dietary intake, may be a factor contributing to the lower incidence of breast cancer in Asian women. Since HER-2 receptor activity is associated with poor prognosis and isoflavones have been implicated to have cancer preventive properties, we hypothesized that treatment of SKBR3 breast cancer cells with Biochanin-A decreases HER-2 receptor activation and inhibits downstream (mitogenic and invasive) signaling pathways. To test our hypothesis we carried out Western Blot analysis to determine the protein levels of phosphorylated and unphosphorylated HER-2 receptor and downstream signaling pathways in SKBR3 breast cancer cells following 48-72 hrs of treatment with various concentrations of Biochanin-A. Our results indicate a decrease in HER-2 receptor phosphorylation (activation) in cells treated with 20, 50 and 100 μM Biochanin-A. Treatment with 50 and 100μM Biochanin-A also suppressed phosphorylation of Akt, Erk1/2 and mTOR. Reduction in levels of Membrane-type 1 MMP (MT1-MMP), which activates the matrix metalloproteases (MMPs) was also observed in cells treated with 50 and 100 μM Biochanin-A. MMPs are matix degrading enzymes which result in cancer cell motility and invasion into surrounding areas. Our results indicate that Biochanin-A suppresses activation of mitogenic (HER-2, Erk1/2), survival (Akt/mTOR), and invasive (MT1-MMP) pathways. These observations suggest Biochanin-A may be a unique natural agent to be used for cancer treatment in combination with other drugs as well as for prevention. (This work was supported by grants NIH/NCRR INBRE grant #P20R16454 and Mountain State Medical Research Institute grant #R2002-34, Boise, Idaho.)

#4843 Analysis of transcription factor expression after homoharringtonine treatment. Shawnya Michaels[1], Dennis Brown[1], David Segal[2]. *[1]ChemGenex Pharmaceuticals, Inc., Menlo Park, CA and [2]Deakin University, Geelong, Australia.*

Homoharringtonine (HHT) is a natural product alkaloid from the Cephalotaxus evergreen family. HHT is currently being evaluated clinically for the treatment of CML resistant to current tyrosine kinase (TK) inhibitor therapies, including imatinib mesylate and dasatinib. Additional phase II activity in AML and MDS has been reported. Identified initially for its inhibitory effects on protein synthesis (elongation step), recent laboratory studies have also demonstrated that HHT upregulates apoptotic pathways and inhibits angiogenic processes. In addition, clinical studies in CML have demonstrated that HHT treatment reduces bcr/abl mRNA transcript levels in patients resistant to imatinib mesylate suggesting that more specific molecular targets may be affected by this agent. To further delineate the cellular pathways affected by HHT, *in vitro* studies examining the expression and nuclear levels of various transcription factors (TF) after HHT treatment were performed. Using the K562 leukemia cell line and a multiplex transcription factor assay (Marligen Biosciences, Ijamsville, MD) the expression of 50 TF was examined after exposure to 10nM HHT for 15 min, 30 min, 2 hr, 4 hr and 24hr. Several TF involved in differentiation were overexpressed relative to untreated controls. These included GATA, PPAR, EGR, PAX6, AR and AP2. In contrast, treatment of the mouse macrophage cell line, RAW 264.7, with 10 nM HHT for the same treatment exposures, did not induce any increased expression of these particular TF. However, treatment of RAW 264.7 cells with HHT followed by LPS stimulation did induce overexpression of some other TF compared to treatment of cells with LPS alone. These included EGR, NF-kappaB, PBX, CEBPa, CEBPd, ER, TRE-AP1, LEF1 and HIC1. These studies indicate that HHT may affect some cellular processes at the level of transcriptional control. Furthermore, differences in the expression of certain TF between malignant and normal cells may exist after HHT treatment. These differences may be of therapeutic value allowing for the opportunity to further optimize the therapeutic use of HHT.

#4844 Mitochondrial peroxiredoxin-3 is selectively oxidised in cells treated with pro-apoptotic isothiocyanates. Kristin K. Brown and Mark B. Hampton. *Free Radical Research Group, Christchurch School of Medicine and Health Sciences, Christchurch, New Zealand.*

The isothiocyanates are a class of natural products derived from cruciferous vegetables. We have shown that aromatic isothiocyanates, in particular phenethyl isothiocyanate, possess an ability to induce apoptosis in cells that overexpress the anti-apoptotic protein Bcl-2. Many isothiocyanates, including sulforaphane, were unable to induce apoptosis in the cells expressing Bcl-2, despite sharing other biological properties. In this study we focussed on identification of the cellular targets of the pro-apoptotic isothiocyanates that may play a role in mediating apoptosis. The predominant intracellular reaction of the isothiocyanates is with cysteine residues, therefore, using a sensitive proteomic technique to label oxidised thiol proteins a preliminary investigation of the targets of the pro-apoptotic isothiocyanates was undertaken. We have shown that a selective pool of thiol proteins are modified following exposure to phenethyl isothiocyanate, but are not affected by exposure to sulforaphane. One thiol protein that consistently changed when cells were exposed to phenethyl isothiocyanate was identified as mitochondrial peroxiredoxin-3. The oxidation of peroxiredoxin-3 was validated by western blotting and shown to occur before the activation of apoptosis. This effect may bypass the anti-apoptotic action of Bcl-2 and promote apoptosis.

#4845 Curcumin potentiates the growth inhibitory effect of paclitaxel through suppression of nuclear factor-kappa B in breast cancer. Hee Jeong Kang[1], Sang Hun Lee[1], Hwa Seon Lee[1], Janet. E. Price[2], Lee-Su Kim[1]. *[1]Hallym University Sacred Heart Hospital, Anyang, Republic of Korea and [2]UT M.D. Anderson Cancer Center, Houston, TX.*

Cancer chemotherapeutic strategies should be devised to provide higher tumor response and lower toxicity. Paclitaxel is an effective anticancer agent for breast cancer, but its major disadvantage is dose-limiting toxicity. Most anticancer agents activate nuclear factor kappa B (NF-κB), which can mediate cell survival, proliferation, and metastasis. Curcumin has been shown to inhibit the growth of various cancer cells, without toxicity to normal cells. The antitumor effects of curcumin could be due in part to the inactivation of NF-κB. We hypothesize that blocking NF-κB activity may augment paclitaxel cancer chemotherapy. In this study, we investigated whether the inactivation of NF-κB by curcumin would enhance the efficacy of paclitaxel for inhibiting breast cancer growth *in vitro* and *in vivo*. We confirmed that curcumin showed inactivation of NF-κB induced by paclitaxel through IκBα western blor analysis. Next, MDA-MB-231 breast cancer cells were treated with 10 uM paclitaxel, 10 μM curcumin, 10 uM paclitaxel and 10 μM curcumin combined, and 10uM paclitaxel plus IKKβ dominant negative (DN) transfection, as a positive control that totally inhibits NF-κB activation. The combination of 10 μM curcumin with 10 uM paclitaxel elicited significantly greater inhibition of cell growth and more apoptosis, compared with either agent alone. Inactivation of NF-κB by IKKβ DN enhanced the anti-tumor effect of paclitaxel as much the same manner as curcumin.. In an experimental breast cancer murine model using MDA-MB-231 cells, combination therapy with paclitaxel and curcumin significantly reduced tumor size and decreased tumor cell proliferation, increased apoptosis and decreased the expression of MMP-9 compared with either agent alone. These results clearly suggest that a curcumin-paclitaxel combination which inactivates NF-κB activity, may contribute to increased cell growth inhibition and apoptosis, thus augmenting paclitaxel chemotherapy, could be a novel strategy for the treatment of breast cancer.

#4846 The Mcl-1L protein confers resistance of human non-small cell lung carcinoma NL20TA cells to arsenic trioxide-induced apoptosis. Bao-Zhu Yuan[1], Amy M Jefferson[1], Rentian Feng[2], Steven H Reynolds[1]. *[1]Nat'l Institute for Occupational Safety & Health, Morgantown, WV and [2]University of Pittsburgh Cancer Institute, Division of Hematology/Oncology, Pittsburgh, PA.*

Arsenic is an occupational and environmental carcinogen. Arsenic is also used as a therapeutic reagent in treating some hematopoietic malignancies through the induction of tumor cell apoptosis. Two opposing paradigms of arsenic in cancer development and in apoptosis induction support a general view that an alteration in apoptosis develops during tumor formation and confers resistance of the transformed cells to apoptosis from different stimuli. To understand the apoptotic response of normal lung epithelial cells and lung cancer cells to arsenic, we characterized some apoptosis-related biochemical events in NL20, a non-tumorigenic human lung epithelial cell line, and NL20TA, a tumorigenic lung cancer cell line treated with arsenic trioxide (ATO). It was found that NL20 cells exhibited a positive apoptotic response to 10μM ATO through activation of the mitochondria-caspase 3 pathway, as manifested by Bid protein cleavage, selective mitochondrial release of the Smac protein and cleavage of the caspase 3, caspase 9 and PARP proteins. However, NL20TA cells showed no induction of apoptosis and no corresponding biochemical reactions following ATO treatment. The apoptosis in NL20 cells was associated with increased cellular oxidative stress; antioxidant treatment with MnTBAP and NAC inhibited ATO-induced apoptosis and the accompanying protein cleavages. Further investigation showed that the resistance of NL20TA cells to ATO-induced apoptosis was conferred by elevated Mcl-1_L expression coupled with the loss of Mcl-$1_{S/\Delta TM}$ expression, resulting in the inhibition of Smac release and caspase cleavage. This study provides a new insight into the mechanism of ATO-induced apoptosis and forms a basis for developing a novel therapeutic approach for treating lung cancers using a combination of anti-Mcl-1 and ATO treatments.

#4847 A novel EGFR/Neu targeting anticancer drug - 4'-Hydroxygilvocarcin V. Sowmyalakshmi Srinivasan, Madankumar Kharel, Jurgen Rohr, Damodaran Chendil. *University of Kentucky, Lexington, KY.*

Overexpression of Epidermal growth factor receptor (EGFR) is associated with aggressive phenotype and poor prognosis in lung cancer patients. Hence, targeting EGFR is one major step towards enhanced therapeutic benefits in lung cancer patients. A new gilvocarcin derivative, 4'-hydroxygilvocarcin V (4'-OH GV), was

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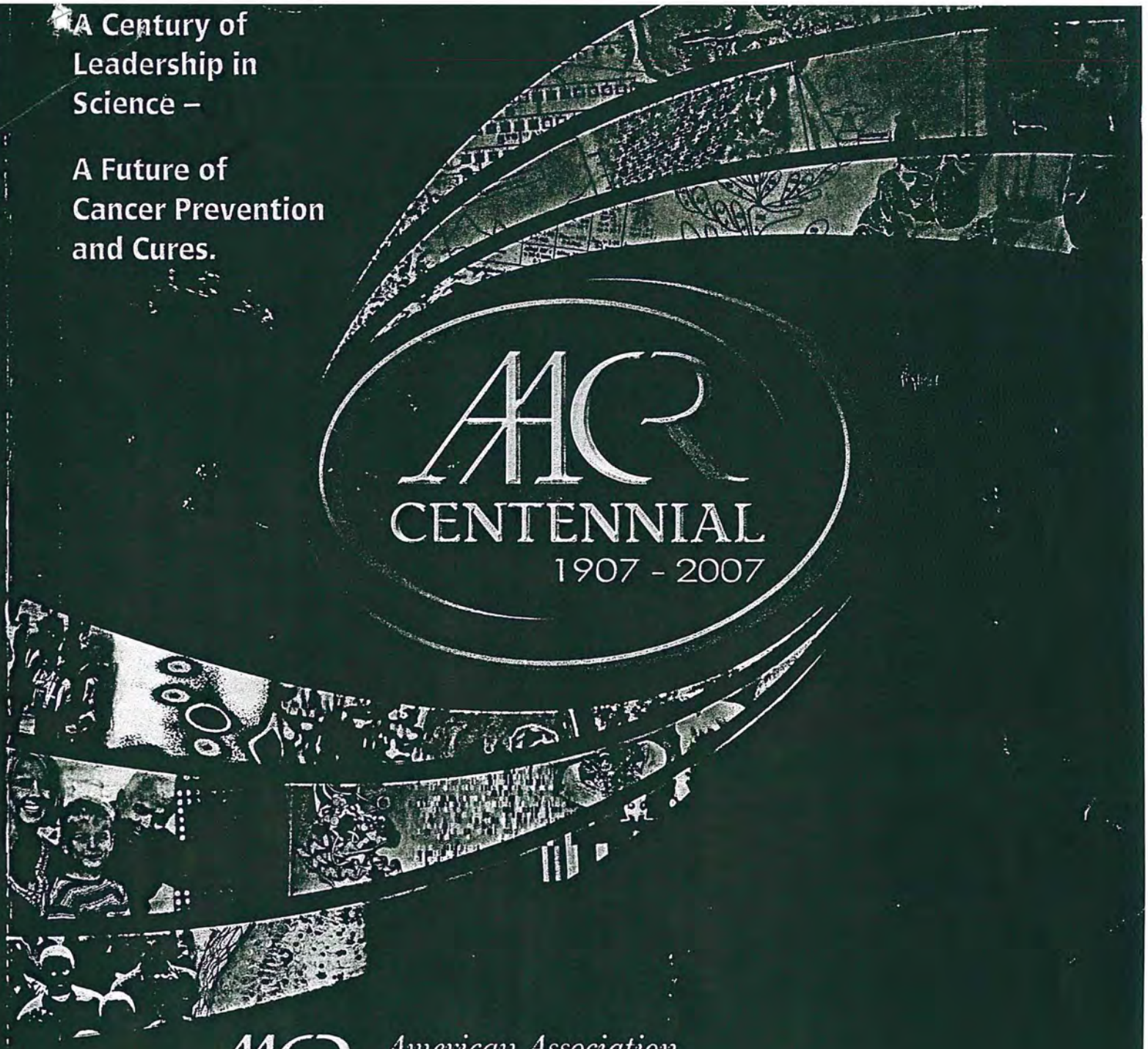

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