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Inflammatory memory of Keratinocytes in psoriasis

Ph.D. Thesis

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LIST OF PUBLICATIONS

Scientific papers included in this thesis

I. **Ghaffarinia A**., Ayaydin F., Póliska S., Manczinger M., Bolla B., Borbála Flink L., Balogh F., Veréb Z., Bozó R., Szabó K., Bata-Csörgő Z., and Kemény L.: Psoriatic resolved skin epidermal keratinocytes retain disease-residual transcriptomic and epigenomic profiles, *Int J Mol Sci 24*(5):4556 (2023).
IF: 5.6 (Scopus - Medicine (miscellaneous) SJR indicator: Q1)

II. **Ghaffarinia A**., Póliska S., Ayaydin F., Goblos A., Parvaneh S., Manczinger M., Balogh F., Erdei L., Veréb Z., Szabó K., Bata-Csörgő Z., Kemény L.: Unraveling transcriptome profile, epigenetic dynamics, and morphological changes in psoriasis-like keratinocytes: "Insights into similarity with psoriatic lesional epidermis", *Cells 2023, 12*(24), 2825; (2023).
IF: 6 (Scopus - Biochemistry, Genetics and Molecular Biology (miscellaneous) SJR indicator: Q1)

Publications not directly related to the thesis

I. Flink L., **Ghaffarinia A**., Papp B., Varga Á., Vigh A., Vidács D., Kui R., Kemény L., Bata-Csörgő Z., Bozó R.: Abnormal basement membrane results in increased keratinocyte-derived periostin expression in psoriasis similar to wound healing, *Sci Rep 13*(1):16386 (2023).
IF: 4.6 (Scopus - Multidisciplinary SJR indicator: D1)

II. Parvaneh S., Kemény L., **Ghaffarinia A**., Yarani R., Veréb Z.: Three-dimensional bioprinting of functional β-islet-like constructs, *Int J Bioprint 9*(2): 665 (2023).
IF: 8.4 (Scopus - Materials Science (miscellaneous) SJR indicator: Q1)

1. INTRODUCTION

Psoriasis, a chronic autoimmune skin disorder influenced by genetic, epigenetic, and environmental factors, affects about 125 million people globally. It occurs equally in both genders and can start before or after age 40. The most prevalent subtype of psoriasis is chronic plaque psoriasis, characterized by symmetrical lesions that can occur throughout the body, with a predominant presence on the knees, elbows, trunk, and scalp.

The disease involves complex interactions between the innate and adaptive immune systems dysregulation and epidermal keratinocytes responses. Histologically, psoriasis is characterized by increased epidermal thickness, dilated capillaries, and inflammatory infiltrates, resulting in red patches covered by scales, commonly referred to as lesions.

Triggers for psoriasis include genetic predisposition and various environmental factors such as trauma, obesity, stress, infection, smoking, alcohol, drugs, and sunlight. Psoriasis development is also influenced by epigenetic changes, particularly DNA methylation and hydroxymethylation. DNA methylation involves the addition of a methyl group to cytosine, a process regulated by DNA methyltransferases. DNA demethylation occurs through passive or active mechanisms, including the involvement of TET enzymes.

Psoriasis treatment options range from topical treatments for mild cases to systemic therapies for severe cases. Ustekinumab, an IL-

12/23 inhibitor, has effectively treated moderate-to-severe plaque psoriasis by targeting cytokines involved in inflammatory processes.

Successful psoriasis treatment results in normalized epidermal thickness, reduced white blood cell infiltration, and clinically and histologically restored skin, known as "resolved" skin. Despite initial success, many patients face recurrent symptoms within weeks or months after treatment cessation, posing the primary challenge in psoriasis therapy. Notably, these lesions reappear on resolved regions rather than never-lesional skin.

One approach to studying the contribution of keratinocytes in local psoriasis relapse is to induce psoriasis-like inflammation in keratinocytes derived from both never-lesional and resolved skin. By examining their responses to the same inflammatory conditions, we can gain insights into the role of keratinocytes in the recurrence of psoriatic lesions. However, previous studies often introduced high cytokine concentrations to induce psoriasis-like inflammation in healthy human epidermal KCs, potentially differing from in vivo conditions. Microarray analyses comparing psoriatic lesional skin and cytokine-stimulated KCs showed limited transcriptome overlap. The literature lacks exploration of "mild inflammation" at physiologically relevant levels, and no research has investigated inducing psoriasis-like inflammation in KCs from never-lesional and resolved psoriatic skin. Studying these responses could reveal insights into mechanisms behind lesion recurrence, particularly in resolved psoriatic skin.

2. AIMS

Part I of our study focused on investigating the residual disease transcriptome, along with DNA methylation and hydroxymethylation profiles in resolved skin versus never-lesional skin. Additionally, we assessed the overlap between differentially expressed genes (DEGs) in resolved versus never-lesional skin from our study and DEGs in lesional versus healthy skin from available datasets.

In Part II, we aimed to create a novel cytokine mixture (CytoMix) for inducing mild and severe psoriasis-like inflammation in healthy human epidermal keratinocytes. Inducing psoriasis-like inflammation in resolved and never-lesional KCs is part of a separate project beyond the scope of this PhD thesis. The effectiveness of CytoMix was validated through comprehensive examinations, including studying the transcriptome of mildly and severely inflamed KCs and comparing transcriptome similarities with psoriatic-lesional epidermis. We also investigated gene expression alterations in epigenetic modifiers during inflammation.

3. MATERIALS AND METHODS

3.1. Psoriatic Skin Samples Collection and Ethics

Seven psoriasis patients with moderate-to-severe plaque-type psoriasis were recruited. Skin samples, including full-thickness 6 mm paired never-lesional and resolved skin punch biopsies (PBs), were collected. Informed consent was obtained, and the Regional and Institutional Research Ethics Committee approved protocols.

3.2. Isolation and culture of skin epidermal KCs

KCs were isolated from healthy abdominal skin samples and cultured in keratinocyte serum-free medium (KSFM) until around 70% confluency.

3.3. Cytokine mixture (CytoMix) and reagents

A novel CytoMix was created using IL-17A, IL-22, TNF-α, IFN-γ, and KGF/FGF7. We induced severe inflammation using the same mixture but at 10× higher concentrations.

3.4. Total RNA isolation and real-time RT-PCR

RNA was isolated from skin samples and cultured KCs. Real-time RT-PCR was performed for gene expression analysis, with genes normalized to 18S rRNA.

3.5. IL-8 ELISA assay

IL-8 production in KCs exposed to CytoMix was quantified using an ELISA kit.

3.6. Immunofluorescence staining

Staining for co-localization of 5-mC and 5-hmC, epigenetic marks indicating methylated and hydroxymethylated DNA, was performed on sections from never-lesional and resolved skin.

3.7. Confocal microscopy

Fluorescence imaging was conducted using a confocal microscope for immunolocalization analysis.

3.8. High-throughput RNA sequencing

RNA sequencing was performed on never-lesional and resolved skin epidermal and dermal samples and on KCs treated with mild and severe CytoMix and analyzed using StrandNGS software.

3.9. RNA sequencing data analysis

DEGs were identified using a moderated t-test, with genes having a p-value < 0.05 considered significant.

3.10. Examining the overlap between resolved vs. never-lesional DEGs and lesional vs. healthy DEGs

We assessed the overlap in DEGs between resolved versus never-lesional skin in our study and lesional versus healthy skin in external datasets.

3.11. Comparing the transcriptome similarity between mild and severely inflamed KCs and psoriatic-lesional epidermis

DEGs from mildly and severely inflamed KCs were compared with those from the psoriatic-lesional epidermis to uncover similarities.

3.12. Statistical analysis

Statistical analyses were conducted using GraphPad Prism 8.0.2 for real-time RT-PCR and ELISA results.

4. Results

In **part I** of the study, we found both 5-mC and 5-hmC levels were lower in the resolved epidermis than in the never-lesional epidermis, as observed through immunofluorescence staining and confocal microscopy. The decreased 5-hmC intensity in the resolved epidermis was associated with a significant decline in TET3 mRNA expression, while TET1 and TET2 levels remained unchanged.

Transcriptome analysis revealed 476 DEGs in the epidermis and 2966 DEGs in the dermis when comparing resolved to never-lesional skin. In the resolved epidermis compared to the never-lesional epidermis, NEAT1_3, SAMHD1, and HOXB2 genes showed significant down-regulation ($|FC| \geq 2.5$). Notably, NEAT1 and SAMHD1 have been previously implicated in psoriasis. Six genes, including STAC2, AQP5, FAM25C, ELOVL3, C10orf99, and AKR1B10, exhibited substantial up-regulation ($|FC| \geq 2.5$) in resolved epidermis. In the resolved dermis, genes such as SPRR4, ATP12A, CST6, SPRR1A, CRCT1, MSMB, KRT34, and GJB4 demonstrated significant down-regulation ($|FC| \geq 10$), with only MSMB previously associated with psoriasis. Importantly, among the 25 most up-regulated DEGs in resolved dermis, 14 transcripts, accounting for 56%, belonged to immunoglobulin coding gene segments, suggesting potential B cell involvement in local psoriasis relapse.

The comparative transcriptomic analysis aimed to identify the residual disease profile in resolved versus never-lesional skin. Since

no significant overlap was found in dermal samples, our focus concentrated on the outcomes from epidermal samples. In epidermal samples, 102 overlapping genes were found between resolved and lesional psoriatic skin with 95% of overlapping genes showed the same direction of fold change in both comparisons and degree of change was significantly correlated. Notably, AKR1B10 emerged as the top up-regulated overlapping gene between resolved and lesional epidermis DEGs. Protein-protein interaction (PPI) networks revealed associations of overlapping genes with TNF and WNT signaling pathways. KEGG pathway analysis highlighted enrichment in WNT and mTOR signaling, with the most significant enrichment in the basal cell carcinoma pathway.

In **part II**, we investigated the impact of mild psoriasis-like inflammation on cytokeratin gene expression in healthy human KCs. Notable changes included down-regulation of KRT1 and KRT10, transient shifts in KRT15, and up-regulation of KRT17. Severe inflammation induced higher levels of pro-inflammatory cytokines (IL-8 and IL-23A) in KCs. IL-8 protein level and IL-23A mRNA level were notably elevated in mildly and severely inflamed KCs compared to controls, with more pronounced changes in the severely inflamed group.

RNA sequencing at 48 hours post-CytoMix treatment revealed more DEGs in severely inflamed KCs than in mildly inflamed KCs. The top 20 genes with significant expression changes in mildly and severely inflamed KCs were identified. Shared up-regulated genes

(e.g., S100A7, DEFB4A, LCE3A) mainly play roles in KCs terminal differentiation, cornification, and antimicrobial defense. Shared down-regulated genes in both mild and severe inflammation, such as KRT1 and EPHB6, demonstrate decreased levels in psoriatic lesions.

Epigenetic modifiers exhibited significant changes in inflamed KCs, including down-regulation of HAT1, DNMT1, and DNMT3B and up-regulation of HDAC9. TET3 and DNMT3A showed opposing fold changes in severely inflamed KCs compared to mildly inflamed ones.

Transcriptomic analysis indicated that the gene expression profile of mildly inflamed KCs closely resembled that of psoriatic lesional epidermis, with 89% overlapping genes showing the same direction of fold change while severely inflamed KCs exhibited a weaker correlation. KEGG pathway analysis revealed enrichment of the IL-17 signaling pathway in the overlapping gene set of mildly inflamed KCs and psoriatic lesional epidermis, suggesting shared molecular mechanisms.

5. DISCUSSION

This study is the first to underscore the potential role of epidermal keratinocytes in psoriasis local relapse by examining DRTP and methylation/hydroxymethylation profile in never-lesional and resolved epidermis. Our transcriptional findings confirmed the distinction between transcriptomic profiles of never-lesional and resolved uninvolved skin in psoriasis.

We defined the DRTP as a set of 102 expressed genes that overlapped between the DEGs of resolved vs. never-lesional and the DEGs of lesional vs. healthy epidermis. Remarkably, we found that the AKR1B10 transcript was not only among the top up-regulated DEGs in the resolved epidermis compared to the never-lesional epidermis, but was also the top up-regulated transcript overlapping between resolved and lesional epidermis. This finding suggests that the retinoic acid signaling pathway plays an essential role in the local recurrence of psoriatic lesions. Our analyses, including STRING and KEGG pathway assessments, revealed the up-regulation of genes associated with the Wnt pathway, supporting the hypothesis that WNT signaling may significantly contributes to psoriatic lesion recurrence. Additionally, TNF and mTOR signaling pathways, known for their roles in psoriasis pathogenesis, emerged as prominent disease-residual pathways in our analyses.

Furthermore, we demonstrated a clear difference in the 5-mC and 5-hmc general pattern between psoriatic never-lesional and resolved, uninvolved skin. In the resolved epidermis, compared to never-lesional epidermis, we found reduced 5-hmC levels in addition to altered 5-mC and TET3 mRNA expression. Notably, previous studies have reported disruptions in the TET-5-hmC pathway in the epidermis of psoriatic lesions.

Concurrently, the study introduces novel CytoMix, for inducing psoriasis-like inflammation. In vitro experiments with CytoMix-treated KCs replicate psoriasis effects, demonstrating down-

regulation of cytokeratin genes (e.g., KRT1, KRT10), partially mirroring psoriatic lesional epidermis. Severe inflammation induces a more significant number of DEGs than mild inflammation. Overlapping genes with psoriatic lesional epidermis show a strong correlation in mild inflammation, emphasizing that subjecting human epidermal KCs to 10× CytoMix or severe inflammation may not accurately replicate the conditions in psoriatic lesions. Mildly inflamed KCs' overlapping genes, primarily enriched in the IL-17 pathway, highlight our model's accuracy in replicating and targeting this key pathway in psoriasis. Transcriptomic profiles show alterations in crucial epigenetic modifiers (HAT1, HDAC9, DNMTs, TETs) affecting histone modification and DNA methylation. This highlights how short-term and even mild inflammation can activate gene regulation in these key epigenetic modifiers.

6. SUMMARY

Our results suggest that epigenetic changes detected in epidermal KCs of resolved skin may be responsible for the DRTP in the same regions. Thus, the DRTP of keratinocytes may contribute to site-specific local relapse. This study also emphasizes how inflammation severity affects the transcriptomic similarity of KCs to psoriatic epidermis and proves dynamic epigenetic regulation in psoriasis-like inflamed KCs.

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