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Integrative methylome and transcriptome analysis to dissect key biological pathways for psoriasis in Chinese Han population

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ABSTRACT

Background: Recent studies have revealed that DNA methylation (DNAm) could modulate gene expression in psoriasis (Ps). However, the relationship between whole-genome DNAm and gene expression in Ps has not been studied yet.
Objectives: To better characterize the relationship between DNAm and gene expression, and to identify biological pathways triggered by changes in methylation involved in the pathogenesis of Ps.
Methods: Differentially methylated sites (DMSs) and differentially expressed genes (DEGs) were analysed by comparing 20 involved psoriatic (PP) skin, 20 uninvolved psoriatic (PN) skin and 20 normal (NN) skin biopsies. DEGs in negative correlation with the methylation were entered into further Gene Ontology (GO) and pathway analysis by clusterProfiler package in R program.
Results: A total of 290 genes with reverse correlation overlapped in PP vs PN and PP vs NN comparisons. GO categories of reversely-associated genes mainly enriched in T cell activation, type I interferon signaling pathway and defense response to other organism. Pathway analysis revealed superior NOD-like receptor signaling pathway and Measles enriched in the differentially up-regulated transcripts and regulation of lipolysis in adipocytes in the down-regulated transcripts.
Conclusions: Our results provided a comprehensive correlation analysis of transcriptome and methylome in Ps. Increased innate immunity and decreased lipid biosynthesis play important roles in the development of psoriatic skin. This integrated analysis shed light on novel insights into the pathogenic mechanisms involved in Ps.

1. Introduction

Psoriasis (Ps) is a chronic recurring hyperproliferative and inflammatory skin disease with a diverse prevalence rate ranging from 0.17%–4% of the people worldwide [1]. The pathogenesis of Ps involves a complex interplay of genetic and environmental factors. Genetic linkage analysis, candidate gene association study, genome-wide meta-analysis and genome-wide association studies (GWAS) have found numerous susceptibility genes or loci associated with Ps in different populations [2–7]. However, analyses based on sequence variation like gene mutation, single nucleotide polymorphism (SNP) and copy number variation (CNV) demonstrated that only about 13% of the total proportion of heritability for diseases could be explained [8,9], suggesting that non-DNA sequence changes such as epigenetic, gene–gene interactions, epigenetic and genetic synergies may have important effects on disease developing [10–12].

It is generally believed that abnormal gene expression is a key process in disease initiation and progression. Epigenetic variation of DNA, particularly the CpG DNA methylation (DNAm), is a major epigenetic modification that controls gene expression in physiologic and pathologic states. Studies have been focusing on the remarkable role of aberrant DNAm in the pathogenesis of cancer [13,14], neurological disease [15], metabolic disease [16], and autoimmunity

Abbreviations: CNV, copy number variation; DEGs, differentially expressed genes; DMSs, differentially methylated sites; DNAm, DNA methylation; FDR, false discovery rate; GO, Gene Ontology; GWAS, genome-wide association studies; KEGG, Kyoto Encyclopedia of Genes and Genomes; NN, normal; PN, uninvolved psoriatic; PP, involved psoriatic; Ps, psoriasis; RNA-seq, RNA sequencing; SNP, single nucleotide polymorphism.

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[17]. Likewise, evidences have shown that abnormal DNAm is involved in the development of Ps [10,18,19]. The expression of genes mapped to confirmed susceptibility loci in Ps [2] as well as the profile of differential gene expression levels [20] have been investigated before. Recently, RNA sequencing (RNA-seq) has been proven to be a new alternative to microarrays, and four RNA-seq studies on Ps have been published so far [21–24]. However, the relationship between whole-genome DNAm and gene expression levels in Ps has not been studied yet. In our previous studies, we conducted a three-stage epigenome-wide association study using a combination of 262 skin samples and performed a RNA-seq analysis on 60 skin biopsies from Han Chinese individuals [24,25]. Herein, we further integrated the high-resolution methylome and transcriptome, followed by the biological function/pathway analysis, with a view to further explore the potential mechanisms driven by aberrant methylation involved in Ps.

2. Materials and methods

2.1. Study subjects

Infinium HumanMethylation 450 K BeadChip (Illumina, San Diego, CA) was used previously on 41 paired PP/PN and 73 PP/62 NN skin samples from a Han Chinese population. RNA-seq analysis was performed on 60 biopsies (20 paired PP/PN and 20 NN) and sequenced on the Illumina HiSeq 2500 Sequencer (Illumina, Inc., San Diego, CA). The features of samples, details of methylation and transcriptome sequencing were previously described elsewhere [24,25]. In this study, we paid main attention to the correlation between methylome and transcriptome datasets and performed further integrative analyses.

2.2. Identifying correlation between gene expression and DNA methylation

The ChAMP package was used for the analysis of methylation data. Gene expression levels were compared in PP vs PN (by using one-sample *t*-tests on the differences between paired PP and PN samples) or PP vs NN (by using two-sample *t*-tests) [24]. The false discovery rate (FDR) adjusted p-value was calculated to test the null hypothesis of zero correlation. Due to the limited sample size, we used less strict criteria and set the threshold for the FDR adjusted p-value < 0.01 in the analysis of DEGs. And DMSs was defined as follows: 1) FDR adjusted p-value < 0.01; 2) the CpG site with the minimum p-value in a gene; 3) |deltaBeta| ≥ 0.01, and deltaBeta is measured as median beta difference between PP and NN or between PP and PN. The correlation between DNAm and expression for each gene was assessed by Pearson's correlation test with the FDR adjusted p-value < 0.05. All analyses were performed with the R statistical package (https://www.r-project.org/).

2.3. Gene Ontology and KEGG pathway analysis

We used an R package, clusterProfiler to analyze the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways for the adjusted p-value < 0.05. The clusterProfiler package offers a gene classification method (groupGO) to classify genes based on the projection at a specific level of the GO corpus, and provides functions (enrichGO and enrichKEGG) based on hyper-geometric distribution to calculate enrichment test for GO terms and KEGG pathways. Furthermore, clusterProfiler supplies a function (compareCluster) to automatically calculate enriched functional categories of each gene clusters and provides methods for visualization. It automates the process of biological-term classification and the enrichment analysis of gene clusters. The function calls of groupGO, enrichGO, enrichKEGG are consistent and all the output can be visualized by bar plot, enrichment map and category-gene-network plot. The analysis module and visualization module were combined into a reusable workflow. This package is released under Artistic-2.0 License within Bioconductor project. The source code and vignette are freely available at http://bioconductor.org/packages/release/bioc/html/clusterProfiler.html

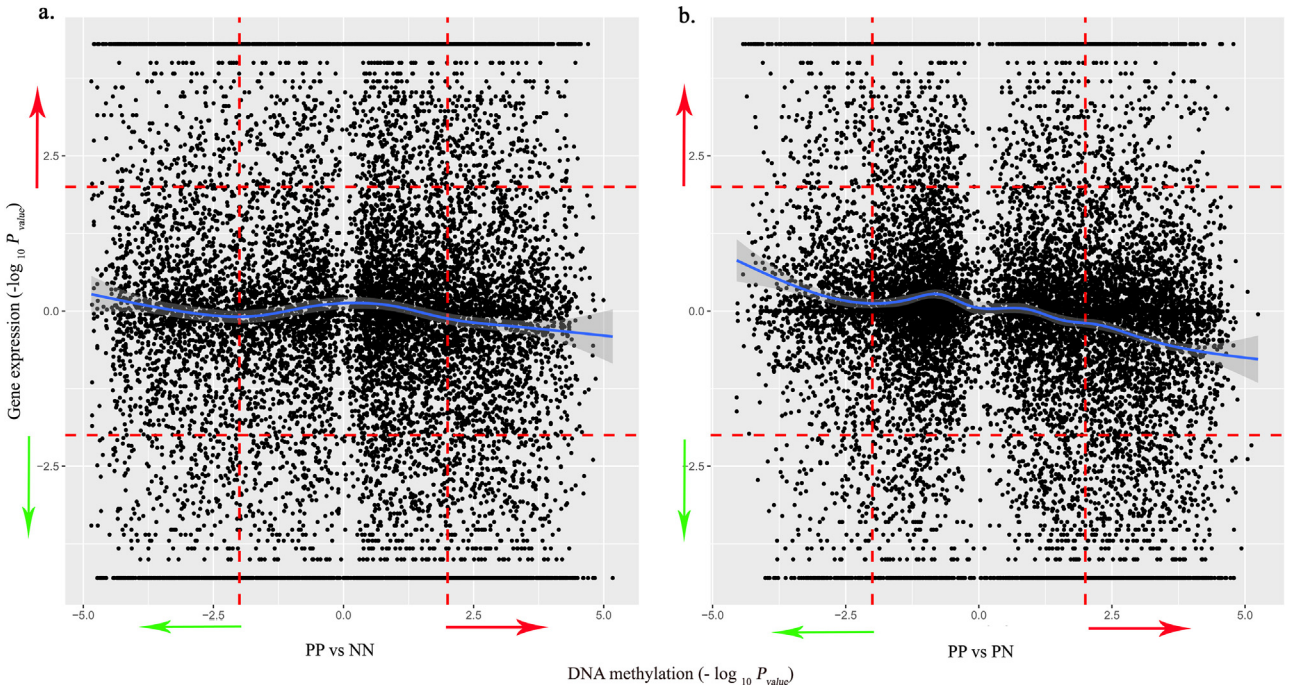

Fig. 1. Expression of genes correlated with CpG site methylation in skin tissue. Comparison of the RNA-seq-derived gene expression levels (FPKM) and methylation of target genes across the PP vs NN (a) and PP vs PN (b). x-axis: DNA methylation levels; y-axis: relative gene expression levels; black line: linear regression.

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