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 hypoplasminogenemia occurred following burn injury and persisted through 3-days post burn when compared to controls (p<.05). The extent of hypoplasminogenemia in these patients correlated with the TBSA (r= 0.0014; Spearman r= -0.70, Image B). Similar to human patients, mice who sustained a 30% TBSA burn were also hypoplasminogenemic 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 controlASO-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

Lei Chen, MD, PhD1, Qi Xing, PhD2, Qiyi Zhai, MS1, Mitchell Tahtinen, BS2, Fei Zhou, MD1, Shaohai Qi, MS1, Mohamed Magdy Ibrahim, MD3, Feng Zhao, PhD2

1First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China, 2Michigan Technological University, Houghton, MI, 3Duke University Medical Center, Durham, NC

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
evaluated and the implants were harvested at predetermined time points for analysis (n=6 animals per group). Histology and immunohistochemistry were performed to test if PHCS can enhance the therapeutic effects of STSG.

RESULTS: The hMSCs in HCS still maintain self-renewal and differentiation capacity after extended periods of culture. The TGF-β1, ANG1 and ANG2 amount in PHCS was higher than HCS (p<0.05) whereas the VEGF amount in PHCS was significantly lower than that in HCS (p<0.05). The HCS and the PHCS implantation significantly reduced skin contraction and improved cosmetic appearance relative to the STSG control group. The PHCS group experienced the least hemorrhage and necrosis, and lowest inflammatory cell infiltration. It also induced the highest neovascularization in early stages, which established a robust blood microcirculation to support grafts survival and tissue regeneration. Moreover, the PHCS grafts preserved the largest amount of skin appendages (including hair follicles and sebaceous glands) and developed the smallest epidermal thickness. The collagen deposition and fibril morphology in PHCS grafts were also similar to normal skin, indicating a lower degree of skin fibrosis. The superior therapeutic effect seen in PHCS groups was attributed to the elevated presence of growth factors and cytokines in the pre-vascularized cell sheet, which exerted a beneficial paracrine signaling during the early stage of wound repair.

CONCLUSION: The strategy of combining autologous STSG with PHCS implantation appears to be a promising approach in regenerative treatment of full thickness skin wounds.

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Requirement Of talin1 For Cell Proliferation During Palate And Mandibular Development In Zebrafish

Kana Ishii, MD, Kusumika Mukherjee, PhD, Eric C. Liao, MD, PhD

Massachusetts General Hospital, Boston, MA

PURPOSE: The role of cytoskeletal proteins in craniofacial morphogenesis remains understudied. Critical cellular processes like cell morphological change, migration, proliferation and differentiation are coordinated to mediate craniofacial morphogenesis. These cellular processes require extensive interactions between cellular junctions and the extracellular matrix. Adhesion of cells to extracellular matrix is necessary for the development of multicellular organisms both functionally and structurally during embryonic development. The integrin family of cell adhesion molecules regulates interactions between cells and extracellular matrix. Talin (tln), an adaptor protein, is one of the several proteins that link an integrin subunit to the actin filaments. This link is essential to transmit force from the actin cytoskeleton to the extracellular matrix. Gene knockout studies in mice imply that tln1 plays an important role in the early morphogenetic events during embryonic development. However, the role of tln1 in the molecular and cellular mechanisms involved in craniofacial morphogenesis is poorly understood. Given the central role of tln as a cytoskeletal protein that interface with a number of key regulatory pathways, we hypothesize that tln1 is critical in regulating cellular processes that underlie craniofacial morphogenesis.

METHODS: The tln1 mutant line was generated from an insertion mutagenesis screen. The expression of tln1 was determined by whole mount in situ hybridization (WISH) during embryogenesis, and the level of expression was analyzed by quantitative and non-quantitative PCR. Craniofacial cartilaginous structures and muscles were examined by Alcian Blue stain and in the mylz2:mCherry transgenic respectively. Cell lineage tracing, morphology characterization, proliferation and apoptosis assays were performed in sox10:kaede and sox10:mCherry transgenic animals.

RESULTS: The tln1 homozygotes exhibit microcephaly and pericardial edema, and survive until 5 days post fertilization (dpf). WISH analysis shows that tln1 is expressed in the craniofacial region starting at 48 hours post fertilization (hpf). The mutant is a loss-of-function (LOF) allele demonstrated by quantitative PCR. Analysis of the craniofacial cartilage and muscles show that the lower jaw is shorter and highly malformed, the palate is shorter and the craniofacial muscles are disorganized. There is no observed defect in cranial neural crest cell (CNCC) migration and no increased CNCC death in the mutants. Pulse-chase analyses suggest that defects in directive cell proliferation in the CNCCs may be causing the palate anomalies. Further cell proliferation experiments via EdU and BrDu labeling are ongoing to validate these results.

CONCLUSIONS: Tln1 is required for craniofacial development: in formation of the palate, Meckel’s cartilage and a number of other ventral cartilage structures and the craniofacial muscles. We hypothesize that the cytoskeleton