CircRNA expression profile and circRNA-miRNA-mRNA crosstalk in allergic rhinitis

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To the Editor,

Allergic rhinitis (AR) is a chronic multi-factorial inflammatory disease, with a prevalence on the rise in China[1]. CircRNAs, a newly discovered family of endogenous non-coding RNAs, function in various biological processes[2]. For instance, circRNAs are involved in neuronal activities, tumor development, and innate immune responses, inflammatory diseases[3-4]. However, the roles of circRNAs in AR remain unclear. In this study, using high-throughput sequencing (HTS), we explored the circRNA, miRNA, and mRNA expression profiles in nasal mucosa from AR patients, and constructed a differentially expressed (DE) circRNA-DEmiRNA-DEmRNA network to illustrate the molecular mechanism in AR.

This study was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University and each patient provided written informed consent. Patients and methods are detailed as online data.

RESULTS

CircRNA expression profile in nasal mucosa

Three pairs of nasal mucosa tissue of middle turbinate were randomly selected from ten patients with perennial AR and ten healthy controls for HTS analysis. The clinical characteristics of the patients are shown in Table S1. All normalized sample datasets displayed similar circRNA distribution intensities (Fig. S1A). A total of 30,936 circRNAs were identified, including 12,376 known and 18,560 novel ones (Fig. S1B). CircRNA transcripts were widely distributed on all chromosomes (Fig.S1C). Spliced reads per million (RPM) revealed that almost all circRNA transcripts were expressed at low levels (Fig.S1D).

Among these circRNA, 1,119 were antisense and 29,335 were sense, including 28,694 exonic, 641 intronic, and 1,119 intergenic circRNAs. In particular, 93% of the circRNAs were sense exons (Fig. S2A) and most circRNA transcripts carried 1–7 exons (Fig.S2B), with a length of 200–400bpor >2000bp (Fig.S2C). Hierarchical clustering revealed the DEcircRNAs between samples (Fig. S2D) and HTS analysis identified 264 DEcircRNAs (120 upregulated and 144 downregulated). The top 300 relevant circRNA-miRNA pairs were screened and their network constructed with Cytoscape software (Fig.S3).

MiRNA and mRNA expression profiles in nasal mucosa

Of the 1,784 miRNAs, 13 were upregulated and 15 downregulated. Of the 2,030 mRNAs,93 were upregulated and 79 downregulated. Hierarchical clustering revealed that these DEmiRNAs (Fig. S4A) and DEmRNAs (Fig. S4B) were distinct between samples.

DEcircRNA-DEmiRNA, DEmiRNA-DEmRNA, and DEcircRNA-DEmRNA interactions

Using miRanda software, 911 DEmiRNA-DEcircRNA pairs and 379 DEmiRNA-DEmRNA pairs were detected. Next, we calculated their Pearson correlation coefficients (PCCs), with p -value <0.05 and an absolute PCC of [?]0.7. We screened out 941 DEmiRNA-DEcircRNA and 865 DEmiRNA-DEmRNA pairs with negative correlation, and 116 DEmiRNA-DEcircRNA and 379 DEmiRNA-DEmRNA pairs were screened by miRanda and correlation analyses. Then a DEcircRNA-DEmRNA network was made based on 11 circRNAs and 29 mRNAs that were positively correlated (Fig. S5).

DEcircRNA-DEmiRNA-DEmRNA network and functional annotation

Next, we identified17 miRNAs interacting with both circRNAs and mRNAs, and used them to construct a DEcircRNA-DEmiRNA-DEmRNA network (Table S2) containing 64 interaction pairs (Fig.1).

According to GO and KEGG analyses, the genes in the network were enriched in 159 terms, including 172 biological processes, 47 cell components, and 46 molecular functional components. The GO terms mainly involved the Wnt signaling pathway, lung epithelial cell development, and TNF biosynthesis(biological processes), endoplasmic reticulum lumen, flotillin complex, and intercellular canaliculus (cell components), fibronect in binding, collagen binding, and calcium:sodium antiporter activity (molecular functions; Fig.2A). In addition, these genes were enriched in the inflammatory response and various signaling pathways (such as cell surface receptor signaling pathways), indicating that circRNAs may participate in multiple pathways related to AR. KEGG analysis revealed 24 significantly involved pathways, mainly about adhesion, ECM receptor interaction, protein degradation and absorption, and PI3K-Akt signaling (Fig. 2B). However, other pathways, such as the Toll-like receptor signaling pathway, suggested that circRNAs may act as competing endogenous RNAs (ceRNAs) in AR to regulate mRNAs by competitively binding to miRNAs.

Sanger sequencing verification and RT-PCR quantification of circRNAs

Of the 11 circRNAs in the network (Table S2), two were known (hsa_circ_0008668 and hsa_circ_0029853) and nine were novel. Then, hsa_circ_0008668, hsa_circ_0029853, circTRIQK (a sense-overlapping circRNA), and circRNA_01002 (an intergenic circRNA) were subjected to RT-PCR amplification and Sanger sequencing to confirm their back-splicing sites (Fig.S6A-D). The RT-PCR dissolution curve had a single peak, suggesting that the primers had good repeatability and yielded a single type of product. In addition, RT-PCR revealed that hsa_circ_0008668 (Fig. S6E) and circTRIQK (Fig. S6G) were upregulated in AR, whereas hsa_circ_0029853 (Fig. S6F) and circRNA_01002 were downregulated (Fig.S6H). These findings were consistent with the HTS data.

DISCUSSION

CircRNAs have been shown to regulate mRNAs by sponging miRNA[5]. Fang *et al* . reported that circANKRD36 expression positively correlated with the level of IL-6 and might be involved in type 2 diabetes mellitus and inflammation-related pathways[6]. Similarly, Ddx17 was found to exert protective effects by inhibiting miR-17-5p expression in ovalbumin -induced AR mice[7]. To the best of our knowledge, this is the first study to investigate circRNA expression profiles and functions in AR.

We predicted DEmiRNAs and DEcircRNAs using miRanda and screened out 116 pairs with negative relationships, including 17 miRNAs (like hsa-miR-98-5p and hsa-miR-455-5p). Similarly, we identified 379 DEmiRNA-DEmRNA pairs and many DEgenes (such as DOCK8 and MUC19) that were significantly associated with allergic inflammation[8]. Finally, we constructed a novel DEcircRNA-DEmiRNA-DEmRNA network consisting of 64 ceRNA pairs. In this network, 11 circRNAs bound to 17 miRNAs, such as hsa-miR-98-5p, to regulate the expression of 29 genes. Previous studies have found that AR is regulatedby many signaling pathways, including PI3K-Akt and TLR signaling pathways[9]. Consistent with these findings, we found that the 29 AR-related genes also were involved these signaling pathways, suggestive of the key roles of these genes in AR development.

In summary, crosstalk between circRNAs, miRNAs, and mRNAs is essential for AR development and the

crosstalk network identified in this study provides new directions for targeted treatments against AR.

KEYWORDS

Allergic rhinitis; Circular RNA; MicroRNA; mRNA; Nasal mucosa

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interests.

AUTHOR CONRTIBUTIONs

Chang-Yu Qiu performed data analysis and wrote the draft manuscript. Qing Yang performed RT-PCR. Mei-Ping Lu, Min Yin, Wan-Yun Xu, Xin-Jie Zhu, and Xin-Yan Cui collected samples and performed specimen quality screening. Lei Cheng designed the study and finalized the manuscript. All authors approved the final version of the manuscript.

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Figure legends

Figure 1. DEcircRNA-DEmiRNA-DEmRNA crosstalk network. The network includes 17 miRNAs (red nodes), 11 circRNAs (green nodes), and 29 mRNAs (yellow nodes). CircRNAs and miRNAs were annotated using circBase and miRbase, respectively. Novel circRNAs and miRNAs were described in terms of their genome location. circRNA, circular RNA; miRNA, microRNA; DE, differentially expressed.

Figure 2. Bioinformatics analysis of the DEcircRNA-DEmiRNA-DEmRNA network. (A) GO terms pertaining to biological processes, cell components, and molecular functions, respectively.**(B)** KEGG analysis of the transcripts involved in different biological pathways. Bar plot depicting the enrichment score of significantly enriched pathways. circRNA, circular RNA; miRNA, microRNA; DE, differentially expressed; GO, gene ontology; KEGG, Kyoto encyclopedia of genes and genomes.

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