ORIGINAL ARTICLE

A systematic integrative approach reveals novel microRNAs in diabetic nephropathy

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Background: Despite huge efforts, the underlying molecular mechanisms of diabetic nephropathy (DN) are yet elusive, and holistic views have rarely been generated. Considering the complexity of DN pathogenesis, the integration of datasets from different molecular types to construct a multilayer map of DN can provide a comprehensive insight toward the disease mechanisms and also can generate new knowledge. Here, we have re-analyzed two mRNA microarray datasets related to glomerular and tubulointerstitial compartments of human diabetic kidneys. **Materials and Methods:** The quality of the datasets was confirmed by unsupervised hierarchical clustering and principal component analysis. For each dataset, differentially expressed (DE) genes were identified, and transcription factors (TFs) regulating these genes and kinases phosphorylating the TFs were enriched. Furthermore, microRNAs (miRNAs) targeting the DE genes, TFs, and kinases were detected. Based on the harvested genes for glomeruli and tubulointerstitium, key signaling pathways and biological processes involved in diseases pathogenesis were recognized. In addition, the interaction of different elements in each kidney compartment was depicted in multilayer networks, and topology analysis was performed to identify key nodes. Central miRNAs whose target genes were most likely to be related to DN were selected, and their expressions were quantitatively measured in a streptozotocin-induced DN mouse model. **Results:** Among the examined miRNAs, miR-208a-3p and miR-496a-3p are, for the first time, found to be significantly overexpressed in the cortex of diabetic kidneys compared to controls. **Conclusion:** We predict that miR-208 is involved in oxygen metabolism and regulation of cellular energy balance. Furthermore, miR-496 potentially regulates protein metabolism and ion transport. However, their exact functions remain to be investigated in future studies. Taken together, starting from transcriptomics data, we have generated multilayer interaction networks and introduced novel players in DN.

Key words: Diabetic nephropathy, gene expression profiling, gene regulatory networks, microRNAs, systems biology

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INTRODUCTION

Diabetic nephropathy (DN) is the most common complication of diabetes mellitus and a leading cause of end-stage renal disease.[1,2] Although many studies have shown the role of individual genes in DN pathogenesis,[3] the molecular mechanisms of this divesting disorder are not fully understood. Systems biology provides an invaluable opportunity to process omics-scale data to obtain a holistic view of the complex interactions underlying chronic diseases. Although this approach has been employed in a few recent studies on DN,[4,5] the construction of multilayer networks has rarely been performed. Using multi-omics integration approaches, the flow of biological information can be explored and also novel interacting genes that drive DN disease can be identified.[6]

In this study, we reanalyzed two microarray datasets of kidney glomerular and tubulointerstitial compartments. The differentially expressed (DE) genes were identified and exploited to infer transcription factors (TFs), kinases, and microRNAs (miRNAs) related to this disorder. Finally, the multilayer interaction maps of these different elements were constructed and analyzed to identify the

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central nodes and predict the main signaling pathways and biological functions. Considering the key function of miRNAs in the pathogenesis of DN,[7-9] a systematic framework is followed to select important miRNAs in the integrative networks of kidney glomerule and tubulointerstitium and the expression alternation of two novel miRNAs is experimentally confirmed in an animal model of DN. This study suggests an innovative strategy for the investigation of complex disorders to identify novel players. The approach employed in this study is shown in Figure 1.

MATERIALS AND METHODS

Microarray data analysis

GSE30528 and GSE30529 datasets deposited by Woroniecka *et al.*,[10] were obtained from the Gene Expression Omnibus (GEO) database.[11] GSE30528 includes the mRNA expression profiles of kidney glomeruli from 9 DN patients and 13 healthy individuals. GSE30529 contains the data of kidney tubulointerstitium from 10 DN and 12 controls. To evaluate microarray data quality, we performed unsupervised hierarchal clustering using ClusterMaker[12] application of Cytoscape 3.5.1[13] and principal component analysis (PCA) using ggplot2 package[14] of R software. GEO2R tool of GEO database was used for the identification of DE genes, and *P* value was corrected using Benjamini–Hochberg method. Volcano plots were prepared using the ggplot2 R package.

Enrichment analyses

To identify the regulators of the DE genes, TF enrichment analysis was performed using ChEA tool of Enrichr database.[15] Furthermore, kinase enrichment analysis was performed using KEA application of Enrichr to harvest kinases that regulate the identified TFs. In addition, gene ontology (GO) terms related to all gene sets were identified by GO term enrichment analysis using ClueGO v3.2.2 plugin of Cytoscape[16] and the parents of enriched GO terms were detected using REVIGO tool.[17] In addition, pathway enrichment analysis was performed by ClueGO, which retrieved data from KEGG and REACTOME databases with medium network specificity. In addition, the ggplot2 R package was used to visualize the enriched pathways as scatter plots. Rich factor was calculated as the number of genes enriched in a certain pathway relative to the total number of genes in the pathway. miRNA enrichment analysis was done using TargetScan 2017 application of Enrichr. For all these analyses, adjusted $P \leq 0.05$ was considered as statistical significance threshold.

Molecular interaction network

Cytoscape CluePedia v1.3.3[18] application was used to construct protein–protein interaction networks encompassing DE genes, TFs, and kinases. For network construction, the interaction confidence cutoff was set at 0.6, and only edges with experimental validation evidence were retrieved from STRING v10.0.[19] Next, miRNA-target interactions were merged into the networks. Network topology analysis was performed using Network Analyser tool of Cytoscape.

MicroRNA-mRNA interaction

The predicted and validated targets of selected miRNAs were harvested using TargetScan[20] and miRTarBase,[21] respectively. The miRNA seed conservation was evaluated using TargetScan.

RNA extraction

The DN animal model was established using multiple low dose of streptozotocin injection, and the model was validated using biochemical and histopathological assessments in our previous study (unpublished). The kidney tissues of DN and normal mice were lysed with RNX (CinnaGen,

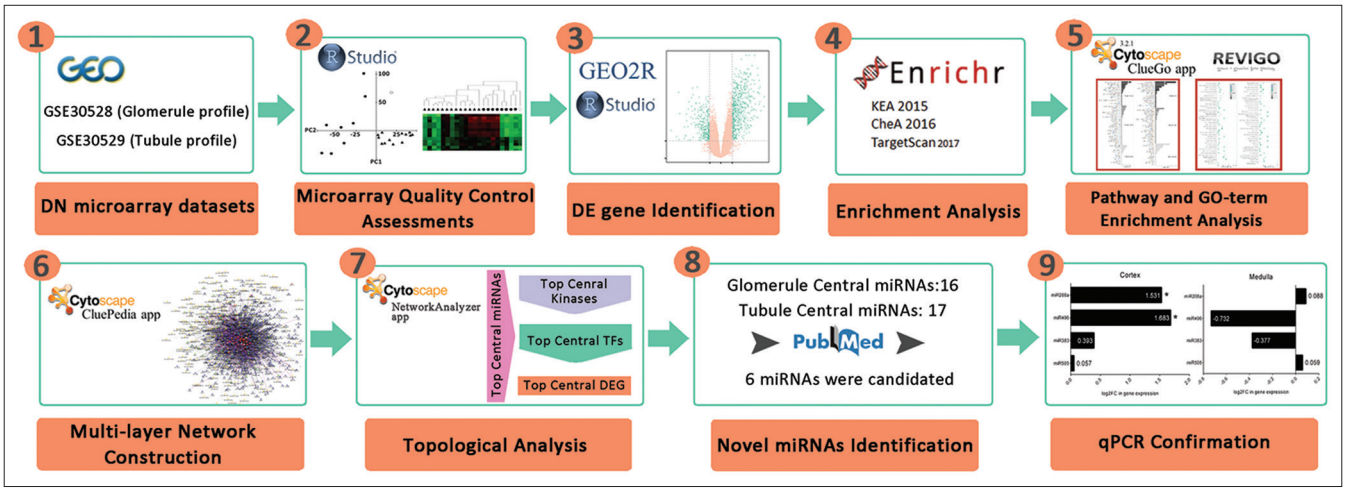

Figure 1: The schematic representation of proposed approach. In this study, we employed a holistic integrative approach to identify novel player in diabetic nephropathy pathogenesis

Tehran, Iran) using the micro smash machine (TOMY Digital Biology, Tokyo, Japan). The homogenized tissues were transferred to new tubes, and 250 µl of chloroform (Merck, Darmstadt, Germany) was added and incubated at room temperature for 15 min. Then, samples were centrifuged at 4°C, 12,000 rpm for 20 min. The supernatant was transferred to new tubes, and 100% cold ethanol (Merck, Darmstadt, Germany) was added and gently mixed. The samples were stored overnight at −20°C. Next, samples were centrifuged at 4°C, 14,000 rpm for 45 min, and 1 ml 70% cold ethanol was added to the platelets. Samples were centrifuged at 4°C, 12,000 rpm for 10 min. Then, the supernatant was discarded, and 50 µl of distilled water was added to the platelets.

cDNA synthesis

For cDNA synthesis, 10 µl RNA and 1 µl of specific RT primer for each miRNA were mixed, and double-distilled water was added up to a total volume of 13.4 µl. The tubes were placed at 70°C for 5 min and then mixed with 4 µl first strand buffer, 1 µl dNTP, 0.5 µl RNase, and 1 µl reverse transcriptase M-MLV enzyme (YektaTajhiz, Tehran, Iran). The samples were incubated in polymerase chain reaction (PCR) machine (Eppendorf, Hamburg, Germany) at 37°C for 60 min, followed by 70°C for 5 min.

Quantitative polymerase chain reaction

Specific primers were designed [Supplementary Table 1] using AlleleID[22] and GeneRunner,[23] and their specificity was assessed using the NCBI-BLAST database sno202 and sno234 were chosen as references. For real-time PCR, 1 µl cDNA, 5 µl high ROX™ SYBR Green master mix (Ampliqon, Herlev, Denmark), 0.5 µl forward and 0.5 µl reverse primers, and 3 µl double distilled water were mixed and then the reaction was carried out using Applied Biosystems Real-Time machine (Carlsbad, USA). The temperature profile consisted of an initial step at 95°C for 15 min and then 40 cycles at 95°C for 15 s and 60°C for 1 min. REST software[24] was used to analyze the results.

RESULTS

In order to explore DN transcriptomics profile, two microarray datasets related to human kidney glomerular (GSE30528) and tubulointerstitial (GSE30529) compartments were retrieved, and their quality was assessed using PCA and hierarchical clustering. Most samples were separated according to the study groups in an unsupervised manner, indicating the acceptable quality of both datasets. However, few samples not following the expected segregation were excluded to enhance the reliability of downstream analyses [Figure 2a and b]. Genes with adjusted $P \leq 0.05$ and absolute logarithmic fold change ($|\log_2 FC|$) ≥1 were assumed as DE [Figure 2c and d].

In the glomerule dataset, we identified 709 DE genes. TF enrichment analysis revealed that 61 TFs potentially regulating these genes. In turn, these TFs are proposed to be controlled by 91 kinases according to kinase enrichment

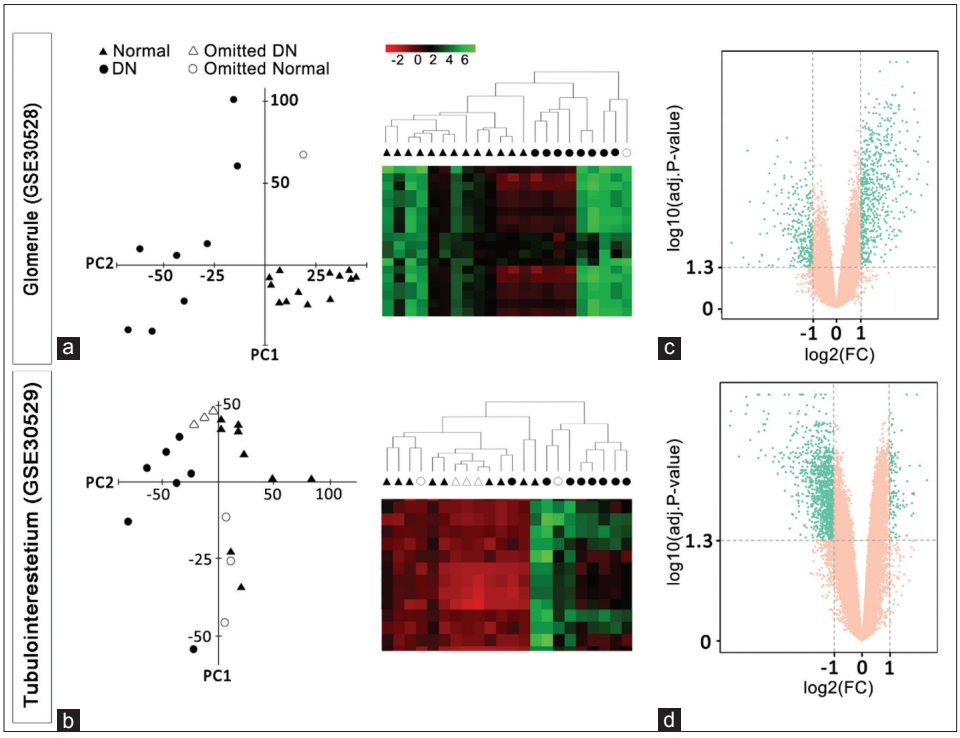

Figure 2: Datasets quality assessments. Principle component analysis and hierarchical clustering with all genes revealed an acceptable quality of both microarray datasets (a and b). The genes with adj. p-value ≤ 0.05 and $|\log FC| \geq 1$ are considered as differentially expressed (DE) and depicted as green dots in the volcano graphs (c and d)

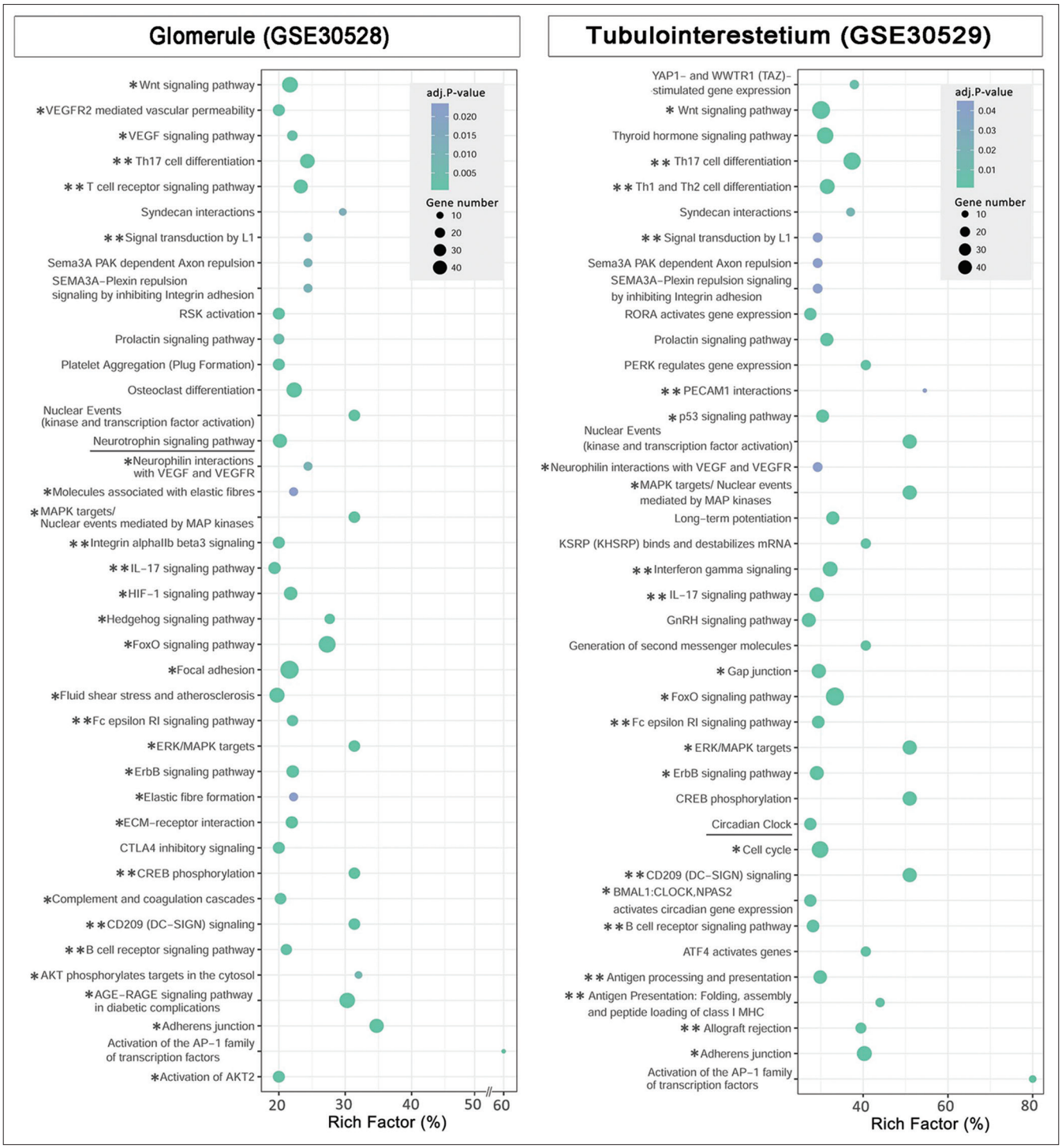

Figure 3: Signaling pathways related to the glomerule and tubulointerstitium networks. Pathway enrichment analysis was performed with the differentially expressed genes, transcription factors, and kinases in each network. The horizontal axis is rich-factor, and pathways with adjusted $P \leq 0.05$ are shown. The pathways with one star are known to be involved in the pathogenesis of diabetic nephropathy. Specifically, the pathways associated with immune response and inflammation are marked with double starts. The underlined pathways have not been previously described to be involved in diabetic nephropathy

analysis. Furthermore, 174 miRNAs regulating the DE genes, TFs, and kinases were identified by miRNA enrichment analysis [Supplementary Table 2]. Similarly, 1372 DE genes, 91 TFs, 92 kinases, and 181 miRNAs were determined for the tubule dataset [Supplementary Table 3]. In order to avoid the bias caused by the focus of previous studies on certain sets of miRNAs, the TargetScan algorithm was used for miRNA enrichment, which is based on bioinformatics predictions rather than experimental data for miRNA-mRNA interactions.

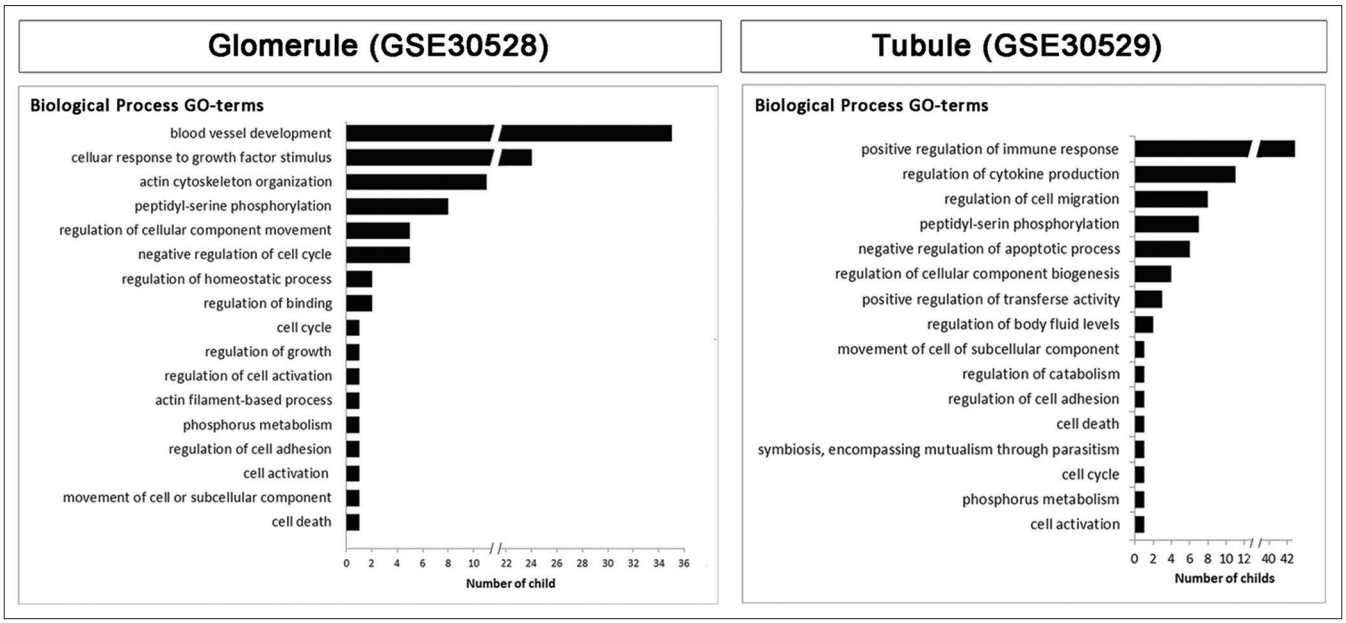

Figure 4: The ontology of the nodes in the glomerule and tubulointerstitium networks. Gene ontology enrichment analysis was performed with the differentially expressed genes, transcription factors and kinases in each network. Gene ontology biological process parents are illustrated. The horizontal axis is the numbers of children for each parent term. adjusted $P \leq 0.05$ is considered as the threshold of statistical significance

In order to explore the key underlying molecular and cellular phenomena in DN, the signaling pathways associated to DE genes, TFs, and kinases were obtained by pathway enrichment analysis [Supplementary Tables 4 and 5]. A considerable fraction of the pathways with the highest rich factors is related to immune responses and tissue fibrosis [Figure 3]. In addition, some well-known pathways in DN, including hypoxia, FoxO, VEGF, and AGE-RAGE signaling pathways, as well as platelet aggregation[25-28] are identified. In addition, ErbB signaling pathway is enriched in the glomerule dataset, which is in line with the identification of EGF as a promising urinary DN biomarker.[29] As expected, the complement cascade,[30] which is underscored by the initial developers of the current datasets, is among the top pathways in the glomerule dataset. Interestingly, the Hedgehog signaling pathway whose role in DN is just recently shown is also enriched.[31] In addition to the previously recognized pathways, some novel interesting ones such as circadian clock and neurotrophin pathway are also enriched. Although the importance of the circadian rhythm in normal kidney function and nephrectomy-induced fibrosis is just studied,[32] its involvement in DN remains an interesting topic for future studies. Similarly, neurotrophin signaling is studied in diabetes, and its some other complications,[33] however, to the best of our knowledge, it is not yet investigated in DN. Moreover, the GO terms were identified and summarized as parent terms [Figure 4 and Supplementary Figures 1 and 2]. Interestingly, the majority of biological process terms for the glomerule and tubule genes are related to blood vessels and immune responses, respectively. This is in accordance with the histopathological features of DN, including glomerular capillary injuries and tubulointerstitial inflammation.

The harvested DE genes, TFs, kinases, and miRNAs were employed to construct four-layer molecular interaction networks for the glomerule and tubulointerstitial sets. The connected networks were analyzed, and topology parameters were determined [Supplementary Tables 6 and 7]. It is supposed that the central genes in protein–protein interactions are critical for disease pathogenesis.[34,35] Hence, we identified the nodes with the highest degree and betweenness centralities in DE gene, TF, kinase, and miRNA layers of the glomerule and tubule networks [Figure 5].

Considering the critical role of miRNAs in DN pathogenesis,[7] we focused on the most central 16 and 17 miRNAs identified in the networks of glomerule and tubulointerstitium, respectively. Remarkably, among them is miR-21, a well-known player in DN.[36] To concentrate on miRNAs that are most likely involved in DN, the validated and predicted targets of each miRNA were determined and compared with the list of genes known to be involved in DN manually retrieved from literature [Supplementary Table 8]. The targets of miR-505-3p, miR-590-3p, miR-496a-3p, miR-208a-3p, miR-921, and miR-383-5p had the most overlap with DN-related genes. We hypothesized that these miRNAs are involved in DN albeit not described by previous investigators. Therefore, we planned to experimentally assess their expressions in diabetic kidneys. A mouse model of DN previously established in our laboratory was exploited, and after RNA extraction and miRNA-specific cDNA synthesis, the expression of the candidate miRNAs was quantified. Notably, for miR-921 no ortholog is reported in mouse, and quantitative PCR with human primers was not successful. Five miRNAs which have murine ortholog with

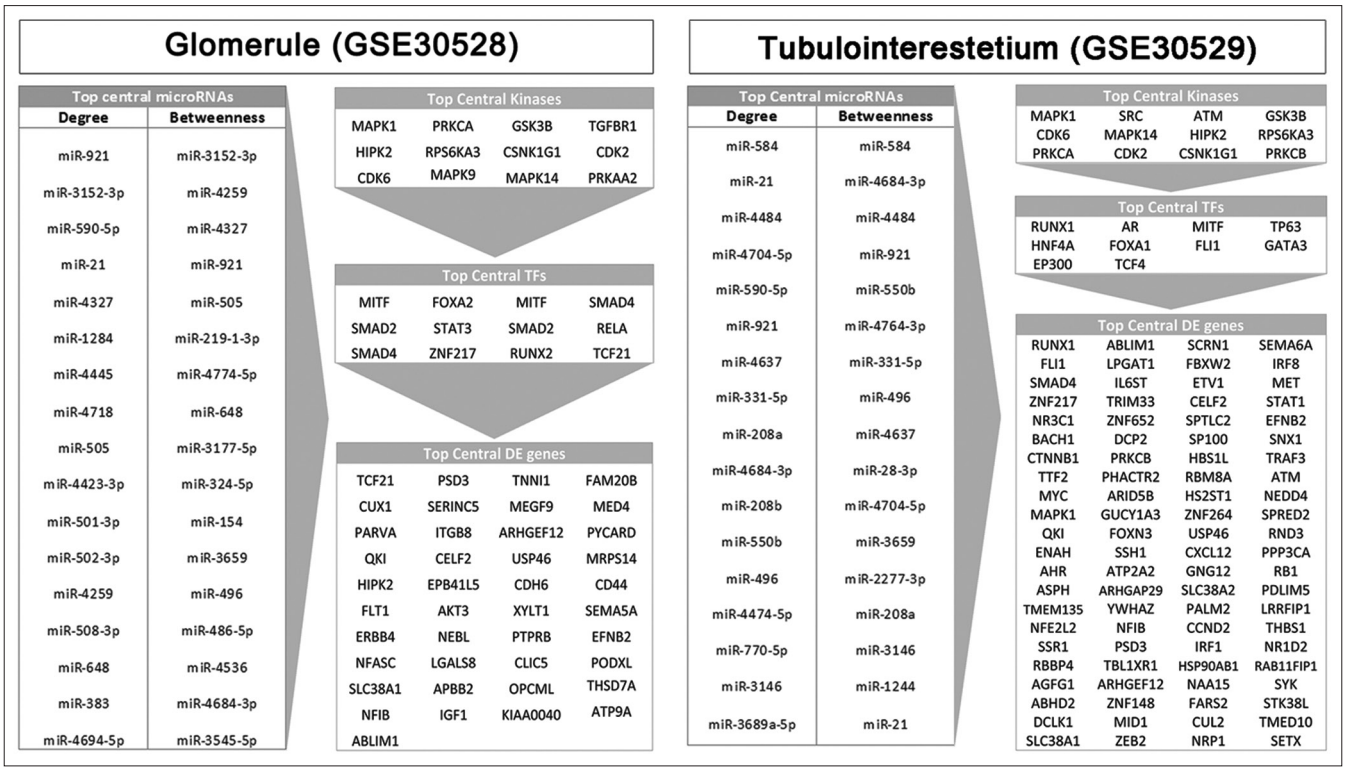

Figure 5: Central nodes in the glomerule and tubulointerstitium multilayer networks. The topology of the networks is analyzed, and top 5% differentially expressed genes as well as top 10% transcription factors, kinases, and microRNAs that are most central based on degree and betweenness are shown

conserved seeds were used for specific primer design. In the examined kidney tissues, miR-590-3p was undetectable. The expressions of miR-505-3p, miR-496a-3p, miR-208a-3p, and miR-383-5p were measured in the cortex and medulla compartments [Figure 6a]. Remarkably, miR-208a-3p and miR-496a-3p were almost three-fold overexpressed in the cortex of diabetic kidneys ($P \leq 0.05$).

The role of miR-208a-3p and miR-496a-3p in DN has not been investigated so far. In order to predict the biological processes in which these two novel miRNAs are involved, the ontology of their validated targets was determined [Figure 6b]. Among the GO terms enriched for miR-208a-3p are well-known DN-associated cellular functions including oxidative stress,[37] response to oxygen levels,[37] carbohydrate and protein metabolism,[38] response to nutrient levels,[39] regulation of transforming growth factor-beta signaling pathway,[40] apoptosis,[37] and tissue remodeling.[41] Furthermore, miR-496a-3p is mainly related to transcriptional, translational, and posttranslational gene expression control and anion transmembrane transport.

DISCUSSION

Chronic noncommunicable diseases are the main challenge of current medicine. Systems biology with its holistic view may assist to reveal the complex pathogenesis of these disorders. In order to provide an inclusive map of DN molecular pathogenesis, we have here re-analyzed two microarray datasets initially generated by Woroniecka *et al*.[10] These datasets have prominent advantages such as being derived from human subjects and separate profiling of glomerular and tubulointerstitial sections. Indeed, the diverse expression profiles of different anatomical kidney compartments are shown in previous studies.[42] Moreover, we have shown that these datasets fulfill the quality control criteria.

Biomedical phenomena are the result of complex interactions between thousands of molecules from different entities. Although data integration approach is underscored as a critical step to generate inclusive maps of complex biomedical phenomena,[43] it is commonly ignored in omics data analysis. In this study, the primary data were at the transcriptome level, and other potential interacting levels such as TFs, kinases, and miRNAs were predicted to generate multilayer networks. These integrated elements were found to be potentially involved in well-known DN-associated pathogenic processes such as Wnt, VEGF, FoxO, hypoxia, P53, AGE-RAGE signaling pathways, angiogenesis, and immunological reaction.[44-51] Pathway enrichment analysis also suggests that circadian clock is important in this disorder. This idea is not yet comprehensively investigated and can be an interesting subject for future studies.

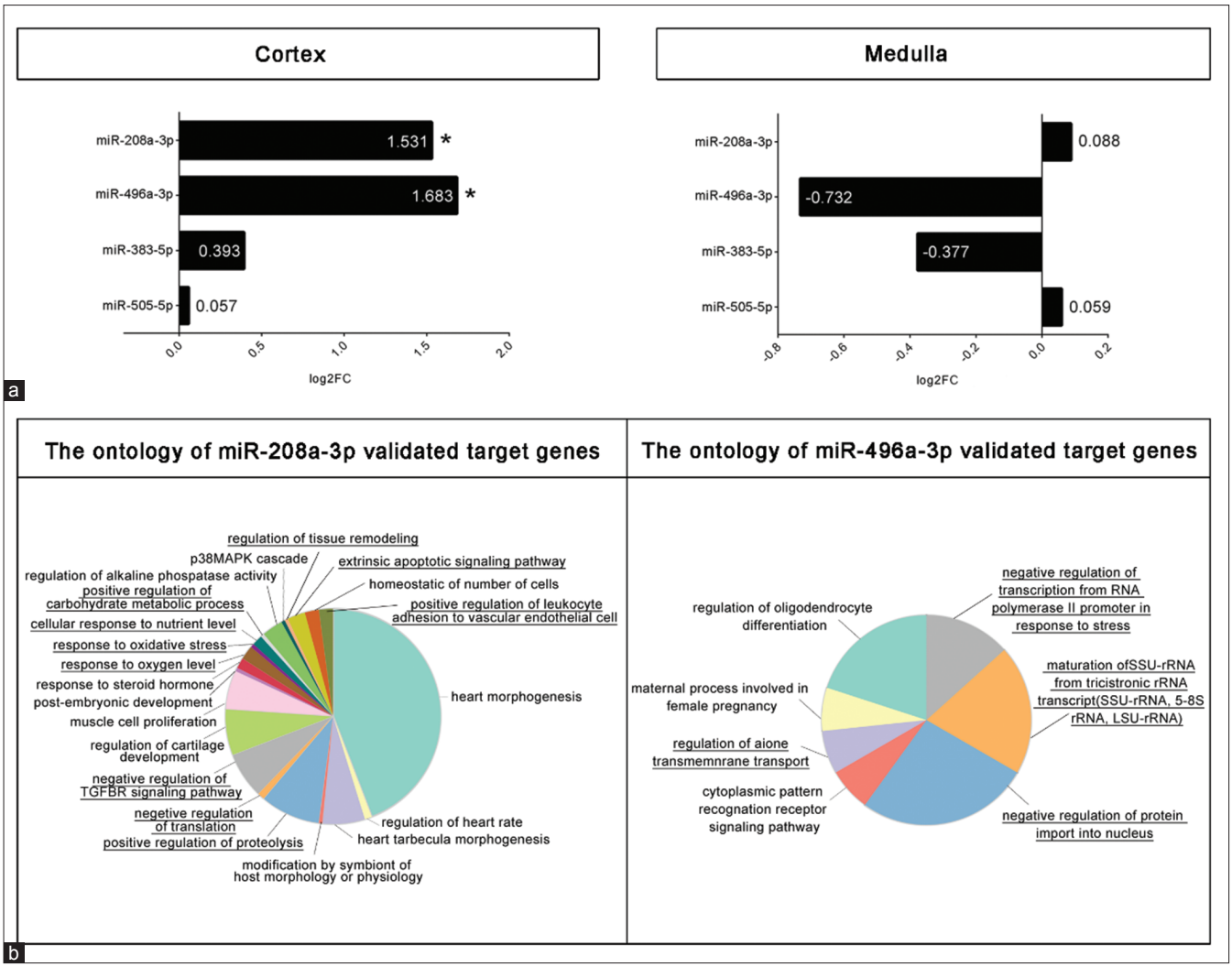

Figure 6: Expression assessments and functional analysis. The expressions of selected microRNAs with the greatest centrality values were assessed by quantitative polymerase chain reaction. Asterisks indicate $P \leq 0.05$ (a). The Gene ontology biological process terms enriched with the validated targets of miR-208a-3p and miR-496b-3p are demonstrated. The terms that are most related to diabetic nephropathy are underlined (b)

Considering the pivotal role of miRNAs in the regulation of DN pathogenesis, we focused on the miRNA layer of the networks. miRNAs with the greatest network centrality whose target genes were most likely to be involved in the disease were selected for the experimental study. Gene expression quantification in tissue samples of an experimental animal model revealed that miR-208a-3p and miR-496a-3p are significantly overexpressed in the kidney cortex in DN. To the best of our knowledge, this is the first time that the involvement of these two miRNAs in DN is reported.

GO term enrichment analysis revealed that miR-208a-3p could be involved in response to oxygen levels and metabolic pathways, indicating the plausible key function of this miRNA in cellular energetics. In agreement with this assumption, miR-208 is extensively shown to be involved in myocardial ischemia.[44-48] In addition, circulating miR-208a is a candidate biomarker of coronary artery diseases.[44,45] Considering the fact that chronic kidney disease significantly increases the risk of cardiovascular disorders,[49] it is interesting to investigate if miR-208a secreted from injured kidneys can partly mediate this association. Remarkably, it is shown that miR-208 is associated with angiotensin-mediated blood pressure control in the heart.[46] Based on GO results, miR-496 is related to protein metabolism. In addition, this miRNA is reported to be involved in cell proliferation,[50] aging,[51] apoptosis,[52] and response to vasopressin in kidney collecting ducts.[53] However, it is a new identified miRNA, and further studies are definitely required to disclose its functions.

Taken together, based on a system approach, we have explored the underlying molecular mechanisms of DN and proposed two novel miRNAs. The top-down framework exploited in this study is of potential value for the investigation of other complex disorders.

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Conflicts of interest

There are no conflicts of interest.

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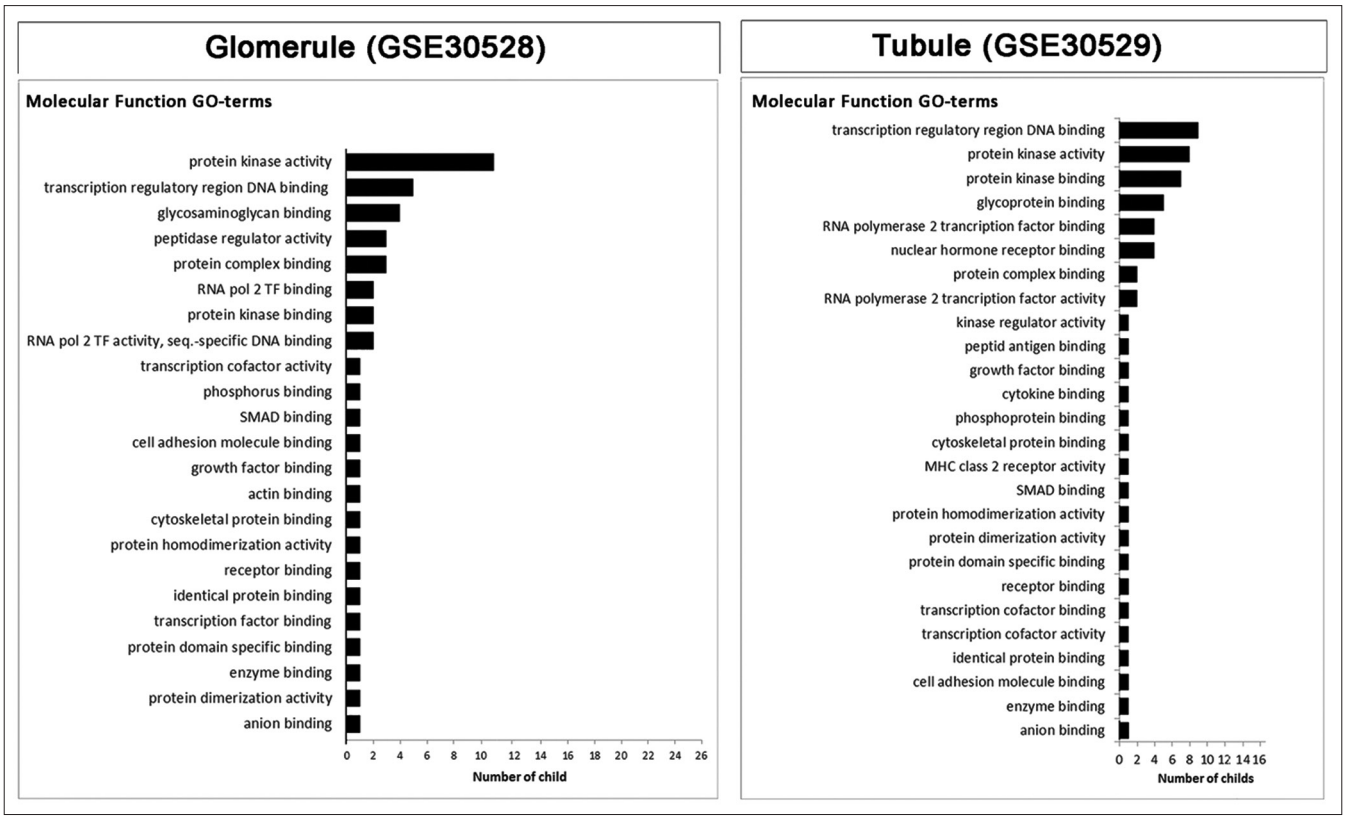
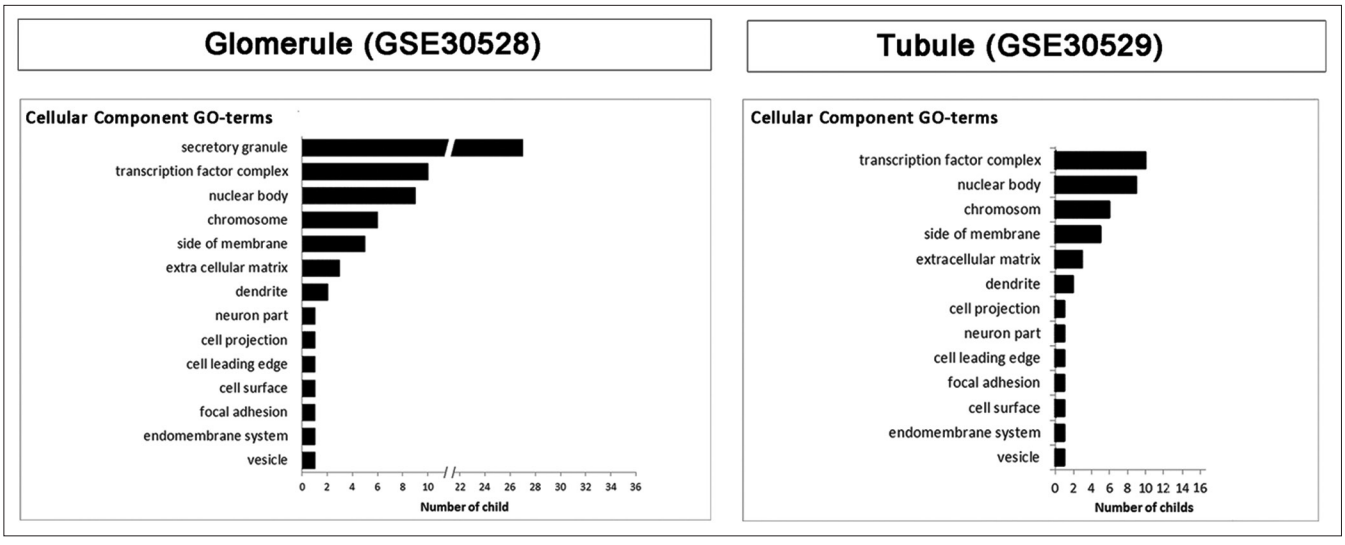


Supplementary Figure 1: The ontology of the nodes in the glomerule and tubulointerstitium networks. Gene ontology enrichment analysis was performed with the differentially expressed genes, transcription factors and kinases in each network. Gene ontology molecular function parents are illustrated. Horizontal axis is the numbers of children for each parent term. adjusted $P \leq 0.05$ is considered as threshold of statistical significance



Supplementary Figure 2: The ontology of the nodes in the glomerule and tubulointerstitium networks. Gene ontology enrichment analysis was performed with the differentially expressed genes, transcription factors and kinases in each network. Gene ontology cellular component parents are illustrated. Horizontal axis is the numbers of children for each parent term. adjusted $P \leq 0.05$ is considered as threshold of statistical significance

Supplementary Table 1: Specific primers were designed to evaluate the expression of candidate miRNAs

miR name	Sequence
miR-921	RT: GTCGTATGCACAGCAGGGTCCGAGGTATTCGCAGTGCATACGACGAATCC
	F: CTAGTGAGGGACAGAACCA
	R: CAGCAGGGTCCGAGGT
miR-505	RT: GTCGTATGCACAGCAGGGTCCGAGGTATTCGCAGTGCATACGACAACATC
	F: AGGGAGCCAGGAAGTATT
	R: CAGCAGGGTCCGAGGT
miR-590-5p	RT: GTCGTATGCACAGCAGGGTCCGAGGTATTCGCAGTGCATACGACCTGCAC
	F: GGTCCGAGCTTATTCATAAAA
	R: CAGCAGGGTCCGAGGT
miR-383-5p	RT: GTCGTATGCACAGCAGGGTCCGAGGTATTCGCAGTGCATACGACAGCCAC
	F: GGCGAGATCAGAAGGTGACT
	R: CAGCAGGGTCCGAGGT
miR-208a-3p	RT: GTCGTATGCACAGCAGGGTCCGAGGTATTCGCAGTGCATACGACACAAGC
	F: GCCGATAAGACGAGCAAAAA
	R: CAGCAGGGTCCGAGGT
miR-496a-3p	RT: GTCGTATGCACAGCAGGGTCCGAGGTATTCGCAGTGCATACGACGAGATT
	F: GCGTGAGTATTACATGGCC
	R: CAGCAGGGTCCGAGGT

Supplementry Table 8: DN-associated genes are manually retrived from literature. For each miRNA, validated and predicted tragets known to be DN-associated are listed.

DN assosiated genes
Vegf
Fox
Hif
Cyp
Ace2
Adipoq
Agt
Akr1b3
Akt1
Bdkrb1
Col1a1
Col2a1
Col3a1
Col4a1
Ctgf
Fn1
Icam1
Jun
Lep
Lepr
Mapk14
Mmp9
Nos3
Pdgfb
Pdgfc
Pdgfd
Ppara
Ppp2ca
Prkca
Pten
Serpine1
Smad2
Smad3
Smad7
Sod2
Sod3
Spp1
Srebf1
Tgfb2
Tgfbr1
Tgfbr2
Tgfbr3
Tnf
Zeb1
Yap
Foxn
Pdcd4
Mmp9
Ogg
Ros

Glomerule Compartment

microRNA	Common genes between validated targets and DN gene list	Common genes between predicted targets and DN gene list
hsa-miR-590-5p	TGFBR2,SMAD3,SMAD7,FOXN2,FOXO3,PDCD4,TGFB1	PPP3CA,SERP1
hsa-miR-921	ANGPTL1,FOXN3,TNFAIP8L1	MAP2K6,MAPK1
hsa-miR-505	PRKCA,ACER2,COL4A1,FOXE1	MAPK1IP1L,PTEN,TNFSF11
hsa-miR-383	ADIPOQ,AGTRAP,ANGEL2,ANGPT4,COL8A1,CYP20A1,CYP51A1	PRKAG1,VEGFA
hsa-miR-3152-3p	PPP2CA,MAPK10,PPP1R16B	SMAD2,TGFBR1,ADI1,MMP16,TGFBR1,TNFSF14
hsa-miR-4259	COL18A1	CYP20A1,HIP1,TNFRSF14
hsa-miR-4327	HIF1AN,LEPROT,MAPK1IP1L	PTEN
hsa-miR-4445	SOD2	PPP2CA
hsa-miR-1284	FGF2	AKTIP
hsa-miR-4718	IGF2BP1,PRKCB	ACER3,MAP2K6
hsa-miR-4423-3p	IGF1R,PPP1R2	AGTRAP,PPP4R1L
hsa-miR-501-3p	SOD2,COL23A1,CYP4F11	COL10A1,PPP2R2C,PPP2R5E,PPP4R2
hsa-miR-502-3p	SOD2	
hsa-miR-508-3p	FLOT2,PPP1R15B	
hsa-miR-3682-3p		COL4A4,MAP2K6,PPP1R12B
hsa-miR-4694-5p		FOXN3,PRKCA,PTEN

Tubulointerstitium Compartment

microRNA	Common genes between validated targets and DN gene list	Common genes between predicted targets and DN gene list
hsa-miR-208a	LEP,CYP1B1,FOXP1,MAK16,MAP3K5,MAPK10,	TGFBR1,COL4A3,FNIP1,FOXG1,FOXP2,MAP3K2,MMP16,PPP3CB,PRKAR1A,ZEB2
hsa-miR-921	ANGPTL1,FOXN3,PRKG1,TNFAIP8L1	MAPK1
hsa-miR-496	AKT1,COL19A1,FOXA1,FOXN2,LEPROTL1,MAPK8,PPP6C	TGFBR2,FOXN2,PPP6C,TNFRSF10D
hsa-miR-590-5p	TGFBR2,SMAD3,SMAD7,FOXN2,FOXO3,TGFB1	PPP3CA,SERP1
hsa-miR-3146	IGF2R,PPP1R15B	COL4A4,SMAD9
hsa-miR-331-5p	SOD2,PPP1R1A	MAP2K6,MAP3K1,PDGFD,PRKAB2,SMAD2
hsa-miR-4484	MAPKAPK5	SOD3,FOXE1,SMAD4,SOD3,
hsa-miR-4637		TMEM236,PPP1R2
hsa-miR-4684-3p	SOD2,FOXN3,PPP1R3G	PPP1R15B,PRKAA2,ZEB2
hsa-miR-4704-5p	PPP3R1	MAP2K4,SMAD9
hsa-miR-550b	MAP10,TNFSF15	
hsa-miR-584	PPP2CA,FOXA1,IGFBP5,MAPK1	PPP2CA
hsa-miR-208b	LEP,CYP1B1,FOXP1,MAP3K5,MAPK10	COLQ,FNDC3A,PPP6C
hsa-miR-4474-5p	SOD2,COL23A1,HIF1AN	COLEC10,SMAD4
hsa-miR-770-5p	MAP9	PPP1R12B
hsa-miR-3689a-5p	COL19A1,TNFAIP1,	COL4A4,MAP2K6,PPP1R12B
hsa-miR-4445	SOD2	