adipocytes that may play a key role in regulation of hair follicle and skin appendage regrowth.

**Methods:** Murine hypertrophic scar (HTS) model and porcine deep partial-thickness excisional wound model were studied to investigate the effects of FAK inhibition on regeneration of intradermal adipocytes. 1) Mouse model: For HTS model, full-thickness dorsal incisional wounds (2 cm) were immediately suture closed and left untreated until D4. On D4, sutures were removed and the healing tissue was subject to consistent mechanical loading until D14 to induce HTS. A small molecule FAK inhibitor (FAK-I; VS-6062, Verastem Oncology) was locally injected to the wound site daily until the animals were euthanized on D14. 2) Red Duroc model: Female red Duroc swine was used to create deep partial-thickness excisions (25 cm² in size and 0.07 inches in depth). Wounds were immediately treated with FAK-I-releasing hydrogel for 90 days. FAK-I-releasing hydrogels were replenished twice a week until D90. Animals were euthanized on D180. 3) Tissue analyses: Specimens were collected at various time points after the initial injury. The presence of intradermal adipose tissue, hair follicles, and other skin appendages were visualized by histology and immunofluorescent techniques.

**Results:** In the murine HTS model, healed scar lesions without FAK-I treatment displayed apparent fibrotic tissue lacking hair follicles and intradermal adipocytes positive for Perilipin A, a surface marker for fully differentiated adipocytes. Healed wounds treated with FAK-I showed an intact intradermal adipocyte layer positive for Perilipin A (N=3 each), similar to unwounded mouse skin. In the red Duroc model, intradermal adipocytes typically cluster around hair follicles and other cutaneous glands. With FAK-I treatment throughout early wound healing and remodeling phase, hair follicles, glands, and intradermal adipocytes positive for Perilipin A in the newly formed scar lesions began to reappear approximately at D28 (N=4 each) and increased in size thereafter. Regeneration of intradermal adipocytes and skin appendages were not found in untreated scar lesions at these time points examined.

**Conclusion:** The mechanisms by which inhibition of FAK-mediated mechanotransduction signaling results in wound tissue regeneration has not been identified previously. We used two distinct animal models to demonstrate that disruption of FAK leads to regeneration of intradermal adipocytes that may play a key role in regrowth of hair follicles and other skin appendages upon deep dermal injury. Our therapy, therefore, holds great potential as a unique therapeutic strategy for management of post-injury scar formation.

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**Single Cell RNA-Seq Reveals Molecular Signatures Of Heterogeneous Populations Of Dendritic Cells And Macrophages In Murine Wound Healing And Hypertrophic Scarring**

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**Purpose:** Defining cellular sub-populations within the skin is critical to understand wound healing and scarring and develop novel therapeutic approaches. Dendritic cells (DC) and macrophages (MF) are mediators of the inflammatory response to skin injury and are involved in tissue homeostasis, wound healing and fibrosis. However, their cellular sub-populations in the skin remain poorly characterized. Here, we use single-cell RNA sequencing to analyze sub-populations of DCs and MFs in murine excisional wounds and hypertrophic scars over time.

**Methods:** Cells were isolated from stented excisional wounds in C57/BL6J mice 7 and 14 days after wounding as well as from a murine hypertrophic scar (HTS) model 14 days after external mechanical loading. Unwounded skin and scars without mechanical loading served as controls. DCs and MFs were enriched in single cell suspensions using flow cytometry, gating for live, CD45+ cells and excluding B cells, T cells, NK cells and granulocytes. Cells were further processed for droplet-based microfluidic
single cell analysis using the 10X Genomics platform, in which individual cells are barcoded, lysed, and sequenced. Read depth was 50,000 reads per cell. Data were log-normalized and partitioned using UMAP based density mappings.

**Results:** We identified 7 distinct clusters of DCs, MFs and tissue monocytes. Two MF sub-populations (MHCII+,CD11cLo,CD11b+,CD103- and MHCII+,CD115+F4/80Int) were most abundant in excisional wounds, whereas HTS and control scars exhibited an enrichment of CD103-CD11b+CD24+ DCs and tissue monocytes which were specifically enriched in HTS. Unwounded skin showed an abundance of CD11c+CD11b+ DCs and B220-F4/80Lo,CD115+MHCII+ macrophages. An enrichment of transcriptomic signatures of hypertrophy, fibrosis, and cytokine signaling was found in DC and MF subpopulations which were identified in wounds and HTS.

**Conclusion:** Our study demonstrates that wound healing and scarring induces a high degree of heterogeneity among sub-populations of DCs and MFs and thus expands our knowledge on cellular diversity of these cell types identifying novel targets for the development of therapies for chronic wounds and hypertrophic scars.

**ICG-fluorescence Imaging With Lymphoscintigraphy For Sentinel Node Biopsy In Melanoma: Reducing The False Negative Rate**

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**Purpose:** In melanoma, sentinel lymph node (SLN) status has shown to be the best predictor of disease-free survival and identifying the correct SLN is of paramount importance to enable appropriate adjuvant work-up and treatment. Despite the advances in cutaneous melanoma management, the false negative rate (FNR) of sentinel lymph node biopsy (SLNB) is still reported to be as high as 29.8%. The majority of studies examining ICG-guided SLNB in melanoma are retrospective studies with inadequate sample size and follow-up time, making calculation of FNR difficult. Amongst studies with large cohorts of patients (>300) and at least two-years mean or median follow-up, the FNR ranges from 13.4-29.8%. Indocyanine green (ICG) fluorescence imaging has improved identification of SLNs in numerous cancers. The goal of this study was to analyze the largest cohort of patients with primary cutaneous melanoma who underwent a SLNB with the combination of lymphoscintigraphy and ICG-based fluorescence to determine the FNR.

**Methods:** Consecutive primary cutaneous melanoma patients who underwent radioisotope lymphoscintigraphy and ICG-based fluorescence imaging for SLNB by the senior author from 2012-2018 were prospectively enrolled. All patients were staged according to AJCC version 8. The FNR was calculated based on the formula FN/TP+FN.

**Results:** 594 melanomas were analyzed, of which there were 130 T1a, 114 T1b, 148 T2a, 34 T2b, 46 T3a, 56 T3b, 22 T4a and 43 T4b. At least one SLN was identified in every patient. 1827 nodes were sampled. 1556 (85.2%) were identified by radioactivity/fluorescence, 255 (14%) by radioactivity only and 16 (0.9%) with fluorescence only. There were 163 positive sentinel nodes. 147 (90.2%) were identified by radioactivity/fluorescence, 13 (8%) by radioactivity only and 3 (0.6%) with fluorescence only. Of the 128 true positive patients, 116 (90.6%) had at least one positive node identified by radioactivity/fluorescence, 8 (6.3%) by radioactivity only and 4 (3.4%) with fluorescence only. There were 128 true positive, 454 true negative and 12 false negative patients. The FNR was 8.6%. Mean follow up was 1030.9 days.

**Conclusion:** In our cohort, while the majority of positive nodes were identified by both radioactivity and fluorescence, for 12 patients, their only positive node was identified by only radioactivity or fluorescence. Thus, without dual modality, these nodes may have been missed and patients may not have been accurately staged and allocated adjuvant therapy. The FNR of our study at 8.6% is lower than that of any large cohort study with at least two-year follow-up, including the Sunbelt Melanoma trial, the largest prospective study examining nodal recurrence in melanoma patients, which demonstrated a FNR of 10.8%. In conclusion, in the study of the largest cohort of patient with primary cutaneous melanoma who underwent a SLNB with radioisotope lymphoscintigraphy and ICG-based technology, we demonstrate the lowest reported FNR amongst large studies with at least two-year follow-up. This has important implications for melanoma patients as the widespread adoption of this technique with subsequent accurate staging, adjuvant work-up and treatment may improve survival outcomes.