Lone Star Regional Chapter of the Society of Toxicology
Annual Regional Meeting
September 7-8, 2012 ▪ Waco, TX

Advances in Toxicology

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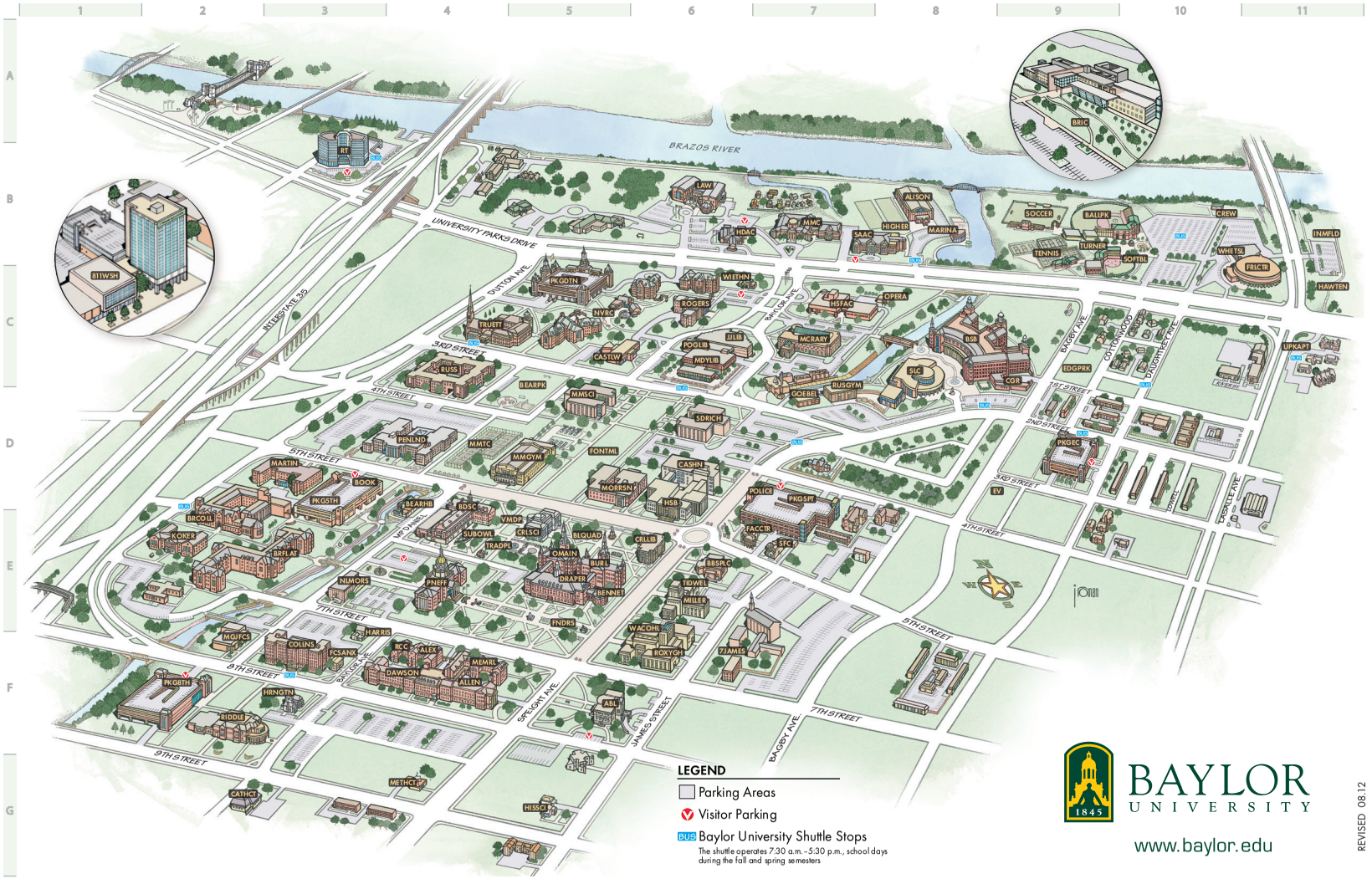

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REVISED 08.12

NAME	GRID	CODE
ACADEMIC/ADMINISTRATION		
Air Force and Army R.O.T.C.	D7	PKGSPT
Baylor Research and Innovation Collaborative *1800 Orchard Ln.*	A9	BRIC
Baylor Sciences Building	C8	BSB
Burleson Hall	E5	BURL
Carlile Geology Research Center	C9	CGR
Carroll Science Hall	E5	CRLSCI
Castellaw Communications Center	C5	CASTLW
Clifton Robinson Tower	B3	RT
Draper Academic Building	E5	DRAPER
Ed Crenshaw Student Foundation Center	E7	SFC
George W. Truett Theological Seminary	C4	TRUETT
Glennis McCrary Music Building	C7	MCRARY
Goebel Building	D7	GOEBEL
Hankamer School of Business	D6	HSB
Cashion Academic Center	D6	CASHN
Harrington House	F3	HRNGTN
Harris House	F3	HARRIS
History Fair & Science Fair Office	G5	HISSCI
Hooper-Schaefer Fine Arts Center	C7	HSFAC
Lewis Art Building		
Marrs McLean Science Building	D5	MMSCI
Martin House	F3	FCSANX
Mary Gibbs Jones Family and Consumer Sciences Building	F2	MGJFCS
Mayborn Museum Complex	B7	MMC
Morrison Hall	D5	MORRSN
Neill Morris Hall	E3	NLMORS
Old Main	E5	OMAIN
Opera Building	C8	OPERA
Pat Neff Hall	E4	PNEFF
Rogers Engineering and Computer Science Building	C6	ROGERS
Sheila and Walter Umphrey Law Center	B6	LAW
Sid Richardson Building (Paul L. Foster Success Center)	D6	SDRICH
Speight Plaza Office and Parking Facility	D7	PKGSPT
Tidwell Bible Building	E6	TIDWEL
Waco Hall	E6	WACOHL
ACADEMIC MAIN OFFICES (Office Number)		
College of Arts & Sciences (210)	E5	BURL
George W. Truett Theological Seminary (105)	C4	TRUETT
Graduate School (200)	D5	MORRSN
Hankamer School of Business (106)	D6	HSB
Honors College (203)	D5	MORRSN
Louise Herrington School of Nursing (C107)	C8	BSB
Office of the Provost	E4	PNEFF
School of Education (119)	E5	BURL
School of Engineering & Computer Science (307)	C6	ROGERS
School of Law (219)	B6	LAW
School of Music (206)	E6	WACOHE
School of Social Work *811 Washington Ave.*	B1	811WSH
University Libraries	C6	MDYLIB
ATHLETICS, RECREATION & SPORTS		
Baylor Recreational Sports Fields (Intramurals)	B11	INMFLD
Carlile Equestrian Building and Willis Family Equestrian Center *3540 S. University Parks Dr.*	Not Pictured	
Hawkins Indoor Tennis Center *under construction*	B11	HAWTEN
Highers Athletic Complex	B8	HIGHER
Allison Indoor Football Practice Facility	B8	ALISON
Simpson Athletics and Academic Center	B7	SAAC
Marina	B8	MARINA
McLane Student Life Center	C8	SLC
McLean Tennis Courts	D4	MMTC
Rena Marrs McLean Gymnasium	D5	MMGYM
Russell Gymnasium	D7	RUSGYM
Turner Riverfront Complex	B9	TURNER
Baylor Ballpark	B9	BALLPK
Baylor Soccer Offices & Betty Lou Mays Soccer Field	B9	SOCCER
Ferrell Center	C11	FRLCTR
Whetsel Basketball Practice Facility	B11	WHETSL
Getterman Softball Stadium & Indoor Practice Facility	B9	SOFTBL
Hurd Tennis Center	B9	TENNIS
AUDITORIUMS & CONFERENCE CENTERS		
Bennett Auditorium	E5	BENNET
Bill Daniel Student Center (SUB)	D4	BDSC
Blume Conference Center	D6	CASHN
Bobo Spiritual Life Center	E6	BBSPLC
Hughes-Dillard Alumni Center	B6	HDAC
Jones Concert Hall	C7	MCRARY
Jones Theater	C7	HSFAC
Kayser Auditorium	D6	HSB
McMullen-Connally Faculty Center	E7	FACCTR
Miller Chapel	E6	MILLER
Paul Powell Chapel	C4	TRUETT
Reynolds Conference Center	F4	RCC
Robbins Chapel	E2	BRCOLL
Roxy Grove Hall	F6	ROXYGH
Stacy Riddle Forum	F2	RIDDLE
Waco Hall	E6	WACOHL
SPIRITUAL LIFE CENTERS		
Bobo Spiritual Life Center	E6	BBSPLC
Methodist Student Center	G4	METHCT
St. Peter's Catholic Student Center	G2	CATHCT
Seventh and James Baptist Church	F6	7JAMES
DINING & FOOD SERVICES		
Atrium Dining (Baylor Sciences Building)	C8	BSB
Brooks Dining Hall	E2	BRCOLL
C3 Convenience Store	D7	PKGSPT
Collins Café	F3	COLLNS
Discovery Center Café	B7	MMC
Food Court (SUB) (Bill Daniel Student Center)	D4	BDSC
Law School Café	B6	LAW
McMullen-Connally Faculty Center	E7	FACCTR
Memorial (RFoC)	F4	MEMRL
Penland Food Court	D4	PENLND
Starbucks in Moody	C6	MDYLIB
Starbucks in PKGDTN	C5	PKGDTN
The Seasons Sushi	C5	NVRC
Terrace Café	B3	RT
INFORMATION & SERVICES		
Baylor Bookstore	D3	BOOK
Baylor Police Department	D7	POLICE
Parking & Transportation Services	B3	RT
Student Financial Services	B3	RT
Wiethorn Visitors Center	C6	WIETHN
LIBRARIES & MUSEUMS		
Armstrong Browning Library	F5	ABL
Carroll Library (Texas Collection)	E6	CRLLIB
Jesse H. Jones Library	C6	JJLIB
Martin Museum of Art	C7	HSFAC
Mayborn Museum Complex	B7	MMC
Moody Memorial Library	C6	MDYLIB
Poage Legislative Library	C6	POGLIB
Sheridan and John Eddie Williams Library	B6	LAW
OUTDOOR AREAS		
Bear Park	C5	BEARPK
Bill and Eva Williams Bear Habitat	D4	BEARHB
Burleson Quadrangle	E5	BLQUAD
Edgefield Park	C9	EDGPRK
Founders Mall	E5	FNDRS
Fountain Mall	D5	FONTML
National Pan-Hellenic Garden	D5	FONTML
SUB Bowl	E4	SUBOWL
Traditions Plaza	E4	TRADPL
Vara Martin Daniel Plaza	E5	VMDP
PARKING FACILITIES		
Dutton Avenue Office and Parking Facility	C5	PKGDTN
East Campus Parking Facility	D9	PKGEC
Eighth Street Parking Facility	F2	PKG8TH
Fifth Street Parking Facility	D3	PKG5TH
Speight Plaza Office and Parking Facility	D7	PKGSPT
RESIDENCE FACILITIES		
Alexander Residence Hall	F4	ALEX
Allen Residence Hall	F4	ALLEN
Brooks Residential College	E2	BRCOLL
Brooks Residential Flats	E3	BRFLAT
Collins Residence Hall	F3	COLLNS
Dawson Residence Hall	F4	DAWSON
East Village Residential Community *under construction*	D9	EV
Kokernot Residence Hall	E2	KOKER
Martin Residence Hall	D3	MARTIN
Memorial Residence Hall	F4	MEMRL
North Village Residential Community	C5	NVRC
Penland Residence Hall	D4	PENLND
Russell Residence Hall	C4	RUSS
University Parks Apartments	C11	UPKAPT

Society of Toxicology
Lone Star Regional Chapter (LSSOT) Annual Meeting
"Advances in Toxicology"
September 7-8, 2012
Baylor Sciences Building (BSB)
Baylor University
Waco, Texas

Friday, September 7, 2012

5:00 PM – Keynote Address, Dr. Stephen Safe, "The long non-coding RNA HOTAIR is pro-oncogenic and a drug target in pancreatic cancer". (Rm. 234, E Wing, Baylor Sciences Building)

Note: Free Parking is available in the Baylor Sciences Building parking lot. There is also a free shuttle available from the hotel to campus.

6:00 PM – Dinner, sponsored by Waters. (Rm. E234)

7:00 – 9:00 PM – Poster Session (Outside Rm. E234 on landing near elevators)

Saturday, September 8, 2012

9 - 9:15am – Welcome from Erica Bruce, LSSOT President (Rm. 110, D Wing, Baylor Sciences Building)

Platform session I

9:15am – Israel Muro, UT Austin, "ARNT Isoforms Differentially Regulate the Proliferation of Cancer Cells by Controlling RelB Heterodimers".

9:35am – Dan Jackson, UTMB, "A Novel Protein Interaction Between the Aryl Hydrocarbon Receptor and the Tumor Suppressor KLF6".

9:55am – Sara Nowinski, UT Austin, "Mitochondrial Uncoupling Protein 3 Antagonizes Epidermal Tumor Promotion and Growth Signaling".

10:15am - Break (Landing near Rm. E234)

10:30 – Jordan Blodgett, Ph.D.– Waters Corporation.

Platform session II

11:15am – Durga Tripathi, Texas A&M Health Science Center, "Reactive Nitrogen Species Regulate Autophagy Through ATM-AMPK-TSC2-mediated Suppression of mTORC1".

11:35am – James Harman, UT Austin, "Impact of Clinically Relevant Cisplatin Treatment on the Undifferentiated Spermatogonia Population and Niche".

11:55am – Tod Harper, Jr., UTMB, "Novel Insights into Normal AhR Biology Through Regulation of Stanniocalcin 2".

12:15pm - Lunch, sponsored by Fisher Scientific and Biomedical Solutions, Inc. (Rm. E234) (Business Meeting and working lunch for LSSOT officers)

Platform session III

1:30pm – Xi Li, Texas A&M Health Science Center, "Sulindac Sulfide Inhibits Colon Cancer Cell Growth Through MicroRNA-27a-ZBTB10-specificity Protein Axis".

1:50pm – Crystal Usenko, Baylor University, "Simplified Analytical Methods for Concentration and Dose Determination in Toxicity Studies".

2:10pm – Tulika Tyagi, Texas Tech University, "Co-treatment of green tea polyphenol and 5-aza-2-deoxycytidine increases growth inhibition of human breast cancer cells".

2:30pm – Catherine Rondelli, UTMB, "DNA Repair Capacity is modulated by *XPC* Haplotypes".

2:50pm – Break (Landing near Rm. E234)

3:30pm – 4:00pm - Award Ceremony and Closing Remarks

Abstracts:

Platform Presentations:

ARNT isoforms differentially regulate the proliferation of cancer cells by controlling RelB heterodimers.

Israel Muro[1], Kacie Gardella[2], and Casey W. Wright[1,2]

Division of Pharmacology and Toxicology, College of Pharmacy[1], and The Institute for Cellular and Molecular Biology[2], The University of Texas at Austin, Austin, TX, USA.

The arylhydrocarbon receptor nuclear translocator (ARNT) is a transcription factor that is integral in the cellular response against xenobiotics and hypoxic stress. ARNT is expressed as two isoforms, isoform 1 and 3, which only differ by an extra 15 amino acids found near the N-terminus of isoform 1. Interestingly, we previously showed that ARNT isoforms also modulate NF-kB signaling as simultaneous suppression of the isoforms leads to increased NF-kB activity. In addition, we found that ARNT isoform 3 directly binds the NF-kB subunit RelB. By designing a small interfering RNA to the extra 45bp in isoform 1, we find here that suppression of ARNT isoform 1 in various cancer cells results in cell cycle arrest. Furthermore, co-immunoprecipitation experiments demonstrate that isoform 1 also binds RelB although at a lower affinity as compared to isoform 3. Using a NF-kB-GFP reporter we determined that expression of ARNT isoform 1 leads to an increase in NF-kB signaling, which is blocked by co-expression of isoform 3. Competition experiments further showed that ARNT isoforms differentially affect the binding of RelB to other NF-kB subunits. Together these observations suggest a model where ARNT isoform 1 binds RelB to increase NF-kB activity but is inhibited by ARNT isoform 3, which binds stronger to RelB and prevents isoform 1 binding. Intriguingly, our findings suggest that small molecules designed to mimic ARNT isoform 3 binding to RelB may be used to prevent isoform 1 from activating NF-kB in cancer cells and inhibit their growth.

A Novel Protein Interaction Between the Aryl Hydrocarbon Receptor and the Tumor Suppressor KLF6

Jackson, D. P.1, Joshi A. D.1, and Elferink, C. J.1 1. Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston Texas 77550

The Aryl Hydrocarbon Receptor (AhR) is a basic helix-loop-helix transcription factor of the Per-Arnt-Sim family. It is ligand activated by structurally diverse polycyclic and halogenated aromatic hydrocarbon (PAH/HAH) compounds. Many of these compounds are potent petrogenic or anthropogenic environmental contaminants, including the prototypical AhR ligand, 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD). In the classically defined canonical pathway, the activated AhR translocates to the nucleus, dissociates from chaperones, and heterodimerizes with the Aryl Hydrocarbon Receptor Nuclear Translocator (Arnt). This heterodimer binds to DNA via the cis acting motif, GCGTG, dubbed the xenobiotic response element (XRE), thereby regulating the transcriptional status of AhR target genes, including the xenobiotic metabolizing enzymes of the cytochrome P450 system. Plasminogen Activator Inhibitor 1 (PAI-1) is a recently characterized AhR target gene, which lacks an XRE. We demonstrated that AhR binding to PAI-1 occurs through a novel non-consensus XRE (NC-XRE) independent of the Arnt protein. Instead, the AhR binding to the NC-XRE occurs in partnership with the tumor suppressor Kruppel-like Factor 6 (KLF6). KLF6 has been implicated in numerous cancers and diseases, including liver cancer. This study sought to identify the gene targets of this novel AhR/KLF6 transcriptional complex using a global strategy based on chromatin immunoprecipitation next-generation sequencing (ChIP-seq) anchored to next generation RNA sequencing. These experiments have identified a number of KLF6/AhR target genes, including the cyclin-dependent kinase inhibitor, CDKN1A, and cell adhesion protein, CDH1. This novel protein-protein interaction between KLF6 and the AhR constitutes a physiologically important regulatory complex likely to play a critical role in cell growth and cancer progression.

Mitochondrial uncoupling protein 3 antagonizes epidermal tumor promotion and growth signaling.

Sara M. Nowinski[1], Joyce E. Rundhaug[2], Okkyung Rho[1], Cory U. Lago[3], John DiGiovanni[1], Edward M. Mills[1]

[1] Pharmacology and Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, TX.
[2] UT MD Anderson Cancer Center, Science Park Research Division, Smithville, TX.
[3] Translational Medicine Branch, NHLBI, NIH, Bethesda, MD 20892

One hallmark of malignancy is increased glycolysis and decreased mitochondrial respiration compared to normal tissues. Mitochondrial uncoupling proteins drive futile mitochondrial respiration that is uncoupled from ATP synthesis. We previously demonstrated that mice expressing a keratin 5 - uncoupling protein 3 (K5-UCP3) transgene in the epidermis were completely protected from the formation of skin carcinomas in response to a two stage chemical carcinogenesis regimen. To explore the mechanisms behind UCP3 induced cancer resistance, we inter-bred K5-UCP3 animals with Tg.AC mice that express a cutaneous oncogenic v-Ha-Ras transgene. Whereas Tg.AC mice formed tumors in response to treatment with the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) alone, bigenic K5-UCP3/Tg.AC animals were protected from TPA-induced tumorigenesis, indicating that UCP3 likely inhibits tumor promotion. Moreover, K5-UCP3 mice exhibited striking resistance to epidermal proliferation induced by TPA as measured using a bromodeoxyuridine (BrdU) based labeling assay. In agreement with these data, K5-UCP3 epidermis failed to show activation of Akt and downstream signaling molecules in response to TPA treatment. This effect corresponded to decreased expression of cyclins D1 and A, and increased expression of the cell cycle inhibitory proteins p21 and p27. This decrease in Akt activation was also observed *in vitro* in response to epidermal growth factor (EGF) treatment of isolated primary keratinocytes. However, treatment with the protein phosphatase 2a (PP2A) inhibitor okadaic acid was able to cause proportional Akt activation in both wild type and K5-UCP3 epidermis. Taken together, these data suggest that mitochondrial uncoupling causes a blockade of EGF receptor signaling, possibly through an increase in PP2A activity.

Reactive Nitrogen Species Regulate Autophagy through ATM-AMPK-TSC2-mediated Suppression of mTORC1

Durga Nand Tripathi[1], Rajdeep Chowdhury[2], Gerald N. Wogan[2],Cheryl Lyn Walker[1#]

1. Institute for Biosciences and Technology, Texas A&M Health Science Center, Houston, TX 77030
2. Massachusetts Institute of Technology, 77 Massachusetts Avenue Cambridge, MA 02139
#Corresponding authors

Reactive intermediates such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) play essential roles in the cell as signaling molecules, but in excess, constitute a major source of cellular damage in both physiological (i.e. mitochondrial respiration) and pathophysiological (i.e. inflammation) settings. Recently, ROS was shown to induce autophagy via a novel ATM signaling pathway in the cytoplasm. Activation of cytoplasmic ATM by ROS engages LKB1 and AMPK kinases to activate the TSC2 tumor suppressor, which represses mTORC1 (a negative regulator of autophagy) to induce autophagy. However, whether RNS engage this or similar signaling pathway(s) to regulate mTORC1 and induce autophagy is unclear. We found under conditions of steady-state nitric oxide (NO) exposure, rapid activation of ATM and downstream signaling by this stress kinase to LKB1-AMPK and the TSC2 tumor suppressor. As a result, mTORC1 was repressed as evidenced by decreased phosphorylation of the direct mTORC1 targets S6K, 4EBP1, and ULK-1, the target for mTORC1 repression of autophagy. Induction of autophagy concordant with decreased ULK-1 phosphorylation by mTORC1 at S757 and increased phosphorylation at S317 by AMPK was demonstrated by increased ratio of LC3-II/LC3-I, formation of GFP-LC3 puncta, increase in acidic vesicles and decreased p62 levels, indicative of increased autophagic flux. While autophagy has been shown to have pro-death and pro-survival functions, induction of autophagy by NO caused loss of cell viability, suggesting it functions primarily as a cytotoxic response to excess nitrosative stress. These data indicate that nitrosative stress, like oxidative stress, regulates autophagy. As cancer cells are particularly sensitive to nitrosative stress, these data open new therapeutic avenues capitalizing on the ability of NO and RNS to induce autophagic cell death.

Impact of Clinically Relevant Cisplatin Treatment on the Undifferentiated Spermatogonia Population and Niche

Harman, James and Richburg, John, The University of Texas at Austin

A typical clinical cisplatin (CDDP) regimen consists of repeated cycles of 5-7 daily low dose injections followed 1-2 week recovery period; and while effective, often results in prolonged, sometimes permanent, infertility. Theoretically, undifferentiated spermatogonia (undiff-Sp), including spermatogonial stem cells (SSCs), should repopulate the testis after exposure has ceased. We hypothesize that SSC mitotic activity increases following the initial exposure to CDDP, rendering them increasingly susceptible to CDDP-induced injury during subsequent cycles. We examined changes in the adult C57 undiff-Sp population and niche at days 1, 8 and 16 of the recovery period following a clinically-relevant course of 1 cycle (at 2.5 or 5.0 mg/kg/d) and 2 cycles (at 2.5 mg/kg/d) of intraperitoneal CDDP. Histological examination of the testis epithelium showed an increase in damage correlating with exposure dose and cycle number. Apoptosis was measured via TUNEL and found to increase during the recovery period following 1 cycle of 2.5mg/kg and 5.0mg/kg (300%), as well as 2 cycles of 2.5mg/kg (400%) CDDP compared to controls. Immunohistochemistry (IHC) was performed using antibodies specific for FOXO1 (undiff-Sp marker) and GDNF (critical SSC niche protein secreted by Sertoli cells). Analysis of FOXO1 showed a reduction in undiff-Sp during the recovery period following 1 cycle of 2.5 (50%) and 5.0mg/kg CDDP (66%), as well as 2 cycles of 2.5mg/kg CDDP compared with controls (50%); followed by a return to numbers equal with or surpassing controls at 16d recovery. IHC analysis of GDNF revealed a general increase in expression, prominently along the basal membrane, during the recovery period in all treatment groups. Western blot analysis of GDNF expression corroborated these findings. These data suggest that a greater dose of CDDP in a single cycle of exposure causes a greater impact on the functional stem cell pool and its niche and multiple cycles of exposure result in a still greater impact than an equivalent cumulative dose. Future experiments focus on whether CDDP exposure targets the SSCs, the Sertoli cells, or both.

Novel Insights into Normal AhR Biology Through Regulation of Stanniocalcin 2

Tod A Harper Jr* and Cornelis J Elferink*

*Department of Pharmacology & Toxicology, University of Texas Medical Branch - Galveston

The aryl hydrocarbon receptor (AhR) is a ligand-activated member of the Per-ARNT-Sim (PAS) superfamily of transcription factors. The AhR is traditionally recognized for its role in the adaptive metabolism of xenobiotics and the toxic responses associated with these compounds. However recent evidence has shown that the AhR is associated with normal physiological processes turning our laboratory's interest toward the understanding of how the AhR mechanistically contributes to liver homeostasis through regulation of genes in the absence of an exogenous ligand. Therefore, the purpose of this study was to identify AhR genes associated with normal AhR biology, and in doing so uncover novel receptor targets. Using primary hepatocytes isolated from an $AhR^{fx/fx}$ (floxed) mouse, we are able to generate AhR null hepatocytes through introduction of Cre recombinase using an adenoviral expression system. To monitor AhR transcriptional control in the absence of an exogenous ligand we performed a comprehensive microarray analysis designed to identify changes in the transcriptome temporally concomitant with loss of the AhR. This analysis generated a unique data set identifying mostly new target genes not detected in previous array studies. Expression of one gene in particular, Stanniocalcin 2 (Stc2), was significantly altered in the microarray analysis and independently validated by qRTPCR. In silico analysis of the Stc2 promoter region revealed 9 putative AhR binding sites (XRE, DRE, AhRE) within the 1 kb region upstream of the Stc2 transcriptional start site. It is noteworthy that although AhRdependent, Stc2 expression is refractory to induction by the exogenous agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin. Stc2 is a secreted glycoprotein with relatively unknown function. However, Stc2 has recently been shown to play a cytoprotective role under conditions that elicit ER stress. To this end we are now characterizing the AhR-Stc2 relationship in order to extend the understanding of normal AhR biology. [ES012018, ES07254, ES006676]

Sulindac Sulfide Inhibits Colon Cancer Cell Growth through MicroRNA-27a-ZBTB10-Specificity Protein Axis

X. Li[1], S. Safe[2,3]

1. College of Medicine, Texas A&M Health Science Center, College Station, TX, USA.
2. Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX, USA.
3. Institute of Biosciences & Technology, Texas A&M Health Science Center, Houston, TX, USA.

Specificity protein (Sp) transcription factors play pivotal roles in maintaining the phenotypes of many cancers. Genetic knockdown of these transcription factors individually or in combination leads to decreased proliferation and even death of cancer cells *in vitro*. In most cases of malignant tumors and in various types of human cancer cell lines, members of the Sp protein family, Sp1, Sp3 and Sp4, are overexpressed. An oncogenic microRNA, mir-27a, targets the Sp repressor ZBTB10 and mir-27a-dependent suppression of ZBTB10 is responsible for the overexpression of Sp transcription factors in cancers. We have focused on investigating drugs that target Sp protein downregulation through the mir-27a-ZBTB10 axis. Sulindac sulfide is a metabolite of sulindac, a non-steroidal anti-inflammatory drug (NSAID), and sulindac sulfide inhibited growth of SW480 and RKO colon cancer cell lines with an IC_{50} value (24 hr) of 50 uM in both cell lines. Treatment with sulindac sulfide increased the expression of ZBTB10 and, in turn, downregulated Sp1, Sp3, Sp4 proteins in SW480 and RKO cells. Sulindac sulfide also decreased expression of several Sp-regulated genes that are critical for cancer cell survival, angiogenesis and proliferation such as survivin, vascular endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR) and cyclin D1. The mechanism of sulindac sulfide-induced anticancer activity was also investigated and antioxidants such as glutathione reversed sulindac sulfide-dependent growth inhibition and downregulation of Sp1, Sp3, Sp4 and Sp-dependent gene products. Sulindac sulfide also induced reactive oxygen species (ROS) and, through a currently unclear mechanism, decreased the levels of mir-27a in colon cancer cells, which resulted in the upregulation of ZBTB10 and downregulation of Sp proteins. In addition, sulindac sulfide greatly reduced the transcriptional activities of Sp1 and Sp3 in a GAL4-UAS assay system in colon cancer cells. The results suggest that the cancer chemotherapeutic effects of sulindac may be due to its major metabolite sulindac sulfide which downregulates Sp transcription factors and pro-oncogenic Sp-regulated genes.

Simplified analytical methods for concentration and dose determination in toxicity studies

Crystal Y. Usenko, Eleanor Robinson, Erica D. Bruce, and Sascha Usenko
Baylor University, Waco, TX

Toxicological studies rely on the purity and accuracy of chemical exposure, yet chemical analysis is often very timely and expensive. Two high-throughput analytical methods have been developed for the rapid analysis of chemical concentrations in 1) water and culture media and 2) zebrafish embryo tissue. These methods were specifically designed for toxicity studies with low intrinsic cost. Chemicals over a wide range of physical-chemical properties were assessed in preliminary studies, primarily polybrominated diphenyl ethers (PBDEs) and pesticides. This method uses as little as 10 µL of exposure solution and up to 100 µL, depending on the solution concentration and method detection limits. By using only 10 µL, exposure solutions from the same well of a 96-well plate can be measured *in situ* multiple times over the course of exposure. Additionally, there is a significant reduction in chemical waste and extraction time. Furthermore, an extraction method was simplified for tissue analysis, requiring as little as 20 mg of zebrafish embryo tissue for detection of PBDEs. Embryos are homogenized and extracted with hexane. The use of non-isotopically labeled standards greatly reduced the cost of analysis and extracts were analyzed using gas chromatography-mass spectrometry in electron capture negative ionization mode. The use of these methods allow for a better understanding of the actual concentration of exposure, and the rate of uptake. While these are preliminary studies, the use of both methods could be transferred to many other applications in toxicology.

Co-treatment of green tea polyphenol and 5-aza-2-deoxycytidine increases growth inhibition of human breast cancer cells

Tulika Tyagi, Kamaleshwar P Singh

Department of Environmental Toxicology, The Institute of Environmental and Human Health (TIEHH), Texas Tech University, Lubbock, TX 79424

Breast cancer is the second most commonly diagnosed cancer in US women, preceded only by skin cancer. Both genetic and epigenetic processes are implicated in carcinogenesis. Studies show that half of all the tumor suppressor genes are inactivated more often by epigenetic, rather than genetic mechanisms in all types of cancer. DNA methylation is an important epigenetic process in carcinogenesis. Aberrant promoter region methylation induced silencing of various genes is seen in breast cancer. As epigenetic changes like DNA methylation are reversible, demethylating drugs, such as, 5-aza -2' deoxycytidine (5-aza-2'dC) are being clinically used in certain cancers. However, there are adverse cytotoxic effects associated with high doses of these drugs .Alternative dietary compounds like EGCG,a green tea polyphenol has been shown to induce apoptosis and cell cycle arrest in cancer cells without affecting normal cells.EGCG is reported to have DNA demethylation and histone modification activity.Therefore,the purpose of this study was to investigate the efficacy of co-treatment of EGCG and 5-aza-2'dC,at low doses in inhibiting growth and survival of breast cancer cell line, MCF-7. MCF-7 cells were treated with EGCG (50μM, 100 μM) and 5-aza-2'dC (5μM), both individually and in combination for 7 days.MTT assay showed that both EGCG and 5-aza-2'dC decreased cell viability significantly as compared to vehicle control (DMSO) treated cells. Co-treatment with EGCG and 5-aza-2'dC lead to a significant decrease in cell viability as compared to individually treated cells. Cell cycle analysis further showed that co-treatment significantly arrests more cells in G0/G1 phase as compared to individual treatments. Real-time PCR analysis revealed that the expression of *BCl2 and Cyclin D1* genes was significantly decreased by co-treatment with EGCG and 5-aza-2'dC.Co-treatment significantly decreased *DNMT1* expression as compared to individual treatments. These data suggest that co-treatment with EGCG and 5-aza-2'dC, at low doses can synergistically reverse methylation induced gene silencing and hence be used for inhibition of breast cancer growth while minimizing cytotoxicity on normal cells.

DNA repair capacity is modulated by *XPC* haplotypes

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University of Texas Medical Branch, Galveston TX[a] and [b]MD Anderson Cancer Center, Houston, TX

The impact of single nucleotide polymorphisms (SNPs) of the DNA repair gene *XPC* on DNA repair capacity (DRC) has not been comprehensively determined. We constructed a haplotype map encompassing all *XPC* SNPs existing with a minor allele frequency ≥0.05. We identified 21 haplotypes that segregated into 6 phylogenetic groups (PGHs A-F). We evaluated the relationship between these PGHs and DNA damage associated with smoking, using chromosome aberrations (CA) as a biomarker. We observed a significant interaction between PGH-C and smoking for baseline CA (P=0.046). Using mutagen-sensitivity as a biomarker of cancer risk, we observed significant interactions between smoking and PGH-D (P=0.023) and -F (P=0.007). To provide mechanistic explanations to our findings, we exposed human lymphoblastoid cells representing different *XPC* haplotypes to UV radiation to generate the NER substrates 6-4 photoproducts (6-4PP) and cyclopyrimidine dimers (CPD). We then evaluated the effect of *XPC* haplotypes on DRC using ELISA. We hypothesized that if *XPC* haplotypes have functional effects, there would be a correlation between these haplotypes and repair of 6-4PP and CPD. We found significant differences in 6-4PP repair as a function of *XPC* haplotypes. For example, cells with the PGH-EE repaired 6-4PP more efficiently than cells with -DD or -FF. At 30 min post-UV exposure, levels of 6-4PP in cells with the PGH-EE returned to near baseline levels while adduct levels in cells with -DD and -FF remained significantly high. Additionally, we observed that 6-4PP and CPD are not necessarily repaired at the same rate depending on *XPC* haplotype. For example in cells with -DD, 6-4PP levels were significantly repaired at 1 hr after UV exposure while CPD levels remained high up to 3 hrs. These data provide initial mechanistic explanations to our earlier findings indicating an effect of *XPC* haplotypes on smoking-induced genetic damage and support reported associations between *XPC* SNPs and cancer risk.

Posters:

Conditional Knockout of the Aryl Hydrocarbon Receptor in the Liver Alters Mouse Phenotype as Well as Glucose and Lipid Homeostasis

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The aryl hydrocarbon receptor (AhR) is a ligand activated transcription factor historically known for its role in the adaptive metabolism of xenobiotics. However, generation of the AhR knockout mouse (AhR-KO) has provided evidence of a physiological role for the AhR. To further study this physiological role, AhR conditional knockout (AhR-CKO) mice were utilized by crossing AhR fx/fx (control) mice with mice that express Cre recombinase under the control of the albumin promoter, resulting in loss of AhR expression specifically in the liver parenchyma. Our experiments indicate a novel sex dependent phenotype wherein; AhR-CKO females showed significant reduction body weight at 10 weeks old (*$p < 0.0108$), loss of adipose tissue and increased basal glucose levels (218 ± 19.73 mg/dl). Therefore, our goal is to characterize the AhR-CKO mouse phenotype and identify potential AhR-dependent mechanisms responsible for lipodystrophy and abnormal glucose homeostasis. AhR-CKO and control mice were subjected to glucose and insulin tolerance tests. Our data suggests decreased glucose tolerance and increased insulin sensitivity in AhR-CKO females compared to their controls. Plasma analyses suggest alterations in leptin (control 3.227 ± 0.27 vs CKO 1.028 ± 0.129), FFA (control 0.3630 ± 0.026 vs CKO 0.594 ± 0.02) and insulin levels (control 0.495 ± 0.008 vs CKO 0.253 ± 0.004). Immuno-Histochemical staining for insulin revealed reduced insulin content and smaller pancreatic islets in AhR-CKO mice. We also conducted histopathological analysis using Oil red O staining, which revealed decreased lipid content in AhR-CKO mice livers. Our experiments strongly suggest that AhR activity in the liver can somehow communicate with extra-hepatic tissues to alter their function. AhR-CKO mice may offer an elegant model to study the link between endogenous AhR activity liver and clinically relevant metabolic disorders such as diabetes, lipodystrophy and obesity.

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Colon cancer stem cell markers are modulated by Sp (specificity protein) transcription factors.

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Emerging evidence indicates that colon cancer stem cells (CSCs), a small subset of cells within a tumor, are responsible for its malignancy, chemo-resistance and metastasis. Previous studies support the essential role of specificity protein (Sp) transcription factors as important therapeutic targets in colon cancer and other tumor types due to their over-expression in various tumors including colon cancer cells and minimal expression in non-tumor tissue. Sp proteins also have an important functional role in cancer cells and tumors and are responsible for expression of various critical genes required for tumor survival (survivin), growth (cyclin D1), inflammation (NFkBp65) and angiogenesis (VEGF/VEGF receptors). Earlier reports from our laboratory have identified novel drugs including some that are derived from phytochemicals that target and decrease Sp transcription factors in pancreatic, prostate, colon and bladder cancer cell types. One of these drugs methyl 2-cyano-3,11-dioxo-18 -olean-1,12-dien-30-oate (CDODA-Me), a synthetic derivative of glycyrrhetinic acid, a triterpenoid is found in licorice extracts. The anticancer activities of CDODA-Me and its effects on Sp1, Sp3 and Sp4 proteins and Sp regulated genes was investigated in a panel of four colon cancer cells, SW480, RKO, HCT116 and HT29 cells. Initial studies focused on the expression of several cancer stem cell maker proteins and the ability of these cell lines to form stem cell-enriched spheres in ultralow attachment plates. Results of these experiments show that only HT29 colon cancer cells form spheres, which exhibited several cancer stem cell markers including increased expression of CD44, CD105, CD133, aldehyde dehydrogenase (ALDH1). HT29 cells also formed a significant number of primary and secondary spheres in ultra-low attachment plates. The results with HT29 cells are characteristic of a population enriched in colon cancer stem cells. Results of initial studies show that CDODA-Me decreased HT29 adherent cell survival, expression of Sp1, Sp3 and Sp4 proteins, and stem cell sphere formation. In the same cell line, CDODA-Me also decreased CD44, CD105 and ALDH1 expression, which are cancer stem cell markers. Preliminary data using RNA interference studies suggests that the CD44 stem cell marker is also an Sp-regulated gene in HT29 cells. These results suggest that colon cancer stem cells also overexpress Sp1, Sp3 and Sp4 proteins that can be targeted by anticancer agents. The anticancer activity of CDODA-Me and Sp protein knockdown and effects on other cancer stem cell markers and sphere forming ability are currently being investigated.

Age-Dependent Alterations in Spermatogenesis in *Itchy* Mice

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Spermatogenesis is an intricate process in which spermatogonial stem cells divide and differentiate to produce mature sperm cells under the guidance and support of somatic Sertoli cells. This process strongly depends on the rapid turnover of proteins, both in the remodeling germ cells and in the supportive Sertoli cells. Recent evidence has demonstrated the importance of ubiquitin-proteasome for this protein turnover, and several testis-specific enzymes involved in the ubiquitin-proteasome system have already been identified. This system is a three-step enzymatic process that covalently links ubiquitin to a target protein, with the final enzymatic E3 ligase providing the target specificity. One E3 ligase, Itch, has been well characterized in the immune system, and has also been reported to act at the blood testis barrier where is regulates the junctional protein occludin. Although the specific role of Itch during spermatogenesis is not yet well understood, its ubiquitous expression and wide array of functional targets suggest multiple tissue-specific roles. Here we evaluate the testes of mice that lack Itch protein at two developmental time points, the peri-pubertal period at postnatal day (PND) 28 and adult PND 56. We found that *itchy* mice had an increased apoptotic index compared to wild type C57BL/6J mice at both PND 28 (8.9±0.43% *vs* 6.9±0.58%) and PND 56 (7.2±0.50% *vs* 4.1±0.34%). A corresponding 27% reduction in the total number of spermatid heads produced in PND 56 *itchy* mice was also evident. A histological evaluation revealed a delay in spermatogenesis at PND 28 and disorganization of late stage spermatids at PND 56. The breeding success of the *itchy* mice was also significantly decreased compared to C57BL/6J mice (5.15±0.23 *vs* 7.92±0.36 pups/litter), possibly due to a developmental defect. Taken together, these findings indicate that Itch is required for functional spermatogenesis, and that it may play differing cellular roles during different periods of spermatogenesis.

ARNT isoforms mediate opposing effects on NF-kB signaling

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We have previously shown that the arylhydrocarbon receptor nuclear translocator (ARNT) regulates the chromatin binding activity of the RelB NF-kB subunit. ARNT is a transcription factor that is integral in the regulation of xenobiotic and hypoxic responses but our studies suggest that ARNT also participates in NF-kB signaling. ARNT is expressed as two isoforms, isoform 1 and 3, but whether these isoforms differentially regulate NF-kB activity is unclear. Isoform 1 is identical to isoform 3 except for an additional 15 amino acids near the N-terminus. The extra amino acids in isoform 1 provide a phosphorylation site, which has been shown to inhibit its DNA binding. Interestingly, we have observed in lymphoid malignancies that ARNT isoform 1 is expressed at much higher levels than isoform 3 as compared to normal lymphocytes where the ARNT isoforms are expressed at equal levels. We find that ARNT isoform 1 potentiates while ARNT isoform 3 abrogates NF-kB activity. In light of our previous ARNT-RelB model, these opposing effects of the ARNT isoforms in NF-kB signaling appear to hinge on their ability to associate with RelB as ARNT isoform 3 binds much more strongly than isoform 1. Lastly, we found that the co-expression of a GFP-tagged p100 in combination with the ARNT isoforms promoted p100 nuclear translocation, possibly through a RelB bridge, resulting in diminished transactivation of NF-kB responsive genes. Thus, our current working model is one in which lymphoid malignancies shift toward the production of ARNT isoform 1, which in turn enhances NF-kB activity, as a contributing factor to their growth and survival.

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Measuring 5-Halocytosines in Mammalian DNA as a Marker of Inflammation Mediated DNA Damage

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The DNA of mammalian cells is persistently damaged by oxidation, methylation and hydrolysis. Emerging evidence indicates that cellular DNA can also be halogenated by reactive species generated by neutrophil myeloperoxidase and eosiniophil peroxidase. Halogenated pyrimidines in DNA could cause polymerase-mediated miscoding and act as fraudulent epigenetic signals, explaining in part the association between inflammation and the development of cancer. In addition to endogenous causes of inflammation, many environmental toxicants are responsible for chronic inflammation and cancer development as well. In this poster, we present a gas chromatography/mass spectrometry method designed to measure halopyrimidines in DNA as a marker for inflammation mediated DNA damage. To demonstrate the utility of this method the levels of halopyrimidines in the DNA of mammalian cells grown in culture was measured. Significant levels of both 5-chlorocytosine and 5-bromocytosine are identified, with the highest levels in the myeloperoxidase expressing HL-60 cell line. Surprisingly, no 5-chlorouracil or 5-bromouracil was found. The data reported here suggest that inflammation-mediated DNA damage could contribute to human disease.

STAT3 regulation by Sp protein transcription factors in pancreatic cancer cells

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Signal Transducer and Activator of Trancription 3 (STAT3) is constitutively activated in most cancer types including pancreatic cancer and plays a significant role in cancer cell survival and inflammation. There is limited data on regulation of STAT3, however, several cis-elements such as CRE/ATF, SBE and GC boxes are involved in STAT3 gene expression and specificity proteins (Sp) Sp1, Sp3 and Sp4 bind and activate GC-rich promoters and regulate genes involved in cell growth, angiogenesis and metastasis. We investigated the effects of individual knockdown of Sp1 (iSp1), Sp3 (iSp3), and Sp4 (iSp4) and their combination (iSp) on expression of phospho-STAT3 (p-STAT3) and STAT3 proteins in Panc28 and L3.6pL pancreatic cancer cells. Transfection of Panc28 and L3.6pL cells with iSp1 or iSp4 significantly decreased expression of pSTAT3 and STAT3 but iSp3 had no effect on STAT3 expression. The effect of combined knockdown (iSp) of all 3 Sp proteins was more effective than individual Sp knockdown in both cell lines. Compounds that degrade Sp proteins such as betulinic acid (BA), a naturally occurring pentacyclic triterpenoid are highly effective anticancer agents. In this study we observed that 10-25 μM BA not only inhibited cell proliferation but also decreased expression of both p-STAT3 and STAT3 in Panc28 and L3.6pL cells. BA and iSp also decreased expression of STAT3-regulated genes such as cyclin D1 and VEGF and luciferase activity of STAT3 promoter. Results from chromatin immunoprecipitation show that Sp1 binds to STAT3 promoter and the binding activity is decreased after treatment of L3.6pL cells with BA (25 μM) for 12 hr. STAT3 is also involved in transcriptional regulation of several pro-oncogenic microRNAs (miRs) such as miR-21, 181b-1 in breast and miR-17 in lung cancer cells and knockdown of Sp proteins (iSp) decreased both STAT3-regulated miR-21 and 181b-1 in Panc28 and L3.6pL cells. Similar results were observed with BA. These data show that BA-mediated downregulation of Sp proteins decreased expression of STAT3 and its downstream pathways including mRNA and miRs that are involved in cancer cell growth and inflammation.

The Putative IAP-Binding Motif of NIK is Involved in the Regulation of Non-Canonical NF-κB Signaling.

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The non-canonical nuclear factor-κB (NF-κB) signaling pathway plays a central role in the development of lymphoid cells. However, deregulation of the non-canonical NF-κB pathway can lead to lymphoid tumorigenesis. Thus, it is important to understand mechanisms that control the activation of non-canonical NF-κB signaling. It has been shown that NF-κB inducing kinase (NIK) through interaction with cellular inhibitor of apoptosis (cIAP) proteins, tumor necrosis factor receptor-associated factor 2 (TRAF2) and TRAF3 regulates the activity of the non-canonical pathway. The cIAPs inhibit the activation of the non-canonical pathway by ubiquitinating and degrading NIK. Moreover, the function of cIAPs can be antagonized by their interactions with proteins that possess a conserved IAP binding motif (IBM), which induces apoptosis. Interestingly, we found the presence of a putative IBM at the amino-terminus of NIK. However, the functional significance of the NIK IBM in cIAP-mediated NIK turnover has not yet been studied. Therefore, we hypothesized that cIAPs partly recognize NIK through this putative IBM, facilitating the destabilization of NIK followed by the inhibition of the non-canonical NF-κB pathway. To investigate the implication of NIK IBM, we introduced point mutations in two initial peptide sequences of NIK (Ala-Val to Gly-Gly) and examined interactions between NIK and cIAPs (cIAP1 and cIAP2) by co-immunoprecipitation. The mutation in the NIK IBM resulted in decreased binding of NIK with cIAPs albeit with more stabilized expression of NIK. To gain insight into the mechanism of the inhibitory function of the NIK IBM on non-canonical NF-κB signaling, the level of ubiquitination of NIK was examined after overexpressing cIAP1. Interestingly, we found that the NIK IBM was involved in promoting the cIAP1-dependent ubiquitination of NIK. Our results suggest that cIAP1 recognizes the putative IBM of NIK, in turn facilitating ubiquitination and degradation of NIK, which inhibits non-canonical NF-κB activation.

Deciphering the Role of TRAIL in Altering Spermatogenesis by Controlling Germ Cell Apoptosis

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TRAIL (TNFSF10/Apo2L) is a member of the tumor necrosis factor (TNF) superfamily of proteins, expressed in human and rodent testis. TRAIL is a ligand known to induce apoptosis via binding to receptors DR4 (TRAIL-R1/TNFRSF10A) and DR5 (TRAIL-R2/ TNFRSF10B) in humans, and TRAIL-R (MK/mDR5) in mice. TRAIL-R is equally homologous to both human death receptors. TRAIL is found in Leydig cells and germ cells during development and TRAIL-R is predominantly expressed in post-meiotic germ cells in the rat. The major role of TRAIL in male reproduction is still unclear, even though a recent report shows that the TRAIL gene has higher expression in potentially fertile than infertile human populations. In this study, we investigated TRAIL gene deficient mice and evaluated the possible role of TRAIL in germ cell development by measuring testis weight, germ cell apoptosis, and spermatid head count in peripubertal (28 day-old) or young adult ages (44 day-old). Our results revealed that though there was no significant difference in testis to body weight ratio between C57 and $TRAIL^{-/-}$ mice, peripubertal $TRAIL^{-/-}$ mice show a dramatic increase in the basal germ cell apoptotic index (A.I., 21.54%) as compared to the C57BL/6J wild-type strain (5.16%). The A.I. in adult C57 mice had dropped to 1.5%, but remained elevated in adult $TRAIL^{-/-}$ mice (20.2%); indicating a sustained high incidence of germ cell apoptosis. Spermatid head counts in adult $TRAIL^{-/-}$ were dramatically reduced compared with C57 mice (39%), indicating these animals suffer a marked decline in the production of mature spermatozoa. We hypothesize that TRAIL is an important factor for maintaining germ cell homeostatsis during germ cell development via a death receptor-dependent mechanism.

Overexpression of CRM1 in Normal Human Lung Epithelial Cells Changes Cellular Morphology and Cytotoxic Responses to Tobacco-specific Carcinogen NNK

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Chromosome region maintenance 1 (CRM1), the major nuclear export receptor with a broad substrate range, is not only required for transport of many RNAs and proteins but also involved in various modulations within the cell such as mitosis, cell arrest, and apoptosis. Our recently published study showed that CRM1 played critical roles in response to tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), in BEAS-2B cells (a normal human lung epithelial cell line). The objective of the present study was to further examine the significance of CRM1 in lung cancer development using BEAS-2B cells stably overexpressing CRM1 (BEAS-2B$_{CRM1+}$ cells). The overexpression of CRM1 in BEAS-2B$_{CRM1+}$ cells was confirmed by real-time PCR and western blot. As compared to BEAS-2B cells, BEAS-2B$_{CRM1+}$ cells were prone to form colonies. Soft-agar assay further demonstrated increased colony formation and larger colony size in BEAS-2B$_{CRM1+}$ cells in comparison with BEAS-2B cells. In addition, the cytotoxic effects in response to NNK was measured by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay in both cells. Cells were treated with 0-500 μM NNK for 24, 48, and 72 h. The inhibitory effects were dose and time dependent in both cells ($p<0.05$). However, BEAS-2B$_{CRM1+}$ cells showed different sensitivity to NNK as compared to BEAS-2B cells. Taken together, our results indicate that CRM1 overexpression changes cellular morphology and cytotoxic response to tobacco carcinogen in human lung epithelial cells. The potential molecular mechanisms involving these changes are being evaluated to better understand the critical role of CRM1 in chemical carcinogenesis after NNK exposure.

Role of Bcl-2 Proteins in Heat Shock-Induced Apoptosis.

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Apoptosis is a programmed form of cell death executed by caspases (cysteine proteases) that cleave substrates exclusively after aspartic acid residues. In response to stress, cells often activate the intrinsic apoptotic pathway, wherein mitochondrial outer membrane permeabilization (MOMP) initiates the release of proapoptogenic proteins, such as cytochrome c (cyt c) and Smac, into the cytosol. In particular, cyt c promotes formation of a caspase-activating complex known as the Apaf-1-caspase-9 apoptosome. Heat shock (HS) is an ancient stress that activates both prosurvival (thermotolerance) and prodeath cellular responses, and we have previously shown that HS induces apoptosis through pathways that involve MOMP and downstream effector caspase-3 activation, but do not require activation of the apoptosome. HS-induced MOMP is strictly controlled by BCL-2 family members, which include both pro-apoptotic (e.g. Bax, Bak, Bim, Bid, etc.) and anti-apoptotic (e.g. Bcl-2, Mcl-1, etc.) proteins. Other studies suggest that HS initiates MOMP following cleavage of Bid by caspase-2. However, we observe that immortalized mouse embryonic fibroblasts (MEFs), lacking either caspase-2 or Bid, remain sensitive to HS. In contrast, we find that *bim*$^{-/-}$ MEFs are highly resistant to HS-induced apoptosis and exhibit significantly decreased levels of MOMP. Thus, our findings indicate that Bim is essential for HS-induced death, while caspase-2 and Bid function as part of an amplification loop. Interestingly, Bim is known to induce MOMP through direct activation of Bax and/or Bak, but we find that *bax*$^{-/-}$/*bak*$^{-/-}$ MEFs are only partially resistant to HS-induced cell death, implying the existence of a Bim-dependent, Bax/Bak-independent pathway. We speculate that another proapoptotic Bcl-2 family, namely Bok, may functionally substitute for Bax and Bak, or alternatively that Bim may directly permeabilize lysosomal membranes, resulting in the release of proapoptotic cathepsins. (These studies were supported by grants, CA129521 and GM096101, from the NIH.)

Metformin Causes Degradation of Fatty Acid Synthase and PI3 kinase Signaling Proteins in Pancreatic Cancer Cells Through Downregulation of Specificity Protein (Sp) Transcription Factors

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Metformin or N,N'-dimethyl biguanide is an oral hypoglycemic drug with a remarkable record of safety that has been prescribed worldwide for treatment of Type II diabetes. In addition to its antidiabetic property it also exhibits antineoplastic effects which include inhibition of angiogenesis through decreased levels of vascular endothelial growth factor (VEGF) and blocks cell cycle progression through decreased expression of cyclin D1. Fatty Acid Synthase (FAS) a key enzyme in lipid metabolism is overexpressed in cancer cells, which is one of the phenotypic alterations in cancer progression. The differential expression of FAS between normal and neoplastic tissues makes it a potential tumor biomarker. Here we show that expression of FAS was significantly decreased when pancreatic cancer cells were treated with metformin. FAS expression is stimulated by growth factors and their receptors, like EGFR and IGFR. Effects of these receptors on FAS complex involve cross talk between various signal transduction pathways. One such signaling pathway include PI3kinase/ mTOR pathway. FAS protein level was significantly decreased when cells were treated with PI3kinase inhibitor (LY294002). Specificity protein (Sp) transcription factors - Sp1, Sp3 and Sp4 play a complex role in malignant transformation of pancreatic cancer cells. In this study we investigated the effects of metformin on levels of Sp transcription factor which are normally overexpressed in cancer cells compared to their low levels of expression in normal cells. We report for the first time that metformin downregulates specificity protein (Sp) transcription factors (Sp1, Sp3, Sp4) in cancer cells. Furthermore metformin downregulates the expression of growth factor receptors like EGFR, IGFR and phosphorylation/expression of major PI3K effectors like AKT and mTOR. Knockdown of Sp1, Sp3, and Sp4 proteins by RNA interference decreased the expression of FAS pmTOR and pAKT indicating that FAS and these signaling proteins are regulated by Sp proteins. However when cells were pretreated with SOV a phosphatase inhibitor, there was a reversal of Sp downregulation and phosphorylation of mTOR which indicates that phosphatases has major role in Sp downregulation by metformin. The effects of metformin on downstream targets of PI3 kinase pathway, the potential use of FAS as a biomarker and the specific phosphatases which play a major role in the antineoplastic effects of metformin are currently being investigated.

Aspirin Inhibits Colon Cancer Cell and Tumor Growth and Downregulates Specificity Protein (Sp) Transcription Factors

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Background and Aims: Acetylsalicyclic acid (aspirin) is a chemotherapeutic agent for colon cancer patients; however, the mechanisms are not well defined and were investigated in this study.

Methods: Inhibition of colon cancer cell growth and induction of apoptosis by aspirin and sodium salicylate were investigated using cell counting and Annexin V staining, and modulation of specificity protein (Sp) transcription factors Sp1, Sp3, Sp4 and Sp-regulated gene products was determined by western blot analyses of nuclear, cytosolic and whole cell lysates. Mechanisms of aspirin-induced Sp downregulation were investigated using pathway-specific inhibitors and the in vivo anticancer activity of aspirin was determined in athymic nude mice bearing RKO cells as xenografts.

Results: Aspirin and its major metabolite sodium salicylate induced apoptosis and decreased colon cancer cell growth and this was accompanied by caspasedependent proteolysis of Sp1, Sp3 and Sp4 and decreased expression of Sp-regulated gene products including bcl-2, survivin, VEGF, VEGFR1, cyclin D1, c-MET, p65 and b-catenin. Aspirin also inhibited colon tumor growth and decreased Sp1, Sp3 and Sp4 expression in tumors.

Conclusions: Aspirin-induced repression of Sp transcription factors and Sp regulated genes play a role in the cancer chemotherapeutic effects of aspirin. Since patients administered aspirin exhibit high salicylate/aspirin serum ratios, results of this study suggest for the first time that salicylate may be the major contributor to the anticancer activity of aspirin in colon cancer.

Regulation of Alcohol Consumption by NF-κB signaling.

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Alcohol is known to induce neuroinflammation, which in turn contributes to drinking-related brain damage. It has been shown recently that the production of neuroinflammation by the bacterial lipopolysaccharide (LPS) enhances alcohol consumption. LPS can activate the transcription factor NF-κB. Furthermore, the origin of many inflammatory associated diseases has been linked to deregulated NF-κB. For this reason NF-κB signaling has become an intense area of investigation to identify the regulators and context of stimulation of the NF-κB pathway. We have assessed the contribution of NF-κB signaling in mice brain microglia to potentiating alcohol intake. The immortalized murine microglial cell line BV-2 has been used frequently as a substitute for primary microglia. We stimulated the BV-2 cells with LPS for different time points and treated the cells with ethanol at different time points. The cell lysates were harvested for western blot and real time PCR. We have observed that ethanol is inhibitory to the production of inflammatory cytokines upon stimulation with LPS, thus suggesting the role of ethanol in regulating neuroinflammation.

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Cannabinoids Induce Phosphatases and Decrease Expression of Pro-oncogenic Phospho-eIF4E in Colon and Prostate Cancer Cells

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The phosphorylated form (serine-209) of the eukaryotic translational initiation factor eIF4E exhibits pro-oncogenic activity and plays a critical role in cancer cell growth, transformation and metastasis. Ongoing studies in this laboratory demonstrate that the anticancer activity of several agents is accompanied by induction of multiple phosphatases and some of the effects of these drugs are blocked by the phosphatase inhibitor sodium orthovanadate (SOV). For example, treatment of SW480 colon and LNCaP prostate cancer cells with synthetic cannabinoid WIN-55-212 (WIN) (7.5 uM) and 15 uM cannabidiol (CBD) inhibit cancer cell growth, induce apoptosis and multiple phosphatase genes and this was accompanied by decreased expression of several phospho-kinases and also phospho-eIF4E, in both cell lines. Treatment of SW480 cells with WIN or CBD alone decreased levels of phospho-eIF4E protein and co-treatment with 0.35 mM SOV significantly blocked cannabinoid-induced dephosphorylation of phospho-eIF4E. In contrast SOV did not block cannabinoid-induced dephosphorylation of phospho-eIF4E in LNCaP cells. Protein phosphatase 2A (PP2A) has previously been linked to dephosphorylation of both eIF4E and Mnk-1, an upstream kinase that catalyzes phosphorylation of eIF4E. Treatment of SW480 cells with 7.5 uM WIN and knockdown of PP2A by RNA interference block dephosphorylation of phospho-eIF4E; however, the effects of CBD on dephosphorylation of phospho-eIF4E were PP2A- independent. Using a similar approach in LNCaP cells we showed that WIN and CBD also induced dephosphorylation of phospho-eIF4E but this response was not affected by PP2A knockdown. Ongoing studies are focused on characterizing other cannabinoid-induced phosphatases required for dephosphorylation of phospho-eIF4E and their role in the antitumorigenic activity of cannabinoids, and other anticancer drugs.

Alternative Splicing of ATG5 In DU145 Human Prostate Cancer Cells Inactivates Autophagy and Promotes Murine Xenograft Tumor Growth

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Autophagy is a highly conserved pathway that targets cytoplasmic cargo to the lysosome for degradation. Excess or aberrant organelles, large protein aggregates and non-selective portions of the cytosol can be targeted by the autophagosome and delivered to the lysosome in order to maintain cellular homeostasis or survival in response to stress. In this study, we show that DU145 human prostate cancer cells alternatively splice Autophagy-related protein 5 (ATG5), a protein essential for formation of the autophagosome. These novel ATG5 splice forms are unable to conjugate normally to ATG12 and instead are rapidly ubiquitinated and turned over by the proteasome. The absence of ATG5-ATG12 conjugate in DU145 cells prevents autophagosome formation and autophagic degradation. Stable expression of full-length ATG5 using lentiviral infection rescued ATG5-ATG12 conjugation and autophagic degradation. By elimination of potentially genotoxic damaged organelles and protein aggregates, autophagy is currently thought to be tumor suppressive. However, the role autophagy plays in tumor progression and metastasis remains unclear. To address this question, we performed subcutaneous injections of autophagy-deficient and autophagy-proficient DU145 cells into immunodeficient mice and monitored tumor formation and growth. In initial experiments, autophagy deficient tumors grew at a significantly faster rate despite having a significantly longer latency period. These results suggest that autophagy can promote initial establishment of the tumor, while suppressing later tumor growth. Currently there are numerous clinical trials investigating the therapeutic potential of drugs that inhibit autophagy. Our results suggest that these drugs may actually be detrimental to the treatment of prostate cancer. (These studies were supported by grants, CA129521 and GM096101, from the NIH and by start-up funds from UTMD Anderson Cancer Center.)

Influences of Capping Agents on Cellular Uptake of Silver Nanoparticles

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Silver nanoparticles (AgNPs) have been used in numerous commercial products, including textiles and food packaging, to prevent bacterial growth. Meanwhile, there are increasing concerns that exposure to these nanoparticles may cause potential adverse effects on humans as well as the environment. The most common route of occupational exposure to AgNPs is inhalation. The toxicity has been attributed to nanoparticle-cell interactions, such as the adsorption and internalization of nanoparticles, however, little is known about the mechanisms of these interactions. The experiment was carried out to quantitatively and mechanistically investigate the role of capping agents of AgNPs on cellular uptake by cells in the epithelial lining of the human lung. *In vitro* studies of human bronchoalveolar carcinoma derived cells (A549) are used to quantify the uptake kinetics and the extent of internalized AgNPs and to investigate the uptake mechanism among different capping agents. The results of this study can be applied to manage the risk associated with occupational inhalation exposure to silver nanoparticles, limiting the risk of being exposed to the silver nanoparticles for extended periods of time.