2018 ANNUAL

CANCER RESEARCH

SYMPOSIUM

FRIDAY | FEB. 2 | 2018

HOSTED BY STEPHENSON CANCER CENTER

The Stephenson Cancer Center wishes to recognize and thank the Oklahoma Tobacco Settlement Endowment Trust (TSET) for co-sponsoring the 2018 Stephenson Cancer Research Symposium.

In 2012 TSET awarded a five-year, $30.25 million grant to the Stephenson Cancer Center to establish the Oklahoma TSET Cancer Research Program. In 2017 TSET renewed this award for an additional five year period.

The mission of the Oklahoma TSET Cancer Research Program is to decrease the burden of cancer in Oklahoma and nationally through promoting, coordinating and supporting innovative cancer research. It seeks to accomplish this mission through:

- Attracting cancer researchers with grant funding from the National Cancer Institute and other national sponsors to Oklahoma
- Developing trans-disciplinary, collaborative cancer research programs
- Promoting inter-institutional partnerships to leverage unique strengths at research institutions in Oklahoma
- Enhancing research infrastructure and shared resources to enable and support innovative and nationally-competitive cancer research
- Serving as a statewide resource for researchers and institutions that conduct cancer research

The Oklahoma TSET Cancer Research Program supports a wide range of programs, shared resources and initiatives designed to accomplish these goals.

FIVE YEAR HIGHLIGHTS

With support from the Oklahoma TSET Cancer Research Program the Stephenson Cancer Center accomplished the following:

- Increased cancer center membership from 75 to 254 members at ten academic institutions across Oklahoma
- Recruited twenty five new cancer researchers to Oklahoma
- Funded fifty seed and directed-research grants to cancer investigators in Oklahoma
- Enhanced five Shared Resource facilities
- Hosted over 200 research seminar speakers
- Hosted annual statewide Cancer Research Symposium that bring together over 250 researchers from around the state
- Hosted over 50 undergraduate students from 20 different universities for a summer cancer research experience
- Opened 627 new cancer clinical trials
- Enrolled 4114 patients to interventional clinical trials
- Enrolled 5089 patients to non-interventional clinical trials
- Opened 114 new Phase I and Phase I/II clinical trials
- Enrolled 786 patients to Phase I clinical trials

OTRC
OKLAHOMA TOBACCO RESEARCH CENTER

Stephenson Cancer Center wishes to recognize and thank the Oklahoma Tobacco Research Center (OTRC) for co-sponsoring the 2018 Stephenson Cancer Research Symposium.

The mission of the Oklahoma Tobacco Research Center (OTRC) is to reduce, and ultimately eliminate, tobacco-related morbidity and mortality in Oklahoma through research that informs interventions and policies with a particular emphasis on addressing tobacco-related health disparities.

The following goals help drive our mission:

1. To be a leading tobacco research program with a focus on the entire translational continuum – from the discovery of basic mechanisms of tobacco use, cessation, and relapse, to the development and evaluation of novel tobacco treatments, to the dissemination and implementation of treatments, policies, and education throughout Oklahoma.
2. To effectively and efficiently deliver state-of-the-science, evidence-based tobacco treatment to Oklahomans throughout the State.
3. To train the next generation of tobacco researchers.

In addition, the OTRC provides tobacco cessation services across the state through its Tobacco Dependence Treatment Program, and educates and trains health providers in state-of-the-art cessation services.

The OTRC was established in 2007 with funding from the Oklahoma Tobacco Settlement Endowment Trust (TSET). Recognizing the investments that TSET has made in statewide and community-based cessation and intervention projects, a key feature of the OTRC is establishing partnerships with existing and future TSET-funded projects and the Oklahoma State Department of Health (OSDH) tobacco-related programs. Those partnerships directly link OTRC researchers with tobacco-related issues and initiatives in Oklahoma.

OTRC DIRECTOR

Jennifer Vidrine, PhD

OTRC ASSOCIATE–DIRECTORS

Steven Gillaspy, PhD
D. Robert McCaffree, MD, MSHA
Damon Vidrine, DrPH
Theodore Wagener, PhD

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CANCER RESEARCH SYMPOSIUM

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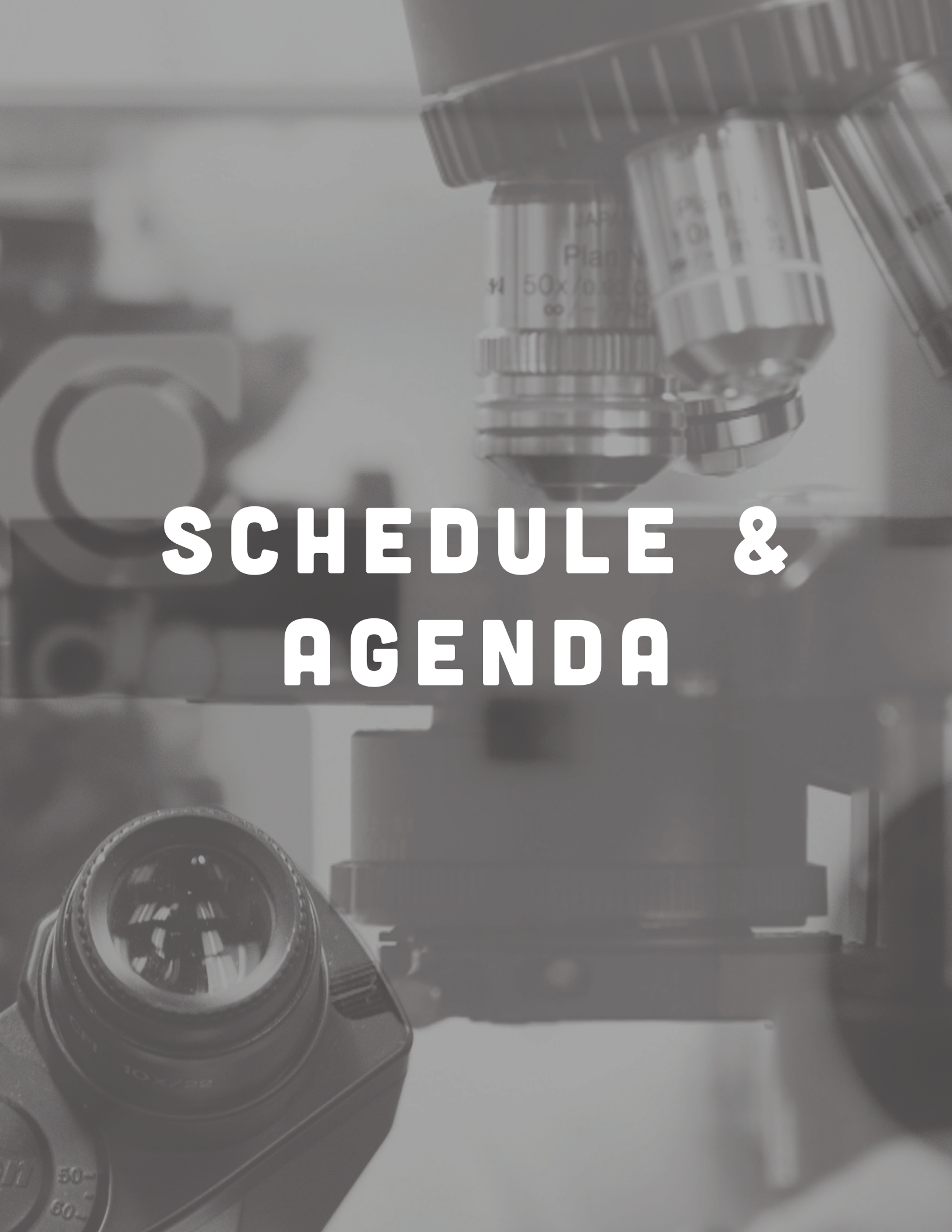

SCHEDULE & AGENDA

2018 ANNUAL

CANCER RESEARCH SYMPOSIUM

8:00 – 8:45 AM	**Registration, Continental Breakfast & Poster Set-Up**
8:45 – 9:00 AM	**Welcome & Opening Remarks**
9:00 – 9:15 AM	Break
9:15 – 10:15 AM	**Session I (All Tracks)**
10:15 – 11:15 AM	**Session II (All Tracks)**
11:15 AM – 1:15 PM	Lunch & Poster Sessions
1:15 – 2:15 PM	**Session III (All Tracks)**
2:15 – 3:15 PM	**Session IV (All Tracks)**
3:15 – 3:30 PM	Break
3:30 – 4:30 PM	**Session V (All Tracks)**
4:30 – 4:45 PM	Break
4:45 – 5:00 PM	**Awards & Closing Remarks**
5:00 – 6:00 PM	**Reception**

SCHEDULE AT A GLANCE

PRECLINICAL TRANSLATIONAL RESEARCH SESSIONS

PRECLINICAL TRANSLATIONS RESEARCH AUDITORIUM

9:15 – 10:15 AM

SESSION I
TUMOR CELL BIOLOGY

Moderators: Maria Ruiz-Echevarria, PhD

9:15 – 9:35 AM

MECHANISTIC INSIGHTS INTO THE ONCOGENIC POTENTIAL OF THE ETV1 TRANSCRIPTION FACTOR AND ITS COFACTOR JMJD2A

Ralf Janknect, PhD
Department of Cell Biology
The University of Oklahoma Health Sciences Center

9:35 – 9:55 AM

ELUCIDATING THE ROLE OF XRN2 IN TUMOR CELL MOTILITY AND CHEMO-RESISTANCE.

Julio Morales, PhD
Department of Neurosurgery
The University of Oklahoma Health Sciences Center

9:55 – 10:15 AM

DOES PROTEOLYTIC ACTIVITY OF FIBROBLAST ACTIVATION PROTEIN-ALPHA DEGRADE NK-CELL RECEPTORS AND THEREBY DIMINISH IMMUNE SURVEILLANCE OF CANCER CELLS?

Victoria Christiansen, PhD
Department of Internal Medicine
The University of Oklahoma Health Sciences Center

10:15 – 11:15 AM

SESSION II
TARGETED THERAPY

Moderators: Jie Wu, PhD

10:15 – 10:35 AM

TARGETING RIBOSOME BIOGENESIS IN CANCER

Lawrence Rothblum, PhD
Department of Cell Biology
The University of Oklahoma Health Sciences Center

10:35 – 10:55 AM

TARGETING MYOCARDIN-RELATED TRANSCRIPTION FACTOR-A IN PROSTATE CANCER

Bojie Dai, PhD
Department of Cell Biology
The University of Oklahoma Health Sciences Center

10:55 – 11:15 AM

HUR TARGETED THERAPY INDUCES THE IMMUNE-SUPPRESSOR PD-L1 (B7-H1): AN ESCAPE MECHANISM IN LUNG CANCER

Rajagopal Ramesh, PhD
Department of Pathology
The University of Oklahoma Health Sciences Center

PRECLINICAL TRANSLATIONS RESEARCH AUDITORIUM

1:15 – 2:15 PM

SESSION III
NOVEL THERAPEUTICS & DIAGNOSTICS

Moderators: Resham Bhattacharya, PhD

1:15 – 1:35 PM

MASS SPECTROMETRY DETECTION OF CHEMOTHERAPY DRUGS IN SINGLE BLADDER CANCER CELLS IN PATIENTS

Anthony W.G. Burgett, PhD
Department of Chemistry and Biochemistry
The University of Oklahoma

1:35 – 1:55 PM

A DIFFUSE OPTICAL SPECTROSCOPY IMAGING TECHNOLOGY THAT IS POTENTIALLY COMPATIBLE WITH MINIMALLY-INVASIVE-PROCEDURES FOR INTRA-OPERATIVE IDENTIFICATION OF ANATOMIC STRUCTURES AND ASSESSMENT OF PARENCHYMAL PATHOLOGY

Daqing Piao, PhD
School of Electrical and Computer Engineering & Department of Veterinary Clinical Sciences
Oklahoma State University

1:55 – 2:15 PM

ANALYSIS OF NANOPARTICLE DELIVERY TO SOLID TUMORS

Stefan Wilhelm, PhD
Stephenson School of Biomedical Engineering
The University of Oklahoma

2:15 – 3:15 PM

SESSION IV
NOVEL THERAPEUTICS & DIAGNOSTICS II

Moderators: Joe Zhao, PhD

2:15 – 2:35 PM

OKN-007: A MULTI-PURPOSE AGENT FOR THE TREATMENT OF BRAIN TUMORS AND POSSIBLY MORE

Rheal Towner, PhD
Advanced Magnetic Resonance Center
Oklahoma Medical Research Foundation

2:35 – 2:55 PM

THERANOSTIC WITH RADIATION-INDUCED ULTRASOUND EMISSION (TRUE)

Liangzhong Xiang, PhD
Department of Electrical and Computer Engineering
The University of Oklahoma

2:55 – 3:15 PM

NOVEL DRUGS FOR CANCER TREATMENT - A JOURNEY FROM THE SEA

Natarajan Aravindan, PhD
Department of Radiation Oncology
The University of Oklahoma Health Sciences Center

3:30 – 4:30 PM **SESSION V: TUMOR MICROENVIRONMENT**
Moderators: Xin Zhang, PhD

3:30 – 3:50 PM **EXOSOME MICRORNAS IN CANCER: BIOLOGY AND POTENTIAL APPLICATIONS**
Wei-Qun Ding, PhD
Department of Pathology
The University of Oklahoma Health Sciences Center

3:50 – 4:10 PM **CIRCULATING EXTRACELLULAR VESICLES TARGET T CELLS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA**
Asish Ghosh, PhD
Department of Pathology
The University of Oklahoma Health Sciences Center

4:10 – 4:30 PM **IMMUNE RESPONSE TO COLORECTAL CANCER DIFFERS BY SEX**
Katherine Morris, MD
Department of Surgery
The University of Oklahoma Health Sciences Center

MECHANISTIC INSIGHTS INTO THE ONCOGENIC POTENTIAL OF THE ETV1 TRANSCRIPTION FACTOR AND ITS COFACTOR JMJD2A

Sangphil Oh, Sook Shin, Hoogeun Song, Tae-Dong Kim, Ralf Janknecht
Department of Cell Biology, OUHSC, and Stephenson Cancer Center

ETV1 is a DNA-binding transcription factor, whose overexpression leads to the development of prostatic intraepithelial neoplasia (PIN). Likewise, overexpression of JMJD2A, a coactivator of ETV1 and histone demethylase, leads to PIN formation in transgenic mice. However, ETV1 and/or JMJD2A overexpression does not induce the progression of PIN to the carcinoma stage, possibly suggesting that some barrier has to be overcome to fully realize the oncogenic potential of these two proteins. Here, we discuss molecular changes that may be required to enhance the oncogenic potential of ETV1 and JMJD2A in prostate and other cancers.

ELUCIDATING THE ROLE OF XRN2 IN TUMOR CELL MOTILITY AND CHEMO-RESISTANCE

Tuyen T. Dang and Julio C. Morales

XRN2 is a 5'-3' exo-nuclease traditionally associated with transcription elongation and termination. Recently, we uncovered a role for XRN2 in the DNA damage response. Loss of XRN2 lead to increased double strand breaks, RNA:DNA hybrids (R-loops), radiation sensitivity, chromosomal aberrations and replication stress. We also found that loss of XRN2 impairs the cells ability to perform non-homologous end joining. Additionally, loss of XRN2 sensitizes cells to PARP1 and DNA repair inhibition. Interestingly, the presence of R-loops dictated whether XRN2 deficient cells where sensitive to certain genotoxic stresses. Surprisingly, we also found that XRN2 plays a role in tumor cell motility. Loss of XRN2 decreases tumor cell motility. RNA-seq experiments demonstrated a role for XRN2 in maintaining the expression levels of several proteins involved in cell motility and invasion. Thus, we hypothesize that XRN2 mediates DNA repair and cell invasion, possibly through R-loop formation.

DOES PROTEOLYTIC ACTIVITY OF FIBROBLAST ACTIVATION PROTEIN–ALPHA DEGRADE NK–CELL RECEPTORS AND THEREBY DIMINISH IMMUNE SURVEILLANCE OF CANCER CELLS?

Victoria J Christiansen[1], Kenneth W. Jackson[1], Susan Kovats[2] and Patrick A. McKee[1]

[1]University of Oklahoma Health Sciences Center, Dept. of Medicine/Warren Medical Research and [2]Oklahoma Medical Research Center

Contact: victoria-christiansen@ouhsc.edu

Fibroblast Activation Protein-alpha (FAP) is a prolyl specific endoproteinase expressed on activated fibroblasts. It is thought to be involved in the growth and metastasis of cancer. FAP is expressed during embryogenesis and in wound healing, but is rarely expressed in normal human adult tissues. It is, however, significantly overexpressed in stroma of over 90% of epithelial-derived cancers and is believed to be associated with tumor immune tolerance, but the mechanism remains unclear. No effort has been made to link immune tolerance to FAP proteolytic function. We designed and synthesized a FAP inhibitor termed M83 that is specific and does not inhibit DPPIV, a prolyl specific dipeptidase. We showed that M83 significantly suppresses colon cancer growth in an athymic mouse xenograft model. While these mice lack adaptive immunity, their innate immune system, which mostly depends on NK cells, remains intact. NK cells serve as the sentry for identifying non-self virally-infected or tumor derived invading cells. We posit that FAP interacts with NK cells to reduce their function and induce immune tolerance for cancer growth. Proteolytic degradation of NK receptors could easily lessen NK cell function and thus impair immune surveillance. FAP's active-site has a highly specific substrate scissile bond preference for x-Gly-**Pro–x**-. We identified 17 NK cell receptors with at least one Gly-Pro bond. To test our hypothesis that FAP may play a significant role in reducing tumor immune surveillance by cleaving NK cell receptors, we purified human NK cells and exposed them to a low concentration of soluble FAP, trypsin, chymotrypsin, elastase, prolyl oligopeptidase (POP) or plasmin. After incubation with each enzyme, NK cells were assayed for cytotoxicity using a standard NK cell target, K562 leukemia cells. All of these enzymes except POP markedly reduced the NK cell cytotoxicity, while FAP did so at ~10X lower concentration than the others. Important to note is that FAP has no known biologic inhibitor whereas the other proteolytic enzymes studied have highly specific and efficient natural inhibitors. Our studies indicated that the effect of FAP on NK cells is concentration and time dependent, with FAP 55 nM abolishing NK cell activity in 1 hour. Preliminary results show that the 40% loss of NK cell function that occurs in just 5 minutes of incubation with FAP is totally reversed by M83. In addition, DFP, which inactivates serine proteinase enzymes by covalently binding to their active-sites, eradicated FAP's ability to destroy NK cytotoxic function. Flow cytometry patterns changed when FAP-treated NK cells that lost all cytotoxic function were analyzed using antibodies to two NK cell proteins: namely, NKG2D, an activating receptor and CD11a, a subunit of LFA-1a adhesive protein. Our studies are consistent with low concentrations of FAP proteolytic activity causing structural degradation and loss of NK cell receptor function, events that would clearly diminish immune surveillance, amplify tolerance, and favor cancer growth.

Funding provided by Warren Medical Research Center – Tulsa, OK and Presbyterian Health Foundation Bridge grant 1-11-17 thru 1-10-18.

TARGETING RIBOSOME BIOGENESIS IN CANCER

William Placzek, Priyabatra Mukherjee, Mohammed Nair Hossen, Katrina Rothblum, and Lawrence Rothblum

Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham School of Medicine, Department of Pathology, *Department of Cell Biology, University of Oklahoma College of Medicine.

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Tumor cell growth results from the accumulation of protein and requires an increased amount of ribosomes. In fact, the pleomorphic nature of nucleoli of tumor cells was one of the first cytological hallmarks of cancer (Pianese, 1896). The rate-limiting step in ribosome biogenesis is rDNA transcription by RNA polymerase I (Pol I). There are several molecular components of the transcription apparatus that are targets for regulating rDNA transcription, including a transcription factor referred to as UBF and even the assembly of the polymerase itself. For example, we have found that the fraction of RNA polymerase I that contains a protein required for transcription initiation, Rrn3, is subject to regulation.

Studies from our laboratory have demonstrated that transcription initiation is dependent upon the assembly of several polymerase associated proteins with the core polymerase. One of these proteins is Rrn3. The assembly of Rrn3 with core Pol I is required for the formation of a polymerase complex that is capable of specific transcription initiation. The rpa43 subunit of Pol I is essential for the recruitment of Rrn3. The detailed molecular study of the interaction between Rrn3 and rpa43, has led to the discovery of a twenty-two amino acid long peptide within rpa43 that can inhibit cell proliferation and, in the case of many cancer cells, cause cell death. In some cancer cell lines we have observed >90% cell death within 8-24 hr. These observations are consistent with reports from other laboratories that have demonstrated the death of tumor cells in response to an inadequate rate of ribosome biogenesis, *e.g., J Cell Sci.* 124:3017 (2011). Interestingly, the resulting cell death is p53 independent and does not necessarily occur through apoptosis. The 22mer is a small molecule inhibitor of rDNA transcription that is based on our understanding of the mechanism of rDNA transcription and appears to be specific for the rDNA transcription apparatus. As such, we hypothesize that it represents a novel way to interfere with cell growth, and we believe that it demonstrates a potentially novel pharmaceutical target for the treatment of cancer cells.

Ongoing studies are designed to enhance the efficacy of the peptide and to determine the structure of the 22mer in order to define a pharmacophore. To this end we have carried out NMR studies of the interaction of the peptide with Rrn3 and have confirmed that the peptide is assuming a beta sheet and are focusing on determining the essential amino acids in the structure. We have also found that packaging the peptide in an organic nanocomposite enhances the efficacy approximately 40-60X. In these assays we see cell death from micromolar amounts of the peptide.

TARGETING MYOCARDIN-RELATED TRANSCRIPTION FACTOR-A IN PROSTATE CANCER

Bojie Dai, James Griffith, William Berry, James J. Tomasek
Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma

Myocardin-related transcription factor (MRTF) -A and -B are transcriptional co-activators of serum response factor (SRF). Previous studies have demonstrated that depletion of MRTFs reduces cell invasion and motility without affecting proliferation in MDA-MB-231 breast carcinoma and B16F2 melanoma cells. However, the role of MRTF in prostate cancer still remains largely unknown. Recently, through bioinformatical analyses of *Gene Expression Omnibus (GEO)* data sets and published microarray data, we have discovered that the transcript level of MRTF-A, but not MRTF-B, is upregulated in prostate cancer progression and metastasis. Our immunohistochemical analysis of human tissue microarrays has revealed that MRTF-A protein expression is elevated in human prostate tumors and positively correlated with the Gleason grades of the tumor samples. Moreover, we found that MRTF-A protein expression increases in prostate carcinoma tissue from the well-defined mouse model with heterzygous Pten deletion compared with wild-type controls. Based upon these results we hypothesize that MRTF-A plays a critical role in prostate cancer development. Supporting this hypothesis, we found that MRTF-A overexpression in prostate epithelial cells induces epithelial-to-mesenchymal transition and triggers the differentiation of epithelial cells into myofibroblasts, while the downregulation of MRTF-A activity by its pharmacological inhibitor abrogates cell proliferation and migration in prostate cancer cells. In addition, the signaling mechanisms underlying the novel function of MRTF-A involve the activation of ERK. Treatment with a combination of MRTF-A inhibitor and ERK inhibitor synergistically abrogates prostate cancer cell proliferation and migration compared with treatment of either drug alone *in vitro*. Using an *in vivo* prostate cancer xenograft model, we have demonstrated that the MRTF-A inhibitor significantly suppresses tumor growth, while concurrent administration of the ERK inhibitor enhances the antitumor activity of the MRTF-A inhibitor. Our data reveal a previously unidentified role of MRTF-A in prostate cancer development and support the potential combined therapeutic targeting of MRTF-A and ERK in human prostate cancer.

HUR TARGETED THERAPY INDUCES THE IMMUNE-SUPPRESSOR PD-L1 (B7-H1): AN ESCAPE MECHANISM IN LUNG CANCER

Ranganayaki Muralidharan[1,3], Akhil Srivastava[1,3], Anupama Munshi[2,3], Rajagopal Ramesh[1,3,4]

Department of [1]Pathology, and [2]Radiation Oncology; [3]Stephenson Cancer Center, and [4]Graduate Program in Biomedical Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK

HuR is an mRNA-binding protein that post-transcriptionally regulates the expression and stability of several oncoproteins. We recently demonstrated tumor-targeted HuR siRNA nanoparticle (NP) delivery significantly suppressed lung tumor growth both *in vitro* and *in vivo*. While our study results were exciting, we serendipitously identified siHuR-NP therapy modulates PD-L1 expression in lung cancer cells.

Expression of Programmed Death Ligand 1 (PD-L1; B7-H1, CD274) was detected in human lung cancer cell lines. siRNA-NP treatment increased PD-L1 expression in H1299, A549, HCC827, and H1975 cell lines. Increase in PD-L1 was confirmed by western blotting, and flow-cytometry. Cell fractionation studies showed PD-L1 expression increased on cell membrane. Molecular studies showed HuR transcriptionally regulated PD-L1. Overexpression of HuR reduced endogenous PD-L1 expression in A549 and HCC827 cell lines indicating an inverse correlation between HuR and PD-L1. Immunohistochemical staining of human lung tissue microarray (TMA) for both HuR and PD-L1 showed a strong inverse correlation and concurred with the *in vitro* cell line study results.

To understand the functional importance of PD-L1 upregulation by HuR-NP treatment, we co-cultured activated T-(Jurkat) cells with tumor cells and measured PD-L1 expression on tumor cells. Co-culturing of tumor cells with Jurkat cells resulted in increased PD-L1 expression on tumor cell surface while increased apoptosis in T-cells. Interestingly, co-culturing of HuR-NP-treated tumor cells with T-cells resulted in reduction in PD-L1 expression in tumor cells and increase in T-cell survival. Analysis for interleukin (IL)-2 showed a marked increase in the supernatant collected from HuR-NP-treated tumor cells suggesting that the increase in T-cell survival observed in co-culture studies is likely supported by the IL-2. Our data shows HuR-NP-mediated upregulation of PD-L1 could attenuate the efficacy of HuR-based therapy. In conclusion, a better understanding of the HuR/PD-L1 interaction will provide a rationale for combining HuR-NP with PD-L1 inhibitors for improving therapeutic outcomes.

Acknowledgments. This work was supported in part by grants received from the National Institutes of Health R01 CA167516, and by funds received from the Presbyterian Health Foundation Seed Grant, Presbyterian Health Foundation Bridge Grant, Stephenson Cancer Center Seed Grant, and Jim and Christy Everest Endowed Chair in Cancer Developmental Therapeutics, The University of Oklahoma Health Sciences Center.

MASS SPECTROMETRY DETECTION OF CHEMOTHERAPY DRUGS IN SINGLE BLADDER CANCER CELLS IN PATIENTS

Shawna Standke*, Ning Pan*, Naga Rama Kothapalli*, Ryan Bensen*, Jonathan E. Heinlen‡, Zhibo Yang*,§,¶ and Anthony W.G. Burgett*,§,¶

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Abstract: The development of precision cancer medicine will require the capability to administer drug treatments in an individually tailored manner that will maximize the benefit to the patient. Currently in the clinical treatment of cancer, there is a lack of bioanalytical methodology capable of providing information of the effectiveness of chemotherapy treatment on a real time, day-to-day basis. Cancer as a disease state is increasingly understood as a process defined and propagated at the single cancer cell biology level. A major unmet bioanalytical need at the interface of precision medicine and single cell analysis will be the capability to monitor the dosing and effectiveness of patient-administered chemotherapeutic therapies on the single cancer cell level. Currently, there are no clinical bioanalytical methods capable of determining the concentration of chemotherapeutic agents inside of a patient's individual cancer cells, and such a method would be a powerful tool in establishing ideal dosing regiments that deliver effective chemotherapeutic concentrations with minimal toxicities. We have developed a novel first-in-class mass spectrometry (MS) technology—the Single-probe—capable of performing single cell mass spectrometry (SCMS) of compounds inside of living single cancer cells under ambient cell culture conditions. In our published results, we have used this device to detect the presence of anti-cancer compounds inside of singe cancer cells cultured and dosed *in vitro,* with a sampling time of ~3 minutes per cell with no sample preparation required. The Single-probe is ideally suited for the rapid SCMS analysis of cancer cells, including patient isolated cancer cells. In our current NCI funded research project, we are developing the capability of quantitating the amount of standard-of-care chemotherapy drugs in bladder cancer cells, including in both laboratory cultured cell lines and bladder cancer cells isolated from patient urine samples. Our SCMS method also allows compound pharmacology, including the kinetics of cellular uptake and clearance, to be measured. Additionally, our SCMS analysis allows for lipidomic/metabolomic profiling of cancer cells, including cancer cells isolated from patients. Our overall research progress provides a foundation for potential deployment of novel single cell mass spectrometry analytical methods to personalize and guide chemotherapy drug administration in the clinical treatment of cancer.

Funding provided by NCI IMAT R21 Grant (CA204706)

A DIFFUSE OPTICAL SPECTROSCOPY IMAGING TECHNOLOGY THAT IS POTENTIALLY COMPATIBLE WITH MINIMALLY-INVASIVE-PROCEDURES FOR INTRAOPERATIVE IDENTIFICATION OF ANATOMIC STRUCTURES AND ASSESSMENT OF PARENCHYMAL PATHOLOGY

Daqing Piao,[1,2,*] Erin Rubin,[3] Halen Borron,[4] Alan Hawxby,[5] Harlan Wright, 5 Mohammad Ramadan,[6,7] and Sanjay Patel[6]

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[2] Department of Veterinary Clinical Sciences, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK
[3] Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK
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Abstract (2600 characters, not including spaces)

Minimally invasive surgery (MIS) procedures such as robotic-assisted partial nephrectomy requires intraoperative identification of anatomical structures including major blood vessels and ureters, as well as continuous monitoring of organ conditions such as ischemia. Intra-operative decision-making during MIS procedures on organs such as the liver could be enhanced by real-time assessment of the parenchyma pathology.

Although there is not currently a methodology to easily assess anatomic structures, monitor organ viability, or image tissue histopathology, we demonstrate a simple technology of diffuse optical spectroscopy imaging (DOSi) for intraoperative use. DOSi is capable of identifying vascular structures and potentially other major anatomic heterogeneity several millimeters below the overlying tissue surfaces. Using a pig model *in vivo* (OUHSC ACUP #101489-16-017-NS), seven operative procedures were performed on four pigs by two surgeons independently. A probe operable in either a drop-in fashion or by affixing to an 8mm laparoscopic instrument was used. Consecutive dynamic DOS spectra images were acquired with only standard room lighting during freehand movement of the probe. When the probe was placed on peritoneal fat up to a thickness of 4mm, the vena cava and aorta were clearly identified when imaged. In addition to intraoperative identification of underlying anatomic structures, this technology is able to assess ischemic conditions. DOS spectra images acquired from one kidney before and after vessel clamping differentiated between normoxic and hypoxic renal parenchyma.

DOSi assessment of parenchymal pathology was demonstrated *ex vivo* on 12 livers (OUHSC IRB #8155). A portable lab-on-a-crater DOSi device has been developed for DOSi measurements on the capsular surface of livers rejected from transplantation. These images of optical spectroscopic patterns were compared to the gold standard of formalin-fixed paraffin embedded (FFPE) H&E tissue sections. A global DOSi pattern corresponding to increased tissue scattering indicates fibrosis. A quantitative indexing method assessing the global elevation of tissue scattering has separated 3 livers with fibrosis stage greater than 2 (Ishak scale) from 9 livers with fibrosis stage 1 or lesser. A local DOSi pattern in the vicinity of 900nm is projected to specify pathologic hepatocyte ballooning that was not previously assessable by surface measurements. Most notable was a case of carbon monoxide inhalation. Unremarkable on histopathological examination, DOSi measurements showed patterns false positive for fibrosis and other pathology, suggesting the spectral changes caused by carboxyhemoglobin formation.

In conclusion, DOSi assessment is capable of use during intraoperative MIS procedures to identify anatomic structures, to monitor ischemic conditions, and to assess parenchymal pathology.

Funding: OSU Technology Development Center and OUHSC Summer Pathology Research Fellowship (Halen Barron).

ANALYSIS OF NANOPARTICLE DELIVERY TO SOLID TUMORS

Stefan Wilhelm, Stephenson School of Biomedical Engineering
Anthony Tavares, IBBME, University of Toronto
Qin Dai, IBBME, University of Toronto
Seiichi Ohta, Center for Disease Biology and Integrative Medicine, The University of Tokyo
Julie Audet, IBBME, University of Toronto
Harold Dvorak, Center for Vascular Biology Research, Department of Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School
Warren Chan, University of Toronto

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The concept of "targeting" nanoparticles into tumors for improved diagnosis and therapy of cancer is attractive but challenging. The premise of this concept is to specifically deliver imaging and/or therapeutic nanoparticles to the target (*i.e.*, the tumor) while minimizing off-target accumulation.

Recently, we quantified the nanoparticle tumor delivery efficiency, *i.e.*, the number of systemically administered nanoparticles that accumulate in the target tumor tissue by surveying manuscripts published from 2005 to 2015 [1]. Surprisingly, we found that only 0.7% of the injected nanoparticle dose actually interacts with solid tumors (median value derived from 232 data sets) in preclinical studies. Our results suggest that only 7 out of 1,000 systemically administered nanoparticles reach the targeted tumor tissue.

This presentation will explore the potential causes of the poor delivery efficiency from the perspective of the tumor biology and organs that compete for administered nanoparticles, followed by a discussion of the impact on the clinical translation of nanomedicines. The lack of translational progress is currently impeded by the inability to control the nanoparticle transport inside the body due to the complexity of these biological systems.

Reference:

[1] S. Wilhelm, A.J. Tavares, Q. Dai, S. Ohta, J. Audet, H.F. Dvorak, W.C.W. Chan; Analysis of nanoparticle delivery to tumours; Nature Reviews Materials; 1; 16014; 2016.

OKN-007: A MULTI-PURPOSE AGENT FOR THE TREATMENT OF BRAIN TUMORS AND POSSIBLY MORE

Rheal A. Towner, Nataliya Smith, Debra Saunders
Advanced Magnetic Resonance Center, Oklahoma Medical Research Foundation

OKN-007 (OKlahoma Nitrone 007) is currently a new investigational drug in phase II clinical trial for recurrent glioblastoma (GBM), which has demonstrated an increase in overall survival, compared to current standard-of-care therapies (surgical resection, radiation- and chemo-therapies, and anti-VEGF antibody therapy). Recent *in vitro* and *in vivo* preclinical studies were carried out to establish if OKN-007 can be effective against TMZ (temozolomide)-resistant glioma tumor cells. TMZ is currently used as the SOC chemotherapeutic agent for newly-diagnosed GBM patients.

OKN-007 was found to significantly decrease IC50 (drug concentration causing 50% inhibition of the desired activity) values in both TMZ-resistant (T98G (>2-fold), G55 (<20-fold)) and TMZ-sensitive (U251 (>10-fold)) human glioma cells, as well as significantly increase animal survival ($p<0.01$) and significantly decrease tumor volumes ($p<0.0001$) in a human G55 xenograft nude mouse model, compared to untreated glioma-bearing controls. TMZ-resistance is one of the major reasons for GBM recurrence. OKN-007 may be able to reduce the TMZ-resistant glioma cell population when combined with current SOC therapies. It was also recently discovered by our group that OKN-007 has the ability to temporarily open up the blood-brain barrier (BBB) for a 3 h window. OKN-007 allowed both small (~500) and large (~400 kDa) molecular weight (MW) agents into normal rat brains. The small MW agent tested was a MRI contrast agent, Gd-DTPA (546 MW), which normally can't cross the BBB. Large MW molecules included the enzyme, β-galactosidase (465 kDa), and an antibody (150 kDa) against the neuronal marker, EphB2, which were both tagged with the MRI contrast agent, Gd-DOTA, for *in vivo* MRI visualization of uptake into the brain. For the EphB2 probe, *ex vivo* fluorescence imaging also confirmed uptake into brain tissue. OKN-007 can therefore be used to augment the delivery of therapeutic agents that normally can't cross the BBB, such as anti-Her2nue antibody treatment for HER2+ breast cancer cells that metastasize to the brain, or for various neurological diseases that have had limited therapeutic options.

Funding was obtained by the Oklahoma Medical Research Foundation, Oblato, Inc., and the Presbyterian Health Foundation.

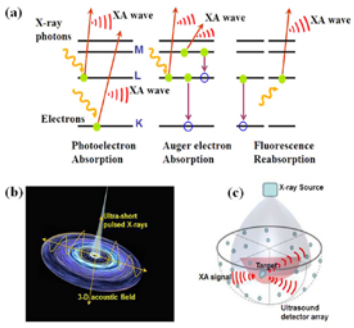
THERANOSTIC WITH RADIATION–INDUCED ULTRASOUND EMISSION (TRUE)

Liangzhong Xiang

My laboratory mainly focuses on the development of ultrasound imaging techniques with new imaging contrast mechanisms including X-ray-induced acoustic computed tomography (XACT), nanoscale photoacoustic tomography (nPAT), and other radiation induced acoustic emission for cancer diagnosis and therapy monitoring. We expect those new imaging techniques to find widespread applications in both pre-clinical and clinical biomedical research.

I. X-RAY-INDUCED ACOUSTIC COMPUTED TOMOGRAPHY (XACT)

Laser-induced photoacoustic tomography (PAT) overcomes the high degree of scattering of optical photons in biological tissue by making use of the photoacoustic effect[1]. PAT can create multiscale multicontrast images of living biological structures ranging from organelles to organs[2]. However, optical attenuation limits the penetration of PAT to ~5–7 cm in tissue when <1 mm resolution is desired. To break through the imaging depth limit, we extend the excitation source from visible light to X-rays, to develop a new imaging modality called x-ray-induced acoustic computed tomography (XACT)[3, 4]. The X-ray-induced effect process is intrinsically three-dimensional (3D), as the XA waves are spherical in nature and propagate in all directions from their point of generation (Fig. 1b). The use of XA signals for volumetric imaging is uniquely advantageous: a single projection X-ray exposure is sufficient to generate acoustic signals in 3D space (Fig. 1c)[5]. Both X-rays and ultrasound can penetrate deep in tissue. The super-depth of the XACT imaging enables the visualization of deep-seated micro-calcifications in breast imaging that are beyond the limitation of emerging non-ionizing imaging strategies. XACT imaging has great potential for breast cancer screening, bone density measurement, and radiation therapy dosimetry.



II. NANOSCALE PHOTOACOUSTIC TOMOGRAPHY (NPAT)

PAT imaging resolution currently is limited by the optical diffraction to approximately 220 nm. We are developing a super-resolution PAT to push the imaging resolution down to 20 nanometers. An ultra-short (<ps) pulsed laser is used for the generation of GHz ultrasound waves[6]. The ultrahigh frequency acoustic waves are detected by a pump-probe technique to achieve nanoscale tomographic imaging. The advances of the nPAT imaging technique not only will benefit the biomedical imaging sciences, but also fundamental understanding in biological processes (hemoglobin in red blood cells, cytochromes in mitochondria, and DNA/RNA within the cell nucleus) and medicine (pharmaceutical molecule).

Figure 1. (a) Schematic of the main processes which contribute to XA signals. (b) Schematic of the 3D acoustic field generated by ultrashort-pulsed X-rays. (c) Schematic diagrams of 3D XACT.

Acknowledgment

The authors gratefully acknowledge the University of Oklahoma Research Council, College of Engineering, and IBEST-OUHSC seed grant, PHF grant for funding. The authors would like to thank my lab memebers Shanshan Tang, Ali Zarafshani, Jian Chen, Pratik Samant, Rowzat Faiz, Siqi Wang, and the undergraduate research assistants for their contributions in the TRUE lab.

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NOVEL DRUGS FOR CANCER TREATMENT – A JOURNEY FROM THE SEA

Natarajan Aravindan, PhD

Nature has been instrumental as a source for therapeutics with more than 50% of drugs in clinical use. In particular, the upsurge of marine derived therapeutics for cancer cure and beyond is evident with many drugs in clinical use and, many more in the clinical trials. Seaweeds are known to harbor large amounts of polyphenols that possess anticancer properties and, are linear to its anti-oxidant activity. We characterized a panel of polarity-gradient polyphenols from an array of seaweeds and investigated their anti-tumor potential. Weightage-factor analysis across all fractions, systems and endpoints identified three superlative drug candidates.

With genetically diverse *in vitro* systems and preclinical mouse model of residual disease we defined the adjuvant benefit of such candidates in mitigating the acquisition of molecular rearrangements that governs tumor relapse/recurrence viz., adaptive survival, invasive phenotype and stemness maintenance after therapy. Lead agents targeted the acquired activation of autophagy machinery, and signify their efficacy in the mitigation of autophagy driven residual cell survival. In addition to the inflicted attenuation of the acquired invasion/metastasis transcriptional-translational rearrangements in residual tumors, the marine drugs selectively targeted the functional drivers that determine the dissemination destiny of surviving tumor cells.

The drug deliverables exhibited a clone-independent inhibition of cancer stem cell signaling events and annulled stemness maintenance in residual tumor cells. This study identified novel drug candidates that could target acquired molecular rearrangements that drive clonal selection, survival, dissemination in residual disease after current standard of care and, thereby could mitigate tumor relapse/recurrence.

EXOSOME MICRORNAS IN CANCER: BIOLOGY AND POTENTIAL APPLICATIONS

Wei-Qun Ding, PhD
Department of Pathology, University of Oklahoma Health Sciences Center

Exosomes are endosome-derived membrane vesicles that are secreted from different cell types and present in all biological fluids. These membrane vesicles contain proteins, lipids, and nucleotides that mediate intercellular communication. Previous studies have shown that cancer cells secrete exosomes that are constantly released into the tumor microenvironment. Importantly, exosome microRNA signaling has been demonstrated to promote tumor progression in various cancer model systems. However, exosome microRNA signaling events in cancer and their potential applications for cancer management remain poorly understood. In pursuing this line of research, we have recently discovered that exosomes selectively encapsulate and enrich microRNAs in human cancer cells.

These selectively enriched exosome microRNAs are released and transferred to recipient cells and the circulation. The selective exosome microRNA encapsulation and enrichment in cancer cells seemed to be related to specific microRNA sequence motifs and certain RNA binding proteins. Moreover, the highly enriched exosome microRNAs were significantly elevated in plasma exosome preparations from patient-derived tumor explants or human cancer cell line xenograft nude mouse models, indicating their potential to serve as biomarkers for cancer diagnosis or prognosis. In two human subject pilot studies, we have demonstrated that the highly enriched exosome microRNAs in the plasma are indicative of breast cancer and early stage pancreatic cancer, strongly supporting the development of exosome microRNAs as non-invasive biomarkers for these malignancies.

CIRCULATING EXTRACELLULAR VESICLES TARGET T CELLS IN B–CELL CHRONIC LYMPHOCYTIC LEUKEMIA

Hasan Mahmud, Guru P. Maiti, Bedabrata Mukhopadhyay and Asish K. Ghosh
Stephenson Cancer Center, OUHSC

B-cell chronic lymphocytic leukemia (CLL) is the most common mature B-cell neoplasm in Western countries. It is characterized by the accumulation of monoclonal CD5+/CD19+ mature B-cells in the peripheral blood, lymphoid system and bone marrow. While initial therapies of CLL are effective in most, essentially all patients relapse where options available are limited. There are also evidences on the possible role of T cell dysregulation in the pathogenesis and development of CLL. The immune deficiency seen in CLL is wide-ranging, resulting in increased susceptibility to bacterial, viral and fungal infections and failure to mount an effective antitumor immune response. Increase of circulating T cells in CLL which was primarily accounted for by an increased number of CD8+ T cells, resulting in fall in the CD4:CD8 ratio. These T cells show a variety of phenotypic and functional abnormalities. In addition, expansion of CD4+/CD25+ regulatory T cells (Tregs) also may contribute to the immune defect in CLL which have been shown to correlate with decreased T cell responses against viral and tumor antigens. We now know that CLL plasma contain elevated levels of extracellular vesicles including microvesicles (MVs) and exosomes (Exos). These EVs carry cargo of bioactive molecules including various kinases, phosphatases and microRNAs from the originator cells and are able to modulate target cells' functions. We hypothesize that EVs generated by malignant B-cells target T cell function thus, mounting a defective T cell immune response in CLL. To address this, we purified MVs/Exos from various sources including CLL plasma, purified CLL B-cells and megakaryocytic cells (Meg-01).

Our results suggest that both MVs and Exos from all the sources are able to integrate into normal, primary T cells in a time-dependent manner. Interestingly, MVs/Exos obtained from majority of CLL patients showed inhibition of the T cell receptor (TCR) signaling in normal T cells in vitro, albeit at variable degrees. To further validate that the inhibition of TCR signal in normal T cells was due to increased accumulation of CLL B-cell-derived EVs in CLL plasma, we examined the impact of EVs generated in vitro from purified, primary CLL B-cells or megakaryocytes/platelets (Meg- 01 cells) on TCR activation. Indeed, while the CLL B-cell derived EVs showed inhibition of TCR activation in normal T cells in vitro, Meg-01-derived EVs did not exhibit any significant effect on TCR signaling.

Further analysis suggests that purified CLL B-cell derived Exos preferentially bind CD4+ T cells as compared to MVs and MVs/Exos from CLL plasma or megakaryocytes. Together, these results suggest that CLL B-cell derived circulating EVs may interrupt T cell immune response by inhibiting TCR signal preferentially in CD4+ T cells. Further studies are underway to dissect the mechanism of EV-mediated TCR signal inhibition both in vitro and in vivo.

Funding sources: NIH CA170006; CLL Global Research Foundation

IMMUNE RESPONSE TO COLORECTAL CANCER DIFFERS BY SEX

Katherine Morris, MD
Department of Surgery, OUHSC

Women have better overall survival from both early-stage and metastatic colorectal cancer. The reason for the differences in outcome is not known. However, immune signaling and cell activity is crucial to development and progression of colorectal cancer. Sex-based differences in the peritoneal immune response to stressors such as sepsis have been described.

These differences suggest that the immune environment may differ as colorectal cancer advances, and help explain observed clinical outcomes disparities. We will discuss recent *in vitro* and *in vivo* data that suggest an immunologic mechanism underlying the survival benefit noted in women with colorectal cancer.

CANCER PREVENTION & CONTROL SESSIONS

CANCER PREVENTION & CONTROL — BASEMENT, ROOM B

9:15 – 10:15 AM

SESSION I

ENHANCING AMERICAN INDIAN CANCER DATA IN THE SOUTHERN PLAINS AREA

Moderators: Mark Doescher, MD

PANEL DISCUSSION

Tracy Prather, Southern Plains Tribal Health Board, OK Area Tribal Epidemiology Center Travis Watts, OKC Area Indian Health Services
Derek Pate or Raffaella Espinoza, OK State Dept. of Health
Sohail Khan, Cherokee Nation
Mark Doescher, Stephenson Cancer Center Amanda Janitz, OUHSC College of Public Health
Michael Peercy, Chickasaw Nation

10:15 – 11:15 AM

SESSION II

OPEN DISCUSSION & NETWORKING

Moderators: Paul Spicer, PhD

1:15 – 2:15 PM

SESSION II

TOBACCO RESEARCH: NOVEL BEHAVIORAL & RISK ASSESSMENT APPROACHES

Moderators: Michael Businelle, PhD

1:15 PM– 1:35 PM

PILOT RANDOMIZED CLINICAL TRIAL OF AN AUTOMATED SMARTPHONE BASED SMOKING CESSATION TREATMENT

Emily Hébert, DrPH
Oklahoma Tobacco Research Center
The University of Oklahoma Health Sciences Center

1:35 – 1:55 PM

PREDICTING RISK OF IMMINENT SMOKING LAPSE IN REAL TIME USING MA–CHINE LEARNING

Dwayne Geller
Oklahoma Tobacco Research Center
The University of Oklahoma Health Sciences Center

1:55 – 2:15 PM

ELECTRONIC CIGARETTE AEROSOLS INDUCE DNA DAMAGE AND DECREASE DNA REPAIR

Lurdes Queimado, MD, PhD
Department of Otorhinolaryngology
The University of Oklahoma Health Sciences Center

CANCER PREVENTION & CONTROL — BASEMENT, ROOM B

2:15 – 3:15 PM

SESSION IV
TOBACCO PREVENTION & CHEMOPREVENTION

Moderators: CV Rao

2:15 – 2:20 PM

INTRODUCTION OF CHEMOPREVENTION AND EARLY DETECTION METHODS

C.V. Rao, PhD
Department of Hematology Oncology
The University of Oklahoma Health Sciences Center

2:20 – 2:45 PM

CHEMOPREVENTION OF PANCREATIC CANCER: PRECLINICAL PROGRESS AND CHALLENGES

Venkateshwar Madka, PhD
Department of Medicine
The University of Oklahoma Health Sciences Center

2:45 – 3:10 PM

SINGLE CELL METABOLOMICS USING MASS SPECTROMETRY: UNDERSTANDING THE NEXUS BETWEEN CELL–TO–CELL HETEROGENEITY AND METABOLIC PHENOTYPES OF LIVE CANCER CELLS

Renmeng Liu
Department of Chemistry and Biochemistry
The University of Oklahoma

3:30 – 4:30 PM

SESSION V
CHEMOPREVENTION

Moderators: CV Rao, PhD

3:30 – 4:00 PM

PRO–CARCINOGENIC FIELD EFFECTS CAUSED BY GENOMIC INSTABILITY IN CO–LON, LUNG, AND LIVER

Hiroshi Yamada, PhD
Department of Hematology Oncology
The University of Oklahoma Health Sciences Center

4:00 – 4:25 PM

DECITABINE REINSTATE RADIOTHERPY–INDUCED HYPERMETHLATION ME–DIATED RD3 TRANSCRIPTIONAL REPRESSION AND INHIBITS SURVIVAL AND STEMNESS OF RESISTANT NEUROBLASTOMA CELLS

Dinesh Babu Somasundaram, PhD
Department of Radiation Oncology
The University of Oklahoma Health Sciences Center

4:25 – 4:30 PM

CONCLUDING REMARKS

C.V. Rao, PhD
Department of Hematology Oncology
The University of Oklahoma Health Sciences Center

PILOT RANDOMIZED CLINICAL TRIAL OF AN AUTOMATED SMARTPHONE BASED SMOKING CESSATION TREATMENT

Emily T. Hébert, DrPH; Darla E. Kendzor, PhD; Angela Helt, MA; Rachel Moisiuc, BS; Damon J. Vidrine, DrPH; & Michael S. Businelle, PhD

Oklahoma Tobacco Research Center, Stephenson Cancer Center University of Oklahoma Health Sciences Center

Contact Email: emily-hebert@ouhsc.edu

SIGNIFICANCE: Smoking among those in poverty is twice as high as those above the poverty threshold (26.1% vs. 13.9% smoke). Aided and unaided cessation attempts are less likely to be successful among lower socioeconomic status (SES) adults, due to a variety of psychosocial and contextual factors. Thus, smoking is becoming increasingly concentrated among individuals with the lowest levels of income, education, and occupational status. Highly flexible and low burden technology-based treatment approaches may overcome many of the barriers (e.g., transportation, time constraints) that have limited the use and effectiveness of traditional smoking cessation treatments among low SES adults. Research has indicated that momentary changes in key variables can be tracked using ecological momentary assessments (EMA), and used to initiate interventions as they are needed.

METHODS: The current pilot study is a 3-armed randomized clinical trial that aims to determine the initial utility of an automated smartphone based smoking cessation intervention (i.e., Smart-T2) compared with standard in-person smoking cessation clinic care and the free NCI QuitGuide smoking cessation app. Smokers who attend a clinic based tobacco cessation program are randomized to groups and followed for 13 weeks (1 week pre-quit through 12 weeks post-quit). All participants are asked to complete EMAs on study provided smartphones for 5 weeks.

RESULTS: Study recruitment is ongoing. To date, participants (N=24) are mostly male (62.5%), white (70.8%), and report smoking an average of 25 cigarettes per day at baseline. Participants have completed 77% of all EMAs, with no significant differences in compliance across groups. Within the Smart-T2 treatment group, a majority of participants feel that the app provides treatment personalized to their needs (87.5%) and will help them quit smoking (75%). Smart-T2 app design considerations (e.g., which variables to intervene upon and when, proximal and distal outcome variables, on demand and prompted intervention content, compensation for completing EMAs, collection of geolocation data, IRB and ethics considerations) and app features (e.g., on demand tips for coping with lapse triggers, messages that are tailored in real-time to address level of lapse risk and present lapse triggers, medication refill request feature) will be discussed.

CONCLUSIONS: Dynamic smartphone apps that tailor intervention content in real-time may reduce smoking in disparities populations.

FUNDING: This work is supported by the University of Oklahoma Health Sciences Center and Stephenson Cancer Center.

ACKNOWLEDGEMENTS: This study utilized the SCC mHealth Shared Resource.

PREDICTING RISK OF IMMINENT SMOKING LAPSE IN REAL TIME USING MACHINE LEARNING

Dwayne Geller, BS, Emily T. Hébert, DrPH, Darla E. Kendzor, PhD, Michael S. Businelle, PhD

Oklahoma Tobacco Research Center, Stephenson Cancer Center, University of Oklahoma Health Sciences Center

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Significance: Smoking lapse is influenced by momentary triggers such as mood and environmental context. Machine learning uses algorithms to automatically learn and make predictions from large datasets, making it suitable for predicting dynamic behaviors. The purpose of this study was to apply machine learning classification methods to the prediction of smoking lapse.

Methods: Participants were socioeconomically disadvantaged adults participating in a clinic-based smoking cessation program. Participants were loaned smartphones and prompted to complete 5 ecological momentary assessments (EMA) each day from 1 week pre-quit to 4 weeks post-quit, and to self-initiate an EMA whenever they lapsed. EMAs evaluated mood, smoking urge, environmental context, and smoking lapses (i.e., smoking after 24 hours of abstinence). Baseline demographics and all EMA variables reported within 4 hours preceding a lapse were included as potential predictors. Time since last cigarette and last quit attempt, number of prior lapses, and deviations from an individual's average mood (e.g. happiness, sadness, anxiety) over the past 2-days were included in the model. XGBoost, a machine learning library that uses boosted tree ensembles combined with a stratified k-fold cross-validation, was used to identify a lapse prediction model. Receiver operating characteristic (ROC) curve analysis was used to evaluate model performance.

Results: Participants (N=31) were on average 48 years old, female (77.4%), white (58.1%), and earned less than $10,000 a year (57.7%). The variables found to be most predictive of imminent smoking lapse (i.e., lapse within the next 4 hours) were related to prior lapse (time since initiation of last quit attempt, time since last cigarette, and prior number of lapses). However, changes in anxiety, sadness, and confidence that smoking would improve mood were also strongly predictive of lapse. The algorithm had a sensitivity of 82.95%, a specificity of 89.04%, and an area under the ROC curve of 0.86.

Conclusions: Accurate prediction of smoking lapse using machine learning methods and EMA data can significantly improve just-in-time interventions for smoking cessation.

Funding: Research reported in this grant is supported by grant number R01CA197314 from the National Cancer Institute.

ELECTRONIC CIGARETTE AEROSOLS INDUCE DNA DAMAGE AND DECREASE DNA REPAIR

Lurdes Queimado[1-5*], Jimmy Manyanga[1,2], Lacy Brame[1] , D McGuire[7,] David Rubenstein[6], Balaji Sadhasivam[1], Daniel Brobst[1], David Rubenstein[6], Evan Floyd[7], Theodore Wagener[5,6,7], Ilangovan Ramachandran[1], and Vengatesh Ganapathy[1]

Departments of [1]Otorhinolaryngology, [2]Cell Biology, and [3]Pediatrics; [4]The Oklahoma Tobacco Research Center and [5]The Peggy and Charles Stephenson Cancer Center, The University of Oklahoma Health Sciences Center, Oklahoma. [6]Department of Biomedical Engineering, Stony Brook University, New York.

Significance: E-cigarettes (ECs) are battery-operated devices that deliver nicotine through inhaled aerosols. The use of ECs has increased sharply since 2003, reaching more than 13% of high school students and 10 % of adults in U.S. Limited data suggest that EC is a less harmful alternative to tobacco cigarettes and a promising smoking cessation aid. Nonetheless, EC aerosols contain unique toxicants, as well as carcinogens and reactive oxygen species (ROS) that are also present in tobacco smoke. Moreover, we have previously reported that EC aerosols can cause DNA damage. Here we aim to determine the sensitivity of diverse methods to detect DNA damage induced by EC aerosols and to investigate the mechanisms by which EC use can contribute to DNA damage.

Methods: Extracts were prepared from two distinct EC brands and a reference combustible cigarette. Nicotine was quantified by gas chromatography mass spectroscopy. Cells were exposed for 10 min, 1 h, or 2 weeks to diverse doses of EC aerosol or tobacco smoke extracts. DNA damage was quantified using the primer anchored DNA damage detection assay (q-PADDA), the comet assay, and an ELISA kit that detects only 8-oxo-7,8-dihydroguanine (8-oxoG). Levels of ROS and total antioxidant capacity (TAC) were evaluated using standard kits. mRNA and protein expression were evaluated by RT-PCR and western blot, respectively. Data were analyzed by Student's *t*-test.

Results: Exposure to EC aerosol caused a significant increase in ROS and in DNA damage detectable by q-PADDA and comet assays. We also observed a significant increase in 8-oxoG, one of the most mutagenic DNA lesions caused by ROS. Exposure to EC aerosol caused a dose-dependent increase in DNA damage detectable only by q-PADDA. Exposure to EC extracts reduced the cellular antioxidant capacity and the expression of proteins essential for DNA damage repair.

Conclusion: EC aerosols can cause significant DNA damage, including lesions that are highly mutagenic, possibly by decreasing the cellular antioxidant and DNA damage repair capability. Our data emphasize the urgent need to further evaluate EC safety to ensure evidence-based public health policies and regulations.

Grant support: Grant support: This work was supported by the Oklahoma Tobacco Research Center (LQ), the Presbyterian Health Foundation (LQ), and by the Oklahoma Center for the Advancement of Science & Technology (LQ). Dr. Queimado holds a Presbyterian Health Foundation Endowed Chair in Otorhinolaryngology.

CHEMOPREVENTION OF PANCREATIC CANCER: PRECLINICAL PROGRESS AND CHALLENGES

Venkateshwar Madka[1], Altaf Mohammed[3], Gaurav Kumar[1], Gopal Pathuri[1], Hari Prasad Gali[2], and Chinthalapally V. Rao[1]

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Pancreatic ductal adenocarcinoma (PDAC) is a fatal malignancy with an overall 5-year survival of 8% for all stages combined. In the US, nearly 53,000 new cases and 43,000 deaths are estimated due PDAC for the year 2017. Unfortunately majority of these patients present with stage IV disease at diagnosis. In spite of aggressive PC research, for the past three decades, there has been limited improvement in the five-year survival for this cancer. Data indicates that the deaths caused by this malignant disease are also rising in parallel with incidence of PDAC largely due to limitations ranging from early detection to effective treatment options. Given the challenges and the rising incidence of PDAC expected to become the second leading cause of cancer-related death by 2030, there is a major unmet need to develop reliable early detection biomarkers and effective interventions, therefore prevention appears to be desirable option.

Our current understanding suggests that, like many other cancers, PDAC also takes several years for normal pancreatic cells to transform into pancreatic precursor lesions and to further progress into invasive carcinoma. Therefore identify and intervening with the factors driving this transformation can significantly reduce the incidences and mortalities associated with this disease. Cancer chemoprevention is a strategy taken to retard, regress, or resist the multistep process of carcinogenesis, including the blockage of its vital morphogenetic milestones viz. normal-preneoplasia-neoplasia-metastasis.

Availability of various genetically engineered mouse (GEM) models of PC has led to accelerated progress in understanding the disease and developing intervention strategies otherwise stalled for a long time. These GEM models develop PDAC spontaneously (p48-$KRAS^{G12D}$; PDX^{cre}-$SMAD4^{-}KRAS^{G12D}$) or tamoxifen inducible ($Ptf1^{cre}$-TP53-$KRAS^{G12D}$; Ela^{Cre}-$KRAS^{G12V}$) in-a-stepwise manner and mimic the disease etiology in humans. Understanding PC development from initiation to progression to metastasis is very important for early detection and prevention of PC. Using some of these GEM models we have identified i) promising mouse model for chemoprevention studies, and ii) potential agents for prevention of PDAC iii) developing agents for early detection of PDAC. Our data suggests the chronic dietary administration of these agents can significantly suppress tumor growth, as well as progression from preneoplastic lesions (PanINs) to carcinoma. Also, we have identified some models representative of IPMNs and MCNs that will be summarized in our presentation.

SINGLE CELL METABOLOMICS USING MASS SPECTROMETRY: UNDERSTANDING THE NEXUS BETWEEN CELL–TO–CELL HETEROGENEITY AND METABOLIC PHENOTYPES OF LIVE CANCER CELLS

Presented by: Renmeng Liu; Renmeng Liu[1], Genwei Zhang[1], Mei Sun[1], Zhibo Yang[1*]

[1]Department of Chemistry and Biochemistry, University of Oklahoma, Norman, OK;
[*]corresponding author

Cancer has been recognized as a major threat to human health for decades and the treatment of cancer is a research field of broad interest which involves modern instrumentation and methodologies to control tumor progression, promote long-term survival rate, and ultimately achieve complete remission. With such aims, there have been numerous methods and techniques developed that focus on the mechanism of malignancy, prognosis at early stage, and therapeutic development on a variety types of cancers. Those methods and techniques can be roughly classified as genetic, proteomic, and metabolomic approaches. Among those, metabolomic profiling of cancer cells provides a direct readout of biological and physiological status of cells, which facilitates further analysis of cellular metabolism under native or stressed microenvironment. Recently, mass spectrometry (MS) based metabolomics gains growing popularity and it has been applied to interrogate cellular contents, investigate drug-induced phenotypes, and interpret cell metabolism both *in vitro* and *in vivo*.

However, cell-to-cell heterogeneity exists within a tumor tissue, which is masked and muted by conventional MS analysis of cell lysate prepared from populations of cells, resulting in inadequate understanding of the cellular metabolism of single cells. In our current work, we utilize the single cell MS (SCMS) experiment combined with statistical data analysis to study cellular metabolism of single cells and further reveal the relationship between cell-to-cell heterogeneity and metabolic phenotypes induced by anticancer drugs. Human cervical cancer cell line, HeLa, treated by mitotic inhibitors, taxol and vinblastine, was selected as a model system to demonstrate our sampling and SCMS data analysis strategies. Particularly, individual live cells were sampled using the Single-probe device under ambient conditions and we conducted a comprehensive data analysis procedure that includes a series of statistical tests and visualization techniques. Through such procedure, we distinguished single cells with distinct phenotypes, studied the abundance change and heterogeneity alternation of cellular metabolites after drug treatment, and found rapid and vigorous cellular response towards changes of microenvironment. In addition, we discovered potential biomarkers that are sensitive to drug treatment and those species are potentially of clinical interest as indicators for therapeutic assessment. Based on those biomarkers, we computed for underlying biological pathways which regulate the overall xenobiotic metabolism of single cancer cells.

PRO-CARCINOGENIC FIELD EFFECTS CAUSED BY GENOMIC INSTABILITY IN COLON, LUNG, AND LIVER

Chinthalapally V. Rao, Farooqui Mudassir, Gaurav Kumar, Yuting Zhang, Adam S. Asch, Hiroshi Y. Yamada

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Background:
Genomic instability and resulting aneuploidy is associated with cancer. However, if genomic instability is causal to cancer, and the molecular link between genomic instability and carcinogenesis, had been under-investigated. Genomic instability is among an earliest event occurring in carcinogenesis. Thus, we reasoned that identifying gene expression signatures of genomic instability and enabling to distinguish normal tissue and cells with genomic instability would lead to effective cancer prevention through vetted targeting approach.

Methods:
To investigate the link between genomic instability and cancer, we used two-pronged approach; use of bioinformatics data from human cancer genome database, and use of preclinical transgenic mouse models of genomic instability.

Results:
By analyzing functions of frequently mutated genes in human cancers in colon, we reached progressive genomic instability model in colonic carcinogenesis. In human liver, the same approach suggested clusters of genes whose mutations would synergistically contribute to hepatic carcinogenesis.

One of the genomic instability mouse models, Sgo1 model, has shown proneness to spontaneous cancers in lung and liver by the middle age. With colonic carcinogen Azoxymethane challenge, the model showed enhanced developments of colonic precancerous lesions and adenocarcinomas. Using the model, we identified differentially expressed genes compared with wild type in the colon, lung, and liver. We will discuss pathways affected by the genomic instability-inducing mutation in the model.

Conclusion:
Genomic instability affects carcinogenesis in critical organs including lung and liver. Gene expression signatures for genomic instability in the preclinical model indicated similarities with those of human cancers, validating the model. Early targeting approach for those pathways would prove usefulness in preventing cancers.

The study was funded by NIH NCI R01CA094962 (C.V. Rao), V.A. merit (grant no. 1I01BX003198-01 to C.V.Rao), and NCI (R03CA162538 to H.Y. Yamada). H.Y. Yamada was also supported by a Chris4Life Colon Cancer Foundation pilot study grant and research support fund from Stephenson Cancer Center.

DECITABINE REINSTATE RADIOTHERAPY–INDUCED HYPERMETHYLATION MEDIATED RD3 TRANSCRIPTIONAL REPRESSION AND INHIBITS SURVIVAL AND STEMNESS OF RESISTANT NEUROBLASTOMA CELLS

Dinesh Babu Somasundaram, Sheeja Aravindan, Terence S. Herman, Natarajan Aravindan

Recently, we defined the novel tumor evolution stabilization role of Retinal degeneration protein 3 (RD3), regulating the metastatic state of neuroblastoma (NB) cells *in vitro* and their metastatic potential *in vivo.* Our earlier studies in various tumor models recognized the role of therapy resistant residual cells in tumor recurrence and relapse. Herein, we investigated the ongoing acquisition of RD3 transcriptional regulation in radiotherapy surviving NB cells and further identified the potential drug candidates that can reprogram and reinstate RD3 transcription in these cells. Human NB (SH-SY5Y, SK-N-AS) cells exposed to single dose (10, 20, 50cGY, 1, 2, 4Gy) or fractionated (FIR, 2Gy/Day for 5 days) irradiation resulted in significant transcriptional repression of RD3. Drug screening with a panel of drugs that are in current clinical use, those in pipelines or in developmental stages by exposing radioresistant cells identified many potential drug candidates that could effectively restore RD3 transcription in this setting.

Evidently, we found RD3 promoter region hypermethylation drives its transcriptional regulation in therapy resistant cells. To that note, FDA approved hypomethylating agent decitabine significantly reinforced RD3 transcription in this setting and further promoted cellular differentiation and completely inhibited the formation of organized tumorosphere under stem-cell culture conditions. Together, these results demonstrate the ongoing acquisition of RD3 loss in therapy resistant cells and further recognized the mechanism of how RD3 is regulated in this setting. More importantly, this results of this study identified a drug candidate, that has been already in clinical use, that could be beneficial in mitigating therapy resistance and better clinical outcomes for the patients presented with high-risk advanced disease.

Funding: NIH-COBRE-IP20GM103639-01, OUHSC-COM-Radiation Oncology Research Development Funds

CLINICAL & CORRELATIVE RESEARCH

CLINICAL & CORRELATIVE RESEARCH BASEMENT, ROOM D

1:15 – 2:15 PM

SESSION III
CLINICAL & CORRELATIVE RESEARCH

Moderators: Kathleen Moore, MD

1:15 PM– 1:35 PM

IMPROVING FLAP RECONSTRUCTION SURGICAL OUTCOMES IN HEAD AND NECK CANCER PATIENTS BY ENHANCING PERIOPERATIVE NUTRITION

Crista Horton
College of Medicine
The University of Oklahoma Health Sciences Center

1:35 – 1:55 PM

NON–INVASIVE LIQUID BIOPSY ANALYSIS OF EXOSOMAL MIRNA FOR MONITORING AND PREDICTING RESPONSE TO CHEMOTHERAPY IN LUNG CANCER PATIENTS

Rajagopal Ramesh, PhD
Department of Pathology
The University of Oklahoma Health Sciences Center

1:55 – 2:15 PM

PARTICIPATORY RESEARCH IN ALASKA NATIVE AND AMERICAN INDIAN COMMUNITIES: A SCOPING REVIEW OF CURRENT PRACTICES AND RECOMMENDATIONS FOR FUTURE RESEARCH

Scott Ketchum, PhD
Center for Applied Social Research
The University of Oklahoma

1:15 – 2:15 PM

SESSION IV
CLINICAL & CORRELATIVE RESEARCH II

Moderators: Kathleen Moore, MD

2:15 – 2:35 PM

2A COMPARATIVE META–ANALYSIS OF CLINCAL STAGE I NONSEMINOMATOUS TESTICULAR CANCER TREATMENT

Chance Morris
Department of
The University of Oklahoma Health Sciences Center

2:35 – 2:55 PM

TARGETING BMI–1 IN GYNECOLOGIC CANCERS: PRE–CLINICAL DATA

Aleia Crim, MD
Department of Obstetrics & Gynecology
The University of Oklahoma Health Sciences Center

2:55 – 3:15 PM

TARGETING BMI–1 IN ENDOMETRIAL CANCER

Megan Buechel, MD
Department of Obstetrics & Gynecology
The University of Oklahoma Health Sciences Center

IMPROVING FLAP RECONSTRUCTION SURGICAL OUTCOMES IN HEAD AND NECK CANCER PATIENTS BY ENHANCING PERIOPERATIVE NUTRITION

Crista E. Horton BS, Ben Collins MD, Landon Massoth MD, Paula Guillion RN, Greg A. Krempl MD, FACS

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Background

Patients with head and neck cancer (HNC) often require complex excision and reconstructive surgery for treatment, including the use of free and adjacent tissue flaps. At least 50% percent of these patients suffer from malnutrition. Institutional review in 2015 showed increasing rates of fistula/flap failure within the ENT department. Malnutrition was identified as a common denominator in a population with a fistula rate of 30%. A process improvement project was designed to determine whether nutritional intervention could improve the rate of surgical site complications.

Methods

HNC patients scheduled for flap reconstruction over an 18-month period voluntarily enrolled in a nutritional intervention quality improvement project. A process improvement protocol and clinical pathway was implemented to improve perioperative nutrition, facilitate education, and provide supplies to adult patients. Nutritional consults and the immunonutrition supplement Impact Advanced Recovery® (NestleHealthScience) was provided at no cost, to be taken five days preoperatively three times daily in addition to their home diet. Five days postoperatively, the subjects were maintained on continuous tube feeds with Impact Advanced Recovery® (NestleHealthScience). Nutritional status was assessed using weight changes and prealbumin levels at baseline, day of surgery, and POD 2-5. Postoperative outcomes included return to the operating room, dehiscence, flap failure, fistula, and hematoma compared to previously reported norms.

Results

Sixteen patients completed the program and all reported using the preoperative supplement; 81.25% were men and 18.75% were women, with an age range of 47-78 (avg 64.4). Eight (50%) had received prior radiation therapy. Thirteen (81.25%) healed well without complications. Of the 6 patients who experienced a complication, 2 (12.5%) were potentially related to nutritional status: 1 had minor dehiscence requiring a revision procedure, 1 had flap necrosis and required a second flap. This rate of fistula formation is less than published rates and our historical rate. There were 4 patients requiring treatment for hematoma/seroma formation not related to nutrition. The patient experiencing flap failure lost 0.08 kg from first nutritional assessment to discharge. Patients experiencing complications had smaller increases in prealbumin from baseline to day of surgery (9.09%) than those with no complications (63.6%). Protocol compliance for labs was poor (29%) and despite aggressive nutritional supplementation, most patients experienced drops in prealbumin during hospitalization.

Conclusions

Perioperative nutritional intervention is recommended for patients undergoing HNC surgery with flap reconstruction. While this did not eliminate lab evidence of malnutrition, the incidence of fistula/flap failure was reduced by 58%.

NON-INVASIVE LIQUID BIOPSY ANALYSIS OF EXOSOMAL MIRNA FOR MONITORING AND PREDICTING RESPONSE TO CHEMOTHERAPY IN LUNG CANCER PATIENTS

Akhil Srivastava[1,4], Mohammad Razaq[2,4] Allison Gillaspy[3,4], Rajagopal Ramesh[1,4,5]

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Background: Conventional approaches to monitor response to chemotherapy and prediction of drug resistance are laborious, time-consuming and often involve invasive procedures. Developing a non-invasive technique will offer a rapid prediction of treatment outcomes benefiting both oncologists and patients. In the present study we examined a non-invasive liquid biopsy approach for isolating exosomes from the urine of lung cancer patients and analyzing them for micro (mi) RNA signatures before and after receiving chemotherapy.

Methods: Five lung cancer patient's urine was collected pre- and post-chemotherapy and used for isolating exosomes. miRNA libraries were constructed using 100 ng of total RNA isolated from exosomes and sequenced on the Illumina MiSeq platform. A minimum of 20 million 50 base pair sequencing reads were collected and data analyzed using CLC Genomics Workbench Software. miRNAs that was significantly changed (i.e. up- or down-regulated) in the matched samples were identified as candidate biomarkers. Bioinformatics tools were applied for performing pathway analysis.

Results: miRNA sequencing (RNAseq) showed 111,416 sequence hits for small non-coding RNAs and after aligning with miRBase21, 548 mature miRNAs were identified. The differentially expressed miRNAs clustered separately in pre- and post-treatment samples in all five-patients. Four miRNAs (hsa-miR-6842, hsa-miR-2957, hsa-miR-143 and hsa-miR-543) showed marked reduction in post-chemotherapy samples compared to pre-treatment samples. Validation studies by quantitative (q)PCR using miRNA specific primers confirmed the RNAseq results. *In silico* target analysis identified RAS oncogenic family as a prominent target for these miRNAs. Additionally, Gene Ontology terms, and KEGG pathways associated with cancer were identified for candidate miRNAs.

Conclusions: Our study demonstrates development of non-invasive liquid biopsy method for predicting treatment response and for development of drug resistance. This technology is also being tested in our laboratory for predicting response and drug resistance in gynecologic malignancies like ovarian and endometrial cancer.

Acknowledgments. The study was supported in part by funds received from the Stephenson Cancer Center (SCC) Seed Grant, SCC Multi-PI Grant, Presbyterian Health Foundation Seed Grant, and Jim and Christy Everest Endowed Chair in Cancer Developmental Therapeutics, the University of Oklahoma Health Sciences Center.

PARTICIPATORY RESEARCH IN ALASKA NATIVE AND AMERICAN INDIAN COMMUNITIES: A SCOPING REVIEW OF CURRENT PRACTICES AND RECOMMENDATIONS FOR FUTURE RESEARCH

Scott Ketchum [1], Brian Woodbury [2], Justin Lund [1], Vanessa Hiratsuka [2], Julie Beans [2], Kyle Wark [2], Jae Swainston [1], Paul Spicer [1]

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American Indian and Alaska Native communities (AI/AN) have consistently been placed in a subject role with limited to no value given to their local contexts and meanings in past health research. Trust issues surrounding data collection and the use of data as exampled by the Havasupai case (1990s/2004) is one of the numerous issues investigators may face when working with AI/AN communities.

In order to better understand and inform contemporary practices in engaging AI/AN communities in participating in health research, this presentation/paper presents findings from a scoping review over existing literature on community engagement focusing on AI/AN populations. Peters et al. (2015) describe scoping reviews as an exercise in reconnaissance with the goal of clarifying "working definitions and conceptual boundaries of a topic or field."

This presentation will explore past, present, and emerging approaches in cancer and health research practices that engage Native and Indigenous communities, with special attention to the unique legal and cultural contexts of American Indian / Alaska Native (AI/AN) communities in Native North America.

The intended audience for this session includes researchers, health care providers, policy makers, and others working with AI/AN populations. Preliminary findings have emphasized the significance of understanding community and context when collaborating with AI/AN communities in health research. The goal of the session is to cultivate an appreciation for the complexities of authentic and rigorous Community-Based Participatory Research when engaging American Indian / Alaska Native communities in health research.

Acknowledgement of Funding:

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A COMPARATIVE META-ANALYSIS OF CLINICAL STAGE I NONSEMINOMATOUS TESTICULAR CANCER TREATMENT OPTIONS

Daniel C. Parker MD, Chance Morris*, Shari Clifton, Christopher Aston, Michael S. Cookson MD, and Kelly Stratton MD

Department of Urology, The University of Oklahoma Health Sciences Center & The Stephenson Oklahoma Cancer Center, Oklahoma City, Oklahoma

Purpose: Clinical guidelines provide several treatment strategies for patients with Stage 1 nonseminomatous germ cell tumors (NSGCT). The purpose of this study is to conduct a meta-analysis of the available evidence examining outcomes associated with each treatment option for patients with Stage 1 NSGCT.

Materials and Methods: A systematic review of studies containing primary data on clinical Stage I NSGCT patients was conducted using articles from the Medline database, as well as prospective articles from the Embase and Medline databases. All studies published between January 1995 and July 2016 with a minimum of one-year median follow-up were included. The primary endpoint evaluated was disease-specific mortality (DSM).

Results: Data were obtained from 74 studies, representing >10000 Stage I NSGCT patients. DSM rates for each treatment option were: 0.80±0.12% (47/5850) for active surveillance (AS), 0.34±0.14% (6/1776) for chemotherapy (CT), and 0.20±0.14% (2/996) for retroperitoneal lymph node dissection (RPLND). Comparisons before risk stratification demonstrated significant differences in DSM rates between AS and CT (p = 0.039) and between AS and RPLND (p = 0.037), but not between CT and RPLND (p = 0.52). The DSM rates among high risk patients did not differ significantly (p > 0.05). Among low risk patients there were no disease-specific deaths associated with any treatment option (n = 1440).

	Relapse Rates Total	Relapse Rates High Risk	Relapse Rates Low Risk
Active Surveillance	24.0±0.5% (1705/7094)	44.1±1.6% (410/929)	17.7±0.7% (571/3217)
Primary Chemotherapy	1.8±0.3% (36/2040)	1.4±0.3% (22/1590)	2.6±1.1% (5/196)

Primary RPLND	7.9±0.8% (97/1223)	11.4±2.4% (20/176)	2.3±1.6% (2/87)

Conclusions: AS is associated with a higher DSM rate than both CT and RPLND for Stage 1 NSGCT. No significant difference in DSM exists between CT and RPLND patients. No disease-specific deaths were reported among low risk patients.

	Overall Mortality Total	Overall Mortality High Risk	Overall Mortality Low Risk
Active Surveillance	2.77±0.22% (149/5378)	0.90±0.90% (1/111)	1.07±0.32% (11/1031)
Primary	0.56±0.20%	0.60±0.27%	1.16±0.82%

	Disease-Specific Mortality Total	Disease-Specific Mortality High Risk	Disease-Specific Mortality Low Risk
Active Surveillance	0.80±0.12% (47/5850)	0.97±0.56% (3/309)	0% (0/1131)
Primary Chemotherapy	0.34±0.14% (6/1776)	0.56±0.23% (6/1076)	0% (0/211)
Primary RPLND	0.20±0.14% (2/996)	0.57±0.57% (1/175)	0% (0/98)

POSTER PRESENTATIONS & ABSTRACTS

CANCER RESEARCH SYMPOSIUM POSTER SESSION
11:15 AM - 1:15PM
LEVEL 1

POSTER PRESENTATION LIST (listed A-Z by presenter)	TRACK & BOARD NUMBER
BRG1 (SMARC4) INFLUENCES THE RESPONSE TO EGFR INHIBITORS IN LUNG CANCER Ahmed, Rebaz	CPC 1
FEXOSOMES AS A THERANOSTIC FOR LUNG CANCER Amreddy, Narsireddy	PTR 19
ZEBRAFISH T LYMPHOBLAST CANCERS INVADE THE CENTRAL NERVOUS SYSTEM Baiamonte, Matteo	CPC 2
MECHANISMS OF ESCO-DEPENDENT COHESIN REGULATION Bender, Dawn	PTR 20
THE ROLE OF GENETICS AND GENOMICS RESEARCH IN THE CHEROKEE NATION: INITIATING COMMUNITY DIALOGUE ABOUT GENOMICS RESEARCH IN TRIBAL COMMUNITIES Blanchard, Jessica	CPC 42
DEVELOPING PRECISE ANATOMIC MODELS OF KEY CORTICAL NETWORKS Briggs, Robert	CCR 15

BRG1 (SMARC4) INFLUENCES THE RESPONSE TO EGFR INHIBITORS IN LUNG CANCER

Authors: Rebaz Ahmed[1,4], Janani Panneerselvam[1,3], Ranganayaki Muralidharan[1,3], Meghna Mehta[2,3], Rajagopal Ramesh[1,3,4], Anupama Munshi[2,3]

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Background: Epidermal growth factor receptor (EGFR) is overexpressed in several human cancers including non-small cell lung cancer (NSCLC) and has become an important molecular target for therapy. The occurrence of gain of function *via* activating mutations in the intracellular kinase domain (IKD) of EGFR provides signaling cues for cell proliferation, differentiation, and survival. Gefitinib (GEF) is an orally available, selective EGFR-tyrosine kinase inhibitor (TKI) that results in clinical benefit in NSCLC patients with EGFR mutations, but not in majority of NSCLC patients with wild-type EGFR (wt-EGFR), implying the existence of unexplored mechanisms that contribute to resistance to GEF. Treatment with GEF over a period of time results in drug resistance thereby leading to treatment failure. Recent studies indicate a role for BRG1 and other subunits of large SWI/SNF complex in modulating the response to anticancer therapies including TKIs. However, little is known whether BRG1 status influences the response to EGFR-TKIs. In the present study we therefore investigated how BRG1 influences the response to EGFR TKI in lung cancer.

Methods: NSCLC cells A549 (mt-BRG1, wt-EGFR) and H358 (wt-BRG1, wt-EGFR) tested for sensitivity to GEF (0.5, 1, and 2 µm) by performing cell viability assay at 24 hours. Lipid-based nanoparticle was used to deliver BRG1-siRNA to H358 and BRG1-overexpression-plasmid to H1299 (mtBRG1, wtEGFR), followed by 1 µm GEF-treatment at 24 hours. Changes in expression of phosphorylated (p) EGFR (Tyr1173), EGFR and BRG1 in the GEF-treated cells were examined by western blot analysis.

Results: Results showed that H358 cells were relatively resistant (86.48%, 78.40% and 68.46% viability at 0.5, 1, and 2 µm GEF, respectively) than A549 cells (78.5%, 44.88% and 27.66% viability at 0.5, 1, and 2 µm GEF, respectively) towards GEF-treatment. Molecular studies showed upregulation of BRG1 with concomitant reduction in EGFR in GEF-treated-H358 cells. In contrast, mt-BRG1-A549 cells showed no change in EGFR expression levels upon GEF-treatment. SiRNA-mediated BRG1 knockdown in H358 increased the sensitivity to (1 µm) GEF-treatment from 78.40% cell viability to 49.20% at 24 hours whereas overexpression of BRG1 in mt-BRG1-H1299 showed trend towards increased resistance, from 76.13% cell viability to 88.06% to (1 µm) GEF-treatment.

Conclusion: The upregulation of BRG1 in GEF-treated-H358 cells suggests possible molecular interaction between EGFR and BRG1 and that BRG1 possibly influences resistance to EGFR-TKIs. Demonstrating the role of BRG1 role in resistance to EGFR-TKIs will allow the development of new combinatorial treatment strategies targeting both BRG1 and EGFR.

Keywords: Non-small cell lung cancer, epithelial growth factor receptor, tyrosine kinase inhibitors, drug resistance, molecular targeted therapy, SWI/SNF complex, BRG1

Funding: The study was supported in part by funds received from the Presbyterian Health Foundation Bridge Grant (AM) and the Experimental Therapeutics Seed Grant from Stephenson Cancer Center (AM).

FEXOSOMES AS A THERANOSTIC FOR LUNG CANCER

Narsireddy Amreddy[1,5], Akhil Srivastava[1,5], Ali Khan[1,8], Natascha Riedinger[6], Mohammad Razaq[3,5], Yan D. Zhao[2], Rheal Towner[7], Anupama Munshi[4,5], Rajagopal Ramesh[1,5,9].

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Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA; [6]Boone Pickens School of Geology, Oklahoma State University, Stillwater, OK, USA; [7]Advanced Magnetic Resonance center, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA; [8]Department of Biology, University of Oklahoma, Norman, Oklahoma, USA; and [9]Graduate Program in Biomedical Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA.

Introduction: Enabling technologies that can deliver cancer drugs to the tumor with simultaneous monitoring of drug delivery and response to therapy will make a significant impact in cancer treatment. In the present study we have developed Fexosomes as a theranostic for cancer imaging and therapy. Fexosome were prepared by loading exosomes with magnetic nanoparticles (MNP) conjugated to a chemotherapeutic via a pH-responsive linker. The MNP enables Magnetic Resonance Imaging (MRI)-based T2 contrast imaging while the pH-linker provides controlled drug release.

Methods: Fexosomes were produced by loading the exosomes, isolated from normal lung fibroblast cells (MRC-9), with MNPs conjugated to Doxorubicin (Dox) via a pH cleavable hydrazone (-C=N-) linker. The Fexosomes were characterized by Transmission Electronic Microscope (TEM), Zeta-Potential and Nanotracker-Analysis (NTA). The Dox release studies from Fexosomes were conducted in PBS (pH 7.4) and acetate buffer (pH 5.5). The Fexosomes cell uptake studies were performed in A549 cells by fluorescence microscopy, Prussian blue staining and ICP-MS. MR imaging and therapeutic effect of Fexosomes was analyzed in A549 lung cancer cells by phantom study, cell viability and western blot assay respectively.

Results: The average size and concentration of exosomes was 134.7 nm ± 2.0 nm and 1.25e+008 ± 2.2e+007 per mL respectively. Surface charge of exosomes changed from -18.29 mV to -1.96 mV when loaded with MNP confirming the formation of Fexosomes. TEM showed lipid bilayer morphology of Fexosomes. The Fexosomes showed more Dox release in acetate buffer than PBS, which confirms the pH sensitive release. The Dox fluorescence studies, Prussian blue and ICP-MS analysis confirmed the uptake of Dox and Fe nanoparticles into the cells. MRI phantom study showed cell uptake of the Fexosomes as relaxation time values increased with concentration (T2 values, 200 µg of MNP in Fexosomes = 26.11 $msec^{-1}$ and untreated control = 76.11 $msec^{-1}$). Finally, Fexosomes effectively inhibited A549 cell proliferation with an IC_{50} around 4 µg/mL Dox concentration and showed increased apoptosis which confirmed by cleavage of caspase9, PARP and induction of DNA damage by γH2AX activation.

Conclusion: Fexosomes are successfully used for therapy and diagnostic for lung cancer in vitro and in vivo planning to do experiments.

Funding Support. The work was supported in part by a grant received from the National Institutes of Health (NIH), R01 CA167516 (RR), an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences (P20 GM103639) of the National Institutes of Health (RR & AM), and by funds received from the Stephenson Cancer Center Seed Grant (RR), Presbyterian Health Foundation Seed Grant (RR), Presbyterian Health Foundation Bridge Grant (RR) and Jim and Christy Everest Endowed Chair in Cancer Developmental Therapeutics (RR) at the University of Oklahoma Health Sciences Center. Rajagopal Ramesh is an Oklahoma TSET Research Scholar and holds the Jim and Christy Everest Endowed Chair in CancerDevelopmental Therapeutics.

Zebrafish T Lymphoblast Cancers Invade the Central Nervous System

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Acute lymphoblastic leukemia (ALL) infiltrates the central nervous system (CNS) in 5-10% of pediatric cases, and the CNS is also an important site of ALL relapse. Few drugs used for ALL treatment cross the blood brain barrier; therefore, current therapeutic regimens utilize intrathecal chemotherapy, high-dose systemic administration of CNS-penetrating drugs, and/or cranial irradiation to treat CNS+ cases. CNS- cases also receive such treatments, to provide prophylaxis against CNS spread. All of these measures can cause neurologic damage and other short- and long-term health sequellae for patients. CNS homing mechanisms and survival pathways of ALL cells are poorly understood, but if these were known, could conceivably lead to less-harmful, yet effective, treatment approaches for ALL CNS prophylaxis and treatment.

Most work investigating CNS+ ALL has used murine models, which are costly and difficult to study, or clinical samples, which are scarce. We propose the use of zebrafish (*Danio rerio*) to study CNS infiltration, taking in account the several advantages that this model has. Our lab studies four zebrafish models of T cell lymphoblast malignancies, but whether cancers in these models invade the CNS is unknown. We screened each mutant line at stages of advanced tumor progression (i.e., disseminated T-ALL), and detected CNS infiltration by the vast majority of T-ALL. We have thoroughly studied the widely-used *MYC*-transgenic line, and performed gene expression studies to compare CNS+ versus CNS- cases. We have also studied three ENU mutants we created, *hulk*, *shrek*, and *oscar the grouch*, and CNS involvement in these lines' T-ALL is also prevalent. Results of these ongoing studies will be presented.

MECHANISMS OF ESCO-DEPENDENT COHESIN REGULATION

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Cohesin is a protein complex that tethers sister chromatids together, promotes normal chromosome organization, and mediates DNA repair. The association of cohesin with chromatin is tightly regulated. Members of the Eco family of acetyltransferases stabilize the interaction of cohesin with chromatin and are essential for cohesion between sister chromatids. Vertebrates express two different Eco enzymes, Esco1 and Esco2 that have distinct roles in cohesin regulation. Esco2 promotes the establishment of sister chromatid cohesion while Esco1, which does the bulk of cohesin acetylation, is involved in cohesin-mediated processes relevant to chromosome structure, organization and repair. We hypothesize that the unique functions of Esco1 and Esco2 reflect their interactions with different partners through their distinct N terminal regions. I have implemented a protein identification system known as BioID in which a promiscuous biotin protein ligase is used to identify protein interacting partners *in vivo*. In this strategy the biotin ligase is fused to the protein of interest, resulting in biotinylation of proximal endogenous proteins. Subsequent streptavidin affinity and mass spectrometry allow identification of proteins that interact with and control unique functions of each Esco enzyme *in vivo*. Protein interactions identified by mass spectrometry are then investigated further using fluorescently labeled proteins and immunofluorescence microscopy. Preliminary data suggests that sister chromatid cohesion is established through acetylation of cohesin by Esco2 through interactions at the replication fork. A deeper look into these protein interactions will provide insight into the mechanisms of Esco-mediated cohesin regulation.

THE ROLE OF GENETICS AND GENOMICS RESEARCH IN THE CHEROKEE NATION: INITIATING COMMUNITY DIALOGUE ABOUT GENOMICS RESEARCH IN TRIBAL COMMUNITIES

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This is a pilot study designed to assess community perceptions and future directions for genetic and genomics research related to the Cherokee Nation of Oklahoma. The objective of this pilot project is to identify community, knowledge, perceptions and concerns related to genomics research in order to anticipate future research needs and to develop appropriate community-engaged protocols for addressing these concerns. Cherokee Nation considers It is important for tribal nations to be at the forefront of the impending changes to health prevention and treatment options and policies impacting health research in their communities.

The impetus behind this collaboration is the National Institutes of Health's latest initiative called the *All of Us* Research Program, a program designed to accelerate research and promote the inclusion of diverse communities by building a database of over 1 million participants to learn about "how individual differences in lifestyle, environment and biological make-up can influence health and disease" (NIH,gov, 2017). This massive participant "cohort" would be asked to "give consent for extensive characterization of biologic specimens…(-including whole-genome sequencing…) and behavioral data" to be shared with researchers from many organizations (Collins and Varmus, 2015). The groundbreaking nature of this initiative stands to rewrite the very processes whereby researchers collect, share, regulate and analyze highly individualized and community-based health data. The *All of Us* program highlights the expanding possibilities for individuals and communities to actively engage with new biotechnologies. These new forms of engagement pose a number of challenges for Native American and other minority communities that have been historically underrepresented, and often exploited, in biomedical research (Bussey-Jones, 2010; Dalton, 2004; Di Chiro, 2007; Drabiak-Syed, 2010; Harry & Dukepoo, 1998; Harry & Kaneche, 2006; Pacheco, 2013). The linking of individualized health information to community-based research practices requires that communities contemplate their positions and interests vis-à-vis the changing landscape of biotechnologies and health disparities research.

As part of a collaborative partnership between the Cherokee Nation of Oklahoma and researchers at the University of Oklahoma, the primary goal for this pilot project was to initiate a dialogue among Cherokee Nation citizens about the imminent role of genetics research in their communities. A series of 14 focus groups were conducted across diverse communities within Cherokee Nation to understand the nature of tribal members' concerns and interests in pursuing new directions in genetics research. This poster addresses the comprehensive and multifaceted nature of tribal perspectives related to the value and utility of genomics research, and highlights some methodological considerations for implementing community-based discussions about the changing landscape of genomic research in tribal communities.

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DEVELOPING PRECISE ANATOMIC MODELS OF KEY CORTICAL NETWORKS

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Introduction: As knowledge of cortical function has increased, clinicians have learned that the cerebrum is composed of complex networks that interact to execute key functions. These networks have been identified and studied using novel technologies such as functional magnetic resonance imaging (fMRI) under both resting-state and task-based conditions. However, no one has attempted to describe these networks in terms of newly published parcellated brain maps.

Methods: Using meta-analytic software provided by BrainMap, we reviewed the relevant fMRI studies related to 9 different cerebral networks: dorsal and ventral attention, neglect, semantic, auditory, motor, default mode, salience, and central executive. The Montreal Neurologic Institute (MNI) coordinates from these studies were used to generate anatomic likelihood estimates (ALEs) of each network. ALEs were then displayed using the Multi-image Analysis GUI (Mango). Pre-constructed regions of interest (ROIs) corresponding to the 180 cerebral parcellations published under the Human Connectome Project were used to identify the parcellations comprising each network. These ROIs were overlaid on the relevant ALE for consideration in the network model. After deriving the parcellations for each network, DSI-based fiber tracking was performed to establish the connectivity between parcellations.

Results: The relevant cortical parcellations of 9 different cerebral networks have been identified. The connectivity between these cortical regions has been used to construct anatomically precise models of the networks. Models are proposed for the dorsal attention, ventral attention, neglect, semantic, auditory, motor, default mode, salience, and central executive networks.

Conclusions: We have sought to specify the cortical regions of interest in nine networks responsible for both resting-state and task-based functions of the cerebrum. Our goal is to move towards more precise, anatomically specific models of these networks for use in future studies. We believe access to this information may provide a foundation for improving neurosurgical outcomes through the preservation of key cortical networks.

INVESTIGATION OF CLINICAL AND PATHOLOGIC FEATURES ASSOCIATED WITH UNDIFFERENTIATED AND DEDIFFERENTIATED ENDOMETRIAL ADENOCARCINOMAS: A MULTI-INSTITUTIONAL STUDY.

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OBJECTIVES: Undifferentiated endometrial adenocarcinoma (UEC) and dedifferentiated endometrial adenocarcinomas (DEC) are rare histologic subtypes that are often misdiagnosed. DEC is characterized by an undifferentiated carcinoma with an associated low grade endometrioid component (FIGO I or II). There is limited literature to help guide treatment decisions and provide prognostic information for these patients. Our primary objective was to investigate survival and clinical outcomes in a cohort of patients with UEC and DEC.

METHODS: A retrospective analysis of patients with UEC and DEC from 2004-2017 was performed at three institutions. Demographic, clinical, and pathologic data were collected and analyzed with appropriate statistical methods. Progression free and overall survival (PFS and OS) were estimated using the Kaplan-Meier method.

RESULTS: Of the 72 pts included in this study, 33 (46%) had DEC and 39 (54%) had UEC. Median age was 61 and median BMI was 31. There was no difference in baseline demographics between the two groups. Only 48% of DEC and 44% of UEC presented with stage I or II disease. The majority underwent primary surgery (91% DEC and 92% UEC) with lymphadenectomy (73% DEC and 75% UEC) and were resected to no gross residual disease (77% DEC and 89% UEC). Adjuvant treatment varied widely with 15% of pts receiving radiation, 23% receiving chemotherapy, and 23% receiving both chemotherapy and radiation. Median PFS was 15 mo and OS 19 mo. DEC pts had worse PFS and OS compared to the UEC pts although PFS did not meet statistical significance (5 vs 21 mo (p=0.11) and 7 vs 37 mo (p=0.02). While there was a trend towards chemotherapy improving survival for UEC pts this did not meet statistical significance (Table 1). Adjuvant radiation significantly improved both PFS for DEC pts but did not meet statistical significance for UEC pts (Table 1).

CONCLUSIONS: Both UEC and DEC confer a poor prognosis, are frequently diagnosed at an advanced stage, and have a high risk of recurrence DEC had a significantly worse prognosis than UEC. While adjuvant radiation improved PFS and OS for DEC pts, neither chemotherapy nor radiation was associated with a statistically significant improved survival for UEC pts. Further understanding of these aggressive subsets of endometrial cancer is imperative to improving treatment regimens and outcomes for our pts.

Table 1: Impact of chemotherapy and radiation treatment on median PFS and OS in DEC and UEC patients.

	Dedifferentiated		Undifferentiated	
	PFS (mo)	OS (mo)	PFS (mo)	OS (mo)
Radiation				
Yes	21	26	23	37
No	3	5	17	32
P-Value	*0.003*	*0.002*	*0.98*	*0.44*
Chemotherapy				
Yes	5	6	23	38
No	8	9	16	24
P-Value	*0.42*	*0.75*	*0.66*	*0.25*

ASSOCIATIONS BETWEEN INTRAVAGINAL PRACTICES AND GENITAL HUMAN PAPILLOMAVIRUS INFECTION AMONG FEMALE SEX WORKERS IN CAMBODIA

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Abstract:

Background: Intravaginal practices (IVPs) include washing, wiping, or inserting something inside the vagina. The very few studies that have examined the association between intravaginal washing and genital human papillomavirus (HPV) infection have generated contrary results and did not scrutinized the effects timing on these practices; the effects of other IVPs on HPV infection have been underexplored. This study investigates the associations between IVPs and HPV infection with one or multiple genotypes.

Methods: We conducted a cross-sectional study of 200 female sex workers aged 18–35 years in Phnom Penh, Cambodia from August–September 2014. Data on sociodemographic characteristics, IVPs, and other behaviors were collected through face-to-face interviews. We asked about IVPs in general, and specifically shortly before, and shortly after, vaginal sex. Genital samples were collected by self-sampling, and were tested for 37 HPV genotypes using Roche Linear Array HPV Genotyping Test.

Results: No participants had received HPV vaccines. The overall prevalence of HPV infection with any type was 47.0%. The prevalence of HPV infection with one, two, three, and 4–8 types was 17.0%, 11.0%, 12.0%, and 7.0%, respectively. The most common types were HPV-62 (10.5%), HPV-16 (7.5%), HPV-18 (6.0%), HPV-52 (6.0%), HPV-53 (6.0%), and HPV-68 (6.0%). Among those who had any HPV type detected, 77.0% harbored at least one oncogenic type. Ninety percent of participants had ever performed intravaginal cleansing, 28.5% performed intravaginal wiping, and 5.5% performed intravaginal insertion. Among those who performed intravaginal cleansing, 92.8% used water or water mixed with soap, salt, or lemon; about half of these also used other solutions such as commercial products. Multivariable Poisson regression models showed that a lower number of infecting HPV genotypes (excluding HPV 6 and 11) were associated with intravaginal washing in the past 3 months (incident rate ratios [IRR] = 0.65, 95% confidence interval [CI]: 0.46–0.94) and often performing intravaginal washing shortly after sex (IRR = 0.89, 95% CI: 0.81–0.99) (adjusted for venues of sex work, alcohol use, self-reported HIV status, number of paying partners

in the past 90 days, and condom use with all partners in the past 90 days). Intravaginal washing before vaginal sex, intravaginal wiping, and intravaginal insertion were not associated with HPV infection.

Conclusion: These findings provide a new angle for and challenge the existing view that all types of vaginal cleansing are harmful. Specifically, intravaginal washing shortly after sex (mainly with water) may help prevent HPV infection in female sex workers, who have several partners and thus frequently expose to sources of HPV infection with different genotypes. Future studies are needed to investigate the potential effect of intravaginal cleansing in cervical intraepithelial neoplasia.

DEFINING LEUKEMIA STEM CELLS IN A NOVEL PRE-B-ALL ZEBRAFISH MODEL.

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Leukemias are hematologic malignancies that can present with various clinical features. The most common leukemia in children is acute lymphoblastic leukemia (ALL). This cancer is characterized by the malignant transformation and proliferation of lymphoid progenitors in the bone marrow, and can develop in lymphoblasts of either the B or T cell lineage. Precursor-B cell acute lymphoblastic leukemia (pre-B-ALL) is the most common ALL subtype in both children and adults, and the most prevalent childhood cancer overall, representing more than 25% of all pediatric malignancy. A major clinical challenge for pre-B ALL patients is relapse. Most pre-B ALL patients respond to chemotherapy, achieving remission, but in a significant group of patients, pre-B ALL recurs. Such patients have much worse prognosis. In many cases, relapse is thought to occur because therapy failed to eliminate leukemia stem cells (LSC), a type of cancer stem cell. LSC are believed to represent a rare and unique cell population capable of self-renewal, which allows re-growth of the cancer. LSC are also thought to be drug resistant. Unfortunately, LSC biology is poorly understood, limiting our ability to identify new therapies to inhibit their self-renewal and prevent pre-B ALL relapse.

Zebrafish (*Danio rerio*) are a powerful vertebrate model to study hematologic cancers, and several *D. rerio* leukemia models closely mimic their human disease counterpart. Our lab recently discovered the only potent zebrafish pre-B ALL model; a human MYC transgene (*hMYC*) induces pre-B ALL in these fish. My project investigates LSC in zebrafish pre-B-ALL using allo-transplantation. We have purified and serially transplanted pre-B-ALL cells into immunosuppressed wild-type recipients. Our data indicate pre-B ALL LSC are present and capable of self-renewal, allowing engraftment in host fish. In addition, we have found that LSC can be enriched by treating donor fish with dexamethasone prior to transplantation. Collectively, our data demonstrate zebrafish pre-B-ALL driven by *hMYC* is a powerful model to study LSC and we next seek to identify their unique gene expression profile, allowing us to answer fundamental questions about the biology of this crucial, yet rare, population of cancer cells.

COUNTY LEVEL CLUSTERS OF BREAST, COLORECTAL, LUNG AND PROSTATE CANCER IN OKLAHOMA 2010-2014

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Introduction

Geography or place is a critical part of the epidemiological and medical research, in addition to planning population-based health care needs. The identification of clusters have often resulted in heightened levels of fear both publically and among healthcare providers. While a few high profile geographic clusters of cancer have been related to occupational hazards, the vast majority of clusters are related to lifestyle choices and the distribution of socio-economic characteristics. A cancer cluster is defined as a greater-than-expected number of cancer cases that occurs within a group of people in a geographic area over a period of time.

Methods

For this study, we obtained age-adjusted incidence rates by county for female breast, lung and bronchus, prostate, and colorectal cancers diagnosed in Oklahoma from 2010-2014 using OK2Share. Global cluster analysis (Getis-Ord General G, and Global Moran's I) and local indicators of spatial association (LISA) (Optimized Hot Spot Analysis, Anselin's Local Moran's I and Local G and G*) were calculated.

Results

Using Getis-Ord General G, we observed global clustering of lung ($z=3.013$ $p=0.003$) and prostate ($z=2.673$ $p=0.008$), but not for female breast ($z=0.560$ $p=0.57$) and colorectal ($z=0.788$ $p=0.43$). Using global Moran's I we observed clusters of female breast ($z=2.239$ $p=0.03$), lung ($z=4.585$ $p<.00001$), prostate ($z=4.823$ $p<.00001$), and colorectal ($z=3.239$ $p=0.001$).

We then conducted LISA using Anselins Local Moran's I for all cancers and Optimized Hot Spot Analysis (Getis-Ord Gi*) for lung and prostate cancers, based on the results the global test methods. For lung cancer we observed clusters using both methods in the southeast central part, with high rates surrounded by low rates, and a low cluster in the northwestern part of the state surrounded by high rates. For prostate cancer, we observed clusters using both methods, with a cluster of high rates in the west central part and a low cluster in the southeastern part of the state. For breast cancer, we observed a two county cluster of high rates in the west north central part of the state and a low cluster in the southeastern part of the state. For colorectal cancer we observed a low cluster in the eastern surrounded by counties with very high rates and in the western part of Oklahoma we see high clusters surrounded by low counties.

Conclusion

Our analyses revealed spatial clusters in Oklahoma that need to be investigated further. Evaluating clustering of cancer at the county level is important to generate hypotheses related to cancer prevention, screening and control efforts. We used multiple geospatial analyses to define an aggregated cluster of counties suffering from high or low cancer rates. We will continue our data exploration by conducting geographically weighted regression using a range of socio-economic and healthcare related independent variables to investigate local relationships.

DIFFERENCE OF MODIFIED FRAILTY INDEX IN HPV (+) AND HPV (-) OROPHARYNX SCCA AND OTHER SITES

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Purpose/Objectives

The Modified Frailty Index (mFI) was developed to attempt to better predict outcomes based upon 11 variables. It has been used in a limited number of studies but has shown value in predicting morbidity and mortality in Inpatient Head and Neck surgery patients. The human papilloma virus (HPV) has been associated with up to 80% of current oropharyngeal Squamous Cell Carcinoma (SCCa), while sites other than the oropharynx have much lower rates. HPV (+) patients have improved outcomes with better survival than HPV(-) oropharyngeal cancer patients or patients with SCCa at other primary head and neck sites. HPV(+) oropharyngeal cancer patients are more likely to be male and on average, younger. HPV(+) oropharyngeal cancer patients are less likely to smoke or consume alcohol. No prior studies have evaluated the prognostic value mFI in patients with oropharynx cancer based upon HPV status. Oropharyngeal SCCa is often treated with chemotherapy and radiation, where successful timely completion of therapy influences outcomes. If there is a significant difference in frailty between the groups, then the mFI may be studied for it's predictive value in successful outcomes with chemoradiation. We hypothesize that oropharyngeal HPV(+) patients will have a lower mFI than oropharyngeal HPV(-) patients and other head and neck cancers.

Materials/Methods

A retrospective chart review of patients identified from weekly tumor board lists at our institution from January 2013 through June 2017 was conducted. Eighty-six previously untreated patients were identified with confirmed HPV status for oropharyngeal tumors. Thirty-five were diagnosed with HPV (+) oropharyngeal SCCa, while 20 were HPV (-) oropharyngeal SCCa, and 31 were diagnosed with other upper aerodigestive tract SCCa. The mFI was calculated for all patients and results stratified and compared between these 3 groups.

Results

The mean mFI was different between the groups; HPV(+) oropharynx cancer was 0.075 (0.10), HPV(-) 0.13 (0.091), and other sites 0.13 (0.12). The difference in mFI between HPV(+) and HPV(-) groups was significant ($p=0.048$). The difference in mFI between HPV(+) and other sites was significant ($p=0.047$).

Conclusion

There is a statistically significant difference in mFI between patients based on HPV status. Since treatment outcome in head & neck cancer depend on the ability to tolerate timely treatment, mFI should be studied as a potential predictor of timely completion/outcomes of chemo-radiation therapy in oropharyngeal cancer patients as frailty may be a factor in the improved survival seen in HPV (+) patients. The significance of mFI may evolve and should be evaluated in ongoing clinical trials to determine it's overall predictive value.

Funding for this project was provided by the Department of Otolaryngology at the University of Oklahoma Health Sciences Center

SINGLE-PROBE MASS SPECTROMETRY OF NON-ADHERENT LEUKEMIA CANCER CELLS

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Intracellular compounds are traditionally analyzed through cell lysate experiments, which extract content from a large population of cells. This masks cellular heterogeneity, so to explore the differences of each individual cell single-cell analysis must be used. The current method employed by the Yang lab is Single-probe mass spectrometry. The Single-probe is a multifunctional device with a tip of ~10 µm to puncture individual cells. It easily couples to a mass spectrometer for the analysis of intracellular compounds (including cellular metabolites and drug compounds) from live, single cells under ambient conditions. However, a drawback of this method is that it is primarily used on adherent cells, which is not representative of patient cell samples.

This project adapts Single-probe mass spectrometry for use on cells in solution. Non-adherent single cells are analyzed by suctioning a single cell with a single-bore glass probe and transferring it to the Single-probe for analysis. The human leukemia cell line, K562, was used as a model for the suspension cell setup. In the future, testing will be done on samples obtained from bladder cancer patients to explore cellular differences between cancer cells and healthy cells. The amount of drug and its active metabolites can be monitored to determine the effectiveness of chemotherapeutic agents from patient to patient and lead to more individualized cancer treatment therapies in the future.

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DELINEATING THE ROLE OF THE TMEFF2 TRANSCRIPT IN ANDROGEN SIGNALING

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Funding sources: SCC start-up

Prostate cancer (PCa) is a common malignancy that is one of the leading causes of cancer related deaths in men. The majority of PCa cells have a dependency on androgen signaling for proliferation and survival. This dependency is exploited in the treatment of PCa with androgen deprivation therapies. While androgen deprivation is initially successful in the treatment of PCa, the majority of patients eventually relapse with an aggressive, resistant and fatal form of the disease, castration-resistant prostate cancer (CRPC). Many CRPC cells are able to maintain androgen signaling in the presence of castrate androgen levels, via mechanisms that include: constitutively active alternative spliced isoforms of the androgen receptor (AR) (including AR variant 7 (AR-V7)), intratumoral hormone production, AR mutations and amplification, and altered expression of AR coregulators. For this reason, the identification of genes that act as coregulators of androgen signaling is likely of great importance for future treatment strategies in advanced PCa.

The transmembrane protein with EGF-like and two follistatin like domains 2 (TMEFF2) is an androgen regulated gene that is primarily expressed in the adult brain and prostate, and its expression is altered during the progression of PCa. Our lab has demonstrated that TMEFF2 can function as a tumor suppressor through modulating PCa cell migration and invasion, and by inhibiting tumor growth in mouse allografts. Here, using both antisense-mediated knockdown and CRISPR-mediated knockout approaches, we show data indicating that the TMEFF2 RNA, but not the protein, functions as a vital activator of androgen signaling in PCa cells. Repression of the TMEFF2 transcript, but not the protein, results in lower AR protein levels and a potent inhibition of androgen regulated gene expression. Importantly, this critical function of the TMEFF2 transcript in androgen signaling is maintained in CRPC cells and in AR-V7 expressing cells, indicating that the TMEFF2 transcript could be a potential therapeutic target for inhibiting androgen signaling in CRPC. We suggest that in addition to its well characterized function as a coding mRNA, the TMEFF2 transcript performs a critical function in androgen signaling as a coregulatory long non-coding RNA.

DEVELOPMENT OF A SPATIOTEMPORAL DATABASE OF POTENTIALLY CARCINOGENIC ENVIRONMENTAL EXPOSURES

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The purpose of this project was to develop a spatiotemporal database of potentially carcinogenic exposures for the state of Oklahoma. The main categories of data collected included exposures found in water (n=91), air (n=236), land (n=26), and industry (n=32). Data collected for the exposures database originated from a variety of local, state and national government departments and agencies. Most of the data from the governmental departments and agencies were in spatial format. Extracted data not available in this format was spatialized using latitude and longitude information provided. We organized information of the data collected into a metadata document, which included the spatial representation, attributes, temporal extent, special extent, source, publication date, data extraction location, and our processes (e.g. spatialization). The temporal information in the data, if available, was important due to its use in determining when exposure began. Several administrative and natural boundaries that are helpful in analyses were also included in the database. The primary result of the project was the collection of a wide range of GIS exposure data from a variety of sources that would assist investigators in identifying the geographic and temporal distribution of potential carcinogens and agents that might cause other diseases in Oklahoma. We plan to use this information to analyze the relationship between environmental exposures and cancer in Oklahoma in future studies.

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ENHANCING EXECUTIVE FUNCTIONS AND PREVENTING CANCER-RISK BEHAVIORS AMONG HISPANIC ADOLESCENTS: NCI RESEARCH PROPOSAL

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It is generally agreed that adolescents engage in cancer-risk behaviors (CRB) such as smoking, drug use, risky sex, etc., partly because of inadequate self-control and more specifically of executive-function (EF) constraints such as impulsivity or poor inhibitory control, limitations in working-memory capacity and in cognitive flexibility. To some extent, these limitations reflect the protracted development EFs, reaching full maturity by the mid-twenties. Accordingly, a number of recent studies have attempted to enhance or augment the adolescents' EFs by means of training in order to improve CRB prevention, and the results are quite promising. One variable that seems to determine the effectiveness of EF training is the relevance or similarity (physical or semantic) of the stimuli presented in the EF task (e.g. beer picture) to the stimuli that activate the specific CRB (e.g., drinking) in real-life situations. To regulate CRBs, role models (e.g., parents, physicians) or cohorts rely mainly on verbal directives intended to impede (e.g., quit smoking!) or instigate (e.g., smoking is cool!) such behaviors. The directives voice tone (lenient, stern, angry, etc.) signifies whether compliance is optional or mandatory, and the implicit assumption is that these directives will activate the adolescents' EFs (e.g., inhibitory control), which in turn determine whether or not adherence is achieved. Because the cognitive processing of verbal directives depends heavily on language skills, one hypothesis in this study is that, in speakers of English as second language (e.g., Hispanic adolescents), the activation of executive functions by English verbal directives may be deficient, resulting in inadequate regulation of CRBs. A second hypothesis is that this inadequacy can be reduced by training Hispanic adolescents to perform executive-function tasks in which the stimuli consist of verbal directives similar to those used by role models to prevent CRBs (e.g., "quit smoking!"). The present project has three main goals. 1) To measure and compare inhibitory-control, selective- attention, and cognitive-flexibility skills of Hispanic and white adolescents in EF tasks presenting verbal directives with conflicting loudness, laterality, or stimulus-response mapping; one set of the verbal directives deals with smoking behavior and the other with risky sex behavior. 2) To train Hispanic adolescents in the EF tasks to determine if executive-function performance improves with training. 3) To develop fully automated EF testing and training modules that can be accessed from distant locations via internet connectivity, so that adolescents could get training at home, school, or elsewhere. The results of the proposed study could help to improve the prevention of CRBs in Hispanic adolescents.

RATES OF DEPRESSION IMPROVE AFTER TREATMENT COMPLETION AMONG PATIENTS WITH CERVICAL CANCER

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Objectives: Though underdiagnosed, depression is associated with poorer survival in cancer patients. Among women with cervical cancer (CC), rates of depression are high. This study sought to identify risk factors for depression and to characterize the course of depression throughout treatment among patients with CC.

Methods: We conducted a retrospective IRB-approved analysis of patients with CC at a single institution from June 2016 to April 2017. All patients were screened for depression with the Primary Care Evaluation of Mental Disorders' Patient Health Questionnaire-9 (PHQ-9) at diagnosis and after treatment. A score of 10 or greater was considered a positive screen. Demographic, clinical, and pathologic data were analyzed with standard descriptive statistics.

Results: Of the 39 pts who met inclusion criteria, median age was 46 (range 25-67), 32 (82.1%) were white, and 13 (33.3%) were stage IB2. At the time of diagnosis, the median PHQ-9 score was 9 (range 0-21) and 18 patients (46.1%) screened positive for depression with a PHQ-9 score $\geq$ 10. Risk factors for a positive screen at diagnosis include a prior history of major depressive or anxiety disorder ($p<0.05$). There was no difference in PHQ-9 scores when stratified by age, race, cancer stage, performance status, or treatment received (all $p>0.05$). Following treatment completion, the median PHQ-9 score was 7.5 (range 0-17), which was significantly lower than the score at the time of diagnosis ($p=0.028$). There were no identified risk factors for a positive screen after treatment completion (all $p>0.05$).

Conclusions: A large number of CC patients have depressive symptoms at the time of diagnosis, and a prior history of depression or anxiety increases the risk. A In our population, depression screening scores improved following treatment completion. Further research is warranted regarding how to improve the psychosocial welfare of CC patients during their therapy.

X -RAY-INDUCED ACOUSTIC TOMOGRAPHY (XACT): 3D IMAGING FROM SINGLE X-RAY PROJECTION

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The detection of cancer early in its development remains to be a pervasive challenge of modern medicine. Current cancer screening methods such as mammography have been extremely useful in the detection of cancer in its preliminary stages, but these methods have unfortunate limitations such as the shear amount of false-positive results, particularly in the case of ductal carcinoma in situ (DCIS), where the cancer is detected by the presence of micro-calcifications, small accumulations of calcium. According to the National Cancer Institute, more than 50% of women screened annually in the United States will at some point in their lives receive a falsepositive result for breast cancer. Improved screening methods are therefore essential to preventing these unfortunate calls. Also, absorption based CT imaging has been an invaluable tool in medical diagnosis such as bone density mapping. However, CT requires a large set of projection data and high radiation dose to achieve superior image quality. Taking up this challenge, we present a new imaging technique, X-ray induced Acoustic Computed Tomography (XACT), which, unlike mammography, creates three- dimensional images of tissue, yielding vastly more information than conventional mammography. XACT runs off a groundbreaking new physics discovery, that X-rays can generate ultrasound waves within tissue. In conventional mammograms, X-rays are used to generate mere two-dimensional images of breast tissue. XACT, in contrast, uses a much smaller X-ray dose to generate three-dimensional images via acoustic waves. X-ray Induced Acoustic Tomography (XACT) as a new imaging modality takes advantages of high sensitivity to X-ray absorption and high ultrasonic resolution in a single modality. The deeper penetration of X-ray induced acoustic wave has brilliant potential to get the bone density mapping with less radiation dose.

Using XACT, we have demonstrated the successful reconstruction of gold fiducial markers and a lead image of the OU logo using an effective new system. Simulation results have also illustrated that the XACT system has the ability to detect micro-calcifications as small as 100μm in size within breast tissue. The dose required by the proposed XACT configuration was calculated to be 0.4mGy for a 4.5cm-thick compressed breast. This is one-tenth dose level of a typical two-view mammography for the breast with same compression thickness. The secondgeneration XACT system has been built to improve the imaging speed of the system. The fast XACT system employs ring array consisting 128 transducers as well as 128 channel pre-amplifier which effectively reduce the imaging speed by eliminating the mechanical scanning process.

Our study results indicate that this imaging device and methodology provide a rapid and high-resolution approach for imaging dynamic information, and may have potential for becoming a new promising noninvasive imaging modality used in future application The authors gratefully acknowledge the PHF Team Science Grant Program, and the Diabetes CoBRE Pilot Project from University of Oklahoma health sciences center, and College of Engineering for funding.

RECENT TRENDS IN PANCREATIC CANCER INCIDENCE

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PURPOSE: The aim of this study was to describe the recent trends in the pancreatic cancer incidence in the United States (US) and, in particular, to focus on regional disparities among American Indians/Alaska Natives (AI/AN) compared to whites in different Indian Health Service (IHS) regions.

METHODS: We obtained the age-adjusted pancreatic cancer incidence data and the corresponding confidence intervals by race for the years 1999-2013 from CDC Wonder, an online data web application from the Centers for Disease Control and Prevention (CDC). The JoinPoint Regression program was used to determine the changes in trends over time and to compute the Annual Percent Change (APC) across the entire period. A log-linear model was used to approximate a normal distribution for rates from a small population, and to interpret trends in terms of a rate change at a constant percent per year through APC. We defined IHS regions according to IHS Service Areas in the US (Southern Plains, Northern Plains, Pacific Coast, Southwest, East Coast).

RESULTS: We observed that the incidence rates of pancreatic cancer among the AI/ANs varied in different IHS regional plains. Moreover, the results indicated that there are regional differences in the age-adjusted incidence rates in the AI/AN population, ranging from 10.2 per 100,000 in the Southern Plains to 4.9 per 100,000 in East Coast. Joinpoint models for the years 1999-2013 suggested an increased APC for pancreatic cancer incidence rates in AI/ANs living in Southern Plains (APC: 2.22) and South West (APC: 0.98), decreased APC in Northern Plains (APC: -1.72) and Pacific Coast (APC: -1.82), and similar APC in the East Coast (APC: 0.21), though these were not statistically significant changes.

DISCUSSION: The observed results provide a characterization of the nation-wide incidence in the pancreatic cancer incidence rates by race. These results suggest that the AI/ANs residing in the Southern Plains had higher annual percent increase in pancreatic cancer incidence rate compared to other racial groups. The regional differences may provide an opportunity to further explore other factors and measures of prevention related to pancreatic cancer incidence.

CANCER SURVIVAL DISPARITIES BY RACE AND ETHNICITY IN THE U.S., 2004-2012: AN ANALYSIS OF THE NATIONAL CANCER DATA BASE

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Introduction – The National Cancer Data Base (NCDB) is the largest cancer database in the United States. The NCDB contains information from patients seen in American College of Surgeons Commission on Cancer accredited facilities which make up approximately 30% of the hospitals in the U.S. They treat, however, nearly 70% of all new cancer cases. In efforts to expand research, a de-identified database, the Participant User File (PUF), was made publicly available. In this study, we use the 2013 PUF to evaluate cancer survival among cases in the NCDB from 2004-2012. We focused on survival disparities by race and ethnicity for cancers with a range of survival outcomes. These cancer types include breast, ovarian, pancreatic, and non-Hodgkin lymphoma.

Methods - Data were obtained from the 2013 version of the NCDB PUF, which includes data from cases diagnosed from 2004 to 2012. Race was categorized as white, African American, or other. Ethnicity is dichotomized as Hispanic or non-Hispanic and those without a response were categorized as "other/unknown". Descriptive statistics were calculated for certain demographic variables. Survival time was calculated by subtracting the date of diagnosis from date of last contact or death. The vital status variable was used to indicate censoring. Kaplan-Meier survival curves were created with corresponding log rank tests to compare (1) estimated survival by cancer site, (2) estimated survival by race for each cancer site, and (3) estimated survival by ethnicity for each cancer site. The median survival time and the 5-year survival were calculated and reported for each scenario mentioned above.

Results - For patients with breast, ovarian, and pancreatic cancers and non-Hodgkin lymphoma, who received care at Commission on Cancer accredited facilities from 2004 to 2012, we found that the estimated 5-year survival was 85.8%, 64.0%, 46.8%, and 9.0%, respectively. There were significantly different survival distributions between at least two of the cancer sites (log rank test, $p < 0.0001$). For Kaplan-Meier survival curves stratified by race, we found significant differences between at least two of the survival distributions f breast cancer ($p < 0.0001$), ovarian cancer ($p < 0.0001$), and pancreatic cancer ($p = 0.0021$). For Kaplan-Meier survival curves stratified by ethnicity, we found significant differences between the survival distributions for breast cancer ($p = 0.01$), ovarian cancer ($p < 0.0001$), and pancreatic cancer ($p < 0.0001$).

Conclusion - When stratified by race, we observed survival disparities among African Americans as compared to whites for breast, ovarian, and pancreatic cancer. Survival disparities were also observed for non-Hispanic ethnicities as compared to Hispanics for breast, ovarian, pancreatic cancers and non-Hodgkin lymphoma. No survival disparities were observed for non-Hodgkin lymphoma patients by race or ethnicity.

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SYNTHESIS AND IN VIVO EVALUATION OF NOVEL TC-99M LABELED PEPTIDES FOR IMAGING EGFR EXPRESSION ON PANCREATIC CANCERS BY SPECT

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Background: Epidermal growth factor receptor (EGFR) is a ubiquitous 170-kd transmembrane tyrosine kinase receptor that regulates cell proliferation and survival. It is overexpressed and activated on many epithelial tumor cells including pancreatic cancer cells. Specific EGFR inhibition has been one of the key targets for cancer therapy and has lead to development of several approved anti-EGFR drugs. Thus, imaging EGFR expression in pancreatic patients would allow selecting appropriate patients who could benefit from the anti-EGFR therapy. Our approach for targeting EGFR uses recently identified 12-mer (GE11, YHWYGYTPQNVI) and 16-mer (P75, KYFPPLALYNPTEYFY) peptides that have been shown to bind EGFR with a nanomolar affinity. The main objective of this project was to evaluate rationally designed technetium-99m (140 KeV gamma-ray emitter; half-life – 6 hr) labeled GE11 and P75 peptides for imaging of pancreatic cancers by single photon emission computed tomography (SPECT).

Methods: The GGC-PEG_2-GE11 and GGC-PEG_2-P75 peptides containing a N_3S chelating group and a PEG linker were synthesized on an inhouse developed automated peptide synthesizer. Re(V)-complexation was achieved by transchelation with Re(V)-gluconate. All peptides were purified by HPLC and characterized by high resolution mass spectrometry. The Tc-99m labeling was achieved by transchelation with ^{99m}Tc(V) gluconate and purified by HPLC. Biodistribution and whole body planar imaging studies of the two Tc-99m labeled peptides were performed in Panc1 tumor-bearing nude mice.

Results: The free and Re(V)-complexed peptides were obtained in about 40% and 70% yield respectively. Mass spectral analyses of Re(V)-complexed peptides were consistent with the formation of neutral Re(V)O-N_3S complexes. The Tc-99m labeled peptides were obtained in a decay-corrected radiochemical yield of about 40% with a radiochemical purity of >98%. The Tc-99m labeled peptides were cleared efficiently from blood predominantly by renal-urinary pathway with <2 %ID/g remained at 1 hr p.i. A tumor uptake of <0.4 ID/g was observed with significant difference between the peptides. This tumor uptake was lower than expected, which could be due to under developed vasculature in these tumors. To determine the EFGR specific uptake in any tissue, we conducted a blocking biodistribution study by co-injecting an excess of P75 (100 μg) along with ^{99m}Tc-GGC-PEG_2-P75 at 1 hr p.i. No significant reduction of uptake in tumor or any tissue except stomach was observed. Planar imaging conducted in Panc-1 tumor bearing mice at 1 hr p.i. with both the Tc-99m labeled peptides did not show any accumulation in tumors, which also confirms the biodistribution studies.

Conclusion: Results obtained in this project indicate that the expression of the EGFR in Panc- 1 tumors may be low for using peptide-based nuclear imaging methods.

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EPIGENETIC CONTROL OF DNA REPAIR

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Chromosome translocations are the most common genetic aberrations found in human cancer. These translocations start with the formation of persistent DNA double-strand breaks (DSBs) at actively transcribed areas of the genome, and can be repaired by two distinct pathways, homologous recombination (HR) and non-homologous end joining (NHEJ). However, DSBs formation and DNA repair occur in the context of chromatin, a structure that impedes efficient recognition and repair of damaged DNA. How chromatin characteristics such as histone epigenetic marks, and chromatin remodeling control the recruitment and activity of DNA repair factors at genomic fragile sites is unknown. Previous studies showed that PBAF chromatin remodeling complexes function as tumor suppressor and promote DNA repair through HR. Here we focus on the role of PBAF (Brg1 catalytic, and Baf200/Baf180 regulatory subunits) in DSB repair efficiency and pathway choice. Our studies employ a recently developed cell system in which DSBs can be induced at hundreds of defined sites in diverse chromatin environments. We generated genome-wide maps of PBAF subunits and HR (Rad51) and NHEJ (Xrcc4) DNA repair factors. Comparison of these maps allows us to determine whether specific repair factors are recruited to PBAF-bound sites. We found that PBAF is present at promoter regions of actively transcribed genes even in absence of DSBs (196 sites analyzed). Both Rad51 and Xrcc4 were also recruited to these sites after DSB induction. To assess the role of PBAF in the choice of DNA repair pathway at specific DSBs, we generated genome-wide profiles of Rad51 and Xrcc4 in Brg1-depleted cells, created with RNAi, and compare them with profiles from wild type cells. We observed that the loss of Brg1 near abolish the recruitment of Rad51 but not Xrcc4 at the selected DSBs, suggesting that Brg1 specifically promotes HR repair of DSBs at active transcribed genomic areas. We will discuss these results and other ongoing experiments in the context of the following model: chromatin characteristics, such as epigenetic marks, around DSBs control DNA repair pathway choice and repair efficiency by affecting the recruitment and stability of PBAF.

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PATIENT AND TUMOR CHARACTERISTICS BETWEEN CARCINOEMBRYONIC ANTIGEN-PRODUCING AND NON-PRODUCING COLORECTAL TUMORS

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Serum Carcinoembryonic Antigen (CEA) is a tumor marker often found to be elevated in colorectal cancer patients. Elevated CEA has been strongly associated with poor prognosis. However, not all colon cancer tumors produce CEA, and little is known about the patient and tumor characteristics between CEA-producing and non-CEA-producing tumors.

We performed a retrospective analysis of all patients (n=120,571) diagnosed with colorectal adenocarcinoma from 2010 to 2014 in the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) database. All patients were designated as either positive/elevated (C1) or negative/normal (C0) based on the pretreatment serum CEA level.

We performed univariate and multivariate analyses to identify variables associated with CEA-producing tumors.

Of the 68,856 (57.1%) with available CEA information, 33,426 (48.5%) were C1. Median age was 65 years and 36,479 (53.0%) were male. Compared to C0 cancers, C1 cancers were significantly more likely (P<0.001) to be female (49.3%% vs. 47.9%), black (58.4%), separated or never married (55.0% and 54.8%, respectively), higher grade (39.0%, 46.2%, 51.1% and 52.3% of well, moderately, poorly, and undifferentiated cancers, respectively), and of signet ring cell histology (61.1%). Multivariate analysis showed age, sex, race, marital status, and TNM stage to be independent prognostic factors associated with the diagnosis of CEA-producing tumors. This is the first report to describe differences in patient and tumor characteristics between CEA-producing and non-CEA-producing tumors in a large American population.

About half of all colorectal adenocarcinomas are associated with elevated pre- treatment serum CEA levels. CEA-producing tumors are associated with female gender, non- Caucasian race, separated or never married marital status at diagnosis, and more extensive local, regional and metastatic disease.

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AN INITIAL EXAMINATION OF MICROFLUIDIC DEVICES AND THEIR ROLE IN STUDYING GLIOBLASTOMA

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Introduction. Glioblastoma multiforme (GBM) remains the most common primary brain cancer in adults. With current treatment protocols, the median survival for patients remains dismal at 15 months. The National Cancer Institute estimates that 15,000 individuals previously diagnosed with GBM died in 2015 alone. Tumor heterogeneity and aggressive migration are two distinct characteristics of GBM that are directly related to the high mortality seen in GBM cancer patients. To enhance our knowledge of the disease and pave the way for new therapies to target this malignancy, new technologies are necessary. Here we report the use of microfluidic devices to study GBM.

Methods. Microfluidic devices consist of a seeding chamber with three or more receiving chambers connected by microchannels, also known as a flower design. Using commercially available GBM cell lines (G55, U87, U251) as well as patient-derived tumor cell lines, we have used these devices to perform cell sorting, develop assays to measure migration velocity, monitor the effects of chemo-radiation, and study the genomic and proteomic characteristics of cells in transit.

Results. In one assay using (Z)-3, 5, 4'- Trimethoxystilbene (Z-TMS), an inhibitor of microtubule polymerization, G55 cell migration slowed considerably compared to control. In another assay, U87 cells transfected with EMR3, a gene known to promote an aggressive invasive phenotype in GBM, migrated faster than control. Other assays looking at the effects of cell sorting, chemo-radiation and the genomic and proteomic profiles of migrating cells are ongoing.

Conclusions. Microfluidic devices are very useful tools for separating and characterizing migrating cells. With the advent of microfluidic devices, new experiments related to GBM heterogeneity and migration can be explored, paving the way for new therapies to treat this deadly disease.

CHEROKEE NATION NATIVE AMERICAN RESEARCH CENTER FOR HEALTH

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Overview: Cherokee Nation and the University of Oklahoma Health Sciences Center (OUHSC) are collaborating on a National Institutes of Health-funded Native American Research Center for Health (NARCH) grant. The Cherokee Nation and its research intensive partner, OUHSC, are working collaboratively to help the Tribe gain the analytical capacity that is needed to monitor and ultimately reduce health disparities and conduct community-based research to improve the health and wellbeing of patients served by the Cherokee Nation Health System. In addition to an Administrative Core (Project Directors: Dr. Khan and Dr. Doescher), the Cherokee Nation NARCH includes two projects: 1) Cherokee Nation Health Analytics Core (CNHAC) (Project Directors: Dr. Janitz and Dr. Khan), and 2) Breast cancer patterns of care and outcomes by diabetes status among American Indians in the Cherokee Nation (Project Directors: Dr. Martinez and Ms. Deerinwater).

Cherokee Nation Health Analytics Core (CNHAC): Cherokee Nation is collaborating with investigators from the OUHSC College of Public Health to build capacity for the Cherokee Nation to conduct comprehensive cancer research. The collaborative partners will link Cherokee Nation Cancer Registry data with Cherokee Nation electronic health records and the MyHealth Access Network, Inc. records to evaluate cancer treatment, comorbidities, behavioral factors, and outcomes. This innovative project will build the capacity of Cherokee Nation to monitor and better address the burden of cancer within its service population.

Breast Cancer Patterns of Care: Evidence suggests the prognosis of breast cancer in women with type 2 diabetes mellitus (T2D) is worse than that of women without T2D. This pilot study will link data from the Cherokee Nation Cancer and Diabetes Registries to medical records through Cherokee Nation Electronic Medical Records and a large health information exchange, which will establish a new and comprehensive database that will provide the opportunity to explore these comorbidities and study the diabetes-related factors placing American Indian/Alaska Native women at highest risk of poor breast cancer outcomes

Summary: Successful attainment of these goals will provide a platform for future interaction among the Cherokee Nation, the University, and the larger community of population health researchers. Specific, empirically based, scientific investigation of health issues affecting Cherokees will provide accurate and meaningful data that will directly and positively impact health status and health care delivery among Cherokee citizens. This model will in turn guide researchers and the medical community in addressing health disparities among American Indians/Alaska Natives and other underrepresented minorities throughout the country.

UNDERSTANDING THE ROLE OF ALCOHOL CONSUMPTION IN WATERPIPE TOBACCO SMOKING TOPOGRAPHY, TOXICANT EXPOSURE, AND SMOKING EXPERIENCE

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Introduction: Concurrent alcohol consumption and waterpipe (WP) tobacco smoking is common. WP smokers are more than twice as likely to use alcohol and alcohol use during WP sessions is associated with greater smoke exposure. However, no research has directly examined the impact of alcohol consumption on WP smoking topography, toxicant exposure, and subjective smoking experience in a controlled laboratory setting.

Methods: Dyads of WP smokers (N = 32) completed two in-laboratory smoking sessions (placebo vs. active drink [sustained BrAC = .08]) in a randomized crossover design. Following drink consumption, participants smoked WP for up to 2 hours. Exhaled carbon monoxide (eCO) was assessed pre- and post-smoking session. Questionnaires assessed subjective smoking experience at post-session during each visit and smoking topography was measured continuously throughout each smoking session.

Results: When consuming active drinks, participants reported a greater desire/urge and need to experience the session again (ps < .05) compared to when consuming placebo drinks. While eCO boost did not differ significantly between sessions, participants smoked significantly longer during the active session (p = .001). While not significant, measures of total number of puffs, average flow rate, and total inhaled volume were also approaching significance (ps < .10) such that all measures were greater during the active drinks session compared to the placebo drinks session.

Discussion: The current study is the first to assess the impact of alcohol consumption on WP smoking topography, toxicant exposure, and subjective smoking experience. Findings that alcohol consumption was consistently associated with an enhanced and longer smoking experience, indicate a potential need for regulations of alcohol sales in WP lounges. The findings also have implications for individual interventions targeting WTS and may suggest a need to incorporate a discussion of alcohol use during WP smoking sessions.

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THE IMPACT OF SURGICAL DELAY ON BREAST CANCER PROGNOSIS: A SEER-MEDICARE ANALYSIS

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Delays in initial surgical intervention for breast cancer patients are increasingly common. Although physicians recognize delays in care are to be avoided whenever possible, there are currently no consensus guidelines regarding the allowable time between diagnosis and initial surgical treatment, and important questions regarding the prognostic impact of this interval remain.

We analyzed the presence and effect of surgical delay in breast cancer using a retrospective cohort from the SEER-Medicare Database who were diagnosed with early stage invasive breast cancer between January 1, 2003 and December 31, 2013 and received surgery as their first treatment. An adjusted Cox proportional hazards regression model was used to analyze the relationship between Breast Cancer Specific Mortality (BSCM) and time to surgery (TTS). We examine whether the prognostic implications of TTS are function of tumor characteristics, such as nodal or hormone receptor status.

We found that TTS significantly impacted BCSM, with a cumulative increase in risk. Clinical guidelines for TTS should be implemented to improve breast cancer survival among early stage women.

OPTIMIZATION OF FOLATE RECEPTOR-TARGETED DELIVERY OF FAR-RED LIGHT-ACTIVATABLE PRODRUGS TO OVARIAN CANCER CELLS

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Background and Objectives: We developed a far-red light-activatable prodrug which combines photosensitizer (phthalocyanine, Pc) and Paclitaxel (PTX) with a singlet oxygen-cleavable linker (L). The prodrug was further conjugated with folic acid (FA) using polyethylene glycol (PEG) spacer of different length for better solubility and target delivery to localized tumor. Our prodrug demonstrated fluorescence imaging capability, light-controlled local PTX release, and combined effect of photodynamic damage and site-specific chemotherapy. In this study, we evaluated impact of the length of PEG spacer on folate receptor-mediated uptake in vitro. Then, we investigated in vivo pharmacokinetics (PK) and biodistribution of prodrugs to find out an appropriate formulation.

Methods: An optimal folate conjugated prodrug was selected based on its spacer length (0K to 5K), formulation and particle size (100-180 nm). The time-dependent intracellular accumulation of the folate-conjugate (0.5 - 5 μM) with or without excess FA were measured via fluorescence plate reader to quantify folate receptor-mediated uptake. PK and tissue distribution studies were performed after intravenous dose of 2 μmol/kg in balb/c mice bearing colon 26 cancer cell. The prodrug dispostion and target-mediated intracellular trafficking was characterized by using a physiologically-based pharmacokinetic (PBPK) model.

Results: The receptor mediated uptake was strongly influenced by the length of the spacer. The optimal length was 1K-3K. 2K prodrug showed a 13 fold higher uptake than folate conjugate without a spacer. For 2K folate conjugate, the receptor mediated uptake was more dominant at high dose (83%) than low dose (46%). 2K prodrug showed a long retention (~ up to 48 hr) in tumor probably because of folate receptor mediated uptake. However, high accumulation in liver and spleen was observed which might attibute to heterogeneous distribution of large particle sizes (>100 nm). By make the formulation in smaller size (1-10 nm), we could reduce accumulation in non target tissues such liver, spleen, and muscle.

Conclusions: Our result indicate that the appropriate PEG chain length is around 1K to 3K, with 2K the best. Particle size of prodrug formulation had a great influence on the biodistribution of the prodrug.

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A ZINC-DEPENDENT INTEGRIN-PAXILLIN-GSK-3B SIGNALING AXIS MEDIATES CELL ADHESION AND TUMOR GROWTH OF PANCREATIC CANCER

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Background: Cell adhesion plays a critical role in the development of pancreatic cancer. Integrins have been reported as a mediator of adhesion dependent growth of tumor cells. Cell adhesion could be induced by dose dependent zinc ion. In this study, we aim to investigate whether dysregulated zinc transport impacts the cell adhesion and tumor growth of pancreatic cancer, and whether targeting the zinc transport pathway has therapeutic potential metastatic pancreatic cancer.

Methods: Human pancreatic cancer cells AsPC-1, Panc-1, and MIA PaCa-2 were chosen for adhesion and tumor growth study. ZIP4 overexpression or knocking down stable cell lines were constructed in MIA PaCa-2, AsPC-1 and Panc-1 cells. Zinc-depleted medium was used and exogenous zinc at different concentration (0.25, 0.5, 1 and 2μM of $ZnCl_2$) was supplemented. MTT assay was performed to measure the cell adhesion. Correlations between ZIP4, integrins and downstream targets Paxillin and GSK-3β were investigated with Western blot. Orthotopic xenograft model was used for in vivo studies.

Results: We found that ZIP4 could induce cell adhesion and tumor growth of pancreatic cancer cells. Integrin-α3 and integrin-β1 were upregulated by ZIP4. Knocking down integrin-α3 and integrin-β1 diminished cell adhesion and tumor growth even in the presence of high ZIP4 in MIA-ZIP4 and Panc-ZIP4 cells. We also found that knocking down integrin-α3 and integrin-β1 inhibited pPaxillin but enhanced pGSK-3β which are downstream targets of integrin in MIA-ZIP4 and Panc-ZIP4 cells. We found ZIP4 promoted cell adhesion and tumor growth in a zinc dependent manner: Low concentrations of zinc accelerated ZIP4 induced adhesion and proliferation while high level of zinc attenuated ZIP4 induced cell adhesion and proliferation. And expression of integrin-α3, integrin-β1 was mediated by ZIP4 and zinc is required for ZIP4-integrin upregulation in pancreatic cancer.

Conclusion: High level of zinc transport ZIP4 promotes pancreatic cancer adhesion and proliferation in a zinc-dependent manner. ZIP4-integrin-Paxillin, GSK-3β pathway may serve as a novel therapeutic strategy of pancreatic cancer treatment.

IDENTIFICATION OF EGFR AS A VULNERABILITY OF RET KINASE INHIBITOR- RESISTANT NON-SMALL CELL LUNG CANCER CELLS

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Oncogenic fusions of RET protein tyrosine kinase (PTK) are present in lung adenocarcinoma (LAD) and are being targeted in clinical trials. In addition to known RET PTK inhibitors (TKIs) cabozantinib, lenvatinib, and vandetanib, we identified nintedanib as a potent RET TKI. Previous clinical experience in PTK-targeted therapies of non-small cell lung cancer (NSCLC) with EGFR and ALK inhibitors indicates that acquired resistant to PTK inhibitors (TKIs) usually occur within a year after initiation of therapy. Understanding mechanisms of resistance to targeted therapy and identification of the vulnerability of resistant cells are essential to prolong the therapeutic response. To study the RET TKI-resistant mechanisms in NSCLC, we cultured the nintedanib-sensitive human LC-2/ad cells that harbor a CCDC6-RET fusion gene in the presence of nintedanib. Nintedanib-resistant LC-2/ad cells (LC-2/NR) were established. Analysis of cell lysate indicated that the RET kinase was suppressed in LC-2/NR cells. To discover the vulnerability of LC-2/NR cells, we compared the sensitivities of LC-2/ad and LC-2/NR cells to 26 drugs at three concentrations (0.25, 0.5, 1 μM). Consistent with CCDC6-RET suppression in LC-2/NR cells, these cells were resistant to RET TKIs. Interesting, LC-2/NR cells were hypersensitive to all three EGFR TKIs (gefitinib, erlotinib, lapatinib) included in the panel of 26 drugs. Consistently, erlotinib was more effective in inhibiting pERK in LC-2/NR cells than in LC-2/ad cells and induced apoptosis in LC-2/NR cells. Increased pEGFR and EGFR-bound GRB2 were detected in LC-2/NR cells. These results suggest that EGFR is a reserve for supporting cell survival and growth in LC-2/ad cells and renders RET TKI resistance and identify EGFR as a targetable vulnerability of LC-2/NR cells.

SHetA2 VAGINAL SUPPOSITORIES TO TREAT CERVICAL DYSPLASIA: INFLUENCE OF ESTRUS CYCLE ON SHETA2 DISPOSITION

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PURPOSE: SHetA2 is a water insoluble compound with potential to treat cervical dysplasia. We developed a vaginal suppository to overcome the solubility limitations of SHetA2 and increase drug concentration at the cervix. SHetA2 suppositories administered vaginally, achieved cervix concentrations >100-fold above therapeutic levels. However, variability was observed within the same treatment group and time point, which was due to mice being in different estrus cycle stages. Thus, our goal was to evaluate the influence of the estral cycle on drug disposition after vaginal administration.

METHOD: The estrus cycle of FVB female mice was synchronized using the Whitten effect. Vaginal lavages were employed to monitor estrus cycle stage. The stages were confirmed by histological evaluation of the uterus stained with hematoxylin-eosin. SHetA2 suppositories (15 mg/kg) were administered vaginally to groups of mice in the estrus and diestrus stages and gynecological tissues collected after predetermined time points. Drug concentrations were determined in cervix by HPLC and drug disposition was characterized by pharmacokinetic parameters (WinNonlin).

RESULTS: Regular estrus cycle was established in all mice and the cycle stages were identified by comparing the cell composition of their vaginal smears with those in the literature. The cell composition of the vaginal smears is shown in figure 1A. During diestrus stage, the most cells in the smear were leukocytes; during proestrus stage the smear showed cornified epithelial cells, epithelial cells and a few leukocytes, whereas during estrus stage most cells were epithelial cells, and during metestrus stage most cells were cornified epithelial cell and leukocytes. The stages of the cycle were further confirmed by histology based on cell types and luminal appearance of the uterus (figure 1B). The highest cervix concentration (Cmax) in both, diestrus and estrus group, was observed at 0.5h (Tmax) with slightly higher concentrations observed in mice dosed in estrus than those dosed in diestrus (14.91±3.18µg/g and 23.41±14.51µg/g, respectively) (figure 2). Interestingly, $t_{1/2\text{-cervix}}$ in mice dosed during diestrus was 10.07h compared to 2.51h in estrus. Moreover, 12h after drug administration, drug concentrations in the cervix were below therapeutic level (1.6µg/g) in the estrus group (0.39±0.22µg/g), whereas in the diestrus group they were 6.67±3.3µg/g, which is 4-fold higher than the therapeutic concentration. This indicates that if drug is administered during diestrus, its residence time is longer, resulting in a sustained drug concentration at target site for longer time.

CONCLUSION: Drug disposition in the cervix of mice appears to be significantly influenced by the stage of the estrus cycle. The implications of these findings may be different in humans, as the anatomy of the human vaginal epithelium and endometrium do not change as much during the different stages of the menstrual cycle compared to the estral cycle in mice.

SECONDHAND SMOKE EXPOSURE INCREASES CISPLATIN RESISTANCE IN ORAL CANCER CELLS.

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Significance: About 58 million Americans are exposed to secondhand (SH) smoke, and about 41,000 deaths in non-smoking adults and 900 deaths in infants yearly have been associated with SH smoke exposure. With 40.6% of children and almost half of non-smokers estimated to be exposed to SH smoke, there remains a pressing need to study the short and long term effects of SH smoke exposure. Both active and passive (SH) smoking have been associated with head and neck squamous cell carcinoma and other adult and childhood cancers. Moreover, continued smoking after cancer diagnosis increases drug resistance and reduces overall survival rate by about 50%. Yet, the effects of SH smoke on drug resistance are unknown. Here, we examined the effects of SH smoke exposure on cisplatin resistance in oral epithelial cancer cells.

Methods: To assess cisplatin resistance, oral cancer epithelial cell lines were exposed to 10 puffs or 100 puffs/5L of mainstream (MS) and sidestream (SS) smoke extracts for 48 h followed by exposure to both cisplatin (0.1-100 μM) and extracts for 48 hours. Cell viability was assessed by the MTT assay. Cell survival after cisplatin treatment was evaluated by the clonogenic assay. Apoptosis and necrosis was evaluated using Annexin V/PI staining. Gene expression was assessed by quantitative PCR.

Results: After cisplatin treatment, cells exposed to SS smoke extract showed a significant increase in cell viability and IC50 values. Similarly, after cisplatin treatment, cells exposed to SS smoke had significantly higher numbers of colonies compared to control cells. Cells exposed to SS smoke extract and treated with cisplatin showed significantly less apoptosis and necrosis that non-exposed cells. Key multidrug and DNA repair genes were significantly upregulated in SS smoke exposed cells.

Conclusions: Altogether, our data suggest that even short-term exposure to SH smoke can lead to cisplatin resistance in oral cancer cells by dysregulating the cells multidrug resistance,

DNA repair, and cell death pathways. These data has major clinical implications for cancer patients exposed to SH smoke.

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TARGETED PHOTOTHERMAL ABLATION OF BREAST CANCER

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Introduction: The aminophospholipid phosphatidylserine (Ptd-L-Ser) is actively sequestered to the inner leaflet of the plasma membrane of health cells. In a tumor, this tightly regulated Ptd-L-Ser membrane asymmetry breaks down and Ptd-L-Ser is expressed on the outer leaflet of cancerous cells and the vascular endothelium. The presence of Ptd-L-Ser solely within the tumor serves as a tumor specific antigen which can be used in targeted therapeutics. We employ the natural ligand of Ptd-L-Ser, Annexin V (ANXA5), in a protein-nanoparticle conjugate to sensitize tumors to photothermal ablation. [1, 2] The targeted ablation of the tumor in conjunction with α-CTLA-4 immunotherapy generates a powerful in situ cancer vaccine creating a robust immune response rejecting the tumor.

Methods: Mice with well-developed orthotopic syngeneic EMT6 tumors ($d \geq 5$ mm) were administered an intravenous systemic dose of SWNT-ANXA5 which localized to tumor vasculature. Tumors were then irradiated with mild laser light using a Diodevet-50 NIR system for 175 s, at a power density of 1 W cm^2. Select mice additionally received three doses of 200 µg of the immunostimulant α-CTLA-4 in order to prime the immune system prior to photothermal therapy.

Results: The low energy NIR laser does not harm healthy tissue, but rapidly generates temperatures in excess of 60° C within the tumor. This temperature is sufficient to instantly destroy cancerous cells. Healthy tissue fails to accumulate the SWNT-ANXA5 conjugate and remains unaffected by the NIR laser. The addition of α-CTLA-4 immunotherapy in conjunction with this targeted photothermal ablation modality resulted in a synergistic increase in survival.

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REGORAFENIB, A MULTIKINASE INHIBITOR, ENHANCES THE RADIATION RESPONSE IN BREAST CANCER CELLS BY SUPPRESSING DNA REPAIR ANDVEGF-DIRECTED ANGIOGENESIS

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Breast cancer is a heterogeneous disease composed of several subtypes, of which triple-negative breast cancer (TNBC) is particularly aggressive. Regorafenib is a tyrosine kinase inhibitor that targets the activity of multiple protein kinases involved in the regulation of tumor angiogenesis [VEGFR1-3 and epidermal growth factor homology domain 2 (TIE2)], oncogenesis (KIT, RET RAF-1, BRAF) and the tumor microenvironment [PDGFR-β and FGFR]. In the present study we tested the therapeutic effects of Regorafenib in combination with radiation in two TNBC (MDA-MB-231 and SUM159PT) cell lines. Regorafenib treatment reduced cell proliferation and migration in both the cell lines and increased radiosensitization. Clonogenic assay showed Regorafenib suppressed cell survival in both the cell lines with survival at 2 Gy (SF2) reduced from 66% ± 8.9 in the control MDA-MB-231 cells to 38% ± 4.95 ($p=0.05$) in 10μM Regorafenib-treated cells and from 88% ± 1.76 in the control to 75% ± 1.12 ($p=0.003$) in 5μM Regorafenib-treated SUM159PT cells. Assessment for receptor kinases (VEGFR, PDGFR, EGFR and ERK) that were impacted by Regorafenib treatment when used as monotherapy and in combination with radiation showed a marked reduction in the expression of the receptor kinases analyzed. Since, VEGFR-2 is known to play an important role in angiogenesis we focused on evaluating the effect of Regorafenib on secreted VEGF. VEGF expression highly correlates with tumor progression, invasion, and angiogenesis in TNBCs and acts mainly by binding to VEGF receptor family member 2 (VEGFR-2). A significant reduction in VEGF-A production was observed in both the cell lines upon Regorafenib treatment. Further, addition of conditioned medium collected from Regorafenib-treated tumor cells onto human umbilical vein endothelial cells (HUVEC) showed suppression of tube formation, an indicator of angiogenesis inhibition. This interesting finding suggests that Regorafenib can impact tumor angiogenesis and contribute to TNBC growth suppression. We also investigated the underlying mechanism contributing to radiosenstivity of Regorafenib-treated TNBC cells. Comet assay showed Regorafenib suppressed the repair of radiation-induced DNA damage response (DDR) in a time-dependent manner.

In conclusion our study shows Regorafenib exerts its antitumor activity by inhibiting both DDR and VEGF-mediated angiogenesis. Thus, combining Regorafenib with radiation and antiangiogenic agents will be beneficial and effective in controlling TNBC.

Funding: This study was supported in part by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences (P20 GM103639) of the National Institutes of Health (AM), and by startup funds received from the Department of Radiation Oncology, The University of Oklahoma Health Sciences Center.

DESCRIPTIVE AND INJUNCTIVE NORMS OF WATERPIPE TOBACCO SMOKING AMONG COLLEGE STUDENTS

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Introduction: Smoking tobacco via a waterpipe (WP) is on the rise, particularly among college students. One reason for this may be normative perceptions of WP tobacco smoking among this population. The current study examined the perceived and actual descriptive and injunctive norms of WP tobacco smoking among a college student sample.

Methods: Participants were 894 college students enrolled at a large, Midwestern university. Participants completed measures of WP smoking frequency and quantity and perceived/actual descriptive and injunctive norms of WP tobacco smoking.

Results: Over one-third of the sample reported ever trying WP smoking, while only 2% reported current (past month) use. When comparing ever and never WP smokers, ever WP smokers reported greater perceived peer approval of WP tobacco smoking. Both males and females overestimated WP smoking frequency of same-sex students at their university.

Discussion: The current study is one of the first to investigate descriptive and injunctive norms of WP smoking among college students. Students who report WP smoking are more likely to overestimate descriptive norms of WP smoking among their peers, suggesting corrective normative feedback regarding actual use by peers may be an important target for WP intervention among college students. Future research should investigate the temporal association between normative perceptions and WP smoking behaviors among college student

Modeling of Extracellular Matrix Degradation in a Metastatic Tumor Microenvironment Using Compucell3D

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Introduction: Every year, there are 8.2 million cancer-related deaths worldwide. The spreading of tumor cells in a human body, called metastasis, is known to be the primary cause of death. In the early stages of metastatic invasion, tumor cells and cancer associated fibroblasts (CAFs) produce two primary types of chemicals: matrix metalloproteinases (MMPs) and lysyl oxidase (LOX). MMPs degrade the extracellular matrix (ECM) fibers, perforate the basement membrane, and allow tumor cells to escape. LOX crosslinks and aligns the ECM fibers, which generates a pathway for cancer cells to migrate more easily through the ECM and eventually invade into a blood vessel and spread to other locations of the body. The mechanism of such ECM remodeling in the local tumor microenvironment remains ambiguous. Multi-scale computational models can provide a fundamental understanding of how ECM remodeling impacts the physical properties of metastatic cancer cells and the dynamics of migration from the tumor microenvironment and this understanding is of keen interest in cancer research.

Materials and Methods: A computational simulation predicting the changes in the ECM during metastasis is developed using the open-source modeling software package CompuCell3D (CC3D). The biological aspects of the CC3D simulation are implemented based on the Glazier-Graner-Hogeweg (GGH) model. In the GGH framework, the effective energy or Hamiltonian is utilized to define the thermodynamics of behaviors and interactions among cellular and non-cellular elements in the simulation, while the Metropolis algorithm is applied to model the stochastic changes in the cell dynamics. The evolution of one of the chemical signals, MMP, with respect to time is described and implemented in the simulation via a reaction-diffusion partial differential equation (PDE). The PDE accounts for the secretion of MMP due to tumor cells and CAFs and the diffusion and decay of MMP. The PDE was solved simultaneously with the agent-based model by the CC3D program.

Results and Discussion: Trials of Monte Carlo simulations were used to study the probability of possible trends resulting in the proliferation, interaction, and dissemination of tumor cells and the degradation of the ECM fibers corresponding to the changes in the MMP. Our current CC3D simulation describes when tumor cells proliferate and penetrate the basement membrane (Fig. 1A) and when they secrete MMPs (Fig. 1C) to degrade this barrier of fibers. After almost all the basement membrane underlying the primary tumor is degraded, tumor cells begin to detach away from the primary tumor and disseminate through the ECM (Fig. 1B). MMPs then are no longer
secreted by tumor cells and mostly diffuse and decay, resulting in a lowered MMP concentrations (Fig. 1D). Other later interactions between ECM fibers and tumor cells are simulated with the model (results not shown).

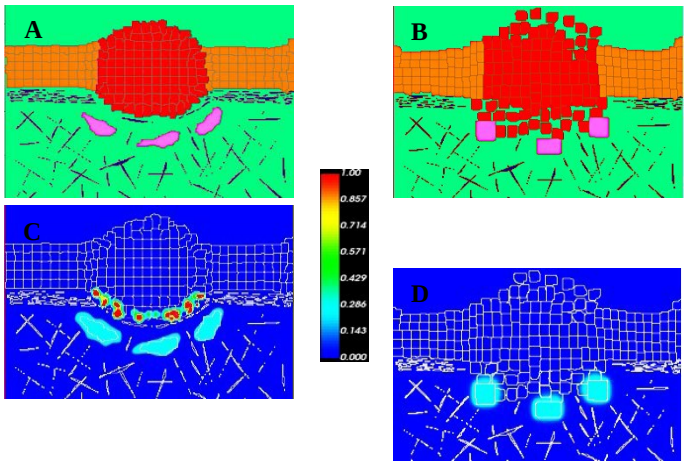

Fig. 1. (A,B) Simulation of the cell fields demonstrates the ECM with a primary tumor (red); an organized normal epithelia (orange); CAFs (pink); basement membrane underlying the primary tumor (horizontal blue lines) and randomly distributed ECM collagen fibers (diagonal blue lines). (C,D) Simulation of chemical field demonstrates the concentration level changes of MMPs over the spatial domain. Note: The rainbow scale shows changes in color go from red to blue corresponding normalized high to low

concentration. (A,C) CC3D simulations at 35 Monte Carlo steps. (B,D) CC3D simulations at 50,000 Monte Carlo steps.

Conclusions: We expect our model could be applicable to potential therapeutics that can prevent or detect such modification in the ECM during cancer metastasis. Unlike experimental approaches, computational models, especially ones built in CC3D, are not difficult to modify. Hence, that allows our CC3D simulation to be easily updated and further developed by adding more complex interactions to the current model implementation considering various cell types, as well as other unaccounted for biological elements and chemical signals and analyzing their effects on the local tumor microenvironment.

HIGH-ENERGY IN-LINE PHASE SENSITIVE X-RAY IMAGING FOR DETECTING BREAST CANCER IN DENSE BREASTS

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Abstract

The objective of this study is to demonstrate the potential of using high energy in-line phase contrast imaging in breast cancer screenings to detect lesions that are indistinguishable by conventional x-ray mammography but are detectable by Supplemental ultrasound screening. Conventional mammography with low energy x-ray is currently being used in clinic and it plays decisive role in cancer detection and subsequent follow-up. On the other hand its effectiveness and performance depends massively on breast density. Therefore it is widely accepted that the screening protocol should include supplemental screening modality when patient has the breasts with higher glandular density. In most of these cases, ultrasound is able to detect the breast lesions which they were subtle in conventional mammography.

We know that when x-ray as an electromagnetic wave passes through the body, it is not only attenuated by tissue but also undergoes a phase change. X-ray phase change in human soft tissue is much greater than x-ray attenuation. Since the difference in attenuation coefficient between normal breast tissue and malignant tissue is very small, adding the phase information to the study seems to be advantageous. In order to explore the advantages of high energy phase contrast imaging to resolve the lesions within the breast with higher density, a custom made introductory x-ray/ultrasound dual-modality phantom that mimics dense breast is used and carbon fiber disks with different diameters and thicknesses were embedded into the phantom. Phase contrast projection was acquired using a prototype at 120kV, focal spot size of 18.3µm with average glandular dose (AGD) of 0.35mGy and R2 approximately equals to 1.5 R1. The conventional x-ray projection was acquired with a bench top system at 40kVp and same AGD.

As the result, the conventional x-ray imaging wasn't able to detect the disks of certain size and thicknesses but phase contrast and ultrasonography were able to detect them under the given experimental condition. This result illustrates that phase contrast imaging was capable of detecting some of the malignant lesions in dense breast which they are not distinguishable by conventional mammography and they need another supplemental screening test. This eventually leads to this fact that phase contrast imaging is most likely satisfactory to utilize the new breast cancer screening test which it merges advantages of both x-ray imaging and ultrasound imaging into one imaging modality with much lower average glandular radiation dose and higher accuracy.

QUANTIFICATION OF ANTICANCER DRUG IN LIVE SINGLE CANCER CELLS USING THE SINGLE-PROBE DEVICE

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Cell-to-cell heterogeneity dictates a multitude of functions for homeostasis and development of disease states. The success in obtaining the concentrations of molecules (e.g. drug compounds) in single cells can potentially revolutionize fundamental research and clinical tests. However, quantitative analysis of species of interest at the single cell level is extremely challenging due to the limited amount of sample present in individual cells and the lack of sensitive bioanalytical techniques. Here, we report a quantitative SCMS (single cell mass spectrometry) method to estimate concentrations of molecules in live individual cells using the Single-probe, a miniaturized sampling and ionization device. In addition, SCMS measurements were compared with results obtained from traditional LC/MS method.

The Single-probe is fabricated by combining a laser-pulled dual-bore quartz needle (tip size smaller than a cell) with a silica capillary and a nano-ESI emitter. In SCMS experiments, the slide containing cells is attached to an XYZ-translational stage for sample movement. Cell insertion is monitored using digital microscopes as the Z-stage is lifted precisely. Solvent containing the deuterated compound (i.e. the internal standard) is continuously pumped into a target cell to extract intracellular compounds. Both target and internal stand molecules are simultaneously detected using MS. Drug concentration in each cell is estimated by accounting for multiple factors, including the relative drug intensities to its internal standard, the concentration of the internal standard, solvent flowrate, data acquisition time, and cell volume.

Plastic slides containing microwells (ID: ~50 μm) were prepared using a micro laser engraving machine. HCT-116 and HeLa cells were used as models, and treated using the anticancer drug Irinotecan. Cell-containing slides were rinsed to remove drug molecules on the cell and slide surfaces prior to measurement. Single cells inside microwells (i.e. one cell/microwell) were selected for quantitative SCMS measurements.

We collected more than 8 sets of data from both cell lines treated under different conditions, and 30-33 cells were measured for each set of SCMS experiment. Drug concentrations from SCMS results exhibit a broad ranges of values. T-test was conducted to find the differences among data sets. A significant difference ($p < 0.001$) was observed between HeLa and HCT-116 cell lines in response to drug treatment. The average Irinotecan concentrations in individual HeLa cells are lower than those in single HCT-116 cells treated under the same conditions (100 nM for 0.5 and 1 hour; 1 μM for 0.5 and 1 hour). This indicates that HeLa cells absorb Irinotecan slower than HCT-116 cells. Furthermore, for HCT-116 cells, the average Irinotecan concentrations in single cells are similar for 0.5 and 1 hour treatments under the same concentrations (100 nM and 1 μM), suggesting that HCT-116 cells establish equilibrium quicker than HeLa cells.

Significantly different results between SCMS and LC-MS experiments were observed. For example, for 100 nM treatment (1 hour), 16.55 ± 13.38 μM and 0.72 ± 0.09 μM were obtained from SCMS and LC-MS experiments, respectively. Similarly, under 1 μM treatment (1 hour), averaged values of 10.82 ± 12.4 μM and 2.01 ± 1.29 μM were obtained from SCMS and LC-MS measurements, respectively. The difference between these two methods is likely due to the heterogeneity of cells, and the possible drug loss during the LC-MS sample preparation.

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REGORAFENIB TREATMENT MODULATES YAP1 EXPRESSION IN LUNG CANCER

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Introduction: Yes-associated protein 1 (YAP1) is a transcriptional effector of the Hippo pathway that regulates organ size at homeostasis and tumorigenesis. Overexpression and nuclear accumulation of YAP1 has been reported in various cancers including non-small cell lung cancer (NSCLC) and is associated with tumor initiation and progression. Further, YAP1 has been implicated in drug resistance and metastasis making it an attractive molecular target for NSCLC treatment. Regorafenib, is an oral multi- kinase inhibitor shown to inhibit angiogenesis, and metastasis in various cancers. However, the underlying mechanism of action of Regorafenib for the treatment of NSCLC remains unclear. Here we investigated the efficacy of Regorafenib and its impact on YAP1 in inhibiting human NSCLC cell growth.

Methods: Human NSCLC cells were treated with Regorafenib and the growth inhibitory activity determined by trypan blue method. Molecular studies focused on determining YAP1 expression level and its sub-cellular localization in Regorafenib-treated cells by western blotting and immunocytochemistry. DMSO-treated cells served as vehicle control.

Results: Regorafenib treatment significantly reduced the cell viability of NSCLC cells compared to vehicle control. Western blot demonstrated that Regorafenib treatment increased phosphorylation of YAP1 at Ser127 ($pYAP1^{Ser127}$), indicating YAP1 inactivation. In accordance with this, immunofluorescence staining showed cytoplasmic accumulation of YAP1 in Regorafenib-treated cells compared with vehicle control.

Conclusions: Our study provides evidence that Regorafenib regulates YAP1 localization in NSCLC cells that contributes to its antitumor activity.

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DISCOVERY OF A MYC-DRIVEN PRE-B CELL ACUTE LYMPHOBLASTIC LEUKEMIA ZEBRAFISH MODEL

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Acute lymphoblastic leukemia (ALL) is a cancer of the bone marrow and blood. ALL develops when the bone marrow over-produces malignant immature lymphocytes. Different ALL subtypes are based on the immunophenotype of the leukemia. Precursor B-cell acute lymphoblastic leukemia (pre-B ALL) is one of these subtypes. Overall, ALL is the most common cancer of childhood, and pre-B ALL represents 80-85% of all pediatric ALL cases. Although pre-B ALL is the most prevalent pediatric cancer, the molecular pathways that drive pre-B ALL development and treatment resistance are poorly understand. Zebrafish (*Danio rerio*) are a powerful animal model to study cancer. *D. rerio* cancers have similar gene signatures when comparing zebrafish and human cancers of the same type. As in humans, over-activity of the *myelocytomatosis oncogene* (*MYC*) can drive zebrafish cancer, including T-cell acute lymphoblastic leukemia (T-ALL). In this model, human *MYC* is regulated by the *D. rerio rag2* promoter (*rag2*:*hMYC*), which causes it to be expressed in T and B lymphoblasts. To detect fish with T-ALL, we use a second transgene, *lck*:*eGFP*, that drives high expression of GFP in T cells.

Recently, we found that *hMYC* fish, which were already known to develop brightly-fluorescent T-ALL, also develop dimly-fluorescent pre-B ALL, a novel and exciting finding.

When *hMYC* fish develop pre-B ALL, these pre-B ALL cells express low GFP levels compared to T-ALL cells. Expression studies of GFP-low ALL of *hMYC* fish revealed that they express B cell specific genes, such as *igic1s1*, *cd79b,* and *pax5*. In contrast, GFP-high ALL of *hMYC* fish express T cell genes, like *cd4, cd8a,* and *il7r*. These studies and others confirm that GFP-low ALL in *hMYC* fish are truly pre-B ALL. Histologic studies show *hMYC* pre-B ALL resembles human pre-B ALL morphologically and in terms of its organ involvement. Pre-B ALL are also sensitive to dexamethasone, a drug used for to treat human pre-B ALL. Incidence studies in *hMYC* fish show pre-B ALL has high incidence and short latency, like T-ALL. Collectively, our results suggest *hMYC* induces not only T-ALL, but also pre-B ALL in zebrafish, making it the first potent zebrafish pre-B ALL model. In addition, *hMYC D. rerio* are the only animal model of any kind to develop both pre-B and T-ALL, the two major subtypes of this important human disease.

ENDOGENOUS PRODUCTION OF UHRF1 AND EFFECTS OF E2F TRANSCRIPTION FACTORS AND FGF9 ON UHRF1 mRNA ABUNDANCE IN OVARIAN CELLS

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Ubiquitin-like with PHD and RING Finger domains 1 (UHRF1) plays an important role in tumorigenesis and in cancer development and progression. In fact, UHRF1 upregulation has been detected in various solid and hematological tumors and it is associated with poor prognosis and clinical outcomes; therefore, it is considered to be a novel diagnostic marker of cancer and a prognostic factor. The present study was performed to evaluate the endogenous production of UHRF1 in bovine ovarian follicles according to cell type and size and to determine if fibroblast growth factor (FGF) 9 and E2F transcription factors affect UHRF1 gene expression in ovarian cells. Ovaries from non-pregnant beef heifers were collected from a local slaughterhouse, granulosa cells (GC) were collected from small follicles (<5 mm) and theca cells (TC) were isolated from large follicles (>8 mm) via dissection and enzyme digestion. Cells were plated on 24-well Falcon multiwell plates in 1 ml of basal medium containing 10% fetal calf serum (FCS) to procure optimal attachment. Plates were maintained in a humidified 95% air and 5% CO_2 environment at 38.5 °C changing medium every 24 h. After 48 h, cells were washed twice with serum-free medium and the different treatments were applied in serum-free medium. Cells were collected after 24 h for RNA extraction.

Experiment 1 was conducted using freshly collected GC and TC from small and large bovine ovarian follicles. UHRF1 expression was greater ($P<0.05$) in GC and TC from small versus large follicles. Also, in small follicles, UHRF1 mRNA abundance was greater in GC compared with TC ($P<0.05$). Greater abundance of UHRF1 mRNA in small versus large follicles suggests UHRF1 may play a role in follicular growth and development while its higher expression in GC indicates that GC might be the main cell type responsible for ovarian follicle production of UHRF1.

In experiment 2, GC from small follicles were exposed to the following treatments: control, E2F inhibitor (E2Fi) (50 μM) and FGF9 (30 ng/ml). When compared to control, UHRF1 expression was increased ($P<0.05$) by FGF9 and decreased ($P<0.05$) by E2Fi. In experiment 3, TC from large follicles were exposed to the same treatments as in Experiment 2. Accordingly, FGF9 increased ($P<0.05$) UHRF1 expression whereas E2Fi decreased ($P<0.05$) UHRF1 expression.

These results taken together suggest that UHRF1 expression in both GC and TC might be regulated by FGF9 and E2F transcription factors, which are overexpressed in ovarian cancer. Further studies are needed in order to better clarify the regulation of these factors and how their interaction might promote and affect ovarian cancer development and progression.

PANCREATIC CANCER IN OKLAHOMA'S AMERICAN INDIAN POPULATION: A SINGLE INSTITUTION, RETROSPECTIVE STUDY.

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Background: Evaluation of racial disparities in cancer reveals that American Indians (AI) demonstrate a higher rate in cancer mortality when compared to the Caucasian population. Pancreatic cancer is expected to be ranked 2nd for cancer related death by 2030. Oklahoma has the 2nd largest number of AI in the nation; therefore, a single institution retrospective study was performed at the University of Oklahoma Health Sciences Center (OUHSC) to identify risk factors in AI affecting survival.

Methods: This is a retrospective chart review of AI adult patients with pancreatic cancer who were treated at OUHSC between 2010 and 2015. The primary objective of this study was to evaluate the risk factors affecting overall survival (OS). We examined how treatment, stage, age, gender, body mass index (BMI), marital status and other covariates were related to OS. Simple descriptive statistics were created for all covariates. Kaplan Meier analysis was performed to assess how categorical variables were related to OS. A cox proportional hazards model was used to assess the association of each covariate with survival. A final multivariate model was then created.

Results: We analyzed 35 patients. Median age was 66 years. Median BMI was 25. Males were 49%, females were 51%. Of the entire cohort, 22% used alcohol, 66% used tobacco, 43% had diabetes, 62% were married; 27% were stage 1, 43% were stage 3, and 30% were stage 4 at diagnosis. Fifty one percent had surgery, 46% had chemotherapy, 14% had radiation and 3% had chemoradiation; 81% had adenocarcinoma and 19% had invasive papillary mucinous carcinoma. Fifty-six percent of the lesions were in the pancreatic head, 22% in the body and 11% in the tail. Median OS for stage 1, stage 3 and stage 4 were 40 months, 14 months and 9 months respectively. Median OS was 15 months vs 3 months for married and unmarried patients, respectively. On multivariate analysis, older age at diagnosis and higher stage at presentation resulted in decreased OS. Interestingly, married patients had better survival ($p = 0.0161$).

Conclusions: Our AI patients had similar survival to the Caucasian patients from historical control. On a multivariate analysis, marriage emerged as a positive prognostic factor for OS.

IMPLEMENTATION OF ASK-ADVISE-CONNECT IN A SAFETY-NET HEALTHCARE SYSTEM: QUITLINE TREATMENT ENGAGEMENT AND CESSATION OUTCOMES

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Background: Ask-Advice-Connect (AAC) was designed by our research team to link smokers in primary care settings with evidence-based tobacco treatment. This approach involves training medical staff to *Ask* about smoking status, *Advise* all smokers to quit, and offer to immediately and electronically *Connect* interested smokers with state quitlines through an automated link within the electronic health record (EHR). In a previous group randomized trial conducted in the same safety-net healthcare system, we found that AAC was associated with a 30-fold increase in quitline treatment enrollment compared to providing smokers with quitline referral cards and encouraging them to call on their own (Ask-Advise-Refer). The current study evaluated the impact of AAC in a 34-month implementation trial on treatment engagement and six-month smoking abstinence outcomes.

Design/Methods: AAC was implemented in 13 community clinics within a large safety- net healthcare system in Houston, Texas. Licensed vocational nurses were trained to implement AAC as part of standard care. The names and telephone numbers of smokers who agreed to be connected were sent electronically to the quitline daily, and patients were proactively called within 48 hours. Treatment enrollment and smoking abstinence, defined as self-report and biochemically confirmed via saliva cotinine, were assessed six months following treatment enrollment among individuals who agreed to be contacted.

Results: Smoking status assessments were recorded for 218,915 patients, and 40,888 reported current smoking. The proportion of all identified smokers who enrolled treatment was 11.8%. Self-reported abstinence six months following treatment enrollment was 16.6%, and biochemically confirmed abstinence was 4.5%.

Conclusions: AAC was successfully implemented as part of standard care, and treatment engagement was high. Although self-reported abstinence was in line with other quitline-delivered treatment studies, biochemically confirmed abstinence, which is not routinely captured in quitline treatment studies, was dramatically lower. This discrepancy challenges the adequacy of self-report for large, population-based studies.

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BROMODOMAIN INHIBITOR JQ1 CAN WORK SYNERGISTICALLY WITH TYROSINE KINASE INHIBITORS TO INHIBIT MYELOID LEUKEMIA

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Introduction. Myeloid leukemia represents a heterogeneous group of cancers that is often aggressive and has a dismal outcome in the relapsed/refractory setting. Genetic alterations in the tyrosine kinase protein are frequently seen in myeloid leukemia, which makes tyrosine kinase inhibitors (TKIs) attractive therapeutic drugs. In acute myeloid leukemia (AML) mutations like *FLT3* internal tandem duplication (ITD) confer a worse prognosis due to more aggressive course. In case of chronic myeloid leukemia (CML) the tyrosine kinase gene fusion (*BCR-ABL*) is the disease defining mutation responsible for malignant transformation. Regardless of the type of myeloid leukemia TKIs are being increasingly used either alone or in combination with conventional chemotherapy. However, development of resistance towards TKIs is a major problem which limits the utility of this class of drugs. The bromodomain and extra-terminal (BET) family of proteins plays an important role in driving aberrant gene expressions that lead to various cancers including leukemia. BET inhibitors have become promising anti-leukemia drug candidates over the past few years. To find rational new therapeutic combinations against myeloid leukemia and overcome the problem of resistance against TKIs, we explored the synergistic effects of BET inhibitor JQ1 and TKIs on inhibition of myeloid leukemia cells.

Materials and methods. MV-4-11 and K562, established AML and CML cell lines respectively, were used for most of our experiments. MV-4-11 cells harbor the *FLT3 ITD* mutation, which makes the cells sensitive to multi-kinase inhibitor sorafenib. K562 cells carry the *BCR-ABL* fusion gene that is pathognomonic of CML and serves as the target of selective tyrosine kinase inhibitor imatinib. Standard cell viability, apoptosis, and flow cytometry assays were performed. Expression and activation of cell signaling components were assessed by western blotting. Similar experiments were conducted on primary leukemia cells obtained from consenting AML patients.

Results. Cell viability assays demonstrated that JQ1 and TKIs (sorafenib and imatinib) synergistically inhibited the growth of leukemia cell lines and primary leukemia cells. The growth inhibition was manifested in induction of apoptosis and cell cycle arrest. Mechanistically, while JQ1 suppressed c-Myc expression, TKIs inhibited activation of ERK1/2 and Akt. Combinations of JQ1 and TKIs exacerbated both c-Myc suppression and inhibition of ERK1/2 and Akt.

Conclusion. Our in-vitro work supports the idea that molecular therapies targeting multiple distinct pathways can be employed to synergistically inhibit myeloid leukemia. We hope that our experiments will add to the growing evidence suggesting synergism between BET inhibitors and TKIs and that eventually these two molecularly targeted therapies will be employed in combination to effectively treat AML and CML.

APPLY TRANSFER LEARNING ON CLASSIFYING EPITHELIUM AND STROMA REGIONS OF BREAST CANCER TISSUE: AN INITIAL STUDY

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In medical image analysis, transfer learning is to repurpose a deep convolutional neural networks (CNNs) which are well trained by a considerably large non-medical image data set to medical image tasks such as tumor classification and segmentation, for which the collected medical image dataset may not be able to sufficiently train and optimize a deep CNN. In this study, we tested the feasibility of applying the transfer learning technology for classifying the regions of epithelium and stroma depicted on digitized histological sections of the hematoxylin and eosin (H&E)-stained tissues acquired from breast cancer patients. For this purpose, we first extracted the features generated at different levels of three deep learning neural networks namely, AlexNet, Places365-AlexNet, and GoogLeNet. Next, the discriminative powers of these features are investigated accordingly, using receiver operating characteristic curve as well as other statistical methods. The experiment involves a total of 19748 regions of interest (ROIs) containing either epithelium or stromal regions. The results indicate that the lower-level features AlexNet and Places365-AlexNet have higher classification performance than the higher-level features. For GoogLeNet, however, the higher level features outperforms the lower level features. In addition, the highest accuracy of 90.4% is achieved by the features acquired from the 4th max-pooling level of GoogLeNet. This study suggests that transfer learning technology may be able to help pathologists improve the diagnostic accuracy in the future clinical practice.

Keywords: Transfer learning; deep convolution neural networks; classification of epithelium and stroma; computer aided diagnosis, quantitative image features

RESEARCH PROGRAM SUMMARY

This study was supported in part by Stephenson Cancer Center Research Award, and grant HR15-016 from the health program, Oklahoma Center for Advancing Science and Technology (OCAST). The Principal Investigators in Norman are Drs. Yuchen Qiu, Bin Zheng and Hong Liu. The purpose of this study is to investigate whether transfer learning based deep neural networks is able to improve the accuracy of epithelium and stroma classification in digital pathology.

The research interest of the PIs and their team are focused on developing artificial intelligence (AI) based quantitative diagnosis schemes as well as novel medical imaging modalities, to facilitate the early stage identification or prediction of different types of tumors (i.e. ovarian cancer, breast cancer, lung cancer, etc). Using state of the art AI technology such as deep learning, we are working on developing new image markers to achieve high accuracy prediction for cancer diagnosis or prognosis.

ABERRANT EXPRESSION AND ALTERED ZONAL DISTRIBUTION OF HEPATIC ORGANIC ANION TRANSPORTING POLYPEPTIDE 1B3 IN CHRONIC HEPATITIS C INFECTION

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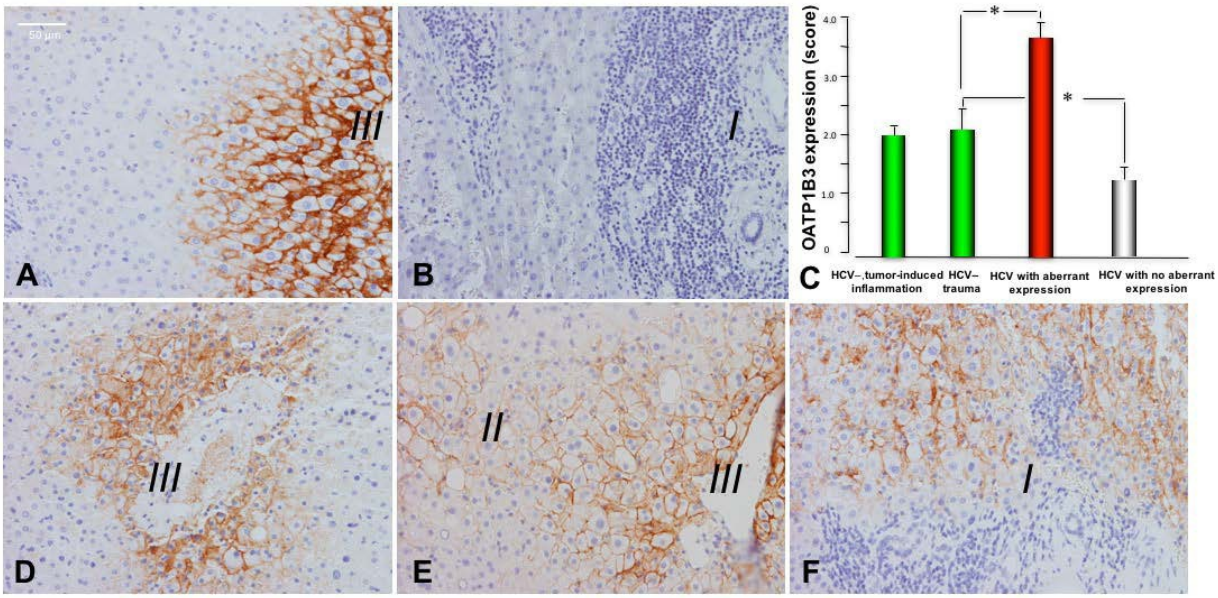
Background: Hepatitis C virus (HCV), the most common cause of chronic hepatitis in the US, infects 4.1 million people. New treatment regimens with nonstructural protein (NS) 3/4A protease inhibitors have reportedly achieved 71-80% sustained virologic response rate. However, a sizeable proportion of treatment-naive patients do not respond to the newer therapy. The mechanism remains unclear. Organic anion transporting polypeptide 1B3 (OATP1B3), exclusively distributed in liver, mediates hepatic uptake of many clinically important drugs including NS3/4A inhibitors simeprevir, paritaprevir and asunaprevir. The expression patterns and regulation of OATP1B3 in the liver of patients with chronic HCV infection are not yet known.

Methods: To study OATP1B3 expression pattern in chronic HCV infection, immunohistochemical stains were performed using formalin-fixed, paraffin-embedded liver specimens from 1) HCV negative (HCV–) trauma patients with no liver inflammation (control, n=12); 2) patients with untreated chronic HCV infection (n=14); 3) HCV– patients with tumor-induced chronic inflammation from metastatic lesions (n=7). Scoring of the OATP1B3 staining was performed based on zonal distribution score x staining intensity score. ANOVA and t-Test were used to compare the differences.

Results: In normal liver and HCV–, tumor-induced lymphocytic inflammation, OATP1B3 has an exclusive pericentral (zone III) expression pattern (Fig.A and B). Aberrant zone I/II expression of OATP1B3 (Fig.E,F) was observed in 64.3% (9/14) liver tissues with untreated chronic HCV infection. In 5/14 patients, OATP1B3 did not show aberrant expression but had significantly weaker staining (Fig. C,D).

Conclusion: This is the first report that drug transport protein OATP1B3 extends its expression from pericentral to periportal areas of the liver in some patients with chronic HCV infection. This expression pattern in not present in HCV–, tumor-induced lymphocytic inflammation, indicating its exclusive HCV association. Aberrant zone I/II expression of OATP1B3 may result in elevated hepatic uptake of the NS3/4A protease inhibitors and therefore predict better therapeutic response.


50 µm
III
A
I
B
OATP1B3 expression (score)
4.0
3.0
2.0
1.0
0
*
*
C
HCV–, tumor-induced inflammation
HCV– trauma
HCV with aberrant expression
HCV with no aberrant expression
III
D
II
III
E
I
F

NANOSCALE PHOTOACOUSTIC TOMOGRAPHY (NPAT) FOR HIGH-RESOLUTION ANALYSIS OF BLOOD CANCERS

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To date, there does not exist any high resolution imaging modality capable of label-free imaging at resolutions surpassing the optical diffraction limit of 200nm. As a result, testing for cancers such as leukemia can be an invasive procedure requiring invasive blood sampling. We here present nanoscale photoacoustic tomography (nPAT), a 3D, label-free, super-resolution imaging modality that can extract biometric data in a noninvasive and painless way. nPAT imaging accomplishes this by taking advantage of the photoacoustic effect, wherein laser-induced thermoelastic expansion results in the emission of detectable ultrasound that can be used for imaging. The ultrasound, distinct from clinical ultrasound used for imaging, encodes information about laser absorption due to its optical origin, and so nPAT imaging contrast is from differences in optical absorption in the sample. In theory, nPAT can be used to image any molecule with a sufficiently high absorption peak at the laser-excitation wavelength relative to surrounding molecules, such as DNA, lipids, and hemoglobin. As compared to photoacoustic microscopy, nPAT achieves orders of magnitude better resolution in the axial direction, surpassing the lateral optical diffraction limit. Ultrasonic axial resolution is largely limited by transducer bandwidth in the context of photoacoustic signal detection, and as a result, conventional piezoelectric transducers are unable to perform axial nanoimaging. However, due to the employment of a pump-probe laser-based method for the detection of acoustic waves, signals with frequencies as high as hundreds of gigahertz (GHz) can be detected. The detection of these high frequency signals enables the high-resolution of nPAT and allows us to push photoacoustic imaging into the nanoscale. We have demonstrated through simulation that nPAT will be thermally safe for imaging of erythrocytes, and that the achievable resolution of nPAT should rival that of x-ray microscopy, while remaining label-free. The applications of nPAT in cancer are varies, but primarily revolve around the efficient and non-invasive diagnosis and biomedical research on leukemias via high-resolution in-vivo rendering of biometrics typically associated with a complete blood count. In this way, we believe that nPAT has the potential to diagnose a series of blood cancers non-invasively and definitively through high-resolution imaging of superficial blood vessels yielding biometric like flow cytometry or clinical blood tests. However, nPAT can achieve this without requiring invasive extraction of blood from patients, and would be painless. As a result, we believe that nPAT, as a 3D in vivo label-free imaging modality with high resolution, can advance the diagnosis of leukemia and a host of blood disorders considerably.

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HIGH INTENSITY FOCUSED ULTRASOUND (HIFU) HEATING OF MURINE MELANOMA INDUCES IMMUNOGENIC CELL DEATH AND ABSCOPAL EFFECT

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Background: Abscopal effect is a rare and unpredictable event, and refers to the generation of systemic antitumor response following localized treatment. Although the mechanisms are poorly understood, it is hypothesized to occur following radiotherapy by "immunogenic cell death" (ICD) that release a number of endogenous damage-associated molecular patterns (DAMPs). Like x-ray irradiation, High Intensity Focused Ultrasound (HIFU) extracorpeal energy delivers focused sound waves non-invasively to induce physical cell damage. The objective of this study was to investigate the role of HIFU heating in ICD and induction of abscopal effect in mouse melanoma.

Method: B16F10 mouse melanoma (140-200mm^3) was established bilaterally in the flank region. A single rastered HIFU heating of the right flank tumors was performed for 15min. 5-7 days later, the ICD marker (calreticulin: CRT), immune cells (M1 & M2 macrophages, T-cells, dendritic cells, and ICAM expressing cells), and tumor necrosis were determined, and correlated with tumor growth delay.

Results: HIFU achieved gradient hyperthermia (~45-50°C) without causing significant necrosis in the treated tumor. Western blot of tumor homogenates revealed a 2 fold increase in the expression of CRT in the treated and abscopal sites compared to control ($p<0.05$; tukeys). Flow cytometry analysis showed a significant enhancement in the infiltration of tumor suppressing M1 macrophages, dendritic and CD4+ T cells. Further, CRT expression and immune cell infiltration correlated strongly with the growth delay of the treated and abscopal sites.

Conclusions: Our in vivo data suggest that localized HIFU heating of melanoma tumors achieves a CRT mediated abscopal effect. This technology has the potential to improve immunotherapeutic outcomes in patients.

Acknowledgement of Funding: This research was supported by the Center for Veterinary Health Sciences seed grant support, the OSU Kerr and McAsland Chair Foundation, and the National Institutes of Health AREA award.

EXPLORING THE ROLE OF A NOVEL EPIGENETIC FACTOR CHD1 IN MYELODYSPLASTIC SYNDROME AND ACUTE MYELOID LEUKEMIA

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Background: Myelodysplastic syndromes (MDS) are a diverse group of hematopoietic clonal disorders initiating in hematopoietic stem and progenitor cells. MDS is characterized by bone marrow dysplasia and ineffective hematopoiesis, leading to cytopenias and progression to acute myeloid leukemia (AML). Human genomics studies reveal that mutations in epigenetic regulators and spliceosomal components are prevalent in MDS patients. Studies in mice have shown that Chromodomain helicase DNA-binding protein 1 (CHD1) is a novel epigenetic factor required for hematopoietic stem cell formation. CHD1 also interacts with the spliceosome. We hypothesize that CHD1 mutations can drive MDS and AML, and that Chd1 depletion synergizes with other known driver mutations of MDS like SF3B1 (Splicing Factor 3B-1) and other epigenetic regulators like TET2 dioxygenase. Zebrafish (*Danio rerio*), are a powerful animal model for cancer genetic studies, and Chd1, Sf3b1, and Tet2 are all conserved in *D. rerio*; therefore, we are testing our hypotheses using zebrafish.

Methods: Initial studies were performed using morpholino-antisense oligonucleotide knockdown of *chd1* in zebrafish, followed by CRISPR/Cas9-targeted mutagenesis to create homozygous mutant *chd1* zebrafish lines. Using whole mount *in-situ* hybridization for *chd1* expression, we confirmed loss of Chd1 in mutants. Whole mount *in-situ* hybridization for embryonic blood cell markers is used to phenotypically characterize hematopoiesis. We are testing stem cell markers *runx1* and *c-myb,* myeloid marker *mpx* and lymphoid marker *rag1* at distinct developmental time points. O-dianisidine staining is used to stain the erythrocyte lineage. In adult fish, marrow evaluation is done to follow mutant *chd1* zebrafish for development of MDS/AML.

Results: No defects in morphology or survival were noted in homozygous *chd1* mutant fish, consistent with prior morpholino studies, but possible functional redundancy from the close Chd1 ortholog Chd1L (Chd1-like) complicates interpretation of this result. To assess for redundancy, we are creating double-homozygous mutants of both *chd1* and *chd1L*. Genetic interactions between *chd1* and other factors during HSPC formation are also being characterized. Morpholino knockdown of *chd1*, alone or together with heterozygous loss of *sf3a3* (another spliceosomal factor), showed no abnormalities in developing embryos, but when *chd1* was diminished in *sf3a3* heterozygous animals, a severe brain necrosis phenotype resembling *sf3a3 homo*zygous mutants occurred.

Conclusion: By taking advantage of zebrafish genetics and CRISPR/Cas9 mediated targeted mutagenesis, we are deciphering the role of a novel epigenetic regulator CHD1 in myeloid neoplasms. We have generated a zebrafish loss-of-function model for Chd1, making it a highly relevant model to study its interactions with other spliceosomal mutations and epigenetic regulators in MDS/AML.

REGRESSION OF TUMOR GROWTH BY THE COMBINED NITRONE OKN-007 AND TEMOZOLOMIDE THERAPY IN PRE-CLINICAL G55 GLIOMA MODEL

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Glioblastoma multiforme (GBM), a World Health Organization grade IV glioma, is the most common, accounting for about 40% of all primary brain tumors. GBM is also one of the deadliest cancers with a very low 5-year survival rate of 6% despite current treatment options. Temozolomide (TMZ) is an alkylating chemotherapeutic agent widely used in anti-glioma treatment. Unfortunately, GBM patients often develop TMZ resistance which results in a common reoccurrence in tumor formation, and hence poor patient outcome. OKN-007, a disulfonyl derivative of α-phenyl-tert-butyl nitrone, has demonstrated anti-glioma effects in several rodent models and is currently in a clinical trial as an investigational drug for recurrent gliomas.

The aim of this study was to assess the effect of combined OKN-007 and TMZ treatment in an orthotopic human xenograft G55 glioma model in nude mice and compare the effect of combination therapy to treatment with OKN-007 or TMZ alone, as well as untreated G55 tumors using Magnetic Resonance Imaging (MRI).

Athymic Nude Foxn1nu mice were intracerebrally injected with human G55 cell and MRI was used to calculate tumor volumes several time points (every 5-10days) following implantation. MRI was done on a Bruker Biospec 7.0 Tesla/30cm horizontal-bore imaging spectrometer. OKN-007 was administered in drinking water. TMZ was given via gavage.

In vitro GBM cells data indicate that combined OKN-007 and TMZ can severely decrease GBM cell viability in different glioma cell lines compared to TMZ treatment alone.

It was found the combination treatment OKN-007-TMZ treatment increased survival, decreased tumor growth and decreased tumor volumes compared to the control untreated group of mice. Thus, the combined therapy has promising hope for the current treatment options.

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NON-INVASIVE LIQUID BIOPSY ANALYSIS OF EXOSOMAL MIRNA AS A SCREENING TOOL FOR ENDOMETRIAL CANCER

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Introduction: Early diagnosis and prediction of response to treatment is critical for deciding the course of therapy for cancer patients. Additionally, a minimally non-invasive procedure of diagnosis will lead to low distress to the patient. In this study, we analyzed the content of exosomes isolated from urine of endometrial cancer patients for its putative use in diagnosis and assessment of treatment response. Studies have shown that exosomes can be isolated from all bodily fluids and their luminal composition (consisting of miRNA, mRNA, proteins and lipids etc.) is reflective of its cell of origin. Based on these reports we hypothesized that the luminal contents of the exosomes before, during and after chemotherapy will present "unique signatures" or "bar codes" that can predict treatment outcomes. To test our hypothesis, we applied non-invasive liquid biopsy approach and isolated exosomes from urine of endometrial cancer patients and analyzed their micro (mi) RNA profile.
Methods: Exosomal miRNA was isolated from urine samples of endometrial cancer patients (n=22). Urine samples from individuals (n=5) mimicking the symptoms of endometrial cancer but not having cancer were used as controls. Subsequently, the exosomes were used for miRNA profiling by quantitative PCR-based miRNA array. The miRNA results were then subjected to hierarchical listing based on the fold difference between cancer exosomes over control exosomes. The top three highly expressed miRNAs in cancer exosomes were identified and validated by conducting miRNA-specific quantitative (q) PCR.
Results. The isolated exosomes were 83-100 nm in size, and exhibited spherical structure. The presence and purity of the exosomes was confirmed by demonstrating the presence of the membrane proteins, tetraspanins CD63 and CD81, but not Hsp90B1 (Grp94) cytosolic protein and fulfilled the International Society of Extracellular Vesicle (ISEV) consortium criteria.
miRNA profiling showed the top three miRNA that were abundant in cancer exosomes were 200c-3p > 23b-3p > 100-5p. Validation of 200c-3p by qPCR exhibited increased expression in cancer exosomes compared to control exosomes confirming the miRNA array results. TCGA data showed miR-200c-3p expression was altered in 2.1% of endometrial cancer patients and patients with miR-200c-3p alterations had poor survival. starBase 2.0 Pan-Cancer TCGA analysis showed approximately a two-fold increase in miR-200c-3p miRNA expression in endometrial cancer patients over normal. Finally, miR target analysis using mirTarBase showed Zeb1, Zeb2 and BMI-1 as molecular targets of miR200c-3p. Zeb1 and Zeb2 are known to play an important role in epithelial-mesenchymal transition (EMT) and metastasis while BMI-1 in drug resistance. The expression of Zeb1, Zeb 2 and BMI-1 mRNA and protein in the cancer exosomes and tumor tissues are currently being investigated.
Conclusions: Our study demonstrates non-invasive liquid biopsy exosomal miRNA as a screening tool and presents unique "miRNA Bar Codes" that could be used for predicting disease progression and treatment outcomes. The technology can also be used longitudinally for monitoring treatment response and in predicting drug resistance.

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QUANTIFICATION OF ANTI-CANCER COMPOUNDS FROM INDIVIDUAL CANCER CELLS

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Abstract: Quantifying the amount of chemotherapeutic agents in individual cells could revolutionize treatments for cancer patients. Both the drug and active forms of its metabolite can be monitored to lead to more personalized care in the future by either adjusting the doses or preventing further harm if the drug is not effective for that patient. The Single-probe is a device capable of penetrating individual cells, and it couples to a mass spectrometer for easy analysis. Using single cell mass spectrometry (SCMS), cellular heterogeneity can be explored. In these studies, we have used SCMS to quantify various anti-cancer compounds in adherent bladder cancer cell line models. We have also expanded the method to work with suspended cell lines as well as with bladder cancer patient samples received from OUHSC.

THINKING THROUGH RISK COMMUNICATION: THE IMPACT OF NEED FOR COGNITION AND MESSAGE EMOTIONALITY AND FRAMING ON RISK PERCEPTIONS, KNOWLEDGE, AND BEHAVIORAL EXPECTATIONS

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Conflict of Interest: The authors declare that they have no conflicts of interest.

Objective: Healthy People 2020's tobacco use goal is to reduce "illness, disability, and death related to tobacco use" through various avenues including health communication. One way to do so may be to tailor health communication risk messages to an individual's level of need for cognition (NC). NC is a stable trait that measures an individual's enjoyment and liking, or disaffection and disliking of processing cognitively effortful information. Effectively communicating risk information to smokers can be an especially useful tactic to influence smoking cessation and understanding how NC effects risk perceptions after exposure to messages may be especially beneficial. This study examined the impact of NC and message emotionality (emotional vs. factual) and framing (gain vs. loss) on smokers' responses to smoking health risk messages.

Method: Current smokers (N = 402) were randomly assigned to one of four message categories: 1) emotional/gain-framed, 2) emotional/loss-framed, 3) factual/gain-framed, and 4) factual/loss-framed. Participants answered a pre- and post-questionnaire assessing knowledge, risk perceptions, and behavioral expectations related to cutting down, limiting, or quitting smoking completely, which also served as the main outcome variables. Multiple linear regression was used to examine the main effects of NC, message emotionality, and message framing on the outcome measures, and interaction effects of NC with message emotionality (factual vs. emotional) and NC and framing (gain vs. loss) on the same variables.

Results: A significant main effect emerged for NC on knowledge retention such that those higher in NC demonstrated greater knowledge retention (p < .001). Additionally, those who viewed emotional (vs. factual) messages perceived higher levels of perceived risk (p < .01) for one of the six risk items. As hypothesized, significant two-way interactions between NC and emotionality emerged for two of the six smoking risk perception items (ps < .05). Smokers lower in NC perceived greater risk after viewing emotional (vs. factual) messages and those with higher NC perceived greater risk when viewing factual (vs. emotional) messages. In addition, a significant interaction emerged between NC and framing on one of the six smoking risk perceptions items (p < .05), such that smokers lower in NC (vs. higher) reported higher perceived risk after viewing gain-framed messages and those higher in NC (vs. lower) reported higher perceived risk after reviewing the loss-framed messages.

Conclusion: NC, message emotionality, and message framing appear to influence levels of perceived risk and, in some cases, smoking knowledge retention. This offers compelling evidence that NC may be an important individual difference factor that should be considered when developing and delivering smoking risk communication.

SYNERGISTIC SEQUENTIAL COMBINATION OF PRE/POST TRANSCRIPTIONAL GENE THERAPEUTICS TARGETING SURVIVIN IN COLORECTAL CANCER CELLS

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Purpose: Combination therapy is an important treatment modality for various diseases, including cancer. In addition, reformulating existing drugs into combination products represents an essential strategy to overcome unmet medical needs. However, the number of possible combinations can be large, and development of models that enhance the understanding of interactions and combination effects can help to select the best combinations for further development. In this study, we aimed to optimize the combinations of agents that reduce the level of a well-validated chemoresistance gene using mechanism-based quantitative pharmacology modeling.

Methods: We chose one of chemoresistance genes, survivin as the target molecule, whose overexpression correlates with poor prognosis in cancer patients. Two agents were selected to target survivin expression/signaling: suramin which is a pre-transcriptional inhibitor of survivin synthesis, and siRNA (delivered as a complex with pegylated cationic liposomes, siRNA-lipoplex) which acts post-transcriptionally to breakdown survivin mRNA. A quantitative pharmacology model was developed to characterize the survivin gene expression in response to different combination strategies.Treatment outcomes of suramin and siRNA-lipoplex alone/combinations were obtained from experimental assay. Model simulations were compared with experimental results to evaluate model performance, and plotted in a three-dimensional response surface. Drug-interactivity was assessed by using combination index, isobologram, and curve shift analysis.

Results: The PD model described the time- and concentration-dependent effects of single agent suramin and siRNA-lipoplex and predicted additional synergistic interactions between these two agents, on survivin gene expression. The predicted interactions were confirmed by the *in vitro* experimental results. The key components governing the synergistic interactions were globally identified at a systems level.

Conclusions: The present study demonstrates the utility of quantitative pharmacology modeling to depict complex PD of gene-targeting agents on cellular and molecular levels; this approach enables us to rule out unproductive combinations and identify potentially useful combinations. The collective modeling and experimental results further indicate combinations of pre-transcriptional inhibitor and post-transcriptional inhibitor yielded synergy in survivin knockdown.

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THE PROTEIN EXPRESSION AND PURIFICATION FACILITY AND THE LABORATORY OF BIOMOLECULAR STRUCTURE AND FUNCTION AT OUHSC

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We would like you to know about the services in protein purification and structural studies available to OUHSC in the Protein Expression and Purification (PEP) Facility and the Laboratory of Biomolecular Structure and Function (LBSF). Dr. Simon Terzyan (BRC 406) manages both facilities. He has 25 years of experience in protein science and crystallography (co-author on 40+ papers, 40+ structures in the Protein Data Bank). The PEP has shakers for bacterial growth and chromatography equipment for non-structural biology labs to make pure protein on the milligram scale. The pure protein can be used for biological, biophysical, or crystallographic studies. Training in the use of this equipment is available from Dr. Terzyan, or he can do this work on a fee-for-service basis. Dr. Terzyan can do standard molecular modeling tasks (e.g., design mutants, compare structures). He can make images of protein structures for your grant applications and manuscripts. For advanced molecular modeling projects (e.g., homology modeling, structure-based drug design), the LBSF has a Molecular Modeling Unit run by Dr. Timothy Mather. This unit has a workstation with four GPUs optimized to run four independent molecular dynamics simulations. Dr. Terzyan can also provide training in dynamic light scattering, protein crystallization (we plan to have a crystallization robot available for soluble and membrane proteins within the next year), X-ray data collection (the LBSF has a new X-ray generator detectors on both ports), structure determination, refinement, and analysis. He can also provide these services on a fee-for-service basis. The X-ray facility is open to use by any academic lab in Oklahoma. Dr. Terzyan serves as an interface with the OU Crystallization facility in Norman and the Physical Biochemistry Laboratory in the Department of Biochemistry and Molecular Biology (BMB). The latter facility has CD spectroscopy, microcalorimetry, and microscale thermophoresis instruments. The LBSF is a Vice President of Research Core Facility and a core facility of the Oklahoma COBRE in Structural Biology. The PEP is supported by the Department of Biochemistry and Molecular Biology.

DEVELOPING AND VALIDATING THE PERSONAL RISK OF ONCOGENIC HUMAN PAPILLOMAVIRUS (PRO-HPV) INFECTION SCORE IN U.S. WOMEN

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Determining risk scores for genital high-risk (i.e., oncogenic) human papillomavirus (HRHPV) infection in women will allow the development of more efficient cervical cancer screening strategies. We aimed to develop and validate point scores to predict the likelihood of any genital HRHPV infection in women. We used data from the 2005-2014 U.S. National Health and Nutrition Examination Survey (7,337 women aged 25-59 years; 6,300 women aged 30-59 years). Predictors were reproductive health practices, risk behaviors, and demographic variables. The outcome was a positive result for any of the 21 genital HRHPV genotypes. The 2005-2012 cohorts were used as training and testing sets to develop scores that best classified women into three risk groups: low risk (<20%), average risk (20-30%), and high risk (>30%). We selected final predictors based on the Akaike Information Criterion. A point score was assigned for each selected predictor based on its β coefficient. The 2013-2014 cohort was used to validate the final scores. From the training and testing sets, two point scores (six self-reported variables for each score) were created to predict any HRHPV risks for the two age groups. Five similar predictors for both scores were age, smoking status, history of other sexual transmitted infections, number of lifetime sexual partners, and race. The sixth predictor was birth control or hormone use in women aged 25-59 years and marital status in women aged 30-59 years. In the validation set, the observed HRHPV prevalence in each risk group fit well into the predicted ranges. The scores had fair discrimination (c-statistics: 0.67 to 0.68), but good calibration between the predicted and observed rates of HRHPV infection. The Personal Risk of Oncogenic HPV (PRO-HPV25 and PRO-HPV30 for women aged 25-59 and aged 30-59 years) scores could help to stratify the risk of genital HRHPV infection, which could be used to personalize cervical cancer screening recommendations.

GTSE1 REGULATES SPINDLE MICROTUBULE DYNAMICS TO CONTROL AURORA B KINASE AND KIF4A CHROMOKINESIN ON CHROMOSOME ARMS

Aaron Tipton

Cell Cycle and Cancer Biology, Oklahoma Medical Research Foundation

In mitosis, the dynamic assembly and disassembly of microtubules are critical for normal chromosome movement and segregation. Microtubule turnover varies among different mitotic spindle microtubules, dictated by their spatial distribution within the spindle and by their interactions with each other and with other organelles such as kinetochores, chromosome arms, and the cell cortex. How turnover among the various classes of spindle microtubules are differentially regulated and the resulting significance of differential turnover for chromosome movement remains a mystery. As a new tactic, we used GAMMA, a bioinformatic method, to identify novel regulators of mitosis. GTSE1 (G2 and S phase expressed protein 1) was selected for further analysis based on previous findings demonstrating GTSE1 association with microtubules. GTSE1 is expressed exclusively in late G2 and M phase. Additionally, GTSE1 has been shown to function as an EB1-dependent plus-end tracking protein. However, following nuclear envelope breakdown in mitosis, GTSE1 microtubule plus-end tip tracking is inhibited until anaphase onset and instead the protein decorates the microtubule lattice. During this time GTSE1 binds preferentially to the most stable populations of mitotic spindle microtubules and promotes their turnover. Cells depleted of GTSE1 show defects in chromosome alignment at the metaphase plate coupled with an increase in the proportion of stable mitotic spindle microtubules. Photoactivation data show that GTSE1 fosters turnover of the most stable population of microtubules within the mitotic spindle from prometaphase to anaphase onset. At anaphase onset, GTSE1 returns to tip tracking and redistributes to the ends of astral microtubules. We speculate that this redistribution prevents GTSE1 from destabilizing midzone microtubules during anaphase and telophase. Cells depleted of GTSE1 display hyper-stabilized spindle microtubules, which in turn affects the activity of the mitotic kinase, Aurora B, on chromosome arms with negligible effects on Aurora B at centromeres. The loss of Aurora B activity on chromosome arms diminishes accumulation of the chromokinesin Kif4A. In sum, we have identified a novel pathway in which GTSE1 is an upstream regulator of microtubule stability, chromosome alignment, spindle pole integrity, and timely mitotic progression.

DEVELOPING PHOTOACOUSTIC MICROSCOPY FOR IN VIVO LABEL-FREE IMAGING OF TUMOR VASCULATURE DEVELOPMENT AND THERAPY

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Photoacoustic microscopy is a novel imaging modality that utilizes a laser to induce heat within a sample which creates pressure waves. Ultrasonic waves are collected by an ultrasonic transducer and synthesized to create an 3D image. The detection of these waves results in the ability to image at depths greater than traditional optical microscopy.

Previously, our system required a laser on one side of the sample and the transducer on the other. This limited our imaging depth as well as limited the sample that we could use including in vivo animal study. Currently we are developing our second-generation system where we have repositioned the laser and ultrasonic transducer to one side along with other modifications. This will allow us to move onto biological samples and image vasculature system in areas relevant to research as well as improving the speed and quality of the imaging.

Photoacoustic imagery is inherently noninvasive. We don't require contrast agents or invasive methods to acquire quality images. This allows for the preservation of a sample in a controlled state and in vivo imaging. We can take images without any collateral damage to the sample.

By using photoacoustic microscopy, we can track the development of a tumor as it induces angiogenesis. Conversely, we can track the effect of tumor targeting drugs that aim to limit angiogenesis and consequently stop the growth of the tumor by restricting the flow of nutrients into it.

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HIFU HEATING AND NANOBUBBLE TREATMENT ENHANCES TUMOR DRUG PENETRATION

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Mild hyperthermia generated using high intensity focused ultrasound (HIFU) and microbubbles (MBs) can improve tumor drug delivery from non-thermosensitive liposomes (NTSLs) and low temperature sensitive liposomes (LTSLs). However, MB and HIFU are limited by the half-life of the contrast agent and challenges in accurate control of large volume tumor hyperthermia for longer duration (>30min.). The objectives of this study were to: 1) synthesize and characterized long-circulating echogenic nanobubble encapsulated LTSLs (ELTSLs) and NTSLs (ENTSLs), 2) evaluate in vivo drug release following short duration (~20min each) HIFU treatments administered sequentially over an hour in a large volume of mouse xenograft colon tumor, and 3) determine the impact of the HIFU/nanobubble combination on intratumoral drug distribution. LTSLs and NTSLs containing doxorubicin (Dox) were co-loaded with a nanobubble contrast agent (perfluoropentane, PFP) using a one-step sonoporation method to create ELTSLs and ENTSLs, which then were characterized for size, release in a physiological buffer, and ability to encapsulate PFP. For the HIFU group, mild hyperthermia (40-42°C) was completed within 90min after liposome infusion administered sequentially in three regions of the tumor. Fluorescence microscopy and high performance liquid chromatography analysis were performed to determine the spatial distribution and concentration of Dox in the treated regions. PFP encapsulation within ELTSLs and ENTSLs did not impact size or caused premature drug release in physiological buffer. As time progressed, the delivery of Dox decreased in HIFU-treated tumors with ELTSLs, but this phenomenon was absent in the LTSL, NTSL, and ENTSL groups. Most importantly, PFP encapsulation improved Dox penetration in the tumor periphery and core and did not impact the distribution of Dox in non-tumor organs/tissues. Data from this study suggest that short duration and sequential HIFU treatment could have significant benefits and that its action can be potentiated by nanobubble agents to result in improved drug penetration.

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LANTHANIDE-DOPED UPCONVERSION NANOPARTICLES ELECTROSTATICALLY COUPLED WITH PHOTOSENSITIZERS FOR NEAR-INFRARED-TRIGGERED PHOTODYNAMIC THERAPY

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Lanthanide-doped upconversion nanoparticles (UCNPs) have recently shown great promise in photodynamic therapy (PDT) for cancer treatment. Herein, we report a facile strategy to fabricate an efficient NIR-triggered PDT system based on $LiYF_4$:Yb/Er UCNPs coupled with a photosensitizer of a β-carboxyphthalocyanine zinc (ZnPc-COOH) molecule via direct electrostatic interaction. Due to the close proximity between UCNPs and ZnPc-COOH, we achieved a high energy transfer efficiency of 96.3% from UCNPs to ZnPc-COOH, which facilitates a large production of cytotoxic singlet oxygen and thus an enhanced PDT efficacy. Furthermore, we demonstrate the high efficacy of such a NIR-triggered PDT agent for the inhibition of tumor growth both in vitro and in vivo, thereby revealing the great potential of the UCNP-based PDT systems as noninvasive NIR-triggered PDT agents for treatment of deep tumors.

REAL-TIME MONITORING OF ELECTROPORATION PROCESS USING ELECTRIC FIELD-INDUCED ACOUSTIC TOMOGRAPHY

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ABSTRACT: Electroporation is a natural phenomenon involving increased cell membrane permeability when the cell is exposed to high voltage nano second electrical pulses (nsEP). Electroporation allows relatively large molecules to diffuse through the nanopores in the cell membrane, this process is not possible under normal circumstances. Electroporation induced by an electric field has the potential to overcome some challenges related to clinical treatment using reversible or non-thermal irreversible electroporation, e.g. it is caused the process of cell apoptosis. A recently developed technique for clinical therapy of electroporation i.e. electrochemotherapy (ECT), uses the local application of short and intensive electric pulses to deliver non-permeant drugs to the cell interior, has increased sharply over the past decade. Although, electroporation has advantages over thermal therapies and is presented as a non-thermal technique, however, during the electroporation, a small temperature rise in the tissue results from the electric pulse excitation. This change in temperature produces pressure waves, which are detectable by ultrasound transducers. In this study, we confirm a linear acoustic signal corresponding to the voltage of the electric field and demonstrate the detection of electric field-induced acoustic emission as a promising tool for the real-time monitoring of the electroporation process. All current imaging techniques used in the clinic to monitor electroporation process are based on pre- and post-stimulation exposure with no real-time monitoring electric field distribution. The proposed technique will address the imaging need during the treatment of for example cancer tumors, which vary in size, location, and shape. In addition, this technique is simple to use for any electrotherapy in future clinical applications. The results of this experimental study suggest that the detection of electric pulse-induced acoustic signals is an innovative methodology for *in situ* monitoring of electrotherapy procedures, especially for processes in which cell apoptosis occur. The real-time, *in situ* monitoring of electric field distribution provide valuable experimental results and has the potential to further enable real time monitoring electroporation therapy.

PUFF3RD - A HIGH VOLUME, HIGH FLOW RATE PUFFING DEVICE FOR CURRENT GENERATION ELECTRONIC CIGARETTES.

Tyler Watson, Evan Floyd

Abstract:

Electronic Cigarette use is rapidly spreading within the smoking community and beyond. Vaping devices have evolved rapidly and continue to do so with FDA deeming rules delayed beyond August 2018. Limited research has been performed characterizing physical and chemical attributes of e-cigs with even fewer specifically targeting 3rd generation e-cigs. No studies conducted to date have used flow rates appropriate for 3rd generation devices which may not affect the mass vaporized but will certainly affect particle size distribution, dilution and temperature of aerosol. We believe this is due to the lack of equipment capable of simulating 3rd gen e-cig puffs at a colossal 90-200 mL/sec. The goal of this project was to build a system capable of testing 3rd gen e-cigs at real-world flow rates.

PUFF3rd™ was built from a large calibration syringe driven by a stepper motor and drive platform with electronic control via a Fubarino microcontroller, with custom Arduino programming code. This program allowed control of puff volume, puff rate, number of puffs, and inter-puff-interval. To validate PUFF3rd™, volume and flow rate inputs were compared to measured volume and flow rate output by the machine. A correction regression was applied to increase accuracy.

A blind evaluation of puff volume was performed at 10 points from 50 - 2,000 mL with overall accuracy of 98.7% and highly linear (R^2=0.99998) across this range with repeatability of ≤ 1.1%. Puff rate was assessed from 15-165 mL/s with precision ≤ 2.0%. showing that this large system has the versatility to assess low flow rate cigalike and 2nd generation e-cigs as well as high flow 3rd generation e-cigs. The accuracy of this system far exceeds the ASTM guidelines for cigarette testing machine reproducibility and accuracy. Further controls for atomizer I/O have been integrated since validation and PUFF3rd is being used to evaluate nicotine delivery and aldehyde production of 3rd generation sub-ohm atomizers in the lab.

ADDRESSING TOBACCO USE AND CESSATION OPPORTUNITIES IN FOOD PANTRIES: THE FOOD INDEPENDENCE, SECURITY, AND HEALTH (FISH) STUDY

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Introduction. Tobacco prevalence is higher in low-income populations and food insecurity is associated with cigarette smoking, hence, food pantries may be novel places for tobacco cessation efforts for this hard-to-reach population. Limited research is available on food pantry personnel perceptions about client tobacco use, tobacco control policies in pantries, and referral practices for smokers.

Methods. We surveyed clients (n=376) and personnel (n=129) in from a subset of food pantries and food resource centers (n=67) affiliated with Oklahoma's two Feeding America food banks. Client and personnel surveys assessed food insecurity, current tobacco use, readiness to quit, and cessation behaviors. Personnel surveys included items about perceptions of client tobacco use, food pantry referrals, and tobacco-free policies. Personnel were also asked about willingness/confidence in supporting client behavior change. Chi-squared tests were used to compare strata and log binomial regression was used to examine multiple variable associations. SAS 9.4 was used for all statistical analyses

Results. Almost half of food pantry clients smoked (48.4%), while only 9.9% of personnel smoked. Pantry clients experiencing food insecurity were more likely to smoke (50.8%) than those experiencing food security. In contrast to most personnel perceptions that smoking clients weren't interested in quitting (67.2%), the majority of client smokers reported contemplating quitting (80.5%) and half recently tried to quit (50.3%). Additionally, over half of personnel (53.9%) wanted to help clients quit, over one-third (39.4%) thought their job should include referring smokers to quit resources, and most pantry organizations reported tobacco-free policies (75.6%). However, few pantry personnel (9.8%) regularly asked clients about smoking, and many lacked confidence to ask about tobacco (58.1%) or to refer clients to a quit-line (48.4%).

Conclusion. Although food pantry personnel have some misconceptions about client smoking, many want helping clients quit, making food pantries novel partners in cessation services and referrals for tobacco use.

THE BIOGENESIS OF EXOSOME MIR-1246 IN HUMAN PANCREATIC CANCER CELLS

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Background: miR-1246 has been considered an oncomiR in various cancer types. We have recently reported that exosome miR-1246 is a potential circulating biomarker for early detection of pancreatic cancer. However, the biogenesis of miR-1246 remains controversial which often leads to misinterpretations of its detection and biological function. The aim of this study was to elucidate the cellular origin of exosome miR-1246 in human pancreatic cancer cells.

Method and result: Human pancreatic cancer cell lines, PANC-1 and MIA-PaCa-2, and the normal pancreas epithelial cell line, served as model systems. Next generation small RNA sequencing, CRISPR-Cas9 knockout technology, the poly-A tailing SYBR qRT-PCR, and siRNA/shRNA knockdown were applied to this study. We found that miR-1246 is highly enriched in exosomes derived from human pancreatic cancer cells and is originated from RNU2-1, a small nuclear RNA and essential component of the U2 complex in spliceosome. Knockdown of Drosha and Dicer did not reduce exosome miR-1246 levels, indicating that exosome miR-1246 is generated in a Drosha- and Dicer-independent manner. The GCAG motif present in the RUN2-1 transcript was shown to mediate miR-1246 enrichment in pancreatic cancer exosomes.

Conclusion: We conclude that exosome miR-1246 is derived from RNU2-1 through a non-canonical miRNA biogenesis pathway. Our findings suggest that most of the recent reports regarding miR-1246 detection or biological function in cancer cells need to be cautiously interpreted.

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ZIP4 DOWNREGULATES MICRORNA-224 AND PROMOTES ANTI-APOPTOSIS, MIGRATION OF PANCREATIC CANCER CELLS THROUGH ZN-CN-PCREB-RREB1 CASCADE.

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Background: The essential trace element Zinc functions as a catalytic cofactor for multiple enzymes, also plays important roles in cancer development. We found that the aberrant expression of zinc importer ZIP4 plays a critical role in PC growth, negatively correlated with microRNA-224 (miR-224) in pancreatic cancer cell. We investigated the mechanism of how ZIP4 negatively regulates miR-224 and downstream target genes in PC.

Method: In vivo tumor growth was performed in nude mice to validate the anti-tumor effect of miR-224; reporter and expression assay were applied to study the miR-224 target genes; promoter analysis, CHIP and enzyme assay were performed to study the mechanism how ZIP4 regulate miR-224.

Result: Our data reveal that ZIP4 activates zinc-dependent transcription factor CREB via suppressing CREB dephosphorylation through inhibiting phosphatase calcineurin (CN) activity caused by increased Zinc, which induce the level of negative transcription factor RREB1, leads to the down-regulation of miR-224 in pancreatic cancer cells. Inhibition of miR-224 is required for efficient ZIP4-dependent enhancement of cancer cell migration and anti-apoptosis in mouse models. Detailed analysis revealed that the anti-tumor function of miR-224 is mediated in part through its negative regulation of API5, which are known to be associated with cancer development and progression.

Conclusion: Our results indicate a novel ZIP4-CN-CREB-RREB1-miR-224 signaling cascade that promotes pancreatic cancer, providing mechanistic insights on how a zinc transporter functions in cancer. Targeted inhibition of the ZIP4 and/or activating miR-224 may be a novel cancer therapy and may have broader implications as inappropriate zinc regulation plays an important role in many other diseases.

BREAST CANCER PRESCREENING METHOD USING TISSUE ELECTROCHEMICAL CHARACTERISTICS

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ABSTRACT: Based on the breast cancer registry data, the successful treatment of breast cancer and chances of survival are highly dependent upon early detection and intervention. Mammography screening program is the gold standard technique for detecting early breast cancer along all existing modalities; however, it is only recommended for women with an average risk of breast cancer of a certain age range (i.e., 45 to 69 years old in the United States). Due to the low cancer detection yield of ≤0.5% and the high false-positive recall rate of ≥10% in the population-based mammography screening environment, controversy regarding the efficacy of mammography screening exists. Many other imaging modalities, such as ultrasound and magnetic resonance imaging, have been recommended as supplementary screening tools; however, they have little impact on improving the efficacy of breast cancer screening. Thus, an alternative but more effective approach to improve the efficacy of breast cancer screening is to identify high risk women before mammography screening, especially young women who are unlikely to attend screening programs. This study aims to develop a low-cost, non-invasive, and non-imaging technique to quantitatively assess bilateral asymmetry of the electrochemical characteristics of the breast tissue. Bilateral asymmetric electrochemical properties of the different sub-regions of the breasts has the potential to introduce a personalized risk factor associated with breast cancer susceptibility. We assume that the tissue composition and distribution of the left and right breasts (in selected sub-regions) are normally symmetrical. We demonstrate this new technique by first acquiring the bilateral electrochemical characteristics of different breast sub-regions and then comparing in a mirror structure to present a risk factor related to having or developing breast cancer. We consider four electrochemical features, which are intra- and extracellular conductivities, relaxation frequency and phase information. We then provide a prediction model based on an Artificial Neural Network (ANN). Results from this study suggest that the bilateral electrochemical characterization of breast sub-regions is a feasible technique to achieve personalized risk assessment as a pre-screening method to improve the sensitivity and specificity of current breast cancer screening. In addition, this unique pre-screening approach may attract more high risk women, especially young women, to the screening programs. That means it will increase the cancer detection yield and reduce false-positive recall. Bilateral electrochemical characterization of the breast sub-regions has proven its potential for a low cost, non-invasive, and portable pre-screening device to improve breast cancer screening and, ultimately, early breast cancer detection.

RGD PEPTIDE-TARGETED POLYETHYLENIMINE-ENTRAPPED GOLD NANOPARTICLES RADIOLABELED WITH ^{99M}TC FOR TARGETED SPECT/CT IMAGING OF AN ORTHOTOPIC MODEL OF HUMAN HEPATIC CARCINOMA

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Abstract

It is essential to combine structural and functional imaging to improve the accuracy and sensitivity of tumor diagnose. In this study, we report the construction and characterization of RGD peptide-targeted polyethylenimine (PEI)-entrapped gold nanoparticles (AuNPs) radiolabeled with ^{99m}Tc (RGD-^{99m}Tc-Au PENPs) for targeted micro SPECT/CT and CT imaging of hepatic carcinomas *in situ.* In this work, PEI sequentially modified with diethylenetriaminepentaacetic acid (DTPA), polyethylene glycol (PEG), and arginine-glycine-aspartic acid (RGD) linked-PEG was used as a nanoplatform to prepare AuNPs, followed by ^{99m}Tc labelling. We show that the designed RGD-^{99m}Tc-Au PENPs are colloidally stable and biocompatible. HCC-LM3 cells (a line of human hematoma cells) could overexpress $\alpha_v\beta_3$ integrin as illustrated by cell immunohistochemistry and were able to specifically uptake the RGD conjugated AuNPs. *In vivo* CT and SPECT imaging results indicated that the particles displayed good contrast enhancement of hepatic carcinomas region, and could target hepatic carcinomas region *in situ*. With the proven biocompatibility and *in vivo* histological examinations, the designed RGD-^{99m}Tc-Au PENPs show a great potential to be used as a contrast agent for targeted SPECT/CT imaging of different $\alpha_v\beta_3$ integrin receptor-overexpressing tumors.

THE T-PROBE: A NOVEL DEVICE FOR MS ANALYSIS OF NON-ADHERENT LIVE SINGLE CELLS

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Single cell mass spectrometry (SCMS) can identify and characterize different subpopulations of cells. Some types of cells are difficult to obtain or culture (e.g. primary cell lines, stem cells, and patient-derived sample), and SCMS is a powerful tool to analyze these cells. In addition, SCMS analysis can avoid the inherent drawbacks of lysate preparation. We have developed a new type of T-probe, a micro-scale sampling device, for MS analysis of live non-adherent single cells. Comparing with the previous T-probe, using this T-probe is more efficient, and it can be applied to broad ranges of cell types. Instead of extracting cytoplasm, the entire single cells are sampled for MS analysis, and no cellular content is lost during the experiment.

T-probe consists of three fused silica capillaries sandwiched by two polycarbonate slides. The solvent-providing capillary provides solvent (Acetonitrile with 0.1 % Formic Acid), the sampling probe (with a length of 0.8 cm and a tip size around 14 μm) can withdraw single cells (due to the Venturi effect), and the nano-ESI emitter (with a length of 5 cm) allows for online cell lysis in acetonitrile followed by ionization and MS analysis. The T-probe is coupled with a Thermo LTQ Orbitrap XL mass spectrometer. The X, Y, Z translation stage system can control the cell samples which were placed on a glass slide. The Z stage can be lift precisely and allows the T-probe tip withdraw a whole single cell.

The T-probe has been used to analyze the lipids and anticancer compounds from single HCT-116 cell after Irinotecan treatment. The HCT 116 cells were cultured in treated McCoy's 5A Medium. Cells were treated with anticancer drug Irinotecan (18 μM for HCT 116) for 45 min. The untreated cells were used in control experiments. Before the SCMS experiments, cells were rinsed with fresh DMEM for three times to remove the remaining drug molecules and reagents on cell surface. The T-probe was positioned above the cell containing plate. Using two digital cameras as visual guide, individual HeLa or HCT-116 cells were selected for MS analysis. A single cell was withdrawn by manipulating the computer-controlled stage system as the T-probe inserted into the cell-containing solution. After observing a single cell withdrawn into the sampling tip, the T-probe tip was raised out from the solution before the analysis of next cell. We have detected some common lipids (e.g. PC (36:2), PC (36:1), etc.) in the control. Irinotecan ($[C_{33}H_{38}N_4O_6 + H]^+$, m/z = 587.2881) was only detected in the treated samples. Some common lipids (PC (34:1), PA(12:0), etc.) were also detected in cells upon drug treatment. The detection of Irinotecan has been further confirmed by conducting tandem MS analysis (MS/MS). The preliminary results from our proof-of-concept experiments indicate that the new design of T-probe has been successfully used for MS analysis of non-adherent cells. Our techniques can be potentially used for high-throughput SCMS analysis of patient derived cells and primary cells, which are non-adherent and vary in sizes.

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