WOUND HEALING DYNAMICS THROUGH DEVELOPMENT AND AGING

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Introduction

Wound healing is a dynamic process that declines with age, resulting in slower recovery times and an increased prevalence of chronic wounds. We are interested in examining this process to explore how our tissues change with age. This pilot study was done using single-cell RNA sequencing (scRNA-seq) to reanalyze datasets from Phan et al., 2020[1] and Vu et al., 2022[2]. Combined, these datasets provided four distinct mouse timepoints – neonatal, young-adult, adult, and aged – each representing an important stage in the lifespan of mice. Our reanalysis consisted of two main methodologies: (1) parallel analysis, where each mouse timepoint was reanalyzed individually, and (2) combined atlas analysis, where all four timepoints were combined into one large dataset to be reanalyzed. These dual approaches to reanalysis are complementary, allowing for both specific and holistic resolution. UMAPs produced from both the parallel and atlas reanalysis can be assessed based on unwounded and wounded states, inspiring possible future directions of our research into how our research into how wound healing changes with age.

Methods

Reanalysis of scRNA-seq Data

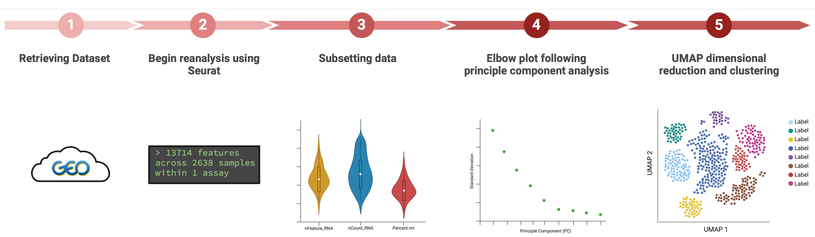


Figure 1. Summary computational workflow used to produce scRNA-seq UMAPs. scRNA-seq datasets were retrieved from NCBI's GEO database and the Seurat package was used for analysis. The analysis begins by initializing the Seurat object and removing cells from the dataset with characteristics of failed or dead status. Normalization and PCA tests are run to identify the ideal dimensionality for UMAP clustering. UMAP dimensional reduction test is run, yielding unbiased clustering of cells.

References

[1] Phan, Q. M. (2020). **Lef1 expression in fibroblasts maintains developmental potential in adult skin to regenerate wounds**. *eLife.*

[2] Vu, R. (2022). **Wound healing in aged skin exhibits systems-level alterations in cellular composition and cell-cell communication**. *Cell Reports (Cambridge).*

[3] Schultz, G. S. (2011). **Principles of Wound Healing**. *Mechanisms of Vascular Disease: A Reference Book for Vascular Specialists.*

[4] Amuso, V. M. (2024). **Deep skin fibroblast-mediated macrophage recruitment supports acute wound healing**. *BioRxiv.*

[5] Kovtum T. (2015) **Cavin family proteins and the assembly of caveolae.** *J Cell Sci*

Preliminary Results

Figure 2. Parallel Reanalysis of Wounded and Unwounded Mouse Back Skin at Four Timepoints

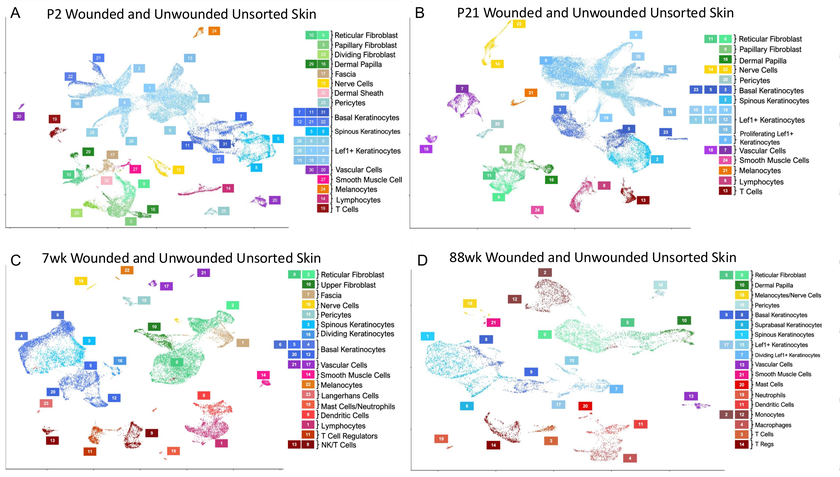


Figure 3. Combined Atlas Reanalysis of Wounded/Unwounded Mouse Back Skin Across Four Timepoints

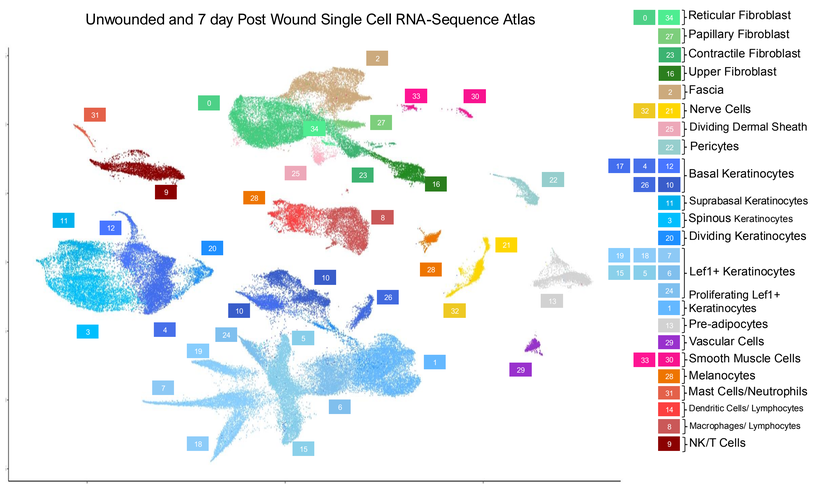
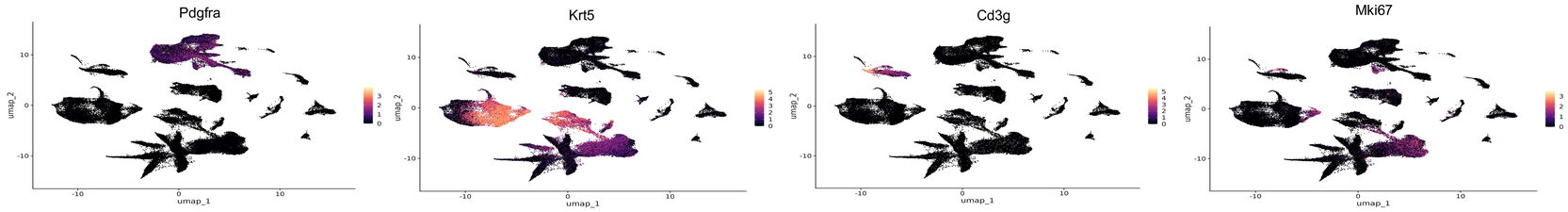


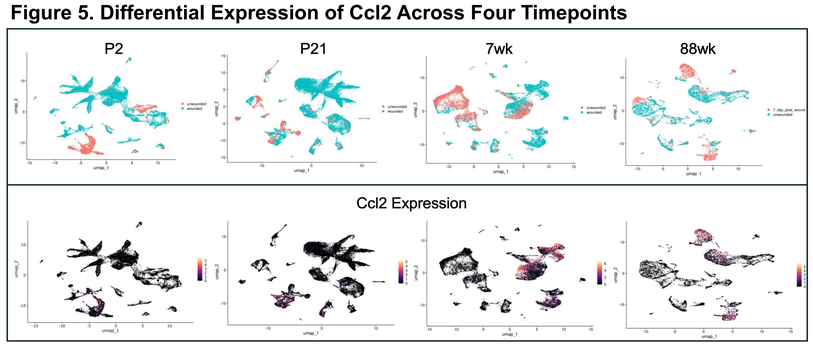
Figure 4. Differential Markers for Labeling Combined Atlas



Future Directions

Some initial observations were made using differential expression testing (DE) to compare gene expression in wounded and unwounded reticular fibroblast populations. One such observation involves Ccl2 expression in wounded reticular fibroblasts. Collection of wounded tissue at 7wk and 88wk time points occurred seven days after wounding, meaning the tissue was likely in the inflammatory stage of wound healing[3]. Fibroblasts are essential for activating the pro-inflammatory immune response to wounding, with Ccl2 being notably upregulated in response to wounding[4]. Our reanalysis of p2 mice failed to exhibit significant upregulation of Ccl2 among other pro-inflammatory markers in wounded fibroblasts (Figure 5). DE testing was performed using parallel analysis datasets and quantitative connections of gene expression between timepoints cannot be drawn.

Figure 5. Differential Expression of Ccl2 Across Four Timepoints



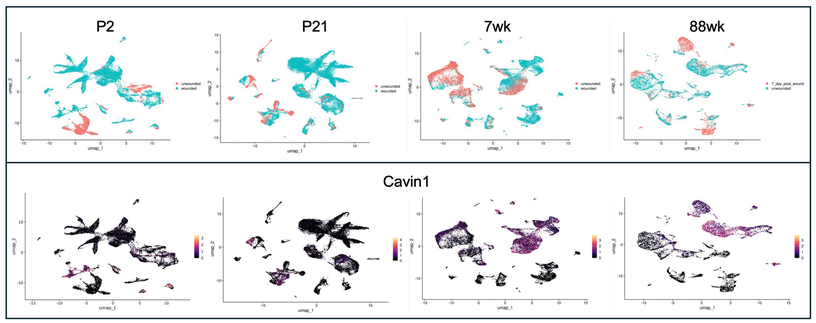


Figure 6. Differential Expression of Cavin1 Across Four Timepoints

Another notable observation involves Cavin1 expression in wounded reticular fibroblasts. In our DE analysis, we found Cavin1 expression only in the p2 and p21 wounded fibroblasts, compared to unwounded fibroblasts. However, in wk7 and wk88, Cavin1 was expressed in both wounded and unwounded reticular fibroblasts. Cavin1 is a protein that functions alongside Caveolin and other Cavin proteins to form caveolae. These proteins are essential for caveolae development, acting as a scaffold to absorb mechanical strain. Given this role, our future investigations are interested in exploring how Cavin1 may be involved in wounding and potentially even wound healing.