ELISA. COX-2 mRNA expression was determined by real-time PCR in time course analyses utilizing cells incubated with IL-4 (10 ng/mL). COX-2 mRNA expression was decreased in response to IL-4; at 6 and 30 hours, the reduction was 50% and 80%, respectively. To determine whether IL-4 is able to regulate COX-2 transcription, transient transfection assays were performed utilizing a reporter gene construct containing the COX-2 promoter sequence (-1437/+127bp). IL-4 inhibited COX-2 transcription. Because signal transducer and activator of transcription 6 (STAT6) is the major transcription factor activated by IL-4, we determined the tyrosine phosphorylation status of STAT6 following IL-4 stimulation. Western blot showed that STAT6 was phosphorylated upon IL-4 stimulation. To determine whether IL-4 inhibition of COX-2 is mediated by STAT6, we utilized STAT6 siRNA to knock down STAT6 expression and then determined COX-2 expression by Western blot. IL-4 suppression of COX-2 is alleviated when STAT6 is knocked down, suggesting that STAT6 at least partially mediated IL-4 inhibition of COX-2. Our experiments demonstrate that IL-4 is able to inhibit COX-2 expression in NSCLC cells. The inhibition acts partly by suppressing COX-2 mRNA transcription and is partly mediated by STAT6. This is the first evidence that IL-4 is able to down-regulate COX-2 in NSCLC cells, and the first documentation that STAT6 is related to COX-2 expression. Our current investigations are also defining these relationships in normal bronchial epithelial cells. Collectively, these studies will help to identify targets that can be exploited for eventual use in cancer prevention.

#B53 Epigallocatechin-3-gallate Mediated Reversal of GSTP1 CpG Island Hypermethylation leads to Reactivation of GSTP1 Expression in Human Prostate Cancer Cells. Sanjeev Shukla, Shawn Trokhan, Martin I. Resnick, Sanjay Gupta. Case Western Reserve University, Cleveland, OH.

Epigenetic mechanisms through aberrant silencing of critical genes are central to the development and progression of prostate cancer. These epigenetic abnormalities are often associated with methylation of cytosine to 5-methylcytosine within a CpG dinucleotide. Such alterations may occur due to genomic imprinting, aging, environmental factors and/or lifestyle patterns. Silencing of glutathione S-transferase-pi (GSTP1) gene encoding the pi-class glutathione-S-transferase, a critical enzyme of carcinogen defense, through methylation of deoxycytidine residue in CpG islands in the 5'-regulating region has been frequently observed in human prostate carcinomas, prostatic intraepithelial neoplasia, and prostate cancer cell lines. GSTP1 plays an important role in the conjugation and detoxification of potential carcinogens and early loss of GSTP1 expression could lead to increased susceptibility to carcinogens, promoting mutation and prostate cancer progression. Unlikely mutated genes, epigenetically silenced genes are intact and are attractive targets for agents that could reactivate these dormant genes. Reactivation of such genes could be accomplished by DNA methylation inhibitors however; clinical utility of these inhibitors has been limited due to severe side-effects and toxicity. In recent years, green tea and its major polyphenolic constituent, epigallocatechin-3-gallate (EGCG) has received much attention as a promising chemopreventive agent for prostate cancer. Extensive laboratory studies in cell culture systems and in limited animal models have demonstrated that green tea polyphenols afford protective effects from diverse types of carcinogens and induce phase II enzyme activity that could lead to enhanced detoxification process. In the present study, we report that EGCG could reactivate GSTP1 gene and repress DNA methyltransferase (DNMT) expression in human prostate cancer LNCaP cells, which possess hypermethylated GSTP1 CpG island alleles, devoid of GSTP1 mRNA and protein expression. Treatment of LNCaP cells with EGCG (5-20 μM) for 3, 7 and 14 days resulted in a dose- and time- dependent reactivation of GSTP1 activity and expression as observed by ELISA, mRNA and protein expression. These results correlated with decrease in DNMT1 protein expression. Further, EGCG treatment of LNCaP cells resulted in increased expression of unmethylation-specific GSTP1 band observed after bisulfite modification and methylation-specific PCR. Taken together, these results suggest that EGCG, the major polyphenolic constituent of green tea has potential to modulate epigenetic mechanism(s) by reactivating GSTP1 gene and could be developed as a promising agent for prevention and/or therapy of prostate cancer in humans. (Supported by USPHS grant R21 CA 109424).

#B54 Induction of Signature Molecular ER Stress Responses by Selenium: A New Look into the Anticancer Mechanism of Selenium Action. Yue Wu, Ke Zu, Young-Mee Park, Clement Ip. Roswell Park Cancer Institute, Buffalo, NY.

Recently, a monomethylated selenium metabolite called methylseleninic acid (MSA) has been shown to cause global thiol redox modification of proteins in PC-3 human prostate cancer cells (see abstract by Eun-Mi Park et al in this conference). Extensive gain or loss of reactive protein thiols is a form of cellular stress because these alterations are known to lead to protein misfolding or unfolding. The concept of quality control monitoring of newly synthesized proteins in the endoplasmic reticulum (ER) is well described. An accumulation of aberrantly folded proteins triggers a defined set of sensor and modulator signals in an attempt to correct the defects so that the cells may continue to survive. We found that exposure of PC-3 cells to MSA induced a number of signature ER stress molecular markers, including phospho-PERK, phospho-eIF2α and GRP-78, in a dose- and time-dependent manner. Interestingly, GADD153 and caspase 12 activation were also greatly increased by MSA. The latter two markers are usually associated with cell cycle exit and/or apoptosis when ER stress due to protein unfolding is too severe and beyond repair. These same cellular changes have been demonstrated by numerous studies to occur in cancer cells (but not necessarily normal untransformed cells) treated with selenium. Based on the above information, we are proposing a novel hypothesis that typical ER stress signaling responses governing the balance between survival and death may also be initiated by selenium as a result of damage to mature proteins. Since metabolic oxidative stress is lower in normal cells compared to cancer cells, normal cells may be better equipped to manage redox stress than cancer cells. This might be one reason why normal untransformed cells are not nearly as sensitive as cancer cells to selenium inhibition of growth.

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#B55 The Chemopreventive Activity of Chlorogenic Acid Is Mediated by Inhibiting AP-1- MAPKs Pathway and Inducing Phase II Detoxifying Enzyme through Stimulating Nrf2 Signaling. Rentian Feng. CDC-NIOSH, Morgantown, WV.

Chlorogenic acid, one of the most abundant polyphenols in the human diet, inhibits chemical-induced carcinogenesis in animal studies. However, little is known about the molecular mechanisms through which chlorogenic acid prevents carcinogenesis. In this study, we report that chlorogenic acid inhibited the proliferation of A549 human cancer cells in vitro. Results of soft agar assays indicated that chlorogenic acid suppressed 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced neoplastic transformation of JB6 P+ cells in a dose-dependent manner. Pretreatment of JB6 cells with chlorogenic acid blocked UVB- or TPA-induced transactivation of activator protein-1 (AP-1) and NF-κB over the same dose range. At low concentrations, chlorogenic acid decreased the phosphorylation of c-Jun NH2-terminal kinases as well as mitogen-activated protein kinase (MAPK) kinase 4 induced by UVB/TPA, while higher doses are required to inhibit p38 kinase and extracellular signal-regulated kinases. Chlorogenic acid also increases the enzymatic activity of glutathione S-transferases (GST). Further study indicated that GST induction by chlorogenic acid may be through stimulating the nuclear translocation of NF-E2-related factor as well as subsequent induction of antioxidant response element (ARE)-mediated GST expression, which may be blocked by inhibition of PI-3 kinase pathway. These results provide the first evidence that chlorogenic acid could protect against environmental carcinogen-induced carcinogenesis and suggest that the chemopreventive effects of chlorogenic acid may be through its up-regulation of cellular antioxidant enzyme and suppression of reactive oxygen species-mediated NF-κB and AP-1-MAPKs activation.

#B56 Anticancer Activity of Capsaicin against Pancreatic Cancer. Sanjay K. Srivastava, Ian Humphreys. University of Pittsburgh, Pittsburgh, PA.

Capsaicin, a major ingredient in red chilli pepper of the genus capsicum, is commonly found in the human diet. Studies have shown that it is protective against experimentally induced mutagenesis and tumorigenesis. Interestingly, capsaicin has also been found to be antiproliferative against various transformed and malignant cells. However, its anticancer activity against pancreatic cancer has not been investigated. Here we report that capsaicin inhibits the growth of human pancreatic cancer cells AsPC-1, in culture, with an IC50 of approximately 150μM. Capsaicin exposure for 24h induced apoptosis

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