Bioinformatic Analysis Reveals VEGFA Promotes the Occurrence of PLA2R-associated Idiopathic Membranous Nephropathy by Angiogenesis via PI3K/AKT Signaling Pathway

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Research

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Abstract

Background: PLA2R-associated IMN covers 70% of IMN, which is one of the main types of chronic kidney disease in adults and one of the most common causes of end-stage renal disease. Vascular endothelial growth factor A (VEGFA), a homodimeric vasoactive glycoprotein, is the key mediator of angiogenesis, which lead to numerous kidney diseases, including TSHD7A-associated IMN. However, the role of VEGFA in PLA2R-associated IMN is still poorly understood.

Methods: We downloaded the microarray data GSE115857 from GEO. The DEGs were identified with R software, and the functional and pathway enrichment analysis of DEGs was performed utilizing the DAVID and Cytoscape ClueGo plug-in. A comprehensive list of interacting DEGs was constructed using the STRING database and visualized by Cytoscape software. The Cytoscape MCODE and cytoHubba plug-in were used to identify clustered sub-networks, and hub genes from the protein-protein interaction network. Gene set enrichment analysis (GSEA) was used to identify signaling pathway in IMN.

Results: There were 1422 genes (952 up-regulated genes and 470 down-regulated genes) were identified as DEGs in GSE115857. The BP of DEGs in GSE115857 was clustered in regulation of transcription from RNA polymerase II promoter, positive regulation of nuclear-transcribed mRNA poly(A) tail shortening, cell adhesion et al. The KEGG pathway of DEGs in GSE115857 was clustered in Rheumatoid arthritis, ABC transporters, PI3K/AKT signaling pathway et al. Then we got a huge PPI network from STRING. 6 modules were screen out to study the functional changes in IMN. The KEGG pathway of module 3 was enriched in soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) interactions in vesicular transport. There were 3 hub genes screened out, namely, *VEGFA*, *JUN*, and *FOS*. Following the random walk, all genes were ranked and GSEA analysis showed that the signaling pathway of DEGs in GSE115857 was focused on angiogenesis, in which *VEGFA* acts as a core gene.

CONCLUSION: In summary, this study reveals VEGFA promotes PLA2R-associated IMN by stimulating angiogenesis via PI3K/AKT signaling. Moreover, SNARE interactions in vesicular transport was involved in the development of PLA2R-associated IMN, which may offer a novel therapeutic strategy in treatment of IMN.

Background

Membranous nephropathy (MN) is one of the most common causes of nephritic syndrome in adults and is the second commonest glomerulopathy to progress to end stage renal disease (ESRD). Most cases are idiopathic (IMN), but ~ 20% are secondary to various causes, including cancers, infections, autoimmune diseases, and medications [1]. The M-type phospholipase A2 receptor (PLA2R) and thrombospondin type1 domain-containing 7A (TSHD7A) are two major autoantigens against podocyte antigens in idiopathic membranous nephropathy (IMN) [2, 3]. PLA2R-associated IMN is a primary membranous nephropathy with positive serum anti-PLA2R antibody, which covers 70% of IMN [2]. The characteristic feature of IMN is immune complex (IC) formation/deposition on the sub-epithelial side of the glomerular

basement membrane resulting in podocyte injury, in which membrane attack complex C5b-9 induces a variety of downstream pathways, including protein kinases, lipid metabolism, reactive oxygen species, growth factors/gene transcription, endoplasmic reticulum stress, and the ubiquitin-proteasome system [4].

The vascular endothelial growth factor A (VEGFA), a homodimeric vasoactive glycoprotein, is the key mediator of angiogenesis. Angiogenesis, the formation of new blood vessels, is responsible for a wide variety of physio/pathological processes in kidney diseases, such as diabetic kidney disease, and polycystic kidney disease [5]. In the renal glomeruli, VEGFA is mainly expressed in and secreted from podocytes and tubular epithelial cells, which induces renal injury through binding to receptor VEGFR, mainly VEGFR2, which expressed on the surface of endothelial cells [6]. In recent decades, studies that investigated dysregulation of VEGFA expression during kidney diseases have led to the identification of a crucial role of this proangiogenic factor in the renal capillary network [7, 8]. *Ayumi Matsumoto et al.* reported that VEGFA may be important for TSHD7A-associated IMN pathogenesis [9]. However, the role of VEGFA in PLA2R-associated IMN is poorly studied.

The phosphoinositide 3-kinase (PI3K)/AKT signaling plays an important role in physiological and pathological conditions of the organism. In the present study, we attempted to identify crucial genes and pathways that are involved in the pathogenesis of PLA2R-associated IMN. We found that VEGFA is highly expressed in kidney tissue from PLA2R-associated IMN patients. VEGFA may promote the occurrence of PLA2R-associated IMN by stimulating angiogenesis via PI3K/AKT signaling. Moreover, we found six modules that closely related to the development of PLA2R-associated IMN. In addition, SNARE interactions in vesicular transport was involved in the occurrence and progression of PLA2R-associated IMN, which has not been reported before. These results may provide new targets for the treatment of IMN.

Methods

Microarray data information

We used “membranous nephropathy” as the keyword to search the Gene Expression Omnibus (GEO) database. We selected the expression profiling of the array: the attribute name was tissue, and the organisms were *Homo sapiens*, and only the array with high expression of M-type phospholipase A2 receptor 1 (PLA2R1) was chosen to further analysis (Accession number: GSE115857). GSE115857 contains 11 renal biopsys from IMN patients, and 7 renal biopsys from living donors, which is based on the GPL14951 (Illumina HumanHT-12 WG-DASL V4.0 R2 expression beadchip) platform.

Because the data in this experiment were all from public databases, there was no need for approval by the ethics committee.

Identification of differentially expressed genes

The R software (Version 3.6.2) was utilized to screen differentially expressed genes (DEGs) between the IMN group and living donors group. An adjusted p-value of <0.05 and $|\log FC| > 1.0$ were considered statistically significant. The ggplot2 and RColorBrewer package in R was used to plot the volcano map and the heat map in the two groups.

Gene ontology and pathway enrichment analysis

Gene Ontology (GO) includes three categories: molecular function (MF), biological process (BP), and cellular component (CC). GO and KEGG pathway analysis were performed via Database for annotation, Visualization and Integrated Discovery (DAVID) [10] and Cytoscape ClueGo plug-in. Gene Set Enrichment Analysis (GSEA) determines whether an a priori defined set of genes has statistically significant differences in expression under two different biological conditions [11]. This analysis, performed using GSEA software 4.0.3 from the Broad Institute. The gene set of “h.all.v7.1.symbols.gmt”, which summarizes and represents specific, well-defined biological states or processes, was downloaded from the Molecular Signatures Database (<http://software.broadinstitute.org/gsea/msigdb/index.jsp>). The normalized enrichment score (NES) was determined by analysis of 1000 permutations. A gene set was considered significantly enriched when the P-value was less than 0.05 and the false discovery rate (FDR) was less than 0.25.

Protein–protein interaction network and hub gene identification

To evaluate the interactive relationships among DEGs, the STRING online database (<https://string-db.org>) was used [12]. The protein–protein interaction (PPI) network of DEGs was visualized with Cytoscape software, and central genes were identified by cytoHubba to select the hub genes, which were intersected by Betweenness, Bottleneck, Closeness, and Degree method [13]. Significant modules in the PPI network were identified by Molecular Complex Detection (MCODE), a plug-in of Cytoscape software that clusters a network based on topology to recognize closely connected regions [14]. The MCODE algorithm sorts and identifies each identified module. The higher the score, the stronger the genes association in this module. The parameters of DEGs clustering and scoring were set as follows: MCODE score ≥ 4 , degree cutoff =2, node score cut-off=0.2, max depth=100 and k-score=4. Pathway, and BP enrichment analysis was performed for DEGs in the identified modules using ClueGo, a plug-in of Cytoscape software.

Results

Identification of DEGs

A total of 1422 DEGs were identified in GSE73953, including 952 up-regulated genes and 470 down-regulated genes. The volcano plot of all DEGs and heat map of the top 50 up-regulated genes and the top 50 down-regulated genes are shown in Figures 1 and 2, respectively.

Pathway and GO enrichment analysis of DEGs

GO enrichment and KEGG pathway analysis was performed with DAVID, which consists of three terms, as shown in Figure 3(a), (b), (c), and (d).

The top 15 biological process (BP) of DEGs in GSE115857 was clustered in regulation of transcription from RNA polymerase II promoter (GO:0006357, 54DEGs), positive regulation of nuclear-transcribed mRNA poly(A) tail shortening (GO:0060213, 7DEGs), cell adhesion (GO:0007155, 48DEGs), positive regulation of nuclear-transcribed mRNA catabolic process, deadenylation-dependent decay (GO:1900153, 6DEGs), response to cytokine (GO:0034097, 11DEGs), extracellular matrix organization (GO:0030198, 25DEGs), signal transduction (GO:0007165, 99DEGs), cellular response to fibroblast growth factor stimulus (GO:0044344, 8DEGs), cell migration (GO:0016477, 22DEGs), positive regulation of I-kappa B kinase/NF-kappa B signaling (GO:0043123, 21DEGs), response to drug (GO:0042493, 33DEGs), response to organic cyclic compound (GO:0014070, 10DEGs), response to mechanical stimulus (GO:0009612, 11DEGs), positive regulation of positive chemotaxis (GO:0050927, 5DEGs), and negative regulation of cell proliferation (GO:0008285, 40DEGs) (Table 1).

The top 15 cellular component (CC) of DEGs in GSE115857 was clustered in postsynaptic density (GO:0014069, 27DEGs), cytosol (GO:0005829, 246DEGs), nucleus (GO:0005634, 380DEGs), nucleoplasm (GO:0005654, 207DEGs), endoplasmic reticulum (GO:0005783, 72DEGs), focal adhesion (GO:0005925, 37DEGs), membrane (GO:0016020, 160DEGs), CCR4-NOT complex (GO:0030014, 5DEGs), SNARE complex (GO:0031201, 9DEGs), cell projection (GO:0042995, 11DEGs), nuclear membrane (GO:0031965, 23DEGs), mitochondrion (GO:0005739, 99DEGs), extracellular space (GO:0005615, 100DEGs), phagocytic vesicle (GO:0045335, 7DEGs), intracellular (GO:0005622, 98DEGs), and mast cell granule (GO:0042629, 5DEGs).

The top 15 molecular function (MF) of DEGs in GSE115857 was clustered in protein binding (GO:0005515, 620DEGs), transcription factor activity, sequence-specific DNA binding (GO:0003700, 92DEGs), identical protein binding (GO:0042802, 68DEGs), neurotrophin TRKA receptor binding (GO:0005168, 4DEGs), ATPase activity, coupled to transmembrane movement of substances (GO:0042626, 9DEGs), steroid hormone receptor activity (GO:0003707, 10DEGs), sequence-specific DNA binding (GO:0043565, 47DEGs), transcription factor activity, RNA polymerase II core promoter proximal region sequence-specific binding (GO:0000982, 6DEGs), transcription factor binding (GO:0008134, 29DEGs), zinc ion binding (GO:0008270, 93DEGs), syntaxin-1 binding (GO:0017075, 5DEGs), palmitoyl-CoA hydrolase activity (GO:0016290, 4DEGs), anion transmembrane-transporting ATPase activity (GO:0043225, 4DEGs), RNA polymerase II activating transcription factor binding (GO:0001102, 7DEGs), and beta-catenin binding (GO:0008013, 11DEGs).

The top 15 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway of DEGs in GSE115857 was clustered in Rheumatoid arthritis (hsa05323, 15DEGs), ABC transporters (hsa02010, 10DEGs), TNF signaling pathway (hsa04668, 16DEGs), SNARE interactions in vesicular transport (hsa04130, 8DEGs), HTLV-I infection (hsa05166, 28DEGs), Ubiquitin mediated proteolysis (hsa04120, 17DEGs), Salmonella infection (hsa05132, 12DEGs), Cell adhesion molecules (CAMs) (hsa04514, 17DEGs), Osteoclast

differentiation (hsa04380,16DEGs), NF-kappa B signaling pathway (hsa04064,12DEGs), PI3K-Akt signaling pathway (hsa04151,32DEGs), ECM-receptor interaction (hsa04512,11DEGs), AMPK signaling pathway (hsa04152,14DEGs), Amphetamine addiction (hsa05031,9DEGs), and Chagas disease (American trypanosomiasis) (hsa05142,12DEGs) (Table 2).

PPI network and hub gene analysis

To explore the relationship between these DEGs and to identify hub genes, a PPI network of DEGs was constructed using the STRING online database and visualized using Cytoscape. There were 1199 nodes and 5490 edges in the PPI network, including 760 up-regulated genes, 350 down-regulated genes. In addition, 6 modules from the PPI network were selected using the MCODE plug-in of Cytoscape: module 1 (score=24), consisting of 24 nodes and 276 edges (Figure 4 (a)), module 2 (score=13.04), consisting of 26 nodes and 163 edges (Figure 4 (b)), module 3 (score=8.078), consisting of 52 nodes and 206 edges (Figure 4 (c)), module 4 (score=5.971), consisting of 71 nodes and 209 edges (Figure 4 (d)), module 5 (score=5.051), consisting of 60 nodes and 149 edges (Figure 4 (e)), module 6 (score=4.654), consisting of 53 nodes and 121 edges (Figure 4(f)). Then, GO and pathway enrichment analysis of these module genes were performed by CluGo plug-in of Cytoscape.

For the BP, genes from module 1 were most significantly clustered in positive regulation of mitotic metaphase/anaphase transition (GO:0045842, 5DEGs), there is no BP enrichment in module 2, genes from module 3 were most significantly clustered in organelle fusion (GO:0048284, 15DEGs) and regulation of glomerular filtration (GO:0003093, 4DEGs), genes from module 4 were most significantly clustered in ribonucleoprotein complex export from nucleus (GO:0071426, 10DEGs), genes from module 5 were most significantly clustered in cell adhesion mediated by integrin (GO:0033627, 8DEGs) and maturation small subunit ribosomal RNA (SSU-rRNA) (GO:0030490, 6DEGs), genes from module 6 were most significantly clustered in pentose-phosphate shunt, oxidative branch (GO:0009051, 4DEGs) (Figure 5, Table 3).

For CC, genes from module 1 were most significantly clustered in cullin-RING ubiquitin ligase complex (GO:0031461, 12DEGs), genes from module 2 were most significantly clustered in clathrin-coated vesicle (GO:0030136, 13DEGs), genes from module 3 were most significantly clustered in SNARE complex (GO:0031201, 9DEGs), specific granule (GO:0042581, 15DEGs), phagocytic vesicle (GO:0045335, 11DEGs), and mast cell granule (GO:0042629, 4DEGs) (Figure 6).

For MF, there is no MF enrichment in module 1, genes from module 2 were most significantly clustered in clathrin adaptor activity (GO:0035615, 3DEGs), genes from module 3 were most significantly clustered in SNAP receptor activity (GO:0005484, 7DEGs), glucocorticoid receptor binding (GO:0035259, 3DEGs), and thrombin-activated receptor activity (GO:0015057, 3DEGs), genes from module 4 were most significantly clustered in extracellular matrix structural constituent conferring tensile strength (GO:0030020, 5DEGs), genes from module 6 were most significantly clustered in phosphatidate phosphatase activity (GO:0008195, 3DEGs) (Figure 7).

For KEGG enrichment, genes from module 1 were clustered in ubiquitin mediated proteolysis (KEGG:04120, 15DEGs), genes from module 3 were clustered in soluble NSF Attachment Protein Receptor (SNARE) interactions in vesicular transport (KEGG:04130, 8DEGs), genes from module 4 were clustered in extracellular matrix (ECM)-receptor interaction (KEGG:04512, 8DEGs), there is no KEGG enrichment in module 2,5 and 6 (Figure 8, Table 4).

In the present study, we used cytoHubba to choose hub genes. According to the five classification methods in cytoHubba, the top 30 hub genes selected by these ranked methods in cytoHubba are shown in Table 5. Finally, three central genes were identified by overlapping the first 30 genes, as shown in Figure 9. VEGFA is the most excellent central genes based on five ranked methods. JUN and FOS were also selected as hub genes (Table 6).

GSEA identifies signaling pathways in IMN

We compared the data sets for IMN and living donors using GSEA to identify signaling pathways. The results indicated significant differences (FDR < 0.25, NOM P-value < 0.05) in the enrichment of the MSigDB collection (h.all.v7.1.symbols.gmt). We selected the most significantly enriched signaling pathways, based on normalized enrichment score (NES) (Figure 10, Table 7). The results indicated the data set with IMN was enriched for angiogenesis. 13 core genes were found, including *PTK2*, *SLCO2A1*, *COL3A1*, *FSTL1*, *CCND2*, *SERPINA5*, *VEGFA*, *VAV2*, *OLR1*, *APP*, *POSTN*, *VCAN*, and *NRP1* (Figure 11).

Discussion

In recent years, with the use of vascular endothelial growth factor A (VEGFA) inhibitor in oncology, the number of patients with kidney disease is increasing, mainly manifested as thrombotic microangiopathy, minimal change nephropathy, and collapsing focal segmental glomerulosclerosis [8, 15, 16], however, there is no membrane nephropathy reported. Additionally, rituximab, a B cell-target monoclonal antibody, which has become the first line therapy in IMN according to KDIGO CLINICAL PRACTICE GUIDELINE ON GLOMERULAR DISEASES 2020, showed its ability to decrease the VEGFA level in plasma in patients with recurrent mantle cell lymphoma [17]. These data suggest that VEGFA may get involved in the development of MN. In this study, we identified 1422 genes as DEGs in kidney from IMN patients compared to living donors, 3 hub genes, one up-regulated gene *VEGFA*, two down-regulated genes *JUN* and *FOS*, and six significant modules selected from a PPI network, which partially revealed the molecular mechanism in IMN and may be used to develop novel targets for IMN treatment. Moreover, we found that soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) interactions in vesicular transport involved in the occurrence and progression of PLA2R-associated IMN, which was first reported in this paper.

Angiogenesis is a process of new blood vessel formation from existing vessels, which are essential for the formation of normal kidney structures and for the functions of glomeruli [18]. Abnormal capillary formation in the kidney may cause morphological changes in glomeruli, as well as infiltration of inflammatory cells [19]. Angiogenesis is tightly regulated by a balance of proangiogenic and

antiangiogenic factors, in which the most potent proangiogenic factor, vascular endothelial growth factor A (VEGFA), has been intensively investigated [15]. VEGFA is the predominant isoform of VEGF in humans and one of the most potent proangiogenic factors, which is mainly expressed in and secreted from podocytes [7] and renal tubular epithelial cells in glomeruli [5]. Expression of VEGFA promotes endothelial cell proliferation, migration, and survival, but can also be associated with vascular hyperpermeability in inflammatory conditions including inflammatory kidney diseases via binding to receptor VEGFR2 [20]. Neuropilin 1 (NRP1) functions as a co-receptor for VEGFA and is required for complete activation of VEGFR2 [21]. As VEGFR2 is expressed on the surface of endothelial cells, VEGFA-mediated epithelial-endothelial crosstalk in the glomeruli plays an essential role in numerous renal injury [22]. In the present study, we found that VEGFA and NRP1 are highly expressed in kidney tissue from IMN patients. GSEA analysis confirmed that the up-regulated VEGFA promotes angiogenesis in IMN group. Pathway enrichment analysis found that the biological process induced by VEGFA is associated with PI3K/Akt signaling. In addition to, the biological process of modules 3 was clustered in cell migration involved in sprouting angiogenesis, which is a main type of angiogenesis. Thus, VEGFA activates PI3K/Akt signaling via binding to VEGFR2 to accelerate angiogenesis, and leads to vascular hyperpermeability, which result in increased filtration of inflammatory factors, complement, and cytokines, eventually lead to immune complex (IC) and MAC formation. These results suggest that regulation of the VEGFA/PI3K/Akt signalling pathway may be a treatment strategy for IMN.

Complement-mediated cell injury has been demonstrated to contribute to IMN [4]. With complement activation, the formation of membrane attack complex C5b-9 in plasma membranes on the soles of the foot processes of visceral glomerular epithelial cells, which has been shown to induce kidney cell injury through activating a variety of downstream pathways including focal adhesion kinases [23], lipid metabolism, reactive oxygen species, growth factors/gene transcription, endoplasmic reticulum stress, the ubiquitin-proteasome system [4] and DNA damage [24], and it impacts the integrity of the cytoskeleton and slit diaphragm proteins, and contributes to the pathogenesis of mesangial-proliferative glomerulonephritis, thrombotic microangiopathy, and acute kidney injury [4]. In our study, six modules were found from PPI network. Our finding remains consistent with previous studies. The biological process of genes from six modules were clustered in positive regulation of mitotic metaphase/anaphase transition, regulation of glomerular filtration, cell adhesion mediated by integrin, pentose-phosphate shunt, oxidative branch, intrinsic apoptotic signaling pathway in response to endoplasmic reticulum stress, nucleotide-excision repair, DNA incision, and fatty acid metabolism. The KEGG pathway enrichment was clustered in ubiquitin mediated proteolysis, and ECM-receptor interaction. Thus, it seems that the vascular hyperpermeability induced by VEGFA/PI3K/Akt signaling plays an initial role in the development of IMN (Figure 12).

Notably, we found that SNARE interactions in vesicular transport was involved in the development of IMN, which was the first reported in this paper. Soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins are essential for exocytosis, mediating the fusion of vesicles with their target membrane. Study reported that SNARE proteins mediate fusion of autophagosomes with endolysosomal vesicles, which mediates autophagosome maturation, resulting in autophagy [25]. And inhibiting

autophagosomes induced excessive autophagy ameliorates proteinuria and protect against glomerular and podocyte injury in nephrotic syndrome rats [26]. Moreover, with accumulation of autophagosomes, cell viability altered, which directly induces cellular toxicity [27]. In our report, we found that genes, including *STX6*, *STX3*, *VAMP1*, *VAMP3*, *VAMP4*, *STX1B*, and *SEC22B*, encoding SNARE proteins are highly expressed in kidney biopsies from IMN patients, which means inhibitor of SNARE interaction may provide a new therapeutic strategy in treating IMN, however, it needs to be verified experimentally.

It should be noted that there were some limitations to our research. First, recent study demonstrated that there are three non-HLA loci, namely, *NFKB1*, *IRF4* and *PLA2R1* revealed in IMN, which means that the canonical NF- κ B pathway plays an active role in IMN [28], however, there is no significantly different expression of *NFKB1* and *IRF4* in this study. The shortage may be that the samples from these two groups are too small. Second, focusing on validated target genes may exclude potential targets that have not been validated by the experiment. Finally, due to its descriptive nature, we were not able to validate our hypothesis in this study.

Conclusion

The present study suggests that the PI3K-Akt signaling pathway is involved in the pathogenesis of IMN. VEGFA overexpressed in IMN, and is an important therapeutic target for IMN, which may lead to vascular hyperpermeability via PI3K-Akt signaling pathway, resulting in increased filtration of inflammatory factors, complement, and cytokines, eventually contribute to immune complex (IC) and MAC formation in IMN. Moreover, SNARE interactions in vesicular transport involved in the development of IMN, which needs further basic studies to validate our results and to illuminate the molecular mechanism in IMN.

Abbreviations

PLA2R	The M-type phospholipase A2 receptor
IMN	Idiopathic membranous nephropathy
VEGFA	Vascular endothelial growth factor A
VEGFR2	Vascular endothelial growth factor receptor 2
GEO	Gene Expression Omnibus
DEGs	Differentially expressed genes
DAVID	Database for annotation, Visualization and Integrated Discovery
STRING	Search Tool for the Retrieval of Interacting Genes
GSEA	Gene set enrichment analysis
GO	Gene Ontology
BP	Biological process
CC	Cellular component
MF	Molecular function
KEGG	Kyoto Encyclopedia of Genes and Genomes
MCODE	Molecular Complex Detection
SNARE	Soluble <i>N</i> -ethylmaleimide–sensitive factor attachment protein receptor
ECM	Extracellular matrix
SSU-rRNA	Small subunit ribosomal RNA
PI3K	Phosphoinositide 3-kinase
NES	Normalized enrichment score
FDR	False discovery rate
PPI	Protein–protein interaction
CAMs	Cell adhesion molecules
AMPK	Adenosine 5'-monophosphate (AMP)-activated protein kinase
KDIGO	Kidney Disease Improving Global Outcomes
NRP1	Neuropilin 1
IC	Immune complex
MAC	Membrane attack complex
NFKB1	Nuclear factor kappa B 1

Declarations

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Contributions

Ben Ke and Wen Shen outlined the manuscript, conceived the project and wrote the manuscript. Ben Ke designed and performed data analysis, drafted the manuscript with additional input from all authors and wrote the manuscript. Zhibing Duan designed and performed the data analysis. Ben Ke and Wen Shen performed the data analysis. Ben Ke and Zhibing Duan contributed to the literature search and data collection. All authors read and approved the final manuscript.

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Ethics declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of supporting data

Not applicable.

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Tables

Table 1 The top 15 biological process of DEGs

Description	P Value	Count	Gene Symbol
GO:0006357 regulation of transcription from RNA polymerase II promoter	4.80E-06	54	FOSL2, MED24, MED23, FOXO3, FOS, ATP2B4, TRAK2, SMARCD3, ZNF444, FOSL1, PITX2, NFKBIZ, SATB2, ARID5A, PKN1, RB1, FOSB, HNF4G, FOXJ3, PURA, RBBP8, SLTM, MED7, VEGFA, SMARCAL1, GLIS3, BBS7, TADA3, ZNF367, SOX7, ABCA2, FOXQ1, LYL1, JUND, CHD1, SUPT4H1, NFATC3, TFDP1, MAFG, SREBF1, KAT2A, MAFF, BRD3, TBX3, MAFB, ZMYM6, CREB1, TFCEP2, ECM1, ZBED1, ATF3, RFX1, MAFA, RFX2
GO:0060213 positive regulation of nuclear-transcribed mRNA poly(A) tail shortening	2.20E-05	7	ZFP36, TNRC6C, BTG2, CPEB3, CNOT1, CNOT7, TNRC6B
GO:0007155 cell adhesion	7.30E-04	48	ITGAL, SPG7, CCL2, SCN1B, NINJ2, ITGB5, CLDN10, POSTN, PCDHGC3, NEO1, CD151, PCDHAC2, SCARF1, VCL, APLP1, VCAM1, CD47, LPXN, WISP2, LAMB3, COL6A6, SORBS2, COL12A1, LAMB1, COL8A1, SPON1, DSCAM, ICAM1, SVEP1, ICAM4, PTPRF, ICAM3, ITGA2, CCL4L1, IGFALS, PCDH17, TPBG, SIRPA, COL4A6, ARVCF, CDH13, PRKD2, SIGLEC5, CD34, CX3CR1, NPHS1, DSC2, CFDP1
GO:1900153 positive regulation of nuclear-transcribed mRNA catabolic process, deadenylation-dependent decay	0.001235	6	ZFP36, TNRC6C, CPEB3, CNOT1, CNOT7, TNRC6B
GO:0034097 response to cytokine	0.00135	11	FNTB, FOS, IFI27, REL, JUN, JUND, ITIH4, PML, RARA, BCL2L1, FOSL1
GO:0030198 extracellular matrix organization	0.001421	25	ITGAL, PDGFB, LUM, ADAMTSL4, ITGB5, POSTN, VCAM1, CD47, LAMB3, POMT1, SERPINE1, BCL3, AGRN, LAMB1, COL8A1, VWA1, COL4A4, ICAM1, ICAM4, ICAM3, OLFML2A, ITGA2, NID1, COL4A6, EGFLAM
GO:0007165 signal transduction	0.001834	99	IL9R, TNFSF15, TNFSF14, IL15, CNOT7, CD2AP, ADORA1, UNC5B, CHRNA9, LILRA2, MIER1, PPP1R1B, PDE4B, RARA, CCL4L1, GEM, IL21, TANK, VEGFC, ARRB1, CD34, CSNK1G3, EXT2, ITGAL, CCL2, SP110, MDK, GPR27, TEK, AGRN, NPHP1, MAP2K3, SMAD5, MRC2, SPHK1, NR4A2, NR4A1, ECM1, TRADD, ZNF217, PLCG1, PDE7A, IL5RA, GRB7, PLAU, PDIA3, HINT1, RASSF9, KCNK10, ARHGAP12, LNPEP, WISP2, MKLN1, NR2F6, SHC1, CASP1, LTB, IFNGR1, IRAK1, AVP, ARHGAP28, IGFALS, PKN1, ARRDC3, PRKCD, OR51G2, DAPK1, CD83, TNFRSF10B, TNFRSF10D, ZDHHC13, RIN2, MAP3K10, GNB5, NRG1, RASD1, SRGAP2, THOC1, PRKCZ,

			GULP1, NR3C2, TRIM13, SCARF1, LPXN, IL10RB, PDE1A, PLA2R1, SECTM1, CAP2, CREB1, ANXA1, MAPK11, DPYSL2, RALGDS, TEX2, SYDE1, IGFBP1, IGFBP2, ARAP2
GO:0044344 cellular response to fibroblast growth factor stimulus	0.002221	8	ZFP36, HYAL1, EGR3, CCL2, NR4A1, POSTN, KDM5B, GCLM
GO:0016477 cell migration	0.002833	22	PRKCZ, ARC, FGFR4, FMNL3, CCDC88A, PTPRF, CD151, MDK, SDC2, PDPK1, SDC1, PLCG1, HOXA5, SORBS2, GSK3B, ARPC5L, SH3KBP1, TGFB3, TNN, CSK, USP33, SPATA13
GO:0043123 positive regulation of I- kappaB kinase/NF- kappaB signaling	0.002881	21	IRAK1, SECTM1, F2RL1, TRIM13, PARK2, PIM2, ECM1, TRADD, TRAF3IP2, CASP10, CTH, TNFRSF10B, REL, MIER1, ZDHHC13, TICAM2, TGM2, PELI2, CASP1, IL1A, F2R
GO:0042493 response to drug	0.003154	33	XPO1, SORD, ABCA2, PPOX, GCLM, MDK, HADHA, B2M, FOS, GAD2, JUND, PEMT, DHODH, CCNO, DNMT3B, FOSL1, SREBF1, ICAM1, SLC8A1, CREB1, ANXA1, ITGA2, DPYSL2, FOSB, CPT1A, SS18, VEGFC, JUN, ABCC4, ABCC1, IGFBP2, HTR2A, ABCC6
GO:0014070 response to organic cyclic compound	0.003223	10	KAT2A, FNTB, ICAM1, CD83, G6PD, BTG2, LUM, ABCC4, CPT1A, POLR2A
GO:0009612 response to mechanical stimulus	0.003595	11	CCL2, PTGER4, BTG2, JUN, JUND, POSTN, FOSB, IGFBP2, MBD2, PSPH, FOSL1
GO:0050927 positive regulation of positive chemotaxis	0.003653	5	CDH13, VEGFA, F2RL1, ITGA2, F7
GO:0008285 negative regulation of cell proliferation	0.003913	40	RARRES1, PML, SOX4, SOX7, MXI1, CNOT7, ADORA1, KANK2, LIF, FNTB, CCL3L1, CCL3L3, PEMT, RARA, AXIN2, CSK, FOSL1, IL1A, CTBP1, DAB2IP, NACC2, PDS5B, CGRRF1, JARID2, VHL, KLF10, RB1, PIM2, FRZB, SLIT3, CDH13, TNFRSF9, VEGFC, BTG2, JUN, SCIN, GDF11, RBM38, MDM4, F2R

Table 2 The top 15 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway of DEGs.

Description	P Value	Count	Gene Symbol
hsa05323 Rheumatoid arthritis	0.001819	15	TCIRG1, ICAM1, ITGAL, CCL3, CCL2, IL15, ATP6V1C1, FOS, CCL3L1, JUN, TEK, VEGFA, CCL3L3, LTB, IL1A
hsa02010 ABC transporters	0.002041	10	ABCB8, TAP1, ABCC4, ABCC1, ABCA2, ABCB6, ABCA6, ABCG1, ABCC5, ABCC6
hsa04668 TNF signaling pathway	0.004494	16	ICAM1, CCL2, SOCS3, MAP2K3, CREB1, CREB5, MAPK11, IL15, TRADD, VCAM1, RPS6KA5, LIF, CASP10, FOS, JUN, BCL3
hsa04130 SNARE interactions in vesicular transport	0.006102	8	STX6, STX3, BET1, SEC22B, VAMP4, VAMP3, VAMP1, STX1B
hsa05166 HTLV-I infection	0.009722	28	ITGAL, XPO1, PDGFB, PPP3R2, ANAPC11, IL15, BCL2L1, VCAM1, FOS, POLE3, NFATC3, FOSL1, TBPL1, NFATC1, ZFP36, KAT2A, ICAM1, EGR2, ANAPC5, CREB1, ANAPC4, CDC23, RB1, CDC27, ATF3, CCND2, JUN, GSK3B
hsa04120 Ubiquitin mediated proteolysis	0.018961	17	ANAPC5, SOCS3, VHL, ANAPC4, PML, CDC23, KEAP1, ANAPC11, PARK2, UBE2Q2, CDC27, PRPF19, WWP2, UBA3, SIAH1, PIAS2, CUL4B
hsa05132 Salmonella infection	0.020675	12	FOS, CCL3, CCL3L1, JUN, ARPC5L, CCL3L3, PKN1, CCL4L1, MAPK11, CASP1, IL1A, IFNGR1
hsa04514 Cell adhesion molecules (CAMs)	0.025698	17	ICAM1, ITGAL, PTPRF, VTCN1, ICAM3, CD276, NTNG1, NTNG2, CLDN10, NEO1, CLDN11, SDC2, CLDN15, VCAM1, SDC1, CD34, ICOS
hsa04380 Osteoclast differentiation	0.026566	16	FOSL2, SOCS3, CREB1, PPP3R2, MAPK11, FOSB, SIRPA, FOS, LILRA2, JUN, JUND, FOSL1, IFNGR1, IL1A, SYK, NFATC1
hsa04064 NF-kappa B signaling pathway	0.028321	12	VCAM1, ICAM1, IRAK1, PLCG1, TICAM2, CCL4L1, TNFSF14, BCL2L1, LTB, PLA2, TRADD, SYK
hsa04151 PI3K-Akt signaling pathway	0.050168	32	FGFR4, PDGFB, ITGB5, BCL2L1, FOXO3, PDPK1, LAMB3, COL6A6, TEK, GYS1, TNN, LAMB1, PPP2R2B, SYK, PPP2R1B, COL4A4, SGK1, CREB1, NR4A1, ITGA2, PKN1, CREB5, COL4A6, VEGFC, CCND2, GSK3B, VEGFA, GNB5, EFNA5, THEM4, PPP2R3C, F2R
hsa04512 ECM-receptor interaction	0.061638	11	COL4A4, CD47, LAMB3, SDC1, COL6A6, ITGB5, ITGA2, TNN, AGRN, LAMB1, COL4A6
hsa04152 AMPK signaling	0.063824	14	PPP2R1B, SREBF1, CPT1C, SCD, CREB1, ACACA, CREB5, FOXO3, CPT1A, PDPK1, GYS1, PPP2R2B, RAB10,

pathway			PPP2R3C
hsa05031 Amphetamine addiction	0.06951	9	FOS, ARC, PPP1R1B, JUN, CAMK2G, CREB1, PPP3R2, CREB5, FOSB
hsa05142 Chagas disease (American trypanosomiasis)	0.083357	12	PPP2R1B, IRAK1, FOS, CCL3, CCL2, CCL3L1, JUN, CCL3L3, SERPINE1, MAPK11, PPP2R2B, IFNGR1

Table 3 The most significant biological process enriched for the genes involved in six modules.

Description	<i>P</i> Value	Count	Gene Symbol	Module
GO:0045842 positive regulation of mitotic metaphase/anaphase transition	0.00	5	ANAPC11, ANAPC4, ANAPC5, CDC23, CDC27	Module 1
GO:0048284 organelle fusion	0.00	15	ANXA1, BET1, CPLX2, PRKN, RAB20, RAB8B, SEC22B, STX1B, STX3, STX6, SYT9, TFRC, VAMP1, VAMP3, VAMP4	Module 3
GO:0003093 regulation of glomerular filtration	0.00	4	ADORA1, F2R, F2RL1, PDGFB	Module 3
GO:0071426 ribonucleoprotein complex export from nucleus	0.00	10	AGFG1, MAGOHB, NUP153, NUP155, RAE1, RIOK2, SRRM1, THOC1, WDR33, XPO1	Module 4
GO:0033627 cell adhesion mediated by integrin	0.00	8	ICAM1, ITGA2, ITGB5, LPXN, PLAU, PLPP3, SERPINE1, SYK	Module 5
GO:0030490 maturation small subunit ribosomal RNA (SSU-rRNA)	0.00	6	HEATR1, KRI1, NOL11, RIOK2, SRFBP1, WDR3	Module 5
GO:0009051 pentose-phosphate shunt, oxidative branch	0.00	4	G6PD, PGD, PHGDH, UBL4A	Module 6

Table 4 The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enriched for genes involved in 6 modules.

Description	<i>P</i> Value	Count	Gene Symbol	Module
KEGG:04120 ubiquitin mediated proteolysis	0.00	15	ANAPC11, ANAPC4, ANAPC5, CDC23, CDC27, CUL4B, KEAP1, PML, PRKN, PRPF19, SIAH1, SOCS3, UBA3, UBE2Q2, VHL	Module 1
KEGG:04130 soluble NSF Attachment Protein Receptor (SNARE) interactions in vesicular transport	0.00	8	BET1, SEC22B, STX1B, STX3, STX6, VAMP1, VAMP3, VAMP4	Module 3
KEGG:04512 extracellular matrix (ECM)-receptor interaction	0.00	8	CD47, COL4A4, COL4A6, COL6A6, ITGA2, ITGB5, LAMB1, SDC1	Module 4

Table 5 The top 30 hub genes rank in cytoHubba.

Betweenness	Bottleneck	Eccentricity	EPC	MNC
ALB	ALB	NACC2	ALB	ALB
VEGFA	VEGFA	JUN	JUN	JUN
JUN	EPRS	POLR2A	VEGFA	VEGFA
EPRS	FOS	ANAPC4	FOS	FOS
POLR2A	JUN	RPL10	SOCS3	POLR2A
ALDH18A1	ALDH18A1	CDC27	CCL2	HIST2H2BE
GSK3B	GSK3B	FOS	ICAM1	CREB1
CREB1	CREB1	RPSA	CREB1	CCL2
FOS	RB1	PLCG1	KEAP1	ICAM1
RAB11A	VCL	RB1	POLR2A	EPRS
TFRC	BCL2L1	CASP2	VCAM1	SOCS3
BCL2L1	RAB11A	AXIN2	PARK2	POLR2L
VCL	LMNA	G6PD	HIST2H2BE	TFRC
RB1	CD34	PPP2R1B	VHL	B2M
HIST2H2BE	SLC35A2	HIST2H2BE	BCL2L1	RAB11A
B2M	POSTN	VEGFA	GSK3B	KEAP1
RPSA	SREBF1	CCND2	B2M	BCL2L1
SOCS3	GAD2	CD34	CDC27	HIST1H2BN
G6PD	G6PD	SMARCD3	SHC1	VCAM1
KEAP1	IRAK1	XPO1	SIAH1	RPSA
ARRB1	RTN4	MAPK11	CDC23	CDC27
CTTN	CCL2	DPYSL2	TFRC	GSK3B
CCL2	CYFIP1	RTN4	FBXL19	SHC1
EIF4G1	SPHK1	NR4A2	ANAPC11	ARRB1
SREBF1	KEAP1	KDM1A	CD34	PARK2
CD34	PSMA4	HIST1H2BN	FBXL5	VHL
XPO1	CTTN	CASP1	KCTD6	RPLP0
RPLP0	CCND2	EPRS	ASB6	CTTN

KDM1A	SUCLG2	EFNA5	ANAPC4	FBXL19
SYK	POU5F1	PML	FBXW2	AVP

Table 6 More Information of The Hub genes.

Gene	Function	Auhtors	PMID
VEGFA	Activates VEGFR2 and promotes endothelial proliferation, migration and survival as well as vascular hyperpermeability	<i>Katsuyuki Tanabe, Jun Wada, Yasufumi Sato</i>	32144398
JUN and FOS	AP-1 up-regulate proinflammatory and profibrogenic genes AP-1 transcription activity is associated with fibrosis	<i>S.A. Mezzano, M.Barria, M.A. Droguett</i> <i>Z. Yang, F. Xiong, Y. Wang</i>	11576350 27317945

Table 7 Gene sets enriched in phenotype high.

Name	ES	NES	NOM p-val	FDR q-val
HALLMARK_ANGIOGENESIS	0.4953875	1.7208529	0.012320329	0.1586698

FDR: false discovery rate; NES: normalized enrichment score; NOM: nominal. Gene sets with NOM P-val <0.05 and FDR q-val <0.25 are considered as significant.

Figures

Volcano

Figure 1

Identification of DEGs Volcano plot of DEGs in IMN. The cut-off criteria were $|\log_2 F_c| > 1$ and P value < 0.05 . The red dots represented the up-regulated genes, and the blue dots denoted the down-regulated genes. The grey dots indicated the genes with a $|\log_2 F_c| < 1$ and/or P value > 0.05

Figure 2

The heat map of the top 50 up-regulated genes and the top 50 down-regulated genes, gray indicates a relatively low expression and red indicates a relatively high expression.

Figure 3

Gene Ontology (GO) and KEGG enrichment analysis of DEGs. GO enrichment analysis of DEGs showed (a) biological process (BP); (b) cellular component (CC); (c) molecular function (MF); and (d) KEGG enrichment analysis of DEGs.

Figure 4

6 modules identified by MCODE 6 modules from the PPI network were selected. The red nodes represented the up-regulated genes, the green nodes represented the down-regulated genes: (a) module 1; (b) module 2; (c) module 3; (d) module 4; (e) module 5; (f) module 6.

Figure 5

Biological process enrichment analysis of modules genes.

Figure 6

Cellular component enrichment analysis of modules genes.

Figure 7

Molecular function enrichment analysis of modules genes.

Figure 8

Figure 9

Hub genes identified by different methods. The hub genes in PPI network screened out via intersected by Betweenness, Bottleneck, Eccentricity, EPC, and MNC method.

Figure 10

Signaling pathway activated in kidney biopsies from IMN patients.

Figure 11

Expression profile of core genes involved in angiogenesis in GSE115857. The red nodes represent up-regulated DEGs with a p-value of <0.05 and $\log FC > 1.0$; the green nodes represent down-regulated DEGs with a p-value of <0.05 and $\log FC < -1.0$.

Figure 12

The possible mechanisms by which VEGFA/PI3K/Akt is involved in IMN.