Transcriptional analysis-based alterations affecting neuritogenesis of the peripheral nervous system in psoriasis

Dóra Romhányi1, Kornélia Szabó1,2,3, Lajos Kemény1,2,3, Endre Sebestyén4, Gergely Groma1,3,*

1Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary.
2Hungarian Centre of Excellence for Molecular Medicine - University of Szeged Skin Research Group (HCEMM-USZ Skin Research Group), Szeged, Hungary.
3Eötvös Loránd Research Network, MTA-SZTE Dermatological Research Group, Szeged, Hungary.
41st Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary.
Corresponding: groma.gergely@med.u-szeged.hu

Abstract
An increasing amount of evidence indicates the critical role of the cutaneous nervous system in the initiation and maintenance of psoriatic skin lesions by neurogenic inflammation. However, molecular mechanisms affecting cutaneous neurons are largely uncharacterized. Therefore, we reanalyzed a psoriatic RNA sequencing dataset from published transcriptome experiments of nearly 300 individuals. Using the Ingenuity Pathway Analysis software, we associated several hundreds of differentially expressed transcripts (DETs) to nervous system development and functions. Since neuronal projections were previously reported to be affected in psoriasis, we performed an in-depth analysis of neurite formation-related processes. Our in silico analysis suggests that SEMA-PLXN and ROBO-DCC-UNC5 regulating axonal growth and repulsion are differentially affected in non-lesional and lesional skin samples. We identified opposing expressional alterations in secreted ligands for axonal guidance signaling (RTN4/NOGOA, NTNs, SEMAs, SLITs) and non-conventional axon guidance regulating ligands, including WNT5A and their receptors, modulating axon formation.
These differences in neuritogenesis may explain the abnormal cutaneous nerve filament formation described in psoriatic skin. The processes also influence T cell activation and infiltration, thus highlighting an additional angle of the crosstalk between the cutaneous nervous system and the immune responses in psoriasis pathogenesis, in addition to the known neurogenic pro-inflammatory mediators.

Keywords:
psoriasis, cutaneous nervous system, axon development, myelination
Introduction

Psoriasis is a chronic inflammatory skin disease affecting approximately 1-3% of the human population worldwide. It is characterized mainly as an abnormal skin reaction to various internal and external stimuli, leading to keratinocyte hyperproliferation and chronic immunological responses. Despite the large amount of work, the exact pathomechanism of psoriasis remains unclear, and currently available therapies only manage the symptoms. Therefore, a deeper understanding of the disease-causing alterations is important, to develop new treatment options that not only treat the existing symptoms but also interfere with their development.

In psoriasis, the macroscopically healthy-looking non-lesional (NL) skin already carries alterations that in combination with various abiotic and biotic stimuli lead to the appearance of symptoms. One of the widely known characteristics of the NL skin is the Köbner phenomenon, the development of lesions in response to mechanical provocations or stress due to elevated immune response and increased keratinocyte proliferation. External, potentially dangerous stimuli are not only sensed by keratinocytes but also by cutaneous neurons, among other cells. Skin cells become activated by these insults, produce pro-inflammatory cytokines, and may also activate and modulate the neuronal functions of nociceptors. An example of this is an altered thermosensation in psoriatic tissues.

Peripheral nervous system (PNS) abnormalities resulting in the loss of sensory abilities can lead to the remission of psoriatic lesions, including I. superficial cutaneous nerve injury, II. poliomyelitis associated flaccid paralysis, III. loss of intercostobrachial nerve function, IV. permanent severance of the left lateral cutaneous nerve, V. traumatic unilateral brachial plexus palsy, VI. loss of finger sensation due to peripheral denervation and VII. partial axonal and demyelinating neuropathy. Damages affecting the central nervous system (and thereby also the PNS) e.g. hemiparesis and hemiplegia or stroke were reported to cause the clearance of psoriatic plaques at the neuronal dysfunction-affected peripheral areas. Moreover, in cases when the nerve injury-associated anesthesia was only temporary, psoriatic symptoms reappeared following nerve function recovery. These case reports were recently summarized in depth by Bi Qin and colleagues and by Tian Hao Zhu and coworkers.

Apart from the nervous system-related injuries, several studies showed near-complete remission of psoriatic lesions following botulinum toxin treatment that further supports the role of the nervous system both in the formation, as well as in the maintenance of psoriatic plaques. In a psoriasiform animal model, botulinum toxin treatment was suggested to exert its effect through the inhibition of neuropeptides. In 1986, researchers suggested the influence of
cutaneous neurons and neuro-immune factors in the pathogenesis of psoriasis\textsuperscript{25}. Since then, numerous studies indicated the role of neuropeptides both in the inflammatory and the proliferative processes in psoriasis pathogenesis. As a result, we may consider psoriasis, at least in part, as a neurogenic inflammatory disease\textsuperscript{26}. Studies reported increased expression of several neuropeptides in the lesional (L) skin, including CGRP (calcitonin gene-related protein)\textsuperscript{27,28}, NGF (nerve growth factor)\textsuperscript{29}, SP (substance P)\textsuperscript{30,31}, VIP (vasoactive intestinal peptide)\textsuperscript{32}. Apart from their neural functions, these molecules also display pro-inflammatory activities and thereby may contribute to inflammation\textsuperscript{33}, highlighting an important role of the nervous system in psoriasis pathomechanism.

The majority of psoriatic patients are troubled by itch at their L skin\textsuperscript{34,35}. In these areas, neurogenic pro-inflammatory mediators, e.g., CGRP, NGF, and SP can contribute to itching (pruritus) development\textsuperscript{36,37,38}. The patients may also suffer from aching, burning, cramping, stinging, tenderness, and tingling at the L areas\textsuperscript{39}, suggesting that cutaneous neuronal sensation mechanisms are affected at multiple levels.

While our knowledge of how neurons affect the immune system is continuously increasing, there is much less know about how the cutaneous nervous system itself is affected in psoriasis. Several large-scale studies, including proteomics\textsuperscript{40,41}, RNA microarray\textsuperscript{42} and sequencing\textsuperscript{43,44}, GWAS\textsuperscript{45}, and DNA methylation profiling\textsuperscript{46} analyses have been performed to gain a deeper insight into the pathomechanism of the disease\textsuperscript{47}. However, our mechanistic knowledge on how the peripheral nervous system is involved and how the cutaneous nervous system is affected in psoriasis remains limited.

We combined the transcriptome sequencing results of nearly 300 individuals from three major published psoriatic datasets, uniformly reanalyzed the data and used the Ingenuity Pathway Analysis software for their downstream enrichment analysis\textsuperscript{44,48,49}. Using an unbiased annotation, we found 347 and 885 differentially expressed transcripts (DETs) in NL and L skin samples, respectively. They were associated with nervous system development and functions, in particular, with neuritogenesis regulating mechanisms. This may seem peculiar, knowing that the cell body of neurons does not locate in the skin. However, earlier studies indicate that a large quantity of RNA transport and translation is taking place in the axons that runs in the cutaneous tissues\textsuperscript{50,51}. This may explain why and how it is still possible to pinpoint transcriptome differences of different nervous system functions. Therefore, we decided to focus on these mechanisms and analyzed neuritogenesis-related alterations in depth.
Materials and Methods

RNA sequencing data processing

The RNA sequencing datasets from three papers were uniformly reprocessed. We downloaded the data from SRA (SRP035988, SRP050971 and SRP055813) using SRA-tools (version 2.9.2). We quantified transcript level expression using Kallisto (version 0.43.0) and the full GENCODE v27 transcriptome annotation. Kallisto was run with the following options: --bias --single -l 120 -s 20 -b 100.

Differential expression analysis

Transcript-level length-scaled TPM expression estimates from Kallisto were imported into the R statistical environment (version 3.4.3), using the tximport package (version 1.6.0). The data was TMM normalized and voom transformed. We used edgeR (version 3.20.9) for the TMM normalization and the voomWithQualityWeights() function from limma (version 3.34.9) for the voom transformation. Limma was also used to test for differential expression between lesional and non-lesional, lesional and healthy, or non-lesional and healthy sample groups. A linear model was fitted with the limma lmFit function, and the moderated t-statistics was calculated with the eBayes function. Transcripts were defined as differentially expressed if they had an FDR corrected p-value < 0.05 and an absolute log2 fold-change larger than 1.

Functional annotation, enrichment analysis and statistics

Differentially expressed transcripts (DETs) from NL vs. H and L vs. H comparison was analyzed using Ingenuity Pathway Analysis (IPA) software (IngenuityH Systems, www.ingenuity.com) to identify pathways that are enriched. DET sets were mapped to the HUGO gene symbols within IPA software and those that did not map to any HUGO gene were discarded. For ‘Diseases and Biological funtions’ annotation, the p-value was calculated using Fisher's exact test to measure the significance of DET enrichment of a given pathway. For the Gene Ontology enrichment analysis and visualization (Gorilla) tool, the enrichment analysis p-value was calculated according to the mHG or HG model; p-value correction for multiple testing was done according to the Benjamini and Hochberg method (FDR correction). Enrichment was defined as: (b/n) / (B/N), where N: total number of genes, B: total number of genes associated with a given specific GO term, n: number of genes in the top of the user's input list or in the target set when appropriate, b: number of genes in the intersection.
Results

Peripheral nervous system-associated transcript expression alterations in psoriasis

Based on our psoriasis transcriptome analysis, 2681 transcripts showed altered expression level in the NL and healthy (H) skin comparison (Supplementary Table 1. A), whereas the number of transcripts with altered expression in L vs. H skin was 12314 (Supplementary Table 1. B). Ingenuity Pathway Analysis (IPA) software identified DETs coded by 347 and 885 genes in association with nervous system development and function in NL and L skin, respectively (Supplementary Table 1. C and 1. D). These DETs are predicted to affect neuronal morphogenesis, including neuritogenesis, which represented the most specific group in the analysis (Table 1. and Supplementary table 1. E and F).

Table 1. Functional annotation of nervous system related DETs in non-lesional and lesional psoriatic skin. (H: healthy, L: lesional, NL: non-lesional skin).

| Categories                                      | Functions                  | Comparison  | P-value  | Number of Molecules |
|-------------------------------------------------|----------------------------|-------------|----------|---------------------|
| Nervous System Development and Function         | Morphology of nervous system | NL vs. H    | 4.11E-17 | 236                 |
|                                                 |                            | L vs. H     | 5.28E-32 | 637                 |
| Nervous System Development and Function, Neurological Disease | Abnormal morphology of nervous tissue | NL vs. H    | 4.95E-13 | 188                 |
|                                                 |                            | L vs. H     | 2.80E-20 | 495                 |
| Nervous System Development and Function, Tissue Morphology | Morphology of nervous tissue | NL vs. H    | 1.11E-12 | 165                 |
|                                                 |                            | L vs. H     | 5.25E-22 | 439                 |
| Nervous System Development and Function, Organismal Development, Tissue Development | Morphogenesis of nervous tissue | NL vs. H    | 4.70E-10 | 144                 |
|                                                 |                            | L vs. H     | 4.46E-22 | 405                 |
| Cell Morphology, Cellular Assembly and Organization, Cellular Development, Cellular Function and Maintenance, Cellular Growth and Proliferation, Nervous System Development and Function, Organismal Development, Tissue Development | Neuritogenesis | NL vs. H    | 5.26E-10 | 142                 |
|                                                 |                            | L vs. H     | 6.62E-22 | 399                 |
| Cell Morphology, Cellular Development, Cellular Growth and Proliferation, Nervous System Development and Function, Organismal Development, Tissue Development | Morphogenesis of neurons | NL vs. H    | 6.60E-10 | 143                 |
|                                                 |                            | L vs. H     | 6.85E-22 | 403                 |
| Cellular Development, Cellular Growth and Proliferation, Nervous System Development and Function, Tissue Development | Development of neurons | NL vs. H    | 1.12E-09 | 177                 |
|                                                 |                            | L vs. H     | 2.14E-24 | 517                 |

Differentially expressed transcripts affecting axon-related alterations in non-lesional and lesional psoriatic skin

Since only neurites penetrate the skin, we wanted to gain further insight into how neuron projections are likely to be affected in the skin. For this, we performed gene ontology (GO) functional enrichment analysis using neuron projection GO:0043005 as a background in Gorilla (Gene Ontology enrichment analysis and visualization tool) on the neuritogenesis-associated DETs from the original IPA analysis. This analysis revealed biological processes linked to the
regulation of neuron projection development and the semaphorin-plexin signaling pathway. According to our results, these pathways are likely to be affected already in the NL skin, and to a greater extent in L samples, as suggested by a higher number of DETs in the latter group (Table 2. and Supplementary Table 1. G and H).

Table 2. Gene ontology (GO) functional enrichment analysis of DETs associated with neuritogenesis in non-lesional and lesional skin. (H: healthy, L: lesional, NL: non-lesional skin).

| GO Term                        | Description                                  | Comparsion     | P-value   | FDR q-value | Enrichment (N, B, n, b) |
|-------------------------------|----------------------------------------------|----------------|-----------|-------------|------------------------|
| GO:0010975                    | regulation of neuron projection development  | NL vs. H       | 1.98E-4   | 2.74E-2     | 1.72 (1442, 229, 139, 38) |
|                               |                                              | L vs. H        | 6.66E-10  | 2.79E-7     | 1.64 (1594, 260, 389, 104) |
| GO:0045664                    | regulation of neuron differentiation          | NL vs. H       | 2.68E-4   | 3.35E-2     | 1.67 (1442, 249, 139, 40) |
|                               |                                              | L vs. H        | 6.63E-11  | 4.07E-8     | 1.64 (1594, 285, 389, 114) |
| GO:0071526                    | semaphorin-plexin signaling pathway           | NL vs. H       | 4.09E-4   | 4.07E-2     | 5.19 (1442, 12, 139, 6) |
|                               |                                              | L vs. H        | 2.3E-5    | 1.37E-3     | 2.96 (1594, 18, 389, 13) |

In addition, neuron projection morphogenesis, development, and guidance (Table 3. and Supplementary Table 1. I and J) were predicted to be affected only in psoriatic lesions (Table 4. and Supplementary Table 1. K and L). Among axon formation-associated regulatory processes, negative regulation of axonogenesis and axon guidance are predicted to be affected in psoriatic lesions (Table 4. and Supplementary Table 1. K and L).

Table 3. Gene ontology (GO) functional enrichment analysis of DETs associated with neuritogenesis-related biological processes in lesional but not in non-lesional skin. (H: healthy, L: lesional).

| GO Term                        | Description                                  | Comparsion | P-value | FDR q-value | Enrichment (N, B, n, b) |
|-------------------------------|----------------------------------------------|------------|---------|-------------|------------------------|
| GO:0048812                    | neuron projection morphogenesis              | L vs. H    | 2.94E-10| 1.43E-7     | 1.96 (1594, 138, 389, 66) |
| GO:0097485                    | neuron projection guidance                   | L vs. H    | 8.37E-7 | 9.17E-5     | 1.78 (1594, 124, 389, 54) |
| GO:0031175                    | neuron projection development                | L vs. H    | 9.31E-7 | 9.74E-5     | 1.68 (1594, 159, 389, 65) |
| GO:0010976                    | positive regulation of neuron projection development |         | 3.15E-6 | 2.54E-4     | 1.69 (1594, 141, 389, 58) |
| GO:0010977                    | negative regulation of neuron projection development |       | 9.99E-5 | 4.76E-3     | 1.74 (1594, 87, 389, 37) |
Axon formation is strongly associated with Schwann cell myelination in the peripheral nervous system. Despite that functional enrichment analysis did not reveal any associated processes, skin tissue expression analysis (tissues.jensenlab.org) integrated into the STRING database (version:11.5) revealed some interesting associations. Four molecules (MBP, MPZ, PMP22, and EGR2) out of the DETs coded by 347 genes in NL were assigned to Schwann cells (BTO:0001220, 4 of 6 molecules), and another four (MBP, MPZ, PMP22, and RTN4) to myelin (BTO:0000894, 4 of 6 molecules). A similar analysis also pointed out four (MBP, MPZ, EGR2, and PRX) Schwann cell-associated molecules in L skin samples (out of the DETs coded by 885 genes), while myelin-related molecules were MBP, MPZ, PLP1, and RTN4 (Supplementary Table 1. M). Our analysis suggests that a common molecule that emerges is RTN4 (also known as Nogo), thus myelin-associated inhibitory regulation of axon formation via RTN4 appears as a general mechanism both in NL and L skin samples.

**Semaphorin-Plexin signaling, an important regulator of axon formation, is differentially affected in non-lesional and lesional psoriatic skin**

Since both IPA and GOrilla enrichment analysis suggested that Semaphorin-Plexin signaling is affected (Semaphorin Neuronal Repulsive Signaling Pathway: p-value\textsubscript{NL} vs. H=1.52E-03 and p-value\textsubscript{L} vs. H=1.45E-02 and Table 2., respectively) in psoriasis pathogenesis, we analyzed these mechanisms in depth. Type 3 semaphorins (Sema3) play a role in neurite formation by regulating axon attraction and repulsion. Among the Sema3 family members that inhibit axon extension, we found DETs coded by Sema3B and Sema3F genes both in NL and L skin, while in L skin, we also detected Sema3D, Sema3E, and Sema3G expression (Figure 1. and
Supplementary Table 1. N). Sema3A is not affected by DETs in NL or L skin. Among semaphorin3 receptors and coreceptors, L1CAM, Nrp1 and PlxnD1 are only affected by DETs in NL skin, while in L samples gene expressional differences are associated with Nrp2 and PlxnA3 (Figure 1.). Transcripts of downstream signaling molecules Fyn, Crpm1, Mapk3, Mnk1, and Paks are differentially expressed both in NL and L skin. Fes and AKT expression are altered only in NL, while DETs of eIF4E, Farp2, Limk2, MsrB1, PI3K, and Rnd1 are present in lesions (Figure 1.). These abnormalities may suggest that axon repulsion and the negative regulation of axon attraction is likely to be highly affected in L in contrast to NL skin, where PI3K mediated negative regulation of axon attraction does not seem to play a role when compared to H skin samples.

**Figure 1.** *In silico* model of how Sema3 signaling alterations regulate axon morphogenesis in NL and L psoriatic skin.

Sema4D is important in axon regeneration not only by modulating axon elongation but also by inhibiting neuron myelination\(^2\). SEMA4D encoding DETs are present in L but not in NL skin. Sema4D cell surface receptors (PlxnD1 and ErbB2), as well as downstream signaling proteins (Paks, Cfl1, and Cfl29) expression is altered in NL and L skin. Whereas in NL skin, AKT, Arhgef11, and RAF, while in L samples Mlc1, PI3K, Rnd1, Rock2, and Shc are affected by DETs (Figure 2. and Supplementary Table 1. O).
Sema6A and 6D gene expression is affected in lesions that share receptors of Sema3A, as well as CSPG, the receptor of Sema5A. These alterations may also affect axon repulsion. The schematic in silico model of the potential crosstalk between Sema3-Sema4-Sema5-Sema6 signaling is shown in (Supplementary Figure 1. and Supplementary Table 1. P), while (Supplementary Figure 2. and Supplementary Table 1. Q) shows the interaction of Sema4 and Sema7A signaling, which only affected in L samples.

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

Axon dynamics is also regulated through Slit and Ntn signaling via Robo and Dcc, respectively. Slit and Ntn signaling via Robo and Dcc were found as part of the general canonical signaling pathway term Axonal Guidance Signaling that also included Wnt5a and semaphorins and were suggested to be affected both in NL and Lesional skin (p-value=3.21E-5 and 5.03E-06, respectively). SLIT2 and its receptor ROBO2 are affected only in L skin, while ROBO1 expression is altered in NL and L samples (Figure 3. and Supplementary Table 1. R). The expression of NTN1, as well as its receptors DCC (Figure 3.) and UNC5A (Figure 4. and Supplementary Table 1. S) are affected in L but not in NL skin, where only some of the downstream proteins may be differentially expressed.
Abnormal WNT5A signaling potentially affect cutaneous axon growth in psoriasis

We found that WNT5A is affected in psoriatic lesions, and the FZD3 and FZD5 receptor-mediated (also affected in L skin) signaling pathway may play a role in axon growth/repulsion (Figure 5. and Supplementary Table 1. T). In contrast, we only found DETs of downstream molecules in the NL skin, and these were mostly affecting axon outgrowth (Figure 5.).
Figure 5. *In silico* model of the effect of Wnt5a signaling on axon growth and retention in psoriasis.
Discussion

Psoriasis is a chronic inflammatory skin disease where interleukin (IL)-17 is the major driver of inflammatory responses\(^ {53}\). Studies, including the imiquimod-induced psoriasis-like skin inflammation models in mice\(^ {54}\), suggest that the peripheral nervous system may have a role in the initiation and maintenance of the inflammatory and hyperproliferative responses through the release of neuropeptides\(^ {30,26}\). Cutaneous nerves can activate dermal dendritic cells’ IL-23 production, and IL-23 triggers IL-17 expression and release by T cells\(^ {54}\). Therefore, peripheral nervous system-related abnormalities may be important to understand the sequence of events in the pathomechanism of psoriasis.

During the reanalysis of public RNA-sequencing data, we identified that neuritogenesis is one of the processes that is affected in the patients. Semaphorin-Plexin signaling cascades regulate various features of neuronal projection formation-related processes \(^ {55}\). Semaphorins were originally identified as neuronal and axon growth guidance molecules. Today it is clear that the superfamily of semaphorins counting over 20 members of soluble extracellular and cell surface transmembrane signaling proteins, can modulate the development and function of several organs, including the cardiovascular\(^ {56}\), immune\(^ {57,58}\), and the nervous system\(^ {59}\), among others\(^ {60,61}\). Despite their massive role in innate immune responses and inflammation\(^ {62}\), we have limited data about the semaphorins’ involvement in psoriasis pathogenesis\(^ {63,64,65}\), but no information on axon formation-related processes in the context of this disease. Since neuritogenesis is affected\(^ {66}\) in psoriasis, it is not surprising that DETs of semaphorins (Semas) were identified in our study, given their clear role in axon guidance. Most of the molecules, like SemaB and SemaF, were implicated in both axon attraction or repulsion\(^ {67}\). These antagonistic functions may be due to the differences in their local concentrations, or the receptor repertoire on the interacting cells. For example, Sema3E stimulates axon growth of PLXND1 and NRP1 expressing neurons, but when PlexinD1 is expressed without NRP1, Sema3E has an opposite effect\(^ {68}\). In addition, Sema3E interaction with its co-receptor VEGFR2 may also stimulate axon extension\(^ {69}\). Despite that, VEGFR2 was not affected in our analyzed dataset, the expression of other Sema receptors, including NRP1, NRP2, PLXNA3, PLXNB1, PLXNB3, PLXND1, as well as L1CAM and ERBB2 were differentially expressed in psoriatic samples. The decrease in Sema3D can negatively influence both the numbers and the branching of peripheral axons\(^ {70}\). Interestingly, the expression of this molecule was affected in lesions, which may be a reason why the number of neurites and axonal branching is reduced in the patients\(^ {66}\). NRP1 was suggested to play in the pathomechanism of psoriasis by several studies in context with
keratinocyte proliferation and differentiation, angiogenesis and lymphangiogenesis among others (reviewed by Sunhyo Ryu and colleagues\textsuperscript{65}).

Class IV semaphorins are transmembrane proteins\textsuperscript{62}. The cell surface Sema4D is known to influence axon regeneration, and its overexpression can inhibit neuron myelination\textsuperscript{52}. Sema4D is also expressed by various immune cells, including T cells, and can modulate dendritic cell functions\textsuperscript{71}. In psoriasis, T cells infiltrate not only to the dermis, where they may interact with dermal dendritic cells, but also the epidermis, where they can come into contact with Langerhans cells. Sema4D was also suggested to induce keratinocyte mediated inflammatory responses in psoriasis\textsuperscript{64}. Moreover, myelination of neurites is the least pronounced in the epidermis, where Sema4D(+) T cells may interrupt the myelination processes, and thereby inhibit axon regeneration. In line with this concept, we found several major myelin-associated proteins\textsuperscript{72}, including MBP, MPZ, PMP22, and RTN4 with altered expression in psoriatic lesions. RTN4 (also known as NOGOA) is a myelin-associated inhibitor of axon growth and regeneration following nerve injury\textsuperscript{73} and may contribute to the reduction of neurites\textsuperscript{66} in lesions.

We also found SLIT2 and its receptor ROBO1, previously shown to be expressed by both axons and Schwann cells, and ROBO2 that is mainly expressed by axons in mice\textsuperscript{74} in the lesional samples of our dataset. Schwann cell-expressed NTN1, which participates in axon regeneration following nerve injury\textsuperscript{75}, is also affected in psoriatic lesions. NTN1 can also influence neutrophil, macrophage, and T cell infiltration\textsuperscript{76}. In addition, dendritic cell-originated Sema4A may play a role in the activation of both Th1 and Th17 cells in the neuroinflammatory demyelinating autoimmune disease, multiple sclerosis\textsuperscript{77,78}. This molecule is also affected by DETs in psoriatic patients.

In the nervous system, Sema4B plays a role in synapse formation and maintenance and may influence post-synaptic density\textsuperscript{79}. We found altered expression of this molecule only in NL skin. In addition, Sema4B may inhibit basophil-mediated Th2 skewing\textsuperscript{80} and contributes to the developing Th1/Th2 imbalance in psoriasis\textsuperscript{81}. Apart from this, circular SEMA4B RNA may decrease the effect of IL-1β through Wnt signaling\textsuperscript{82}. This pathway may also influence axon growth/repulsion via WNT5A (and its receptors FZD3 and FZD5) that we found to be affected in psoriatic lesions which is in line with previous observation\textsuperscript{83}. It may also act as a suppressor of axonal regeneration\textsuperscript{84}, and at the same time, facilitate CXCL12-CXCR4-mediated T cell infiltration\textsuperscript{85}, with the latter being known to be important in chronic inflammatory skin diseases\textsuperscript{86}.
Therefore, we suggest that the expressional dysregulation in 12 different semaphorins and many of their main receptors and co-receptors can contribute to the abnormal neuron projection formation described earlier in psoriasis\textsuperscript{66}. Semaphorin signaling can also greatly influence other major hallmarks of psoriasis, the innate immune and inflammatory processes\textsuperscript{62}. Therefore, our study can highlight an additional angle of the crosstalk between the neuro-immune system, another important mechanism in psoriasis pathomechanism, in addition to the neurogenic pro-inflammatory mediators. Our study provides a strong base and novel directions in psoriatic research since due to the discrepancy between RNA and protein level our results must be examined and validated by future studies.

It is important to note that the vast majority of semaphorin signaling cascades, as well as SLIT-ROBO and NTN-DCC signaling, exert their effect through the small GTPase RAC1\textsuperscript{73}. This molecule not only connects the cutaneous nervous system and the immune cells but also keratinocytes, where it can influence proliferation, differentiation, and innate immune processes\textsuperscript{87}. Based on these features, RAC1 is likely to be an important molecule in psoriasis. RAC1 is also known as a Ras-Related C3 Botulinum Toxin Substrate 1, as it is the primary target of botulinum toxin.

Our results together with previous observations provide an explanation why botulinum toxin treatment of patients is so effective and argues for its more extensive clinical application in psoriasis therapy.
Supplementary Information

Supplementary Figure 1. *In silico* model of the potential crosstalk between Sema3-Sema4-Sema5-Sema6 signaling in NL (left panel) and L (right panel) psoriatic skin. DETs are colored according to the extent of the difference compared to healthy samples. Green color depicts decreased and red increased expression levels. (We used QIAGENs Ingenuity Pathway Analysis software to generate the images.)

Supplementary Figure 2. *In silico* model of the interaction between Sema4 and Sema7A signaling in NL (left panel) and L (right panel) psoriatic skin. DETs are colored according to the extent of the difference compared to healthy samples. Green color depicts decreased and red increased expression levels. (We used QIAGENs Ingenuity Pathway Analysis software to generate the images.)
References

1. Chiricozzi, A., Romanelli, P., Volpe, E., Borsellino, G. & Romanelli, M. Scanning the Immunopathogenesis of Psoriasis. *Int. J. Mol. Sci.* **19**, 179 (2018).

2. Gubán, B. *et al.* Abnormal regulation of fibronectin production by fibroblasts in psoriasis. *Br. J. Dermatol.* **174**, 533–541 (2016).

3. Szlavicz, E. *et al.* Splicing factors differentially expressed in psoriasis alter mRNA maturation of disease-associated EDA+ fibronectin. *Mol. Cell. Biochem.* **436**, 189–199 (2017).

4. Eyre, R. W. & Krueger, G. G. Response to injury of skin involved and uninvolved with psoriasis, and its relation to disease activity: Koebner and ‘reverse’ Koebner reactions. *Br. J. Dermatol.* **106**, 153–159 (1982).

5. Gudjonsson, J. E. *et al.* Global Gene Expression Analysis Reveals Evidence for Decreased Lipid Biosynthesis and Increased Innate Immunity in Uninvolved Psoriatic Skin. *J. Invest. Dermatol.* **129**, 2795–2804 (2009).

6. Regulatory Networks Contributing to Psoriasis Susceptibility.

   http://www.medicaljournals.se/acta/content/abstract/10.2340/00015555-1708
doi:10.2340/00015555-1708.

7. Oh, S. *et al.* Effect of Mechanical Stretch on the DNCB-induced Proinflammatory Cytokine Secretion in Human Keratinocytes. *Sci. Rep.* **9**, 5156 (2019).

8. Baumbauer, K. M. *et al.* Keratinocytes can modulate and directly initiate nociceptive responses. *eLife* **4**, e09674.

9. Yosipovitch, G., Chan, Y. h., Tay, Y. k. & Goh, C. l. Thermosensory abnormalities and blood flow dysfunction in psoriatic skin. *Br. J. Dermatol.* **149**, 492–497 (2003).

10. Sulzberger, M. B. BUGS. *Arch. Dermatol.* **104**, 220 (1971).

11. Weiner, S. R., Bassett, L. W. & Reichman, R. P. Protective effect of poliomyelitis on psoriatic arthritis. *Arthritis Rheum.* **28**, 703–706 (1985).
12. Farber, E. M., Lanigan, S. W. & Boer, J. The Role of Cutaneous Sensory Nerves in the Maintenance of Psoriasis. *Int. J. Dermatol.* **29**, 418–420 (1990).

13. Raychaudhuri, S. P. & Farber, E. M. Are sensory nerves essential for the development of psoriatic lesions? *J. Am. Acad. Dermatol.* **28**, 488–489 (1993).

14. Joseph, T., Kurian, J., Warwick, D. j. & Friedmann, P. s. Unilateral remission of psoriasis following traumatic nerve palsy. *Br. J. Dermatol.* **152**, 185–186 (2005).

15. Kane, D. et al. Protective effect of sensory denervation in inflammatory arthritis (evidence of regulatory neuroimmune pathways in the arthritic joint). *Ann. Rheum. Dis.* **64**, 325–327 (2005).

16. Keçici, A. S., Göktay, F., Tutkavul, K., Güneş, P. & Yaşar, Ş. Unilateral improvement of nail psoriasis with denervation injury. *Clin. Exp. Dermatol.* **43**, 339–341 (2018).

17. Sowell, J. K., Pippenger, M. A. & Crowe, M. J. Psoriasis Contralateral to Hemiparesis Following Cerebrovascular Accident. *Int. J. Dermatol.* **32**, 598–599 (1993).

18. Veale, D., Farrell, M. & Fitzgerald, O. Mechanism of joint sparing in a patient with unilateral psoriatic arthritis and a longstanding hemiplegia. *Br. J. Rheumatol.* **32**, 413–416 (1993).

19. Stratigos, A. J., Katoulis, A. K. & Stavrianeas, N. G. Spontaneous clearing of psoriasis after stroke. *J. Am. Acad. Dermatol.* **38**, 768–770 (1998).

20. Qin, B. et al. The nerve injuries attenuate the persistence of psoriatic lesions. *J. Dermatol. Sci.* **102**, 85–93 (2021).

21. Zhu, T. H. et al. The Role of the Nervous System in the Pathophysiology of Psoriasis: A Review of Cases of Psoriasis Remission or Improvement Following Denervation Injury. *Am. J. Clin. Dermatol.* **17**, 257–263 (2016).

22. González, C., Franco, M., Londoño, A. & Valenzuela, F. Breaking paradigms in the treatment of psoriasis: Use of botulinum toxin for the treatment of plaque psoriasis. *Dermatol. Ther.* **33**, e14319 (2020).

23. Aschenbeck, K. A. et al. Neuromodulatory treatment of recalcitrant plaque psoriasis with onabotulinumtoxinA. *J. Am. Acad. Dermatol.* **79**, 1156–1159 (2018).
24. Amalia, S. N. et al. Suppression of neuropeptide by botulinum toxin improves imiquimod-induced psoriasis-like dermatitis via the regulation of neuroimmune system. *J. Dermatol. Sci.* **101**, 58–68 (2021).

25. Farber, E. M., Nickoloff, B. J., Recht, B. & Fraki, J. E. Stress, symmetry, and psoriasis: Possible role of neuropeptides. *J. Am. Acad. Dermatol.* **14**, 305–311 (1986).

26. Saraceno, R., Kleyn, C. e., Terenghi, G. & Griffiths, C. e. m. The role of neuropeptides in psoriasis. *Br. J. Dermatol.* **155**, 876–882 (2006).

27. Legat, F. J. et al. Repeated subinflammatory ultraviolet B irradiation increases substance P and calcitonin gene-related peptide content and augments mustard oil-induced neurogenic inflammation in the skin of rats. *Neurosci. Lett.* **329**, 309–313 (2002).

28. Vasoactive properties of calcitonin gene-related peptide in human skin - International Angiology 2011 October;30(5):424-8. https://www.minervamedica.it/en/journals/international-angiology/article.php?cod=R34Y2011N05A0424.

29. Raychaudhuri, S. P. & Raychaudhuri, S. K. Role of NGF and neurogenic inflammation in the pathogenesis of psoriasis. in *Progress in Brain Research* vol. 146 433–437 (Elsevier, 2004).

30. Glinski, W., Brodecka, H., Gлинска-Ференц, M. & Kowalski, D. Neuropeptides in Psoriasis: Possible Role of Beta-Endorphin in the Pathomechanism of the Disease. *Int. J. Dermatol.* **33**, 356–360 (1994).

31. Naukkarinen, A., Nickoloff, B. J. & Farber, E. M. Quantification of Cutaneous Sensory Nerves and Their Substance P Content in Psoriasis. *J. Invest. Dermatol.* **92**, 126–129 (1989).

32. Siiskonen, H. & Harvima, I. Mast Cells and Sensory Nerves Contribute to Neurogenic Inflammation and Pruritus in Chronic Skin Inflammation. *Front. Cell. Neurosci.* **13**, 422 (2019).

33. Choi, J. E. & Di Nardo, A. Skin Neurogenic inflammation. *Semin. Immunopathol.* **40**, 249–259 (2018).

34. Szepietowski, J. C. & Reich, A. Itch in Psoriasis Management. *Itch - Manag. Clin. Pract.* **50**, 102–110 (2016).
35. Szepietowski, J. c. & Reich, A. Pruritus in psoriasis: An update. *Eur. J. Pain* **20**, 41–46 (2016).

36. Tsianakas, A. & Mrowietz, U. Pruritus bei Psoriasis. *Hautarzt* **67**, 601–605 (2016).

37. Arck, P. & Paus, R. From the Brain-Skin Connection: The Neuroendocrine-Immune Misalliance of Stress and Itch. *Neuroimmunomodulation* **13**, 347–356 (2006).

38. Leon, A. *et al.* Itching for an answer: A review of potential mechanisms of scalp itch in psoriasis. *Exp. Dermatol.* **28**, 1397–1404 (2019).

39. Pithadia, D. J., Reynolds, K. A., Lee, E. B. & Wu, J. J. Psoriasis-associated cutaneous pain: etiology, assessment, impact, and management. *J. Dermatol. Treat.* **30**, 435–440 (2019).

40. Li, Y. *et al.* Quantitative analysis of differentially expressed proteins in psoriasis vulgaris using tandem mass tags and parallel reaction monitoring. *Clin. Proteomics* **17**, 30 (2020).

41. Szél, E. *et al.* Comprehensive Proteomic Analysis Reveals Intermediate Stage of Non-Lesional Psoriatic Skin and Points out the Importance of Proteins Outside this Trend. *Sci. Rep.* **9**, 11382 (2019).

42. Manczinger, M. & Kemény, L. Novel Factors in the Pathogenesis of Psoriasis and Potential Drug Candidates Are Found with Systems Biology Approach. *PLoS ONE* **8**, e80751 (2013).

43. Li, B. *et al.* Transcriptome analysis of psoriasis in a large case-control sample: RNA-seq provides insights into disease mechanisms. *J. Invest. Dermatol.* **134**, 1828–1838 (2014).

44. Tsoi, L. C. *et al.* Analysis of long non-coding RNAs highlights tissue-specific expression patterns and epigenetic profiles in normal and psoriatic skin. *Genome Biol.* **16**, 24 (2015).

45. Tsoi, L. C. *et al.* Large scale meta-analysis characterizes genetic architecture for common psoriasis associated variants. *Nat. Commun.* **8**, 15382 (2017).

46. Zhou, F. *et al.* Epigenome-Wide Association Analysis Identified Nine Skin DNA Methylation Loci for Psoriasis. *J. Invest. Dermatol.* **136**, 779–787 (2016).

47. Calautti, E., Avalle, L. & Poli, V. Psoriasis: A STAT3-Centric View. *Int. J. Mol. Sci.* **19**, 171 (2018).

48. Liang, Y. *et al.* A gene network regulated by the transcription factor VGLL3 as a promoter of sex-biased autoimmune diseases. *Nat. Immunol.* **18**, 152–160 (2017).
49. Li, B. et al. Transcriptome Analysis of Psoriasis in a Large Case–Control Sample: RNA-Seq Provides Insights into Disease Mechanisms. *J. Invest. Dermatol.* 134, 1828–1838 (2014).

50. Sahoo, P. K., Smith, D. S., Perrone-Bizzozero, N. & Twiss, J. L. Axonal mRNA transport and translation at a glance. *J. Cell Sci.* 131, jcs196808 (2018).

51. Costa, I. D. et al. The functional organization of axonal mRNA transport and translation. *Nat. Rev. Neurosci.* 22, 77–91 (2021).

52. Zhang, H.-L., Wang, J. & Tang, L. Sema4D Knockdown in Oligodendrocytes Promotes Functional Recovery After Spinal Cord Injury. *Cell Biochem. Biophys.* 68, 489–496 (2014).

53. Furue, K., Ito, T., Tsuji, G., Kadono, T. & Furue, M. Psoriasis and the TNF/IL23/IL17 axis. *G. Ital. Dermatol. E Venereol. Organo Uff. Soc. Ital. Dermatol. E Sifilogr.* 154, 418–424 (2019).

54. Riol-Blanco, L. et al. Nociceptive Sensory Neurons Drive Interleukin-23 Mediated Psoriasiform Skin Inflammation. *Nature* 510, 157–161 (2014).

55. Worzfeld, T. & Offermanns, S. Semaphorins and plexins as therapeutic targets. *Nat. Rev. Drug Discov.* 13, 603–621 (2014).

56. Adams, R. H. & Eichmann, A. Axon Guidance Molecules in Vascular Patterning. *Cold Spring Harb. Perspect. Biol.* 2, a001875 (2010).

57. Suzuki, K., Kumanogoh, A. & Kikutani, H. Semaphorins and their receptors in immune cell interactions. *Nat. Immunol.* 9, 17–23 (2008).

58. Kumanogoh, A. & Kikutani, H. Immunological functions of the neuropilins and plexins as receptors for semaphorins. *Nat. Rev. Immunol.* 13, 802–814 (2013).

59. Yoshida, Y. Semaphorin Signaling in Vertebrate Neural Circuit Assembly. *Front. Mol. Neurosci.* 5, 71 (2012).

60. Messina, A. et al. Dysregulation of Semaphorin7A/β1-integrin signaling leads to defective GnRH-1 cell migration, abnormal gonadal development and altered fertility. *Hum. Mol. Genet.* 20, 4759–4774 (2011).
61. Giacobini, P. & Prevot, V. Semaphorins in the development, homeostasis and disease of hormone systems. *Semin. Cell Dev. Biol.* **24**, 190–198 (2013).

62. Kanth, S. M., Gairhe, S. & Torabi-Parizi, P. The Role of Semaphorins and Their Receptors in Innate Immune Responses and Clinical Diseases of Acute Inflammation. *Front. Immunol.* **12**, 1610 (2021).

63. Sabag, A. D. *et al.* Altered expression of regulatory molecules in the skin of psoriasis. *Immunol. Res.* **66**, 649–654 (2018).

64. Zhang, C. *et al.* CD100–Plexin-B2 Promotes the Inflammation in Psoriasis by Activating NF-κB and the Inflammasome in Keratinocytes. *J. Invest. Dermatol.* **138**, 375–383 (2018).

65. Ryu, S. *et al.* Therapeutic Effects of Synthetic Antimicrobial Peptides, TRAIL and NRP1 Blocking Peptides in Psoriatic Keratinocytes. *Chonnam Med. J.* **55**, 75–85 (2019).

66. Casper, M. 3-Dimensional Imaging of Cutaneous Nerve Endings. *J. Invest. Dermatol.* **139**, 999–1001 (2019).

67. Julien, F. *et al.* Dual Functional Activity of Semaphorin 3B Is Required for Positioning the Anterior Commissure. *Neuron* **48**, 63–75 (2005).

68. Chauvet, S. *et al.* Gating of Sema3E/PlexinD1 Signaling by Neuropilin-1 Switches Axonal Repulsion to Attraction during Brain Development. *Neuron* **56**, 807–822 (2007).

69. Bellon, A. *et al.* VEGFR2 (KDR/Flk1) Signaling Mediates Axon Growth in Response to Semaphorin 3E in the Developing Brain. *Neuron* **66**, 205–219 (2010).

70. Liu, Y. & Halloran, M. C. Central and Peripheral Axon Branches from One Neuron Are Guided Differentially by Semaphorin3D and Transient Axonal Glycoprotein-1. *J. Neurosci.* **25**, 10556–10563 (2005).

71. Simona, M., Antonio, P., Massimo, B. & Alfonso, C. Neuronal Semaphorins Regulate a Primary Immune Response. *Curr. Neurovasc. Res.* **3**, 295–305 (2006).

72. Siems, S. B. *et al.* Proteome profile of peripheral myelin in healthy mice and in a neuropathy model. *eLife* **9**, e51406.
73. Kalpachidou, T., Spiecker, L., Kress, M. & Quarta, S. Rho GTPases in the Physiology and Pathophysiology of Peripheral Sensory Neurons. *Cells* **8**, 591 (2019).

74. Carr, L., Parkinson, D. B. & Dun, X. Expression patterns of Slit and Robo family members in adult mouse spinal cord and peripheral nervous system. *PLoS ONE* **12**, e0172736 (2017).

75. Dun, X.-P. & Parkinson, D. B. Role of Netrin-1 Signaling in Nerve Regeneration. *Int. J. Mol. Sci.* **18**, E491 (2017).

76. Boneschansker, L. *et al.* Netrin-1 Augments Chemokinesis in CD4+ T Cells In Vitro and Elicits a Proinflammatory Response In Vivo. *J. Immunol. Baltim. Md 1950* **197**, 1389–1398 (2016).

77. Nakatsuji, Y. *et al.* Elevation of Sema4A Implicates Th Cell Skewing and the Efficacy of IFN-β Therapy in Multiple Sclerosis. *J. Immunol.* **188**, 4858–4865 (2012).

78. Koda, T. *et al.* Sema4A is implicated in the acceleration of Th17 cell-mediated neuroinflammation in the effector phase. *J. Neuroinflammation* **17**, 82 (2020).

79. Burkhardt, C. *et al.* Semaphorin 4B interacts with the post-synaptic density protein PSD-95/SAP90 and is recruited to synapses through a C-terminal PDZ-binding motif. *FEBS Lett.* **579**, 3821–3828 (2005).

80. Nakagawa, Y. *et al.* Identification of Semaphorin 4B as a Negative Regulator of Basophil-Mediated Immune Responses. *J. Immunol.* **186**, 2881–2888 (2011).

81. Zhu, K., Ye, J., Wu, M. & Cheng, H. Expression of Th1 and Th2 cytokine-associated transcription factors, T-bet and GATA-3, in peripheral blood mononuclear cells and skin lesions of patients with psoriasis vulgaris. *Arch. Dermatol. Res.* **302**, 517–523 (2010).

82. Wang, X. *et al.* CircSEMA4B targets miR-431 modulating IL-1β-induced degradative changes in nucleus pulposus cells in intervertebral disc degeneration via Wnt pathway. *Biochim. Biophys. Acta BBA - Mol. Basis Dis.* **1864**, 3754–3768 (2018).

83. Romanowska, M. *et al.* Wnt5a Exhibits Layer-Specific Expression in Adult Skin, Is Upregulated in Psoriasis, and Synergizes with Type 1 Interferon. *PLoS ONE* **4**, e5354 (2009).
84. Clark, C. E. J., Liu, Y. & Cooper, H. M. The Yin and Yang of Wnt/Ryk axon guidance in development and regeneration. *Sci. China Life Sci.* **57**, 366–371 (2014).

85. Ghosh, M. C. et al. Activation of Wnt5A signaling is required for CXC chemokine ligand 12–mediated T-cell migration. *Blood* **114**, 1366–1373 (2009).

86. Zgraggen, S., Huggenberger, R., Kerl, K. & Detmar, M. An Important Role of the SDF-1/CXCR4 Axis in Chronic Skin Inflammation. *PLoS ONE* **9**, e93665 (2014).

87. Winge, M. C. G. et al. RAC1 activation drives pathologic interactions between the epidermis and immune cells. *J. Clin. Invest.* **126**, 2661–2677.