

Single-cell transcriptomic analysis identifies infiltrating plasmacytoid dendritic cells in psoriasis epidermis

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Dear Editor, Psoriasis is a chronic skin inflammatory skin disease resulting from complex keratinocyte–immune cell interactions. Previous single-cell sequencing approaches have identified the heterogeneity of cell populations in psoriasis skin;^{1,2} however, the cell subsets, differentiation paths and interactions in the epidermis in psoriasis remain incompletely understood. In order to create an atlas of the cell subsets and their physical interactions in the epidermis in psoriasis, epidermal sheets from lesional and nonlesional skin from patients with psoriasis and healthy donors were obtained and subjected to a partial dissociation protocol to preserve physical cell–cell interactions. CD45[−] and CD45⁺ subsets were obtained by flow cytometry, and single-cell RNA sequencing (scRNA-Seq) and cell interaction by multiplexed sequencing (CIM-Seq) analysis were performed.³ This approach allowed us to analyse the cellular composition specifically in the epidermis, and cell–cell interactions at an unprecedented resolution.

Our findings reveal altered differentiation trajectories (Figure 1a) and dynamic changes in epidermal cell states in psoriasis, characterized by activated basal, mitotic and suprabasal keratinocytes (ACT-BAS, ACT-MIT, ACT-SBAS) (Figure 1a), with high levels of activation markers (psoriasis/wound healing-specific keratins KRT6, KRT16 and KRT17; gap junction proteins GJB2 and GJB6; and S100-alarmins, S100A7/8/9 and other genes related to cytokine response/proliferation) (data not shown). Virtually all keratinocyte cell states in the psoriatic epidermis exhibited a strong interferon- α signature, and displayed disrupted developmental patterns compared with healthy skin. Interestingly, keratinocytes in nonlesional psoriatic skin also displayed transcriptomic alterations with a major histocompatibility complex II signature, and a group of activated basal keratinocytes was found in the trajectory of nonlesional psoriasis epidermis (Figure 1a), suggesting a ‘prepsoriatic’ state in the epidermis.

Moreover, our epidermis-specific scRNA-Seq analysis has identified seven immune cell subsets {innate lymphoid cell [ILC] and natural killer [NK] [CCL5^{high}], T helper [Th]17, cytotoxic T cell [Tc (CCR6^{high})], proliferative CD8, myeloid dendritic cells [mDC], macrophage/monocyte-derived dendritic cells [Mac/moDC] and plasmacytoid dendritic cells [pDCs]} exclusively present in lesional psoriasis epidermis. Analysis of immune cell populations infiltrating the psoriasis epidermis revealed a higher proportion of Th17 compared with Tc17. Additionally, we identified cells expressing markers of tissue-resident memory T cells (CD103, CD69 and ITGA1/CD49a), in agreement with recent studies suggesting their role in recurrence.⁴ Regulatory T cells (Tregs) have long been known to be present at an elevated number in psoriasis, despite chronic inflammation. Interestingly, our results revealed that Tregs in psoriasis exhibited markers associated with exhaustion, potentially contributing to their reduced ability to suppress inflammation. Surprisingly, we identified a cell population expressing markers of pDCs (Figure 1a), a cell type most commonly assumed to be present in the dermis in the acute phase of psoriasis,^{5,6} and not detected in previous scRNA-Seq studies using full-depth biopsies and different dissociation protocols.^{1,2}

Further characterization of pDCs revealed that they expressed transcripts associated with activation [interferon regulatory factor (IRF)7, Toll-like receptor 7/9, leucocyte immunoglobulin-like receptor subfamily A member 4],⁷ and that they are likely to be of myeloid origin, suggested by their elevated expression of myeloid signature genes (*BCL11A*, *SPIB*, *TCF4*) (Figure 1b).^{5,8} Next, we validated the presence of epidermal pDCs in chronic psoriasis plaques using two methods: single-molecule *in situ* hybridization and immunofluorescence staining for the pDC-specific marker CLEC4C/BDCA-2 (Figure 1c). Multiplex immunofluorescence showed coexpression of BDCA2 with IRF7, suggesting an activated state of pDCs (Figure 1c). Although pDCs have been less relevant for lesion persistence and rarely found in chronic psoriasis lesions, these findings suggest that activated pDCs may contribute to sustained inflammation. Given that activated pDCs can process and present antigens in addition to prime T cells in psoriasis,^{5,6} they may play a previously underestimated role in exacerbating skin inflammation.

Most cell–cell interaction analyses in scRNA-Seq studies rely on computational predictions based on receptor–ligand interactions, and thus have limitations. Using CIM-Seq, we demonstrated physical crosstalk among keratinocytes and immune cells in the psoriatic epidermis (Figure 1d), which underscores the complexity of cellular interactions in psoriatic lesions. These interactions, particularly between keratinocytes and pDCs, provide new insights into the cellular dynamics underpinning the pathogenesis of psoriasis.

Limitations of our study include a small sample size, hindering graph-based clustering for exploring interindividual and disease severity differences. Additionally, we could not capture epidermal cells from upper granular layers (e.g. expressing loricrin and the *LCE* gene) owing to cell adhesion challenges. However, these challenges are common in scRNA-Seq studies in skin.

In summary, our study emphasizes the significance of an optimized, gentler, cell dissociation protocol in identifying rare cell populations (i.e. pDCs) in psoriasis, determines the transcriptome and confirms the presence of epidermal

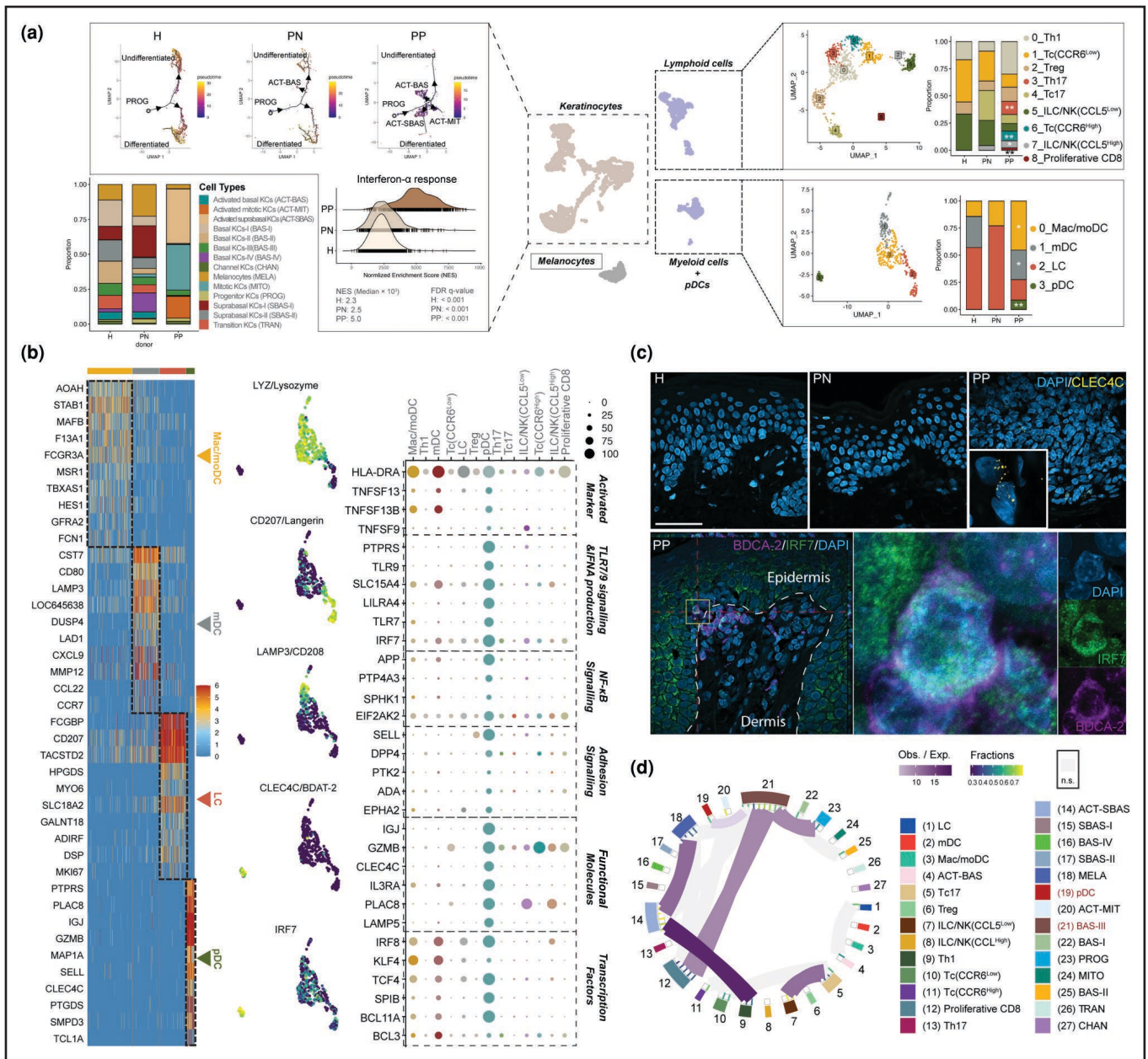


Figure 1 Single-cell sequencing and Cell Interaction by Multiplet Sequencing (CIM-Seq) reveal a unique plasmacytoid dendritic cell population and physical immune cell-keratinocyte interactions in psoriatic epidermis. (a) Epidermal cell subsets and changes in their relative abundance in epidermis from healthy (H) skin ($n=3$), and nonlesional (PN) and lesional (PP) skin from patients with untreated chronic plaque psoriasis ($n=5$), displayed as Uniform Manifold Approximation and Projection (UMAP) plots (CD45⁺ immune cells, middle right; keratinocytes, middle upper left; melanocytes, middle lower left). Trajectory analyses reconstruct distinct keratinocyte differentiation pathways in H, PN and PP epidermis [left upper panel, progenitor cells (PROG); activated basal cells (ACT-BAS); activated suprabasal cells (ACT-SBAS); activated mitotic cells (ACT-MIT)]. Enrichment of interferon- α response genes in PP keratinocytes is shown (GSEA, lower panel). (b) Heatmap showing the top 10 cluster-specific genes for each myeloid cell cluster (left), alongside feature plots for genes characteristic of myeloid and pDCs (middle); dot plot highlights pDC-enriched genes involved in certain biologic processes (right). (c) Single-molecule RNA *in situ* hybridization for *CLEC4C* (the gene encoding BDCA2) highlights epidermal pDCs (upper panels; DAPI, blue; CLEC4C, yellow). Multiplex immunofluorescence of interferon regulatory factor (IRF7) and BDCA2, orthogonal and zoomed views (lower panels: DAPI, blue; BDCA2, violet; IRF7, green). (d) Circos plot displaying multiplet deconvolution of 207 doublets from psoriasis epidermis. Each cell subset is shown as a block, and cell types found together in > 10 multiplets are connected with bands. Purple bands indicate specific interactions, with colour strength representing the fold enrichment of connections compared with that expected by chance. Nonsignificant interactions are depicted in grey. DAPI, 4',6-diamidino-2-phenylindole; ILC, innate lymphoid cell; LC, Langerhans cells; NF, nuclear factor; NK, natural killer; n.s., not significant; mDC, myeloid dendritic cells; Mac/moDC, macrophage/monocyte-derived dendritic cells; pDCs, plasmacytoid dendritic cells; Tc, cytotoxic T cell; Th, T helper cell; Treg, regulatory T cell.

pDCs previously missed in scRNA-Seq studies using full-depth skin biopsies, and represents the first steps towards characterization of physical cell–cell interactions in the epidermis in psoriasis. Further research is needed to reveal the potential roles of epidermal pDCs in psoriasis.

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Data availability: The scRNA-Seq data are available on the National Center for Biotechnology Information's Gene Expression Omnibus, accession number GSE254707. Additional data are available from the corresponding author upon reasonable request.

Ethics statement: The study involving human participants followed the Declaration of Helsinki guidelines and received approval from the Stockholm Ethics Committee.

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