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Lauren Ball, PhD
Associate Professor of Cell & Molecular Pharmacology & Experimental Therapeutics
Director of Core B: Proteomics Core

Craig Beeson, PhD
Professor of Pharmaceutical and Biomedical Sciences
Director of Core C: Bioenergetics Profiling Core

John J. Lemasters, MD, PhD
Professor and GlaxoSmithKlein Distinguished Endowed Chair of Drug Discovery and Biomedical Sciences Professor of Biochemistry and Molecular Biology
Director of Core D: Cell and Molecular Imaging Core

Danyelle Townsend, PhD
Associate Professor of Drug Discovery & Biomedical Science
Director of Core E: Analytical Redox Biology Core

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SC COBRE IN OXIDANTS, REDOX BALANCE AND STRESS SIGNALING

ANNUAL RETREAT
WEDNESDAY, MARCH 7, 2018
MIDDLETON PLACE LAKE HOUSE

AGENDA

8:00- 8:30 Breakfast

8:30- 8:45 Opening Remarks, Chair: Kenneth D. Tew, PhD, DSc

8:45- 9:15 Peggi Angel, PhD — Cobre Project
9:15- 9:25 Discussion

9:25- 9:40 Lauren Ball, PhD — Core Director, Proteomics
9:40- 9:50 Discussion

9:50- 10:05 Craig Beeson, PhD — Core Director, Bioenergetics Profiling
10:05- 10:15 Discussion

Break 15 minutes

10:30- 11:00 Nathan Dolloff, PhD —Cobre Project
11:00- 11:10 Discussion

11:10- 11:40 Seok-hyung Kim, PhD—Cobre Project
11:40- 11:50 Discussion

11:50- 12:45 Lunch

12:45- 1:45 Keynote Speaker: Frank Berger, PhD
"NRF2 and Modulation of Cellular Response to Cancer Chemotherapy: How Robust Is It Really?"

1:45- 2:00 John Lemasters, MD, PhD —Core Director, Cell and Molecular Imaging
2:00- 2:10 Discussion

2:10- 2:40 Gavin Wang, MD, PhD —Cobre Project
2:40- 2:50 Discussion

2:50- 3:00 Break

3:00- 3:15 Danyelle Townsend, PhD — Core Director, Analytical Redox Biology
3:15- 3:25 Discussion

3:25- 3:35 Closing Remarks, Chair: Kenneth D. Tew, PhD, DSc

3:35- 4:15 Executive Advisory Board Meeting

Directions to the Lake House at the Inn at Middleton Place

Follow the signs on Highway 61 to the Inn at Middleton, not Middleton Place. When you pull in the gate, follow the gravel road and stay to the left until you see the small white registration/check in building. To pass through the gate, please enter the following code for entrance: 1860. Please then follow the sign to the Lake House which will be on your left. There are parking spots on the grass located on site next to the building for your convenience.

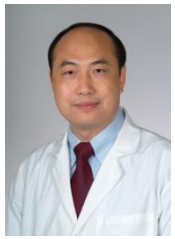
ABOUT COBRE

We are now entering the middle period of Phase II funding of the COBRE program. We continue to bring together junior investigators with interests and expertise in oxidants, redox balance and stress signaling at the Medical University of South Carolina. During the second year of Phase II (2017-2018) we supported four individuals and begin to develop plans for emphasizing eventual transition into phase III, where importance will be placed entirely upon core facilities. Active building of the core infrastructure is a prerequisite to facilitate longer-term planning for a viable Phase III application that focuses only on Institutional support of a "Redox Center." Our short and long-term plans continue to be to develop in South Carolina a Center of Excellence in the scientific discipline of redox biology.

Existing infrastructure has provided a successful mentoring environment for target faculty members with research interests that span a number of human pathologies. The last year has provided funding challenges for the target faculty, where Gavin Wang and Nate Dolloff had RO1 scores that placed them in the 16-17th percentile, but NCI only considers 8th. Peggi Angel has actively applied for a variety of grants and is awaiting results and Seok-Hyung Kim has recently been appointed to the COBRE. Heather Boger has recently submitted an RO1 application. In each instance, the interface of human pathologies with oxidant stress, redox homeostasis and stress signaling provides the fundamentals for the programmatic development of the Center. These projects continue to provide interdisciplinary opportunities and are supported by four scientific cores in Proteomics, Bioenergetics, Cell and Molecular Imaging and Analytical Redox. Our central hypothesis continues to be that redox regulated pathways impact significantly on the pathobiology of diseases such as cancer, aging, diabetes, inflammation and neurodegeneration. We are about to release an RFA for two new junior investigators in the Spring of 2018. We hope to attract external candidates and await a MOU from the College of Medicine Dean to match start-up funding from the COBRE.

In 2017, we were also awarded an Administrative Supplement that has allowed us to purchase new equipment for the Cores and to recruit and support an organizational associate. The administrative core continues to facilitate many functions including business management, faculty development, mentoring, program planning and sustainability. We have appointed oversight committees to include Steering, Internal Advisors and External Advisors. This year we added Dr. Frank Berger to our EAB, and plan to take advantage of his acumen in shepherding a COBRE grant through phase II into the phase III setting. Our advisory groups contain individuals who have broad scientific expertise in chosen disciplines and also considerable mentoring experience. Future development of the program at MUSC is also presently served by existing financial commitments from the Deans of Medicine and Pharmacy and the Provost Office. As we move forward with additional faculty recruitments, our goals will continue to include attainment of peer review support and continued development of the core facilities.

GAVIN WANG, MD, PHD, COBRE PROJECT



Assistant Professor, Pathology and Laboratory Medicine

Targeting Cancer Stem Cells for Breast Cancer Treatment

Breast cancer is the most frequently diagnosed form of cancer and the second leading cause of cancer-related death among women both in the United States and worldwide. Mounting evidence has shown that cancer stem cells (CSCs) are resistant to traditional anticancer therapies, which may contribute to tumor recurrence and metastasis by regenerating new tumors. Therefore, there is a critical need for the development of new therapeutic agents that can effectively eliminate the drug-resistant CSCs and thus improve the efficacy of cancer treatment.

Through unbiased drug screens, we have recently demonstrated that C1572, a small-molecule natural compound, selectively depletes CSCs in a dose-dependent fashion in various human triple-negative breast cancer (TNBC) cell lines. Mechanistically, we found that nanomolar concentrations of C1572 reduced expression and function of c-Myc (MYC) oncoprotein via a proteasome-dependent mechanism. MYC silencing phenocopies the CSC depletion effect of C1572 and induces senescence in TNBC cells. Limited dilution assays revealed that ex vivo treatment with C1572 led to a 28-fold decrease of TNBC CSCs. In xenografts C1572 caused tumor growth suppression and CSC depletion that correlated with marked inhibition of MYC in residual tumor tissues. These results provide the preclinical evidence that C1572 is a promising first-in-class lead compound that eradicates drug-resistant CSCs in TNBC via targeting the MYC oncoprotein.

To understand the mechanisms by which CSCs are resistant to therapy-induced oxidative stress, we investigated reactive oxygen species (ROS) levels in TNBC CSCs compared with non-stem tumor cells (NSCs). We found that CSCs exhibit lower levels of ROS than NSCs do, suggesting a role for redox signaling in CSC drug resistance. Consistent with this hypothesis, our recent studies have demonstrated that knockdown of Nrf2 sensitizes breast CSCs to radiation-induced oxidative stress. These new findings strongly support the hypothesis that the redox signaling pathways may play a pivotal role in modulating the response of CSCs to anticancer treatment. To vigorously test this hypothesis, we will elucidate the mechanisms by which CSCs maintain a lower level of ROS and determine if targeting of selective redox signaling pathways enhances the therapeutic outcome of TNBC therapy. Successful completion of the proposed studies will bring new insights into the role of redox signaling in modulating CSC's response to therapy-induced oxidative stress, which may lead to the development of novel therapeutic approaches to target CSCs for breast cancer treatment.

PEGGI ANGEL, PHD, COBRE PROJECT

Assistant Professor of Cell and Molecular Pharmacology and Experimental Therapeutics

Systems-based analysis of redox activity in aortic valve stenosis

Oxidative stress plays a key but unknown role in fibrocalcific aortic valve stenosis (FAVS). FAVS is a disease of aortic valve (AV), where leaflets progressively stiffen and eventually calcify, causing cardiac malfunction. The only treatment is surgical valve replacement. Increased oxidative stress due to redox imbalance is believed to be an initiating factor for end point calcification of the aortic valve (AV) leaflets, but redox status during FAVS remains undefined. Our current data shows that oxidative stress is increased in a very young adult mouse model of aortic valve stiffening, concomitant with extracellular matrix remodeling, implicating that redox mechanisms play a much earlier role in FAVS than previously described. We hypothesize that early redox imbalances are associated with the ECM disorganization that results in valvular stiffening. To delineate redox mechanisms, Aim 1 will define redox status in bulk, whole mount and histological cross sectional measurements of the AV leaflet during early sequential aortic valve stiffening. Physiological measurements of heart function and biomechanical properties of the AV will be used to define levels of AV stiffening parallel with redox status. Aim 2 will utilize proteomic techniques on extracts from decellularized valve leaflets to identify redox sensitive cellular pathways and protein interaction networks (PINs) during sequential valve stiffening. Bioinformatic approaches will be used to assess PINs regulated during valve stiffening, earmarking new candidates of FAVs. Extracellular matrix (ECM) from the decellularized valve structure will be interrogated by proteomics and immunoblotting to report on ECM changes correlating to increased oxidative stress. Bioinformatics tools and immunoblotting techniques will be used to report on oxidative stress induced post translational modifications. Aim 3 will delineate regulation in protein interaction networks coinciding with increased oxidative stress in de-identified normal and FAVS clinical specimens. A combination of immunohistochemistry, immunoblotting, and proteomics will be used to define the redox status of normal AV compared to FAVS at a cellular level and assess overall contribution to valve structure phenotype. Candidates from the murine model of early valve stiffening will be evaluated for contribution to FAVS in clinical specimens. This investigation relies on the unique resources of MUSC's COBRE and would not be accomplished without use of these resources. This study will allow us to define the role of redox balance and affected protein expression during the early stages of valve stiffening in FAVS, identifying new therapeutic targets that might inhibit disease progression, improve quality of life and decrease mortality.

NATHAN DOLLOFF, PHD, COBRE PROJECT

Assistant Professor of Cell and Molecular Pharmacology and Experimental Therapeutics

Altered redox and cellular bioenergetics drive Velcade resistance in multiple myeloma

Multiple Myeloma (MM) is a plasma cell dyscrasia and the second most common hematological malignancy in the U.S. Patient survival has significantly improved due to treatment advances, such as the development of the proteasome inhibitor bortezomib (Btz). Despite the clinical success of Btz, therapeutic resistance invariably emerges, thus marking the incurable nature of the disease. New molecular targeted treatment strategies are therefore needed, particularly those that enhance the activity of existing drugs like Btz.

Redox signaling is a particularly attractive target in the treatment of MM and other plasma cell disorders for multiple reasons. Plasma cells are the effector cells of the humoral immune response and are tasked with synthesizing and secreting immunoglobulins. The process of mass-producing bulk amounts of protein generates equimolar amounts of reactive oxygen species (ROS), making these cells vulnerable to even the slightest alterations in redox homeostasis. Secondly, the most prevalent genetic abnormality in plasma cell disorders is a chromosomal translocation involving the immunoglobulin heavy chain gene (IgH) and a region of chromosome 11q13. This translocation induces the up-regulation of 11q13 genes, one of which is glutathione S-transferase pi (GSTP), an important regulator of the cellular redox state.

Further evidence comes from drug screening studies that were conducted by our group to identify chemical structures that restore Btz sensitivity to resistant cells. Interestingly, the screening hits were enriched for redox-modulating compounds (4 out of 7), suggesting that disrupting redox signaling reverses proteasome inhibitor resistance. Taken together, the redox pathway is a promising target for the treatment of plasma cell malignancies, and may be an effective therapeutic approach to combating drug resistant MM.

SEOK-HYUNG KIM, PHD, COBRE PROJECT

Assistant Professor of Medicine

Mitochondrial Disease Associated Mutations as novel Genetic Risk Factors to Develop Advanced Fatty Liver Disease

Alcoholic and non-alcoholic fatty liver diseases (FLD) are common chronic liver disorders. A substantial proportion of FLD patients develop an inflammatory response with hepatitis, leading to fibrosis, cirrhosis, liver failure and/or hepatocellular cancer. Increase of oxidative stress caused by accumulation of reactive oxygen/nitrogen species (ROS/RNS) and mitochondrial dysfunction has been implicated in the pathogenesis of both non-alcoholic and alcoholic FLD. The effect of obesity and environmental factors such as alcohol and high-fat diet in FLD are relatively well established. However, genetic determinants of FLD and advanced FLD, including steatohepatitis, fibrosis and hepatocellular carcinoma have not been systematically investigated. Previously, to identify novel genetic variants involved in liver disease, we performed forward genetic screening of zebrafish mutants. Among 19 mutants identified from the screen, four novel mutants (etfavu243, nars2mu101, kdsrmu106, and vmp1mu110) showed hepatomegaly, steatosis, and hepatocellular injury as well as increase of mitochondrial oxidative stress. We found that these proteins are directly or indirectly involved in mitochondrial respiration and maintenance. We also found activation of mTORC1 signaling pathway in livers of all of homozygous mutants. Activation of mTORC1 signaling has been frequently found in FLD patients and might be involved in progression of liver disease, however mechanism by which mTORC1 activation affects progression of liver pathogenesis is not known. Our results showed that mTORC1 activation is associated with increase of oxidative stress and mitochondrial injury in the liver of mutants, which might be associated with liver pathogenesis. We also found severe liver injury phenotype in adult fish with heterozygous mutation of identified genes, supporting the idea that people who carry heterozygous mutations involved in mitochondrial diseases may be susceptible to develop advanced FLD. Thus, investigation of the above identified mutants will identify the mechanism of disease progression in these mutants, and these mutants will be novel vertebrate model of liver disease to develop and test novel therapy to cure advanced FLD. We hypothesize that 1) oxidative stress associated with activation of mTORC1 signaling is important for progression of FLD, from steatosis to hepatocyte injury and 2) mutants which carry heterozygous mutations in genes involved in mitochondrial function are susceptible to the development of severe liver disease caused by a high-fat diet or by chronic alcohol exposure. To explore these hypotheses, in Aim 1, we will investigate whether decreasing mTORC1 signaling using rapamycin or reducing mitochondrial damage using antioxidants such as vitamin E, mitochondria-targeted ubiquinone (Mito-Q), and N-acetylcysteine (NAC) can ameliorate development of liver disease, and in Aim 2, we will investigate whether high fat diet or alcohol exposure can exacerbate liver injury in heterozygous adult zebrafish. Successful completion of these Aims is expected to provide the first in vivo evidence that mutations of proposed genes can be underlying novel genetic risk factors for advanced FLD.