with an average of 13.8 pack-years smoked. There was significantly higher Col6a3 gene expression in the epigastric region superficial to Scarpa’s fascia among former smokers when compared to the same depot in non-smokers (2.1-fold higher expression than the reference depot versus 0.1-fold, p = 0.02). There was no difference in mean adipocyte size between former smokers and non-smokers in this depot (p=0.97).

Conclusion: Col6a3 gene expression in the superficial epigastrium is higher among former smokers than in non-smokers. This finding suggests another possible role of Col6a3, as a mediator of tissue fibrosis in former smokers. This, along with its association with insulin resistance, may implicate Col6a3 as the molecular culprit in wound complications among obese patients who are former smokers. Long-term follow up of this cohort is warranted for evaluation of wound complications.

CD36 Antagonism Minimizes Skin Scarring By Inhibiting JUN-dependent Fibrotic Pathways Within Fibrogenic Fibroblast Subpopulations

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Purpose: Skin fibrosis is the end result of injury in human skin. In the US alone >100 million new scars are formed every year, and with no therapy able to prevent or reverse skin fibrosis the medico-economic burden is enormous. Excessive fibrosis, as seen in hypertrophic scarring (HTS), can lead to devastating disfigurement and permanent functional loss. Incomplete understanding of the key pathogenic mechanisms driving pathological skin fibrosis has significantly hindered development of effective treatment strategies. We recently identified JUN, the AP-1 transcription factor, as a key driver of global tissue fibrosis. Here we investigate the role of JUN in skin scarring.

Methods: Primary cultures of human dermal fibroblasts (HDF) were derived from HTS and unwounded skin. JUN expression was deleted using CRISPR/Cas9, and the downstream genetic/epigenetic consequences were assessed by RNA-sequencing (RNAseq), the Assay for Transposase-Accessible Chromatin with high-throughput sequencing (ATACseq), and gene ontology pathway analysis to identify the genes mediating JUN-dependent fibrosis. HDF were treated with salvianolic acid (SAB), a CD36 antagonist, to determine the in vitro effects on HDF proliferation, apoptosis, and production of collagen and TGFβ. The in vivo effects of CD36 antagonism were explored using a novel inducible mouse model of HTS. Dorsal excisional stented wounds were created in JUN (c-Juncreo R26M2rtTA) mice, and either SAB (1mg/ml) or PBS (control) was administered via intraparietal injection every 24h for 14 days. Wounds were compared macroscopically for scar morphology and re-epithelialization rates. On day-14, wounds were harvested and compared for histological fibrosis as well as for the composition fibroblast subpopulations and immune cells by flow cytometry. PCRs were conducted on genes upregulated in JUN fibroblasts to investigate the mechanism by which CD36 antagonism lead to decreased scarring.

Results: JUN deletion significantly altered HDF gene expression and chromatin accessibility, affecting genes involved in key fibroproliferative pathways (e.g. PI3K/AKT/mTOR, PPARγ, ECM). Targeted exploration of the 100 candidate genes with closing epigenetic landscapes and reduced gene expression following JUN knock-out (KO) in HTS-HDF implicated CD36 as a surface mediating JUN-dependent fibrosis and proliferative pathways. Immunofluorescence showed close association between JUN and CD36 proteins in HDF. In vitro CD36 antagonism using SAB decreased fibroblast proliferation, increased apoptosis, and decreased production of collagen and TGFβ. In vivo, SAB treatment did not alter wound closure rate, but significantly prevented the development of HTS; SAB-treated wounds were less raised, less pigmented, had thinner dermal layers, and more ordered collagen fiber networks. At a cellular level, SAB-treated JUN wounds had fewer immune cells and fewer profibrotic reticular fibroblasts. JUN lipofibroblasts exhibited increased expression of genes in the PPARγ pathway (PLIN1, FABP4, FABP5) linking CD36 signaling to adipocyte differentiation.
Conclusions: JUN drives pathological skin fibrosis following wounding, and CD36 antagonism with SAB can be used to inhibit JUN-dependent fibrotic pathways within key fibroblast subpopulations and decrease pathological scarring.

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Human Cryopreserved Skin Allografts Recruit M2-macrophages And Induce Angiogenesis In A Murine Xenograft Model

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Purpose: For over 50 years, human cryopreserved skin allografts (HCAs) have been used for temporary coverage of major burns when autograft donor sites are insufficient. HCAs have also been shown to promote healing of diabetic foot ulcers and prevent fluid loss and infections. Their mechanism of action, however, remains elusive.

Methods: HCA and human acellular dermal matrix (ADM) grafts were implanted subcutaneously in C57BL/6 (WT) mice and explanted after 1, 3, 7, 14, and 28 days (n=5 per group). A sham surgery served as a control. Immunofluorescent staining (IF) of tissue sections was used to determine the immune reaction against HCA and ADM as well as vascularization and pro-angiogenic signaling within the grafts and the overlaying mouse skin. HCA and ADM grafts were also implanted into WT mice parabiosed to GFP-positive mice to analyze the infiltration of circulating cells by IF and flow cytometry. HCA grafts explanted after 14 days were processed for droplet-based microfluidic single cell RNA sequencing (scRNA seq) of infiltrating cells using the 10X Genomics platform. Data were log-normalized and partitioned using UMAP based density mappings.

Results: Subcutaneous pockets with implanted grafts healed without clinically apparent rejection. Immune cell infiltration, characterized by F4/80, CD11c, and Myeloperoxidase staining, was greater in HCA compared to ADM on post-implantation day 3, whereas no differences were seen on day 7. CD31 staining showed significantly greater vascularization in HCA on day 7 compared to ADM and sham. HCA also demonstrated higher VEGF expression. A greater number of circulating GFP+ cells were found in HCA compared to ADM and sham by IF and FACS. scRNA seq identified 11 distinct cell clusters out of which 2 were defined as macrophage sub-populations, which highly expressed classical M2 macrophage genes (Mrc1, Arg1, Retnla, Cd163). One M2 subpopulation also highly expressed Col3a1 and pro-angiogenic Hif1α.

Conclusion: Our data indicate that the immune reaction to HCA is associated with macrophage polarization towards an M2 phenotype. We have identified a sub-population of pro-angiogenic, collagen-3 expressing macrophages, which may be responsible for increased vascularization after HCA implantation in our xenograft model and may underlie the beneficial clinical effects of HCA transplantation.

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Prrx1 Marks Ventral Fibroblasts With Increased Fibrogenic Potential

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