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SESSION 9: BASIC/TRANSLATIONAL
SCIENCE

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Nrf2 Deficiency is Associated with Endothelial Dysfunction in Pathologic Wound Environments

Darren Sultan, BA, Joshua A. David, BS, Salma Abdou, BA, Jennifer Kwong, BA, Jasmine Lee, BA, Chen Shen, BA, Piul S. Rabbani, PhD, Daniel J. Ceradini, MD

NYU School of Medicine, New York, NY, USA

PURPOSE: Chronic wounds are among the most common complications of diabetes mellitus with 5–10% of diabetics suffering from foot ulcers. Physiologic wound healing involves the complex interplay of multiple cellular components, including endothelial cells as a major angiogenic workhorse. We have previously shown that the absence of effective Nrf2 signaling contributes to pathologic diabetic wounds. The purpose of this study is to analyze the detrimental effects to endothelial cell function that result from a relative loss of Nrf2 within *in vitro* and *in vivo* models.

METHODS: Mouse endothelial C166 cells were transfected with siRNA specific to Nrf2 or a nonsense control. RNA was isolated from cell lysates, and knockdown was confirmed with quantitative RT-PCR. Cell growth was determined by MTS assay comparing transfected populations to appropriate controls over 3 days. *In vitro* angiogenesis measures were carried out by plating cells on Matrigel to induce tube formation under stress conditions. *In vivo* studies were performed by histologic analysis of tissue sections taken from humanized murine wounds.

RESULTS: To recapitulate an environment of Nrf2 dysfunction akin to that seen in chronic diabetic wounds, assays were designed to mimic pathophysiologic defects. Quantitative RT-PCR demonstrated between 70–77% knockdown of *Nrf2* expression compared to non-sense control cohorts ($p < 0.01$). Similarly transfected populations were employed for further *in vitro* study. Nrf2 knockdown was associated with a 25% reduction in relative cell growth as compared to non-sense controls ($p < 0.05$). Untreated cohorts showed a 60% increase in growth, and cells treated with 0.5% triton-x showed a 95% decrease ($p < 0.01$). Plating endothelial cells

on Matrigel was shown to induce tube formation akin to primitive capillary sprouting. In hyperglycemic conditions, transfected cohorts displayed poor migration and fewer ring structures per high-powered-field as compared to silencer controls (average 4.7 versus 13/HPF 10x, $p < 0.01$). Tissue sections were taken from 10-day-old wounds in wild-type and Nrf2 knock-out mice and were stained for CD31 to identify microvessels. Nrf2 KO tissue sections showed fewer than half the number of vessels as compared to the wild-type sections (average 16 versus 34/HPF, $p < 0.01$).

CONCLUSIONS: Employing a number of experimental methodologies, we have highlighted a significant role of Nrf2 as it relates to endothelial cell function and angiogenic potential in wound environments. Our evidence demonstrates that a lack of Nrf2 is associated with stunted endothelial cell growth, migration, capillary tube formation, and angiogenesis. This work sheds new light on the pathophysiologic basis of diabetic wounds and thereby helps to direct future study at potential therapeutic targets.

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Designing a Murine Model of the Foreign Body Reaction

Jagannath Padmanabhan, PhD, Zachary A. Stern-Buchbinder, MS, Teruyuki Dohi, MD, PhD, Peter Than, MD, Geoffrey C. Gurtner, MD, FACS

Stanford University School of Medicine, Stanford, CA, USA

PURPOSE: Biomedical implants such as pacemakers, breast implants, artificial joints, and biosensors have vastly improved quality of life for patients worldwide. Over time, implant performance can be compromised by a fibrotic response known as the foreign body reaction (FBR). The FBR varies in severity with the most serious presentation, referred to here as “Hyper FBR”, involving formation of a proteinaceous capsule leading to implant rejection and failure, often requiring invasive and expensive device removal or replacement procedures. Hyper FBR-mediated implant failure remains the primary challenge in improving biomedical device function and longevity in patients. Development

of an animal model that mimics severe FBR could be used to identify which factors give rise to this exaggerated response and to identify cellular and molecular targets for prophylaxis and therapy. Our lab has previously shown that applied mechanical stress can increase the severity of fibrotic reaction during wound healing by activation of mechanotransduction pathways. Here we hypothesized that application of mechanical stress by vibration of motorized implants might induce hyper FBR.

METHODS: We manufactured cylindrical polydimethylsiloxane (PDMS) implants which could be adapted to house small, prefabricated coin motors. The coin motors can be powered using an external battery to induce vibration of PDMS implants in situ. These vibration-enabled implants were implanted in the subcutaneous space of WT C57/BL6 mice. Non-vibrating PDMS implants were used as controls. Beginning on post-operative day 4, mice with vibration-enabled implants were sedated and their implants vibrated 1 hour daily for 8 days. Subsequently, mice from each group were euthanized at 2-week and 4-week endpoints, and implants were resected en-bloc with surrounding capsule and tissue intact. Fibrotic tissue surrounding the implants was analyzed using: 1. immunohistochemistry to analyze tissue fibrosis, 2. mass spectrometry to analyze protein content of FBR capsules, and 3. single-cell RNA sequencing to identify cells that mediate hyper FBR. Additionally, patient-derived FBR capsules from explanted biomedical devices were analyzed for validation of the animal model.

RESULTS: Histological analyses of tissue around the implants sections revealed that mechanical vibration of PDMS implants leads to an increased fibrotic reaction. At the 2-week timepoint, tissue surrounding control implants was predominantly granulation tissue, characterized by an early inflammatory response with increased vascularization. In contrast, tissue surrounding vibration-enabled implants displayed a more mature collagenous capsule formation. Additionally, analysis of trichrome-stained tissue sections revealed a significant increase in average collagen density around vibration-enabled implants as compared to controls. Comparison of FBR capsules from mice and humans revealed that the vibration-enabled implants in mice more closely resembled the tissue architecture of human FBR capsules than controls.

CONCLUSION: Our data suggests that this novel Hyper FBR mouse model may approximate clinical implant encapsulation and rejection seen in human patients. We are currently investigating specific mechanotransduction pathways activated during hyper FBR, which could serve as potential

targets for therapy. Further research may lead to the development of specialized treatments which attenuate FBR and prolong optimal function of biomedical implants.

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Lymph Node Transplantation Decreases Local Immunosuppression In Lymphedema

Catherine L. Ly, MD, Raghu P. Kataru, PhD, Babak J. Mehrara, MD

Memorial Sloan Kettering Cancer Center, New York, NY, USA

PURPOSE: Lymphedema is a morbid disease that is associated with an increased risk of recurrent infections. Microsurgical lymph node transplantation (LNT) has emerged as a promising treatment option particularly because it may lead to a decrease in the incidence of such complications. Recent research has shown that the local immunosuppression predisposing patients to infections can be at least partly attributed to an increase in T regulatory cells (Tregs). However, the mechanisms underlying the effect of Tregs on immune responses and how such mechanisms may be altered following LNT remain unclear. Using preclinical mouse models, this study therefore sought to further elucidate the means by which Tregs mediate immunosuppression in lymphedema and to determine whether LNT mitigates these changes.

METHODS: To determine the role of Tregs in lymphedema, we performed axillary lymph node dissection (ALND) on transgenic Foxp3-diphtheria toxin receptor (DTR) mice, in which Foxp3⁺ Tregs can be depleted using diphtheria toxin (DT). Six weeks after surgery, these mice were randomized to receive DT or control injection. In other experiments, transgenic FLT4-DTR mice, in which DT can be used to locally ablate the lymphatic vasculature, underwent forelimb lymphatic ablation followed by ALND and were then randomized to undergo either orthotopic LNT or sham surgery. Inflammation, bacterial clearance, and antibody production were analyzed to delineate the effects of Treg depletion and LNT on lymphedema.

RESULTS: The depletion of Tregs in ALND-treated mice resulted in increased inflammation, consistent with the