

**DEPARTMENT OF PATHOLOGY
& LABORATORY MEDICINE**

2011

**STUDENT RESEARCH
OPEN HOUSE
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Presenter Name(s): L .Sutton Boyd, Nabil K. Bissada, Elizabeth Fowler, Philip Sobolesky, Mohamed A. Ghoneim, Thomas E. Keane, Daynna Wolff, Dennis K. Watson, and Omar Moussa

Presentation Title: **Prognostic and Diagnostic Significance of microRNA in Bladder Cancer**

Department: Pathology and Laboratory Medicine

Laboratory (Principal Investigator): Dr. Omar Moussa

Funding Source Name/Number (e.g. NCI R01CA123456):

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Recently the role of microRNA (miRNA) in the development and progression of cancer including genitourinary tumors, and lymphomas to name a few have illustrated specific miRNA's to be significantly dysregulated. Consequently, miRNA's are currently being investigated as potential diagnostic and prognostic markers in neoplastic diseases. Because miRNA's are highly conserved they are promising as a potential biomarker that may be acquired by non-invasive means. This study investigated the presence of miR-21, miR-17-5p, miR-155, miR-26, miR-126, and miR-182 in bladder cancer tumor and tumor normal tissue specimens. We further investigated the presence of miR-21, miR-17-5p and miR-155 in urine sediment of bladder cancer patients. Quantitative Real time (RT-PCR) analysis was utilized for the detection of the precursors of miR-21, miR-17-5p, miR-155, miR-26, miR-126, and miR-182 in 23 RNA samples obtained from bladder cancer tissue specimens. The relative expression values were compared to pooled matched non-tumor tissue. The presence of precursors of miRNA miR-21, miR-17-5p and miR-155 were also tested in 104 urine specimens from patients with active disease following positive cystoscopy, patients previously diagnosed and treated for bladder cancer with negative cystoscopic examination, patients with non-bladder cancer urological disease, and 7 healthy volunteers. Several precursors of the oncogenic miRNAs were up- regulated in the early grades and stages of the disease and maintained high levels throughout high grades and late disease stage. In addition, our data also indicated that the miR-126 is down-regulated in bladder cancer specimens compared to the non-tumor tissues. Our preliminary data have also shown that high levels of the precursors of miR-21, miR-17-5p and miR-155 can be detected in urine sediment collected from patients with active disease in higher levels compared to cystoscopy negative patients, patients with non-malignant urological diseases and healthy controls. Our data have shown that miRNA detection in tissue and urine specimens in patients with bladder cancer has a promising prognostic and diagnostic significance and this preliminary data warranting further investigation to look at miRNA's as potential biomarkers. Illustrating the presence of miRNA in urine sediment of bladder cancer patients may prove useful in noninvasive evaluation of bladder cancer.

Presenter Name(s): Fu-Quan Chen, Carlene Brandon, Su-Hua Sha*
Presentation Title: Noise trauma activates Rac/Rho small GTPases through transient ATP-depletion
Department: Pathology and Laboratory Medicine
Laboratory (Principal Investigator): Su-Hua Sha
Funding Source Name/Number (e.g. NCI R01CA123456): NIH R01 DC009222
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Abstract Narrative: Summary description of research project in 300 words or less (single-spaced, 11 point Arial)

As molecular switches, small GTPases act as major mediators in transmembrane signaling and as key regulators of the actin cytoskeleton in all eukaryotic cells. In this study, we investigated the role of Rac GTPase-linked signaling pathways and NADPH oxidase in noise trauma. The exposure of CBA/J mice to broadband noise (2-20 kHz, 106 dB for 2 hr) disturbed the actin arrangement of the stereocilia, and resulted in a permanent threshold shift of 35, 60, and 65 dB at 8, 16, and 32 kHz, respectively, with corresponding hair cell loss. The hair cell loss initially appeared in the basal turn and spread apically with time after exposure. Apoptotic and necrotic hair cell death was detected in the basal region and mitochondrial-mediated caspase-dependent and -independent hair cell death markers were observed 1 and 3 hr after the noise exposure. Noise trauma increased levels of active Rac1, decreased those of active RhoA, and promoted the formation of a Rac1-p67phox complex. Additionally, NOX3 expression was induced, indicating the involvement of NADPH oxidases. We further hypothesized that overstimulation after noise trauma may cause intracellular ATP depletion and that this imbalance in energy metabolism could activate Rac/GTPase pathways. We found that the concentration of ATP in cochlear tissue dropped immediately after noise exposure and reached a minimum around 1 hr post-exposure. In order to directly address the question of whether energy depletion leads to activation of small GTPase pathways and mitochondrial-mediated cell death, we used HEI-OC1 cells treated with the energy-depleting agent oligomycin. It was found that ATP depletion enhances Rac1 activity and promotes rearrangement of the actin cytoskeleton. Our results suggest that noise trauma-induced transient ATP depletion activates small GTPase signaling pathways that regulate the actin cytoskeleton and activate NADPH oxidase, leading to structural disruptions and increased ROS formation in the inner ear.

Presenter Name(s): Victoria J Findlay
Presentation Title: The Role of MicroRNA 510 in breast cancer progression
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MicroRNAs (miRNAs) are endogenous 19-25 nucleotide non-coding RNAs that have recently emerged as a novel class of small, evolutionarily conserved gene regulatory molecules involved in many critical developmental and cellular functions. Through specific base pairing with target mRNA sequences in the 3' untranslated (3'UTR) region, miRNAs induce mRNA degradation, translational repression, or both. Individual miRNAs can target numerous mRNAs, often in combination with other miRNAs, thereby providing a mechanism for controlling complex regulatory networks. Mounting evidence indicates that miRNAs may also play a significant role in cellular transformation and carcinogenesis acting either as oncogenes or tumor suppressors. To date, there are a limited number of studies in the literature on the role of miRNAs in breast cancer.

We identified miR-510 as a novel oncogenic miRNA (or 'oncomir'). Our studies show that miR-510 is elevated in human breast cancer cell lines and human breast tumor samples when compared with non-tumor samples. Over-expression of miR-510 results in increased migration, invasion, cellular transformation and an altered morphology, similar to a cell that has undergone epithelial-mesenchymal-transition (EMT). Furthermore, over-expression of miR-510 in a non-transformed immortalized breast cell line results in tumor formation in SCID mice. We show that miR-510 is regulated by the PI3K/Akt pathway resulting in a positive feedback loop. In addition, we functionally validated PDEF, a potential tumor suppressor, as a direct target of miR-510. Interestingly, miR-510 is located on chromosome Xq27, a region that is reported to be amplified in breast cancer. Based on these observations, we hypothesize that miR-510 is a critical mediator of breast cancer development and progression and that the mRNAs targeted by miR-510 collectively influence major tumorigenic pathways including the PI3K/Akt growth/survival pathway, epithelial-mesenchymal transition (EMT) and the route to metastasis.

Anti-apoptotic genes Family as a Novel Diagnostic Markers of Bladder Cancer

Fowler, E.B., Sobolesky, P.M., Shao, Y., Woolworth J.A., Keane T.E., Wolff D, Watson DK, Moussa, O.

Early diagnosis of genitourinary malignancies is a very challenging process. Currently, FISH is the gold standard for bladder cancer as researchers found that utilizing fluorescence in situ hybridization (FISH) technology produced sensitivity from 60-75%. Specifically our institution utilizes the UroVysion® test which has been documented to have a 72% sensitivity. This test has replaced the traditional cytology analysis, which only produced results with a sensitivity ranging from 30 – 50% depending on disease state. Therefore, for diagnostic purposes, molecular analysis of gene expression in urine sediment could be useful. Since the distinction between healthy and diseased individuals may depend on the definition of a standard discriminative mRNA threshold level a group of markers is needed to provide a convincing screening tool. Our aim was to elucidate antiapoptotic protein expression in patient bladder cancer urine samples and to determine their usefulness as bladder cancer diagnostic markers. To perform this study we analyzed 50 urine samples by real-time PCR. The markers analyzed included BIRC3, BIRC4, SURVIVIN, BIRC6, BIRC8, BFAR, NAIP, STANNIN, and API5. API5, BIRC3, BIRC4, SURVIVIN, and BFAR provided the most promising data with sensitivity and specificity percentages all ranging from 82.3% to 94.4%. The remaining markers showed an extreme favor of either specificity or sensitivity discrediting its effectiveness as a marker at this time. From this study of bladder cancer markers we initiated a functional study to characterize the novel antiapoptotic protein API5 and its role in bladder cancer initiation and progression. We have characterized API5 expression in human bladder cancer tissues using immunohistochemical analysis. Our data indicated that API5 is expressed early in tumorigenesis, which makes it an ideal early diagnostic marker. We have noticed increase in the level of API5 protein associated with tumor grade and stage which also suggest that API5 might be a potential prognostic marker. In conclusion, detection of the inhibitor of apoptosis proteins is a promising diagnostic and prognostic approach.

Presenter Name(s): Kayla Hill¹, Fu-Quan Chen¹, Ya-Jun Guan², Jochen Schacht², Su-Hua Sha^{1*}

Presentation Title: Activation of apoptotic pathways without cell death in an inner-ear immortal mouse cell line
Department: Pathology and Laboratory Medicine

Laboratory (Principal Investigator): Su-Hua Sha

Funding Source Name/Number (e.g. NCI R01CA123456): Sub/NIH R01 DC-03685

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Abstract Narrative: Summary description of research project in 300 words or less (single-spaced, 11 point Arial)

Aminoglycoside antibiotics and cisplatin are the major ototoxic drugs resulting in permanent hearing loss due to the inability of mammalian sensory cells to regenerate. Understanding the mechanism of pathogenesis is the first step in designing effective treatment and prevention of drug-induced hearing loss. In-vitro systems greatly enhance the efficiency of biochemical and molecular investigations through easy access and manipulation. HEI-OC1, an inner ear cell line from the immortal mouse, expresses markers for auditory sensory cells and, therefore, is a potential tool to study the ototoxic mechanisms of drugs like aminoglycoside antibiotics or cisplatin. We are currently investigating aminoglycoside-induced signaling pathways and cell death using HEI-OC1 cells. HEI-OC1 cells efficiently took up fluorescently tagged gentamicin and responded with changes in a variety of cell death and survival signaling pathways. Within hours, the c-Jun N-terminal kinase pathway and transcription factor AP-1 were activated. At later times, the “executioner caspase”, caspase 3, was activated. These responses were robust and elicited by both gentamicin and kanamycin. However, despite the initiation of apoptotic pathways and transient changes in nuclear morphology, cell death was not observed. Furthermore, β -galactosidase measurements ruled out senescence in gentamicin-treated cells. The ability to withstand treatment with aminoglycosides but not with cisplatin suggests that this cell line may be helpful in providing some insight into the differential actions of the two ototoxic drugs and possibly into mechanisms of intrinsic repair capabilities after aminoglycoside insult.

2011 Abstract

Age-related changes in myelin basic protein expression and glial cell numbers in the human auditory nerve

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Glial cells are non-neural cells that provide support and nutrition, participate in signal transmission and form myelin in the nervous system. The integrity of the compacted multi-lamellar myelin sheath surrounding most auditory nerve axons is a primary determinant of the transmission speed of action potentials. Myelin basic protein (MBP) is one of the most abundant proteins in the nervous system and is used as a biomarker for myelin. The aims of the present study were to: 1) identify the pattern of MBP expression in the human auditory nerve, and 2) assess the effects of age on myelin and the number of glial cells in the inner ear. We examined 13 temporal bones from 10 human subjects including 4 subjects aged 38-46 (two female and two male; middle-aged group) and 6 subjects aged 63-91 (two female and four male; older group). The temporal bones were removed and fixed by perilymphatic perfusion less than 6 hours after death. Each bone was decalcified, embedded in Paraplast X-TRA® embedding medium, and sectioned serially in the horizontal plane at a thickness of 6 µm. Every 10th or 20th section was stained with hematoxylin and eosin. Selected sections were immunoassayed with anti-MBP, anti-neurofilament 200 (NF200, neuronal marker) and anti-class III β-Tubulin (TuJ1, neuronal marker). Intense MBP immunostaining was present throughout the auditory nerve including its peripheral component within the osseous spiral lamina and Rosenthal's canal and its central component within the modulus and internal auditory canal. MBP⁺ auditory fibers were present in both the middle-aged and older groups, however, marked losses and/or thinning of MBP⁺ fibers occurred in certain segments of the auditory nerve in the older ears. In the middle-aged group, MBP expression was absent around the perikarya of most spiral ganglion neurons, but was expressed around the cell bodies of about 7-10% of spiral ganglion neurons in the middle and basal turns. A significant reduction of MBP immunostaining associated with the perikarya of the spiral ganglion neurons was seen in the older group. Importantly, a loss of glial cells occurred in the auditory nerve in 4 of the 6 older ears. The glial cell phenotype was identified by unique morphological characteristics and glial cell markers including Sox2 and Sox10. In addition, a marked decrease of NF200⁺ or TuJ1⁺ neuronal cells was seen in older ears. The present study provides the first evidence that declines in MBP expression and reduced glial cell numbers occur in the human auditory nerve of older adults. Supported by NIH DC00422 (H.L., J.R.D); NIH DC00713 (B.A.S.)

Hematopoietic Stem Cell-Derived Fibroblasts Promote Tumor Cell Migration and Invasion

Lindsay T. McDonald^{1,2,3,5}, Daniel J. Neitzke³, Victoria J. Findlay^{2,5}, David P. Turner^{2,5}, Patricia M. Watson^{4,5}, Dennis K. Watson^{2,5} and Amanda C. LaRue^{1,2,5}

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Cells and paracrine factors of the tumor microenvironment play a central role in tumor angiogenesis, invasion, migration and proliferation, making the tumor microenvironment an exciting therapeutic target. Among the most prominent cell types in the tumor stroma are fibroblasts, termed carcinoma-associated fibroblasts (CAFs). We have identified a novel population of CAFs and CAF precursors (circulating fibroblast precursors, CFPs) that are of hematopoietic stem cell (HSC) origin. These cells preferentially migrate and differentiate in response to tumor and their inhibition results in decreased tumor size. While these studies have identified a unique HSC-derived CAF population, the mechanisms by which these cells promote tumorigenesis are unknown. Previous studies have indicated a critical role for Fli1, an Ets family transcription factor, in regulation of HSCs and differentiation/maturation of hematopoietic lineages. Additionally, loss of Fli1 has been associated with cancer progression. Based on these findings, we hypothesize that HSC-derived CFPs/CAFs directly affect tumor cell migration and invasion and that loss of Fli1 in CFPs/CAFs enhances these effects. To address this hypothesis, we examined the ability of HSC-derived fibroblast populations to affect tumor cell migration and invasion in vitro. Fibroblasts were established from peripheral blood and bone marrow of normal mice and mutant Fli1 mice that express a truncated Fli1 protein (Fli1^{ΔCTA}) lacking the carboxy-terminal regulatory (CTA) domain. The effects of conditioned media from these fibroblast populations on tumor cell migration and invasion were then examined in chemotactic transwell migration assays and matrigel-based invasion assays, respectively. Data show that conditioned media from all HSC-derived fibroblast populations promoted migration and invasion of tumor cells versus control, including conditioned media from fibroblasts derived from Fli1^{ΔCTA}/Fli1^{ΔCTA} mice. Ongoing studies are directed at identifying the mechanisms by which Fli1 target genes affect HSC derived fibroblast modulation of tumor cell migration and invasion.

Amelioration of a Mouse Model of Osteogenesis Imperfecta with Hematopoietic Stem Cell Transplantation: Micro-Computed Tomography Studies

Meenal Mehrotra^{a, b}, Michael Rosol^{c, d}, Makio Ogawa^{a, b, d} and Amanda C. LaRue^{a, b, d, *}

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Science, and ^d Hollings Cancer Center, Medical University of South Carolina.

Abstract

Osteogenesis Imperfecta (OI) is a genetic disorder resulting from abnormal amount and/or structure of Type I collagen and is characterized by osteopenia, fragile bones and skeletal deformities. We wanted to test the hypothesis that hematopoietic stem cells (HSCs) generate bone cells using bone marrow (BM) cell transplantation in a mouse model of OI. Homozygous OI mice (*oim*; B6C3Fe *a/a-Col1a2^{oim}/J*) offer excellent recipients for transplantation of normal HSCs, because fast turnover of osteoprogenitors has been shown. We transplanted BM mononuclear cells or 50 BM cells highly enriched for HSCs from transgenic enhanced green fluorescent protein (EGFP) mice into irradiated *oim* mice and analyzed changes in bone parameters using longitudinal Micro-Computed Tomography (micro-CT). Dramatic improvements were observed in 3D micro-CT images of these bones 3 to 6 months post-transplantation when the mice showed high levels of hematopoietic engraftment. Histomorphometric assessment of the bone parameters such as trabecular structure and cortical width supported observations from 3D images. There was an increase in bone volume, trabecular number and trabecular thickness with a concomitant decrease in trabecular spacing. Analysis of a non-engrafted mouse or a mouse that was transplanted with BM cells from *oim* mice showed continued deterioration in the bone parameters. The engrafted mice gained weight and became less prone to spontaneous fractures while the control mice worsened clinically and eventually developed kyphosis. These findings strongly support the concept that HSCs generate bone cells. Furthermore, they are consistent with observations from clinical transplantation studies and suggest therapeutic potentials of HSCs in OI.

Presenter Name(s): **Chandrakala Puligilla**
Presentation Title: **Sox2 signaling specifies neuronal and sensory cell formation in the developing mouse cochlea**
Department: **Section on Developmental Neuroscience, NIDCD**
Laboratory (Principal Investigator): **Matthew W. Kelley**
Funding Source Name/Number (e.g. NCI R01CA123456): **NIDCD Intramural Program Funds**
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Abstract Narrative: Summary description of research project in 300 words or less (single-spaced, 11 point Arial)

Poster #1

Sox2 signaling specifies neuronal and sensory cell formation in the developing mouse cochlea

The mammalian cochlea is comprised of three main components; mechanosensory hair cells, nonsensory cells, and primary afferent neurons all of which are derived from cells within the otocyst. Sox2, which is a high-mobility transcription factor, is one of the earliest markers of developing inner ear. In humans, mutations in SOX2 cause sensorineural hearing loss and a loss-of-function study in mice showed that Sox2 is required for prosensory formation. Another transcription factor Atoh1, which belongs to bHLH family, has been shown to be essential and sufficient for hair cell formation. Based on these results, *Atoh1* has emerged as a powerful candidate for gene-based treatment to restore auditory and vestibular function. However, the inductive signals for Atoh1 function are still unknown. While Sox2 is required for Atoh1 expression and hair cell formation (Kiernan et al., 2005), our previous study has demonstrated that prolonged expression of Sox2 inhibits Atoh1 expression and hair cell formation (Puligilla et al., 2008). Here we demonstrate that Sox2 is sufficient to induce ectopic hair cell formation and is essential for Atoh1-induced hair cell formation. These results suggest a dynamic role of Sox2 in hair cell formation. Moreover, we demonstrate the role of Sox2 in neurogenesis in the developing inner ear using loss and gain-of-function assays *in vivo* and *in vitro*. The spiral ganglion neurons were completely absent in *Sox2^{Lcc/Lcc}* cochleae indicating that Sox2 is necessary for spiral ganglion formation. Furthermore, non-sensory epithelial cells within the developing inner ear can be converted to neurons through over-expression of Sox2 indicating that Sox2 is sufficient for neuron formation. These ectopic neurons acquired morphological and electrophysiological properties characteristic of neurons. Together, these data suggest that a complex network of context-dependent transcriptional regulators is responsible for specification of neuronal versus sensory cell fates within the otocyst.

Presenter Name(s): **Chandrakala Puligilla**

Presentation Title: **Molecular and cellular factors that regulate morphogenesis of the mouse cochlea – Linking the positional identity and patterning of sensory cells**

Department: **Section on Developmental Neuroscience, NIDCD**

Laboratory (Principal Investigator): **Matthew W. Kelley**

Funding Source Name/Number (e.g. NCI R01CA123456): **NIDCD Intramural Program Funds**

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Poster #2

Molecular and cellular factors that regulate morphogenesis of the mouse cochlea – Linking the positional identity and patterning of sensory cells

Following the specification of the prosensory domain, the prosensory precursors then give rise to cochlear sensory patches that assume their final fates and develop as hair cells or support cells. Once differentiated, hair cells and support cells become arranged into highly ordered rows. The correct cellular patterning is crucial for proper functioning of the cochlea; however it's not clear how a homogeneous group of prosensory cells undergoes patterning to give rise to distinct cell types in distinct positions within the developing inner ear. In addition, factors that regulate this alignment are largely unknown. Cell-to-cell adhesion is a key process that not only underlies the patterning of different cell types but also important in establishing connections between different cell types. We are currently studying the function of cell adhesion molecules, Epithelial (E) and Neural (N)-cadherin that direct the characteristic patterning of hair cells and support cells and possibly cell fate, to generate a precisely patterned structure. N- and E-cadherins are transmembrane proteins that mediate calcium-dependent intercellular cell adhesion. Our results reveal that N-cadherin is expressed in the developing inner hair cells and inner phalangeal cells while E-cadherin is expressed in the pillar cells, outer hair cells and Deiters' cells within the mouse organ of Corti. The expression of these two molecules in a complementary fashion suggests their equivalent and distinct roles in specific cell adhesion and in the determination of two different cellular domains. Preliminary results indicate that loss of E-cadherin leads to disruption in the pattern of outer hair cells leading to patchy rosettes of cells suggesting loss of adhesion between outer hair cells. In addition to their role in the assembly of adherens junctions, cadherins also function in mediating transcription by sequestering cytoplasmic components, β -catenin and p120, of canonical wnt signaling pathway. Studies are now underway to examine whether these cadherins modulate Wnt pathway thereby permitting the transcriptional activity affecting cellular fate.

Increased neurogenesis after acute auditory nerve injury in the inner ear of young adult mouse.

Devadoss J Samuvel¹, Juhong Zhu¹, Lauren A. Kilpatrick², Jeremy L. Barth³, Edward L. Krug³, Hainan Lang¹

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Spiral ganglion neurons (SGNs) are primary auditory afferent neurons that deliver signals from the inner ear to the brain. Loss of SGNs can occur with exposure to ototoxic drugs and noise, genetic mutations and age, resulting in permanent sensorineural hearing loss. Recent studies have shown that neural stem cells are able to be isolated from vestibular and auditory sensory epithelia, and spiral ganglia of postnatal mice. However, it is unknown whether the neural stem/progenitor cells are present in the adult auditory nerve. The goal of the present study is to evaluate the potential of adult neurogenesis in the mouse inner ear. We have established an animal model of acute SGN degeneration by applying ouabain to mouse inner ear. The transcription factor Sox2 is predominantly expressed in undifferentiated neural precursors during adult neurogenesis in the brain. A subset of glial cells up-regulated Sox2 and de-differentiated shortly after ouabain treatment, suggesting that glial cells may be a source for neurogenesis after acute SGN injury in the adult mouse inner ear (Lang et al., 2011). In this study, we examined the capability of neurogenesis in adult mouse ear with and without ouabain exposure. Using a modified neurosphere assay, neurospheres were generated from the auditory nerve of young adult mouse. The cells derived from these neurospheres were stained positively for nestin, a neural stem/progenitor cell marker. The majority of the cells in the neurospheres were also BrdU positive indicating their capability for self-renewal. In addition, more neurospheres were seen when cultured from ouabain-injured mice than from normal controls. These results demonstrate that neural stem/progenitor cells present in the adult auditory nerve and suggest that the acute SGN injury by ouabain exposure enhances the capability of auditory nerve for regeneration. Supported by NIH DC00422 (H.L.); NIH DC00713 (B.A.S.); NIH RR16434 and RR RR16461 (J.L.B).

Presenter Name(s): Philip Sobolesky, Julie Woolworth, Yuan Shao, Elizabeth Fowler, Kristina Hang, Dennis Watson, Perry Halushka, and Omar Moussa

Presentation Title: FOXO3a Transcription Factor Regulation by Thromboxane Receptor- β Signaling: Novel Mechanism for Malignant Transformation

Department: Pathology

Laboratory (Principal Investigator): Omar Moussa

Funding Source Name/Number (e.g. NCI R01CA123456): NIH

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Abstract Narrative: Summary description of research project in 300 words or less (single-spaced, 11 point Arial)

Abstract:

The mechanisms of malignant transformation of urothelial cells is currently unknown. Previous data from our group has shown thromboxane receptor isoform β (TP- β) is overexpressed in 80% of bladder cancer patients and TP- β can induce oncogenic transformation of the immortalized urothelial cell line SV-HUC. Thromboxane signaling occurs through $G_{\alpha 12}$ and β -arrestin 2, which in turn activates AKT and ERK. The aim of the current studies is to elucidate the mechanism of cellular transformation induced by activated TP- β signaling. We utilized a protein-protein interaction array approach to screen for TP- β binding partners. Forkhead box transcription factor 3a (FOXO3a) was identified as one of the proteins that directly interact with TP- β . Immunoprecipitation studies followed by western blot analysis confirmed the interaction. FOXO3a is a transcription factor with tumor suppressor activities and regulates genes involved in cellular processes such as cell cycle arrest, apoptosis, metabolism, DNA repair, and cellular stress resistance. Here we show that shRNAi knockdown of FOXO3a was sufficient to transform SV-HUC cells in vitro. We have found that TP- β agonist stimulation with U46619 activated AKT and ERK which resulted in increased phosphorylation of FOXO3a at both Ser253 and Ser294 AKT and ERK phosphorylation sites respectively. Phosphorylation of FOXO3a causes transcriptional inactivation and transportation out of the nucleus where it becomes targeted for degradation. In our bladder cancer model we have shown that FOXO3a regulates the transcription of manganese superoxide dismutase (MnSOD). MnSOD has been shown to remove reactive oxygen species (ROS) from the cell. Previous studies have shown that ROS can stabilize TP- β by changing the subcellular localization. Our proposed model is that malignant transformation could be a result of feedback loop mechanism where TP- β stimulation causes decreased FOXO3a transcriptional activity thereby decreasing MnSOD levels, and enhancing ROS levels which in turn stabilize TP- β .

Presenter Name(s): Alina G. Sofronescu

Presentation Title: XRCC1 399 Arg>Gln (28152G>A) variation correlates with deterioration in Quality of Life induced by radiotherapy in prostate cancer patients

Department: Pathology and laboratory Medicine

Laboratory (Principal Investigator): Yusheng Zhu

Funding Source Name/Number (e.g. NCI R01CA123456):
Supported in part by grant M01 RR001070 from the National Center for Research Resources, NIH, and grant #21648 from the Departments of Pathology and Laboratory Medicine and Radiation Oncology, MUSC.

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Abstract Narrative:

Background: It is estimated that there were over 200,000 new prostate cancer (PCa) cases diagnosed in the U.S. in 2010 and approximately 48% of patients diagnosed with PCa received some form of radiotherapy for their initial treatment. However, some patients develop severe adverse radiotherapeutic effects (AREs), such as severe lower urinary tract irritation, erectile dysfunction, or rectal bleeding, ulceration or dysfunction. Factors associated with AREs have not been well defined, although some genetic and non-genetic variables have been implicated. X-ray repair cross complementing protein 1 (XRCC1) is one of enzymes involved in DNA repair. A previous study has shown that XRCC1 399 Arg>Gln (28152G>A) is associated with increased risk of radiation-induced late injuries in breast cancer patients. The objective of this study is to identify a potential association between XRCC1 399 Arg>Gln (28152G>A) and deterioration in Quality of Life (QoL) due to urinary symptoms caused by radiotherapy in patients with PCa.

Methods: A cohort of 67 PCa patients treated with radiotherapy was observed for one year following the treatment. The QoL due to urinary symptoms was assessed by grading urinary conditions using the following scores recommended by the American Urological Association: 0, delighted; 1, pleased; 2, mostly satisfied; 3, mixed; 4, mostly dissatisfied; 5, unhappy; 6, terrible. It was considered a deterioration in QoL if a patient's baseline score before treatment was less than or equal to 3, but greater than or equal to 4 after treatment. Peripheral blood samples were used to isolate genomic DNA. XRCC1 399 Arg>Gln (28152G>A) genotypes were determined using TaqMan SNP typing assay (Applied Biosystems). The association between deterioration in QoL and XRCC1 399 Arg>Gln (28152G>A) was analyzed using two-sided Fisher's exact test. The differences were considered statistically significant if $p < 0.05$.

Results: In these 67 patients, 41 were wild-type (GG) and 26 were variant (GA + AA) of XRCC1 399 Arg>Gln (28152G>A), and the frequencies of wild-type and variant were 0.60 and 0.40, respectively. In the wild-type group, only one patient experienced deterioration in QoL due to urinary symptom induced by radiotherapy, while in the variant group, 8 patients had deterioration in QoL. The incidences of deterioration in QoL

were 0.024 and 0.307 in the wild-type and variant groups, respectively, and the difference between these two groups were statistically significant ($p = 0.0016$).

Conclusion: Our data suggest that the variant genotype of XRCC1 399 Arg>Gln (28152G>A) is associated with a higher incidence of deterioration in QoL due to urinary symptoms in patients with prostate cancer post-radiation treatment. Further studies are needed to discover other genetic variations and non-genetic factors that are associated with the risk of deterioration in QoL induced by radiotherapy in PCa patients.

Presenter Name(s): Alina G. Sofronescu

Presentation Title: Effects of temperature and light on the stability of bilirubin in plasma samples

Department: Pathology and laboratory Medicine

Laboratory (Principal Investigator): Yusheng Zhu

Funding Source Name/Number (e.g. NCI R01CA123456): N/A

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Abstract Narrative:

Background: Although it is known that bilirubin is photo-sensitive, detailed effects of both temperature and artificial light exposure on bilirubin stability in plasma have not been well investigated. The aim of present study is to determine the impacts of temperature and artificial light on bilirubin stability in plasma.

Methods: Total and direct bilirubin in plasma was analyzed using a Diazo method. The aliquots of 38 samples were stored at 3°C and 22°C with light protection for 2, 4, 8, 24 h respectively before analysis. The aliquots of 20 samples with normal bilirubin and additional 20 with elevated bilirubin were exposed to artificial light for 2, 4, 8, 24, 48 h at 22°C, and total and direct bilirubin was measured. The differences between the baselines and subsequent measurements were analyzed with analysis of variance.

Results: The baseline total bilirubin was 9.6 ± 8.1 mg/dL (mean \pm SD) and the concentrations were 9.6 ± 8.2 , 9.0 ± 7.4 , 9.0 ± 7.5 , 8.8 ± 7.5 mg/dL at 3°C and 9.5 ± 8.1 , 9.0 ± 7.4 , 9.6 ± 8.1 , 9.5 ± 8.0 mg/dL at 22°C after 2, 4, 8, 24 h. ($p > 0.05$, $N=38$). The baseline direct bilirubin was 1.3 ± 1.2 mg/dL and the concentrations after 2, 4, 8, 24 h were 1.4 ± 1.2 , 1.4 ± 1.2 , 1.5 ± 1.2 , 1.3 ± 1.1 mg/dL at 3°C and 1.4 ± 1.1 , 1.3 ± 1.1 , 1.3 ± 1.1 , 1.3 ± 1.0 mg/dL at 22°C ($p > 0.05$, $N=19$). In the samples with elevated bilirubin exposed to light at 22°C, the baseline total and direct bilirubin concentrations were 10.2 ± 1.7 mg/dL and 5.0 ± 1.9 mg/dL, respectively. After 2, 4, 8, 24, 48 h, the total bilirubin concentrations were 10.1 ± 1.8 , 10 ± 1.8 , 10.0 ± 1.8 , 9.3 ± 2.0 ($p > 0.05$, $N=20$), 8.4 ± 2.3 ($p < 0.01$, $N=20$) mg/dL and direct bilirubin concentrations were 4.9 ± 1.8 , 4.9 ± 1.9 , 4.8 ± 1.8 , 4.2 ± 1.6 ($p > 0.05$, $N=20$), 3.5 ± 1.5 ($p < 0.01$, $N=20$) mg/dL. For samples with normal bilirubin levels under the same conditions, the average baseline total and direct bilirubin concentrations were 0.7 ± 0.1 mg/dL and 0.1 ± 0.0 mg/dL, respectively. After 2, 4, 8, 24, 48 h, the average total bilirubin concentrations were 0.7 ± 0.1 , 0.6 ± 0.1 , 0.6 ± 0.1 ($p > 0.05$, $N=20$), 0.5 ± 0.1 , 0.4 ± 0.1 mg/dL ($p < 0.01$, $N=20$) and direct bilirubin concentrations were the same as the baseline.

Conclusions: Bilirubin in plasma is stable in refrigerator or at room temperature without light exposure for at least 24 h. In normal laboratory environment, a delay of up to 8 h in the measurement of bilirubin left unprotected from light at room temperature does not significantly affect the results. Under these conditions, the changes in bilirubin concentration are not clinically significant until after 24 h.

Presenter Name(s): **Lisa L Steed**

Presentation Title: **Risk Assessment and Mitigation of Microbial Reservoirs Associated with ICU Hospital Beds: Effects of Disinfection on Population Recovery**

Department: Micro and Immuno, Path & Lab Medicine, and Infectious Diseases at MUSC

Laboratory (Principal Investigator): **M.G. Schmidt**

Funding Source Name/Number (e.g. NCI R01CA123456): **US Army Medical Research and Materiel Command under Contracts No. W81XWH-07-C-0053**

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Abstract Narrative: Summary description of research project in 300 words or less (single-spaced, 11 point Arial)

Risk Assessment and Mitigation of Microbial Reservoirs Associated with ICU Hospital Beds: Effects of Disinfection on Population Recovery

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Background: The CDC estimates that more than 2,000,000 hospital-acquired infections (HAI) occur each year in the US adding an approximate \$45 billion to the cost of providing care. At issue is the source of the microbes responsible for these infections. Much work has focused on the transfer of microbes from patients to healthcare workers and vice versa. Commonly touched items are likely to serve as reservoirs from which patients, healthcare workers, and visitors may be exposed and/or transfer suspect microbes. In this study we conducted a quantitative assessment of the risk that the bed, the principal object in the patient's room, represents and how disinfection mitigates this risk. **Methods:** Rails from 36 beds housing patients in the medical intensive care unit were sampled and the bacterial burden was enumerated immediately prior to routine disinfection and subsequently at 0.5, 2.5, 4.5 and 6.5 hours using a pre-moistened sterile rayon/polyester wipe. Half of the beds were sanitized using a commercially prepared, bottled disinfectant while the others were sanitized using an automatically diluted disinfectant from a concentrate. The samples were suspended, diluted, and plated on various microbiological media in order to facilitate characterization and enumeration of the samples. **Results:** The average bacterial bioload for the rails treated with the bottled disinfectant was 5,800 CFU/100 cm². The disinfection step reduced the bioload by 99%. By hour 6.5 the bacterial population returned to an average level of 1,750 CFU/100 cm². Rails treated with the concentrate-diluted disinfectant showed a mean reduction of 45% and returned to pre-disinfection levels by hour 2.5. However, statistical consideration of the two data sets using median values reflected that the initial effectiveness of the two disinfectants were equivalent providing reductions of 98.23% and 94.59% respectively. The majority of bacteria from the rail surfaces were staphylococci. MRSA was isolated from one bed while VRE was recovered from 5 separate beds during the study. **Conclusion:** Disinfection reduced the bacterial burden on bed rail surfaces up to 98%. However, the bacterial population, principally Gram + cocci, returned to levels between 30 -100% of the initial bioloads observed within 6 hours of disinfection.

Acknowledgments/References: Work supported by the US Army Medical Research and Materiel Command under Contracts No. W81XWH-07-C-0053

Presenter Name(s): Roger Stone, Dr. Zhu
Presentation Title: **Positive Propylene Glycol in a Patient with Ethylene Glycol Toxicity**
Department: Pathology
Laboratory (Principal Investigator): N/A
Funding Source Name/Number (e.g. NCI R01CA123456): N/A
Telephone Number: 843-793-8000
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Abstract Narrative: Summary description of research project in 300 words or less (single-spaced, 11 point Arial) :

Background: Both ethylene glycol and propylene glycol can be used as antifreeze. Propylene glycol is also used as a solvent in many medications. Ingestion of both can cause increased serum osmolality and anion gap acidosis, but ethylene glycol is much more toxic. However, large doses of propylene glycol can be toxic. We report a case in which the patient initially presented with ethylene glycol poisoning, but later showed a high level of propylene glycol.

Methods: The patient was a 61 year old female who was found unconscious. Upon arrival to our hospital, the patient was in respiratory failure with evidence of an increased anion gap metabolic acidosis, increased serum osmolal gap, and negative volatiles. Ethylene glycol and propylene glycol in the patient's serum were analyzed with a laboratory developed capillary column gas chromatography assay. The patient was treated with emergent hemodialysis, continuous veno-venous hemofiltration and fomepizole. The patient also received phenytoin and a high dose of lorazepam for a witnessed seizure. Ethylene glycol and propylene glycol were subsequently measured 13 hours and 38 hours later.

Results: On admission, ethylene glycol was elevated at 22 mg/dL and propylene glycol was negative. Thirteen hours later, the ethylene glycol level was undetectable but propylene glycol was detected at a level of 27 mg/dL. The medication list revealed that the patient was given phenytoin and a high dose lorazepam drip which contain propylene glycol. The lorazepam was discontinued and the following day the propylene glycol level decreased to 13 mg/dL and ethylene glycol was still undetectable.

Conclusion: The positive propylene glycol in this patient is caused by medications. This case study supports the notion that propylene glycol accumulation is a common phenomenon that is becoming increasingly recognized in the ICU. Furthermore, it highlights the importance of identifying and reporting this potentially harmful compound whenever glycols analysis is performed.

ETS1 transcriptional activity is increased in advanced prostate cancer and promotes the castrate resistant phenotype

Smith A.M., Findlay V.J., Bandurraga, S.G., Kistner-Griffin E., Spruill L., Liu A., and Turner D.P.

Advanced disease accounts for the majority of prostate cancer related deaths and is a result of lymphatic, local or contiguous spread. Androgen deprivation therapy (ADT) (medical castration, bilateral orchiectomy, or both) targets androgen receptor (AR) activity by reducing available ligand (the hormone androgen) and is the standard of care for men with advanced prostate cancer. ADT decreases PSA levels, promotes tumor regression and improves patient symptoms. However, many patients undergoing ADT become resistant to its effects and progress to castrate resistant prostate cancer (CRPC). Due to inadequate treatment strategies, progression-free survival rates for CRPC patients are as low as 2 months. The molecular events that augment the change from castrate sensitive prostate cancer (CSPC) to CRPC are poorly understood but AR activity remains critical.

Levels of the archetypical ETS factor ETS1 are increased in epithelial tumors, leukemia's, astrocytomas and sarcomas and are increased in clinical and latent prostate cancer relative to benign prostatic hyperplasia and normal prostate. Increased ETS1 activity is associated with aberrant transcriptional regulation of multiple cancer associated genes which result in enhanced energy metabolism, matrix degradation, cell survival, angiogenesis, as well as migration and invasion. ETS1 activity has recently been associated with the castrate resistant phenotype through its transcriptional regulation of the angiogenic factor angiotensin II in castrate resistant prostate cancer cells.

Our studies have found that ETS1 expression is highest in high grade prostate cancer (Gleason 7 and above) and correlates with lower AR levels in human patient samples. ETS1 expression and nuclear phosphorylation at its Thr38 residue correlated with an aggressive and castrate resistant phenotype in the LNCaP model of prostate cancer progression. Elevated AKT activity was demonstrated to increase ETS1 protein levels specifically in castrate resistant cells and exogenous ETS1 expression was sufficient to rescue invasive potential in AKT inhibited cells. Significantly, targeted AR activity altered ETS1 expression levels which in turn altered the castrate resistant phenotype. Elevated expression of the matrix degradation protein MMP1 also correlated with high grade prostate cancer tissue and high ETS1 expression. Direct ETS1 transcriptional regulation of the MMP1 promoter was highest in castrate resistant background compared to castrate sensitive. In combination these data strongly suggest a role for ETS1 transcriptional activity in promoting aggressive prostate cancer and the castrate resistant phenotype.

Ionizing radiation induces senescence in lung cancer cells via activation of the p53-p21 pathway

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Radiation therapy is used in over 50% of patients during the course of cancer treatment and is effective both as a curative modality and for palliation. However, the molecular mechanisms whereby irradiation suppresses tumor growth are incompletely understood. In the present study, we show that although ionizing radiation (IR) kills human non-small cell lung cancer (NSCLC) cells in a dose-dependent manner, it induces only a slight apoptosis response in these tumor cells. These results demonstrate that induction of apoptosis is not the primary mechanism underlying IR-induced cell killing in NSCLC cells. Further investigations revealed that the lung cancer cell killing effects of irradiation correlate well with IR-induced cellular senescence, as evidenced by increased senescence-associated β -galactosidase (SA- β -gal) staining, decreased BrdU incorporation and elevated expression of p16^{INK4a} (p16). Mechanistic studies showed that the induction of senescence in irradiated cells is associated with activation of the p53-p21 pathway, and that knockdown of p53 expression by shRNA attenuates IR-induced cell killing and senescence. Furthermore, our data indicate that treatment with Nutlin-3, a small molecule inhibitor of MDM2, sensitizes lung cancer cells to IR-induced cell killing and senescence by stabilizing the activation of the p53-p21 pathway in irradiated lung cancer cells. Taken together, these studies provide new insight into the role of IR-induced senescence in lung cancer radiotherapy and suggest that pharmacological stabilization of p53 activation with Nutlin-3 may represent a novel approach to sensitize lung cancer to radiation therapy by enhancing IR-induced cellular senescence.

Presenter Name(s): Yusheng Zhu
Presentation Title: THE DIFFERENCE IN THE INTERFERENCE OF FETAL HEMOGLOBIN BETWEEN CATION-EXCHANGE HPLC AND TURBIDIMETRIC INHIBITION IMMUNOASSAY for HbA1c TESTING
Department: Pathology
Laboratory (Principal Investigator): Yusheng Zhu
Funding Source Name/Number (e.g. NCI R01CA123456):
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Abstract Narrative:

Background: Studies have shown that significantly increased HbF interferes with certain HbA1c assays, but the degrees of interference vary in different methods. The objective of this study is to determine if there is a bias in HbA1c values in patients with elevated HbF between a cation-exchange (CE)-HPLC and an immunoassay and if the bias correlates with HbF and HbA1c levels.

Methods: Thirty-four EDTA whole blood samples with elevated HbF were tested with Bio-Rad Variant II TURBO Link CE-HPLC and Siemens Dimension turbidimetric inhibition immunoassay (TINIA) HbA1c methods. HbF was quantified by Bio-Rad Classic Variant CE-HPLC with Beta-thalassemia Short Program. The differences in HbA1c results between these two methods were compared using paired, two-tailed Student's t-Test and the differences were considered statistically significant if the p values were less than 0.05. Linear regression analysis was used to determine the association between the inter-method HbA1c bias and HbF as well as HbA1c levels.

Results: The HbF levels in these 34 samples ranged from 7.8% to 35.7% with an average of $23.5\% \pm 7.3\%$ (mean \pm SD). The average HbA1c values with CE-HPLC and TINIA were $7.4\% \pm 2.2\%$ and $6.4\% \pm 1.9\%$ (mean \pm SD) with an average bias of 1.0% ($p = 0.0005$). Linear regression analysis showed a proportional relationship between the bias of HbA1c and the levels of HbF: y (HbA1c bias) = $0.06x$ (HbF) - 0.36 ($R = 0.3$, SEE = 1.4). Linear regression analysis also showed a proportional relationship between the %bias of HbA1c and HbA1c levels with CE-HPLC: y (%Bias) = $3.5x$ (HbA1c, CE-HPLC) - 14.8 ($R = 0.5$, SEE = 15.3).

Conclusions: There is a significant bias in HbA1c levels between Bio-Rad Variant II TURBO Link CE-HPLC and Siemens Dimension TINIA HbA1c assays in patients with elevated HbF. The bias correlates with HbF values and the %bias correlates with HbA1c values at small ($R = 0.3$) and medium ($R = 0.5$) correlation levels, respectively.