INTRODUCTION

Immunoglobulin A nephropathy (IgAN) is the most common form of primary glomerulonephritis worldwide and is characterized by predominant IgA deposition in the mesangial area. Approximately, one-third of patients with IgAN progress to the end stage of renal disease (ESRD) within two decades and need renal replacement therapy (RRT), which brings heavy health and eco-social burdens. However, the exact pathogenesis of IgAN has remained obscure until now. IgAN is a multifactorial disease, and both environmental...
and genetic effects contribute to it. Recently, the multihit pathogenesis model of IgAN has been widely accepted, which indicates that circulating galactose-deficient IgA1 is the cause. The contribution of galactose-deficient IgA1 to IgAN pathogenesis has been validated in many studies. Moreover, the presence of galactose-deficient IgA1(Gd-IgA1) in the glomerular deposits of patients with IgAN has been proven by immunohistochemical staining using the galactose-deficient IgA1-specific monoclonal antibody KM55, which reinforces the important role that galactose-deficient IgA1 plays in IgAN.

Previous studies have shown that the aberrant deposition of glycosylated IgA1 in the renal mesangial area was from circulation. The production of IgA1, including the O-glycosylation status of IgA1, was regulated not only by B cells but also by some cytokines secreted by T cells, dendritic cells, and monocytes, all of which make up the majority of peripheral blood mononuclear cells (PBMCs). Moreover, previous studies have identified many key proteins that are involved in the O-glycosylation of IgA1, including APRIL and BAFF, and all of these proteins were expressed in PBMCs, indicating that PBMCs are a whole cell population and that further investigation is needed.

MicroRNAs (miRNAs) are a class of single-stranded, short RNA molecules that down-regulate gene expression by binding to specific sites within the 3′ untranslated regions (UTRs) of mRNAs to promote mRNA degradation or to interrupt translation processes. MiRNAs can exist in the cell or be secreted selectively out of the cell, with the regulatory function of gene expression and cell-to-cell communication. In recent years, great progress regarding miRNAs has been achieved in the field of nephrology. Many differentially expressed miRNAs in several kinds of human samples were identified as biomarkers or participants in IgAN pathogenesis, indicating the important pathophysiological role of miRNAs in IgAN.

In the present study, we summarized and reanalysed the previously reported microarray data of PBMCs in IgAN to explore the miRNAs and target genes that are associated with IgAN.

2 | MATERIALS AND METHODS

2.1 | Microarray data preprocessing

All microarray data derived from PBMCs in IgAN were searched in the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/). We obtained three datasets, including two mRNA arrays, GSE14795 and GSE73953, and one miRNA array, GSE25590.

The data of patients with IgAN and controls were extracted in GSE14795 and GSE73953 for subsequent analysis (the data analysis pipeline is shown in Figure 1). The samples included in each dataset and the corresponding annotations for the array platform are listed in Table 1. All data were Log2 transformed to achieve normality. In addition, data normalization was performed with the linear models for the microarray data (limma, http://www.R-project.org) package in R. Principal component analysis (PCA) and clustering were also performed for the data quality control. The samples that did not reach the quality control standards were excluded, as shown in Table 1.

2.2 | DEG analysis and functional enrichment analysis

First, the samples in GSE14795 were used as the discovery cohort, and GSE73953 was used as the validation cohort to obtain the shared differentially expressed genes (DEGs). Second, the target genes of the differentially expressed miRNAs of GSE25590 were predicted using 4 miRNA databases: MiRDB, Tarbase, miRTarBase, and TargetScan. Finally, we merged the above results together and obtained the gene-miRNA network. QuickGO, a web-based tool for gene ontology searching, was utilized for identifying the enriched functions in ‘shared DEGs’, and the Kyoto Encyclopedia of Genes and Genomes (KEGG), a reference resource for gene and protein annotation, was used to assign ‘shared DEGs’ to specific pathways. P < .05 was used as the threshold value.

2.3 | IgAN-associated gene-miRNA network analysis

The Search Tool for the Retrieval of Interacting Genes (STRING) database was used to provide information on the different proteins, including the predicted and experimental interactions and the direct (physical) and indirect (functional) interactions of the proteins. The ‘IgAN-associated genes and relative miRNAs’
Table 1: Information on the three gene expression profile dataset

| platforms                  | GSE14795 | GSE73953 | GSE25590 |
|----------------------------|----------|----------|----------|
| Affymetrix Human Genome U133A Array | 12       | 15       | 7        |
| Agilent-014850 Whole Human Genome Microarray 4x44K G4112F | 8        | 16 (pooled) | 7        |
| GPL7731 Agilent-019118 Human miRNA Microarray 2.0 | 8        | 16 (pooled) | 4        |

were mapped into the network. Cytoscape software, a software for the integrated models of biomolecular interaction networks, was used to construct the IgAN-associated gene-miRNA network.

2.4 | Luciferase reporter assays

HMGB2 and hsa-miR-590-3p were chosen for further validation of the IgAN-associated gene-miRNA network. The dual-luciferase reporter system was applied to verify the interaction between hsa-miR-590-3p and its target gene HMGB2. Briefly, the 3’UTR sequences of the HMGB2 gene were amplified from genomic DNA and were subcloned directly downstream of the Renilla luciferase gene in the pmir-GLO vector. A mutant version of the 3’UTR sequences of HMGB2 in the ‘seed region’ was also generated as the mutant control. Both constructs were verified by DNA sequencing. To determine whether hsa-miR-590-3p could bind to the 3’UTR sequences of the HMGB2 gene, HEK293T cells were seeded into 24-well plates. After 24 hours, the cells were cotransfected with 25 nmol of hsa-miR-590-3p mimics or the negative control (NC) mimics, along with 800 ng per well of pmir-GLO-3’UTR-HMGB2 wild-type construct (pmir-GLO-3’UTR-HMGB2wt) or the mutant version (pmir-GLO-3’UTR-HMGB2mut) performed with Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol. After 48 hours of culture, the cells were lysed, and the firefly and Renilla luciferase activities were quantified using the Dual-Luciferase Reporter Assay System (Promega). The reporter activity of each well was expressed as the relative luciferase expression normalized to the Renilla activity.

2.5 | Study population

Thirty-seven primary IgAN patients diagnosed at The First Affiliated Hospital of Zhengzhou University were recruited between February 2018 and June 2018. The granular deposition of IgA in the glomerular mesangium by immunofluorescence detection and the deposition of electron-dense material in the mesangium by ultra-structural examination confirmed the diagnosis of IgAN. Patients were excluded if the following items are met: (a) patients with Henoch-Schönlein Purpura, systemic lupus erythematosus or chronic hepatic diseases; (b) patients had used corticosteroids or immunosuppressors; (c) IgAN was suspected secondary to other diseases. At the same time, nine age- and gender-matched healthy controls in the physical examination center of this hospital were recruited, after carefully checking the examination results. For each recruited individual, 10-ml peripheral blood was obtained, for patients on the morning of renal biopsy and for controls on the day of recruitment. RNA extraction was performed to determine the expression of HMGB2 and hsa-miR-590-3p, the plasma samples were divided into aliquots and stored in −80°C for subsequent detection of Gd-IgA1 protein levels. The clinical and pathological data at the time of renal biopsy were collected from medical records for further analysis. Notably, all the patients did not receive any special treatment before, such as immunosuppressants, ACEI/ARB.

The Medical Ethics Committee of The First Affiliated Hospital of Zhengzhou University approved the study protocol, and informed written consent was obtained from all participants.

2.6 | Detection of the expression of HMGB2 and hsa-miR-590-3p

Peripheral blood mononuclear cells (PBMCs) were prepared by density gradient centrifugation performed with Ficoll-Paque Plus (GE). After cell isolation, the total RNA was extracted using the commercial TRizol Reagent (Invitrogen). The cDNA for miRNA and mRNA detection was synthesized from 1 μg total RNA using the Reverse Transcription System (for miRNA: TIANGEN, Beijing, China; for mRNA: Promega, Wisconsin, USA) and was stored at −20°C for the following amplification. The expression levels of HMGB2 and hsa-miR-590-3p were measured by semiquantitative reverse transcription-PCR performed with AceQ® qPCR SYBR® Green Master Mix (Takara), and this experiment was performed on an Applied Biosystem 7500 Real-Time PCR System (the primers are shown in Table 2). U6 snRNA and GAPDH were used for normalization. The expression fold change between the patients and the controls was expressed by the 2−ΔΔCT method.

2.7 | Detection of plasma Gd-IgA1 levels

Plasma Gd-IgA1 levels were detected performed with a commercial enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer’s specifications (IBL).
TABLE 2 The primer pairs for HMGB2, GAPDH and hsa-miR-590-3p

|          | Sequence                      |
|----------|-------------------------------|
| HMGB2    | F 5'-GTGGGCTTAGCTCGTCAAGTT-3' |
|          | R 5'-GGGTGAGCAGTTTCCGCGC-3'   |
| GAPDH    | F 5'-GGGAAACTGTGGCCGTGAT-3'   |
|          | R 5'-GAGTGGGTTGCATTGGA-3'     |
| hsa-miR-590-3p | F 5'-TAATTTTATGTATAAAGCTAGT-3' |
|          | R 5'-GTGCAGGGTCCGAGGT-3'      |

2.8 | Statistical analysis

Statistical analyses were performed with SPSS software (version 16.0; SPSS). For continuous variables, data with normal distribution were expressed as the mean ± SD and were compared by an independent-sample t test, and Pearson Correlation was used if need; the other data were expressed as the median (first quartile and third quartile) and were analysed by the Mann-Whitney U test, and the Spearman Correlation was utilized for the correlation analysis. A two-tailed P-value < .05 was considered statistically significant. The graphs were plotted with GraphPad Prism.

3 | RESULTS

3.1 | Screened miRNAs and target genes associated with IgAN

First, 894 DEGs (168 up-regulated and 726 down-regulated genes) and 2668 DEGs (355 up-regulated and 2313 down-regulated genes) were obtained from GSE14795 and GSE73953, respectively. Then, 129 ‘shared DEGs’ were obtained; finally, 38 differentially expressed miRNAs in the GSE25590 dataset were merged with 129 ‘shared DEGs’ from GSE14795 and GSE73953, and 7 miRNAs and 19 target genes were identified (as shown in Table 3). Then, the genes were constructed with functional and pathway enrichment analysis by QuickGO and KEGG (shown in Table 4). We set the gene number above 6 for each GO term and a p-value less than 0.01 as the cutoff values, and we obtained 25 GO terms. Regarding the KEGG pathway, 2 pathways were identified with P < .05, but each included only 1 gene.

IgAN is a multifactorial disease with unclear pathogenesis. Previous studies have demonstrated that immune system disorders, susceptible genes and inflammation are the contributing factors. The IgAN-associated gene-miRNA network was constructed using Cytoscape (shown in Figure 2). In total, five enriched pathways containing six genes, HMGB2, MAN1A1, NAMPT, NPTN, RASA1, and GCC2 and 2 miRNAs, hsa-miR-590-3p and hsa-miR-648, were identified to be potentially involved in the pathogenesis of IgAN. From the IgAN-associated gene-miRNA network, HMGB2 was the core and most weighted gene among the 6 genes because of its significantly different expression in the two mRNA datasets (GSE14795 and GSE73953) and because of its involvement in four IgAN-associated pathways (inflammatory response to antigenic stimulus, defense response to bacteria, somatic diversification of immune receptors and cell surface receptor signalling pathway). Therefore, HMGB2 was chosen for further validation.

3.2 | HMGB2 is a direct target of hsa-miR-590-3p

The 3’UTR of HMGB2 was predicted to have a conserved binding site for hsa-miR-590-3p (363th-370th bp), as shown in Figure 3A. To further confirm HMGB2 as the putative target of hsa-miR-590-3p, an hsa-miR-590-3p mimic or a negative control (NC) sequence was cotransfected with constructs containing the wild-type or mutant HMGB2 3’UTR into HEK293T cells. As shown in Figure 3B, the HEK293T cells that were cotransfected with hsa-miR-590-3p mimics and pmiR-GLO-3’UTR-HMGB2wt displayed significantly reduced luciferase activity levels compared with those cotransfected with NC and pmiR-GLO-3’UTR-HMGB2mut (4.20 ± 0.30 vs 7.16 ± 0.51, P = .001). The luciferase activity of cells cotransfected with hsa-miR-590-3p mimics and pmiR-GLO-3’UTR-HMGB2mut displayed no significant changes compared with the luciferase activity of cells cotransfected with NC and pmiR-GLO-3’UTR-HMGB2mut, indicating that hsa-miR-590-3p binds to the 3’UTR of HMGB2 and down-regulates its expression.

3.3 | Increased hsa-miR-590-3p expression and decreased HMGB2 expression in IgAN

To validate the contribution of HMGB2 and has-miR-590-3p in IgAN, 37 patients with IgAN and 9 healthy controls were
## TABLE 4  GO and KEGG enrichment analysis results for DEGs identified in the three microarray

| Description                                      | gene count | Pvalue     | Genes                                                                 |
|--------------------------------------------------|------------|------------|-----------------------------------------------------------------------|
| GO:0044699-single-organism process               | 18         | 1.00 × 10⁻³| ZFR;TMEM57;MAN1A1;HABP4;FT5;TGFA;CETN3;GCC2;JMJD1C;NAMPT;RASA1;MAPK6;RAB1A;ARID4A;TUBGCP3;HMGB2;NPTN;CNOT3 |
| GO:0044707-single-multicellular organism process | 13         | 4.07 × 10⁻⁶| ZFR;TMEM5;HABP4;TGFA;JMJD1C;NAMPT;RASA1;MAPK6;RAB1A;ARID4A;HMGB2;NPTN;CNOT3 |
| GO:0032501-multicellular organismal development  | 13         | 7.82 × 10⁻⁶| ZFR;TMEM57;HABP4;TGFA;JMJD1C;NAMPT;RASA1;MAPK6;RAB1A;ARID4A;HMGB2;NPTN;CNOT3 |
| GO:0048856-anatomical structure development       | 11         | 5.60 × 10⁻⁵| ZFR;TMEM57;NAMPT;RASA1;MAPK6;RAB1A;ARID4A;HMGB2;NPTN;CNOT3            |
| GO:004767-single-organism developmental process   | 11         | 1.13 × 10⁻⁴| ZFR;TMEM57;TGFA;NAMPT;RASA1;MAPK6;RAB1A;ARID4A;HMGB2;NPTN;CNOT3       |
| GO:0032502-developmental process                 | 11         | 1.32 × 10⁻⁴| ZFR;TMEM57;TGFA;NAMPT;RASA1;MAPK6;RAB1A;ARID4A;HMGB2;NPTN;CNOT3       |
| GO:0060255-regulation of macromolecule metabolic process | 11         | 1.57 × 10⁻³| HABP4;TGFA;JMJD1C;NAMPT;RASA1;MAPK6;RAB1A;ARID4A;HMGB2;NPTN;CNOT3    |
| GO:0019222-regulation of metabolic process        | 11         | 6.12 × 10⁻³| HABP4;TGFA;JMJD1C;NAMPT;RASA1;MAPK6;RAB1A;ARID4A;HMGB2;NPTN;CNOT3    |
| GO:0007275-multicellular organism development     | 10         | 1.02 × 10⁻⁴| ZFR;TMEM57;TGFA;NAMPT;RASA1;MAPK6;ARID4A;HMGB2;NPTN;CNOT3            |
| GO:0048518-positive regulation of biological process | 10         | 5.60 × 10⁻⁴| IFIT5;TGFA;NAMPT;RASA1;MAPK6;RAB1A;ARID4A;HMGB2;NPTN;CNOT3          |
| GO:0016043-cellular component organization        | 10         | 5.45 × 10⁻³| CETN3;GCC2;JMJD1C;RASA1;MAPK6;RAB1A;ARID4A;TUBGCP3;HMGB2;NPTN       |
| GO:0080090-regulation of primary metabolic process | 10         | 6.26 × 10⁻³| HABP4;TGFA;JMJD1C;NAMPT;RASA1;RAB1A;ARID4A;HMGB2;NPTN;CNOT3          |
| GO:0071840-cellular component organization or biogenesis | 10         | 6.85 × 10⁻³| CETN3;GCC2;JMJD1C;RASA1;MAPK6;RAB1A;ARID4A;TUBGCP3;HMGB2;NPTN       |
| GO:0031323-regulation of cellular metabolic process | 10         | 6.93 × 10⁻³| HABP4;TGFA;JMJD1C;NAMPT;RASA1;RAB1A;ARID4A;HMGB2;NPTN;CNOT3          |
| GO:0048522-positive regulation of cellular process | 9          | 5.74 × 10⁻⁴| IFIT5;TGFA;NAMPT;MAPK6;RAB1A;ARID4A;HMGB2;NPTN;CNOT3                |
| GO:0048731-system development                     | 8          | 1.59 × 10⁻³| TMEM57;TGFA;NAMPT;RASA1;MAPK6;ARID4A;HMGB2;NPTN                     |
| GO:0066996-organelle organization                 | 8          | 4.66 × 10⁻³| CETN3;GCC2;JMJD1C;RASA1;RAB1A;ARID4A;TUBGCP3;HMGB2                   |
| GO:006464-cellular protein modification process   | 8          | 9.25 × 10⁻³| MAN1A1;TGFA;JMJD1C;RASA1;MAPK6;RAB1A;ARID4A;NPTN                   |
| GO:0036211-protein modification process           | 8          | 9.25 × 10⁻³| MAN1A1;TGFA;JMJD1C;RASA1;MAPK6;RAB1A;ARID4A;NPTN                   |
| GO:0048468-cell development                       | 7          | 9.85 × 10⁻⁵| RASA1;MAPK6;RAB1A;ARID4A;HMGB2;NPTN;CNOT3                          |
| GO:009893-positive regulation of metabolic process | 7          | 3.75 × 10⁻⁵| TGFA;NAMPT;RASA1;RAB1A;ARID4A;HMGB2;NPTN                           |
| GO:0030154-cell differentiation                   | 7          | 3.83 × 10⁻³| RASA1;MAPK6;RAB1A;ARID4A;HMGB2;NPTN;CNOT3                         |
| GO:006008-regulation of biological quality        | 7          | 4.32 × 10⁻³| HABP4;JMJD1C;RASA1;RAB1A;ARID4A;HMGB2;NPTN                          |
| GO:0048669-cellular developmental process         | 7          | 7.37 × 10⁻³| RASA1;MAPK6;RAB1A;ARID4A;HMGB2;NPTN;CNOT3                         |

### KEGG pathways

- hsa00760-Nicotinate and nicotinamide metabolism
  - NAMPT
- hsa00510-N-Glycan biosynthesis
  - MAN1A1
recruited to detect the expression of hsa-miR-590-3p and HMGB2 in PBMCs. In the IgAN patients (the baseline data are shown in Table 5), the expression of HMGB2 in PBMCs was significantly down-regulated compared with that in the healthy controls (0.91 (0.80, 1.13) vs 1.34 (1.10, 2.54), \( P = .003 \), Figure 4A). Meanwhile, the expression of has-miR-590-3p in PBMCs was significantly up-regulated in the IgAN patients (1.55 (0.96, 1.90) vs 0.70 (0.45, 1.30), \( P = .012 \), Figure 4B). Moreover, we also found a significant negative correlation between the expression of HMGB2 and that of hsa-miR-590-3p in the whole population of IgAN patients and healthy controls (\( r = -0.386, P = .008 \), shown in Figure 4C).

3.4 | Increased plasma Gd-IgA1 levels and correlation with hsa-miR-590-3p as well as HMGB2 expression in IgAN patients

Compared with healthy controls, patients with IgAN showed significantly higher levels of plasma Gd-IgA1 (3612.67 (2310.67, 5883.02) vs 1981.79 (1406.55, 2487.19) ng/mL, \( P = .004 \); Figure 4D). Furthermore, the slight correlation tendency was found between serum Gd-IgA1 levels and hsa-miR-590-3p as well as HMGB2 expression (\( r = 0.270, P = .073 \), Figure 4E; \( r = -0.236, P = .119 \), Figure 4F) in the whole population.

3.5 | HMGB2 was correlated with the severity of IgAN

After the validation of the decreased expression of HMGB2 in IgAN patients, which was down-regulated by the increased has-miR-590-3p levels, we further explored their association with clinical findings and the pathological lesions in patients with IgAN. In the patients with IgAN, the expression of HMGB2 showed a significantly positive correlation with age and serum C3 levels (age: \( r = 0.336, P = .042 \); serum C3: \( r = 0.416, P = .020 \); Figure 5A, 5) and a significantly negative correlation with the serum creatinine, serum BUN (serum creatinine: \( r = -0.335, P = .043 \); BUN: \( r = -0.414, P = .011 \); Figure 5B, 5). Meanwhile, HMGB2 expression also had a negative correlation trend with diastolic blood pressure (\( r = -0.320, P = .053 \); Figure 5E). These results indicate that lower HMGB2 expression correlated with more severe clinical manifestations of IgAN. However, has-miR-590-3p expression was not found to be correlated with the clinical or pathological manifestations of IgAN patients.

4 | DISCUSSION

IgAN is a complex multifactorial disease with an unclear pathogenetic mechanism. For the first time, we integrated the published
microarray data from PBMCs to find more clues to uncover the pathogenesis of IgAN.

In the present study, a total of 19 DEGs (all down-regulated) were identified through the comparison between the IgAN patients and the healthy controls in GEO datasets. The DEGs and differentially expressed miRNAs were mainly mapped to 5 IgAN-associated terms (Figure 2). Among them, ‘defense to response to bacteria’ and ‘cell surface receptor signaling pathway’ were down-regulated; however, ‘inflammatory response to antigenic stimulus’, ‘protein N-linked glycosylation’ and ‘somatic diversification of immune receptors’ were up-regulated. These results suggest that the immune system, inflammatory response and N-glycosylation modifications were associated with IgAN. As seen in patients with IgA nephropathy, the external defense system to bacteria was weakened; on the other hand, once stimulated by an antigen, the responses of the immune system and the inflammatory system were enhanced, which was consistent with the previously reported results of the genome-wide association studies (GWAS) of IgAN. Moreover, although O-linked glycosylation has been widely accepted to be a key step in the initiation of IgAN, a few studies have indicated that N-linked glycosylation is also an important factor in the biological properties of IgA1. There were a total of 6 genes and 2 miRNAs in the 5 terms. Both GCC2- and RAB1A-encoded proteins are required for transport from endosomes to the Golgi. Moreover, RAB1A was reported to act as an oncogene to regulate cellular proliferation, growth, invasion and metastasis via the activation of the mTORC1 pathway in triple-negative breast cancer. MAN1A1 encodes a class I mammalian Golgi 1,2-mannosidase to catalyze the hydrolysis of three-terminal mannose residues from peptide-bound Man(9)-GlcNAc(2) oligosaccharides. The NAMPT-encoded protein is thought to be involved in many important biological processes, including metabolism, stress response and aging. The protein encoded by RASA1 is associated with cellular proliferation and differentiation. HMGB2, which has a high degree of similarity to HMGB1, was reported to be associated with cell viability, invasion and the chemotherapy resistance of glioblastoma and was reported to have antimicrobial activity.

Little is known about hsa-miR-648, and hsa-miR-590-3p was reported to be related to Lynch syndrome in a published research paper. From the IgAN-associated DEG-miRNA network, it is clear that HMGB2 was in the core of the regulation network; the interaction between hsa-590-3p and HMGB2 was verified by the luciferase reporter assay and was validated in our IgAN cohort, in which miRNA-hsa-590-3p and HMGB2 showed a significant negative correlation. In fact, we also explored the HMGB2 protein levels in 5 patients with IgAN and 7 healthy controls, and found the HMGB2 expression levels in PBMCs were significantly lower in patients than healthy.

| TABLE 5 | The baseline data of 37 patients with IgAN |
|---------|------------------------------------------|
| Characters | Mean ± SD or median(IQR) or n |
| Male/female | 23/14 |
| Age (year) | 42.11 ± 14.80 |
| With hypertension (%) | 26(70.27%) |
| SBP (mmHg) | 135.97 ± 17.61 |
| DBP (mmHg) | 86.00 ± 10.39 |
| 24 h proteinuria (g/d) | 1.75 (0.75,4.09) |
| SCR (μmol/L) | 117 (82,189.50) |
| BUN (mmol/L) | 6.60 (5.00,11.61) |
| Uric Acid (μmol/L) | 342.19 ± 82.37 |
| Albumin (g/L) | 35.23 ± 8.98 |
| TCHO (mmol/L) | 4.40 (3.59,5.43) |
| TG (mmol/L) | 1.93 (1.17,2.60) |
| C3 (mg/L) | 1.21 (1.04,1.38) |
| C4 (mg/L) | 0.30 (0.25,0.34) |
| Blood cell count in urine | 24.50 (5.25,48.75) |
| Oxford classification (n) | |
| M score (M0/M1) | 28/9 |
| E score (E0/E1) | 29/8 |
| S score (S0/S1) | 16/21 |
| C score(C0/C1/C2) | 17/18/2 |
| T score (T0/T1/T2) | 20/8/9 |
controls, which was consistent with our previous results (Figure S1A, 1B). These findings suggest that HMGB2, which is targeted by hsa-miR-590-3p, may be associated with the pathogenesis of IgA nephropathy.

The high-mobility group box (HMGB) family consists of HMGB1-HMGB4, which are related to inflammatory diseases by binding to DNA; these proteins induce large-angle DNA bends, enhance the flexibility of DNA and perform numerous important biological processes. HMGB1 is secreted by the cells once it is stimulated by an antigen, and the secreted HMGB1 activates the innate immune system by binding to the cell surface receptors on various cell types.47 HMGB2 and HMGB1 are almost identical to each other and have often explored in the same study.48 Previous studies have shown that HMGB2 and HMGB1 share some functions, including the control of apoptosis by regulating the transcriptional activity of different members of the p53 family.49 Since HMGB1 is a multifunctional alarmin that drives autoimmune and inflammatory diseases, HMGB2 may also have a similar effect in IgAN. Serum complement 3 is one of the most important components of natural immunity, our result showed it had significantly positive correlation with HMGB2 expression levels, indicating the natural immunity system of patients. Robert Küchler et al and Takaishi H et al proved that HMGB2 has the function of antimicrobial activity against different commensal and pathogenic bacteria in the intestinal tract,45,50 which has been proven to influence the pathogenesis of IgAN; at the same time, this finding is consistent with our results in the present study. In addition, the expression level of HMGB2 was found to be related to the severity of IgAN, and both HMGB2 and hsa-miR-590-3p expression levels had a correlation tendency with serum Gd-IgA1, indicating that the elevated expression of hsa-miR-590-3p down-regulates HMGB2 expression and participates in the pathogenesis of IgAN. However, our study based on PBMCs, which include a range of different cells all with different immunological functions, different cells may had different expression levels of HMGB2 and hsa-miR-590-3p, it is better to sort the PBMCs into subsets and then examine HMGB2 and hsa-miR-590-3p expression levels and study the functions in each cell subset; what is more, the molecular mechanism by which HMGB2 regulates downstream factors to participate in IgAN is still unclear and requires further research.

In conclusion, we used bioinformatic analyses to analyse the published microarray data from PBMCs, and for the first time, we verified HMGB2 as the target gene of hsa-miR-590-3p; we also

**FIGURE 4** Compared with healthy controls, patients with IgAN presented significantly lower HMGB2 expression (A: 0.91 (0.80, 1.13) vs 1.34 (1.10, 2.54), P = .003), and higher hsa-miR-590-3p expression (B: 1.55 (0.96,1.90) vs 0.70(0.45,1.30), P = .012); The scatter plot showed significantly negative correlation between the expression of hsa-miR-590-3p and HMGB2 (C: r = −0.386, P = .008); Serum Gd-IgA1 levels were significantly elevated in IgAN patients (D: 3612.67(2310.67, 5883.02) vs 1981.79(1406.55, 2487.19) ng/mL, P = .004), and the slight correlation tendency was found with hsa-miR-590-3p and HMGB2 expression (D: r = 0.270, P = .073; F: r = −0.236, P = .119) in the whole population.
ZHAI et al. identified that HMGB2 was correlated with the severity of IgAN, which provides more clues about the pathogenesis of IgAN.

ACKNOWLEDGEMENTS

The work was supported by the Natural Science Foundation for young scientists of China (Grant No. 81600555), the National Science Foundation for Post-doctoral scientists of China (Grant No. 2018M640684), the National Natural Science Foundation of China (Grant No. 81873611), Science and Technology Innovation Team of Henan (Grant No. 17IRTSTHN020) and Foundation for Leading Personnel of Central Plains of China (Grant No. 194200510006).

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest regarding the publication of this paper.

AUTHOR CONTRIBUTION

Conceived and designed the experiments: Yaling Zhai and Zhanzheng Zhao; performed experiments: Yanna Dou and Xiaoqing Long; analysed the data: Yuanyuan Qi and Genyang Cheng; contributed reagents/materials/analysis tools: Jing Xiao, Dong Liu and Zhangsuo Liu; wrote the paper: Yaling Zhai. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

Raw data used during the current study are available from the corresponding author on reasonable request for non-commercial use.

ORCID

Yaling Zhai https://orcid.org/0000-0001-8638-708X
Zhanzheng Zhao https://orcid.org/0000-0001-6079-1631

REFERENCE

1. D’Amico G. The commonest glomerulonephritis in the world: IgA nephropathy. Q J Med. 1987;64:709-727.
2. Galla JH. IgA nephropathy. Kidney Int. 1995;47:377-387.
3. Lai KN. Pathogenesis of IgA nephropathy. Nat Rev Nephrol. 2012;8:275-283.
4. Placzek WJ, Yanagawa H, Makita Y, et al. Serum galactose-deficient-IgA1 and IgG autoantibodies correlate in patients with IgA nephropathy. PLoS ONE. 2018;13:e0190967.
5. Novak J, Tomana M, Brown R, et al. IgA1-containing immune complexes in IgA nephropathy differentially affect proliferation of mesangial cells. Kidney Int. 2005;67:504-513.
6. Coppo R, Fonsato V, Balegno S, et al. Aberrantly glycosylated IgA1 induces mesangial cells to produce platelet-activating factor that mediates nephritis loss in cultured podocytes. Kidney Int. 2010;77:417-427.

7. Suzuki H, Yasutake J, Makita Y, et al. Aberrant glycosylation of IgA1 in IgA nephropathy. Nephrol Dial Transplant. 2015;30:1315-1321.

8. Yasutake J, Suzuki Y, Suzuki H, et al. Novel lectin-independent approach to detect galactose-deficient IgA1 in IgA nephropathy. Kidney Int. 2018;93(3):700-705.

9. Suzuki H, Yasutake J, Makita Y, et al. Aberrant glycosylation of IgA1 in IgA nephropathy. Nephrol Dial Transplant. 2015;30:1315-1321.

10. Sanfilippo F, Croker BP, Bollinger RR. Fate of four cadaveric donor renal allografts with mesangial IgA deposits. Transplantation. 1982;33:370-376.

11. Brinkmann V, Muller S, Heusser CH, T cell dependent differentiation of human B cells: direct switch from IgM to IgE, and sequential switch from IgM via IgG to IgA production. Mol Immunol. 1999;29:1159-1164.

12. Endsley MA, Njongmeta LM, Shell E, et al. Human IgA-inducing protein from dendritic cells induces IgA production by naive IgD+ B cells. J Immunol. 2009;182:1854-1859.

13. Zhai Y-L, Zhu L, Shi S-F, Liu L-J, Lv J-C, Zhang H. Increased APRIL expression induces IgA1 aberrant glycosylation in IgA nephropathy. Medicine (Baltimore). 2016;95:e3099.

14. Zheng N, Fan J, Wang B, et al. Expression profile of BAFF in peripheral blood from patients of IgA nephropathy: Correlation with clinical features and Streptococcus pyogenes infection. Mol Med Rep. 2017;15:1925-1935.

15. Li W, Peng X, Liu Y, et al. TLR9 and BAFF: their expression in patients with IgA nephropathy. Mol Med Rep. 2014;10:1469-1474.

16. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116:281-297.

17. Tan K, Chen J, Li W, et al. Genome-wide analysis of microRNAs expression profiling in patients with primary IgA nephropathy. Genome. 2013:56:161-169.

18. Dai Y, Sui W, Lan H, et al. Microarray analysis of micro-ribonucleic acid expression in primary immunoglobulin A nephropathy. Saudi Med J. 2008;29:1388-1393.

19. Liang Y, Zhao G, Tang L, et al. miR-100-3p and miR-877-3p regulate overproduction of IL-8 and IL-1beta in mesangial cells activated by secretory IgA from IgA nephropathy patients. Exp Cell Res. 2016;347:312-321.

20. Serino G, Gallustio F, Cox SN, Pesce F, Schena FP. Abnormal miR-148b expression promotes aberrant glycosylation of IgA1 in IgA nephropathy. J Am Soc Nephrol. 2012;23:814-824.

21. Bao H, Chen H, Zhu X, et al. MiR-223 downregulation promotes glomerular endothelial cell activation by upregulating importin alpha4 and alpha5 in IgA nephropathy. Kidney Int. 2014;85:624-635.

22. Wang G, Kwan BC, Lai FM, et al. Urinary miR-21, miR-29, and miR-93: novel biomarkers of fibrosis. Am J Nephrol. 2012;36:412-418.

23. Cox SN, Sallustio F, Serino G, et al. Altered modulation of WNT-beta-catenin and PI3K/Akt pathways in IgA nephropathy. Kidney Int. 2010;78:396-407.

24. Nagasawa Y, Okuzaki D, Muso E, et al. IFI27 Is a Useful Genetic Marker for Diagnosis of Immunoglobulin A Nephropathy and Membranous Nephropathy Using Peripheral Blood. PLoS ONE. 2016;11:e0153252.

25. Wang X. miRDB: a microRNA target prediction and functional annotation database with a wiki interface. RNA. 2008;14:1012-1017.

26. Vergoulis T, Vlachos IS, Alexiou P, et al. TarBase 6.0: capturing the exponential growth of miRNA targets with experimental support. Nucleic Acids Res. 2012;40:D222-D229.

27. Hsu SD, Tseng YT, Shrestha S, et al. miRtarBase update 2014: an information resource for experimentally validated miRNA-target interactions. Nucleic Acids Res. 2014;42:D78-D85.

28. Garcia DM, Baek D, Shin C, et al. Weak seed-pairing stability and high target-site abundance decrease the proficiency of lsi-6 and other microRNAs. Nat Struct Mol Biol. 2011;18:1139-1146.

29. Huntley RP, Binns D, Dimmer E, et al. QuickGO: a user tutorial for the web-based Gene Ontology browser. Database (Oxford). 2009;2009:bap010.

30. Kaneshia M, Sato Y, Kawashima M, et al. KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res. 2016;44:D457-D462.

31. Szklarczyk D, Franceschini A, Wyder S, et al. STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res. 2015;43:D447-D452.

32. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13:2498-2504.

33. Magistroni R, D’Agati VD, Appel GB, Kiyok K. New developments in the genetics, pathogenesis, and therapy of IgA nephropathy. Kidney Int. 2015;88:974-989.

34. Floge J, Moura IC, Daha MR. New insights into the pathogenesis of IgA nephropathy. Semin Immunopathol. 2014;36:431-442.

35. Yu XQ, Li M, Zhang H, et al. A genome-wide association study in Han Chinese identifies multiple susceptibility loci for IgA nephropathy. Nat Genet. 2011;44:118-126.

36. Iwanami N, Iwase H, Takahashi N, et al. Similarities between N-glycan glycoform of tonsillar IgA1 and that of aberrant IgA1 abundant in IgA nephropathy patient serum. J Nephrol. 2008;21:118-126.

37. Chuang PD, Morrison SL. Elimination of N-linked glycosylation sites from the human IgA1 constant region: effects on structure and function. J Immunol. 1997;158:724-732.

38. Derby MC, Liao ZZ, Goud B, Gleeson PA. The trans-Golgi network golgin, GCC185, is required for endosome-to-Golgi transport and maintenance of Golgi structure. Traffic. 2007;8:758-773.

39. Xu H, Qian M, Zhao B, et al. Inhibition of RAB1A suppresses epithelial-mesenchymal transition and proliferation of triple-negative breast cancer cells. Oncol Rep. 2017:37:1619-1626.

40. Bieberich E, Bause E. Man9-mannosidase from human kidney is abundant in IgA nephropathy patient serum. J Biol Chem. 1995;269:644-649.

41. Wang B, Hasan MK, Alvarado E, Yuan H, Wu H, Chen WY. NAMPT overexpression in prostate cancer and its contribution to tumor cell survival and stress response. Oncogene. 2011;30:907-921.

42. Zhang R-L, Yang J-P, Peng L-X, et al. RNA-binding protein QKI-5 inhibits the proliferation of clear cell renal cell carcinoma via post-transcriptional stabilization of RASA1 mRNA. Cell Cycle. 2015;16:3094-3104.

43. Wu ZB, Cai L, Lin SJ, et al. High-mobility group box 2 is associated with prognosis of glioblastoma by promoting cell viability, invasion, and chemotherapeutic resistance. Neuro Oncol. 2013:15:1264-1275.

44. Küchler R, Schroeder BO, Jaeger SU, Stange EF, Wehkamp J. Antimicrobial activity of high-mobility group box 2: a new function to a well-known protein. Antimicrob Agents Chemother. 2013;57:4782-4793.
47. Harris HE, Andersson U, Pisetsky DS. HMGB1: a multifunctional alarmin driving autoimmune and inflammatory disease. Nat Rev Rheumatol. 2012;8:195-202.

48. Davies JE, Apta B, Harper MT. Cross-reactivity of anti-HMGB1 antibodies for HMGB2. J Immunol Methods. 2018;456:72-76. https://doi.org/10.1016/j.jim.2018.02.006.

49. Stros M, Ozaki T, Bacikova A, et al. HMGB1 and HMGB2 cell-specifically down-regulate the p53- and p73-dependent sequence-specific transactivation from the human Bax gene promoter. J Biol Chem. 2002;277:7157-7164.

50. Takaishi H, Kanai T, Nakazawa A, et al. Anti-high mobility group box 1 and box 2 non-histone chromosomal proteins (HMGB1/HMGB2) antibo-odies and anti-Saccharomyces cerevisiae antibodies (ASCA); accuracy in differentially diagnosing UC and CD and correlation with inflammatory bowel disease phenotype. J Gastroenterol. 2012;47:969-977.

51. McCarthy DD, Kujawa J, Wilson C, et al. Mice overexpressing BAFF develop a commensal flora-dependent, IgA-associated nephropathy. J Clin Invest. 2011;121:3991-4002.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Zhai Y, Qi Y, Long X, et al. Elevated hsa-miR-590-3p expression down-regulates HMGB2 expression and contributes to the severity of IgA nephropathy. J Cell Mol Med. 2019;23:7299–7309. https://doi.org/10.1111/jcmm.14582