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Pannexin 3 Deficiency in Mice Delays the Wound Healing Process

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PURPOSE: Pannexin 3 (Panx3) is a gap junction protein. We have previously shown that Panx3 plays multiple channel functions: 1) as a hemichannel to regulate intracellular ATP/cAMP levels between cells and the extracellular space, 2) as an ER calcium channel to regulate calcium flux within the cell, and 3) a gap junction to exchange ions and small molecules between cells. However, the role of Panx3 in skin tissue regeneration, proliferation, and/or differentiation is unclear. Here, we demonstrate that Panx3 plays a role in the skin wound healing process by controlling the inflammatory response, epidermal-mesenchymal transition (EMT), keratinocyte proliferation, and collagen deposition.

METHODS: To identify Panx3 functions in the skin wound healing process, two 8 mm diameter full-thickness skin punches were made in Panx3 knockout (Panx3 -/-) mice and heterozygous knockout (Panx3 +/-) siblings as controls. The wound healing process was analyzed by evaluating the remaining wound area in a time course. The wound area skin samples were collected on the 5th and the 10th day post-surgery for histological and immunohistochemical analysis. The levels of inflammation, EMT and signaling pathway markers were analyzed by RT-PCR. To investigate whether Panx3 promotes keratinocyte differentiation, the human keratinocyte line HaCaT cells were transfected with a human Panx3 cDNA expression vector. Low (0.03mM) or high (1.2mM) Ca²⁺ concentrations in the medium were used to induce differentiation in mock- and Panx3-transfected HaCaT cells. Cell proliferation rates, cell cycle and keratinocyte differentiation markers were then analyzed.

RESULTS: At 10 days post wound surgery, Panx3 -/- mice showed slower healing rates than control Panx3 +/- mice. The most obvious difference was on the 5th day post-surgery, which is associated with the inflammatory stage of wound healing. The cell infusion and collagen deposition rates were less in Panx3 -/- mice than in the Panx3 +/- mice. On the 5th day post-surgery, inflammatory markers including CD4, CD68, IL1-beta, IL1R1, IL6, and IL6R, as well as EMT markers such as MMP9, snail, and N-cadherin demonstrated a decrease in their expression levels in Panx3 -/- mice compared to Panx3 +/- mice. In addition, markers for several cell signaling pathways, such as Wnt, BMP2, BMP4, Shh and TGF-beta2, were reduced in Panx3 -/- mice. In contrast, TGF-beta1 was reduced in Panx3 +/- mice when compared with Panx3 -/- littermates. Using in vitro cell culture assays, we found that low concentrations of Ca²⁺ in Panx3-transfected HaCaT cells promoted cell proliferation. But high Ca²⁺ concentrations inhibited cell proliferation. Cell cycle analysis showed that Panx3 maintained HaCaT cells in the S-phase, indicating active DNA replication. Keratinocyte differentiation markers such as K10, K14, filaggrin, and involucrin were not altered by over-expressing Panx3 in HaCaT cells.

CONCLUSION: Panx3 promotes keratinocyte proliferation but not differentiation. Panx3 deficiency reduces the expression of epithelial-mesenchymal transition markers, inflammatory markers and collagen deposition. This suggests Panx3 is involved in EMT and inflammatory processes. Panx3 plays a critical role in the skin wound healing process.

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Severe Injury Leads to Plasmin Consumption Below a Critical Threshold Required to Heal Soft Tissue Injury

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PURPOSE: Severe burn injury is associated with delayed or failed repair of skin and soft tissue. Following burn injury, impaired tissue healing occurs both at the site of injury and in tissue concurrently injured with, but anatomically remote from, the injury. Thus, the predominate hypothesis is that severe injury provokes derangements in circulating acute phase reactants necessary for soft tissue repair, contributing to pathologic changes in tissue healing. It has been determined that failure to remove fibrin, the major constituent of the coagulation matrix, impairs healing of all tissues. Plasmin is the principle protease responsible for removing fibrin and is essential for the regeneration and healing of all tissues in genetically modified mouse models. It is unknown whether plasmin levels are consumed following significant injuries or whether changes in plasmin levels below a critical threshold contribute to pathologic healing. The purpose of this study is to determine whether plasmin levels are consumed following burn injury in humans and whether plasmin levels are associated with pathologic tissue healing and regeneration in mouse models following a burn injury. We postulate that a significant burn injury influences plasmin levels contributing to poor healing at both the site of burn injury and in tissue anatomically remote from the injury.

METHODS: All human and animal studies were approved by Vanderbilt IRB and IACUC respectively. Circulating plasminogen levels were determined using a commercial sandwich ELISA (Molecular Innovations; Novi, MI) from human burn patient plasma samples obtained one and three days following burn injury. All murine burn studies were conducted in 6-week-old male mice. After adequate anesthesia, a full thickness cutaneous burn covering 30% of the total body surface area (TBSA) was induced. To simulate the clinical scenario of superimposed burn and muscle injury, WT mice assigned to the injury cohort were injected with CTX in the lower extremity prior to sustaining a burn injury. Dystrophic calcification at the site of muscle injury was evaluated as a marker of pathologic muscle healing.

RESULTS: In human patients, significant hypoplasmogenemia occurred following burn injury and persisted through 3-days post burn when compared to controls (p<.05). The extent of hypoplasmogenemia in these patients correlated with the TBSA (p= 0.0014; Spearman r= -0.70, Image B). Similar to human patients, mice who sustained a 30% TBSA burn were also hypoplasmogenemic 3-days post injury. Combined burn and CTX-injected WT mice showed significant radiographic evidence of heterotopic ossification compared to burn alone (p<0.05). The incidence and severity of HO in α2APASO-treated mice was significantly decreased compared to control ASO-treated combined burn/muscle injury mice (p<0.001).

CONCLUSION: This data provides evidence that significant burn injury leads to derangements in systemic plasmin levels that result in poor tissue healing both at the site of injury and in an anatomically remote site. Pharmacologic intervention to preserve circulating plasmin levels may drastically improve outcomes in patients sustaining significant injuries.

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Enhancement of Therapeutic Benefits of Split Thickness Skin Grafts using Pre-vascularized Human Mesenchymal Stem Cells
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PURPOSE: Split thickness skin graft (STSG) implantation is one of the standard therapies for full thickness wound repair when autologous skin full thickness graft (FTG) or skin flap transplants are inapplicable. However, STSG are more fragile than FTG and can contract significantly during the healing process. Human mesenchymal stem cells (hMSCs) are capable of accelerating the wound healing process. So we use pre-vascularized hMSC sheets (PHCS) to study if they would further improve the repair quality of STSG.

METHODS: In vitro cultured control hMSC cell sheets (HCS) were obtained after four weeks culture in complete culture medium (CCM), then endothelial cells (ECs) were cocultured on top of the hMSC sheets for 1 week to get PHCS. Immunofluorescence staining was used to characterize both cell sheets. The progenitor population and multi-lineage differentiation ability of hMSCs inside the cell sheets were tested. The angiogenic growth factor amount present in the cell sheets was also analyzed using an enzyme-linked immunosorbent assay (ELISA). The cell sheets were applied in combination with an autologous STSG in rats with full thickness skin wound. Graft survival and contraction was