increase in the G_2/M accumulation in irradiated cells overexpressing MnSOD. There was a suppression of radiation-induced ROS in MnSOD overexpressing cells and strikingly increased clearance of γH2AX protein, a sensor for responding to DNA double-strand breaks and proposed predictor of radiosensitivity. Mitochondrial catalase overexpression increased the enzymatic activity 4 to 5 folds which resulted in the reversal of the radioresistance, G_2 accumulation, ROS suppression and γH2AX clearance in irradiated MnSOD overexpressing cells. These results indicate a role for superoxide signaling in radiation-induced cell cycle checkpoints and radiosensitivity in human pancreatic cancer cells and suggest that H_2O_2 signaling could act as a protective agent in irradiated MnSOD overexpressing cells. (Supported by CA111365, Carver Trust Fund, and Training Grant -CA078586-0651)

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Mechanistic Studies on the Anti-Cancer Activity of α-Phenyl Tert Butyl Nitrone (PBN)

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We have discovered that PBN has potent anti-cancer activity in hepatocellular carcinoma (HCC) models and is now being tested in a colon cancer model. in glioblastoma models we have also shown that the PBN natural metabolite 4-hydroxy-PBN also has potent activity in HCC models and is now being tested in other models. We have conducted extensive studies directed toward understanding the mechanistic basis of the anti-cancer nitrone activity. Considerable evidence indicates that nitric oxide production from iNOS activity plays an important role in the development of cancer in HCC models and glioblastoma models. Our data indicate that the action of PBN may be due to inhibition of iNOS induction. the role of nitric oxide in cancer development may be due to its action in s-nitrosation of active site cysteine residues thereby inactivating crucial enzymes. These include; A) specific caspases that play a role in cancer cell apoptosis and thus its inactivation would prevent the death of cancer cells; B) OGG1, the DNA repair enzyme that removes 8-OHdG and thus its inactivation would cause enhanced mutations and C) PTEN, the tumor suppressor gene protein, and thus its inactivation would cause enhanced oncogenic growth. We have examined whether PBN suppresses the induction of HIF-1α. This is because iNOS gene as well as several other important genes; including VEGF and many glycolytic enzymes are very important in cancer development are up regulated by the Hypoxia Inducing Factor 1 (HIF-1) acting as transcription factor. Our results in cancer cell models indicate PBN suppresses HIF-1α induction. the specific mechanistic basis of how this occurs is now being investigated. This research is supported in part by NIH RO1CA82506, OARS AR05.2-041 and OARS AR05.2-132.

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Dietary Blueberry in Hemangioendothelioma Therapeutics

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Introduction: a murine model utilizing subcutaneous injection of spontaneously transformed endothelial (EOMA) cells results in development of hemangioendothelioma (HE) tumors with 100% efficiency. Development of HE represents pure angiogenesis since EOMA cells connect with host vasculature to create blood filled, endothelial cell lined, vascular spaces. Agents that inhibit HE growth stop the angiogenesis inherent in its development. We have previously noted that standardized blueberry extract (BBE) has anti-angiogenic properties in vitro, presumably related to its antioxidant properties. Studies were performed to determine whether oral feeding of blueberry extract could inhibit HE growth by inhibiting the transcriptional activation of MCP-1 via the JNK signaling pathway. **Methods:** EOMA cells were grown in DMEM + 0.5% FCS +1% pen/strep. BBE was treated in vitro at 150 mg/ml. Lack of toxicity was confirmed by propidium iodide exclusion assay. BBE inhibition of TNFα inducible JNK phosphorylation, AP-1 activation and MCP-1 expression in EOMA cells was analyzed by ELISA for MCP-1 and JNK and luciferase reporter assay for AP-1. the ability of BBE to inhibit angiogenic activity in EOMA cells was confirmed in vitro using a Matrigel assay. HE were generated by injecting 100ul of EOMA cells ($5x10^7$ cells/ml PBS) subcutaneously in to mice. BBE daily gavage feedings at 5, 20, 200, or 1000 mg/kg were done starting at day 0 to test anti-angiogenic efficacy in vivo. HE specimens were collected day 7 post-injection. **Results:** BBE treatment of EOMA cells resulted in a statistically significant ($p<0.05$) inhibition of TNF-α inducible MCP-1 expression by inhibiting JNK and AP-1 signaling activities. BBE, apocynin and DPI all inhibited EOMA cell angiogenic activity in Matrigel. Oral feeding of BBE resulted in a statistically significant ($p<0.05$) decrease in tumor volume compared to vehicle treated controls. **Conclusions:** Oral intake of BBE can inhibit angiogenesis dependent endothelial cell neoplasm growth in vivo by inhibiting expression of MCP-1, which is required for HE growth. BBE with its strong antioxidant properties represents a potential therapeutic anti-angiogenic strategy for treating vascular tumors. [GMG Supported by K08 GM066964 NIGMS]

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The Ultra-Weak Luminescence of Human Leukemia Cells After Treated with Adriamycin

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Objective: to explore the correlation of the ultra-weak luminescence with biological characteristics of human leukemia cells. **Methods:** HL-60 and HL-60/ADM resistant cells were treated with different dose of adriamycin (ADM). the cell cycle expression of P-glycoprotein (P-gp170), SOD activity, MDA content, index of apoptosis, and the intensity of the ultra-weak luminescence were examined after the treatment. **Results: the** sensitivity of HL-60/ADM cells to ADM was lower than that of HL-60 cells; the percentage of HL-60/ADM cells in G_0/G_1 phase increased to 44.80±1.97% and in G_2/M phase increased to 9.90±0.27%, while the percentage of HL-60/ADM cells in S phase decreased to 45.30±1.93%. the expression of P-gp was found in HL-60/ADM cells; the SOD activity, MDA content and index of apoptosis were lower in HL-60/ADM cells than that of HL-60 cells; the intensity of ultra-weak luminescence of HL-60 cells was higher than that of HL-60/ADM cells ($P<0.05$). the intensity of ultra-weak luminescence was increased distinctly with the increasing of SOD activity and MDA content. **Conclusion:** the sensitivity of human leukemia cells to ADM is correlated well with the intensity of ultra-weak luminescence.

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Silica Carcinogenicity Analysis in a Susceptible Mouse Model

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In 1997 IARC classified crystalline silica as a Group I human carcinogen despite disagreements in the scientific community related to the lack of a mouse model for silica-induced carcinogenicity. Since mutations of the p53 tumor suppressor

gene are the most frequently observed genetic changes in human and animal cancers, this study was designed with mice deficient in p53 to evaluate the potential carcinogenicity of crystalline silica. Specifically, these experiments examined the effects of freshly fractured silica exposure (0 or 2mg) on various biochemical, molecular and genomic changes and incidence of preneoplastic lesions in the lungs of wildtype, heterozygous and homozygous mice after 2 and 6 months. Analysis performed on bronchioalveolar lavage fluid markers (albumin, lactate dehydrogenase), cells (differential cell counts, apoptosis, cell cycle analysis), and lung tissue (microarray, DNA damage) have shown differences related to exposure in levels for albumin, lactate dehydrogenase, apoptosis, cell cycle parameters as well as oxidative stress. Histopathologic alterations associated with silica exposure included alveolar epithelial cell hyperplasia, peribronchiolar bronchiolization and lipoproteinosis in the lungs of silica-exposed animals. Microarray analysis demonstrated alterations in genes related to cell cycle control, DNA damage repair and apoptosis, consistent with alterations seen in the cellular analysis. the data described here shows an effect of p53 status on response to silica exposure as early as 2 months following the initial exposure. This susceptible mouse model may provide insights into the critical role of p53 in silica-induced lung injury.

The findings and conclusions of this abstract are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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2-Deoxy-D-Glucose Sensitizes Human Breast Cancer Cells to the Toxicity of Taxol via Increases in Metabolic Oxidative Stress

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2-Deoxy-D-glucose (2DG) is a glucose analog that inhibits glucose metabolism by competing for uptake into glycolytic metabolic pathways and is believed to selectively induce metabolic oxidative stress in cancer cells. Taxol (TX) is a commonly used chemotherapeutic agent that is believed to kill cancer cells by hyperstabilizing microtubules and preventing cytoskeleton restructuring, as well as, causing oxidative stress. in the current study, human breast cancer cells were found to demonstrate greater than additive toxicity when treated with 2DG+TX. to confirm that oxidative stress was involved in 2DG+TX-induced cytotoxicity, total glutathione (GSH) and oxidized glutathione (GSSG) were measured and found to be increased 3.5- to 9-fold. Consistent with these findings, steady-state levels of prooxidants, as assayed using an oxidation sensitive fluorescent probe ($CDCFH_2$), were significantly enhanced in the presence of 2DG+TX. Furthermore, when human breast cancer cells were treated with 2DG+TX in the presence of the thiol antioxidant, N-acetyl-cysteine (NAC), toxicity was inhibited and parameters indicative of oxidative stress were suppressed. the antioxidant enzyme, catalase, conjugated to polyethylene glycol (PEG-CAT), also inhibited the toxicity of 2DG+TX. Collectively, these results strongly support the hypothesis that metabolic oxidative stress caused by increased steady-state levels of hydroperoxides mediates the synergistic interaction of 2DG+TX in human breast cancer cells. These results also suggest that combined modality cancer therapy designed to inhibit glucose and hydroperoxide metabolism, while increasing prooxidant production, may provide a useful biochemical rationale for the design of clinical protocols for the treatment of breast cancer.

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Histone Modifications Participate in the Transcriptional Regulation of Manganese Superoxide Dismutase

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Genetic information is packaged into higher order structures by histones. the conformation of these structures is modulated through the post-translation modification of histone tails which in turn can influence gene expression. Actively transcribed genes are generally associated with the acetylation of lysines on histone H3 and the methylation of lysine 4 of histone H3. Conversely, silenced genes are often associated with di-methylation of lysine 9 of histone H3. These modifications function as an epigenetic mechanism. Breast cancer cells typically exhibit lower activity and expression of manganese superoxide dismutase (MnSOD) than their normal counterparts. However, the mechanism responsible for this change in expression remains unclear. *SOD2*, the gene encoding MnSOD, contains an expansive CpG island extending from the 5' end of its promoter though its second intron. This characteristic makes *SOD2* an excellent candidate for control by epigenetic mechanisms including DNA methylation and histone deacetylation. We hypothesized that decreased expression of MnSOD in breast cancer cells is associated with altered histone modifications compared to non-transformed breast epithelial cells. to test this hypothesis we quantified the level of different histone modifications located at the *SOD2* locus by chromatin immunoprecipitation in cells with varying degrees of MnSOD expression. Using a primer tiling approach encompassing approximately 3000 bp of the *SOD2* gene we measured histone H3 acetylation, H3 lysine 4 methylation, and H3 lysine 9 di-methylation at key regulatory regions within *SOD2*. Results of these experiments revealed significant changes in the levels of these different histone modifications in breast cancer cells compared to immortalized human mammary epithelial cells. Preliminary evidence also supports that altered histone modifications in breast cancer cell lines might correlate with their level of MnSOD expression. Thus, our studies indicate that epigenetic silencing of *SOD2* could be facilitated by changes in histone modifications and constitute one mechanism leading to the altered expression of MnSOD observed in many breast cancers. (Supported by NIH CA73612 and CA66081)

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Studies on the Molecular Mechanisms of Nicotinic Receptors-Mediated Carcinogenic Effects on Human Breast Cancer Cells

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Tobacco-smoking is one of the well understanding carcinogenic factors involved in breast cancer formation. in this study, we first demonstrated that the several types of nicotinic acetylcholine receptors (nAchRs) were detected in breast cancer cell lines including MCF-7 and MDA-MB-231. the expression of the nAchR mRNA levels in tumor tissues from 157 cases of breast cancer patients in Taiwan were determined and found that the α5, α9 and α10 subunits of the nAchRs were the most prevalence in both normal and tumor tissue. Quantitative assays of the nAchRs mRNA levels was performed by real-time PCR analysis which revealed that the expression levels of α9-nAchR was higher in most of the tumor tissue when compared to the normal tissues which dissected form the tumor margin. the receptor binding activity assay was performed and demonstrated that α9-nAchR significant binding to its ligand (nicotine) in a concentration as low as 7 μM and reached the maximal level as soon as 60 minutes. the α9-nAchR expression in breast cancer cells was knock-