, and sex hormone binding globulin (SHBG) by immunoassay. Insulin, CRP, and leptin were measured previously. Free hormones were estimated from measured total hormones, albumin, and SHBG. Height, weight and waist circumference were measured. Percent body fat was calculated from bioelectrical impedance and anthropometric data. Data on alcohol, smoking, and physical activity were obtained by interview. Using linear regression, we estimated geometric mean concentrations of hormones, insulin, and CRP by quartiles of leptin, applying sampling weights and adjusting for age, alcohol, smoking, physical activity, waist circumference, percent body fat and race/ethnicity.

**Results:** Men who had higher leptin concentrations had lower testosterone (Q1: 5.7, Q2: 5.3, Q3: 4.8, Q4: 4.7 ng/mL; p-trend = 0.02). Although the trend was not statistically significant, men who had higher leptin had lower free testosterone (0.109, 0.106, 0.098, 0.096 ng/mL; p-trend = 0.10). Men who had higher leptin had higher estradiol (34.7, 34.8, 36.4, 38.5 pg/mL; p-trend = 0.03), free estradiol (0.86, 0.89, 0.94, 1.01 pg/mL; p-trend = 0.007), and insulin (43.7, 50.1, 56.3, 60.0 pmol/L; p-trend = 0.0006) levels. Men in the lowest quartile of leptin had the highest SHBG; levels were the same in the upper 3 quartiles (37.7, 34.0, 33.1, 32.5 nmol/mL; p-trend = 0.14). Leptin did not appear to be associated with AAG or CRP. These patterns were similar within age strata.

**Conclusions:** Even after taking into account extent of body fat and other factors, leptin appears to be inversely associated with total and free testosterone but positively associated with estradiol, free estradiol, and insulin. In analysis of the independent association of leptin and prostate and other cancers, whether overall or by stage/grade, in addition to adiposity, adjustment for other hormonal and metabolic correlates of leptin may also be necessary.

**A97 Obesity in relation to serum folates, plasma B12 and homocysteine in premenopausal African American women.** Somdat Mahabir,<sup>1</sup> Michele R. Forman,<sup>1</sup> Richard Hajek,<sup>1</sup> Sanjay Shete,<sup>1</sup> Yong Q. Dong,<sup>1</sup> Abenaa M. Brewster,<sup>1</sup> Beverley J. Gor,<sup>1</sup> Christine M. Pfeiffer,<sup>2</sup> Lovell A. Jones<sup>1</sup>. <sup>1</sup>UT M. D. Anderson Cancer Center, Houston, TX; <sup>2</sup>Centers for Disease Control and Prevention, Atlanta, GA.

**Background:** Lower folate status is a risk factor for cardiovascular disease and certain cancers. Since pre-menopausal African American women have high rates of obesity, breast and colorectal cancers, understanding the link between obesity and folate metabolic status offers a mechanistic insight into the obesity-cancer connection.

**Objective:** We assessed the associations between serum total folate, 5-methyltetrahydrofolic acid (5MeTHF), pteroylmonoglutamic acid (PGA), 5-formyltetrahydrofolic acid (5FoTHF), tetrahydrofolic acid (THF), plasma vitamin B12, and total homocysteine concentrations and obesity in pre-menopausal AA women (n=113).

**Design:** This was a cross sectional study conducted at the baseline segment of a dietary intervention study in which fasting samples were used for folate, vitamin B12, and homocysteine measurements.

**Results:** In multivariable analysis, serum total folate concentrations were 14.5% lower in the overweight (BMI 25-29.9) and obese (BMI 30-39.9) women, and 25% lower in the extremely obese (BMI  $\geq$ 40) compared to normal weight (BMI  $\leq$ 24.9) women (p-trend=0.08). For 5MeTHF, the major form of folate in circulation, overweight women had 15.2% lower, obese women 15.7% lower, and extreme obese women 26.9% lower concentrations than normal weight women (p-trend=0.06). Also, the

## Obesity, Metabolism, and Cancer

overweight and obese women were more likely to be in the lower 50<sup>th</sup> percentile of serum total folate (p-trend=0.03) and 5MeTHF (p-trend=0.08) concentrations compared to the normal weight women. There were no significant associations between BMI and the minor folate species, plasma B12, and homocysteine.

**Conclusions:** With obesity at epidemic levels, these data, if confirmed by prospective or randomized controlled studies, have important public health implications.

## Tobacco and Cancer

**A98 Cigarette smoking, environmental tobacco smoke exposure, and pancreatic cancer risk in the European Prospective Investigation into Cancer and Nutrition.** Alina Vrieling, H. Bas Bueno-de-Mesquita, Hendrick C. Boshuizen, For the EPIC study group. National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands.

Cigarette smoking is an established risk factor for pancreatic cancer. However, prospective data for most European countries are lacking, and epidemiologic studies on exposure to environmental tobacco smoke (ETS) in relation to pancreatic cancer risk are scarce.

We examined the association of cigarette smoking (current and lifetime intensity, duration, pack-years, time since quitting) and exposure to ETS with pancreatic cancer risk within the European Prospective Investigation into Cancer and Nutrition (EPIC). This analysis was based on 465,910 participants, including 524 first incident pancreatic cancer cases diagnosed after a median follow-up of 8.9 years. Estimates of risk were obtained by Cox proportional hazard models and adjusted for weight, height, and history of diabetes mellitus.

Current cigarette smokers had an elevated risk of pancreatic cancer compared to never smokers (hazard ratio (HR) = 1.71, 95% confidence interval (CI): 1.36, 2.15), and risk increased with greater intensity and pack-years. Former cigarette smokers who quit for less than 10 years were also at greater risk of pancreatic cancer (HR = 1.44, 95% CI: 1.06, 1.95), but risk was comparable to never smokers after quitting for 10 years or more. Further, pancreatic cancer risk was increased among never smokers daily exposed to ETS (for many hours) during childhood (HR = 2.60, 95% CI: 0.95, 7.06) and exposed to ETS at home and/or work (HR = 1.54, 95% CI: 1.00, 2.39).

These results from a large European prospective cohort suggest that both active cigarette smoking, as well as exposure to ETS, is associated with increased risk of pancreatic cancer.

**A99 A prospective cohort study on the associations between alcohol consumption and smoking and risk of subtypes of esophageal and stomach cancer.** Jessie Steevens,<sup>1</sup> Leo J. Schouten,<sup>1</sup> R. Alexandra Goldbohm,<sup>2</sup> Piet A. van den Brandt<sup>1</sup>. <sup>1</sup>Maastricht University, Maastricht, Netherlands; <sup>2</sup>TNO Quality of Life, Leiden, Netherlands.

**Background:** Previous studies have suggested that the etiology of histological subtypes of esophageal cancer and of topographical subtypes of stomach cancer may differ. It was found that risk of esophageal squamous cell carcinoma (ESCC) was strongly associated with alcohol consumption and tobacco smoking. However, esophageal adenocarcinoma (EAC) and gastric cardia adenocarcinoma (GCA) had no association with alcohol and a much weaker association with smoking. Because most studies were cases control studies, and only few cohort studies have been performed, the aim of our study is to investigate these associations within a large-scale prospective cohort study.

**Methods:** The Netherlands Cohort Study was initiated in 1986, when participants (58,279 men and 62,573 women) completed a self-administered questionnaire on risk factors for cancer, including alcohol consumption and smoking habits. Follow-up for incident cancer cases was

established by record linkages to the pathology registry and cancer registries. After 16.3 years, we identified 120 ESCC cases, 168 EAC cases and 187 GCA cases. For reasons of efficiency, we used a case-cohort approach, for which a subcohort (n = 4,438) was randomly sampled from the cohort at baseline. Multivariable Cox proportional hazards models estimated incidence rate ratios (RR) and corresponding 95% confidence intervals (95% CI). We corrected for age, sex, alcohol consumption and smoking status, frequency and duration. The following confounders were added if they changed the RR by >5%: fruit consumption, vegetable consumption, energy intake, non-occupational physical activity, BMI, and socioeconomic status.

**Results:** The multivariable RRs associated with alcohol consumption were 1.30 per 10 g ethanol/day increment (95% CI 1.20-1.42, p<0.001) for ESCC, 1.00 (95% CI 0.90-1.12, p=0.98) for EAC and 0.98 (95% CI 0.89-1.08, p=0.74) for GCA.

The RRs (95% CI) of ESCC for former smokers and current smokers were 1.39 (0.75-2.59) and 2.65 (1.49-4.70), respectively (p-trend<0.001), when compared with never smokers. For EAC, these figures were 1.38 (0.86-2.20) and 1.57 (0.95-2.58), respectively (p-trend=0.09) and the RRs for GCA were 1.35 (0.83-2.19) and 1.56 (0.94-2.56), respectively (p-trend=0.09). When we made further adjustments for frequency and duration of smoking in an analysis excluding never smokers, the differences between current and former smokers became smaller for all three tumors. The frequency of smoking was statistically significantly associated with ESCC and EAC, independently of smoking status and duration. The RRs per 10 cigarettes/day increment were: 1.19 (95% CI 1.01-1.40, p=0.04) for ESCC and 1.23 (95% CI 1.06-1.42, p=0.01) for EAC. No statistically significant association was found with GCA (RR=1.07, 95% CI 0.91-1.25, p=0.40). Smoking duration was not statistically significantly associated with ESCC (RR=1.10, 95% CI 0.93-1.31, p=0.25), EAC (RR=1.03, 95% CI 0.90-1.19, p=0.65) or GCA (RR=1.14, 95% CI 0.98-1.33, p=0.09), after adjustment for smoking status and frequency.

**Conclusions:** The results of this cohort study suggest that alcohol and cigarette smoking are differently associated with ESCC than with EAC and GCA.

**A100 Histological classification of primary lung cancer in relation to age, gender, and types of tobacco smoking.** Mulazim H. Bukhari, Muhammad Riaz Hussain, Samina Naeem. King Edward Medical University, Lahore, Pakistan.

**Background:** According to available data the lung cancer remains the leading malignancy amongst males showing a strongest association with smoking. The study was conducted to analyze the histological classification of primary lung cancer in relation to age, gender and types of Tobacco Smoking

**Methods:** The study was conducted on randomly selected 300 patients of 10-90 years age from Ghulab Devi Chest Hospital Lahore. Light microscopy was used for histopathological classification on the sections stained with haematoxylin and eosin. Special (PAS and Alcian blue) stains were used for subclassification. Literature for WHO classification was reviewed.

**Results:** The primary malignancy of lung was more common in males (male to female ratio was 5.7:1) and in old ages with mean age was 54.17±3.46 years. Significantly large numbers of patients (70%) were smokers. The trend of smoking is changing in Pakistan from old traditional hukka to cigarette. A strong association of smoking was found between small cell carcinoma (SCC), and squamous cell carcinoma (SQCC), and Large cell carcinoma (LCC), (P<0.02, P<0.001 and p<0.02 respectively). No significant difference was seen between smokers and non smokers harboring adenocarcinoma (ADC; P>0.05). According to the WHO classification, SQCC (43%) was found to be the commonest histological variant and SCC (22%) was second in the frequency. The frequency of ADC, LCC and others variants was 18%, 11% and 5% respectively.

**Conclusion:** WHO criteria are the better ways to classify the lung carcinoma with light microscopy and provide a potential widespread and worldwide application of this classification system. A strong association of lung cancer was found with age, sex, and all types of smoking and histological variants.

### Other Epidemiology/Lifestyle Factor Studies

#### A101 Assessment of thyroid carcinoma in long standing goiters. Manish Kaushal. M.G.M. Medical College, Indore, India.

**Aim of Study:** To assess relation of undifferentiated thyroid carcinoma or poorly differentiated thyroid carcinoma with duration of goiters

**Patients and Methods:** Retrospective analysis (Jan 03- Dec 2007) of 116 thyroid carcinoma patients was done. 19 cases found with history of long standing goiter and sudden increase in size of goiter. 12 females & 7 males with mean age was 54.8 yrs (41-70 yrs). On clinical profile, duration of long standing goiter was 10.5 yrs (5 - 34 yrs) and mean rapid increase in size of goiter was 4.8 months (2- 7 months). All cases were euthyroid. Gland status was grade 3 goiter in all cases. Goiter was on both sides in 11 cases. Retrosternal extension present in 7 cases and cervical lymphadenopathy were present in 12 cases. One patient presented with respiratory distress. History of previous surgery was noted in 3 patients. On Fine Needle Cytology poorly differentiated, anaplastic, papillary, follicular neoplasm and medullary were noted in 5, 8, 3, 2 and 1 cases respectively.

On radiological investigations calcifications in goiter was noted in 89.5% cases and IJV thrombus/infiltration was seen in 36.8%. Distant metastases in lung and skeletal region were 42.1 and 15.8% cases. Majority of cases were unresectable and biopsy were done in 9 cases. On histopathology anaplastic carcinoma was noted in 9 cases and poorly differentiated carcinoma in 6 cases.

**Conclusion:** Carcinomas developing in a long standing goiter with history of rapid increase in goiter size in an iodine deficient region have presented a very advanced stage and often unresectable.

### Other Molecular Epidemiology Studies

#### A102 Men with low serum cholesterol have a lower risk of high-grade prostate cancer in the Prostate Cancer Prevention Trial. Elizabeth A. Platz,<sup>1</sup> Cathée Till,<sup>2</sup> Phyllis J. Goodman,<sup>2</sup> Howard L. Parnes,<sup>3</sup> William D. Figgis,<sup>3</sup> Demetrius Albanes,<sup>3</sup> Alan Krista<sup>2</sup>. <sup>1</sup> Johns Hopkins University, Baltimore, MD; <sup>2</sup>Fred Hutchinson Cancer Research Center, Seattle, WA; <sup>3</sup>National Cancer Institute, Rockville, MD.

**Objectives:** Several prospective studies suggest that use of cholesterol-lowering statin drugs is inversely associated with advanced and possibly high-grade prostate cancer. A nested case-control study investigating low circulating cholesterol as a possible mechanism underlying these findings reported that men with a lower cholesterol concentration had a lower risk of high-grade and possibly advanced prostate cancer (Int J Cancer 2008;123:1693-8). Given these findings, we investigated the association of low serum cholesterol with prostate cancer overall and by histologic grade in the Prostate Cancer Prevention Trial (PCPT). Unlike standard cohort studies, men in the PCPT underwent PSA screening annually, cases were biopsy detected, diagnoses and Gleason sum determinations were confirmed centrally, and controls were men who were biopsied at the end of the trial per trial protocol irrespective of indication.

**Methods:** We conducted a prospective cohort study of 5,616 men aged 55+ years old randomized to the placebo arm of the PCPT between 1993 and 1996 and who had serum cholesterol measured at entry. By the end of follow-up, 1,251 cases were confirmed. Serum cholesterol concentration was measured enzymatically. We used logistic regression to calculate the multivariable odds ratio (OR) of total, organ-confined, and Gleason sum  $\geq$

8 (n=59), 7 (n=199), and < 7 (n=993) prostate cancer comparing low (normal: < 200 mg/dL) to high (borderline and elevated cholesterol:  $\geq$  200 mg/dL) serum cholesterol.

**Results:** Men with low cholesterol had a lower risk of Gleason sum  $\geq$  8 prostate cancer (OR=0.41, 95% confidence interval (CI) 0.22-0.77; p-trend across quintiles of cholesterol = 0.01) compared with men with high cholesterol; this association was stronger after restricting to organ-confined disease (OR=0.32, 95% CI 0.15-0.66, p-trend < 0.001). No association was present for prostate cancer overall (OR=0.97, 95% CI 0.85-1.11, p-trend=0.43), or Gleason < 7 (OR=1.03, 95% CI 0.89-1.18, p-trend=0.94) or 7 (OR=0.93, 95% CI 0.69-1.24, p-trend=0.49) disease.

**Conclusion:** These prospective findings from the PCPT, in which the opportunity for detection of high-grade prostate cancer was similar because all men underwent biopsy, suggest that low cholesterol may protect against the development of high-grade prostate cancer. The contemporary body of literature on statins, cholesterol, and prostate cancer suggests that cholesterol metabolism should be investigated further in the etiology of prostate cancer that has a worse prognosis.

**Funding:** NCI/NIH P01 CA108964 (Biology of the PCPT). The content of this work is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

#### A103 Risk of breast cancer with plasma prolactin concentrations by phosphorylated STAT3 and STAT5 status. Shelley S. Tworoger,<sup>1</sup> Megan S. Rice,<sup>1</sup> Nichole A. Belsley,<sup>2</sup> Sarah Walker,<sup>3</sup> Laura C. Collins,<sup>2</sup> Susan E. Hankinson,<sup>1</sup> David A. Frank<sup>3</sup>. <sup>1</sup>Brigham and Women's Hospital, Boston, MA; <sup>2</sup>Beth Israel Deaconess Medical Center, Boston, MA; <sup>3</sup>Dana Farber/Harvard Cancer Center, Boston, MA.

Prolactin, a lactogenic hormone, has been associated with risk of breast cancer in premenopausal and postmenopausal women. Prolactin may act by influencing transcription factors in the Signal Transducer and Activator of Transcription (STAT) pathway. In particular, STAT3 and STAT5 appear to be important in breast carcinogenesis; prolactin is known to activate STAT5 via phosphorylation. To determine whether prolactin may act through STAT signaling, we examined whether prediagnostic prolactin levels were differentially associated with risk of breast tumors based on their expression of phosphorylated STAT3 (pSTAT3) and STAT5 (pSTAT5). Tissue microarrays (TMAs) were constructed from 3,093 breast cancers from women enrolled in the Nurses Health Study. Of these, 443 cases had matching plasma prolactin levels drawn at diagnosis; one or two controls were matched to each case by age, menopausal status/hormone use, and characteristics of the blood draw. Immunohistochemical studies with pSTAT3 and pSTAT5 were performed on the TMAs. Tissue cores were scored from 0-3 based on intensity and quantity of nuclei staining. Relationships between expression of pSTAT3 or pSTAT5 and clinicopathologic features were examined by Chi-squared tests. Polytomous logistic regression was used to evaluate the relationship between prolactin and risk of breast tumors by staining expression, adjusting for matching factors and breast cancer risk factors. Interpretable tissue cores were available for 309 subjects. Of these, 195 (63%) and 114 tumors (37%) showed negative/weak (0-1) and moderate/strong staining (2-3) respectively for pSTAT3. 178 (58%) and 131 (42%) tumors showed negative/weak and moderate/strong staining respectively for pSTAT5. Absence of expression of pSTAT3 was significantly associated with high grade (p=0.04) and invasiveness (p=0.04). Though not statistically significant, pSTAT3- status also was associated with lymph node involvement and larger tumor size. pSTAT5 was not associated with other tumor characteristics. Prolactin levels were similarly associated with pSTAT3+ (RR, top vs. bottom quartile=1.7, 95% CI=1.1-2.8) and pSTAT3- tumors (comparable RR=1.8, 95%CI=1.0-3.3). However, high versus low prolactin levels were more strongly associated with pSTAT5+ tumors (RR=2.3, 95%CI=1.3-4.2) compared to pSTAT5- tumors (RR=1.5, 95%CI=0.9-2.4), although the p-heterogeneity was not statistically significant (p=0.28). Our findings

suggest that prolactin may influence breast cancer risk via activation of STAT5. On-going analyses are exploring the combined role of pSTAT3 and pSTAT5 presence as well as other hormones thought to modulate or reflect STAT activity including IGFs, CRP, and adiponectin.

**A105 Genetic variations in sonic pathway genes as predictors of bladder cancer risk.** Jessica N. Clague, Jie Lin, Joshua Chang, Michelle Hildebrandt, Julie Izzo, Hushan Yang, Ashish Kamat, Xifeng Wu. M.D. Anderson Cancer Center, Houston, TX.

**Background:** The hedgehog (Hh) signaling pathway plays a crucial role in normal embryonic development. However, abnormal activation of the pathway later in life has been demonstrated in a variety of human diseases, including bladder cancer.

**Methods:** We genotyped 171 potentially functional single nucleotide polymorphisms (SNPs) and tagging SNPs among seven Sonic Hedgehog pathway-related genes in a case-control study including 803 Caucasian bladder cancer patients and 803 matched controls. **RESULTS:** The homozygous variant genotype of a SNP located in the Intron of Gli2 (rs4848123) was associated with a significantly increased bladder cancer risk [odds ratios (OR), 4.21; 95% confidence interval (95% CI), 1.15-15.44]. Likewise, the homozygous variant genotype of a 3' UTR SNP (rs3823720) and a SNP in the Intron (rs10951671) of Gli3 was associated with a significantly increased bladder cancer risk (OR, 1.58, 95% CI, 1.10-2.26; and OR, 1.87, 95% CI, 1.14-3.06; respectively). To assess the cumulative effects, we performed a combined unfavorable genotype analysis that included all SNPs showing at least a borderline statistical significance. We found that, compared with the low-risk reference group with less than two unfavorable genotypes, the medium-risk group with two unfavorable genotypes exhibited a 1.29-fold (0.92-1.81) increased risk and the high-risk group with more than two unfavorable genotypes exhibited a 1.92-fold (1.36-2.71) increased risk ( $P(\text{trend}) < 0.0001$ ).

**Conclusion:** To our knowledge, this is the first epidemiologic study showing that sonic pathway-related genetic variants may affect bladder cancer risk individually and jointly.

**A106 Serum testosterone concentrations and risk of testicular germ cell tumors.** Victoria M. Chia,<sup>1</sup> Sabah Quraishi,<sup>1</sup> Barry I. Graubard,<sup>1</sup> Frank Z. Stanczyk,<sup>2</sup> Mark V. Rubertone,<sup>3</sup> Ralph L. Erickson,<sup>4</sup> Katherine A. McGlynn<sup>1</sup>. <sup>1</sup>National Cancer Institute, Rockville, MD; <sup>2</sup>University of Southern California, Los Angeles, CA; <sup>3</sup>U.S. Army Center for Health Promotion and Preventive Medicine, Washington, DC; <sup>4</sup>Walter Reed Army Institute of Research, Silver Spring, MD.

Although the incidence of testicular germ cell tumors (TGCT) has been rising worldwide, little known about the etiology. There is evidence, however, that TGCT is related to other male reproductive disorders, specifically, hypospadias, cryptorchidism and infertility. Because testosterone is responsible for the development and proliferation of testicular cells and testicular descent, it is plausible that testosterone and its binding protein, sex hormone binding globulin (SHBG), may be associated with TGCT risk. Using 512 case and 789 control participants from the U.S. Servicemen's Testicular Tumor Environmental and Endocrine Determinants (STEED) Study, we assessed associations of pre-diagnostic serum SHBG and testosterone (measured total and calculated free) concentrations and risk of TGCT. Odds ratios (OR) and 95% confidence intervals (CI) were estimated using adjusted logistic regression models. Among the controls, SHBG concentrations increased with age ( $p < 0.01$ ), while free testosterone concentrations decreased with age ( $p = 0.02$ ). Total testosterone concentrations, however, were not associated with age ( $p = 0.84$ ). SHBG and total testosterone concentrations decreased with increasing body mass index ( $p < 0.01$ ), however free testosterone did not ( $p = 0.67$ ). Overall, there were no statistically significant associations between SHBG and total testosterone concentrations and TGCT risk. There were suggestions that men had an increased risk of TGCT if they were in

the highest quintile of SHBG (OR=1.41, 95% CI, 0.98-2.02) and free testosterone (OR=1.31, 95% CI, 0.91-1.88), but there were no statistically significant trends. Serum total testosterone concentrations were not associated with TGCT risk. There did not appear to be differences in risk by tumor subtype, seminoma versus nonseminoma, nor effect modification by body mass index. These results suggest that serum testosterone is not associated with risk of TGCT; other endogenous hormones, however, such as gonadotropins and estrogens, may provide additional clues to the etiology of TGCT.

**A107 Polymorphisms in nucleotide excision repair pathway and acute myeloid leukemia outcome.** Sara S. Strom, Sameer B. Gokhale, Elihu H. Estey. UT M. D. Anderson Cancer Center, Houston, TX.

Acute myeloid leukemia (AML), the most common leukemia in adults is frequently associated with genetic abnormalities. Based on pre-treatment cytogenetics, AML patients are classified into favorable, intermediate and poor subgroups. Cytogenetics are good predictors of treatment outcome for the favorable and poor subgroups. However, for patients with intermediate cytogenetics, optimal treatment is uncertain. For these patients incorporating genetic markers to the existing prognostic factors might be helpful to identify subgroups of patients. Variants in genes within the nucleotide excision repair (NER) pathway may lead to inter-individual differences in DNA repair capacity which could eventually influence AML outcome. We studied the role of 5 polymorphisms (ERCC1 *Gln504Lys*, XPD *Lys751Gln*, XPC *Ala499Val*, XPC *Lys939Gln*, and CCNH *Val270Ala*) within this pathway on overall and disease-free survival among 170 adult *de-novo* AML patients with intermediate cytogenetics [diploid karyotypes ( $n = 117$ ) and non-diploid karyotypes ( $n = 53$ )], treated with induction chemotherapy. Kaplan-Meier methods and Cox proportional hazards models were performed. After a median follow-up of 16 months, 56% of diploid patients died compared to 71% among the non-diploid group (Log Rank  $p = 0.03$ ). Among patients with diploid karyotypes, after adjusting for clinical and socio-demographic characteristics (age, sex, ethnicity, WBC, performance status, and smoking), XPD *Lys751Gln* and XPC *Ala499Val* were found to be independently associated with overall survival. Patients with the XPD *Lys751Gln/Gln751Gln* genotype were twice more likely to have died than those with the wild genotype (HR: 1.97; 95% CI: 1.09 - 3.55). XPC *Ala499Val/Val499Val* patients had a 75% increased mortality compared to those with the wild genotype (HR: 1.75; 95% CI: 1.02 - 2.98). Patients carrying both (XPD *Lys751Gln/Gln751Gln* and XPC *Ala499Val/Val499Val*) had significantly shorter survival compared to those with the wild genotype (HR: 3.49; 95% CI: 1.58 - 7.68). 21% of the AML patients with diploid cytogenetics carried both risk variants. No significant associations were observed for disease-free survival in AML patients. Our results suggest that polymorphic variants in NER repair enzymes may modulate AML outcome in patients with diploid cytogenetics. Patients with the high risk genotype combination could have diminished DNA repair capacity, which in turn, could result in greater susceptibility to genotoxic effects of treatment decreasing overall survival. These findings could in the future be used in selecting treatment strategies for patients with normal cytogenetics.

**A108 Predictors of hormone receptor status in ovarian cancer: Results from the Nurses' Health Study.** Joanne Kotsopoulos,<sup>1</sup> Jonathan L. Hecht,<sup>2</sup> Susan E. Hankinson,<sup>3</sup> Shelley S. Tworoger<sup>3</sup>. <sup>1</sup>Brigham and Women's Hospital and Harvard Medical School, Boston, MA; <sup>2</sup>Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA; <sup>3</sup>Brigham and Women's Hospital, Harvard Medical School, and Harvard School of Public Health, Boston, MA.

**Background:** Hormone receptor expression in tumors may offer etiologic information for ovarian cancer, particularly in light of known associations with hormonal and reproductive risk factors.

**Methods:** TMAs were constructed using triplicate core samples extracted from 157 paraffin-embedded blocks of confirmed epithelial ovarian tumors collected from participants in the Nurses' Health Study and stained for estrogen receptor- $\alpha$  (ER $\alpha$ ) and progesterone receptor (PR). We examined receptor expression by stage, grade and histologic subtype. Multivariate unconditional logistic regression was used to evaluate whether various hormonal, reproductive and anthropometric risk factors differed by ER $\alpha$  and PR expression among cases, and whether these exposures are differentially associated with the risk of developing receptor-positive or receptor-negative ovarian cancer compared to controls.

**Results:** PR expressing tumors were less likely to be invasive ( $P = 0.05$ ) and more likely to be of a lower grade ( $P < 0.001$ ) compared with PR- tumors. ER $\alpha$  status was not associated with any pathological features of the tumor ( $P \geq 0.76$ ). In the case-control analysis, increasing age, being postmenopausal, and PMH use were associated with an increased risk of developing ER $\alpha$ +, but not ER $\alpha$ -, ( $P$  for heterogeneity = 0.001, 0.06, and 0.06, respectively) and PR-, but not PR+, tumors ( $P$  for heterogeneity = 0.08, 0.003, and 0.40, respectively), while height was only associated with the risk of developing PR- disease ( $P$  for heterogeneity = 0.08). There were no clear differential associations of OC use, parity, adolescent or adult BMI, or physical activity with expression of either receptor. Results were similar in the case-case analysis.

**Conclusion:** Despite limited statistical power, our findings suggest that various risk factors are only associated with certain tumor subtypes based on hormone receptor expression, thus providing mechanistic evidence regarding these relationships in the etiology of ovarian cancer. Given our small sample size, confirmatory studies are required to further delineate these relationships.

**A109 A prospective study of vitamin D receptor polymorphisms, interaction with vitamin D status, and colorectal cancer risk in men.** Jung Eun Lee,<sup>1</sup> Haojie Li,<sup>2</sup> Edward Giovannucci,<sup>3</sup> Bruce W. Hollis,<sup>4</sup> David J. Hunter,<sup>3</sup> I-Min Lee,<sup>1</sup> Meir Stampfer,<sup>1</sup> Jing Ma<sup>1</sup>. <sup>1</sup>Brigham and Women's Hospital, and Harvard Medical School, Boston, MA; <sup>2</sup>GlaxoSmithKline R&D, Collegeville, PA; <sup>3</sup>Harvard School of Public Health, Boston, MA; <sup>4</sup>Medical University of South Carolina, Charleston, SC.

Growing evidence suggests an elevated risk of colorectal cancer among individuals with low levels of vitamin D, the biological actions of which is mediated by the vitamin D receptor (VDR). We examined the prospective associations between three VDR single nucleotide polymorphisms (SNPs) (BsmI (bb, BB/BB), FokI (FF, Ff/f), and Cdx2 (G>A)) and colorectal cancer risk and their interactions with plasma vitamin D status in a nested case-control study among men in the Physicians' Health Study. A total of 249 incident colorectal cancer cases were identified through 2000 among men who provided blood specimens in 1982-1984 and individually matched to controls by age and smoking. We used conditional logistic regression to calculate the relative risk (RR) and 95% confidence interval (95% CI). We found that men with the VDR Cdx2 variant A allele tended to have a lower risk of developing colorectal cancer compared to men with the homozygous GG genotype (RR=0.71; 95% CI = 0.50-1.02;  $P = 0.06$ ). Moreover, the lower risk of colorectal cancer associated with the VDR Cdx2 A genotype was mainly confined to men with low vitamin D status (plasma 25(OH)D below median level; RR = 0.45; 95% CI = 0.24-0.84;  $P = 0.03$ ) but not among men with vitamin D levels above the median ( $P$  for interaction = 0.04). We found no statistically significant associations for the other two VDR SNPs, FokI or BsmI, or statistically significant interactions between plasma 25(OH)D levels and these two SNPs. Our prospective data suggest the hypothesis that the functional polymorphic Cdx2 A allele variation in the VDR gene, which is linked to a higher transcriptional activity of the VDR, may be protective against colorectal cancer development when vitamin D status is low.

**A110 Kinase gene expression profiles are associated with clinical outcomes in clear cell renal cell carcinoma patients.** Michelle A.T. Hildebrandt, Pheroze Tamboli, Maosheng Huang, Yuanqing Ye, Ju-Seog Lee, Xifeng Wu. M. D. Anderson Cancer Center, Houston, TX.

There are 516 known kinases in the human genome. These proteins are involved in regulating nearly all cellular processes through phosphorylation of target proteins. Because of their important role maintaining proper cellular function, they are often misregulated during tumorigenesis and associated with clinical outcomes in cancer patients. Similarly to other cancers, several kinases have been found to play key roles during development of renal cell carcinoma (RCC). However, less is known about the global expression status of these genes in RCC and their association with clinical outcomes. To identify variation in the RCC kinome, we performed a systematic analysis of gene expression for 503 human kinases using Illumina whole genome arrays in 93 tumor samples. Expression levels were analyzed with regard to associations with recurrence and overall survival. Six kinases were found to be significantly associated with an increased risk developing a recurrence, while two kinases were protective ( $p < 0.01$ ). Furthermore, an expression signature comprised of these eight genes was able to identify low risk and high risk groups of patients (HR: 26.83, 95% CI: 3.77-190.78,  $p = 0.0010$ ). These two risk groups also had significantly different recurrence-free survival times of 67.6 compared to 37.9 months ( $p = 0.0003$ ) for the low and high risk groups, respectively. Expression of one of these eight genes, IKBKE, was also associated with a 5.3-fold increased risk of dying (95% CI: 1.93-14.59,  $p = 0.0012$ ). Interestingly, this kinase was constantly associated with a poor prognosis in both univariable and multivariable analyses with similar HRs, indicating that expression levels of this gene may be a candidate, independent prognostic marker for RCC. IKBKE is a member of the NF $\kappa$ B signaling pathway and is responsible for regulation of NF $\kappa$ B-mediated transcriptional activation of genes involved in inflammation, immunity and stress responses. Overall, these results suggest a major role of global kinase gene expression in modulating clinical outcomes in RCC patients while identifying several candidate genes, including IKBKE, for further study.

**A111 Genetic variations in five estrogen metabolizing genes and endometrial cancer risk.** Hannah P. Yang,<sup>1</sup> Elizabeth A. Platz,<sup>2</sup> Mia Gaudet,<sup>1</sup> James Lacey Jr.,<sup>1</sup> Louise A. Brinton,<sup>1</sup> Jolanta Lissowska,<sup>3</sup> Beata Peplonska,<sup>4</sup> Mark E. Sherman,<sup>1</sup> Stephen Chanock,<sup>1</sup> Montserrat Garcia-Closas<sup>1</sup>. <sup>1</sup>National Cancer Institute, Rockville, MD; <sup>2</sup>Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; <sup>3</sup>M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland; <sup>4</sup>Nofer Institute of Occupational Medicine, Lodz, Poland.

**Background:** Estrogen plays a major role in endometrial carcinogenesis and the balance of estrogen metabolites has been hypothesized to be a determinant of risk. Cytochrome p450 (CYP) enzymes, CYP1A1, CYP1A2, and CYP3A4 catalyze the 2-OHE1 pathway, whereas the CYP1B1 catalyzes the 4-OHE1 pathway. The activity of these enzymes determines the proportion of metabolites and a higher proportion of the 4-OHE1 metabolites is thought to have a higher carcinogenic potential. Although several studies have examined the association between single nucleotide polymorphisms (SNPs) of these estrogen metabolizing genes and endometrial cancer risk, results have been inconsistent and limited to putatively functional variants.

**Methods:** We comprehensively assessed known common genetic variation in these estrogen metabolizing genes by genotyping candidate and tagging SNPs (tagSNPs) in a population-based case-control study of 417 endometrial cancer cases and 407 controls (most of whom were postmenopausal) conducted in Poland (Warsaw and Lodz) between 2001-2003. The following criteria were used to identify tagSNPs: (1) located in the respective gene or within the 5kb flanking regions, (2) with a minor allele frequency greater than or equal to 0.01 in Caucasian populations,

and (3) with pairwise  $r^2=0.90$  between tagSNPs and undetermined SNPs. A total of 5 SNPs for *CYP1A1*, 7 for *CYP1A2*, 9 for *CYP1A1/A2*, 34 for *CYP1B1*, 6 for *CYP3A4*, and 12 for *COMT* were examined. Genotyping was performed using an Illumina Custom Infinium iSelect assay that included nearly 27,000 SNPs with coverage of over 1,300 candidate genes.

**Results:** We did not observe significant associations for candidate variants of *CYP1A1* (rs1048943 (I462V) per C allele: OR (95% CI)=0.98 (0.56-1.72); rs1799814 (T461N) per T allele: 1.75 (0.94-3.24)); *CYP1A2* (rs762551 (IVS1-154C>A) per C allele:0.91(0.74-1.12)); *CYP1B1* (rs1056827 (A119S) per A allele: 0.98 (0.80-1.20); rs1056836 (V432L) per C allele: 0.96 (0.79-1.18); rs1800440 (N453S) per C allele: 1.15 (0.88-1.51)); and *CYP3A4* (rs2740574 (-391A>G) per C allele: 1.46 (0.84-2.54)). In addition, we did not observe any significant association with endometrial cancer risk in haplotype analyses (global p-value>0.3) or the individual tagSNPs analyses (p-trend>0.09). Meta-analysis including our study and previous reports for these SNPs did not show significant overall associations; however, there is substantial heterogeneity across studies.

**Discussion:** Our findings do not provide evidence for a substantial association between common genetic variation of estrogen metabolizing genes with endometrial cancer risk. However weak associations with these genes or association with other genes in the estrogen metabolizing pathway may exist.

**A112 Molecular variants of E7/HPV58 in women with normal cytology from the Colombian cohort. Preliminary results.** Oscar Buitrago, Carolina Martin, Antonio Huertas, Pablo Moreno, Gustavo Hernandez, Teresa Martinez, Monica Molano. National Cancer Institute, Bogota, D.C, Colombia.

**Introduction:** Human papillomavirus type 58 (HPV 58) is the second viral type more prevalent in women with normal cytology from the Colombian population. Also this type is highly prevalent in women with high grade squamous intraepithelial cervical lesions (HGSIL) and in women with invasive cervical cancer worldwide. There are few studies of E7/HPV 58 variants, however some of them have shown an association of variants presence with a higher risk of HGSIL and cervical cancer in Asian populations. In Colombia there are not studies of presence and persistence of E7/HPV58 variants.

**Objective:** To identify molecular variants of E7/HPV58 in cervical scrapes of women with normal cytology that belongs to the base line of the Bogotá, Colombia Cohort.

**Materials and Methods:** 1845 cervical scrapes of women with normal cytology that belong to the base line of the Bogotá, Colombia cohort were analysed. HPV detection was done using the GP5+/GP6+ primers and 37 different HPV types were typed using a PCR-RLB assay. The E7 region of samples HPV 58 positives was amplified using the E7P1- E7P2 primers that amplify a fragment of 316 bp (561 to 877 nucleotides). E7/HPV 58 variants were detected using automated direct sequencing. The reference sequence of HPV 58 was used to compare sequences.

**Results:** 34 samples were positive for HPV 58 (1.84%). Until this moment 13 samples have been analysed for E7/HPV 58 variants. 12 of these samples have the A694/G744/A761 variant (94.7%), one sample have the A599/A694/G744/A761 variant (5.5%), and none of the samples had the reference sequence of HPV58.

**Conclusions:** Two variants have been identified in the samples analysed: 1 previously identified in Asian population and 1 new variant (A599/A694/G744/A761). There are few diversity of E7/HPV58 variants in the analysed samples. The cohort analysis will give important information about the role of these variants in the persistence of HPV 58 infection and in the development of cervical intraepithelial lesions.

## Other Risk Factors

**A113 Association between NSAIDs and PSA, prostate volume, and prostate cancer.** Jay H. Fowke, Sandra Motley, Susan Byerly, Michael Cookson, Sam Chang, Joseph A. Smith Jr.. Vanderbilt University, Nashville, TN.

**Purpose:** NSAIDs such as aspirin are commonly used to prevent cardiovascular disease, and several prior studies suggest NSAIDs may also reduce prostate inflammation and prostate cancer risk. We investigated the association between NSAID use, PSA, and prostate volume, hypothesizing lower PSA and volume levels with NSAID use.

**Methods:** The Nashville Men's Health Study utilizes a multi-centered, rapid-recruitment protocol to collect clinical, biological, behavioral, and body measurement data from 1,277 men over age 40 years and scheduled for diagnostic prostate biopsy. Approximately 95% of eligible men approached for recruitment agree to participate. NSAID use was ascertained by survey and clinical interview, and medical charts were reviewed to ascertain current PSA levels, prostate volume, and clinical diagnoses following biopsy. The distributions of PSA level and prostate volume data were natural log transformed prior to analysis. In a linear model, mean PSA and volume scores across NSAID categories were adjusted for age; race (black/white); family history (yes/no); number of prior PSA tests (1,2, 3 or more); BMI; WHR; height; or treatment for BPH (steroid reductase inhibitors, other), diabetes (Yes/No), CVD (Yes/No), hyperlipidemia (Yes/No); and the diagnosis of PIN, atypical findings suspicious for cancer, low-grade cancer, or high-grade cancer following biopsy. PSA and prostate volume scores were back-transformed and geometric mean values are reported.

**Results:** Approximately 46% of subjects reported taking an NSAID, primarily aspirin (37% of subjects). After adjusting for age, race, family prostate cancer history, obesity, and treatment for BPH, CVD, hyperlipidemia, and diabetes; aspirin was significantly associated with lower PSA levels (7.3 vs. 8.0 ng/ml,  $p=0.01$ ). This effect of aspirin on PSA was greatest among men with a prostate volume of 60 mls or more (8.6 vs. 9.7 ng/ml,  $p=0.06$ ), men diagnosed with prostate cancer (6.1 vs. 7.3 ng/ml,  $p<0.01$ ), or men with both cancer and prostate enlargement (7.3 vs. 12.7 ng/ml,  $p<0.01$ ). NSAID use was not significantly associated with prostate volume (47.6 vs. 46.0 mls,  $p=0.16$ ).

**Conclusions:** Aspirin use was significantly associated with lower PSA levels. Controlling for BMI, WHR, and the use of medications to treat CVD, hyperlipidemia, BPH, and diabetes had little effect on the association between aspirin use and PSA levels, suggesting aspirin was not simply a proxy for these comorbid conditions or their associated treatments. These results may suggest that aspirin use decreases the ability to detect prostate cancer and may contribute to prior investigations reporting a protective association between NSAID use and prostate cancer risk. Implications for prostate cancer screening recommendations and for clinical decision-making will require further investigation.

**A114 No increased risk of cancer after coal tar treatment in patients with psoriasis or eczema.** Judith H.J. Roelofzen,<sup>1</sup> Katja K.H. Aben,<sup>1</sup> Ursula T.H. Oldenhof,<sup>1</sup> Pieter-Jan Coenraads,<sup>2</sup> Hans A.C. Alkemade,<sup>3</sup> Peter C.M. Van de Kerkhof,<sup>1</sup> Pieter G.M. Van der Valk,<sup>1</sup> Lambertus A.L.M. Kiemeny.<sup>1</sup> <sup>1</sup>Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; <sup>2</sup>University Medical Centre Groningen, Groningen, Netherlands; <sup>3</sup>Canisius-Wilhelmina Hospital, Nijmegen, Netherlands.

**Background:** Coal tar is an effective topical treatment for psoriasis and eczema, but it contains several carcinogenic polycyclic aromatic hydrocarbons (PAHs), such as benzo(a)pyrene, benz(a)anthracene and dibenz(a,h)anthracene. Occupational and animal studies showed an increased risk of cancer after exposure to coal tar. Many dermatologists have abandoned this effective treatment for safety reasons, although the risk of cancer after dermatological coal tar therapy is unclear. To study the

risk of cancer after coal tar treatment and other therapies, the LATER study (Late effects of coal tar treatment in eczema and psoriasis; the Radboud study) was conducted.

**Methods:** A large historical cohort study was performed including 13,200 patients diagnosed with psoriasis and eczema between 1960 and 1990 at three large hospitals in the Netherlands. Specific information on skin disease and treatment, risk factors and cancer occurrence was retrieved from medical files, postal questionnaires, the Netherlands Cancer Registry and Causes of Death Registry. Cox Proportional Hazard regression models were used to evaluate differences in cancer risk by treatment modality.

**Results:** Coal tar treatment did not increase the risk of non-skin malignancies (HR=0.92; 95%CI=0.78-1.09), nor the risk of skin cancer (excluding basal cell carcinoma) (HR=1.09; 95%CI=0.69-1.72). Alternative therapies, such as PUVA and systemic therapies appeared to be associated with an increased risk of skin cancer (HR=1.17; 95%CI=0.75-1.82 and HR=2.33; 95%CI=1.60-3.40, respectively), but no increased risk of non-skin cancer was observed (HR=0.91; 95%CI=0.75-1.10 and HR=1.12; 95%CI=0.95-1.32).

**Conclusion:** This study is the first study with sufficient numbers of patients and follow-up to reliably estimate the risk of cancer after coal tar treatment. The use of coal tar was not associated with an increased risk of cancer. PUVA and systemic therapies showed an increased risk of skin cancer, but no increased risk of tumors at other sites was observed. After considering the risks and benefits of coal treatment against the risks and benefits of other therapies, we conclude that coal tar coal tar can be maintained as a safe and effective treatment within dermatological practice.

#### PR-8 *Trichomonas vaginalis* infection and prostate cancer incidence and mortality: A prospective study in the Physicians' Health Study.

Jennifer R. Stark,<sup>1</sup> Gregory Judson,<sup>1</sup> John F. Alderete,<sup>2</sup> Siobhan Sutcliffe,<sup>3</sup> Vasanthakrishna Mundodi,<sup>2</sup> Ashwini S. Kucknoor,<sup>2</sup> Edward L. Giovannucci,<sup>1</sup> Elizabeth A. Platz,<sup>4</sup> Katja Fall,<sup>5</sup> Tobias Kurth,<sup>1</sup> Jing Ma,<sup>6</sup> Meir J. Stampfer,<sup>1</sup> Lorelei A. Mucci<sup>1</sup>. <sup>1</sup>Harvard School of Public Health, Boston, MA; <sup>2</sup>Washington State University, Pullman, WA; <sup>3</sup>Washington University, St. Louis, MO; <sup>4</sup>Johns Hopkins University, Baltimore, MD; <sup>5</sup>Karolinska Institutet, Stockholm, Sweden; <sup>6</sup>Brigham and Women's Hospital, Boston, MA.

This abstract is being presented as a short talk in Concurrent Session 9. A full abstract is printed in the Proffered Papers: Oral Presentation Abstracts section of the conference proceedings. (Presented on board number A115)

#### A116 Nonsteroidal anti-inflammatory drug use and risk of gastric and esophageal adenocarcinoma in a large cohort study.

Christian C. Abnet,<sup>1</sup> Neal D. Freedman,<sup>1</sup> Michael F. Leitzmann,<sup>1</sup> Albert Hollenbeck,<sup>2</sup> Arthur Schatzkin<sup>1</sup>. <sup>1</sup>National Cancer Institute, Rockville, MD; <sup>2</sup>AARP, Washington, DC.

**Background:** Previous studies suggest that the use of aspirin or non-aspirin nonsteroidal anti-inflammatory drugs (NSAIDs) may reduce the risk of gastric and esophageal adenocarcinomas, but few studies have been prospective, have used data collected directly from subjects, and have controlled for the many potential confounders. **Methods:** We examined the association between aspirin or non-aspirin NSAID use and incident gastric noncardia (N=182), gastric cardia (N=178), and esophageal adenocarcinomas (N=228) in the NIH-AARP Diet and Health prospective cohort study with subjects followed for up to 7 years. We estimated the hazard ratios (HR) and 95% confidence intervals (CI) in Cox models for any use of aspirin or non-aspirin NSAIDs in the previous twelve months or for the typical frequency of use (at least monthly, weekly, or daily) with adjustment for age, sex, smoking, alcohol, BMI, education, physical activity, and fruit and vegetable intake.

**Results:** For gastric noncardia cancer, we found that any aspirin use had an HR (95%CI) of 0.64 (0.47-0.86) and for daily use 0.57 (0.39-0.85). The age-standardized incidence rates (95% CI) per 100,000 person years dropped from 11.0 (8.4-13.6) in non-aspirin users to 7.0 (5.7-8.3) in users. For any non-aspirin NSAID use we found an HR (95%CI) of 0.68 (0.51-0.92) and for daily use 0.82 (0.50-1.34). We found no significant association between NSAID use and gastric cardia cancer or esophageal adenocarcinoma. For the latter, any or daily aspirin use had HR (95% CI) of 1.00 (0.73-1.37) and 1.11 (0.78-1.57), respectively.

**Conclusions:** We found a strong association between NSAID use, especially aspirin, and reduced risk of noncardia gastric adenocarcinoma, but not adenocarcinoma of the gastric cardia or esophagus.

#### A117 A meta-analysis of the incidence of non-AIDS cancers in HIV-infected individuals.

Meredith Shiels,<sup>1</sup> Stephen Cole,<sup>2</sup> Charles Poole<sup>2</sup>.

<sup>1</sup>Johns Hopkins School of Public Health, Baltimore, MD; <sup>2</sup>University of North Carolina at Chapel Hill, Chapel Hill, NC.

**Objective:** To compare the incidence rate of all non-AIDS cancers combined among HIV-infected individuals with the general population rate and to estimate modification of the association by gender and AIDS diagnosis.

**Design:** A meta-analysis was carried out using data from 11 studies of non-AIDS cancer incidence in HIV-infected individuals.

**Methods:** Standardized incidence rate ratios (SIR) for non-AIDS cancers in HIV-infected individuals compared to the general population and 95% confidence limits (CL) were abstracted from each study. The results were assessed for between-study heterogeneity and funnel plot asymmetry. Meta-regression was used to estimate modification of the general-population SIR by gender and AIDS.

**Results:** There was pronounced heterogeneity between studies, thus, an overall pooled estimate was not calculated. The SIR of non-AIDS cancers in HIV-infected individuals, adjusted for AIDS and study design was greater among men (2.3; 95% CL: 1.7, 3.1) than women (1.5; 95% CL: 0.9, 2.3; ratio of SIRs = 1.6; 95% CL: 0.7, 3.2). The SIR also appeared slightly greater among those with an AIDS diagnosis; however, when the analysis was adjusted for gender and study design, the SIRs for those with (1.88; 95% CL: 1.34, 2.66) and without AIDS (1.98; 95% CL: 1.35, 2.88) were similar (ratio of SIRs: 0.95; 95% CL: 0.46, 1.96).

**Conclusions:** The incidence rate of non-AIDS cancers was elevated among HIV-infected individuals. Additionally, HIV-infected men appeared to have a greater SIR for non-AIDS cancers than HIV-infected women. However, no difference was seen between the SIRs for non-AIDS cancers among those with and without AIDS.

#### A118 Tea drinking habits and esophageal cancer in a high risk area in northern Iran.

Farhad Islami,<sup>1</sup> Farin Kamangar,<sup>2</sup> Akram Pourshams,<sup>3</sup> Dariush Nasrollahzadeh,<sup>3</sup> Saman Fahimi,<sup>3</sup> Christian C. Abnet,<sup>2</sup> Henrik Moller,<sup>4</sup> Sanford M. Dawsey,<sup>2</sup> Reza Malekzadeh,<sup>3</sup> Paolo Boffetta<sup>1</sup>. <sup>1</sup>International Agency for Research on Cancer, Lyon, France; <sup>2</sup>NCI, Bethesda, MD; <sup>3</sup>Digestive Disease Research Center, Medical Sciences/University of Tehran, Tehran, Iran, Islamic Republic of; <sup>4</sup>King's College London, Thames Cancer Registry, London, United Kingdom.

Golestan Province in northern Iran is a high incidence area for oesophageal squamous cell carcinoma (ESCC). We investigated the association between tea drinking habits and risk of ESCC in a population-based case-control study conducted between 2003 and 2007 in Golestan, with 300 histologically-proven ESCC cases and 571 matched neighborhood controls. In addition, we measured tea temperature and tea drinking patterns among 48582 healthy participants in a cohort study in the same region. Nearly all (98%) of the cohort participants drank black tea regularly, with a mean volume of over one liter/day, and over 60% drank their tea at temperatures over 60°C. The results of the case-control study showed that compared to drinking lukewarm or warm tea, the OR (95%

## Other Risk Factors

CI) for drinking hot tea was 2.07 (1.28-3.35) and for drinking very hot tea was 8.16 (3.93-16.9). Likewise, compared to drinking tea four or more minutes after pouring it, the ORs (95% CIs) for drinking tea 2-3 minutes and less than 2 minutes after pouring were 2.49 (1.62 - 3.83) and 5.41 (2.63 - 11.1), respectively. There was a strong agreement between responses to questions on temperature of tea drinking and time interval from pouring tea to drinking it (weighted kappa = 0.68). In conclusion, drinking hot tea, a habit commonly seen in Golestan Province, was strongly associated with higher risk of ESCC.

**A120 Agricultural pesticide use and pancreatic cancer risk in the agricultural health study cohort.** Gabriella Andreotti,<sup>1</sup> Laura E. Beane Freeman,<sup>1</sup> Lifang Hou,<sup>2</sup> Joseph Coble,<sup>1</sup> Jennifer Rusiecki,<sup>3</sup> Jane A. Hoppin,<sup>4</sup> Debra T. Silverman,<sup>1</sup> Michael Alavanja<sup>1</sup>. <sup>1</sup>National Cancer Institute, Rockville, MD; <sup>2</sup>Northwestern University, Chicago, IL; <sup>3</sup>Uniformed Services University of the Health Sciences, Bethesda, MD; <sup>4</sup>National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Pancreatic cancer is a rapidly fatal disease that has been linked with pesticide use. Previous studies have reported excess risks of pancreatic cancer with organochlorines such as DDT, however, many other commonly used pesticides have not been examined. To further examine the potential associations between the use of a number of pesticides and pancreatic cancer, we conducted a case-control analysis in the Agricultural Health Study, one of the largest prospective cohorts with over 89,000 participants including pesticide applicators and their spouses in Iowa and North Carolina. This analysis included 93 incident pancreatic cancer cases (64 applicators, 29 spouses) and 82,503 cancer-free controls who completed an enrollment questionnaire providing detailed pesticide use, demographic and lifestyle information. Ever use of 24 pesticides and intensity-weighted lifetime days [(lifetime exposure days) x (exposure intensity score)] of 13 pesticides was assessed. Risk estimates were calculated using unconditional logistic regression controlling for age, smoking, and diabetes. Among pesticide applicators, two herbicides (EPTC and pendimethalin) of the 13 pesticides examined for intensity-weighted lifetime use showed a statistically significant exposure-response association with pancreatic cancer. Applicators in the top half of lifetime pendimethalin use had a 3.0-fold (95% CI 1.3-7.2, p-trend=0.01) risk compared to never users, and those in the top half of lifetime EPTC use had a 2.56-fold (95% CI=1.1-5.4, p-trend=0.01) risk compared to never users. Organochlorines were not associated with an excess risk of pancreatic cancer in this study. These findings suggest that herbicides, particularly pendimethalin and EPTC, may be associated with pancreatic cancer.

**A121 The association of menstrual and reproductive factors with the risk of upper gastrointestinal tract cancers in the NIH-AARP cohort.** Neal D. Freedman,<sup>1</sup> James V. Lacey Jr,<sup>1</sup> Michael F. Leitzmann,<sup>2</sup> Albert R. Hollenbeck,<sup>3</sup> Arthur Schatzkin,<sup>1</sup> Christian C. Abnet<sup>1</sup>. <sup>1</sup>NCI, Rockville, MD; <sup>2</sup>University of Regensburg, Regensburg, Germany; <sup>3</sup>AARP, Washington, DC.

Upper gastrointestinal tract cancers (UGI), including cancers of the esophagus, head and neck, and stomach have high worldwide incidence and mortality. Incidence rates are consistently higher among men than women; the male/female ratios are 2:1 for cancers of the stomach and 7:1 for cancers of the larynx. Strong environmental risk factors have been identified for these cancer sites, but do not appear to explain differences in incidence rates between men and women. Differences in sex hormones in men vs. women and among women of different ages might affect UGI risk. In nearly 10 studies of menstrual and reproductive factors with stomach cancer risk in women, significant inverse associations with older age at menopause or use of menopausal hormone therapy (MHT) were observed in some, but not all studies. Only a few studies have investigated the association of these factors with squamous cancers of the esophagus and head and neck. We prospectively investigated the association of menstrual and reproductive factors with UGI cancer risk in 201,506 women of the NIH-AARP Diet and Health cohort study. We used Cox proportional hazard models adjusted for age, alcohol intake, cigarette smoking, body mass index, physical activity, education, and total energy intake and report hazard ratios (HRs) and 95% confidence intervals. During 1,463,551 person years of follow-up from 1995/1996-2003, 162 women were diagnosed with adenocarcinomas (ACs; esophagus and stomach) and 353 women were diagnosed with squamous cell carcinomas (SCCs; oral cavity, pharynx, larynx, and esophagus). We found no significant associations between age at menopause, age at menarche, parity, or age at first birth and AC risk. Ever- versus never-use of MHT showed a borderline non-significant inverse association with adenocarcinoma (HR=0.74, 95% CI 0.54-1.02) in age adjusted models. After multivariate adjustment, the association was attenuated (HR=0.81, 95% CI, 0.59-1.12). For SCCs, we found a significant inverse association with older age at menopause but not with age at menarche, parity, or age at first birth. The HR for age at menopause of >55 years (vs. <45 years) was 0.53 (95% CI 0.28-1.01; p-value for trend=0.019). We also observed an inverse association with ever- versus never-use of MHT (HR=0.77, 95% CI 0.62-0.96). We further examined relations with the type of MHT stratified by hysterectomy status, using data from a subsequent questionnaire (1996-1997) completed by 127,385 women. In 51,515 women with a reported hysterectomy at baseline, the HRs and 95% CIs for <5 years of estrogen-plus-progestin MHT, relative to never MHT users, were 0.74 (0.43-1.27) for ACs (N=38) and 0.51 (0.18-1.46) for SCCs (N=80), respectively; for ≥5 years of use, the HRs and 95% CIs were 0.47 (0.19-1.16) and 0.28 (0.13-0.58), respectively. In 74,372 women with intact uteri, we found no associations between estrogen MHT and ACs (N=49) or SCCs (N=130). Our results suggest that estrogen-plus-progestin therapy is associated with reduced risk of UGI tract cancers, supporting the involvement of sex hormones in the etiology of these cancers. Future studies are needed to replicate these results.

**A122 Trichomonosis and subsequent risk of prostate cancer in the Prostate Cancer Prevention Trial.** Siobhan Sutcliffe,<sup>1</sup> John F. Alderete,<sup>2</sup> Cathie Till,<sup>3</sup> Phyllis J. Goodman,<sup>3</sup> Ann W. Hsing,<sup>4</sup> Jonathan M. Zenilman,<sup>5</sup> Angelo M. De Marzo,<sup>5</sup> Elizabeth A. Platz<sup>6</sup>. <sup>1</sup>Washington University School of Medicine, St. Louis, MO; <sup>2</sup>Washington State University, Pullman, WA; <sup>3</sup>Fred Hutchinson Cancer Research Center, Seattle, WA; <sup>4</sup>National Institutes of Health, Department of Health and Human Services, Bethesda, MD; <sup>5</sup>Johns Hopkins Medical Institutions, Baltimore, MD; <sup>6</sup>Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

We previously observed a positive association between a history of trichomonosis, a sexually transmitted infection caused by the protozoan, *Trichomonas vaginalis*, and prostate cancer risk in the Health Professionals Follow-up Study. To determine the reproducibility of this finding, we conducted a second, prospective investigation of trichomonosis and prostate cancer in the Prostate Cancer Prevention Trial. Participants were men  $\geq 55$  years of age with no evidence of prostate cancer at enrollment (n=18,882). Men were screened annually for prostate cancer, and if not diagnosed during the trial, were offered an end-of-study prostate biopsy. Cases were a sample of men diagnosed with prostate cancer on any biopsy after visit 2 or on their end-of-study biopsy (n=616). Controls were men not diagnosed with prostate cancer during the trial or on their end-of-study biopsy (n=616). Controls were frequency-matched to cases by age, treatment arm, and family history of prostate cancer. Serum from visit 2 was tested for anti-*T. vaginalis* IgG antibodies. No association was observed between anti-*T. vaginalis* antibody serostatus and prostate cancer. 21.5% of cases and 24.8% of controls had low antibody seropositivity, and 15.2% and 15.0% had high seropositivity. Compared to seronegative men, the odds ratio of prostate cancer for men with low seropositivity was 0.83 (95% confidence interval (CI): 0.63-1.09), and that for men with high seropositivity was 0.97 (95% CI: 0.70-1.34). Given the original strong biologic rationale and potential for prevention, additional studies are warranted to help resolve discrepancies between study findings, and further investigate this hypothesis from a variety of different approaches.

Funding: NCI/NIH P01 CA108964 (Biology of the PCPT). The content of this work is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

**A123 Serum levels of hormones and breast tissue composition in young women.** Lisa J. Martin,<sup>1</sup> Sofia Chavez,<sup>2</sup> Anoma Gunasekara,<sup>2</sup> Ayesha Salleh,<sup>1</sup> Olga Melnichouk,<sup>1</sup> Martin Yaffe,<sup>2</sup> Salomon Minkin,<sup>1</sup> Michael Bronskill,<sup>2</sup> Norman F. Boyd<sup>1</sup>. <sup>1</sup>Campbell Family Institute for Breast Cancer Research, Ontario Cancer Institute, Toronto, Ontario, Canada; <sup>2</sup>Imaging Research, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada.

**Background:** Percent mammographic density (PMD) is a strong and heritable risk factor for breast cancer with characteristics that suggest it may be a marker of susceptibility to the disease. We have examined serum hormone levels and other factors associated with breast tissue composition in young women, when susceptibility to breast carcinogens is greatest.

**Methods:** In 400 young women aged 15-30 years we obtained quantitative measures of breast water, which reflects fibro-glandular tissue, and breast fat using magnetic resonance (MR), and collected anthropometric and other data. All examinations were performed in the follicular phase of the menstrual cycle and fasting blood samples for hormone assays were obtained on the morning of the MR examination.

**Results:** Serum levels of growth hormone (GH) and sex hormone binding globulin (SHBG) were positively associated with percent breast water content in all young women aged 15-30, and the associations remained statistically significant after adjustment for height, weight and other covariates. IGF-I was not associated with any MR breast measures. Serum levels of estradiol, progesterone, and testosterone, were not associated with MR breast measures in all young women, or in those aged 20-30, but did show evidence of positive associations with percent water in those aged 15-19 years.

After adjustment for other covariates, interactions of age and serum levels of testosterone ( $p=0.02$ ) and progesterone ( $p=0.03$ ) were associated with percent water, and estradiol with total water ( $p=0.07$ ).

**Conclusions:** Serum levels of sex hormones were associated with breast tissue composition in young women aged less than 20, while GH and SHBG were associated with breast tissue composition in all young women.

**A124 Radiomodulation by rosemary extract.** Dhanraj Soyal, Abhilasha Sharma, Inder Singh, Pradeep Kumar Goyal. Rajasthan University, Jaipur, India.

Rosemary (*Rosmarinus officinalis*, Labiateae) is widely used for its antioxidant activities in many parts of the world. The present study investigates the radioprotective and antioxidative potential of rosemary leaves extract (ROE). Swiss albino mice were administered ROE orally once daily for 5 consecutive days, then exposed to a single dose of 3, 6 and 9 Gy of gamma radiation. lipid peroxidation (LPx), Glutathione (GSH), acid phosphatase estimations in liver were carried out. Radiation-induced increases in the levels of lipid peroxidation (LPO) and acid phosphatase were significantly ameliorated by ROE pre-treatment, and radiation-induced depletion in the level of glutathione (GSH) was significantly inhibited by ROE administration. These alterations were found to be dose dependent. The life-span was increased in the ROE treated irradiated mice in comparison with their respective control mice. Radiation-induced deficits in body and organ weight were significantly reduced or prevented in ROE pretreated mice. The protection afforded by ROE may be attributed to the constituents of the rosemary, which include rosmarinic acid, rosmanol, carnosol which appear to play an important role in free radical scavenging and singlet oxygen quenching. The study does not rule out the possibility of a prophylactic potential of ROE against radiation-induced degenerative changes in liver.

## Other Risk Factors

**A125 Self reported exposure to pesticides and risk of breast cancer.** Umar Farooq,<sup>1</sup> Monika Joshi,<sup>1</sup> Vinod Nookala,<sup>1</sup> Pramil Cheriyaht,<sup>1</sup> Steven D. Stellman,<sup>2</sup> Nora Graber,<sup>3</sup> Joshua E. Muscat<sup>4</sup>. <sup>1</sup>Harrisburg Hospital, PinnacleHealth Systems, Harrisburg, PA; <sup>2</sup>Mailman School of Public Health, New York, NY; <sup>3</sup>Public Health Sciences at Penn State Hershey, Hershey, PA; <sup>4</sup>Penn State Milton S. Hershey Medical Center, Hershey, PA.

**Background:** Several studies have examined the possible role of serum organochlorine pesticide and PCBs levels in the development of breast cancer over the past decade, but there is little data on self-reported residential exposures.

**Methods:** This was a case control study conducted in New York City and the surrounding area that included 1204 patients (446 cases and 758 controls). Cases were defined as women with newly diagnosed breast cancer or carcinoma in situ, while patients with benign breast diseases and women undergoing non-breast related surgery were included in the control group. All patients were asked a series of questions to determine their pesticide exposure, including the type, location (inside vs. outside), who applied the pesticide, and duration of pesticide use. Logistic regression models were used to estimate unadjusted and adjusted odds ratios (OR) and corresponding 95% confidence intervals (CI).

**Results:** The most common pests encountered in the residences were ants, carpenter ants and cockroaches. The adjusted odds ratio for self-application of pesticide and professional application for this group of pests was 1.25(95%CI 0.79-1.98) and 1.06(95% CI 0.65-1.73) respectively. The risk did not vary when comparing inside and outside applications, (OR= 1.13(95% CI 0.75-1.72) and 1.22(95% CI 0.60-2.50) respectively. Tests for trend showed no dose-response relationship with each type of application.

**Conclusion:** Our study did not show an association between self-reported exposure to pesticides and breast cancer risk, which is consistent with the vast majority of studies including our own that showed no increased risk of breast cancer with biological levels of pesticides.

**A126 Characteristics of menstruation and pregnancy and the risk of lung cancer in women.** Anita Koushik,<sup>1</sup> Marie-Elise Parent,<sup>2</sup> Jack Siemiatycki<sup>1</sup>. <sup>1</sup>Universite de Montreal, Montreal, Canada; <sup>2</sup>INRS – Institut Armand-Frappier, Universite du Quebec, Laval, Canada.

Differences between men and women in the descriptive epidemiology of lung cancer suggest that hormonal factors may influence lung carcinogenesis in women. Few epidemiological studies have been conducted on hormone-related variables and lung cancer risk and the findings have not been consistent. We investigated the association between characteristics of menstruation and pregnancy in relation to lung cancer risk in a population-based case-control study carried out in Montreal, Canada. Between January 1996 and December 1997, newly diagnosed lung cancer cases were identified and recruited from 18 Montreal-area hospitals that together diagnose 98% of cases that occur among Montreal residents. Population controls from Montreal were identified from the provincial electoral lists and were randomly selected, stratified to the expected age and sex distribution of cases. The participation rate was 81.7% among cases and 69.4% among controls. Among cases, interviews were conducted an average of 12.1 months after diagnosis. For each variable, odds ratios (OR) and 95% confidence intervals (CI) were estimated using unconditional logistic regression modeling. Each hormone-related variable was modeled separately. Associations were also examined according to age at diagnosis and level of smoking and by lung cancer histology. All statistical tests were two-sided. Among 422 women with lung cancer and 577 controls, we observed that most characteristics of menstruation and pregnancy were not associated with the risk of lung cancer. However, an increased lung cancer risk was observed for women who had had surgical menopause with bilateral oophorectomy compared to women who had had a natural menopause (OR=1.95, 95% CI: 1.29-3.17). These results did not vary by age at diagnosis or level of smoking, and they were similar for different histological types. Our results suggest

that hormonal factors, related to surgical menopause and/or ovary removal, may play a role in the risk of lung cancer. Further studies are needed to confirm these findings, and to assess the possible contribution of hormone replacement therapy.

## Preclinical and Translational Prevention Studies

## Breast Cancer

**PR-12 Is the screening-related increase of breast cancer mainly caused by lesions that would undergo spontaneous regression if left untreated?** Per-Henrik Zahl,<sup>1</sup> Jan M?hlen<sup>2</sup>. <sup>1</sup>Norwegian Institute of Public Health, Oslo, Norway; <sup>2</sup>Ullevål University Hospital, Oslo, Norway.

This abstract is being presented as a short talk in Concurrent Session 13. A full abstract is printed in the Proffered Papers: Oral Presentation Abstracts section of the conference proceedings. (Presented on board number A127)

**A128 Protective effects of nextrutine, a phellodendron amurense extract, against breast cancer is associated with autophagy.** Matthew Rosen, Chenguang Wang, Guang Yan, Wei Zhang, Susan Lanza-Jacoby. Thomas Jefferson University, Philadelphia, PA.

Breast cancer is the most frequent cancer in women with over 40,000 deaths per year in the United States. The prognosis for a subset of these women with breast tumors that are either estrogen receptor (ER)-negative or ER- and overexpress ErbB2 (also known as HER2/Neu) is very poor because these tumor are aggressive and do not respond to standard treatments. This situation emphasizes the importance of developing new agents and protocols for preventing breast cancer. Numerous epidemiological studies suggest that plant-based diets are protective against many types of cancer. However, there is very little known about many of these botanicals for preventing breast cancer. Nextrutine is an herbal extract from the Chinese tree phellodendron amurense that has been used over hundreds of years in traditional Chinese medicine for treatment of inflammation, gastroenteritis, abdominal pain, and diarrhea. Numerous studies indicate a relationship between inflammation and cancer. In particular, many studies have reported that breast tumors have increased expression of cyclooxygenase (COX-2), the rate-limiting enzyme that controls the conversion of arachidonic acid into pro-inflammatory prostaglandins (PG). High levels of PGE2 in breast tumors are associated with increased proliferation, metastasis, resistance to apoptosis, and angiogenesis. Overexpression of COX-2 occurs more frequently in DCIS, which suggests that COX/PG is an important target for preventing the progression of DCIS to metastatic breast cancer. However, considering the recent concerns of the cardiovascular risks associated with the COX-2 inhibitor drugs, there is an urgent need to develop nontoxic approaches to target the PGE pathway. Targeting PGE2 synthase and 15-hydroxy PG dehydrogenase (15-PGDH) provides an alternative strategy to block PGE2 without the risk the cardiovascular risks. SkBr3, MDA-MB 231, and BT474 cells were treated with nextrutine for 24, 48, and 72 h. Nextrutine produced a time and concentration decrease in cell survival, which was accompanied by a G0/G1 cell cycle arrest and decreased protein expression of cyclin D1. Nextrutine reduced the production of PGE2 without altering PGI2. The reducing in PGE2 levels was associated with decreased protein expression of PGE2 synthase and increased 15-PGDH. Peroxisome proliferators-activated receptor (PPAR)  $\gamma$  is also increased in many cancers including breast. Recent studies have suggested that PPAR  $\gamma$  may function as a tumor promoter gene. Upregulation of PPAR  $\gamma$  has been demonstrated in ER- breast cancer tumors and may be a marker for recurrence of DCIS. We show that nextrutine decreased activation of PPAR  $\gamma$ . Nextrutine reduced protein levels of PPAR  $\gamma$  and activation of PPAR  $\gamma$ . Growth inhibitory effects of nextrutine were associated with increased PTEN and Beclin 1 protein

expression, and type-2 cell death known as autophagy. Based upon these findings, we propose that the use of nextrutine could provide a novel approach for protection against breast cancer.

**A129 Alpha-fetoprotein-derived peptide (AFPep): A novel drug in translational studies for the treatment and prevention of breast cancer.** Herbert I. Jacobson, James A. Bennett, Thomas T. Andersen. Albany Medical College, Albany, NY.

It has been well established that parity is associated with reduced lifetime risk for breast cancer. Recent U.S. Census data note that the proportion of American women who remain nulliparous has doubled over the last 30 years. These data suggest that the incidence of breast cancer will increase in the coming years. Is there a molecule in pregnancy that can be isolated, shown to inhibit breast cancer, and be converted into a drug that can be used to treat and prevent breast cancer? The studies reported here demonstrate that a-fetoprotein (AFP) is such a molecule, that the anti-breast cancer site within that molecule is an 8-amino acid sequence in the third domain of the protein, and that this site has been synthesized in a form that can be used as a novel, non-toxic drug for the treatment and chemoprevention of breast cancer. We refer to this agent as AFPep.

AFPep is active after oral administration. It stops the growth of human breast cancers growing as xenografts in immune deficient mice, and it prevents the development of carcinogen-induced mammary cancers in rats. Its anti-breast cancer activity is additive in combination with tamoxifen, and it prevents the uterine hyperplasia induced by tamoxifen. Moreover, AFPep is active against breast cancers which have become resistant to tamoxifen. Its anti-oncotic mechanism is different from that of tamoxifen, aromatase inhibitors, anti-gonadotropins, and cytotoxic chemotherapy. There has been no evidence of toxicity in mice and rats receiving AFPep even when its dose was escalated 100-fold above its effective dose. Grant proposals are currently in review to support further development of AFPep including completion of its preclinical toxicology and initiation of Phase I clinical trials.

**A130 Effect of high-dose estrogen exposure in adolescence on mammographic density in adulthood.** Helen Jordan,<sup>1</sup> Anne Kavanagh,<sup>2</sup> Dorota Gertig,<sup>2</sup> John Hopper,<sup>2</sup> Alison Venn<sup>1</sup>. <sup>1</sup>University of Tasmania, Hobart, Australia; <sup>2</sup>The University of Melbourne, Melbourne, Australia.

**Background:** Since adolescence is an important stage of mammary development, exposure to high-dose estrogens at this time may have long-term effects on breast tissue and therefore mammographic density. It is well-established that women with greater mammographic density for age and BMI are at an increased risk of breast cancer.

**Methods:** We conducted a retrospective cohort study of women from the Australian Tall Girls Study who were 40 years or older and had been assessed for tall stature during adolescence between 1959 and 1993. Eligible women included 263 who had been treated during adolescence with high-dose estrogens and 254 who had not been treated. Treated women had received one of two types of estrogen: 3mg diethylstilbestrol (DES) daily, or 150 µg ethinyl estradiol (EE).

A mammogram within the previous two years was obtained from 167 treated women (mean age 48.4 yrs) and 142 untreated women (mean age 46.2 yrs). The total area of the breast image and the area of mammographically dense tissue (dense area), and hence non-dense area and percent mammographic density (PMD), were calculated from digitally scanned cranio-caudal mammographic films using a computer thresholding technique. Reproductive history, lifestyle factors, hormone exposure, treatment and anthropometric data were collected from medical records and by telephone interview using a structured questionnaire.

**Results:** After adjusting for age and BMI, treated women had, on average, 17% lower dense area ( $p=0.03$ ); adjusted means for treated and untreated women were 24.5 cm<sup>2</sup> (95% CI: 21.8, 27.2) and 29.1 cm<sup>2</sup> (95%

CI: 26.0, 32.4), respectively. There was no difference in adjusted means between treated and untreated women for non-dense area [71.7 cm<sup>2</sup> (95% CI: 66.2, 77.7) versus 70.5 cm<sup>2</sup> (64.7, 76.9);  $p=0.78$ ], PMD [24.8% (95% CI: 22.4, 27.4) versus 27.7% (95% CI: 24.8, 30.7);  $p=0.16$ ], or total area [105.6 cm<sup>2</sup> (95%CI: 100.1,111.4) vs 109.3 cm<sup>2</sup> (95%CI: 103.1,115.8);  $p=0.41$ ].

**Conclusion:** High-dose estrogen treatment for tall stature in adolescence is unlikely to increase risk of breast cancer through mechanisms related to mammographic density.

## Colon and Other Gastrointestinal Cancers

**A131 Molecular events associated with the anti-tumor effects of black raspberries in *N*-nitrosomethylbenzylamine-induced rat esophageal carcinogenesis.** Li-Shu Wang,<sup>1</sup> Alan A. Dombkowski,<sup>2</sup> Bethany Larue,<sup>1</sup> Daniela Cukovic,<sup>2</sup> Anju Mukundan,<sup>2</sup> Cassandra Henry,<sup>1</sup> Claudio Rocha,<sup>1</sup> John F. Lechner,<sup>3</sup> Gary D. Stoner<sup>1</sup>. <sup>1</sup>James Cancer Hospital & Solove Research Institute, Columbus, OH; <sup>2</sup>Institute of Environmental Health Sciences, Wayne State University, Detroit, MI; <sup>3</sup>Maine Center for Toxicology and Environmental Health, University of Southern Maine, Portland, ME.

We reported that the feeding of a diet containing 5 or 10% freeze-dried black raspberries (BRB) to *N*-nitrosomethylbenzylamine (NMBA)-treated rats results in a 39-64% reduction in the number of esophageal papillomas when using either anti-initiation or anti-promotion/progression protocols. The molecular events associated with the effects of BRB on NMBA-induced preneoplastic and papillomatous esophageal lesions however, have not been fully elucidated. In the present study, 4-5 week-old male F344 rats were injected s.c. with NMBA (0.3 mg/kg b.w., 3x/wk for 5 wks) after which they were fed either control diet or diet containing 5% BRB until the end of the study (35 wks). Control rats were injected s.c. with a solution of DMSO/water (20:80), the vehicle for NMBA. Esophagi from vehicle control, NMBA- and NMBA + BRB-treated rats were collected at 35 weeks for histologic grading, and for microarray and Real-Time PCR analyses. Treatment with 5% BRB reduced the number of preneoplastic lesions (dysplasias) and the number and size of papillomas in the esophagus of NMBA-treated rats. When compared to esophagi from vehicle control rats, NMBA treatment alone led to the differential expression of 4,807 genes in preneoplastic esophagus and 17,846 genes in esophageal papillomas. Treatment with 5% BRB resulted in a modulation towards control levels of expression of 626 genes in preneoplastic esophagus and 627 genes in papillomas. In both preneoplastic esophagus and in papillomas, the berry-modulated genes were associated with regulation of cell proliferation, inflammation and receptor-mediated pathways. Twenty-five genes were commonly modulated (down- or up-regulated) by BRB in both preneoplastic lesions and in papillomas. Interestingly, several of these genes are associated with matrix metalloproteinases involved in tissue invasion and metastasis, cell-cell adhesion and with calcium signaling. This is the first report suggesting that berries might influence genes involved in tissue invasion and metastasis in the rat esophagus.

Supported by NCI grant No. CA103180.

## Colon and Other Gastrointestinal Cancers

**PR-13 Inhibition of chronic colitis-induced carcinogenesis in IL-10 knockout mice by dietary supplementation of black raspberries.**

Jie Liao,<sup>1</sup> Yeon Tae Chung,<sup>1</sup> Li-Shu Wang,<sup>2</sup> Allison Yang,<sup>1</sup> Gary Stoner,<sup>2</sup> Guang-Yu Yang<sup>1</sup>. <sup>1</sup>Northwestern University, Chicago, IL; <sup>2</sup>The Ohio State University College of Medicine, Columbus, OH.

This abstract is being presented as a short talk in Concurrent Session 15. A full abstract is printed in the Proffered Papers: Oral Presentation Abstracts section of the conference proceedings. (Presented on board number A132)

**A133 Time-dependent gene expression changes in a human colon polyp cell line induced by a clinically relevant concentration of atorvastatin.** Eugene Elmore,<sup>1</sup> Aarti Jain,<sup>1</sup> Vernon E. Steele,<sup>2</sup> J. Leslie Redpath<sup>1</sup>. <sup>1</sup>UC Irvine, Irvine, CA; <sup>2</sup>National Cancer Institute, Bethesda, MD.

The identification of biomarkers for chemopreventive agent efficacy in cell cultures from relative target tissues is of great importance. We have utilized a cell line from a human precancerous colon polyp cell line, VACO-235, to evaluate the changes in gene expression following continuous exposure to a clinically relevant concentration of atorvastatin (0.3 nM). The changes were monitored relative to the time matched controls at 48 and 96 hours of continuous exposure. The total RNA was isolated from three replicate cultures for each time point and from four replicate cultures of the time matched controls. The RNA was analyzed using Affymetrix human genome U133 plus 2 arrays. The data were analyzed using Genespring GX (Agilent Technologies) software to determine agent induced changes and to determine significant changes ( $P \leq 0.05$ ) that occurred at either 48 or 96 hours (2613 genes) or at both 48 and 96 hours (334 genes). The data were further analyzed using Ariadne Pathway Studio. A number of pathways showed responses that suggest possible changes in the regulation of proliferation and apoptosis. The tumor necrosis factor receptor superfamily, member 1a pathway also showed important changes. Caspase 3 was induced by 5.4 fold at 48 hours and 2.4 fold at 96 hours. Pak1 (P21 activated kinase 1), caspase 8, caspase 2, and caspase 4 were also induced. The p38 MAP kinase pathway showed inhibition with MAP3K4 (MAP/ERK kinase 4), which showed a 5 fold inhibition at 48 hours and 2 fold at 96 hours. Changes in the p38 MAP kinase pathway suggested inhibition of multiple kinase genes. As could be predicted, the most significantly modified pathway was LRP5 (low density lipoprotein receptor-related protein 5) which regulates low density lipoprotein. This finding suggests that atorvastatin was evaluated at a concentration that was similar to *in vivo*. Longer term exposure studies are currently underway to determine the effect of continuous atorvastatin exposure on the culture morphology and growth of VACO 235 cells. These data suggest that the use of a clinically-relevant concentration of atorvastatin can modify the expression of several key potential biomarkers for colon cancer prevention.

## Head and Neck Cancers

**A134 Honokiol targets STAT3 in head and neck squamous cell carcinoma.** Rebecca J. Leeman-Neill,<sup>1</sup> Quan Cai,<sup>1</sup> Jack L. Arbiser,<sup>2</sup> Jennifer R. Grandis<sup>1</sup>. <sup>1</sup>University of Pittsburgh, Pittsburgh, PA; <sup>2</sup>Emory University, Atlanta, GA.

Head and neck squamous cell carcinoma (HNSCC) is a commonly occurring malignancy associated with severe morbidity, persistently high mortality rates, frequent recurrence, and the appearance of second primary tumors. Honokiol, a naturally occurring compound that is derived from the plant, *Magnolia officinalis*, which has been used in traditional Chinese medicine, has been shown to have anticancer activity in various models and to inhibit nuclear factor kappa B (NF $\kappa$ B), an oncogenic transcription factor. Crosstalk between NF $\kappa$ B and another transcription factor, signal transducer and activator of transcription (STAT)-3, has been demonstrated in HNSCC. Furthermore, NF $\kappa$ B and STAT3 have been found to directly interact in certain cell lines and to have various target genes, including proteins that regulate apoptosis, the cell cycle, and tumor angiogenesis, in common. Therefore, we hypothesized that honokiol might be useful in targeting STAT3, a protein that is known to promote growth, invasion, metastasis, and tumor angiogenesis in HNSCC and that shows promise as an important therapeutic target in the treatment of HNSCC. In the current study, 72-hour honokiol treatments inhibited the growth of HNSCC cell lines, Cal-33, UM-22b and 1483 with IC50's ranging from 3.90-7.44  $\mu$ M, as shown through trypan blue dye exclusion assays. Honokiol treatment also decreased expression levels of phosphotyrosine STAT3 in these same cell lines. The growth inhibitory activity of honokiol is associated with apoptosis, as demonstrated by staining for annexin-V. Because clinical data indicates that currently available HNSCC therapies have been inadequate in improving mortality rates over the last three decades, it is of interest that honokiol enhances the activity of the small-molecule receptor tyrosine kinase inhibitor, erlotinib, in HNSCC cells. Finally, honokiol was found to modestly inhibit the growth of tumors in HNSCC xenograft models. These results suggest that honokiol inhibits HNSCC growth in conjunction with downregulation of phosphotyrosine STAT3 in HNSCC.

## Lung Cancer

**A135 Uncovering tumor suppressive and oncogenic microRNAs in lung cancer.**

Xi Liu,<sup>1</sup> Lorenzo Sempere,<sup>1</sup> Fabrizio Galimberti,<sup>1</sup> Sarah Freemantle,<sup>1</sup> Candice Black,<sup>2</sup> Steven Fiering,<sup>1</sup> Charles Cole,<sup>1</sup> Konstantin Dragnev,<sup>2</sup> Vincent Memoli,<sup>2</sup> Murray Korc,<sup>1</sup> Hua Li,<sup>1</sup> James DiRenzo,<sup>1</sup> Mads Bak,<sup>3</sup> Sakari Kauppinen,<sup>3</sup> Ethan Dmitrovsky<sup>1</sup>. <sup>1</sup>Dartmouth Medical School, Hanover, NH; <sup>2</sup>Dartmouth-Hitchcock Medical Center, Lebanon, NH; <sup>3</sup>University of Copenhagen, Copenhagen, Denmark.

MicroRNAs (miRNAs) are noncoding small RNAs that regulate gene expression. Expression profiles of miRNAs are useful to improve classification, diagnosis, and prognostic information of specific human malignancies, including lung cancer. We sought to uncover miRNAs preferentially repressed or over-expressed in pre-malignant and malignant lung lesions in recently described transgenic cyclin E mice. These transgenic mice expressed in the lung under control of the human surfactant C promoter wild-type or proteasome degradation-resistant cyclin E species. These mice developed pulmonary dysplasia and adenocarcinoma, recapitulating pre-malignant and malignant lung lesions frequently found in lung cancer patients. Comprehensive miRNA microarray analyses were conducted using independently harvested normal and malignant lung tissues from these transgenic mice. A cluster of miRNAs was preferentially repressed in transgenic lung cancers versus normal lung tissues including: miR-34c, miR-145, miR142-5p, and other miRNAs previously associated with lung carcinogenesis. Expression profiles were independently validated by semi-quantitative and real-time polymerase chain reaction assays. In transgenic mice, single cell expression profiles were studied in pre-malignant and malignant lung lesions by *in situ* hybridization assays. Concordant results were obtained after analyses of paired normal-malignant human lung tissues representing each histopathologic subtype of non-small cell lung cancer. To address functional roles of repressed miRNAs, novel lung cancer cell lines were derived from murine transgenic wild-type (ED-1 cells) or proteasome-degradation resistant (ED-2 cells) cyclin E expressing lung cancers. ED-1 and ED-2 cells each caused lung adenocarcinomas to form after tail-vein injections into syngeneic FVB mice. Engineered over-expression of each basally repressed miRNA in ED-1 as well as in ED-2 cells markedly ( $P \leq 0.001$ ) repressed cell growth. Anti-miR co-transfections antagonized these effects. A mechanism for this growth suppression was found by showing cyclin E, a predicted miR-34c bioinformatic target, was significantly repressed in miR-34c transfectants. Other miRNAs were selected for knock-down in ED-1 and ED-2 cells by virtue of their high basal expression in these cells as well as in murine transgenic lung cancers and human lung cancers versus normal lung tissues. Knock-down of candidate oncogenic miRNAs repressed lung cancer cell growth, which was antagonized by over-expression of the same miRNA. Experiments are underway in miRNA transfected ED-1 cells to assess *in vivo* tumorigenicity in FVB mice. Taken together, these studies uncovered candidate tumor suppressive and oncogenic miRNAs. We propose these miRNAs are molecular pharmacologic targets for lung cancer therapy and chemoprevention.

**A136 A systems approach to the preclinical evaluation of targeted chemoprevention for lung cancer.**

Saswati Hazra,<sup>1</sup> Kostyantyn Krysan,<sup>1</sup> Tonya Walser,<sup>1</sup> Brian Gardner,<sup>1</sup> Gina Lee,<sup>1</sup> Jerry W. Shay,<sup>2</sup> John D. Minna,<sup>3</sup> Steve Horvath,<sup>4</sup> Steven M. Dubinett<sup>1</sup>. <sup>1</sup>David Geffen School of Medicine at UCLA, Los Angeles, CA; <sup>2</sup>Department of Cell Biology, The University of Texas Southwestern Medical Center, Dallas, TX; <sup>3</sup>Department of Internal Medicine, The University of Texas Southwestern Medical Center, Dallas, TX; <sup>4</sup>Department of Biostatistics & Human Genetics, UCLA, Los Angeles, CA.

The possibility of an "individualized medicine" approach to chemoprevention targeted to the specific molecular abnormalities in the individual at risk has been suggested as a means to tailor prevention strategies (Markowitz, *N Engl J Med.* 2007;356:2195-8). In order to initiate targeted chemoprevention for lung cancer, we have developed a pre-clinical system to assess potential chemoprevention agents against mutated human bronchial epithelial cell lines (HBEC). HBECs are exposed to drugs such as rosiglitazone, erlotinib and celecoxib and selected proliferative responses, gene expression, micro-RNA profiles and protein panels are assessed. This data is then assessed by systems analysis to determine the optimal chemopreventive agents against specific mutations. These data will ultimately be utilized both for agent selectivity and agent specific biomarker identification. In preliminary studies, we evaluated rosiglitazone, erlotinib and celecoxib against three genetically altered HBEC cell lines harboring mutations relevant in the pathogenesis of human lung cancer (p53 knockdown, K-ras<sup>V12</sup> and EGFR L858R mutations). As a model of long-term tobacco exposure, NNK- treated HBEC cells are also being utilized. In addition to selected proliferation assays, we assessed each agent against HBEC cell lines and evaluated a 50 protein luminex profile as well as a panel of more than 700 micro RNAs. These potential chemopreventive agents reveal specific patterns of alterations in proliferation, gene expression, protein secretion and microRNA profiles. Based on these findings, assessment of combinations of preventive agents are in progress. We anticipate that these methods will assist in the development of agent specific biomarkers and a personalized approach to chemoprevention for lung cancer.

This work is supported by the UCLA SPORE in Lung Cancer P50 90388, UT Southwestern SPORE in Lung Cancer P50CA75907 and The Flight Attendant Medical Research Institute (FAMRI) Young Clinical Scientist Awards.

## Melanoma and Skin Cancer

## Melanoma and Skin Cancer

**A137 NO initiates progression of human melanoma via a feedback loop mediated by APE/Ref-1, new opportunities for chemoprevention.** Zhen Yang, Sun Yang, Bobbye J. Misner, Rita Chiu, Frank L. Meyskens, Jr. UC Irvine, Orange, CA.

It is well recognized that nitric oxide (NO) is involved in tumor progression, including melanoma. Measurement of proliferative and metastatic capacity by MTS and Matrigel-invasion assays respectively were done. NO-treated melanoma cells (especially metastatic Lu1205) exhibited a higher capacity compared with control, especially metastatic Lu 1205 cells. Apurinic/aprimidinic endonuclease-1/redox effector factor-1 (APE/Ref-1) is a multifunctional protein, and its role in tumor biology has attracted considerable attention. To determine whether APE/Ref-1 plays a role in mediating NO-stimulation of melanoma progression, we investigated the effect of DETA/NO on levels of APE/Ref-1 and related downstream targets (Activator Protein-1 (AP-1)/Jun D, Matrix Metalloproteinase-1 (MMP-1), Bcl-2, and inducible nitric oxide synthase (iNOS)) by Western Blot and RT-PCR analysis. Following DETA/NO treatment, APE/Ref-1 and other downstream molecules were induced. Knockdown of APE/Ref-1 or AP-1/Jun D by specific siRNA markedly reversed the induction by NO stress of target proteins. These results present evidence for the existence of a functional feedback loop contributing to progression and metastatic capacity of melanoma cells. Resveratrol has been demonstrated to be an APE/Ref-1 inhibitor, and significant decreases in AP-1/JunD, MMP-1, Bcl-2, and iNOS protein levels occurred after exposure to resveratrol. This phenolic antioxidant as well as other inhibitors of Ref-1 may be an appropriate choice for combining with other compounds against melanoma cells that develop resistance by up-regulations of these molecules.

## Ovarian and Other Gynecological Cancers

**A138 Vaccination with HPV 16 L2E6E7 with GPI-0100 adjuvant elicits protective humoral as well as cell-mediated immune responses.** Balasubramanyam Karanam, Richard B.S Roden, Ratish Gambhira, Subhashini Jagu. Johns Hopkins School of Medicine, Baltimore, MD.

L1 virus-like particle (VLP) vaccines provide effective protection against Human papillomavirus (HPV) type from which the L1 was derived via the induction of neutralizing antibodies. While partial protection can occur against very closely related types, it is generally type-restricted and necessitates highly multivalent L1 VLP vaccines to obtain broad coverage. The minor capsid protein, L2, is emerging as an attractive alternative cross-protective antigen. Unfortunately, neither L1 VLP nor L2-based vaccines are effective against pre-existing disease, suggesting the need to develop therapeutic vaccines. Unlike the capsid proteins, the E6 and E7 oncoproteins are both required to maintain and expressed throughout cervical intraepithelial neoplasia (CIN) and cancer. TA-CIN, a vaccine comprising the HPV16 L2, E6 and E7 in a single tandem fusion protein, attempts to combine the advantages of broad cross-protection against HPV transmission with therapeutic responses targeting HPV16 early proteins. Here we test TA-CIN formulated along with GPI-0100, a semi-synthetic quillaja saponin analog, that was developed to promote both humoral and cellular immune responses. TA-CIN administered subcutaneously to mice three times at monthly intervals (125µg of clinical-grade protein) with 50µg GMP-grade GPI-0100 was found to elicit high titer antibodies that effectively neutralized not only HPV16 but also other oncogenic HPV types including HPV18, HPV31, HPV45, HPV58. Similarly, vaccination of pigtail macaques (*Macaca nemestrina*) with TA-CIN (three doses of 125µg of TA-CIN with 1000µg GMP-grade GPI-0100 at monthly intervals) was well tolerated and induced serum antibodies that neutralized HPV16, HPV18, and HPV31 *in vitro*. Notably, vaccination of mice with TA-CIN protected them from cutaneous HPV16 challenge as effectively as HPV16 L1 VLP. Combination of TA-CIN along with adjuvant GPI-0100 enhanced production of E7-specific, interferon gamma producing CD8+ T cell precursors by 20-fold. Vaccination with TA-CIN in adjuvant GPI-0100 completely prevented tumor growth after challenge with 5 x 10<sup>4</sup> HPV16-transformed TC-1 tumor cells. Protection of the mice immunized with TA-CIN plus GPI-0100 against TC-1 in a prophylactic setting was significantly more effective than TA-CIN alone or GPI-0100 alone. Patients vaccinated with TA-CIN alone develop weak HPV neutralizing antibody and E6/E7-specific T cell responses. GPI-0100 has been used safely as an adjuvant in several human trials suggesting that the combination of TA-CIN and GPI-0100 warrants further study.