Identifying altered transcriptional dynamics and signaling pathways in vitiligo affected epidermis

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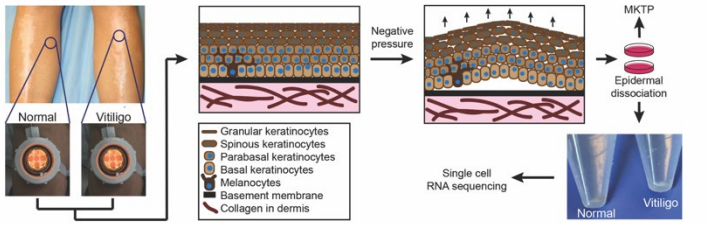
Background

Vitiligo is an autoimmune skin disease that affects 0.5% to 2% of the population globally and is characterized by the progressive destruction of melanocytes by autoreactive T cells[1]. The disease results in chronic disfiguring white patches. Vitiligo patients often experience shame, depression and anxiety, which can lead to social isolation [2, 3]. First line treatment involves narrow band ultraviolet B (NBUVB) [4], which induces the migration of hair follicle melanocyte precursors, in conjunction with topical steroids or calcineurin inhibitors to suppress the local immune response [5]. NBUVB therapy is only 70% effective and requires around 50 treatments and is not feasible for many patients [6].

While the role of the immune system is well defined in disease initiation, factors that drive vitiligo persistence are unknown. In particular, stable lesions with white patches that persist over time have an absence of active, cytotoxic $CD8^+$ T cells. Some regard stable vitiligo as a state of quiescence because the size and number of lesions can remain the same over several years [7, 8].Recent studies demonstrate that keratinocytes secrete chemokines such as CXCL9 and 10 that are important in T cell recruitment and vitiligo pathogenesis[9]. In order to further characterize keratinocyte populations that may affect vitiligo persistence and how they interact with other cell types present in the epidermis, we generated a single cell RNA (scRNA) sequencing data set from matched normal and vitiligo affected epidermis from six patients as illustrated in Figure 1. We previously proposed to characterize pseudotemporal dynamics of keratinocyte differentiation states and determine cell-cell signaling pathways in the epidermis. In this report, we show that compared to patient-matched nonlesional skin, there was an enrichment of a unique population of keratinocytes that exhibited increased expression of keratins associated with cell stress (*KRT6A*) and chemokines known to induce vitiligo (*CXCL9*, *CXCL10*), implicating a role for these cells in disease persistence. Cell-cell communication involving the CXCL9/10 axis was also altered in the epidermis of vitiligo skin compared to normal skin.

A



B

Patient	Date Collected	Normal % Live	Vitiligo % Live	Normal Cells	Vitiligo Cells
A	9/7/18	83%	71%	1000*	1,237
B	9/21/18	88%	89%	* Not enough viable cells	
C	10/12/18	70%	64%	1,264	839
D	12/7/18	52%	76%	2,809	2,651
E	1/22/19	56%	80%	2,630	3,920
F	11/1/19	54%	38%	1,000*	1,000*
G	11/1/19	23%	31%	200*	1,000*

Fig 1. Tissue collection pipeline and breakdown of samples from vitiligo patients for single cell sequencing. A) Overview of collection procedure. The epidermis is lifted off the basement membrane of the skin using a suction blister device that applies negative pressure. After the blister is removed and dissociated, normal cells are prepped for the melanocyte keratinocyte transplant procedure (MKTP). Excess cells from normal and vitiligo skin are processed for single cell RNA sequencing. B) Breakdown of patient matched normal and vitiligo samples.

Results

Using the suction blister approach, we expected to be able to capture the heterogenous mixture of keratinocytes and immune cells present in the epidermis of normal and vitiligo skin after dissociation. Keratinocytes, the predominant cell type in mammalian epidermis, undergo a complex gene differentiation program to give rise to the different layers of the skin. Basal keratinocytes give rise to daughter cells that move up through the epidermis, until they become the fully keratinized layer that provides barrier function for the entire body[10]. scRNA analysis of normal and vitiligo skin from patient matched samples revealed the expected different clusters of keratinocyte (**Fig 2A,B**) with marker gene expression profiles that largely agreed with known keratinocyte states (**Fig 2B,C)**. All patients contributed to different cell clusters (**Fig 2D)** but patient-matched vitiligo samples were enriched in "stressed keratinocytes" **(Fig 2E-G).** Stressed keratinocytes express markers such as *KRT6A*, *KRT6B* and *KRT16* (**Fig 3A)** which are normally only expressed in epithelial appendages [11]. However, UV exposure, wounding, and other stresses can lead to the expression of *KRT6* and *KRT16* in the mature interfollicular epidermis. This stressed keratinocyte population also express high levels of *S100A8* and *A9* as well as increased expression of chemokines known to induce vitiligo (*CXCL9, CXCL10)* [9, 12] (**Fig 3B**).

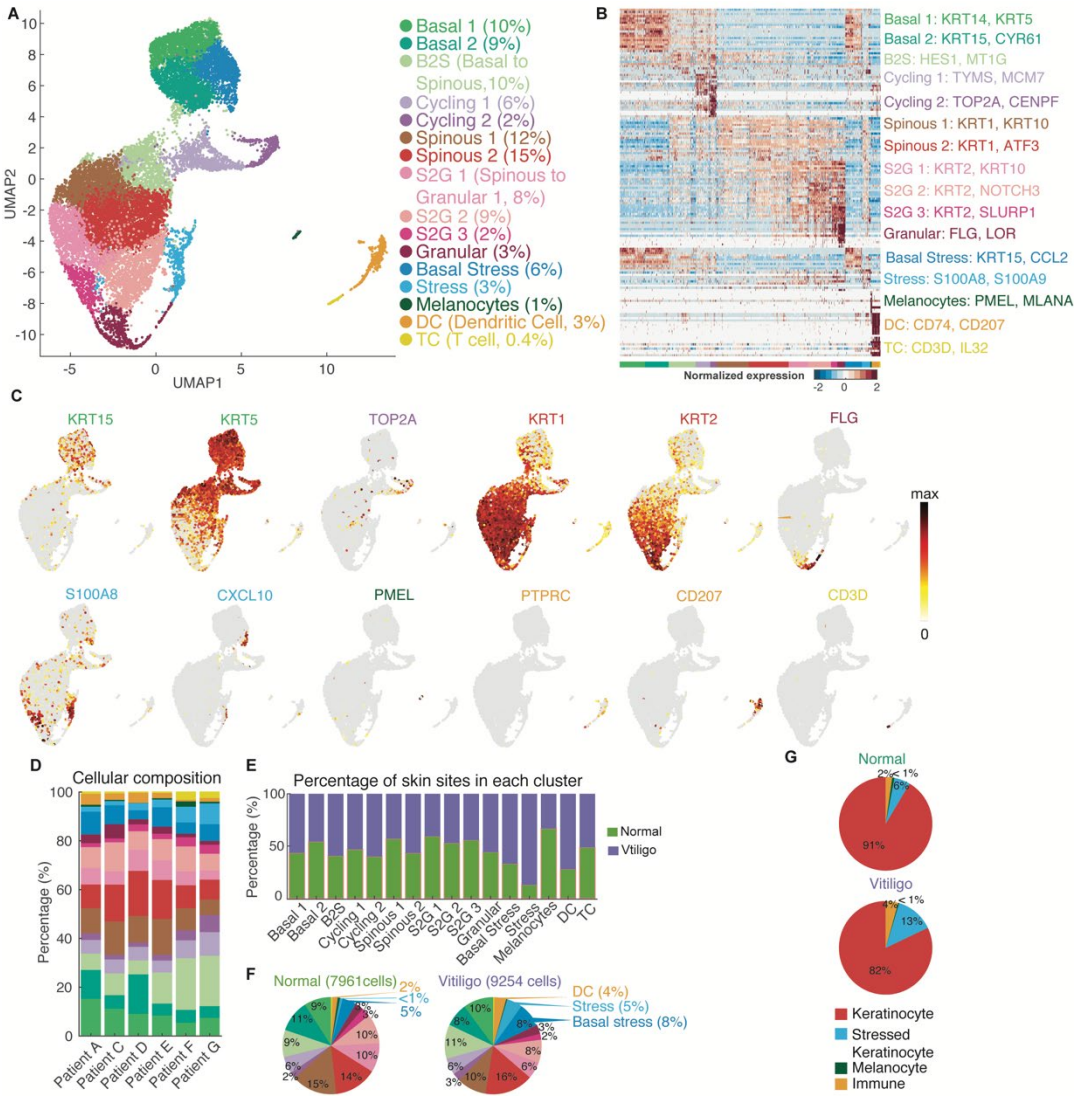


Fig 2. Single cell transcriptomics on human epidermis of normal and vitiligo skin from patient matched samples. A) UMAP plot of combined patient data showing the identified epidermal cell subpopulations in both normal and vitiligo skin. B) Heatmap depicting enriched gene signatures for each cluster of cells. C) Feature plots showing the expression levels of different marker genes with the stressed keratinocyte populations expressing high levels of *S100A8* and *CXCL10*. D) Bar plot showing the proportion of cell clusters from each patient. E) Breakdown of clusters by skin type. F) Pie chart breakdown of different cell subpopulations in normal vs. vitiligo skin. The proportion of keratinocytes expressing certain stress markers significantly increases from normal to vitiligo skin. G) Pie chart indicating the enrichment of stressed keratinocyte and immune cells in vitiligo skin.

To further characterize the cell-cell signaling pathways in patient-matched normal and vitiligo epidermis, we first manually curated a new database of over 2,000 signaling ligand-receptor interactions and then developed a computational framework to infer and analyze intracellular communication networks. In contrast to existing databases, our new database considers multimeric structure of ligand-receptor complexes and their cofactors through secreted, extracellular, and cell-to-cell interactions. We then quantify the cell-to-cell communication probability using the law of mass action based on the average expression values of a ligand by one cell subpopulation and a receptor by another cell subpopulation. Using our computational framework, we predicted that the cell-to-cell communication network architecture of several signaling pathways prominently changed from normal to vitiligo skin, including for CXCL and WNT signaling. In normal skin, CXCL9/10 signaling to its receptor CXCR3 is not seen in keratinocytes (**Fig 4A,B**). In contrast, in vitiligo skin, CXCL9/10 communication among keratinocytes is enhanced with stressed populations as major targets (**Fig 4A,C**). Wnt signaling, a pathway known to affect skin development and homeostasis, was also perturbed in vitiligo lesional skin (data not shown).

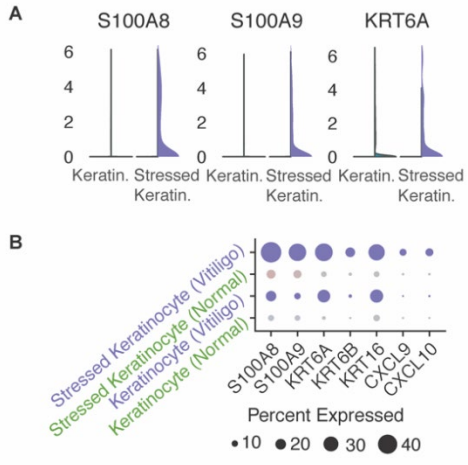


Fig 3. Expression of stress markers in keratinocyte and stressed keratinocyte populations. A) Split violin plot showing increased relative expression of inflammatory markers and stress keratins (*Krt6a*) in the stressed keratinocyte population in vitiligo compared to normal skin. B) Comparison of expression levels of chemokines and stress markers in keratinocytes and stressed keratinocyte between normal and vitiligo samples. *CXCL9* and *CXCL10* are predominantly produced by stressed keratinocytes in vitiligo skin.

Future Directions

This report summarizes some preliminary data of the first scRNA analysis of patient-matched normal and vitiligo skin. We found that unique populations of stressed keratinocytes are enriched in vitiligo skin and express chemokines known to drive disease, suggesting that a subset of keratinocytes play a role in establishing and maintaining vitiligo lesions in the skin. The presence of stressed keratinocytes and the expression of chemokines and stress-related genes appear to affect cell-cell communication in the epidermis. We aim to further characterize pseudotemporal dynamics of keratinocyte differentiation and how stress keratinocytes arise in the future. We will also use RNAscope to validate the predicted ligand-receptor pairs that are altered between normal and vitiligo skin.

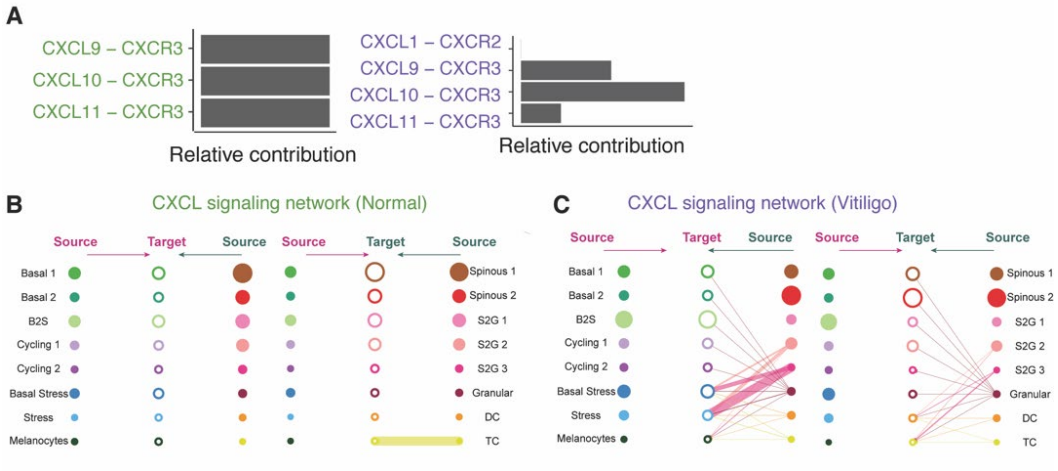


Fig 4. Cell-cell communication analysis between epidermal and immune cells in normal and vitiligo skin. A) Relative contribution of each ligand-receptor pair to the overall communication network of CXCL signaling pathway in normal (green) and vitiligo skin (purple), respectively. B) Hierarchical plot shows the inferred intercellular communication network for CXCL signaling in normal skin. Left and right panels highlight the autocrine and paracrine signaling to basal/stress/melanocytes states and other skin cell states, respectively. Solid and open circles represent source and target, respectively. Circle sizes are proportional to the number of cells in each cell group. Edge colors are consistent with the signaling source. Thicker line indicates a stronger signal. C) Hierarchical plot shows the inferred intercellular communication network for CXCL signaling in vitiligo skin.

References

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