ORIGINAL ARTICLE

Prediction of mRNA Targets of miR-101-3p in Diabetic Kidney Disease by Bioinformatics Tools

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ABSTRACT

Introduction: Diabetic kidney disease (DKD) remains the leading cause of chronic kidney disease (CKD) worldwide. Current biomarkers and treatment still fall short at preventing its progression. In search for a better diagnostic or therapeutic target, much interest in microRNAs, which act as post-translational regulators of gene expression has emerged. An upregulation of miR-101-3p was identified in the sera of type 2 diabetic patients with macroalbuminuria in a selected Malaysian population by profiler RT-PCR array. Using bioinformatics tools, this study aimed to predict the mRNA targets of miR-101-3p. Given the scarcity of bioinformatics studies in DKD, this study also attempted to fill the gap. **Methods:** The mRNA targets were identified from two experimentally validated databases, namely Tarbase and MirTarBase. The commonly identified mRNA targets were submitted to Metascape and Enrichr bioinformatic tools. **Results:** A total of 2630 and 342 mRNA targets of miR-101-3p were identified by Tarbase and miRTarbase, respectively. One-hundred ninety-seven (197) mRNA targets were submitted for functional enrichment analysis. Our bioinformatics and bibliographical analyses suggested that ras-related C3 botulinum toxin substrate 1 (RAC1) and Ras-associated protein-1 b (RAP1b) were the most promising putative mRNA targets of miR-101-3p. The most enriched Gene Ontology term and pathway associated with these putative mRNA targets included Ras protein signal transduction and focal adhesion, respectively. Based on these analyses, their molecular mechanisms were proposed. **Conclusion:** Given the structural heterogeneity of the kidneys and cell type-dependent miRNA modulation, an *in-silico* target prediction of miR-101-3p increases the probability of a successful future *in-vitro* experimental verification.

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Keywords: Diabetic kidney disease, miR-101-3p, mRNA targets, Prediction, Bioinformatics

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INTRODUCTION

Diabetic kidney disease (DKD) is the leading cause of chronic kidney disease (CKD) worldwide, driven by the increasing prevalence of diabetes mellitus (DM) (1). CKD and its sequel, the end-stage-kidney disease (ESKD) constitute higher mortality rates, mainly via cardiovascular disease and is a major cause of morbidity and economic burden (2). The complex pathogenesis of DKD is not fully understood and is still evolving (1). Despite growing knowledge, current biomarkers and treatment still fall short at preventing its progression (1). Hence, there is a need to further unravel the underlying mechanisms of DKD in hope of a better diagnostic or therapeutic target.

Recently, epigenetic modification has been implicated in the early stages of DKD and the phenomenon of metabolic memory which contribute towards self-perpetuation of diabetic complication pathways (3). Epigenetic modification includes microRNAs, a family of small non-coding RNAs which vary from 19 to 22 nucleotides in length, which act as post-translational regulator of gene expression (4). The 5′ end of miRNA binds within the 3′ untranslated region (UTR) of the mRNA target with often a perfect Watson-Crick complementarity to its seed sequence region (4). A perfect complementary binding leads to mRNA destabilization whilst a partial one leads to translational repression (4). Partial complementarity also allows each miRNA to target several mRNAs and likewise, each

mRNA is a potential target of several miRNAs (5).

Identification of their mRNA targets is pivotal in understanding such complex network of regulatory roles of miRNAs in gene expression (5). This may be achieved by bioinformatics analysis using sequence-based and experimentally-validated miRNA:mRNA interaction databases (6). The commonly used web-based databases of the former type include TargetScan (www.targetscan.org) and miRANDA (www.microrna.org). To predict their mRNA targets, these tools utilize miRNA seed sequence plus other factors, such as conservation status and other non-canonical mRNA sites (7). Meanwhile, miRTarBase (http://mirtarbase.mbc.nctu.edu.tw) and TarBase (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=tarbase/index) are two experimentally-validated miRNA target databases commonly available online (6, 7), which curate data on experimental de novo identification of mRNA targets either manually (8, 9) or by text mining (9). A score is given based on the quality of the experimental techniques used, whereby direct low-throughput techniques, such as the reporter assay, ranks the highest, followed by indirect low-throughput methods, such as RT-qPCR (8). Direct high-throughput methods such as CLIP chimeric method is ranked next followed by indirect high-throughput methods such as CLIP-sequencing (8). A higher score also implies that an experiment is more related to a miRNA-target interaction (9).

Subsequent bioinformatics analysis involves Gene Ontology (GO) enrichment analysis which characterizes the selected mRNA targets (genes) in terms of their location in the cell, biochemical activity of a gene product and biological purposes of the gene or its product, denoted by cellular components (CC), molecular functions (MF) and biological processes (BP) GO terms, respectively (10). Functional enrichment analysis then provides an insight into the functional roles of these genes by comparing them to a reference of numerous gene set libraries of known biological function, including protein-protein interactions, transcription factors and biological pathways (10, 11). Several definitions of the latter exist, such as the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Reactome (10). Using statistical methods such as Fisher's exact test, enrichment analysis identifies classes of genes that are significantly enriched or over-represented and these outputs are ranked according to their p-values (10, 11).

Previous studies have demonstrated dysregulation of miRNAs in both *in vitro* and *in vivo* models of DKD (12). Our recent *in vivo* study showed a trend of upregulation of miR-101-3p in type 2 diabetic patients with macroalbuminuria compared to those with normoalbuminuria whereby identification of differential expression of a set of miRNAs involved in human fibrosis was done by a profiler RT-PCR (13). It was concluded that a trend of upregulation of miR-101-3p was observed in these patients, as statistical significance was only reached in the validation cohort and not the screening cohort (13).

Bioinformatic analysis is of particular importance in DKD prior to *in vitro* experimental verification in view of the structural heterogeneity of the kidneys and the pathological complexity of DKD (14) as well as the variable effects of miRNAs observed in different renal cell types (4, 12). As cited in Kato et al., 2014 (12), the functional roles of several DKD-related miRNAs, such as miR-192 and miR-21, have been previously investigated. However, little is known about the putative mRNA targets of miR-101-3p and their roles in DKD. Hence, in this study, we had applied *in silico* bioinformatics analysis in search of its most likely mRNA targets. Furthermore, this study attempted to fill the gap, given the scarcity of utilization of bioinformatics tools in DKD, as recently highlighted (15).

MATERIALS AND METHODS

mRNA targets of miR-101-3p

The putative mRNA targets of miR-101-3p were identified from two experimentally validated miRNA-target gene interactions databases, TarBase v8.0 (8) and miRTarBase release 8.0 beta (9). The mRNA targets commonly identified by these two databases were chosen for further analysis. The most promising mRNA targets of miR-101-3p were chosen based on the principle of reciprocal expression, whereby miRNA-target pair is considered a high probability when a miRNA is upregulated and its mRNA target is downregulated (16). Based on this principle, literature search and matching (15, 16) were performed to identify the most promising mRNA targets of miR-101-3p.

Gene Ontology (GO) and functional enrichment analyses of mRNA targets of miR-101-3p

The commonly identified mRNA targets of miR-101-3p were submitted to web-based enrichment bioinformatic tools Enrichr (11, 17, 18) and Metascape (19). In Enrichr, the input genes are compared to about 300 gene set libraries and the output of enrichment terms are ranked based on p-value derived by Fisher's exact test or the hypergeometric test (11). Metascape uses hypergeometric test and Benjamini-Hochberg p-value correction algorithm, integrating annotation information from over 40 knowledgebases, including Gene Ontology, KEGG and Reactome (19). Its bar graph displays top non-redundant enrichment clusters, ranked according to statistical significance, defined as $p<0.05$ (19).

RESULTS

mRNA targets of miR-101-3p

A total of 2630 and 342 mRNA targets of miR-101-3p were identified by Tarbase and miRTarbase, respectively.

One-hundred ninety-seven (197) commonly identified mRNA targets of miR-101-3p were submitted for enrichment analysis. Our bioinformatics data and literature search suggested that ras-related C3 botulinum toxin substrate 1 (RAC1) and Ras-associated protein-1 b (RAP1b) were the most promising mRNA targets of miR-101-3p.

Gene Ontology (GO) enrichment analysis of mRNA targets of miR-101-3p

Fig. 1 shows Gene Ontology (GO) analysis by Enrichr, for the 197 putative targets of miR-101-3p. For these mRNA targets of miR-101-3p, the most enriched GO Biological Process (BP) terms included negative regulation of gene expression and Ras protein signal transduction (Fig. 1a); and the most enriched GO Molecular Function (MF) terms included RNA binding and transcription coactivator activity (Fig. 1b); whilst the most enriched GO Cellular Component (CC) terms included chromatin and RISC-loading complex (Fig. 1c).

Further GO analysis revealed that the most enriched GO BP term for RAC1 and RAP1b was protein signal transduction (Fig. 1a). The most enriched GO MF terms for RAC1 and RAP1b were guanine diphosphate (GDP) binding (Fig. 1b) and guanosine triphosphate (GTPase) activity; and the most enriched GO CC terms for RAC1 were recycling endosome membrane (Fig. 1c), ruffle membrane and focal adhesion; whilst RAP1B was mostly enriched in lysosomal membrane.

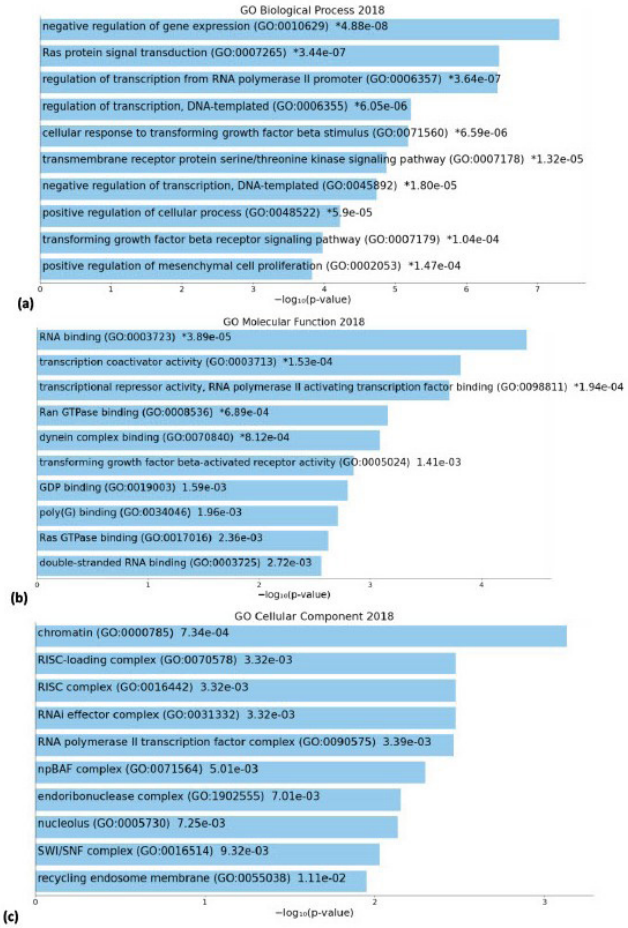

Figure 1: Gene Ontology (GO) analysis of the putative mRNA targets of miR-101-3p (a) Biological Processes; (b) Molecular Functions; (c) Cellular Components

Functional enrichment analysis of mRNA targets of miR-101-3p

Fig. 2 shows the most enriched KEGG pathways, by Enrichr, and Fig. 3 shows the most enriched functional terms, by Metascape, of the 197 putative targets of miR-101-3p. The most enriched KEGG pathways associated with these putative mRNA targets of miR-101-3p included colorectal cancer, renal cell carcinoma and apoptosis (Fig. 2); whilst their most enriched functional terms included cellular response to growth factor stimulus, cytokine signalling in immune system and chromosomal and microsatellite instability in colorectal cancer (Fig. 3). Further analysis showed that the most significant DKD-related pathways associated with RAC1 and RAP1b were focal adhesion, mitogen-activated protein kinase (MAPK) signalling pathway and Ras signalling pathway (Fig. 2 and 3).

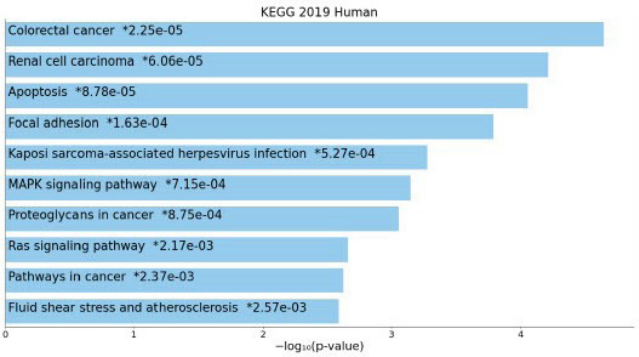

Figure 2: The most enriched KEGG pathways of the putative mRNA targets of miR-101-3p

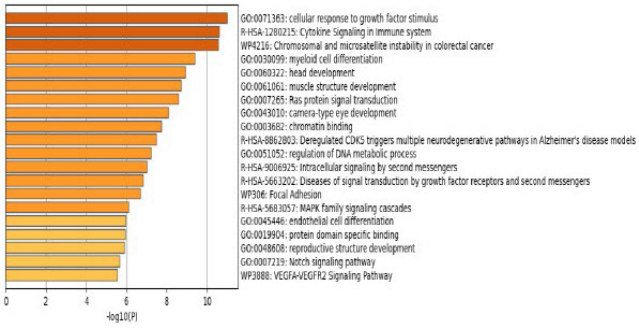

Figure 3: The most enriched terms of the putative mRNA targets of miR-101-3p

DISCUSSION

Bioinformatics analysis in this study indicated that miR-101-3p might act as a negative regulator in the progression of DKD through involvement of its putative targets in the focal adhesion, MAPK signalling pathway and Ras signalling pathway (Fig. 2). Clinically, this finding was supported by a negative correlation of miR-101-3p relative expression with eGFR, observed in our previous study (13). In that study, significant difference

in eGFR was seen across the 3 groups of albuminuria; whereby eGFR was significantly lower in T2DM patients with macro- than in those with normoalbuminuria (13). Thus, the significant correlation between miR-101-3p relative expression and eGFR observed in that study might point towards a possible pathological role of miR-101-3p in DKD, leading to reduced renal function (13).

In this study, the putative mRNA targets of miR-101-3p were identified from experimentally-validated databases to minimize the rate of false positive (6). The bioinformatics tools Enrich and Metascape were utilized as they were the most updated in their databases and visualization summaries compared to other web-based tools. Literature search suggested that RAC1 and RAP1B were the most promising putative mRNA targets of miR-101-3p and will be discussed further.

ras-related C3 botulinum toxin substrate 1 (RAC1)

Although DKD is traditionally regarded as a glomerular disease due to mesangial cell injury, the role of podocyte injury is increasingly recognized. This is in keeping with its clinical signature of proteinuria/albuminuria, signifying perturbed glomerular filtration barrier (14, 20). The latter is mainly attributed to structural and functional changes seen in podocyte injury. Foot process (FP) effacement is one of such changes, mediated by actin cytoskeleton rearrangement (21, 22). The latter is regulated by many factors, including RAC1, a member of Rho small GTPase.

Various other cellular processes are also regulated by these Rho GTPases including adhesion and cell survival (23) many of which involve the three most enriched pathways predicted in this study (Fig. 2). Previous evidence (23-26) was also in keeping with gene ontology description of RAC1, whereby its most enriched GO terms included Ras protein signal transduction, GDP binding, ruffle membrane and focal adhesion, as discussed earlier and shown in Fig. 1.

The leading edge of a foot process, known as lamillepodium (LP) serves as both focal adhesion and migration structure (27). Here, RAC1 activates Dia2 and WAVE [Wiskott-Aldrich syndrome protein (WASP)-family verprolin-homogenous] protein to initiate actin elongation and branching, respectively, therefore driving actin polymerization (28). WAVE promotes actin-related protein 2/3 (Arp2/3)-mediated F-actin branching leading to rapid actin polymerization (28). RAC1 also activates P21-activated kinases (PAKs) and LIM-domain-containing kinases (LIMK) which in turn phosphorylates cofilin, thereby inactivating it (28). Active cofilin would otherwise depolymerize actin by dissociating Arp2/3-derived F-actin branches, therefore block its elongation, and thus destabilize LP (28).

It has been shown that Pak deletion in macrophages reduced LP stability during adhesion (29). The authors concluded that Pak1 could be critical for formatting new adhesion in LP for stable attachment to the substratum (29). A subsequent study concurred (30). It has been shown that miR-101 was highly expressed in kidney (31) and RAC1 was expressed in developing podocytes (24). As discussed earlier, RAC1 is essential for LP formation and its stability. Since Pak is the downstream target of RAC1, we postulate that downregulation of the latter brought about by an upregulation of miR-101-3p would result in reduced LP stability in podocyte FP, contributing towards FP effacement by unstable attachment, as depicted in Fig. 4.

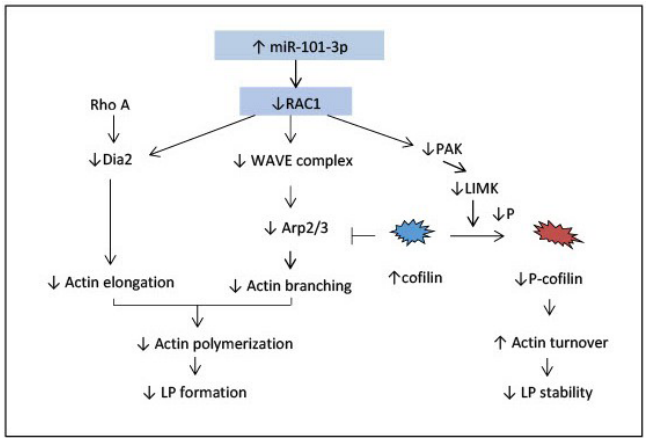

Figure 4: Proposed roles of RAC1 in DKD; PAK: p-21 activated protein kinase; WAVE: [Wiskott-Aldrich syndrome protein (WASP)-family verprolin-homogenous; LIMK: LIM-domain-containing kinases; Arp2/3: actin-related protein 2/3; Ⓟ: phosphorylated; LP: Lamillepodium

Nevertheless, there have been mixed results in studies pertaining to whether RAC1 expression exacerbates (32-36) glomerular disease or otherwise (23, 24, 37-40). It could be possible that both activation and inactivation of RAC1 activity is deleterious, depending on the pathological condition (21, 37,39, 41-42). Thus, a balanced regulation of Rho small GTPase is essential for podocyte integrity (41, 43).

Ras-associated protein-1 b (RAP1b)

Ras-associated protein-1 b (RAP1b) is a small GTPase belonging to the Ras superfamily of G proteins (44). Like Ras and Rho GTPases, RAP1b cycles between an inactive GDP-bound and an active guanine triphosphate (GTP)-bound conformation (44). Transformation into active and inactive form is regulated by guanine nucleotide exchange factor (GEF) and GTPase activating protein (GAP), respectively (44). The location, biological and molecular functions of RAP1b were in keeping with findings in this study (Fig. 1), whereby its most enriched GO terms included Ras signal transduction, GDP binding and lysosomal membrane. RAP1b localizes to membrane and functions to relay signals from extracellular stimuli to intracellular signal transduction pathways (45), including those significantly enriched in this study (Fig. 1b). RAP1b also regulates several cellular processes including cell adhesion, migration,

proliferation, and cell survival (46).

It was first observed that RAP1b expression was increased in hyperglycaemia (44). Subsequently, it was reported that although total RAP1b expression was increased, its activity was reduced (associated with an increased GAP activity) (47, 48). RAP1b is thought to exert an anti-apoptotic effect by binding to BH4 domain of the anti-apoptotic Bcl-2 gene and stabilizing it, protecting tubular cells from mitochondrial dysfunction and eventually apoptosis (47).

RAP1b expression was later shown to be significantly reduced in renal tubules from diabetic patients and the mechanisms underlying mitochondrial dysfunction and apoptosis of the tubular cells were delineated (48). The authors showed that reduced RAP1b led to reduced activation of ERK1/2 activation which in turn led to reduced expression of transcriptional factor and coactivator, CCAAT-enhancer-binding protein beta (C/EBPβ) and peroxisome proliferator-activated receptor γ coactivator 1 alpha (PGC-1α), respectively (48). This led to reduced expression of genes involved in mitochondrial biogenesis and antioxidants (48).

Although glomerular injury is believed to be the primary damage in DKD, recent evidence suggests that mitochondrial dysfunction leading to apoptosis of tubular cells, also contributes towards DKD (48). Taken together, it is conceivable that overexpression of miR-101-3p seen in our previous study (13), firstly, results in downregulation of RAP1b, which then via its effect on extracellular signal–related kinase 1/2 (ERK1/2) pathway which leads to increased ROS production (48). Secondly, by causing instability of Bcl-2 gene (47), this eventually leads to mitochondrial dysfunction and apoptosis of tubular cells possibly via mechanisms discussed above, as depicted in Fig. 5.

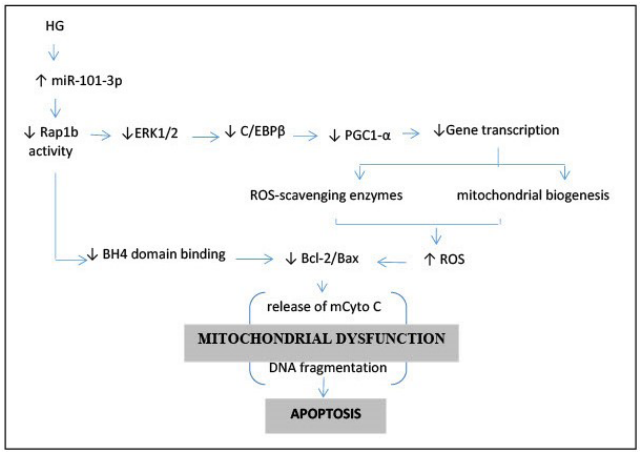

Figure 5: Proposed roles of RAP1b in DKD. HG: Hyperglycaemia; C/EBPβ: CCAAT-enhancer-binding protein; PGC-1α: peroxisome proliferator-activated receptor γ coactivator 1; ROS: reactive oxygen species; mCyto C: mitochondrial cytochrome C

Findings of this study suggested that miR-101-3p might act as a negative regulator in the progression of DKD through involvement of its putative targets in the focal adhesion, Ras and MAPK signalling pathways. The most likely putative mRNA targets of miR-101-3p identified from 2 experimentally-validated miRNA-target databases and in-depth literature review were RAC1 and RAP1b. Their most enriched gene ontology terms which included RAS protein signal transduction, GTPase activity, ruffle membrane and focal adhesion, were in keeping with their predicted roles in DKD.

To verify our *in silico* prediction, suggested *in vitro* experiments included, but not limited to, the following. Firstly, verification that RAC1 and Rap1b are mRNA targets of miR-101-3p should ideally be done by luciferase 3'UTR reporter assay (49). Secondly, an induction of an upregulation of miR-101-3p in specific renal cells in response to diabetic milieu such as hyperglycaemia needs to be verified. Previous studies have shown that miR-101 was highly expressed in kidney (31) and RAC1 was expressed in developing podocytes (24); whilst downregulation of RAP1b has been shown in renal tubular cells (47) and tubules of diabetic patients (48). We therefore suggest such *in vitro* verification to be done in podocytes and tubular cells. Thirdly, a downregulation of RAC1 and RAP1b following an upregulation of miR-101-3p, in podocyte and tubular cells respectively, can be verified either by measuring the expression level of mRNA targets by RT-PCR or that of its target protein by western blotting (47). Alternatively, RAC1 activity may be determined using RAC1 G-LISA Activation Assay (24).

Subsequently, verification of predicted phenotypic and functional changes and reversal of these changes may be achieved by means of miR-101-3p knock-in and knock-out, respectively (49). Following an upregulation of miR-101-3p, proposed downstream experiments include verification of foot processes effacement and apoptosis of podocytes and tubular cells, respectively. Cellular processes quantification study may be done to assess the structure lamellipodia (24). An apoptosis assay may be used to assess the predicted apoptosis; whilst mitochondrial enzyme activities may be determined using a superoxide dismutase or catalase activity assay kit (48). Mitochondrial DNA studies to assess damage to high- and low-molecular-weight DNA by RT-PCR may also be done (48).

Being an *in-silico* analysis, our findings had limitation as they were based on data retrieved from public databases, thus requiring further experimental verification, as outlined earlier. Secondly, finding of the upregulated miR-101-3p was derived from a relatively small sample size, involving 12 and 33 sera in the screening and validation cohorts, respectively, as highlighted in our previous study (13).

CONCLUSION

In conclusion, bioinformatics findings followed by in-depth literature search in this study pave a direction for *in vitro* verification experiments. Such *in silico* analysis is particularly important given the structural heterogeneity of the kidneys, comprising various functional compartments and cell types (14). This is compounded by the complexity of the pathogenesis of DKD, which involves an interplay between several haemodynamic and mechanical factors and signaling pathways, some of which are unique to kidneys (1, 14). Furthermore, diverse effect of miRNAs in different kidney cell types has been well-documented (4). By identifying the most likely mRNA targets and renal cell type, we would increase the probability of successful *in vitro* experimental verification which may lead to new insights into the pathophysiology of DKD.

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REFERENCES

1. Chen Y, Lee K, Ni Z, He JC. Diabetic Kidney Disease: Challenges, Advances, and Opportunities. Kidney Dis. 2020;6(4):215-25. doi: 10.1159/000506634
2. GBD Chronic Kidney Disease Collaboration. Global, regional, and national burden of chronic kidney disease, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet. 2020;395(10225):709-33. doi: 10.1016/S0140-6736(20)30045-3.
3. Barrera-Chimal J, Jaisser F. Pathophysiologic mechanisms in diabetic kidney disease: A focus on current and future therapeutic targets. Diabetes, Obes Metab. 2020;22(S1):16-31. doi:/10.1111/dom.13969
4. Badal SS, Danesh FR. MicroRNAs and their applications in kidney diseases. Pediatr Nephrol. 2015;30(5):727-40. doi:10.1007/s00467-014-2867-7
5. Disner GR, Falcro MAP, Lima C, Lopes-Ferreira M. *In silico* target prediction of overexpressed microRNAs from lps-challenged zebrafish (Danio Rerio) treated with the novel anti-inflammatory peptide TnP. Int J Mol Sci. 2021;22(13):7117. doi: 10.3390/ijms22137117.
6. Mullany LE, Wolff RK, Slattery ML. Effectiveness and usability of bioinformatics tools to analyze pathways associated with miRNA expression. Cancer Inform. 2015;14:121-30. doi: 10.4137/CIN.S32716.
7. Riffo-Campos, Á. L., Riquelme, I., & Brebi-Mieville, P. (2016). Tools for sequence-based miRNA target prediction: What to choose? International Journal of Molecular Sciences, 17(12). https://doi.org/10.3390/ijms17121987 doi:10.3390/ijms17121987
8. Karagkouni D, Paraskevopoulou MD, Chatzopoulos S, Vlachos IS, Tastsoglou S, Kanellos I, et al. DIANA-TarBase v8: A decade-long collection of experimentally supported miRNA-gene interactions. Nucleic Acids Res. 2018;46(D1):D239-45. doi:10.1093/nar/gkx1141
9. Huang HY, Lin YCD, Li J, Huang KY, Shrestha S, Hong HC, et al. MiRTarBase 2020: Updates to the experimentally validated microRNA-target interaction database. Nucleic Acids Res. 2020;48(D1):D148-54. doi:10.1093/nar/gkz896
10. Zhou T, Yao J, Liu Z. Gene Ontology, Enrichment Analysis, and Pathway Analysis. In: Liu Z, editor. Chichester: John Wiley & Sons; 2017. https://doi.org/10.1002/9781118782392.ch10
11. Xie Z, Bailey A, Kuleshov M V., Clarke DJB, Evangelista, JE, Jenkins SL, et al. Gene Set Knowledge Discovery with Enrichr. Curr Protoc. 2021;1(3):1-51. doi: 10.1002/cpz1.90.
12. Kato M, Natarajan R. Diabetic nephropathy-emerging epigenetic mechanisms. Nat Rev Nephrol. 2014;10(9):517-30. doi:10.1038/nrneph.2014.116
13. Zahari Sham SY, Ng CT, Azwar S, Yip WK, Abdullah M, Thevandran K, et al. Circulating miRNAs in type 2 diabetic patients with and without albuminuria in Malaysia. Kidney Blood Press Res. 2022;47(2);1-13. doi: 10.1159/000518866
14. Hewitson TD. Fibrosis in the kidney: Is a problem shared a problem halved? Fibrogenes Tissue Repair. 2012;5(Suppl.1):S14. doi:10.1186/1755-1536-5-S1-S14
15. Oh JW, Muthu M, Haga SW, Anthonydhason V, Paul P, Chun S. Reckoning the dearth of bioinformatics in the arena of diabetic nephropathy (DN)-Need to improvise. Processes. 2020;8(7):808. doi:10.3390/pr8070808
16. Kriegel AJ, Fang Y, Liu Y, Tian Z, Mladinov D, Matus IR, et al. MicroRNA-target pairs in human renal epithelial cells treated with transforming growth factor β1: A novel role of miR-382. Nucleic Acids Res. 2010;38(22):8338-47. doi:10.1093/nar/gkq718
17. Kuleshov M V., Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res. 2016;44(W1):W90-7. doi: 10.1093/nar/gkw377.
18. Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, et al. Enrichr: Interactive and collaborative HTML5 gene list enrichment analysis tool. BMC Bioinformatics. 2013;14:128. doi: 10.1186/1471-2105-14-128.
19. Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi, AH, Tanaseichuk O, et al. Metascape provides a biologist-oriented resource for the analysis

of systems-level datasets. Nat Commun. 2019;10(1):1523. doi: 10.1038/s41467-019-09234-6.
20. Shankland SJ. The podocyte's response to injury: Role in proteinuria and glomerulosclerosis. Kidney Int. 2006;69(12):2131-47. doi: 10.1038/sj.ki.5000410.
21. Perico L, Conti S, Benigni A, Remuzzi G. Podocyte-actin dynamics in health and disease. Nat Rev Nephrol. 2016;12(11):692-710. doi: 10.1038/nrneph.2016.127.
22. Sever S, Schiffer M. Actin dynamics at focal adhesions: a common endpoint and putative therapeutic target for proteinuric kidney diseases. Kidney Int. 2018;93(6):1298-307. doi: 10.1016/j.kint.2017.12.028.
23. Zhang X, Song Z, Guo Y, Zhou M. The novel role of TRPC6 in vitamin D ameliorating podocyte injury in STZ-induced diabetic rats. Mol Cell Biochem. 2015;399(1-2):155-65. doi: 10.1007/s11010-014-2242-9.
24. Attias O, Jiang R, Aoudjit L, Kawachi H, Takano T. Rac1 contributes to actin organization in glomerular podocytes. Nephron - Exp Nephrol. 2010;114(3):e93–106. doi: 10.1159/000262317.
25. He F-F, Chen S, Su H, Meng X-F, Zhang C. Actin-associated Proteins in the Pathogenesis of Podocyte Injury. Curr Genomics. 2013;14(7):477-84. doi: 10.2174/13892029113146660014.
26. Phuyal S, Farhan H. Multifaceted Rho GTPase Signaling at the Endomembranes. Front Cell Dev Biol. 2019;7:1-10. doi: 10.3389/fcell.2019.00127.
27. Perisic L,Lal M, Hulkko J, Hultenby K, Lnfelt B, Sun Y, et al. Plekhh2, a novel podocyte protein downregulated in human focal segmental glomerulosclerosis, is involved in matrix adhesion and actin dynamics. Kidney Int. 2012;82(10):1071-83. doi:10.1038/ki.2012.252
28. Sit ST, Manser E. Rho GTPases and their role in organizing the actin cytoskeleton. J Cell Sci. 2011;124(5):679-83. doi: 10.1242/jcs.064964.
29. Smith SD, Jaffer ZM, Chernoff J, Ridley AJ. PAK1-mediated activation of ERK1/2 regulates lamellipodial dynamics. J Cell Sci. 2008;121(22):3729-36. doi: 10.1242/jcs.027680.
30. Zhu J, Attias O, Aoudjit L, Jiang R, Kawachi H, Takano T. P21-Activated Kinases Regulate Actin Remodeling in Glomerular Podocytes. Am J Physiol - Ren Physiol. 2010;298(4):951-61. doi: 10.1152/ajprenal.00536.2009.
31. Aguado-Fraile E, Ramos E, Conde E, Rodrнguez M, Martнn-Gymez L, Lietor A, et al. A pilot study identifying a set of microRNAs as precise diagnostic biomarkers of acute kidney injury. PLoS One. 2015;10(6):e0127175. doi: 10.1371/journal.pone.0127175.
32. Togawa A, Miyoshi J, Ishizaki H, Tanaka M, Takakura A, Nishioka H, et al. Progressive impairment of kidneys and reproductive organs in mice lacking Rho GDIα. Oncogene. 1999;18(39):5373-80. doi:10.1038/sj.onc.1202921
33. Shibata S, Nagase M, Yoshida S, Kawarazaki W, Kurihara H, Tanaka H, et al. Modification of mineralocorticoid receptor function by Rac1 GTPase: Implication in proteinuric kidney disease. Nat Med. 2008;14(12):1370-76. doi: 10.1038/nm.1879.
34. Akilesh S, Suleiman H, Yu H, Stander MC, Lavin P, Gbadegesin R, et al. Arhgap24 inactivates Rac1 in mouse podocytes, and a mutant form is associated with familial focal segmental glomerulosclerosis. J Clin Invest. 2011; 21(10):4127-437. doi: 10.1172/JCI46458.
35. Babelova A, Jansen F, Sander K, Luhn M, Schдfe, L, Fork C, et al. Activation of Rac-1 and RhoA contributes to podocyte injury in chronic kidney disease. PLoS One. 2013;8(11):e80328. doi: 10.1371/journal.pone.0080328.
36. Schaldecker T, Kim S, Tarabanis C, Tian D, Hakroush S, Castonguay P, et al. Inhibition of the TRPC5 ion channel protects the kidney filter. J Clin Invest. 2013;123(12):5298-309. doi: 10.1172/JCI71165.
37. Blattner SM, Hodgin JB, Nishio M, Wylie, SA, Saha J, Soofi AA, et al. Divergent functions of the Rho GTPases Rac1 and Cdc42 in podocyte injury. Kidney Int. 2013;84(5):920-30. doi: 10.1038/ki.2013.175.
38. Ishizaka M, Gohda T, Takagi M, Omote K, Sonoda Y, Oliva Trejo, JA, et al. Podocyte-specific deletion of Rac1 leads to aggravation of renal injury in STZ-induced diabetic mice. Biochem Biophys Res Commun. 2015;467(3): 49-55. doi: 10.1016/j.bbrc.2015.09.158
39. Tavasoli M, Li L, Al-Momany A, Zhu LF, Adam BA, Wang Z, et al. The chloride intracellular channel 5A stimulates podocyte Rac1, protecting against hypertension-induced glomerular injury. Kidney Int. 2016;89(4):833-47. doi: 10.1016/j.kint.2016.01.001.
40. Gee HY, Sadowski CE, Aggarwal PK, Porath JD, Yakulov TA, Schueler M, et al. FAT1 mutations cause a glomerulotubular nephropathy. Nat Commun. 2016;7:10822. doi: 10.1038/ncomms10822
41. Zhang H, Cybulsky A V, Aoudjit L, Zhu J, Li H, Lamarche-Vane N, et al. Role of Rho-GTPases in complement-mediated glomerular epithelial cell injury. Am J Physiol - Ren Physiol. 2007;293(1):148-56. doi:10.1152/ajprenal.00294.2006.
42. Yu SMW, Nissaisorakarn P, Husain I, Jim B. Proteinuric kidney diseases: A podocyte's slit diaphragm and cytoskeleton approach. Front Med. 2018;5:221. doi: 10.3389/fmed.2018.00221.
43. Falkenberg C V., Azeloglu EU, Stothers M, Deerinck,TJ, Chen Y, He JC, et al. Fragility of foot process morphology in kidney podocytes arises from chaotic spatial propagation of cytoskeletal instability. PLoS Comput Biol. 2017;13(3):1-21.

doi: 10.1371/journal.pcbi.1005433.
44. Lin S, Chugh S, Pan X, Wallner EI, Wada J, Kanwar YS. Identification of up-regulated Ras-like GTPase, Rap1b, by suppression subtractive hybridization. Kidney Int. 2001;60(6):2129-41. doi: 10.1046/j.1523-1755.2001.00061.x.
45. Kanwar YS, Wada J, Sun L, Xie P, Wallner EI, Chen S, et al. Diabetic nephropathy: Mechanisms of renal disease progression. Exp Biol Med. 2008;233(1):4-11. doi: 10.3181/0705-MR-134.
46. Bos JL. Linking Rap to cell adhesion. Curr Opin Cell Biol. 2005;17(2):123-8. doi:10.1016/j.ceb.2005.02.009
47. Sun L, Xie P, Wada J, Kashihara N, Liu FY, Zhao Y, et al. Rap1b GTPase ameliorates glucose-induced mitochondrial dysfunction. J Am Soc Nephrol. 2008;19(12):2293-301. doi: 10.1681/ASN.2008030336
48. Xiao L, Zhu X, Yang S, Liu F, Zhou Z, Zhan M, et al. Rap1 ameliorates renal tubular injury in diabetic nephropathy. Diabetes. 2014;63(4):1366-80. doi: 10.2337/db13-1412
49. Kantharidis P, Hagiwara S, Brennan E, McClelland AD. Study of microRNA in diabetic nephropathy: Isolation, quantification and biological function. Nephrology. 2015;20(3):132-9. doi: 10.1111/nep.12374.