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A differential gene expression study: Ptpn6 (SHP-1)-insufficiency leads to neutrophilic dermatosis-like disease (NDLD) in mice

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ABSTRACT

Background: Irradiated syngeneic wild-type mice developed the same neutrophilic dermatosis-like disease (NDLD) after adoptive transfer of bone marrow cells from *Ptpn6*$^{meb2/meb2}$ mutant mice.
Objective: To analyze differentially expressed genes in the bone marrow of mice with NDLD to gain insight into the role of Ptpn6 in myelopoietic bone marrow pathology, and the mechanisms by which Ptpn6 insufficiency in the hematopoietic cells can lead to the development of skin lesions.
Methods: As Ptpn6 is involved in a myriad of signaling pathways, we used a global approach with microarray technology for the first time to characterize changes in the bone marrow and skin of motheaten-type mice.
Results: A total number of 1,511 probe sets in the bone marrow showed at least two-fold changes with FDR <0.05, of which 256 probe sets had over four-fold changes. A group of 63 genes in the bone marrow of NDLD mice had more than a 4-fold change with FDR <0.0001. From 503 genes encoding proteins with ITIM motif that binds to Ptpn6, 109 were up-regulated and 83 were down-regulated. We found that genes encoding hematopoietic receptors, neutrophil chemoattractants, Toll-like receptors (*Tlr1*, *Tlr2* and *Tlr4*) and C-type lectin innate immunity receptors (*Clec4e*, *Clec4d*, *Clec4n*, *Clec4a2* and *Clec4a3*) were significantly up-regulated in both NDLD bone marrow and skin. The *Il1b* gene was also significantly overexpressed in skin samples, confirming the importance of the IL-1/TLR pathway in the development of early skin inflammation in NDLD mice.
Conclusion: Our results suggest that innate immunity genes play a major role in development of neutrophilic dermatosis-like disease in mice.

1. Introduction

We recently described a spontaneous mutation in the protein tyrosine phosphatase, non-receptor type-6 (*Ptpn6*) gene in mice [1]. All animals with homozygous mutation *Ptpn6*$^{meb2/meb2}$ developed a patchy absence of hair and pigment in the skin, and massive inflammation of the paws with erosions and ulcerations. *Ptpn6*$^{meb2/meb2}$ mice showed clinical and histopathological similarities to human neutrophilic dermatoses (ND). Indeed, for the first time, we have reported *Ptpn6* gene aberrations in patients with pyoderma gangrenosum (PG) and Sweet's syndrome (SS) [2].

To date, four different spontaneous mutations have been described in murine *Ptpn6* gene [1,3–5]. All animal models (*me*, *mev*, *spin and meb2*) develop the characteristic motheaten phenotype, although other clinical symptoms and their life-span

Abbreviations: Ptpn6, tyrosine phosphatase, non-receptor type-6; Hcph, hematopoietic cell phosphatase; Shp-1, Src homology 2 domain-containing tyrosine phosphatase 1; ITIM, immunoreceptor tyrosine-based inhibitory motif; ITAM, immunoreceptor tyrosine-based activation motif; ND, neutrophilic dermatoses; PG, pyoderma gangrenosum; SS, Sweet's syndrome; NDLD, neutrophilic dermatosis-like disease; wt, wild-type; me, motheaten; meb2, motheaten mouse with spontaneous B2 repeat insertion in Ptpn6 gene; RT-qPCR, real time quantitative polymerase chain reaction; FDR, false discovery rate; GEO, gene expression omnibus (database).

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are significantly different, depending upon the type and location of the mutation. In the most severe form of the disease associated with complete phosphatase deficiency in *Ptpn6*$^{me/me}$ mice, the skin symptoms appear as early as 1–2 days of life [3]. In contrast, the *Ptpn6*$^{spin/spin}$ and *Ptpn6*$^{meb2/meb2}$ mice have a significantly longer life-span, thus sufficient time to develop a ND-like disease (NDLD), and they are also more responsive to environmental conditions and treatments [1,5,6].

Mutant mice without B cells (*Ptpn6*$^{me/me} \times$ *Btk*$^{xid/xid}$) [7] or without T cells (*Ptpn6*$^{mev/mev} \times$ *Foxn1*$^{nu/nu}$) [8], or those that have neither T nor B cells (*Ptpn6*$^{mev/mev} \times$ *Rag1*$^{-/-}$ and *Ptpn6*$^{mev/mev} \times$ *Prkdc*$^{scid/scid}$) [9,10], developed the same skin symptoms, providing strong evidence that adaptive immunity does not play a primary role in the development of the motheaten phenotype. Moreover, mice deficient for Ptpn6 only in B-cells (*Ptpn6*$^{f/f}$; *CD19-cre*) do not develop inflamed paws or other obvious skin lesions [11].

A large number of studies has shown that Ptpn6 works as a negative regulator of signaling for multiple receptors of innate and adaptive immunities in both mice and humans [12–17]. Ptpn6 is predominately expressed in hematopoietic cells and plays an important role in differentiation and development of neutrophils, cells that infiltrate the skin and other organs in NDLD mice and patients with ND. Interestingly, ND in humans are frequently associated with multiple hematological abnormalities or malignancies including leukemias [18–21]. Confirming the importance of the bone marrow cells in the development of the skin disease, we were able to transfer the motheaten phenotype with bone marrow cells from *Ptpn6*$^{meb2/meb2}$ mice to irradiated syngeneic wild-type (wt) mice [1].

The goal of this study was to analyze differentially expressed genes in the bone marrow of mice with NDLD to gain insight into the role of Ptpn6 in myelopoietic bone marrow pathology, and the mechanisms by which Ptpn6 insufficiency in the hematopoietic cells can lead to the development of skin lesions.

2. Materials and methods

2.1. Sample and data collection

All the animal studies were approved by the Institutional Animal Care and Use Committee of Rush University Medical Center (Chicago, IL). Bone marrow samples were harvested from 5-week old homozygous Ptpn6$^{meb2/meb2}$ mice with NDLD and their wt littermates. Phenotyping and genotyping were performed as described in our earlier publication [1]. Each group of mice consisted of 5 biological replicates. Total RNA samples from the bone marrow and skin were isolated with Trizol (Life Technologies, Carlsbad, CA) as previously described [1]. The quality of the samples was tested with Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA).

An Affymetrix Mouse Genome 430 2.0 GeneChip Array (Affymetrix, Santa Clara CA) was employed for the study. This array is comprised of 45,037 probe sets representing approximately 34,000 known mouse genes. All labeling reactions and hybridizations were carried out according to the standard GeneChip eukaryotic 3′ target labeling protocol using the One-cycle cDNA synthesis and in vitro transcription (IVT) labeling kit (Affymetrix).

Real-time quantitative polymerase chain reaction (RT-qPCR) was carried out with the iQ5 real-time PCR detection system (Bio-Rad, Hercules, CA). RNA samples from bone marrows and 1×1 cm skin samples from the back of 5 mice with NDLD and 5 wt littermates (Supplementary Fig. 1) were used in reverse transcription reactions. cDNAs were synthesized using an oligo-dT reverse primer and the SuperScript First Strand Synthesis kit (Invitrogen, Carlsbad, CA). Gene-specific PCR primers were purchased from Integrated DNA Technologies, Inc. (Coralville, IA). RT-qPCR data were analyzed with the iQ5 system's software package. Expression of the GAPDH housekeeping gene was used to normalize data. Gene expression was quantified by the $2^{-\Delta\Delta CT}$ method [22], and the results were expressed as fold changes (FC) relative to the corresponding control samples.

2.2. Data analysis and annotation processes

Signal normalization, background correction, additional quality control and all statistical analysis were performed with the use of Affymetrix Transcriptome Analysis Console (TAC) 2.0. Robust Multi-array Average (RMA) method was used for background correction and for the normalization of hybridization signal intensities across all collected samples [23]. ANOVA test was used to calculate the statistical significance of the differential expression. Raw p-values were corrected for False Discovery Rate (FDR) according to the step-up or Benjamini-Hochberg (BH) procedure. The principal component analysis (PCA), hierarchical clustering and chromosomal localization analyses were performed on Affymetrix Transcriptome Analysis Console (TAC) 2.0 [24]. The flow charts of the analysis are shown in Fig. 1B and Supplementary Fig. 2.

We used the Protein ANalysis THrough Evolutionary Relationships (PANTHER) classification system (http://www.pantherdb.org, version 9.0, which includes 7,180 protein families divided into 52,768 functionally distinct protein subfamilies) to annotate genes into three major Gene Ontology (GO) domains: molecular function, biological process and cellular component.

Additional protein-protein interactions were analyzed using MetaCore software (Thomson Reuters, New York, http://thomsonreuters.com/metacore/). All CEL files and the complete gene list of all differentially expressed 9,533 probe sets (FDR <0.05) in Excel format were deposited on Gene Expression Omnibus (GEO) website (http://www.ncbi.nlm.nih.gov/geo/) in GSE73592 series. The comparison between different GEO series were performed by GEO2R interactive web tool from NCBI GEO database.

3. Results

3.1. Differentially expressed genes in the bone marrow of mice with NDLD

A total of 45,037 probe sets on the Affymetrix GeneChip Mouse Genome 430 2.0 Array were analyzed representing approximately 34,000 known mouse genes; one gene was frequently detected with two or three different probe sets. The bone marrow samples of five sick homozygous *Ptpn6*$^{meb2/meb2}$ and five healthy wt animals showed differences in the overall gene expression as presented on the principal component analysis (PCA) plot (Fig. 1A). The distances between samples in the PCA plot, which represent differences in the total gene expression were significantly larger between the samples from the sick and wt groups than between samples of the same group (Fig. 1A). In fact, any sample could be marked as unknown and then correctly assigned to the wt or sick groups by the transcriptome analysis software.

A total of 9,533 probe sets (21%) representing 6,637 genes were differentially expressed in bone marrow samples with the False Discovery Rate (FDR) <0.05. From the 9,533 differentially expressed probe sets, 4,694 were up-regulated and 4,839 were down-regulated. A total number of 1,511 probe sets showed at least two-fold changes (FC) at FDR <0.05, of which 256 probe sets had over four-fold changes (Fig. 1B). From the significantly changed 1,511 probe sets, 479 were up-regulated and 1,032 down-regulated

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