Early transcriptomic profiling variation caused by cluster allergen immunotherapy

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Allergen immunotherapy is executed by the repeated administration of specific allergens to patients with allergic disorders to protect against allergic reactions. It is the only disease-modifying therapy for allergic rhinitis and asthma, and many clinical trials and meta-analyses have demonstrated its beneficial effects.^[1] Immunologic changes associated with immunotherapy are complicated, and the underlying mechanism needs further elucidation. Cluster immunotherapy is an improved immunotherapy with an accelerated build-up schedule that achieves symptom improvement earlier than the conventional way.^[1] This study aimed to investigate the early immune changes caused by cluster immunotherapy by employing transcriptomic profiling analysis and identifying candidate biomarkers for early evaluation of immunotherapy. Approval of the Peking Union Medical College Hospital Ethics Committee had been obtained (No. ZS-1917), and all the subjects had given written informed consent.

Two patients with allergic rhinitis received cluster immunotherapy. Patient 1 was a 55-year-old woman who presented with seasonal sneezing, rhinorrhea, and itchy eyes every August and September for 2 years. Laboratory tests showed a positive intradermal test for *Artemisia* pollen (Allergen extract preparation room, Peking Union Medical College Hospital, Beijing, China) and a serum specific immunoglobulin E (sIgE) against *Artemisia* pollen of 5.61 kUA/L (ImmunoCAP, Thermo Fisher Scientific, Waltham, MA, USA). She was diagnosed with seasonal allergic rhinitis caused by *Artemisia* pollen and was administered cluster subcutaneous allergen immunotherapy with *Artemisia* pollen allergen extract. The specific protocol of the build-up stage of cluster

immunotherapy is shown in Supplementary Table 1, <http://links.lww.com/CM9/A227>. During the build-up stage, the systemic allergic reaction occurred only once after 0.25 mL of maintenance pollen extract was injected. The patient complained of mild nasal obstruction and an itchy nose but showed no wheezing. After anti-histamine treatment, her symptoms were alleviated soon and she reached the maintenance stage successfully.

Patient 2 was a 23-year-old woman with perennial rhinorrhea and sneezing for 4 years. Her intra-dermal test to dust mites was positive, and her serum sIgE level to dust mites was 1.47 kUA/L. She was diagnosed with allergic rhinitis caused by dust mites and received cluster immunotherapy with dust mite allergen extract. The protocol of build-up immunotherapy was similar to that for Patient 1. No large local reaction or systemic allergic reaction was observed in the build-up stage. She entered the maintenance stage as planned.

After 4 months of allergen immunotherapy, the rhinitis symptoms were alleviated in both patients, as evidenced by their improved quality of life evaluated by the rhinoconjunctivitis quality of life questionnaire (RQLQ) score. The RQLQ score of Patient 1 during the *Artemisia* pollen season decreased from 2.50 to 0.86; the score of Patient 2 decreased from 1.14 to 0.46.

Peripheral blood was obtained from both patients at three-time points: 1 day before cluster immunotherapy (Ctrl), 1 month after the start of immunotherapy (1M), and 4 months after the beginning of immunotherapy (4M). Transcriptomic profiling was carried out on peripheral whole blood samples. The gene expression levels at two-

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Figure 1: Genes with more than two-fold increased expression after cluster immunotherapy. (A) Heatmap of normalized expression level (Z score) of genes with more than two-fold increased expression. The gradient color from blue to red represents low to high gene expression level. (B) Biological pathways enriched by genes with more than two-fold up-regulation after cluster immunotherapy. The Benjamini-Hochberg method was employed for the calculation of the FDR value. Gene ratio was calculated by the number of up-regulated genes in a certain term divided by total number of genes of that term. FDR: False discovery rate; Ctrl: 1 day before cluster immunotherapy; 1M: 1 month after the start of immunotherapy; 4M: 4 months after the beginning of immunotherapy.

time points after immunotherapy were compared with those before treatment, and genes significantly up- or down-regulated after immunotherapy were identified [Figure 1A and Supplementary Figure 1A, <http://links.lww.com/CM9/A226>]. Analysis of differentially expressed genes showed that 268 and 520 genes were up-regulated and down-regulated, respectively, more than two-fold after 1 month of allergen immunotherapy in both patients. After 4 months of immunotherapy, 589 and 415 genes were up-regulated and down-regulated, respectively, more than two-fold in both patients. Combining these two datasets resulted in 704 up-regulated genes and 856 down-regulated genes after allergen immunotherapy treatment. Next, we analyzed these 704 up-regulated and 856 down-regulated genes for the enrichment of Reactome pathways using Metascape. The false discovery rate was calculated using the Benjamini-Hochberg method. As shown in Figure 1B, for the up-regulated genes, the top five most enriched pathways were neutrophil degranulation, citric acid (TCA) cycle and respiratory electron transport, signaling by interleukins, platelet degranulation, and interferon signaling. For the down-regulated genes, the top five most enriched pathways were cilium assembly, SUMOylation of ubiquitination proteins, DNA repair, synthesis of plasma membrane intrinsic proteins at the plasma membrane, and low-density lipoprotein clearance [Supplementary Figure 1B, <http://links.lww.com/CM9/A226>]. There were 153 genes up-regulated and 79 genes down-regulated at both time points (1 and 4M). The most enriched pathway for these up-regulated genes was still neutrophil degranulation.

To further investigate the critical factors involved in immunotherapy, we strengthened the screening criteria.

Genes with more than ten-fold up-regulated expression in both patients after 1 month included *LGALS1*, *PSTPIP1*, *GZMM*, *MOB2*, *UBALD2*, *SEPW1*, and *ROMO1*, and genes with more than 50-fold up-regulated expression in both patients after 4 months included *WAS*, *S100A8*, *IFITM3*, *ISG15*, *CMTM2*, *UBC*, and *LGALS1*. Among them, *LGALS1* was the only gene with markedly increased expression at both time points.

Our study adopted cluster allergen immunotherapy to treat two patients with allergic rhinitis effectively and confirmed that gene expression patterns could be changed significantly as early as 1 month after the beginning of immunotherapy. We also identified overt changes in gene expression and immune-related pathways following immunotherapy by differential gene expression and pathway analysis.

The relationship between neutrophils and atopic disorders has been reported. Neutrophils of atopic patients produced more superoxide in the basal condition or after stimulation with formyl-methionyl-leucyl-phenylalanine than cells from control subjects.^[2] Monteseirín *et al*^[2] found a significant reverse correlation between the level of myeloperoxidase released by neutrophils and pulmonary ventilation function in asthma patients. After the patients received allergen immunotherapy, the secretion of myeloperoxidase from neutrophils was significantly reduced, indicating that immunotherapy actively modified neutrophil degranulation. In our study, transcriptomic data analysis showed that the pathway of neutrophil degranulation had the highest degree of enrichment of those genes with more than two-fold up-regulated expression. This finding was consistent with that of the literature report

mentioned above. When the effect of allergen immunotherapy on the immune system is discussed, attention is usually focused on the induction of antigen-specific regulatory T cells (Treg) and immune deviation from Th2 to Th1 responses.^[3] Variations in the neutrophil reaction are ignored. Our study suggests that variations in the neutrophil degranulation pathway might play a role in the mechanism of immunotherapy.

Our study observed that *LGALS1* is the only gene with markedly increased expression in both patients at both testing time points after immunotherapy. The protein encoded by *LGALS1* is Galectin-1, which regulates apoptosis, cell proliferation, and cell differentiation. Published data have already shown that Galectin-1 could suppress mast cell transmigration and degranulation. Yang *et al*^[4] reported that the co-administration of Galectin-1 and allergen immunotherapy significantly inhibited allergic responses in the mouse intestine by suppressing mast cell activation and facilitating Treg development. Our study found that the expression of *LGALS1* was elevated 14 folds as early as 1 month after the beginning of immunotherapy, and this up-regulated trend was sustained and then was amplified several months later, suggesting that the increase in endogenous Galectin-1 might be involved in the central immune mechanism of allergen immunotherapy.

Our study identified differentially expressed genes following allergen immunotherapy through transcriptomic profiling analysis. However, more prospective clinical studies with a large sample size are needed to identify candidate biomarkers with clinical significance among

these genes. The relationships between the differentially expressed proteins and efficacy of immunotherapy at different time points of therapy should be evaluated to investigate their applications in the assessment of efficacy or prediction of early responders.

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Conflicts of interest

None.

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