Gene expression profiles of peripheral blood mononuclear cells from patients with atopic dermatitis stimulated with *Malassezia globosa*

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To the Editor: *Malassezia* located in the skin of patients with atopic dermatitis (AD) might be related to its pathogenesis, consisting of *Malassezia globosa* and *Malassezia restricta* as the dominant species.[1] However, the specific molecular mechanisms of *Malassezia* on host immune cells from AD patients are still unclear. In our study, the Affymetrix Human Prime View array was used to detect the effect of *M. globosa* on the gene expression of peripheral blood mononuclear cells (PBMCs) from AD patients, which was favorable to further understand the role of *Malassezia* colonization in the pathogenesis of AD.

The research participants comprised three male patients with severe AD, whose eczema area and severity index (EASI) scores were greater than 21,[2] and three healthy male controls. The three AD patients were 8, 10, and 12 years old, and EASI scores were 22.7, 24.2, and 55.2, respectively. The three healthy controls were aged 8, 9, and 13, respectively. Exclusion criteria included the following: systematic use of glucocorticoid, immunosuppressive agents, and antihistamines within 2 weeks; local use of drugs within 2 weeks; and participants with any other skin diseases, cancer, or severe illness. All procedures were approved by the Ethics Committee of Clinical Medical College and The First Affiliated Hospital of Chengdu Medical College (No. 2018CYFYHEC-BA-28) and were conducted in accordance with the *Declaration of Helsinki* and subsequent revision. Participants signed an informed written consent form.

About 5 mL of venous blood from each participant was slowly added along the sloping tube wall into the centrifuge tube with 5 mL lymphocyte separation solution (Hao Yang, Tianjin, China), keeping the dimeric interface clear. The solution was centrifuged at 2000 r/min for 25 min, and the upper layer was carefully aspirated. Then PBMCs layer was drawn into a new test tube, and the cells were washed twice with cell washing liquid (Hao Yang). Next, the cells were suspended in the PBMCs culture solution containing 80% Roswell Park Memorial Institute (RPMI)-1640 culture medium (Gibco, Carlsbad, CA, USA), 20% fetal bovine serum (Gibco), 100 U/mL streptomycin (Hyclone, Los Angeles, CA, USA), and 100 μg/mL penicillin (Hyclone), where the density was adjusted to 1×10^6/mL. The upper cell suspension was moved to a six-well plate (6 mL per well) and cultured at 37°C in 5% CO_2 and saturated humidity for 24 h. Afterward, the cell suspension in each well was equally divided into two wells. One was added with 0.6 mL *M. globosa* (CBS7874) suspension (washed twice with the PBMCs culture solution and the density was 1×10^6/mL) and the other was added with an equal volume of PBMCs culture solution. After cultured at 37°C in 5% CO_2 and saturated humidity for 48 h, the cell suspension was centrifuged at 1000 r/min for 10 min. Finally, 1 mL Trizol lysate (Ambion, Austin, TX, USA) was added to the cell precipitation and then frozen at −80°C. The supernatant was retained for later verification by enzyme-linked immune sorbent assay (ELISA).

The samples were delivered to Shanghai OE Biotech Co., Ltd. for Prime View™ Human Gene Expression Array (Affymetrix, Santa Clara, CA, USA) assay. Differentially expressed genes were then identified through fold changes and *P* values were calculated with the *t*-test. The threshold set for regulated genes was a fold change ≥2 and $P < 0.05$. We submitted the raw and analyzed microarray data to the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) with the serial number GSE139247. Interleukin (IL)-4 and IL-13 levels of the cell culture supernatant were measured by the double-antibody sandwich method with the human IL-4 ELISA kit (Abcam, Cambridge, UK) and the human IL-13 ELISA kit (Abcam), respectively.

After stimulation with *M. globosa*, the gene expression profiles revealed a total of 356 differentially expressed (188 up-regulated and 168 down-regulated) genes in

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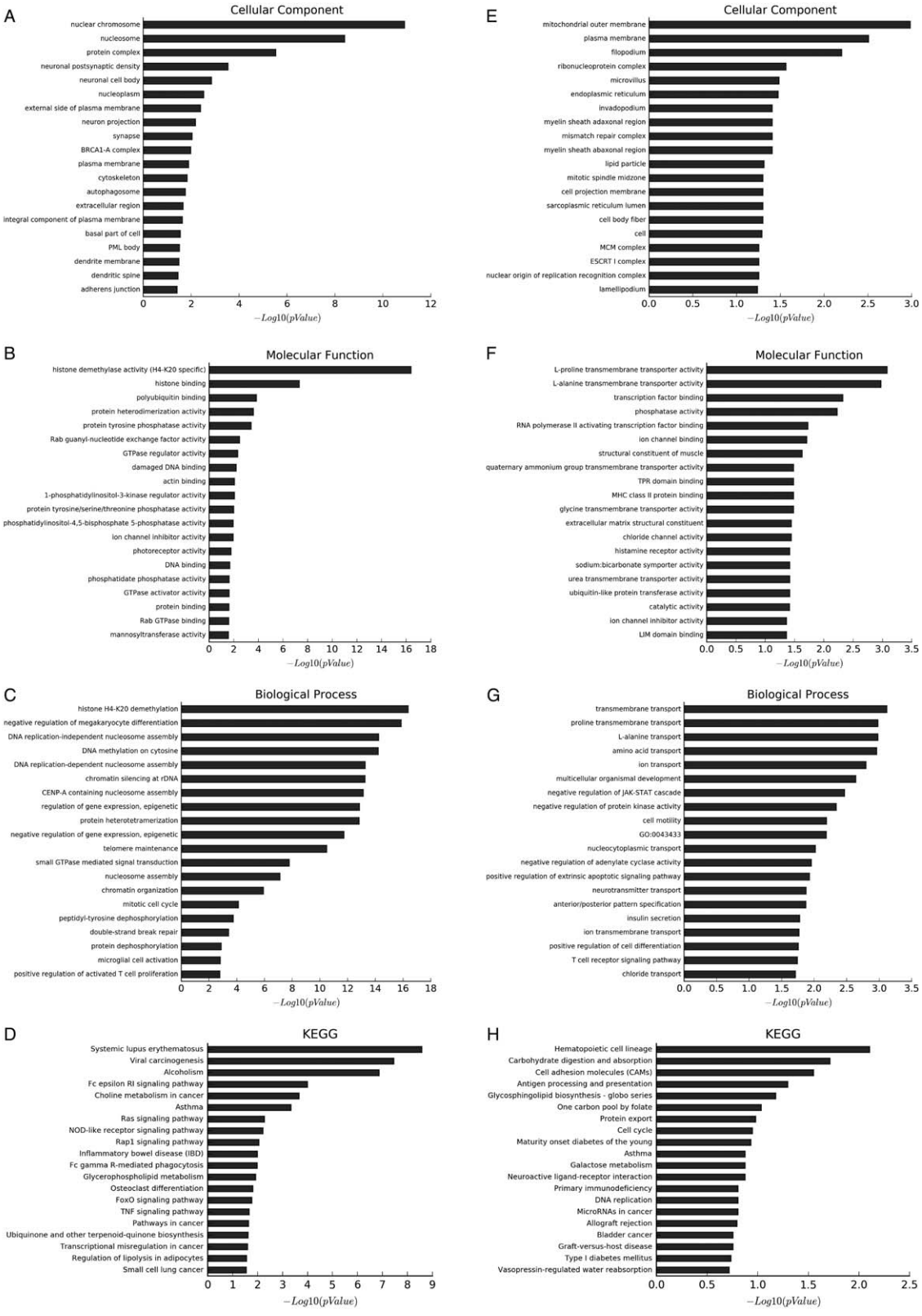

Figure 1: GO terms and signaling pathways significantly enriched in PBMCs stimulated with *Malassezia globosa*. (A–D) The top 20 significantly enriched cell component terms, molecular function terms, biological process terms, and signaling pathways in AD patients. (E) The first 15 cell component terms were significantly enriched in healthy controls. (F and G) The top 20 significantly enriched molecular function terms and biological process terms in healthy controls. (H) The first three signaling pathways were significantly enriched in healthy controls. AD: Atopic dermatitis; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; PBMCs: Peripheral blood mononuclear cells.

PBMCs from AD patients and a total of 110 differentially expressed (60 up-regulated and 50 down-regulated) genes from healthy controls. Among them, only ten identical genes (eight up-regulated and two down-regulated) were detected in both AD patients and healthy controls [Supplementary Figure 1, http://links.lww.com/CM9/A556 and Supplementary Table 1, http://links.lww.com/CM9/A556]. Cluster analysis revealed that the changing trends of each pair of samples were consistent in both AD patients and healthy controls [Supplementary Figure 2, http://links.lww.com/CM9/A556].

Gene ontology (GO) and pathway analysis revealed that 23 and 15 cell component terms, 31 and 24 molecular function terms, 139 and 101 biological process terms, and 31 and three signaling pathways were significantly enriched in AD patients and healthy controls, respectively [Figure 1]. Five cell component terms, two molecular function terms, five biological process terms, and none of the pathways were identical between AD patients and healthy controls. In AD patients, it was worth focusing on the signaling pathways activated by allergen or pathogens, including the IgG Fc receptor (FcγR), high-affinity IgE Fc receptor (FcεRI), rat sarcoma (Ras), and NOD-like receptor (NLR) signaling pathways [Supplementary Figures 3, http://links.lww.com/CM9/A556 and 4, http://links.lww.com/CM9/A556]. Comparison of IL-4 and IL-13 concentrations between the stimulated group and the non-stimulated group of AD patients and healthy controls showed the same changing trend of gene expression by ELISA [Supplementary Table 2, http://links.lww.com/CM9/A556]. This finding suggested that our microarray assay results were probably accurate.

In our study, GO analysis showed that the significantly enriched terms of AD patients were mainly involved in DNA methylation and histone modification. DNA methyltransferase (DNMT) and thymine DNA glycosylase (TDG) are key enzymes of DNA methylation and DNA demethylation, respectively.[3] Previous studies showed that *FCER1G* (FcεRIγ subunit gene) promoter demethylation contributed to FcεRI overexpression related to the pathogenesis of AD, and the covalent modification of histone might lead to the activation of FcγR. Our study showed that DNMT3L was down-regulated by 2.44-fold and TDG was up-regulated by 2.46-fold in PBMCs from AD patients after stimulation with *M. globosa*. These findings indicated that *M. globosa* might participate in the activation of FcεRI and FcγR by inhibiting DNA methylation and activating histone demethylation, respectively.

FcγRIIIA, which is mainly distributed on the surface of monocytes/macrophages, may activate the phosphatidylinositol-3-kinase (PI3K)/protein kinase B signaling pathway, then trigger actin cytoskeleton regulation, cell membrane remodeling, pseudopodia stretching, and so on, eventually ingest the particles by endocytosis, forming phagosomes or endosomes. These bodies are degraded to form immune complexes by the enzymes of lysosomes and released to the outside of cells.[4] Binding of allergen/IgE complexes to FcεRI on mast cells of patients with AD leads to activation of mast cells and synthesis of IL-4, IL-5, and IL-13. The expression of these ILs is closely related to the PI3K pathway.[5] Combined with our results of the FcγR and FcεRI pathways, we speculate that *M. globosa* may be degraded to form immune complexes by monocytes/macrophages endocytosis. This process is initiated through the FcγR pathway, and the immune complexes then up-regulate the expression of IL-4, IL-5, and IL-13 through the FcεRI pathway, leading to the onset or aggravation of AD.

Besides the FcγR and FcεRI pathways, our study revealed that the Ras and NLR pathways were induced by stimulation with allergens or pathogens. Previous studies showed that the Ras pathway was involved in monocytes/macrophages endocytosis, and the NLR pathway participated in the differentiation of T cells and the immune response of lymphocytes mediated by specific antigens. The Ras and NLR pathways were associated with the pathogenesis of AD. Therefore, combined with our results of pathway analysis, we speculate that antigens of *M. globosa* may activate the Ras and NLR pathways by binding to the corresponding receptors on lymphocytes, thereby producing the relevant biological effects.

Our study showed few identical differentially expressed genes, GO terms, and signaling pathways between AD patients and healthy controls. This suggests that under different genetic backgrounds and immunological states, the effects of *M. globosa* on PBMCs from AD patients and healthy controls vary greatly.

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Conflicts of interest

None.

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