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Peripheral nervous system and histone acetylation-related alterations of gene expression in non-lesional psoriatic skin

PhD Thesis

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LIST OF PUBLICATIONS

List of publications related to the thesis

1. Dóra Romhányi, Kornélia Szabó, Lajos Kemény, Endre Sebestyén and Gergely Groma Transcriptional Analysis-Based Alterations Affecting Neuritogenesis of the Peripheral Nervous System in Psoriasis. Life (Basel, Switzerland) 12, no. 1 (2022): 111.

https://doi.org/10.3390/life12010111.

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2. Dóra Romhányi, Kornélia Szabó, Lajos Kemény and Gergely Groma Histone and Histone Acetylation-Related Alterations of Gene Expression in Uninvolved Psoriatic Skin and Their Effects on Cell Proliferation, Differentiation, and Immune Responses International Journal of Molecular Sciences 24, no. 19 (2023): 14551

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List of publications not related to the thesis

1. Márta Kotormán, Dóra Romhányi, Bence Alpek, Orsolya Papp and Katalin Márton

Fruit Juices Are Effective Anti-Amyloidogenic Agents. Biologia Futura 72, no. 2 (2021): 257–62.

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1. INTRODUCTION

1.1. Psoriasis and non-lesional psoriatic skin

Psoriasis is a multifactorial, polygenic chronic inflammatory disease affecting 2-3% of the world's population. Psoriasis is characterized by an altered immunological response to biotic and abiotic stressors, leading to keratinocyte hyperproliferation and massive immune cell infiltration, resulting in the appearance of psoriatic plaques. The most common form of psoriasis is plaque psoriasis, which is characterized by well-defined, silvery, scaly plaques.

Molecular abnormalities are already present in the macroscopically healthy-looking, non-lesional skin. These abnormalities and alternations can be classified into two major groups: "predisposing" alternations that contribute to the formation of lesions and "protective" alterations that participate in the maintenance of the non-lesional stage.

1.2. Abnormalities of the peripheral nervous system in psoriasis

In non-lesional skin, Koebner's phenomenon occurs when psoriatic plaques form in response to mechanical injury or stress, driven by an exaggerated immune response and increased keratinocyte proliferation. External, potentially dangerous stimuli are not only sensed by keratinocytes but also by cutaneous axons of neurons, among other cells. Upon activation, keratinocytes produce pro-inflammatory cytokines and influence neuronal function.

Several studies suggest a link between psoriasis pathomechanism and the peripheral nervous system. Psoriatic plaques may regress in skin areas with peripheral nerve dysfunction and reappear when nerve function is restored. Although neuronal cell bodies are not present in the skin, significant amount of RNA transport and translation occur in the terminal regions of the axons.

Currently, the molecular mechanisms associated with the functional and structural abnormalities of the nervous system are largely unknown. However, recent research suggests that cutaneous nerve endings, which are in close contact with keratinocytes and immune cells, play a significant role in the pathogenesis of psoriasis.

The large number of molecular abnormalities already present in non-lesional skin suggests that epigenetic alterations may occur in different skin cells, potentially affecting the function of dermal nerve endings.

1.3. Epigenetic regulation of histones and their abnormalities in psoriasis

The basic unit of chromatin is the nucleosome, which is composed of DNA and an octamer made up of histones H2A, H2B, H3, and H4. Histones can be categorized as replication-dependent or replication-independent based on their role in DNA replication. Three major histone-related epigenetic regulatory layers can be distinguished. The first layer of histone-related epigenetic modification involves histone chaperones, which transport, exchange and incorporate histone variants, thereby modifying histone composition of the nucleosome. The second layer is the histone (and histone variant) composition of the nucleosome determined by these chaperones. The third layer consists of post-translational modifications of histones, including acetylation, phosphorylation, methylation, and ubiquitination. Histone acetylation is carried out by histone acetyltransferases, leading to transcriptional activation, while deacetylation is mediated by histone deacetylases, resulting in transcriptional repression.

An increasing number of studies are revealing abnormalities in histones and histone acetylation patterns, as well as their potential role in the pathomechanism of psoriasis. During epidermal development, histone H3 acetylation gradients are observed between basal and differentiating cells. This H3 histone acetylation gradient is disturbed in psoriasis and an abnormal H3K27 acetylation level is observed in the psoriatic skin.

Mononuclear cells from the peripheral blood of psoriasis patients exhibit an abnormal acetylation pattern associated with the H4 histone. Furthermore, altered H3 histone acetylation patterns have already been demonstrated during the differentiation of Th17 cells, which play a key role in the pathogenesis of the disease. This indicates an epigenetic dysregulation of both the innate and adaptive immune responses in psoriasis patients.

2. AIMS

I) To explore the molecular mechanisms underlying peripheral nervous system-related abnormalities in psoriatic skin and identify potential signaling pathways affected by altered transcriptional levels.

II) a, To map histone-related epigenetic regulatory abnormalities in non-lesional psoriatic skin, focusing on proteins that govern histone composition and acetylation.

b, To analyze the effect of the identified histone-related epigenetic abnormalities in shaping core psoriatic processes, with an emphasis on their effects on cell proliferation and immune responses.

3. MATERIALS AND METHODS

3.1. Criteria for pooling transcriptome sequencing data from three published psoriasis databases

- Three published studies (Li B. et al., 2014; Tsoi L.C. et al., 2015; Liang Y. et al., 2017) were used, with random inclusion of patients with chronic plaque psoriasis and healthy donors.
- Inclusion criteria: age >18 years; PASI score of at least 1% on total body surface area.
- Skin biopsies: 6 mm biopsies from various regions of the body (hips, buttocks, thighs, back, arms, sides, abdomen, elbows).
- Washout period before sampling: 1 week for local treatments, 2 weeks for systemic treatments.

3.2. Processing of RNA sequencing data

- Data source: Reprocessing of RNA sequencing data from the three published studies.
- Data download: Downloaded from SRA (IDs: SRP035988, SRP050971, SRP055813); SRA-tools were used to download the data.
- Transcript-level of expression was quantified using Kallisto with the full GENCODE v27 transcriptome annotation.

3.3. Differential expression analysis

- Importing data: Transcript-level, length-proportional TPM expression estimates generated by Kallisto were imported into the R statistical environment using the tximport package.
- Normalization and transformation: Data were TMM-normalized and voom-transformed. The edgeR package was used for TMM normalization, and the voomWithQualityWeights() function from the limma package was used for voom transformation.
- Sample handling: The voomWithQualityWeights() function was used to account for lower-quality samples.
- Testing for differential expression: Differential expression was tested using the limma package to fit a linear model and compute moderated t-statistics with the eBayes function.

Transcripts with an FDR-corrected p-value < 0.05 were considered differentially expressed (DETs).

3.4. Analysis of differentially expressed transcripts (DETs) related to neuronal changes: functional annotation, enrichment analysis, and statistics

- Analysis of DETs: Differentially expressed transcripts were analyzed using IPA software to identify enriched pathways.
- Enrichment analysis: For the "Diseases and Biological Functions" annotation, the Fisher exact test was used to calculate the p-value, measuring the significance of DET enrichment in specific pathways.
- Gene Ontology enrichment analysis: The p-value was calculated based on the mHG or HG model, with multiple testing correction performed using the Benjamini-Hochberg method.

3.5. Screening of DETs for histones and histone acetylation

- Data combination: Based on the three main publications, we combined publicly available psoriasis transcriptome datasets and created a database of differentially expressed transcripts from non-lesional skin.
- Molecular database creation: Using online databases (GOEST, STRING and Genecards) supplemented with literature, we compiled a list of molecules related to histones, histone chaperones, acetyltransferases, and deacetylases.
- Screening and matching: Genes for histones, histone chaperones, and acetyltransferases/deacetylases that were differentially expressed in non-lesional psoriatic skin compared to healthy skin were identified through screening.
- Function search: A literature review was conducted to identify functions related to proliferation, differentiation, and immune response from the combined dataset.

4. RESULTS and DISCUSSION

4.1. Axonal abnormalities caused by differentially expressed transcripts in non-lesional and lesional psoriatic skin.

Using IPA software, we identified 347 and 885 genes encoding differentially expressed transcripts (DETs) associated with neurodevelopment and function in non-lesional and lesional skin, respectively. These DETs influence neuronal morphogenesis, particularly neuritogenesis. Since only neurites penetrate the skin, we aimed to gain further insight into how neuronal projections might be affected in the skin. The analysis revealed biological processes involved in the regulation of neurite outgrowth and the semaphorin-plexin signaling pathway. These pathways are already altered in non-lesional skin and further exacerbate in lesional skin.

4.2. Semaphorin-plexin signaling, an important regulator of axon formation, is differentially affected in non-lesional and lesional psoriatic skin.

Both IPA and GOrilla enrichment analyses indicated the involvement of the semaphorin-plexin signaling pathway in the pathogenesis of psoriasis, we perdormed a detailed analysis of these pathways. The Sema3 family members play a role in neurite formation by regulating axon attraction and repulsion. In both non-lesional and lesional skin, we found DETs encoded by Sema3B and Sema3F genes, while in lesional skin, Sema3D, Sema3E, and Sema3G were also involved. Expression of Sema receptors, including NRP1, NRP2, PLXNA3, PLXNB1, PLXNB3, PLXND1, as well as L1CAM and ERBB2, was also differentially regulated in psoriatic samples. SemaB and SemaF are both involved in axon attraction or repulsion, and these antagonistic functions may result from differences in their local concentrations and/or the receptor repertoires of interacting cells. Sema3E can stimulate axon growth in neurons expressing PLXND1 and NRP1, but when PlexinD1 is expressed without NRP1, Sema3E has the opposite effect. Consistent with our findings on axon growth, several studies have implicated NRP1 in the pathomechanism of psoriasis in relation to keratinocyte proliferation and differentiation, as well as angiogenesis and lymphangiogenesis.

SEMA4D, a modulator of axon elongation, shows transcriptional differences in lesional skin, while its receptors and downstream molecules are differentially expressed in both lesional

and non-lesional skin. Consistent with our findings, SEMA4D was previously suggested to play a role in triggering the keratinocyte-induced inflammatory response in psoriasis.

4.3. ROBO-DCC-UNC5 signaling regulates axon formation and differentially affected in non-lesional and lesional psoriatic skin

Slit and Ntn signaling via Robo and Dcc receptors are involved in the regulation of axonal dynamics. These receptors play roles in several pathways including Axonal Guidance Signaling, while SLIT2 and its receptor ROBO2 show alterations only in lesional skin, ROBO1 expression is altered in both non-lesional and lesional skin. NTN1 and its receptors DCC and UNC5A also show alterations exclusively in lesional skin, while the expression of some downstream proteins is affected in both non-lesional and lesional skin. Consistent with our results, NTN1 was suggested to be involved in immune cell infiltration in psoriasis.

4.4. Disruption of WNT5A signaling may affect skin axon growth in psoriasis

We also detected the involvement of signaling pathways mediated by WNT5A and the FZD3/FZD5 receptors in psoriatic lesions, which may affect axon growth and repulsion. Notably, the expression of downstream molecules affecting axon growth was altered in non-lesional skin. In line with this, WNT5A has been shown to contribute to the pathological immune response in psoriasis through its effect on T-cell chemotaxis.

4.5. Altered expression of histone chaperones in non-lesional skin and their role in cell proliferation and immune-related processes

The first layer of histone-related epigenetic regulation is represented by histone chaperones, which influence histone composition and metabolism. Our analysis showed that some members of the CAF-1 and HIRA complexes, which regulate the deposition of histones H3-H4, exhibit abnormal expression in non-lesional skin, potentially contributing to the abnormal keratinocyte proliferation observed in psoriasis. In addition, we detected DETs regulating H2AZ1 (related to

H2A histone) histone removal and deposition, which may modulate inflammatory immune response in psoriasis through their role in regulating immune cell infiltration.

4.6. Altered expression of histones in psoriatic non-lesional skin and their effect on cell proliferation and immune-related processes.

A second layer of epigenetics is constituted by histone variants, which can be divided into replication-dependent and replication-independent groups based on their role in DNA replication. We found that H2AC18 and H4C14 histone variants are abnormally expressed in non-lesional skin and may have a significant impact on abnormal keratinocyte proliferation through their key role in DNA replication. We have identified differentially expressed transcripts associated with replication-independent H2A and H3 histones that play a key role in cell lineage commitment and stem cell renewal, thus potentially contributing to epithelial-mesenchymal transition known to take place in psoriasis.

4.7. Effects of differentially expressed histone acetyltransferases and their complex components on cell proliferation and immune response in non-lesional skin.

Only type A HATs or their modulators show aberrant expression in non-lesional psoriatic skin. In the CBP/CREBBP family, only modulators show aberrant transcription levels, whereas both acetyltransferases and their complexes are affected among members of the GNAT and MYST families. This includes ATAC and SAGA (HAT module) related to the GNAT family, as well as NSL and TIP60 complexes related to the MYST family.

Abnormal expression of GNAT family member ELP3 may affect macrophage polarization and thus can contribute to the abnormal M1/M2 macrophage ratio observed in psoriasis. While, transcriptional abnormalities of the ATAC complex components may be associated with abnormal keratinocyte proliferation through their role in regulating mitosis.

In addition, we found that the MYST family component KAT5 and its TIP60 complex show altered expressions in non-lesional psoriatic skin. KAT5 may play a role in the pathogenesis of the disease, particularly in regulating the IL-9 signaling pathway, angiogenesis, and Th17 responses. Although the catalytic unit of the MYST family NSL complex does not show alterations in non-

lesional skin, some components of the complex show altered expression and may contribute to the development of an abnormal immune response in psoriasis.

4.8. Effect of abnormally expressed histone deacetylases and complexes on cell proliferation and immune response in non-lesional skin.

Our analysis suggests that some members of each of the four histone deacetylases families (HDACI.-IV.) are abnormally expressed in non-lesional skin. In addition, some components of the HDAC I. family complexes also show differences in expression in non-lesional skin, including NCOR/SMRT, NURD, SIN3 and SHIP complexes.

Among HDAC I. family members, we identified abnormal expression of HDAC3 and HDAC8 in non-lesional skin, which may play a role in the development of the skin dryness and inflammatory skin response observed in psoriasis. Differentially expressed components of the SHIP and SIN3 complexes may contribute to the development of hyperkeratosis and abnormal stratification of the epidermis in psoriatic patients. Differentially expressed members of the NURD and SIN3 complexes may not only lead to impaired T cell-induced inflammatory response, but also some components of the NURD complex may play an important role in the development of altered stress response of non-lesional skin.

Among the members of the HDAC II. family, the expression of HDAC4, HDAC5 and HDAC6 is also affected in non-lesional skin. These HDACs are known to affect the regulation of inflammatory cytokines, NF-kB and Foxo3a signaling, Treg cell differentiation, T cell motility, anti-inflammatory processes, chemotaxis and immune synapse organization. Therefore, alterations in their function are likely to play a significant role in the development of psoriasis. In particular, abnormal expression of HDAC5 may influence the imbalance between Treg/Th17 cells in psoriasis. In addition, altered expression of HDAC6 may contribute to the increased rate of wound healing in both lesional and non-lesional skin.

Among class III. HDACs, alterations in the expression of SIRT5 may influence IL17-A-induced inflammation and cell proliferation. In contrast, abnormal expression of SIRT6 may contribute to the abnormal wound healing and dysregulation of macrophage activity observed in psoriasis.

Abnormal expression of HDAC11, a member of the class IV. HDAC family, may affect immune regulation, neutrophil lineage commitment, as well as IL-10 and IL-1β secretion and T cell activation, which are known to be altered in psoriasis.

5. SUMMARY

In summary, our study of complete RNA sequence analysis of more than 300 individuals identifies potential mechanisms that may underlie the morphological changes in the peripheral nervous system within the skin of psoriasis patients. Among these mechanisms, the abnormalities associated with semaphorins, which regulate axon growth and branching while also influencing immune responses, are particularly noteworthy. These findings enhance our understanding of neuro-immune interactions in psoriasis and may open new research options for therapeutic interventions targeting both the immune and nervous system components of the condition.

The axons of the peripheral nervous system closely interact with various skin cells, with the most intimate interactions occurring in the epidermis, where the axons are predominantly demyelinated, allowing direct contact with keratinocytes. Consequently, disturbances in skin cells can affect the functioning and morphology of peripheral nerve axons. Therefore, it is crucial to gain a deeper understanding of the primary regulatory mechanisms behind the abnormalities present in non-lesional skin. Considering the complex abnormalities present in non-lesional skin, we proposed that epigenetic dysregulation may play a role. Our results confirmed several known abnormalities related to epigenetic regulation and revealed new factors that could contribute to already established non-lesional abnormalities. We found that non-lesional skin exhibits differences in expression across all levels of histone-related epigenetic regulation compared to healthy skin, impacting histones themselves, histone chaperones, and histone acetylation processes.

In conclusion, we described several novel alterations related to axon guidance and epigenetic regulation in psoriasis that can influence the formation of immune responses and the functioning of keratinocytes, including their proliferation, which are likely to affect nerve functions (and *vica versa*) before any visible skin changes manifest. However, further studies are required to determine which of these newly described alterations manifest at the protein level and influence their function, as well as to identify the processes that help to maintain the non-lesional state and those that contribute to the disease's progression. Clarifying these questions may provide new targets for future therapeutic options for psoriasis.

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