SATURDAY, MAY 6, 2017
SESSION 7 GROUP B
8:30 AM – 10:00 AM

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Hyaluronan Synthase 2 Knock Down in Epidermis Alter Wound Healing and Hair Follicle Development

Mason Bartels, BS, Yucel Akgul, MD, PhD
University of Texas Southwestern Medical Center, Dallas, TX

PURPOSE: Impaired wound healing is a serious complication and impacts patient’s quality of life adversely. Among extracellular matrix (ECM) molecules, hyaluronan (HA) has been suggested to play a critical role in all phases of wound healing as it modulates cell behavior including adhesion, migration, proliferation, metabolism and differentiation. HA is produced by 3 distinct hyaluronan synthase (Has) and exists in varying sizes. It is widely accepted that large molecular weight HA is involved in structural and anti-inflammatory functions, whereas small molecular weight HA is angiogenetic and pro-inflammatory.

METHODS: To evaluate the cell specific functions of HA in wound healing, human primary cell culture and transgenic mice that lack epidermal Has2 were utilized. Transgenic mice were generated by crossing Keratin-14 CreER with Has2 flox/flox and tamoxifen injection (HA KO). Gene expression and histological evaluation were utilized in this study. For cell culture studies, human dermal fibroblasts and keratinocytes were isolated from the waste skin tissue of patients undergoing plastic surgeries.

RESULTS: Among 3 hyaluronan synthesis, Has3 was responsible for majority of the HA production in differentiated keratinocytes. In contrast, Has2 gene expression and HA production were much higher in epidermal stem cells suggesting that Has2 has a critical role in stem cell function. Interestingly, both epidermal stem cells (first 2 passage after dermal keratinocyte isolation) were highly sensitive to immune stimulation following TLR agonist treatment, however HA production, Has2 expression and immune response decreased with increasing passage number as keratinocytes mature. Furthermore, immune response to TLR agonists was blocked by Has2/Has3 blocking reagent suggesting that HA may have a role in chronic inflammation associated with impaired wound healing like diabetic ulcers. Wound closure especially reepithelization following wounding surgery was not effected in the HA KO mice compare to wild type. However, histological evaluation suggested that the wound repair was much slower in HA KO mice compare to their wild type siblings. Particularly, higher collagen expression in the granulation tissue and epidermis. In addition, HA expression in the granulation tissue of HA KO wounds were much lower compare to wild type. Moreover, hair follicle development was altered in the HA KO mice with larger gland development compare to wild type.

CONCLUSION: Collectively these studies provide unexpected results regarding the roles of HA in the epidermal cells of wound healing. While HA secretion by Has2 in epidermal cells is not obligatory for reepithelization, it is critical for collagen matrix reorganization during wound healing.

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Inhibition of Scleraxis Signaling Provides a Target to Reduce Mesenchymal Cell Inflammation During Wound Healing

Arminder Kaura, BS, Michael Chung, MD, David Cholok, BS, Shawn Loder, BS, Christopher Breuler, BS, John Butts, BS, Joseph Habbouche, BS, John Li, MD, Shuli Li, PhD, Shailesh Agarwal, MD, Benjamin Levi, MD
University of Michigan, Ann Arbor, MI

PURPOSE: Inflammation following trauma is a critical step during both normal and pathologic wound healing. Recently, we have identified Scleraxis, a transcription factor with conserved topoisomerase homology, as a mediator of pathologic wound healing. Here we demonstrate that ciprofloxacin, a known topoisomerase inhibitor, reduces exhibits anti-inflammatory properties during injury through a reduction in Scleraxis signaling.

METHODS: Mesenchymal cells were isolated from mice with genetic loss of Scleraxis (Prx-cre/Scx ΔΔ) and wild type mice. Separately, wild type cells were treated with ciprofloxacin (10 mg/kg) or vehicle control. In vitro assays were performed to quantify proliferation and Scleraxis signaling. Furthermore, mutant or wild type mice underwent hindlimb tendon transection and subsequent flow cytometry and
histologic analysis during the first week after injury. This was similarly performed for mice treated with ciprofloxacin or vehicle control.

RESULTS: Flow cytometry demonstrated that genetic loss of scleraxis among mesenchymal cells significantly reduced the presence of macrophages (F4/80+) and neutrophils (CD11b+Ly6G+) at the injury site within 48 hours after injury. The presence of PDGFRA+ mesenchymal cells was also significantly reduced based on both flow cytometry and histologic analysis. These findings were confirmed with ciprofloxacin treatment. Furthermore, genetic loss of Scleraxis and ciprofloxacin both corresponded with a significant reduction in mesenchymal cell proliferation. Ciprofloxacin treatment led to reduced chondrogenic differentiation and aggregcan expression. Genetic loss of Scleraxis reduced ectopic cartilage formation when compared with wild type controls.

CONCLUSION: These findings indicate that Scleraxis is a potent target to prevent mesenchymal cell proliferation and inflammation. Ciprofloxacin, an FDA-approved drug, has therapeutic efficacy as an anti-inflammatory agent with translational potential to prevent pathologic wound healing.

Identification and Therapeutic Targeting of a Central DNA-Based Mechanism Through Which Movement Augments Inflammation

Shailesh Agarwal, MD, Shawn Loder, BS, David Cholok, BS, Michael Chung, MD, Arminder Kaura, BS, John Li, MD, Kavitha Ranganathan, MD, Christopher Breuler, BS, Joseph Habbouche, BS, John Butts, BS, Hsiao Hsieh Hsung, DDS, Shuli Li, PhD, Yuji Mishina, PhD, Benjamin Levi, MD

University of Michigan, Ann Arbor, MI

PURPOSE: Musculoskeletal trauma and surgery poses a unique challenge to surgeons: early mobilization facilitates rehabilitation but also promotes local inflammation and pathologic wound healing. However the mechanism by which extremity movement promotes local inflammation remains poorly characterized. Recently, discrete structural webs composed of DNA and histones, known as neutrophil extracellular traps (NETs), have been identified as key components of the inflammatory cascade elicited by neutrophils. Here we demonstrate that physically or chemically disrupted NETs are responsible for augmenting local inflammation by directly inducing NET formation (NETosis) yielding a therapeutic target to prevent inflammation caused by early movement.

METHODS: Mice received dorsal hindlimb tendon transection +/- cast immobilization with subsequent treatments to destabilize NETs (+/- DNase I), inhibit NETosis (+/- Cl-Amidine), and/or inhibit NET-induced NETosis (+/- ODN-2088). MicroCT imaging was performed to evaluate In vitro experiments were performed to confirm that NETs produced by neutrophils in response to the inducing agent PMA are able to induce neutrophils to form NETs (NET-induced NETosis). Furthermore, in vitro experiments were performed to confirm that Cl-Amidine but not ODN-2088 inhibits PMA-induced NETosis.

RESULTS: In vitro experiments confirmed that NETs are capable of inducing secondary NET formation (NET-induced NETosis). Both Cl-Amidine and the toll-like receptor 7/8/9 inhibitor ODN-2088 reduced the number of NETs formed through NET-induced NETosis (Cl-Amidine: 46.6 v. 9.1, p<0.05; ODN-2088: 46.6 v. 11.0, p<0.05). Cast-immobilization of mice after tendon transection eliminated ectopic cartilage and heterotropic ossification. Flow cytometry showed that cast-immobilization significantly reduced the normalized presence of neutrophils (1.0 v. 0.08, p<0.05) and macrophages (1.0 v. 0.13, p<0.05) 48 hours after injury. However, treatment of cast-immobilized mice with DNase to destabilize NETs led to a rebound increase in inflammation (neutrophils: 1.0 v. 6.39, p<0.05; macrophages: 1.0 v. 3.0, p<0.05) and caused ectopic cartilage and heterotropic ossification. Furthermore, Cl-Amidine and ODN-2088 both reduced early neutrophil presence in mice with the mobile hindlimb (Cl-Amidine: 1.0 v. 0.4, p<0.05; ODN-2088: 1.0 v. 0.27, p<0.05) and in DNase-treated mice with cast-immobilization (Cl-Amidine: 1.0 v. 0.27, p<0.05; ODN-2088: 1.0 v. 0.28, p<0.05).

CONCLUSION: These results elucidate a central mechanism by which movement induces inflammation - NETs produced during the early inflammation are disrupted and further induce NETosis. These experiments identify a class of toll-like receptors such as ODN-2088, which are capable of reducing inflammation caused by movement by targeting NET-induced NETosis. These findings have immense value in preventing wound healing pathology associated with unchecked...