The landmark observation of phenotype inheritance in peas by Augustinian Mendel’s in 1866 was the first concept that subsequently evolved as a framework for a new science, which is now known as genetics. The relentless search for genetic elements that predispose to a disease state reached a peak when the human genome project was completed in 2003, which led to extensive investigation of genome-wide association with diseases. However, despite the initial excitement, one message became clear: although genetic factors are important; most variants identified so far confer relatively small increments in disease risk, with few exceptions.

Epigenetics is a relatively new science (compared to genetics) that serves as a link between environmental factors and genes. Epigenetics is defined as heritable changes that affect the cellular phenotype (or gene expression) without altering the DNA sequence. The epigenetic mechanisms serve to coordinate the unique gene expression cascade in each cell type through a highly developed regulatory system. Consequently, abnormalities of epigenetic mechanisms or abnormal expression of epigenetic regulators exert a direct impact on gene transcription and may predispose to a disease state, such as cancer, inflammation or autoimmune diseases. There is significant evidence to indicate that environmental factors participate in modulating the epigenome by altering epigenetic regulation. Examples of epigenetic mechanisms include (i) DNA methylation, which is characterized by chemical modification of nucleotides, where a methyl group is added to the Cytosine in CG pairs, (ii) post-translational modification of the histone proteins.

Systemic sclerosis (also known as scleroderma, SSc) is an autoimmune connective tissue disease that is characterized by activation of the immune system, vascular dysfunction, and tissue fibrosis. SSc is considered a devastating disease, not only because it is disfiguring due to skin fibrosis, but also it is associated with significant morbidity and mortality due to failure of the internal organs. The precise etiology of SSc is undetermined, but there is evidence to suggest that the environment significantly contributes to SSc pathogenesis. Thus, there is significant association between specific environmental factors and predisposition to SSc (Silica exposure, UV light, chemical solvents, etc.) and the development of SSc.

For the first time, we have recently characterized the DNA methylation abnormalities in SSc fibroblasts [http://www.ncbi.nlm.nih.gov/pubmed/24812288]. The fibroblast is an important cell that is involved in collagen production and expansion of the extracellular matrix (ECM), which leads to tissue fibrosis. We identified genomewide DNA methylation abnormalities in SSc fibroblasts. Some of these
abnormalities point to an epigenetic defect in key pathways that are involved in activation of fibroblasts such as Wnt/β-catenin, and transforming growth factor-β (TGF-β). Other DNA methylation defects are directly involved in collagen production and expansion of the ECM. Moreover, we demonstrated in this study a good correlation between DNA methylation abnormalities of a subset of differentially methylated genes and gene expression, which indicates that the epigenetic abnormalities that we identified in this study are also functionally relevant.

After characterization of methylation defects in SSc fibroblasts at the genome-wide level (or SSc fibroblast methylome), we sought to expand our work to evaluate genome-wide DNA methylation aberrancies in other cell types that are involved in pathogenesis of SSc, starting with microvascular endothelial cells (MVEC), with an aim to establish a "methylome signature of SSc". Also, we sought to evaluate the role of the environmental-epigenetic factors in pathogenesis of SSc by studying the effect of oxidative stress on DNA methylation machinery. The choice of studying the role of oxidative stress on DNA methylation machinery is based on the following observations; (i) SSc is characterized by an abnormal redox state, and increased markers of oxidative injury; (ii) silica and other environmental exposures can cause an oxidative stress state. Putting all together, we demonstrate our working hypothesis for the development of SSc in Figure 1.

The broad goal of this work is to; (1) comprehensively identify DNA methylation profile in SSc-fibroblasts and endothelial cells compared to control-fibroblasts and endothelial cells, respectively, (2) evaluate DNA methylation profile of SSc-fibroblasts obtained from involved (fibrotic)/uninvolved skin, and (3) study the effect of oxidative stress on DNA methylation profile in healthy fibroblasts and endothelial cells. This will be
undertaken in parallel with evaluation of the functional consequences of DNA methylation alterations on gene expression.

We anticipate that we will identify novel DNA methylation alterations in SSc-fibroblasts and endothelial cells with functional consequences that contribute to fibroblast activation and endothelial dysfunction. Also, this project will improve our understanding of the role of oxidative stress in altering DNA methylation regulation, and therefore, generation of the SSc pathologic cellular phenotype. At the conclusion of this work, we anticipate gaining new information regarding the role of DNA methylation in pathogenesis of the disease that will help in generating new hypotheses and therapeutic approaches to SSc.

The scleroderma research community enthusiastically received our proposal since, to date, there are few mechanistic studies to address the role of environmental-epigenetic factors, and how oxidative stress and tissue hypoxia induce fibroblast activation and endothelial cell dysfunction in SSc. Moreover, the unbiased approach of interrogating the epigenome to identify differentially methylated genes in SSc and functional consequences of DNA methylation aberrancies will uncover new pathogenic pathways in SSc. Our approach to study the effect of oxidative stress provides another novel conceptual framework for integrating current knowledge of SSc pathogenesis with epigenetics, with an aim to identify the trigger behind dysregulation of DNA methylation in SSc.

This work is funded by the Scleroderma Foundation new investigator grant.

New Grant

Restriction of Tick-Borne Flavivirus Infection in the Natural Host

Travis Taylor, Ph.D.
Assistant Professor, Department of Medical Microbiology and Immunology

The arthropod-transmitted members of the Flaviviridae family represent an overwhelming disease burden to humans. Flaviviruses are globally significant human pathogens including dengue virus (DENV), West Nile virus (WNV) and tick-borne encephalitis virus (TBEV) that cause encephalitis and hemorrhagic fever with high mortality rates. Unfortunately, there is no specific treatment for clinically recognized cases and only a few vaccines are available.

While infection in human patients may result in severe disease, an interesting dichotomy exists for the outcome of infection with many of the flaviviruses. Vector-borne flaviviruses routinely cycle in nature between reservoir host animals and arthropod vectors without causing any noticeable disease. One potential explanation is that the natural host has evolved potent antiviral restriction factors. We believe that studying flaviviruses in the natural host will provide an important model to identify factors that allow these animals to be protected from disease development.

We were recently awarded a year-long pilot grant from the Lyme Disease Association (LDA) to establish a model to study reservoir host resistance to the tick-borne Powassan virus (POWV). POWV is found in North America and causes encephalitis in humans. As with other members of the TBEV group, POWV uses wild rodents such as the white-footed mouse, Peromyscus leucopus, as part of its infection cycle in nature.

While the LDA is interested in funding research on non-lyme disease tick-borne pathogens, there is some overlap in the natural biology of POWV and the causative agent of lyme disease, Borrelia burgdorferi. Both pathogens share the same tick vectors and rodent hosts. Neither pathogen causes clinical disease in their natural reservoir host, whereas infected humans develop disease symptoms ranging from mild to quite severe. Thus, resistance factors identified for POWV may be relevant to understanding how B. burgdorferi is maintained in nature.

For the proposed pilot project, we intend to use cell lines derived from P. leucopus to dissect antiviral responses using mass spectrometry. P. leucopus fibroblasts will be infected with tick-borne viruses to identify important factors that bind to viral proteins and subsequently restrict the ability of the virus to multiply and cause disease. The work will also initially focus on the P. leucopus homolog of the TBEV restriction factor TRIM79 (pITRIM79) (Figure 1).
Using the *P. leucopus* fibroblasts, we successfully cloned pTRIM79 and showed that similar to the murine homolog, pTRIM79 also targets the flavivirus virulence factor NS5. Studies are underway to make a gene knock-out to evaluate the importance of pTRIM79 to the antiviral response to POWV in *P. leucopus*. By identifying the critical antiviral factors and how they function during flavivirus infection in natural hosts, our studies may provide new targets for therapies to prevent or cure these tick-borne diseases.

**Department of Medicine, Division Research Highlights**

**Division of Community Internal Medicine**

Created to serve internists within the community holding academic University appointments, the division works to keep its members informed of activities within the Medical School and Medical Center that may be relevant personally or for their patients. These faculty provide a valuable clinical education resource for both the undergraduate and graduate medical education programs. Academic activity also involves the conduct of clinical trial research.

Dr. Lawrence Monger is principal investigator for the currently active FREEDOM study.

**TITLE:** A Randomized, Multicenter Study to Evaluate Cardiovascular Outcomes with ITCA 650 in Patients Treated with Standard of Care for Type 2 Diabetes

**PROTOCOL NUMBER:** ITC A 650-CLP-107

**INVESTIGATIONAL PRODUCT:** ITCA 650 (exenatide in DUROS® device)

**PHASE:** Phase 3/Phase 4

**INDICATION:** ITCA GSO is indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes (T2D).

**CLINICAL GOAL:** This study seeks to achieve 2 distinct clinical goals that are hierarchical in nature. The first goal is to show that for adult patients on Standard of Care for T2D, the risk of a pre-defined composite cardiovascular (CV) event for patients on ITCA 650 is no worse than that for the control group within a confidence limit that does not exceed 1.8 at the upper limit; this will be evaluated by pooling the data from the current Study 107 with other pivotal Phase 3 studies of ITCA 650 to perform a meta-analysis of the combined data. The second goal is to show that for adult patients on Standard of Care for T2D, the risk of a more stringent pre-defined composite CV event for patients on ITCA 650 is no worse than that for the control group within a confidence limit that does not exceed 1.3 at the upper limit; the second goal will be evaluated using data from Study 107 alone.

**Division of Infectious Disease**
The Infectious Disease division receives federal funding through the Ryan White (Comprehensive AIDS Resources Emergency) program to provide medical care to all people in the Northwest quadrant of the state of Ohio infected with HIV. Currently, the Ryan White clinic provides primary care, subspecialty care, and an in-house pharmacy to over 800 patients. Clinical drug trials and pneumococcal vaccine trials are ongoing. Anonymous and confidential HIV testing are available through the clinic. In addition, Dr. Westerink's laboratory studies the immune response to pneumococcal vaccination in various groups of individuals who classically respond poorly to the polysaccharide vaccine including the elderly and HIV-positive individuals. These studies are NIH funded.

**Clinical Research Snippets**

Anand Mutgi, M.D.
Sadik Khuder, Ph.D.

To start a new year, we have decided to launch a new section, termed “Clinical Research Snippets”. We will introduce cutting-edge clinical research articles that are relevant to practitioners and researchers. Our expert faculty and residents will actively participate in this section by selecting articles and providing comments. Our aim is to help disseminate new knowledge rapidly to UT community with balanced criticism. As part of this we bring a recent article from JAMA with a preventive medicine theme.

This study was designed to assess whether primary prevention with once-daily, low-dose aspirin would reduce the risk of death from cardiovascular causes, nonfatal stroke, and nonfatal myocardial infarction in Japanese patients (aged ≥60 years) with hypertension, dyslipidemia, or diabetes mellitus. The study was conducted at 1007 clinics in the 47 prefectures of Japan. A total of 14,658 patients were randomized between March 2005 and June 2007 and with completed follow-up in May 2012. At 5 years after randomization, the cumulative primary event rate was similar in participants in the aspirin group and those in the no aspirin group. Overall, few deaths from cardiovascular causes or nonfatal stroke or myocardial infarction were reported with aspirin or no aspirin. However, aspirin significantly reduced the risk of nonfatal myocardial infarction and TIA. Conversely, the risk of extra cranial hemorrhage requiring transfusion or hospitalization was higher with aspirin than with no aspirin.

In discussing the limitations of the study, the investigators acknowledged the decreasing level of adherence with daily low-dose aspirin in the aspirin group (dropping to 76% in year 5) and increasing uptake of daily aspirin in the no aspirin group (reaching 10% in year 5). Also, this study does not have all the advantages of a double blind, randomized, placebo-controlled trial and does not control for lack of ascertainment. The study participants were not blinded, and it is possible that patients receiving aspirin were more likely to report adverse events believed to be related to aspirin treatment than those not receiving treatment. Moreover, it is possible that enrollment in the study led to patients having more physician contact, resulting in better control of risk factors than the general population. Lower prevalence of current smoking and a lower mean BMI in the Japanese population compared with a Western population, might account for the low observed event rates and early termination of the study.

The findings of this study contradict with recent meta-analyses that suggest beneficial effects for aspirin in the primary prevention of cardiovascular events. Despite inconsistent evidence for the benefit of aspirin in primary prevention, the benefits in secondary prevention of cardiovascular events are well documented. There is also a growing body of evidence to suggest benefits for aspirin in the prevention of colorectal, breast, and other types of cancers, and the prevention of cancer recurrence. Primary prevention of cancers and secondary prevention of cardiovascular events may influence the overall risk-benefit profile of aspirin.

**Use of Single IRB for Multi-site Research**

We all know that duplicated reviews of a multi-site clinical study by individual IRBs of all participating institutions represent a major administrative burden causing waste of time and significant delay of the start-up processes. The US Department of Health and Human Services (HHS) and the Food and Drug Administration (FDA) already have rules allowing institutions to use joint review, but only few institutions are
taking advantage of this option. The National Institutes of Health (NIH) recently released a draft policy to promote the use of a single IRB for NIH-funded multi-site clinical studies (http://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-026.html). Please visit this site to learn more of the new draft policy and to submit your comments.

BRIM Connection Conference - January 26

12:00 - 1:00 PM, 1035 Collier Building

Michael Rees, M.D., Ph.D., Professor, Vice Chair and Medical Dir, Human Donation Science Program
Selection of simultaneous cycles vs. non-simultaneous chains in kidney paired donation

Stanislaw Stepkowski, Ph.D., Professor, Med Microbio & Immunology
New approach for selection of sensitized donor/recipient pairs in kidney paired donation program.

Lunch will be provided

New clinical trials

A Pilot, Phase 2, Randomized, Placebo-Controlled, Double-Blind, Study To Evaluate Efficacy, Safety, Tolerability, Pharmacokinetics, Pharmacodynamics and Pharmacogenetics of CC-220 In Subjects With Systemic Lupus Erythematosus.
Dr. Kahaleh - Medicine

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