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Quantitative Proteomic Analysis of Plasma Extracellular Vesicle from Mite-Induced Allergic Rhinitis Patients

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Keywords:

Allergic rhinitis; Mite; Extracellular vesicle; Proteomic analysis

1. Abstract

1.1 Objective: Allergic rhinitis (AR) is a serious and most common chronic allergic disease. Numerous studies reported that extracellular vesicle (EV) is involved in various physiological and pathological processes.

1.2 Method: In this study, we extracted plasma EV from mite-induced AR patients and healthy controls (HC) for proteomic analysis to screen potential targets for AR diagnosis and treatment. Plasma EV of mite-induced AR patients and HC was separated using the ultracentrifugation method. Quantitative proteomic analysis of plasma EV was performed using LC-MS/MS. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses for differentially expression proteins (DEPs) were performed.

1.3 Results: Isolated plasma EV sizes have no significant difference between AR and HC, both ranging from 30-150 nm, while plasma EV concentrations were higher in AR patients than that in HC. Proteomic analysis yielded 89 DEPs from AR compared with HC. GO analysis showed that 89 DEPs mediated AR may be associated with immune regulation. KEGG enrichment analysis showed that 89 DEPs regulated the pathogenic process of AR through multiple pathways, including complement and coagulation cascades, estrogen and adipocytokine signaling pathway.

1.4 Conclusion: We used proteomic analysis to characterize plasma EV in mite-induced AR patients and obtained potential diagnostic targets for AR.

2. Introduction

Allergic rhinitis (AR), characterized by sneezing, runny nose, or nasal congestion, is a symptomatic disease of the nose induced by IgE-mediated inflammation following exposure to allergens [1]. Reportedly, AR affects the school, work, and life of approximately 400 million people worldwide, and the prevalence is increasing year by year [2]. A variety of factors are important triggers of AR, including house dust mites, pollen, pets, and mold. Among them, house dust mites are the most common allergen in perennial AR in southern regions of China [3, 4]. Currently, the treatment strategies for AR mainly include pharmacological symptomatic treatment and specific immunotherapy, however, their therapeutic effects are limited [5]. Therefore, further studies are needed to reveal the pathogenesis of mite-induced AR to provide new therapeutic strategies for AR.

Extracellular vesicles (EVs), which are produced by all cell types as nanoscale lipid bilayer vesicles, are secreted from a variety of body fluids, including saliva, plasma, and urine [6]. The EVs family includes exosomes, microvesicles, apoptotic bodies, and other types of EVs which have not been fully characterized [7]. EVs play a key role in intercellular communication by transferring its bioactive components, including proteins, RNA, DNA, and lipids [8]. Study has shown that EVs are involved in pathogenic processes of diseases, including AR [9]. For example, human mesenchymal stem cell-derived EV inhibits Th2 cell differentiation by regulating the miR-146a-5p/SERPINB2 pathway [10]. Fang et al. found that plasma EV from AR patients exhibits antigen-presenting features promoting Th2 cell differentiation and IgE secretion [11]. Li et al. demonstrated mesenchymal stem cell-derived EVs containing Linc00632 inhibits GATA-3 expression to suppress Th2 cell differentiation, thereby affecting AR progression [12]. Zhu et al. conducted that long-noncoding RNA GAS5, isolated from nasal epithelial cells of patients with AR, regulates Th1/Th2 differentiation partly via inhibiting T-bet and EZH2 expression [13]. Therefore, the above studies suggest that EVs can be a promising tool in the diagnosis, treatment, and prognosis of AR.

To our knowledge, the disease mechanism between plasma EV and mites-induced AR remains unclear. Therefore, in this study, we used proteomics to investigate the differences in plasma protein composition between AR patients and healthy controls (HC), followed by systematic data analysis to explore the pathological mechanism of mites-induced AR.

3. Materials and Methods

3.1. Subjects

A total of 20 patients with AR (male/female 50%/50%, age 25.05 ± 8.06 years) and 20 HC (male/female 45%/55%, age 29.10 ± 4.62 years) were included in this study. This study was approved by Institutional Review Board of Xiamen Chang Gung hospital (XMCGIRB2020036) and informed consents were provided from all subjects. The study was conducted in accordance with the Declaration of Helsinki. According to the ARIA guidelines [14], the inclusion criteria for AR patients were as follows: patients with a clinical diagnosis of AR; all specific IgE (sIgE) testing for house dust mites; skin testing for various inhaled allergens. Only patients with mite sensitization were included, and AR patients with seasonal onset tendency induced by pollen dust were excluded.

3.2. Plasma Extracellular Vesicle (EV) Extraction

Plasma EV was extracted using differential ultracentrifugation. Briefly, blood samples were centrifuged at 2,000× g for 30 min and 10,000× g for 45 min at 4°C to remove precipitates and obtain supernatants. The supernatants were centrifuged at 100,000× g for 1 h in Optima L-100XP ultracentrifuge (Beckman, IN, USA). EV pellets were resuspended with 10 mL cold phosphate buffered saline (PBS) and ultracentrifuged again at the same conditions. Finally, EV pellets were resuspended with 100 μL PBS and stored at -80°C for subsequent study.

3.3. Transmission Electron Microscopy

The 10 μL EV samples were loaded onto the copper mesh and incubated for 1 min. Then, samples were stained with 2% uranyl acetate for 1 min. After removing excess water with filter paper, EV images were captured using a HT-7700 TEM (Hitachi, Japan).

3.4. Nano-Flow Cytometry

The size and concentration of EVs were measured using N30E nano-analyzer (NanoFCM, Xiamen, China) followed the reported method [15]. EV samples were incubated with fluorescein-conjugated CD9 (1 μL, BioLegend, UK, #209306) and CD81 (1 μL, Invitrogen, CA, USA, MA1-81261) antibodies at 37°C for 30 min and spinning down at 110,000× g at 4°C for 1 h. Data were analyzed by FlowJo software.

3.5. Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

Briefly, isolated EVs were lysed with 1% Rapigest (100 μL/sample), boiled with dithiothreitol (20 mM), alkylated with iodoacetamide (50 mM) for 30 min, and centrifugated at 20,000× g for 10 min. The supernatants were loaded onto spin filters (Millipore, Germany), centrifuged at 13,500× g, and then washed with 8 M Urea for three times, followed by with ammonium bicarbonate twice. Subsequently, trypsin was added to the proteins at a dilution of 1:50. After trypsin digestion overnight at 37°C, the samples were desalted using stage tips.

Digested peptides were analyzed for proteomics using an EASY-nLC 1200UHPLC system (C18 column, 75 μm × 25 cm, 2 μM) with Orbitrap Fusion Lumos. The mobile phase consisted of 2% acetonitrile and 0.1% formic acid. A gradient of 9% to 28% acetonitrile was passed through the sample over 1.5 h at a flow rate of

300 nL/min for 20 min to increase the concentration of acetonitrile to 80%. MS analysis was performed at 2.2 kV spray voltage and 300 °C ion transport capillary. MS raw data were analyzed with Proteome Discoverer (version 2.2), and the processed data were compared with the SwissProt Human Proteome Database.

3.6 Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Enrichment Analysis

GO and KEGG enrichment were analyzed on Database for Annotation, Visualization, and Integrated Discovery (DAVID 6.7) online tool (http://david.abcc.ncifcrf.gov/) with screening threshold set as P < 0.05.

3.7 Statistical Analysis

Differences between two groups were analyzed by Student's t-test. P < 0.05 set as significantly differences.

4. Results

4.1 Clinical Characteristics of Subjects

Information of clinical characteristics are shown in Table 1. Eosinophils and serum IgE levels are considered as preliminary diagnostic criterion of AR [16]. The amounts of eosinophils were significantly higher in the AR group than that in the HC group (P = 0.002). Moreover, the level of total IgE was significantly reduced in the AR group compared with in the HC group (P < 0.001). House dust mites are the most prevalent allergens of patients with AR, including Dermatophagoides pteronyssinus (d1), Dermatophagoides farina (d2), Blattella germanica (i6) [17]. The levels of sIgE d1 (P < 0.001), sIgE d2 (P < 0.001), and sIgE i6 (P = 0.001) were increased, in contrast to total IgE level.

4.2. Characterization of plasma EV

To characterize EV in the plasma of participants, we performed an identification analysis by TEM. Results showed that EVs were observed in participants' plasma with diameters in the range of 30-150 nm (Figure 1a–b) and that the mean size of EVs was no significant difference between AR and HC groups (Figure 1c). However, the concentration of EVs were significantly increased in the AR compared to the HC group (P < 0.05, Figure 1d). Additionally, compared with IgG (a blank control), EV protein markers CD9 and CD81 were detected from plasma samples (Figure 1e).

4.3 Quantitative Proteomic Analysis of Plasma EV

Quantitative proteomic analysis was used to further explore the differences of EV in the plasma of participants with or without AR. Principal component analysis (PCA) showed no significant difference between AR and HC groups (Figure 2a). Interestingly, partial least squares discriminant analysis (PLS-DA) results indicated a clear grouping boundary between AR and HC (Figure 2b). Simultaneously, a total of 664 proteins were identified from the EV of AR and HC, of which 44 up-regulation and 45 down-regulation in AR compared with HC (Figure 2c-d). The information of up- or down-regulated proteins are listed in Table 2 and 3.

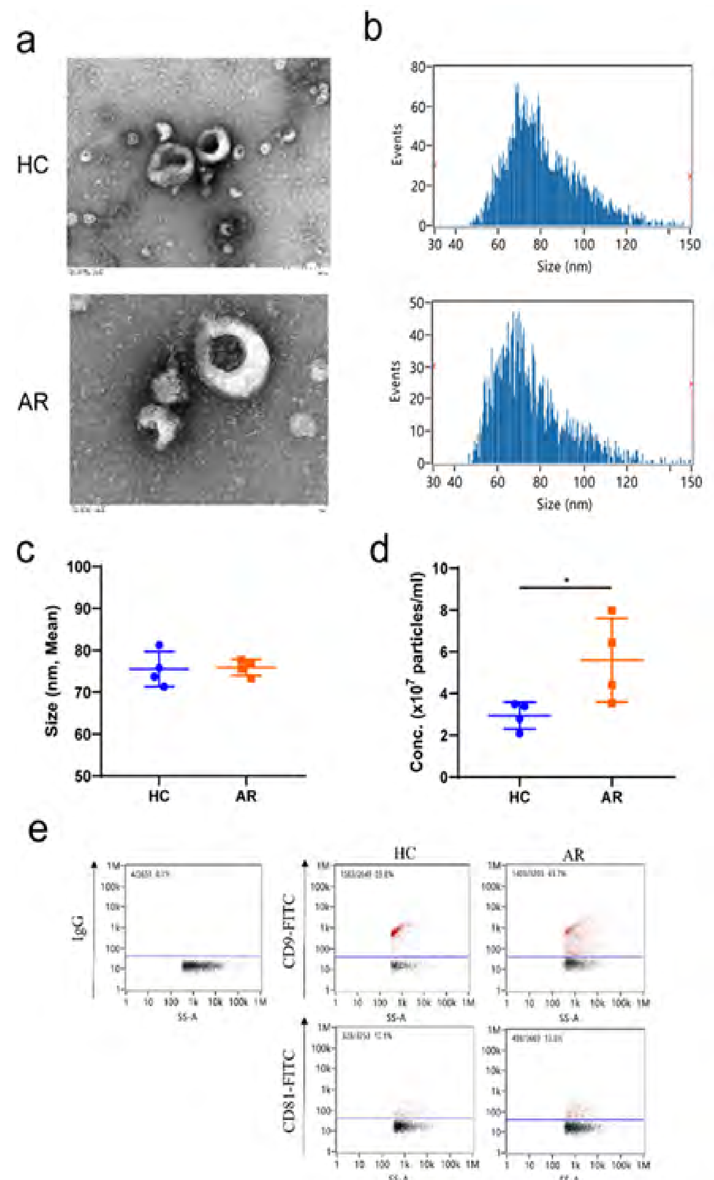

Figure 1: Characterization of plasma extracellular vesicle (EV). a, Isolated EVs were observed using transmission electron microscopy, scale bar = 200 nm. b, Size information of plasma EVs were measured using Nano-flow cytometry. c–d, Mean size and concentration of EVs were from healthy controls (HC) and patients with allergic rhinitis (AR). e, Expression of EV markers CD9 and CD81 were examined using flow cytometry.

Table 1: Clinical characterization of study subjects.

Variable	HC (n=20)	AR (n=20)	P value
Gender (male %)	45%	50%	NS
Age (Year)	29.10 ± 4.62	25.05 ± 8.06	NS
Eosinophils (%)	1.98 ± 0.99	4.21 ± 2.88	0.002
Total IgE (IU/mL)	23.30 ± 22.66	301.89 ± 335.35	<0.001
sIgE dI (KUA/L)	0.03 ± 0.03	25.1 ± 22.50	<0.001
sIgE d2 (KUA/L)	0.02 ± 0.03	26.63 ± 23.97	<0.001
sIgE i6 (KUA/L)	0.07 ± 0.07	0.84 ± 1.02	0.001

Table 2: Up-regulated proteins in plasma extracellular vesicle from AR patients

Number	Protein name	Fold Change	P-value
1	Sodium/potassium-transporting ATPase subunit beta-3	5.572	1.16E-02
2	Ficolin-3	3.098	1.95E-03
3	Filaggrin-2	2.667	4.04E-03
4	Bleomycin hydrolase	2.506	3.88E-02
5	C4b-binding protein alpha chain	2.325	8.83E-05
6	Thymidine phosphorylase	2.268	2.31E-02
7	Solute carrier family 2, facilitated glucose transporter member 1	2.201	1.80E-02
8	RAS protein activator like-3	2.139	9.94E-03
9	Junctional adhesion molecule C	2.115	6.53E-04
10	Immunoglobulin lambda-like polypeptide 1	1.999	3.55E-02
11	Choline transporter-like protein 1	1.961	8.55E-03
12	Keratin, type I cytoskeletal 14	1.918	1.37E-03
13	Inositol-3-phosphate synthase 1	1.909	4.69E-03
14	Leukocyte surface antigen CD47	1.909	3.47E-02
15	Methylcrotonoyl-CoA carboxylase subunit alpha, mitochondrial	1.903	1.18E-02
16	Keratin, type I cytoskeletal 10	1.878	8.24E-03
17	Keratin, type II cytoskeletal 2 epidermal	1.871	7.55E-03
18	Actin-related protein 10	1.845	1.73E-03
19	Eukaryotic translation initiation factor 3 subunit L	1.802	3.88E-03
20	Keratin, type II cytoskeletal 5	1.769	1.29E-02
21	Solute carrier family 2, facilitated glucose transporter member 3	1.752	2.18E-02
22	Keratin, type I cytoskeletal 9	1.719	1.05E-03
23	Dermcidin	1.715	2.64E-02
24	ATP-dependent RNA helicase A	1.713	2.17E-03
25	Ankyrin-1	1.707	4.57E-03
26	Kinesin-like protein KIF2A	1.697	1.38E-02
27	Plexin-B3	1.663	4.10E-03
28	Proteinase-activated receptor 4	1.638	1.78E-02
29	Peroxiredoxin-2	1.616	1.54E-03
30	ATP synthase subunit alpha, mitochondrial	1.615	3.18E-02
31	Coagulation factor XIII A chain	1.608	7.87E-04
32	Adenosylhomocysteinase	1.608	8.24E-03
33	Caveolae-associated protein 2	1.602	4.98E-02
34	ATP-dependent Clp protease ATP-binding subunit clpX-like, mitochondrial	1.578	2.12E-03
35	Inter-alpha-trypsin inhibitor heavy chain H3	1.577	2.19E-02
36	Tetraspanin-14	1.557	2.13E-02
37	14-3-3 protein eta	1.555	1.01E-03
38	Peroxiredoxin-1	1.551	3.00E-02
39	T-complex protein 1 subunit eta	1.545	5.92E-03
40	Fermitin family homolog 3	1.538	1.13E-02
41	Coagulation factor V	1.532	2.27E-05
42	Patatin-like phospholipase domain-containing protein 7	1.51	8.03E-04
43	Septin-9	1.503	8.11E-03
44	Nck-associated protein 1-like	1.502	3.32E-02

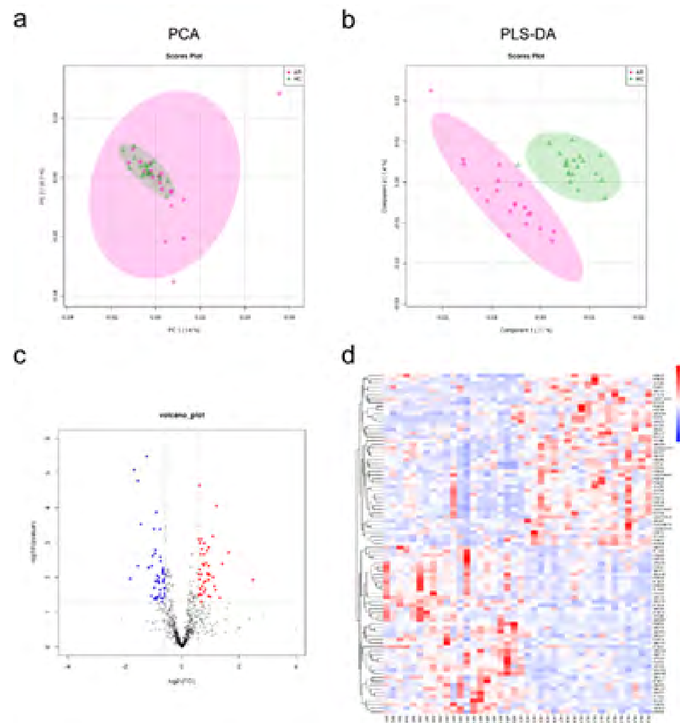

Figure 2: Quantitative proteomic analysis of plasma EV. a–b, Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) of plasma EV in HC and AR groups. c, Volcano plot of differentially expressed proteins (DEPs) in plasma EV between AR and HC. Blue represents down-regulated proteins; red represents up-regulated proteins. d, Hierarchical clustering heatmap of DEPs in plasma EV of HC and AR samples.

Table 3: Down-regulated proteins in plasma extracellular vesicle from AR patients.

Number	Protein name	Fold Change	P-value
1	Delta-aminolevulinic acid dehydratase	-3.502	1.08E-02
2	Fibronectin	-3.17	8.09E-06
3	Protein transport protein Sec31A	-2.906	1.66E-05
4	Dynamin-1	-2.897	4.66E-03
5	Ras-related protein Rab-35	-2.698	2.94E-04
6	T cell receptor alpha chain constant	-2.337	3.29E-06
7	Adiponectin	-2.239	5.23E-03
8	Immunoglobulin kappa variable 6D-2	-2.089	3.40E-02
9	CD5 antigen-like	-2.071	4.66E-03
10	Immunoglobulin heavy variable 1-69	-2.033	2.44E-03
11	Wiskott-Aldrich syndrome protein family member 2	-1.961	4.06E-04
12	von Willebrand factor	-1.955	4.15E-02
13	Dynamin-1-like protein	-1.948	2.01E-02
14	Immunoglobulin lambda variable 3-9	-1.92	2.05E-03
15	Fatty acid synthase	-1.895	1.61E-03
16	Microtubule-associated protein RP/EB family member	-1.889	1.28E-02
17	Coronin-1C	-1.867	3.75E-02
18	Immunoglobulin heavy variable 3-53	-1.845	1.34E-04
19	Purine nucleoside phosphorylase	-1.84	4.29E-02
20	Immunoglobulin lambda variable 2-18	-1.813	8.85E-03
21	Complement C1r subcomponent	-1.787	3.61E-02
22	Immunoglobulin lambda variable 2-8	-1.781	1.20E-02

23	Quinone oxidoreductase	-1.751	1.33E-02
24	14-3-3 protein epsilon	-1.743	1.45E-02
25	Immunoglobulin kappa variable 1-16	-1.742	9.99E-03
26	Proteasome subunit beta type-4	-1.702	3.04E-02
27	Immunoglobulin heavy constant alpha 1	-1.7	4.10E-04
28	Exportin-1	-1.696	2.29E-02
29	Complement C1r subcomponent-like protein	-1.685	2.56E-03
30	Fibrinogen-like protein 1	-1.675	3.40E-03
31	Eukaryotic translation initiation factor 3 subunit G	-1.669	3.83E-02
32	Immunoglobulin heavy variable 3-73	-1.623	4.99E-02
33	Apolipoprotein A-II	-1.595	7.76E-03
34	Immunoglobulin heavy variable 3-7	-1.589	6.32E-03
35	Dopamine beta-hydroxylase	-1.579	4.00E-02
36	Immunoglobulin kappa variable 1-5	-1.577	6.70E-03
37	Immunoglobulin kappa variable 4-1	-1.573	5.50E-03
38	Immunoglobulin heavy variable 4-59	-1.572	1.22E-02
39	Immunoglobulin kappa variable 1D-39	-1.56	1.37E-02
40	Complement C1q subcomponent subunit A	-1.54	2.07E-02
41	Probable non-functional immunoglobulin heavy variable 3-35	-1.528	1.08E-02
42	Phosphoribosyl pyrophosphate synthase-associated protein 1	-1.524	2.80E-02
43	Immunoglobulin kappa variable 1-27	-1.518	3.37E-02
44	Collectin-10	-1.504	2.04E-02
45	Immunoglobulin kappa variable 2-28	-1.5	8.40E-03

4.4. Functional Enrichment Analysis

GO enrichment analysis was performed to illustrate the function of the differentially expressed proteins (DEPs) involved in the AR group. Biological processes (BP) terms were mainly related to vesicle-mediated transport, immune effector process, and regulation of immune system process (Figure 3a). Cellular component (CC) terms were mainly enriched in extracellular region, extracellular region part, and extracellular space (Figure 3b). Molecular function (MF) terms were focused on binding, protein binding, and antigen binding (Figure 3c).

4.5. KEGG Enrichment Analysis

Subsequently, we further analyzed the signaling pathways involved in the DEPs. KEGG enrichment analysis suggested that DEPs were primary involved in complement and coagulation cascades, estrogen signaling pathway, and adipocytokine signaling pathway (Figure 4a). To further elucidate the pathogenic mechanism of DEPs and AR in EV, we constructed protein-protein interaction (PPI) network using STRING database and Cytoscape software (version 3.8.0). The potential targets of AR treatment were described a connectivity PPI network relating to KEGG pathways, including 20 potential marker proteins (ATP1B3, FN1, VWF, C4BPA, F13A1, F5, etc.) and top 10 KEGG pathway (complement and coagulation cascades, estrogen signaling pathway, adipocytokine signaling pathway, and so on) (Figure 4b).

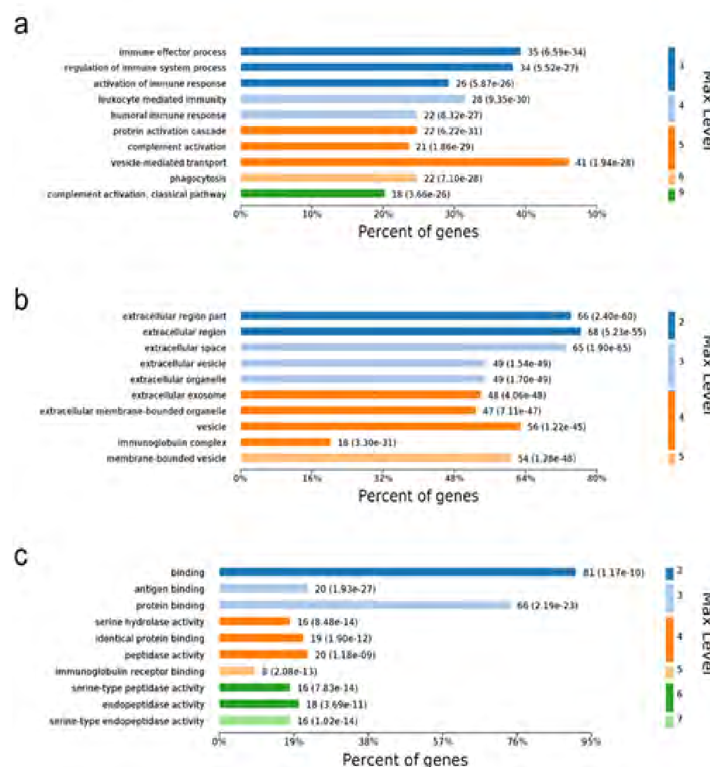

Figure 3: Gene Ontology (GO) enrichment analysis of differentially expressed proteins (DEPs). a, The top 10 biological process (BP) enriched terms. b, The top 10 cellular component (CC) enriched terms. c, The top 10 molecular function (MF) enriched terms.

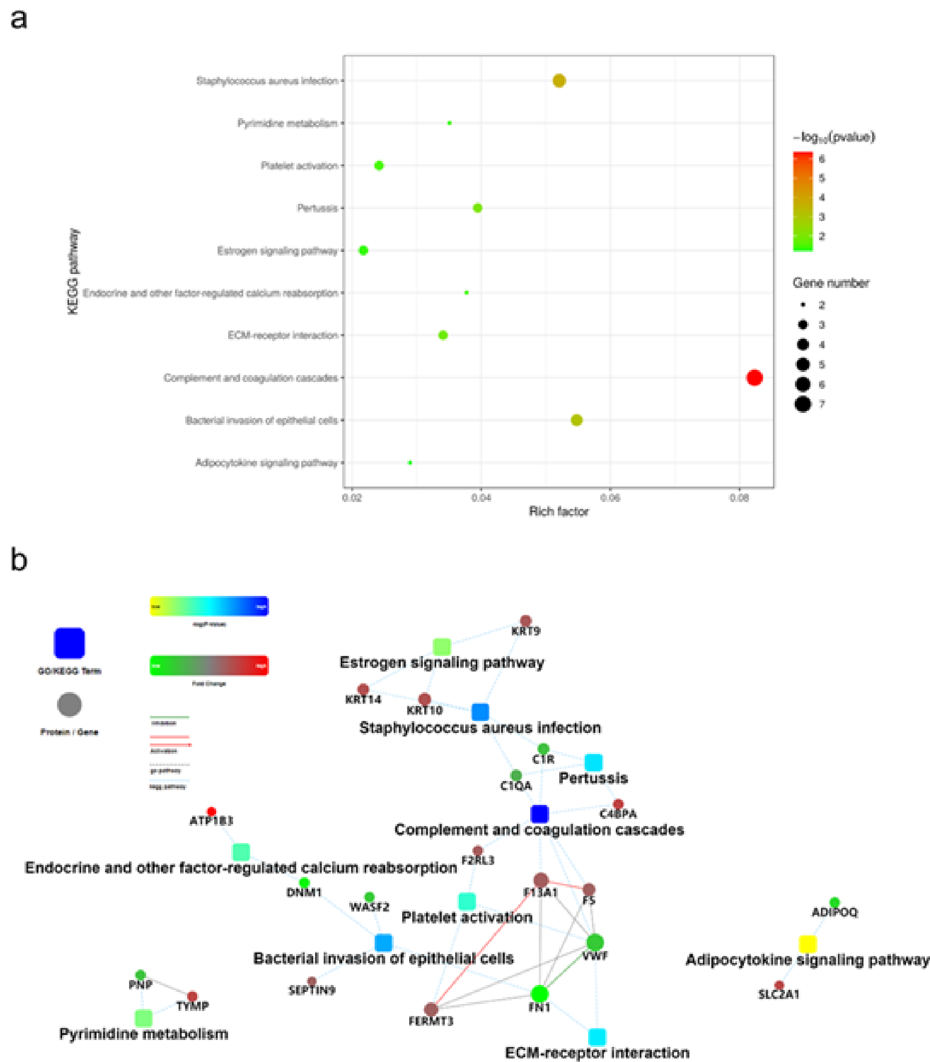

Figure 4: Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis and protein-protein interaction (PPI) network. a, The top 10 KEGG enriched terms. b,PPI network analysis of DEPs. Molecular relationships are indicated by solid lines with or without arrows (inhibition/activation relationships are indicated by green/red, respectively). Gray or blue dashed lines represent GO pathways or KEGG pathways, respectively. Squares or circles represent GO/KEGG terms or proteins, respectively.

5. Discussion

EV is involved in intercellular communication in normal or pathological conditions of the organism [18]. Also, increasing evidences have suggested the involvement of EV in the development and progression of AR [11, 19-21]. In the present study, we characterized the differences of plasma EV between mite-induced AR and HC subjects using proteomic analysis for the first time, and their DEPs were analyzed by GO, KEGG, and PPI network analysis.

Here, we have obtained 89 DEPs from the plasma-derived EV of AR and HC subjects. GO analysis indicated that DEPs as candidates of AR mainly focus on vesicle-mediated transport, immune effector process, and regulation of immune system process. Study have indicated that allergic reactions are characterized by elevated levels of allergen-specific IgE antibodies and disturbances in the balance of the Th1/Th2 immune response [22]. Jacquet stated that mite-mediated AR is caused by the dysregulation of the Th2 biased adaptive immune response [23]. Increasing studies suggest that EVs are involved in the Th2 response. For example, mesenchymal stromal cells-derived small EV regulates dendritic cell (DC) function and suppress Th2 immune responses by reducing IL-4, IL-9, and IL-13 production [21]. Moreover, plasma-derived EV may exhibit antigen presentation characteristics and promote Th2 cell differentiation [11]. From the above findings, the involvement of DEPs in AR is mainly related to the regulation of immune responses.

KEGG and PPI network analysis revealed that DEPs treat AR were involved in various pathways, including complement and coagulation cascades, estrogen signaling pathway, and adipocytokine signaling pathway. Complement system is a major effector in innate immunity and can be synthesized by fibroblasts, T and B cells, adipocytes, and endothelial cells [24]. Down-regulated C1QA and C1R are involved in the complement classical pathway. C1QA is a major component of innate immunity, and Gueguen et al. found

that C1Q is a novel marker of regulatory DC that can be expressed on antigen-presenting cells and contribute to Treg cell responses [25]. It has also been found that C1Q can act by removing infectious agents [26], indicating that C1Q-mediated AR is associated with its regulation of the immune response. Bisphenol A, an estrogen, has been reported to adversely affect AR by impairing Th2 and Treg reactions [27]. Up-regulated KRT9, KRT10, and KRT14 are involved in the complement classical pathway. One study confirmed that the basal epithelial marker KRT14 is upregulated in Canine atopic dermatitis [28]. Study has showed that obesity is associated with a variety of diseases, including allergic diseases [29]. White adipose tissue secretes adipokines (leptin, adiponectin, and resistin) to regulate immunity and inflammation [30]. Reportedly, serum levels of adiponectin were lower, while leptin levels were higher in patients with AR compared with HC [31]. Moreover, Zeng et al. found that leptin alleviates AR through regulating type II innate lymphoid cell differentiation [32]. In animal study, adiponectin suppresses the production of neuropeptides (substance P) to ameliorate AR progress [33]. Notedly, we also found down-regulated ADIPOQ (adiponectin related protein) and up-regulated SLC2A1 (obesity related protein) in plasma EV of AR compared with HC in our study.

In conclusion, our study identified a total of 89 DEPs in plasma EV of mite-induced AR patients compared to HC. GO, KEGG, and PPI network analysis of these 20 DEPs were involved in the progression of AR by modulating individual immune responses through multiple pathways. This is the first study to explore the effect of plasma EV on mite-induced AR patients using proteomic analysis. However, our study also has shortcomings, such as no further analysis of the most likely diagnostic or therapeutic targets for AR. In the future, we will further screen out potential targets and perform experimental validation.

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7. Author Contributions

Conceptualization, TYC and YRY; methodology, CHF and TJL; formal analysis, TYC; investigation, YRY; resources, MYZ, FY and YRY; data curation, TYC; writing-original draft preparation, YRY and LC; writing-review and editing WHC and CJC; supervision, CJC; project administration, CJC. All authors have read and approved the published version of the paper.

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References

1. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2) LEN and AllerGen). Allergy. 2008; 63 Suppl 86: 8-160.
2. Nur Husna SM, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, et al. Allergic Rhinitis: A Clinical and Pathophysiological Overview. Front Med (Lausanne). 2022; 9: 874114.
3. Xie S, Zhang H, Wang F, Xie Z, Jiang W, Gao K. Circulating MIF Associated With Disease Severity and Clinical Response of Sublingual Immunotherapy in House Dust Mite-Induced Allergic Rhinitis. Front Pharmacol. 2021; 12: 681724.
4. Xie S, Zhang H, Xie Z, Liu Y, Gao K, Zhang J, et al. Identification of Novel Biomarkers for Evaluating Disease Severity in House-Dust-Mite-Induced Allergic Rhinitis by Serum Metabolomics. Dis Markers. 2021; 2021: 5558458.
5. Zou W, Liu G, Zhang J. Secretome from bone marrow mesenchymal stem cells: A promising, cell-free therapy for allergic rhinitis. Med Hypotheses. 2018; 121: 124-6.
6. Hovhannisyan L, Czechowska E, Gutowska-Owsiak D. The Role of Non-Immune Cell-Derived Extracellular Vesicles in Allergy. Front Immunol. 2021; 12: 702381.
7. Poon IKH, Parkes MAF, Jiang L, Atkin-Smith GK, Tixeira R, Gregory CD, et al. Moving beyond size and phosphatidylserine exposure: evidence for a diversity of apoptotic cell-derived extracellular vesicles in vitro. J Extracell Vesicles. 2019; 8(1): 1608786.
8. O'Farrell HE, Bowman RV, Fong KM, Yang IA. Plasma Extracellular Vesicle miRNAs Can Identify Lung Cancer, Current Smoking Status, and Stable COPD. Int J Mol Sci. 2021; 22(11).
9. Boukouris S, Mathivanan S. Exosomes in bodily fluids are a highly stable resource of disease biomarkers. Proteomics Clin Appl. 2015; 9(3-4): 358-67.
10. Zhou J, Lu Y, Wu W, Feng Y. HMSC-Derived Exosome Inhibited Th2 Cell Differentiation via Regulating miR-146a-5p/SERPINB2 Pathway. J Immunol Res. 2021; 2021: 6696525.
11. Fang SB, Zhou ZR, Peng YQ, Liu XQ, He BX, Chen DH, et al. Plasma EVs Display Antigen-Presenting Characteristics in Patients With Allergic Rhinitis and Promote Differentiation of Th2 Cells. Front Immunol. 2021; 12: 710372.
12. Li W, Cai CY, Zeng JJ. Mesenchymal Stem Cell-Derived Exosome-Containing Linc00632 Regulates GATA Binding Protein-3 Expression in Allergic Rhinitis by Interacting with Enhancer of Zeste Homolog 2 to Inhibit T Helper Cell 2 Differentiation. International Archives of Allergy and Immunology. 2022; 183(2): 235-45.
13. Zhu X, Wang X, Wang Y, Zhao Y. Exosomal long non-coding RNA GAS5 suppresses Th1 differentiation and promotes Th2 differentiation via downregulating EZH2 and T-bet in allergic rhinitis. Molecular Immunology. 2020; 118: 30-9.
14. Brożek JL, Bousquet J, Agache I, Agarwal A, Bachert C, Bosnic-Anticevich S, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines-2016 revision. J Allergy Clin Immunol. 2017; 140(4):

950-958.

15. Schindler VEM, Alhamdan F, Preußer C, Hintz L, Alhamwe BA, Nist A, et al. Side-Directed Release of Differential Extracellular Vesicle-associated microRNA Profiles from Bronchial Epithelial Cells of Healthy and Asthmatic Subjects. Biomedicines. 2022; 10(3).
16. Sharma M, Khaitan T, Raman S, Jain R, Kabiraj A. Determination of Serum IgE and Eosinophils as a Diagnostic Indicator in Allergic Rhinitis. Indian J Otolaryngol Head Neck Surg. 2019; 71(Suppl 3): 1957-61.
17. Eiringhaus K, Renz H, Matricardi P, Skevaki C. Component-Resolved Diagnosis in Allergic Rhinitis and Asthma. J Appl Lab Med. 2019; 3(5): 883-98.
18. Vandendriessche C, Kapogiannis D, Vandenbroucke RE. Biomarker and therapeutic potential of peripheral extracellular vesicles in Alzheimer's disease. Adv Drug Deliv Rev. 2022; 114486.
19. Liu W, Ota M, Tabushi M, Takahashi Y, Takakura Y. Development of allergic rhinitis immunotherapy using antigen-loaded small extracellular vesicles. J Control Release. 2022; 345: 433-42.
20. Liu Y, Sha J, Meng C, Zhu D. The Role of Small Extracellular Vesicles and MicroRNAs in the Diagnosis and Treatment of Allergic Rhinitis and Nasal Polyps. Mediators Inflamm. 2022; 2022: 4428617.
21. Peng YQ, Wu ZC, Xu ZB, Fang SB, Chen DH, Zhang HY, et al. Mesenchymal stromal cells-derived small extracellular vesicles modulate DC function to suppress Th2 responses via IL-10 in patients with allergic rhinitis. Eur J Immunol. 2022; 52(7): 1129-40.
22. Di Cicco M, D'Elios S, Peroni DG, Comberiati P. The role of atopy in asthma development and persistence. Curr Opin Allergy Clin Immunol. 2020; 20(2): 131-7.
23. Jacquet A. Characterization of Innate Immune Responses to House Dust Mite Allergens: Pitfalls and Limitations. Front Allergy. 2021; 2: 662378.
24. Bajic G, Degn SE, Thiel S, Andersen GR. Complement activation, regulation, and molecular basis for complement-related diseases. Embo j. 2015; 34(22): 2735-57.
25. Gueguen C, et al. Changes in markers associated with dendritic cells driving the differentiation of either TH2 cells or regulatory T cells correlate with clinical benefit during allergen immunotherapy. J Allergy Clin Immunol. 2016; 137(2): 545-58.
26. Nayak A, Pednekar L, Reid KBM, Kishore U. Complement and non-complement activating functions of C1q: a prototypical innate immune molecule. Innate Immun. 2012; 18(2): 350-63.
27. Wang Y, Cao Z, Zhao H, Ren Y, Hao L, Gu Z. Bisphenol A Exacerbates Allergic Inflammation in an Ovalbumin-Induced Mouse Model of Allergic Rhinitis. J Immunol Res. 2020; 2020: 7573103.
28. Theerawatanasirikul S, Sailasuta A, Thanawongnuwech R, Suriyaphol G. Alterations of keratins, involucrin and filaggrin gene expression in canine atopic dermatitis. Research in Veterinary Science. 2012; 93(3): 1287-92.
29. Ciprandi G, Caimmi D, Raschetti R, Giudice MMD, Salpietro C, Caimmi S, et al. Adipokines and their role in allergies. Int J Immunopathol Pharmacol. 2011; 24(4 Suppl): 13-6.
30. Umano GR, Pistone C, Tondina E, Moiraghi A, Lauretta D, Giudice EMD, et al. Pediatric Obesity and the Immune System. Front Pediatr. 2019; 7: 487.
31. Hsueh KC, Lin YJ, Lin HC, Lin CY. Serum leptin and adiponectin levels correlate with severity of allergic rhinitis. Pediatr Aller, gy Immunol. 2010; 21(1 Pt 2): e155-9.
32. Zeng Q, Luo X, Tang Y, Liu W, Luo R. Leptin Regulated ILC2 Cell through the PI3K/AKT Pathway in Allergic Rhinitis. Mediators Inflamm. 2020; 2020: 4176082.
33. Furukawa S, Asano K, Kobayashi H. Suppressive Activity of Adiponectin on the Development of Allergic Rhinitis in Mice. In Vivo. 2019; 33(1): 93-8.