1093 Piperlongumine Targets Sp Transcription Factors in Cancer Cells by ROS-Dependent Epigenetic Repression of cMyc

K. B. Karki[2], U.-H. Jin[2], E. Hederick[2], S. Safe[2], R. Kasiappan[2], G. Chadalapaka[2], and I. Juturoo[1]. [1]*Covance Inc,, Madison, WI; and* [2]*Texas A&M University, College Station, TX.*

Piperlongumine (PL) is a natural product isolated from the plant species *Piper Longum L* that was identified in a cell-based small molecule screening assay for anticancer agents that selectively kill cancer vs non-cancer cells. Piperlongumine enhances cellular stress by induction of ROS and we investigated the effects of PL on ROS-dependent down-regulation of specificity protein (Sp) transcription factors Sp1, Sp3 and Sp4 and pro-oncogenic Sp-regulated gene products. Preliminary studies show that 5, 10 and 15 μM PL inhibited growth of Panc-1 & L3.6PL pancreatic, A549 lung, 786-O kidney and SKBR3 breast cancer cells after treatment for 24 or 48 hours and EC50 values for growth inhibition were 10 & 9.46, 21, 15, 15 μM (24 hours) and 14 & 6.71, 10, 14, 11 μM (48 hours) respectively. These same cell lines were also treated with PL plus the antioxidant glutathione (GSH) and growth inhibition was attenuated at all concentration of PL indicating that piperlongumine-induced ROS was the major induced cytotoxic response. Treatment of the cancer cell lines with 10 μM PL for 24 or 48 hours also downregulated expression of Sp1, Sp3 and Sp4 proteins and co-treatment with GSH reversed this response. PL also downregulated expression of Sp-regulated survival (survivin, bcl-2) growth promoting (cyclin D1, epidermal growth receptor [EGFR]) and angiogenic (vascular epithelial growth factor [VEGF]) genes in several different cancer cell lines. PL also decreases expression of Myc and other Myc regulated genes including miR-17, miR-20a and miR-27a and these effects were also attenuated in cells co-treatment with PL plus GSH. PL also induced ROS-dependent epigenetic repression of cMyc, downregulated miR-27a and miR-20a/miR-17 and induced cMyc regulated transcriptional repressors ZBTB10, ZBTB34 and ZBTB4 in cancer cells. Our results demonstrate that the selective ROS-dependent cytotoxicity of PL is due, in part, to downregulation of Sp proteins and pro-oncogenic Sp-regulated genes via cMyc-miR-ZBTB pathway.

1094 Mitochondrial Roles in Tumorigenesis

W. Irwin. *US EPA, Arlington, VA.*

Mitochondria supply ~90% of the ATP in most eukaryotic cells for normal functions by oxidative phosphorylation. Solid tumors can have hypoxic cores inhibiting oxidative metabolism. Despite their rapid proliferation, paradoxically tumor cells have predominantly glycolytic metabolism even in the presence of oxygen (Warburg Effect), a survival advantage. The mitochondrial electron transport chain is the source of ~90% of the reactive oxygen species in cells (DNA damage linked to carcinogenesis). Apoptosis is a mechanism by which the body kills tumor cells, with key check points in the mitochondria. Induction of the mitochondrial permeability transition pore (PTP) causes mitochondrial damage and induces cell death via apoptosis or necrosis. Some viruses which desensitize apoptosis and citric acid cycle inhibition can promote tumorigenesis. Wistar rats were fed the liver carcinogen acetylaminofluorene (AAF) at 100-400 ppm for up to 16 weeks, then liver cells and mitochondria were assayed for resistance to cell death, TUNEL staining and PTP induction, as described previously (*PNAS* 100: 10014). Also, chemicals with potential effects on mitochondrial biology were screened for induction/inhibition of the PTP/cell death (oligomycin, dantrolene, 4-hydroxynonenal, cyclosporine, etc). Our results showed that AAF induces liver tumors which is preceded by resistance to PTP inducers (i.e. arachidonic acid, calcium overload) and tolerance to cell death/caspase activation *in vivo* by hepatotoxins (lipopolysaccharide + galactosamine). Our unpublished data shows that AAF upregulated the anti-apoptotic mitochondrial cytochrome c oxidase (critical in oxidative phosphorylation) and a modulator of this enzyme abolished AAF PTP inhibition. Importantly, our original results also show that compounds which inhibit the PTP or apoptosis correlate with increased risk for tumorigenesis while compounds which induce the PTP are toxic to tumor cells. Thus, inhibition of the PTP or apoptosis are mechanisms by which abnormal cells can survive and proliferate. Mitochondria generate energy for normal biological functions and are important cell death checkpoints which can be exploited as targets for cancer therapies. Conversely, aberrations in mitochondrial function have pivotal roles in tumor cell survival and proliferation. Disclaimer: *The views on this abstract are those of the authors alone and do not represent Agency policy or endorsement. Mention of trade names of commercial products should not be interpreted as an endorsement by the US Environmental Protection Agency.*

1095 Hydroxyphenyl-Bisindole Isomers As Specific Ligands for Nuclear Receptor 4A1 (NR4A1)

X. Li, E. Hedrick, A. Lacey, and S. Safe. *Texas A&M University, College Station, TX.*

The nuclear receptor 4A1 (NR4A1/Nur77/TR3) plays an important role in normal physiology and several types of diseases and there is evidence that this receptor is overexpressed in multiple tumors and exhibits oncogene-like activity. 1,1-Bis(3′-indolyl)-1-(p-hydroxyphenyl)methane (DIM-C-pPhOH) has previously been identified as an NR4A1 ligand that acts as an NR4A1 antagonist in cancer cell lines and inhibits NR4A1-dependent genes/pathways in colon, pancreatic, breast and kidney cancer cells and in rhabdomyosarcoma (RMS) cells. In this study, we compared the relative activities of DIM-C-pPhOH and the m-hydroxyphenyl (DIM-C-mPhOH) and o-hydroyphenyl (DIM-C-oPhOH) isomers in transactivation assays and in regulation of NR4A1-dependent genes. In transactivation assays, Panc1 pancreatic cancer cells were transfected with a GAL4-NR4A1 (full length) chimera and UAS-luciferase reporter gene and we observed that all of these isomers decreased transactivation and exhibited NR4A1 antagonist activity. In addition, the specificity of DIM-C-pPhOH, DIM-C-mPhOH and DIM-C-oPhOH for NR4A receptors was determined in Panc1 cells transfected with GAL4-NR4A2 and GAL4-NR4A3 where the receptors are also full length. The results show that these compounds were specific for NR4A1 and did not modulate luciferase activity in cells expressing GAL4-linked NR4A2 or NR4A3. We further evaluated the effects of these compounds to downregulate NR4A1-dependent expression of thioredoxin domain containing 5 (TXNDC5) and beta-1 integrin in Panc1 cells and SKBR3 breast cancer cells and PAX3-FOXO1A and TXNDC5 in Rh30 RMS cells. The results demonstrated that all these compounds inhibited expression of these NR4A1-regulated genes in the different cancer cell lines and their order of potency was DIM-C-oPhOH > DIM-C-mPhOH > DIM-C-pPhOH. Current studies are focused on developing more potent NR4A1 ligands and examining their *in vivo* anticancer activities and potential for clinical applications in cancer chemotherapy for patients overexpressing NR4A1.

1096 Dose Response of Multi-Walled Carbon Nanotube-Induced Lung Tumors

L. M. Sargent[2], D. W. Porter[2], D. Lowry[2], L. Battelli[2], K. Siegrist[2], M. L. Kashon[2], B. T. Chen[2], A. F. Hubbs[2], L. Staska[1], W. McKinnney[2], M. Andrew[2], M. Andrew[2], S. Tsuruoka[3], M. Endo[3], V. Castranova[4] , and S. H. Reynolds[2]. [1]*Charles River, Durham, NC;* [2]*NIOSH, Morgantown, WV;* [3]*Shinshu University, Nagano, Japan; and* [4]*West Virginia University, Morgantown, WV.*

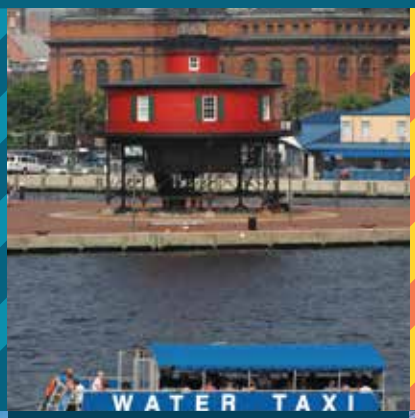
Mitsui-7 MWCNTs are strong lung tumor promoters in B6C3F1 mice. B6C3F1 mouse lung tumors have many molecular and morphological similarities to human pulmonary tumors. In previous work, we have demonstrated that exposure to inhaled Mitsui-7 following exposure to a DNA damaging agent caused potent promotion of lung tumors. To investigate a possible threshold for Mitsui-7-induced carcinogenesis, we exposed B6C3F1 mice to a single dose of either methylcholanthrene (MC, 10 μg/g BW, i.p.) or vehicle (corn oil). One week after i.p. injections, mice were exposed by inhalation to MWCNTs (5 mg/m^3, 5 hours/day, 5 days/week) or filtered air (controls) for a total of 2, 5 or 10 days. At 17 months post-exposure, mice were euthanized and examined for lung tumor formation. Thirty six percent of the filtered air controls, 33% of the MWCNT-exposed, and 47% of the MC-exposed, had a mean of 0.33, 0.33 and 0.4 tumors per mouse, respectively. By contrast, 94% of the mice which received MC followed by 10 days MWCNT had an average of 2.9 tumors per mouse while 81% of the mice exposed to 5 days of MWCNTs had 1.9 tumors per mouse, and 73% of the mice exposed to 2 days of MWCNTs had 1.2 tumors per mouse. Additionally, mice exposed to MWCNTs or MC followed by MWCNTs had larger tumor volumes than their corresponding air-exposed control groups. Preliminary data indicate a dose response in the percent of animals with tumors as well as the number of tumors per animal following exposure to MC and MWCNTs. In this study, mouse MWCNT lung burden approximates feasible human occupational exposures.


The Toxicologist

Supplement to *Toxicological Sciences*

56th Annual Meeting and ToxExpo™

Baltimore, Maryland | March 12–16, 2017

OXFORD
UNIVERSITY PRESS

ISSN 1096-6080
Volume 156, Issue 1
March 2017

www.toxsci.oxfordjournals.org

The Official Journal of the Society of Toxicology

SOT | Society of Toxicology

www.toxicology.org