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Review / *Derleme*

The Emerging Roles of MicroRNAs as Biomarkers in Diabetic Nephropathy

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ABSTRACT

Diabetic nephropathy (DN) is one of the most common complications of diabetes mellitus (DM), as well as the most common health issue among End Stage Renal Diseases (ESRD). Recent studies have shown that this concern will likely to become a global phenomenon in the future. Early diagnosis of DN is vital for its treatment. MicroRNAs (miRNAs or miRs) are the most promising for new biomarker candidates proposed for DN. MiRNAs, known as non protein-coding short-chain RNA sequences, have a regulatory role in many cellular events. Advances in molecular genetics and successful genomic techniques allow miRNAs to be used in the diagnosis of several diseases. As the DN diagnostic markers used today are insufficient for some cases, identifying new diagnostic markers is the basis of recent studies. Successful use of miRNAs in the areas of cancer, immunity and diabetes indicates that the DN perspective should be based on miRNA. In this review, miRNAs and their role in DN will be reviewed.

Keywords: *Diabetic nephropathy, MicroRNAs, miR, Biomarkers.*

MikroRNA'ların Diyabetik Nefropatide Biyobelirteçler Olarak Ortaya Çıkan Rolleri

ÖZ

Diyabetik nefropati (DN), diabetes mellitusun (DM) en sık rastlanan komplikasyonlarından biri olmasının yanı sıra son dönem böbrek hastalıkları (SDBH) arasında da en sık rastlanan sağlık sorunudur. Bu kaygının gelecekte küresel bir fenomen haline geleceği son zamanlarda yapılan çalışmalarda açıkça rapor edilmiştir. DN'nin erken teşhisi, tedavisi için hayati önem taşımaktadır. MikroRNA (miRNA veya miR)'lar, DN için önerilen en umut verici yeni biyobelirteç adaylarıdır. Protein kodlamayan kısa zincirli RNA dizileri olarak bilinen miRNA'ların birçok hücresel olayda düzenleyici rolü olduğu bilinmektedir. Moleküler genetikteki ilerlemeler ve başarılı genomik teknikler, miRNA'ların birçok hastalığın tanısında kullanılmasına olanak sağlamaktadır. Günümüzde kullanılan DN tanı işaretleri bazı durumlar için yetersiz olduğundan yeni tanısal belirteçlerin belirlenmesi son çalışmaların temelini oluşturmaktadır. MiRNA'ların kanser, bağışıklık ve diyabet alanlarında başarılı kullanımı, DN perspektifinin miRNA'ya dayanması gerektiğini göstermektedir. Bu derlemede, miRNA'lar ve DN'deki rolleri gözden geçirilecektir.

Anahtar Sözcükler: *Diyabetik nefropati, MikroRNA'lar, miR, Biyobelirteçler*

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a disease characterized by hyperglycemia and dyslipidemia caused by impaired glucose and lipid metabolism as a result of insulin resistance and defects in insulin secretion from β cells. Today, the number of individuals with T2DM has been increasing all over the world. DN, one of the most common complications of DM, is observed in advanced stages in 40% of patients with type 1 diabetes mellitus (T1DM), defined as insulin-dependent and T2DM, defined as independent of insulin. (1,2). In addition, it ranks first among ESRD worldwide (3). DN is not an inevitable consequence of diabetes, but the probability of its occurrence is very high. Furthermore, it is known that risk factors such as hypertension, poor glycemic control, smoking habit, genetics, age, and race play an important role in the course of DN (1). DN is a diabetic kidney problem with excessive albuminuria, loss of glomerular renal filtration and lesions (1). The daily amount of albumin excreted in the urine is evaluated as 30 mg/24 hours or 200 μg/minute. Microalbuminuria is defined as moderately elevated albuminuria with 30-300 mg/24h urinary albumin excretion. High-level albuminuria with values above 300mg/24h is called macroalbuminuria (4). Glomerular filtration rate (GFR) below 60 ml/min/1.73m^2 is defined as impaired renal dysfunction and starts to pose a risk for ESRD. There is a 2-20 mL/min decrease in the GFR rate every year in patients with ESRD (1,4). Current studies suggest that DM and its complications may be directly related to inflammatory processes. While tumour necrosis factor-alpha (TNF-α) activation is among the known early-stage markers of DN, it has also been reported that there are changes in the levels of C-reactive protein (CRP) serum amyloid A (SAA), fibrinogen and interleukins (ILs). These markers are also considered to be associated with basement membrane thickening and the presence of globular lesions (5-7).

Hyperglycemia observed in individuals with DN has an inducing effect on the activation of transforming growth factor-beta (TGF-β), mitogen-activated protein kinases (MAPK), and protein kinase C (PKC) (7,8). Furthermore, under diabetic states, glucose presents the primary source of carbonyl groups for glycation reactions that lead to the generation of advanced glycation end products (AGEs) (9). Increased AGEs, PKC and MAPKs consequently cause the activation of nuclear factor kappa B (NF-κB), having an important role in the transcription of inflammatory cytokine genes (8). The diagnosis of DN is based on the presence of microalbuminuria, however, microalbuminuria is not specific for DN, and it can be observed in conditions such as many tissue damages or increased glomerular disorder. Therefore, the occurrence of microalbuminuria can not always be used for direct diagnosis of nephropathy (10–12). This situation raises the idea that new biomarkers that are more precise and determinant are needed in the case of DN. At this point, investigating the effects of epigenetic factors in the development of DN, as in many types of cancer, has gained importance.

Recent research has found that nucleic acids reflect potential markers for disease diagnosis, except that serological or pathological proteins. Among them, miRNAs are a new class of small single-stranded RNAs. Thousands of miRNAs have been discovered in the human genome and more than 90% of protein-encoding mRNAs can be regulated by miRNAs in a tissue and/or cell-specific manner. miRNAs are normally 19-25 nucleotide small non-coding RNA molecules that bind to the usually 3'-untranslated region (3'-UTR) of target mRNAs, leading to degradation and/or translational inhibition of the mRNA (10,13–16). miRNAs act as regulators in all downstream pathways of cell change, development, and apoptosis through gene regions to which they are appropriately linked (17). Besides, miRNAs can regulate a wide variety of biological events and the onset/progression of different diseases. Some miRNAs regulate expression specifically for a tissue, whereas some others may increase or decrease in many tissues at the same time. Thus, some inferences can be made through the expression of miRNAs that have been specifically studied for tissues and cells. The simultaneous expression level of one or more miRNAs may vary leading to examine the expression of many miRNAs at the same time. In this way, it can be possible to comment on the physiological state of the organism. MiRNAs have begun to play an important role in the cases of diseases such as cancer, inflammation, and diabetes (10,18). Intervention to the course of DN, one of the most common complications of diabetes, may be possible with early diagnosis and reliable biomarkers. For this reason, miRNAs can play a key role in DN related processes. In this paper, we aimed to examine miRNAs as biomarkers and their role in DN.

MiRNA Biogenesis and Maturation

MiRNA biogenesis starts with the formation of microprecursor miRNA (pri-miRNA) particles via processing the genomic DNA in the nucleus by the core enzyme complex (RNA polymerase II). Pri-miRNA fragments consist of thousands of nucleotides that are precursor of microRNAs with 5'-methylguanosine cap and 3'-poly A tail in the structure of complex hairpin-like loops and stems (19). The resulting pri-miRNA binds to the RNAase III type enzyme (Drosha) and goes through pre-miRNA formation and maturation processes. (20). Drosha and its cofactor (Pasha consists of DiGeorge critical syndrome region 8 DGCR8) located in the core, also known as microprocessor assembly, enable to cut the pri-miRNAs from their appropriate sites and form pre-miRNAs with a length of 70 nt from the hairpin structure. These structures can be described as meaningless and flawed (21) and are transported from the nucleus to the cytoplasm via the RAN-GTP and Exportin 5 complex.

The Dicer enzyme complex again cleaves these structures from the intron regions, forming a double-stranded miRNA (dsRNA) of approximately 22 nt (22). To become functional, a helical enzyme (helicase) breaks down the double-stranded structure and converts it into its single-stranded RNA (ssRNA) form (23). The single-stranded structure to affect the binding site, which is the 3 'end of the appropriate mRNA sequence, must combine with the Argonaut protein to form the RISC (RNA-induced silencing complex) (24). In this way, ssRNA binds to the appropriate mRNA with inserts (seed region) consisting of several nucleotides, causing its suppression and gaining a silencing function in its genomic region (25,26). The classical maturation pathway of miRNAs is shown in Figure 1.

MiRNA Expressions in Albuminuria

The first promising studies of miRNAs have emerged with studies on cancer types. In individuals with chronic lymphocytic leukemia (CLL), some gene regions were deleted on investigating tumor suppressors on chromosomes at the gene level and determined that they generally consist of gene sequences containing miR-15a and miR16-1. The deleted gene region also constitutes the BCL-2 gene region with anti-apoptotic effect (28,29). Cimmino et al. showed that the amounts of miR-15a and miR16-1 are expressed inversely with the BCL-2 gene region in CLL patients (30). However, it has been shown that silencing the BCL-2 gene region except for CLL disease will lead to the emergence of polycystic kidney disease in another study (31). In this context, a useful idea has emerged in silencing the gene regions and using miRNAs in the diagnosis of diseases and elucidating the complications of diseases with each other.

To determine miRNA levels, various in vivo and in vitro studies including DN patients, mice, or isolated cell lines have been conducted and seen that many miRNAs show upregulated or downregulated in states of nephropathy. Balasubramanyam et al. showed that miR-146a expression was considerably reduced in individuals with T2DM (32). Similarly, Motawi et al. investigated the potential of miR-130b to be a DN marker and emphasized that there is a significant decrease in miR-130b level in individuals with

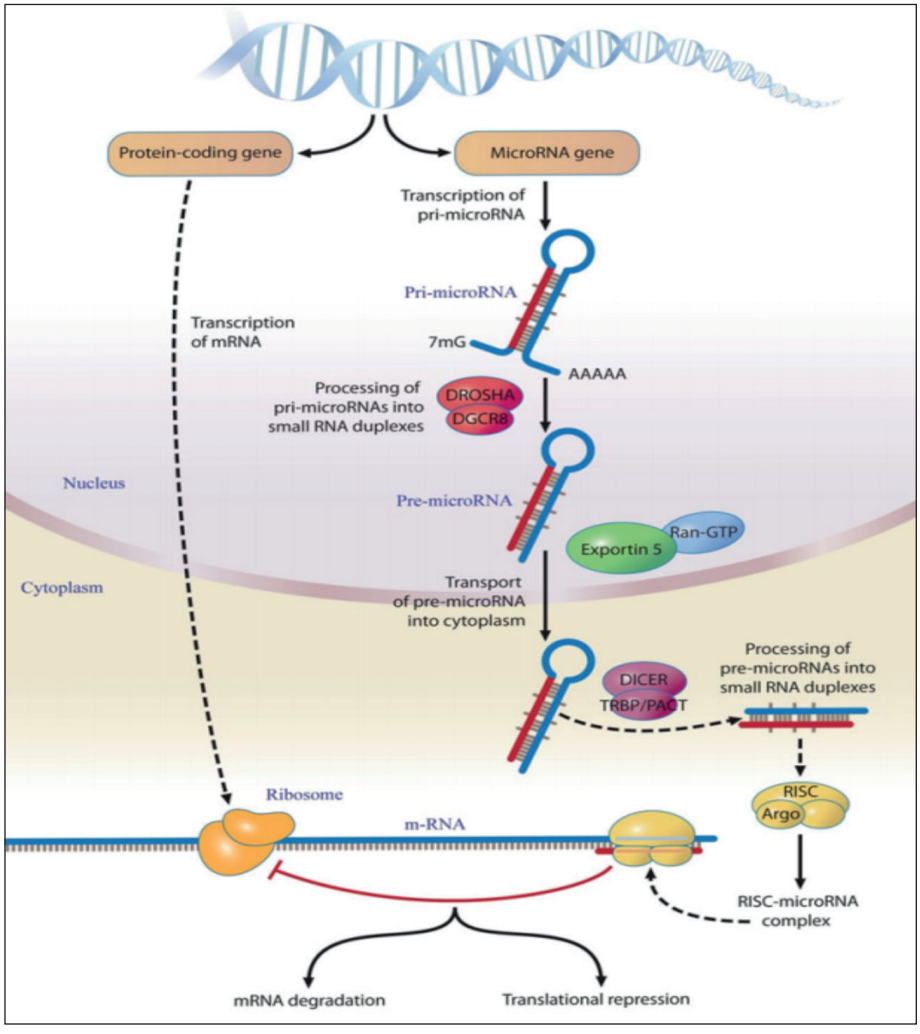

Figure 1: miRNA biogenesis and maturation.
Maturation stages of miRNAs. In the figure, the process of miRNA formation, which begins with genomic DNA transcription and then continues with the maturation stage, ultimately results in degradation or transcriptional suppression of the mRNA (27).

DN, depending on the severity of the disease (33). The specificity of their positive or negative correlation with other diagnostic markers of the disease suggests that the use of miRNA in DN diagnosis should be considered. For example, it has been reported in several studies that miR-29 increased in DN patients with microalbuminuria and showed positive correlation with IL-6 and TNF-α (10,34,35). Saadi evaluated the relationship between miR-192 and the severity of the disease in individuals with DN, low glomerular filtration, high albumin/creatinine ratio and found significantly high miR-192 level (36). In addition to this study, Putta et al. reported that miR-192 inhibition significantly reduced the occurrence of renal fibrosis (37). The study on miR-192 also supports other studies, Kato et al. observed that miR-192 and miR-200 upregulated with TGF- β1(38). In another study, miR-21 and miR-29 family were significantly elevated in individuals with microalbuminuria. Additionally, miR-21 expression caused a decrease in the level of metalloprotease tissue inhibitor 3 (TIMP3), which is one of the causes of rapidly increasing fibrosis in the extracellular matrix in DN (39). Studies on miR-21 also emphasize that its use in the diagnosis of early-stage renal dysfunction is possible (10,40). Effective use of miRNAs in individuals with DN has been tried not only as diagnostic criteria but also as a possible treatment method. Zhang et al. reported that glomerular mesangial cell (MC) proliferation was inhibited by in vivo miR-451 administration to mice (41). In a study on urine samples, Wang et al. demonstrated that miRNAs are specific to the stress experienced by the body. MiR-10 and miR-30d, which are present in certain amounts of urine in animal and human models, rapidly increased in the case of kidney damage. It was shown that this condition was not associated with hyperglycemia, and miR-10 and miR-30d levels did not change as a result of intraperitoneal glucose injection, causing it to be considered as a safe marker for kidney damage (42). Long et al. reported that miR-29 increases podocyte apoptosis leading to protein accumulation in the extracellular matrix (ECM), and its breakdown prevents podocyte apoptosis (43). In an another study, this situation was supported and it was observed that miR-29c inhibitors inhibit TNF-α (44). Previous study on DN patients, it was demonstrated that miR-30c level was decreased and therefore caused inhibition of connective tissue growth factor (CTGF) related to the increased amount of ECM, and it was also reported that renal fibrosis could be reduced by increasing the amount of miR-30c (45). These findings have potentially demonstrated that studies on the targeted miRNAs might have critical importance in the cases of DN. Data obtained from previous studies, concerning the roles of some miRNAs in diabetic diseases are shown in Table 1.

Sun et al. investigated the tissue specificity for miR-192, miR-194, miR-204, miR-215 and miR-216 it was emphasized that miRNAs seen in kidney tissue were also overexpressed in muscle, heart, prostate, spleen, and lung (46). In another tissue-specific study, six precursors were reported that the expression of the miR-17-92 cluster consisting of seven mature miRNAs, was significantly higher in kidney tissue than in other tissues (47). Long et al. demonstrated that miR-29c is significantly existing in the brain, kidney, lung and heart, and there is a significant increase in the amount of it especially in kidney tissues in the case of high glucose intake (43).

MiRNAs as Biomarkers in Diabetic Nephropathy

Metabolic pathways involve a set of reactions that shed light on the diagnosis, treatment, and complications of diseases. Some of these pathways are actively used during the DN and they correlate with miRNAs. The symptoms that several miRNAs are effective in diabetic nephropathy are shown in Figure 2.

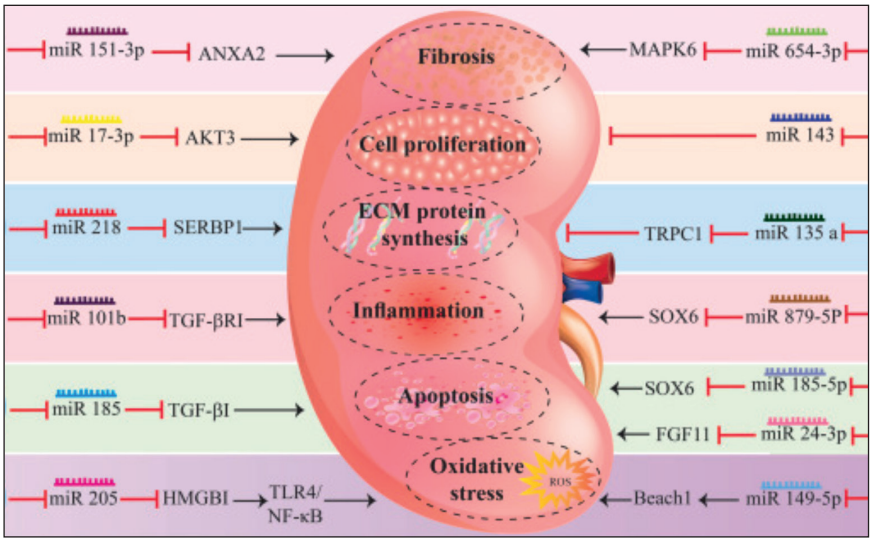

Figure 2: Some miRNAs in development of diabetic nephropathy
miRNAs enfulence in kidney tissue by activating some pathways, increasing the synthesis of some proteins, or acting on gene regions. Figure edited from article published by Mafi et al (48).

The activation of p38 MAPK is effective in processes such as cell differentiation, glucose uptake, and inflammation, and it decreases during the DN accompanied by down-regulation of miR-451 (41). miR-20a inhibits NF-κB, C-X-C chemokine receptor type 4 (CXCR 4), and extracellular-signal-regulated kinase (ERK) signaling pathways in human proximal tubular epithelial cells (49). Previous studies have also shown that the gene region for which miR-21 is responsible is the same as the phosphatase and tensin homologous (PTEN) gene region (50). PTEN is an antagonist of phosphoinositide 3-kinase (PI3K) and is involved in many processes including PI3K cell growth, division, cancer, insulin receptors, glucose metabolism (51). Janus kinase/signal transducer and activators of transcription (JAK/STAT) pathway is involved in the formation of many cytokines and many other cellular processes together with other pathways (52). In a mouse model with chronic renal failure, overexpression of miR-206 has been reported to inhibit glomerulosclerosis by inhib-

Table 1: Some studies investigating the effects of miRNAs in diabetic diseases.

	Regulation Direction	Disease Status	Sample Type	Region of Effect	
miR-29c	Up Regulation	↑ cytokine formation, DN, ↑TNF-a	Blood, Urine	TPP gene region repressor	(34)
miR-29a	Up Regulation	↑ Microalbuminuria, Kidney dysfunction, ↓ eGFR, DN	Blood		(10,35)
miR-21	Up Regulation	↑ Microalbuminuria, Kidney dysfunction, ↓ eGFR, DN	Blood	TIMP3 repressor	(10,35,39)
miR-192	Up Regulation	↑Macroalbuminuria ↓eGRF, DN	Blood	TGF-β1 inductive	(36,38)
miR-451	Down Regulation	↑ Albuminuria, DN	Blood	Twhaz gene region repressor, P38MAK signal pathway repressor	(41)
miR-10a miR-30d	Up Regulation	Acute Chronic Kidney Injury	Blood, Urine		(42)
miR-20a	Up Regulation	Acute Chronic Kidney Injury	Renal proximal tubular epithelial cell	CXCR, NF-KB, ERK1/2 blockage, caspase3,9 repressor	(64)
miR-216a	Up Regulation	DR	Blood	JAK/STAT repressor	(66)
miR-25	Up Regulation	High glucose supplement	Mesangial cell	NOX4 suppressor	(57)
miR-326	Up Regulation	↑LPS stimulation, ↑IL6, ↑IL1, ↑TNF-a	Granulosa cells	TLR-4 gene region repressor	(67)
miR-485	Up Regulation	High glucose supplement ↑ROS, ↑MDA, ↓IL6, ↓IL-1, ↓TNF-a,	Mesangial cell	NOX5 repressor	(60)
miR-326	Up Regulation	DN, ↑UAE ↓TNF-a ve ↓IL-8	Blood	↓NF-κB /P65,	(68)
miR-133b miR-342 miR-30a	Up Regulation	↑Microalbuminuria, ↑Macroalbuminuria, ↓eGRF, , DN, ↑UACR, ↑HbA1c , ↑ LDL	Urine		(69)
miR-130b	Down Regulation	DN, ↑ Microalbuminuria, ↑HbA1c, ↑TG (mg / dl), ACR (mg / g)	Urine	↑NGAL (ng / ml), ↑βTP (ng / ml)	(33)
miR-27a	Up Regulation	DN Hyperglycemia	Podocyte cell	↑PPAR γ	(70)

LDL: Low density lipoprotein, **HDL:** High density lipoprotein, **TG:** Triglyceride, **eGFR:** Estimated glomerular filtration rate, **ACR:** Albumin to creatinine ratio, **NGAL:** Lipocalin associated with neutrophil gelatinase , **βTP:** β-trace protein, **PPAR γ:** Peroxisome proliferator activated receptor γ, **ROS:** Reactive oxygen species, **MDA:** Malondialdehyde.

iting the JAK/STAT signaling pathway (53). Downstream products linked to the Wnt/β-catenin pathway are known to be reduced in individuals with DN. Decreased β-catenin proteins increase apoptosis and mesangial fibrosis (54). In a series of studies on mice Hsu et al. reported that miR-29a positively correlated with stimulation of the Wnt/ β-catenin signaling pathway (55).

Apoptosis observed in glomerular MCs is a process known to exacerbate albuminuria. However, the mechanisms underlying the stimulation of MCs are not fully understood. The Sox4 gene mediates podocyte apoptosis by activating p53 and p21 during DN. miR-130b-3p can reverse the situation by binding to the 3' UTR region of the mRNA of Sox4 in podocytes (56). Oxidative stress observed in the body with DN triggers systems that generate free radicals (57). Suppression of reactive oxygen species contributes to alleviation of glomerulosclerosis by preventing MC apoptosis (58) Wang et al. showed that CTBP1-AS2 overexpression decreased ROS and MDA levels and increased superoxide dismutase (SOD) activity. In the study, it was shown that CTBP1-AS2 can be suppressed by miR-155-5p. Thus, miR-155-5p mediates oxidative stress-induced apoptosis (59). The relationship between nicotinamide adenine dinucleotide phosphate oxidase-4 (NOX4), one of the effective enzymes in the formation of free radicals and miR-25 was investigated and it was reported that expression of the NOX4 enzyme was stopped by miR-25 at the mRNA step (57). In another experimental setup created with high glucose supplementation, it has been reported that miR-485 suppresses proinflammatory processes in human mesangial cells and excessive protein production in ECM by suppressing NOX5 expression. In the study, it was emphasized that miR-485 reduces oxidative stress (60). Nuclear factor erythroid 2 (Nrf2) activates many gene regions in the cellular oxidant defense system and provides an important protection especially in the oxidative stress that occurs in DN. In studies, suppression of miR-200 causes a decrease in Nrf2 and worsening of DN symptoms such as renal fibrosis (61,62).

Acute inflammation is a natural response of the immune system, while chronic inflammation points to a metabolic problem. miRNAs are also directly related to many cytokines known as markers of acute and chronic inflammation (63). It has been reported that miR-20a decreases levels of IL-6, IL-1β, and TNF-α in renal tubular epithelial cells (HK-2) in individuals with acute chronic kidney damage (64). In the study on evaluating miR-31 level in DN patients, the decrease in miR-31 correlated with the increase in IL6, TNF-a, ICAM-1, however, miR-31 decrease was not seen in individuals with retinopathy. It was emphasized that miR-31 level did not change in another complication of DM (65). In this case, it can be said that some miRNAs show changes specific to the complications of DM. In a study in which miR-29c showed its inhibitory effect on Tristetraproline (TTP), Guo et al. reported that miR-29c increased the production of TNF-α by suppressing TTP (44). In a previous study on mice, the amount of miR-29c in the hyperglycemic state was examined and it was reported that miR-29c pairs with the Sprouty homolog 1 (Spry1) gene region which has an important role in kidney development and has a preventive effect. Spry1 is the negative regulator of the Rho kinase cascade responsible for the release of many cytokines (NF-κB, IL-10, IL-17, etc.). Therefore, it has been suggested that the inhibition of Spry1 contributes to the progression of DN (43).

CONCLUSION

Studies on miRNA show that the potential relationship between miRNAs and several diseases is among the remarkable topics of today. The obtained miRNA data demonstrate us that the areas of these unique micro molecules used can be vital in the diagnosis of the diseases. Moreover, the uses of miRNAs are not only limited to the diagnosis of diseases. The researchers also have revealed that miRNAs to be evaluated among the biomolecules can likely to be used in the treatment of DN in the future, by integrating with various techniques treatments. In this context, the research conducted on miRNA-related processes of DN, as one of the most common diseases worldwide, offers the ways in which etiology of the disease can be understood.

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Author Contributions

The article content were conceptualized and designed by **Aysun Hacışevki** and **Destan Kalaçay.** The literature review was conducted by **Destan Kalaçay** under the supervision of **Aysun Hacışevki. Destan Kalaçay** has contributed actively in writing manuscript. Content analysis and interpretation was done by **Aysun Hacışevki** with critical review of content.

Conflict of Interest

The authors declare that they have no conflict of interests.

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REFERENCES

1. Lim AK. Diabetic nephropathy – complications and treatment. Int J Nephrol Renovasc Dis. 2014;7:361-381.
2. Ulu İ, Çakmak Genç G, Karakaş Çelik S. Sirtuin 1 ve sirtuin 2'nin tip 2 diyabet ile ilişkisi. Turk J Diab Obes. 2021;5:81-88.
3. Breyer JA. Diabetic nephropathy in insulin-dependent patients. Am J Kidney Dis. 1992;20:533-547.
4. Roelofs JJ, Vogt L. Diabetic Nephropathy: Pathophysiology and Clinical Aspects, Cham: Springer International Publishing, 2019.
5. Lampropoulou IT, Stangou M, Sarafidis P, Gouliovaki A, Giamalis P, Tsouchnikas I, Didangelos T, Papagianni A. TNF-α pathway and T-cell immunity are activated early during the development of diabetic nephropathy in Type II Diabetes Mellitus. Clin Immunol. 2020;215:108423.
6. Dalla Vestra M, Mussap M, Gallina P, Bruseghin M, Cernigoi AM, Saller A, Plebani M, Fioretto P. Acute-phase markers of inflammation and glomerular structure in patients with type 2 diabetes. J Am Soc Nephrol. 2005;16:S78-82.
7. Miyauchi K, Takiyama Y, Honjyo J, Tateno M, Haneda M. Upregulated IL-18 expression in type 2 diabetic subjects with nephropathy: TGF-β1 enhanced IL-18 expression in human renal proximal tubular epithelial cells. Diabetes Res Clin Pract. 2009;83:190-199.
8. Kato M, Castro NE, Natarajan R. MicroRNAs: Potential mediators and biomarkers of diabetic complications. Free Radic Biol Med. 2013;64:85-94.
9. Sanajou D, Ghorbani Haghjo A, Argani H, Aslani S. AGE-RAGE axis blockade in diabetic nephropathy: Current status and future directions. Eur J Pharmacol. 2018;833:158-164.
10. Chien HY, Chen CY, Chiu YH, Lin YC, Li WC. Differential microRNA profiles predict diabetic nephropathy progression in Taiwan. Int J Med Sci. 2016;13:457-465.
11. Simpson K, Wonnacott A, Fraser DJ, Bowen T. MicroRNAs in diabetic nephropathy: From biomarkers to therapy. Curr Diab Rep. 2016;16(3):35.
12. Mukhadi S, Hull R, Mbita Z, Dlamini Z. The role of MicroRNAs in kidney disease. NcRNA. 2015;1:192-221.
13. Miranda KC, Huynh T, Tay Y, Ang YS, Tam WL, Thomson AM, Lim B, Rigoutsos I. A pattern-based method for the identification of MicroRNA binding sites and their corresponding heteroduplexes. Cell. 2006;126:1203-2017.
14. Guo H, Ingolia NT, Weissman JS, Bartel DP. Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature. 2010;466:835-840.
15. Baek D, Villén J, Shin C, Camargo FD, Gygi SP, Bartel DP. The impact of microRNAs on protein output. Nature. 2008;455:64-71.
16. Kalaçay D, Hacışevki A. miRNA 29 ailesi: Diyabetik nefropatideki rolleri. İçinde: Yücel D. Güncel Biyokimya Çalışmaları III, Ankara: Akademisyen Yayınevi; 2022.
17. O'Connell RM, Rao DS, Chaudhuri AA, Baltimore D. Physiological and pathological roles for microRNAs in the immune system. Nat Rev Immunol. 2010;10:111-122.
18. Iorio MV, Croce CM. MicroRNAs in cancer: Small molecules with a huge impact. JCO. 2009;27:5848-5856.
19. Patel V, Noureddine L. MicroRNAs and fibrosis. Curr Opin Nephrol Hypertens. 2012;21:410-416.
20. Gregory RI, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, Cooch N, Shiekhattar R. The microprocessor complex mediates the genesis of microRNAs. Nature. 2004;432:235-240.
21. Rhoads RE. MiRNA regulation of the translational machinery. New York: Springer; 2010.
22. Yi R. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes Dev. 2003;17:3011-3016.
23. Tesfaye D, Worku D, Rings F, Phatsara C, Tholen E, Schellander K, Hoelker M. Identification and expression profiling of microRNAs during bovine oocyte maturation using heterologous approach. Mol Reprod Dev. 2009;76:665-677.
24. Lin S, Gregory RI. MicroRNA biogenesis pathways in cancer. Nat Rev Cancer. 2015;15:321-333.
25. Bartel DP. MicroRNAs: Target recognition and regulatory functions. Cell. 2009;136:215-233.
26. Pordzik J, Jakubik D, Jarosz-Popek J, Wicik Z, Eyileten C, De Rosa S, Indolfi C, Siller-Matula JM, Czajka P, Postula M. Significance of circulating microRNAs in diabetes mellitus type 2 and platelet reactivity: Bioinformatic analysis and review. Cardiovasc Diabetol. 2019;18:113.
27. Arenz C. miRNA maturation: Methods and protocols. New York: Humana Press; Springer; 2014.
28. Peng Y, Croce CM. The role of MicroRNAs in human cancer. Signal Transduct Target Ther. 2016;1:1-9.
29. Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, Iorio MV, Visone R, Sever NI, Fabbri M, Iuliano R, Palumbo T, Pichiorri F, Roldo C, Garzon R, Sevignani C, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. N Engl J Med. 2005;353(17):1793-1801.
30. Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojcik SE, Aqeilan RI, Zupo S, Dono M, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci U S A. 2005;102:13944-13949.
31. Bouillet P, Cory S, Zhang LC, Strasser A, Adams JM. Degenerative disorders caused by Bcl-2 deficiency prevented by loss of its BH3-only antagonist bim. Dev. Cell. 2001;1:645-653.
32. Balasubramanyam M, Aravind S, Gokulakrishnan K, Prabu P, Sathishkumar C, Ranjani H, Mohan V. Impaired miR-146a expression links subclinical inflammation and insulin resistance in Type 2 diabetes. Mol Cell Biochem. 2011;351(1-2):197-205.

33. Motawi TK, Shehata NI, ElNokeety MM, El-Emady YF. Potential serum biomarkers for early detection of diabetic nephropathy. Diabetes Res Clin Pract. 2018;136:150-158.
34. Guo J, Li J, Zhao J, Yang S, Wang L, Cheng G, Liu D, Xiao J, Liu Z, Zhao Z. MiRNA-29c regulates the expression of inflammatory cytokines in diabetic nephropathy by targeting tristetraprolin. Sci Rep. 2017;7(1):2314.
35. Ibrahim AA, Soliman HM, El-Lebedy D, Hassan M, Helmy NA, Abdel Hamid TA. Expression of exosomal miR-21 and miR-29 in serum of children and adolescents with T1DM and persistent microalbuminuria. Gene Reports. 2019;16:100461.
36. Saadi G, El-Meligi A, El-Ansari M, Alkemary A, Ahmed G. Evaluation of microRNA-192 in patients with diabetic nephropathy. Egypt J Internal Med. 2019;31:122-128.
37. Putta S, Lanting L, Sun G, Lawson G, Kato M, Natarajan R. Inhibiting microRNA-192 ameliorates renal fibrosis in diabetic nephropathy. J Am Soc Nephrol. 2012;23:458-469.
38. Kato M, Arce L, Wang M, Putta S, Lanting L, Natarajan R. A microRNA circuit mediates transforming growth factor-β1 autoregulation in renal glomerular mesangial cells. Kidney Int. 2011;80:358-368.
39. Fiorentino L, Cavalera M, Mavilio M, Conserva F, Menghini R, Gesualdo L, Federici M. Regulation of TIMP3 in diabetic nephropathy: A role for microRNAs. Acta Diabetol. 2013;50(6):965-969.
40. Zhang Z, Peng H, Chen J, Chen X, Han F, Xu X, He X, Yan N. MicroRNA-21 protects from mesangial cell proliferation induced by diabetic nephropathy in db/db mice. FEBS Lett. 2009;583:2009-2014.
41. Zhang Z, Luo X, Ding S, Chen J, Chen T, Chen X, Zha H, Yao L, He X, Peng H. MicroRNA-451 regulates p38 MAPK signaling by targeting of Ywhaz and suppresses the mesangial hypertrophy in early diabetic nephropathy. FEBS Lett. 2012;586:20-26.
42. Wang N, Zhou Y, Jiang L, Li D, Yang J, Zhang CY, Zen K. Urinary microRNA-10a and MicroRNA-30d serve as novel, sensitive and specific biomarkers for kidney injury. PLoS One. 2012;7:e51140.
43. Long J, Wang Y, Wang W, Chang BHJ, Danesh FR. MicroRNA-29c is a signature microRNA under high glucose conditions that targets Sprouty homolog 1, and its in vivo knockdown prevents progression of diabetic nephropathy. J Biol Chem. 2011;286:11837-11848.
44. Guo J, Li J, Zhao J, Yang S, Wang L, Cheng G, Liu D, Xiao J, Liu Z, Zhao Z. MiRNA-29c regulates the expression of inflammatory cytokines in diabetic nephropathy by targeting tristetraprolin. Sci Rep. 2017;7:2314.
45. Wang J, Duan L, Guo T, Gao Y, Tian L, Liu J, Wang S, Yang J. Downregulation of miR-30c promotes renal fibrosis by target CTGF in diabetic nephropathy. J Diabetes Complications. 2016;30(3):406-414.
46. Sun Y, Koo S, White N, Peralta E, Esau C, Dean NM, Perera RJ. Development of a micro-array to detect human and mouse microRNAs and characterization of expression in human organs. Nucleic Acids Res. 2004;32(22):e188.
47. Sawera M, Gorodkin J, Cirera S, Fredholm M. Mapping and expression studies of the mir17-92 cluster on pig Chromosome 11. Mamm Genome. 2005;16:594-598.
48. Mafi A, Yadegar N, Salami M, Salami R, Vakili O, Aghadavod E. Circular RNAs; Powerful microRNA sponges to overcome diabetic nephropathy. Pathol Res Pract. 2021;227:153618.
49. Roy S, Khanna S, Hussain SR, Biswas S, Azad A, Rink C, Gnyawali S, Shilo S, Nuovo GJ, Sen CK. MicroRNA expression in response to murine myocardial infarction: miR-21 regulates fibroblast metalloprotease-2 via phosphatase and tensin homologue. Cardiovasc Res. 2009;82(1):21-29.
50. Carracedo A, Pandolfi PP. The PTEN-PI3K pathway: Of feedbacks and cross-talks. Oncogene. 2008;27(41):5527-5541.
51. Chalhoub N, Baker SJ. PTEN and the PI3-Kinase pathway in cancer. Annu Rev Pathol. 2009;4:127-50.
52. Rawlings JS, Rosler KM, Harrison DA. The JAK/STAT signaling pathway. J Cell Sci. 2004;117:1281-1283.
53. Zhao SQ, Shen ZC, Gao BF, Han P. microRNA-206 overexpression inhibits epithelial-mesenchymal transition and glomerulosclerosis in rats with chronic kidney disease by inhibiting JAK/STAT signaling pathway. J Cell Biochem. 2019;120:14604-14617.
54. Guo Q, Zhong W, Duan A, Sun G, Cui W, Zhuang X, Liu L. Protective or deleterious role of Wnt/beta-catenin signaling in diabetic nephropathy: An unresolved issue. Pharmacol Res. 2019;144:151-157.
55. Hsu YC, Chang PJ, Ho C, Huang YT, Shih YH, Wang CJ, Lin CL. Protective effects of miR-29a on diabetic glomerular dysfunction by modulation of DKK1/Wnt/β-catenin signaling. Sci Rep. 2016;6:30575.
56. Pasupulati AK, Paturi ASV. The sponging effect of a lncRNA on a miRNA contributes to diabetic nephropathy. Mol Ther Nucleic Acids. 2022;28:259-260.
57. Fu Y, Zhang Y, Wang Z, Wang L, Wei X, Zhang B, Wen Z, Fang H, Pang Q, Yi F. Regulation of NADPH oxidase activity is associated with miRNA-25-mediated NOX4 expression in experimental diabetic nephropathy. Am J Nephrol. 2010;32(6):581-589.
58. Wang Y, He Z, Yang Q, Zhou G. XBP1 inhibits mesangial cell apoptosis in response to oxidative stress via the PTEN/AKT pathway in diabetic nephropathy. FEBS Open Bio. 2019;9:1249-1258.
59. Wang G, Wu B, Zhang B, Wang K, Wang H. LncRNA CTBP1-AS2 alleviates high glucose-induced oxidative stress, ECM accumulation, and inflammation in diabetic nephropathy via miR-155-5p/FOXO1 axis. Biochem Biophys Res Commun. 2020;532(2):308-314.
60. Wu J, Lu K, Zhu M, Xie X, Ding Y, Shao X, Chen Y, Liu J, Xu M, Xu Y, Zhou J, Shen X, Zhu C. miR-485 suppresses inflammation and proliferation of mesangial cells in an in vitro model of diabetic nephropathy by targeting NOX5. Biochem Biophys Res Commun. 2020;521:984-990.

61. Wu H, Kong L, Tan Y, Epstein PN, Zeng J, Gu J, Liang G, Kong M, Chen X, Miao L, Cai L. C66 ameliorates diabetic nephropathy in mice by both upregulating NRF2 function via increase in miR-200a and inhibiting miR-21. Diabetologia. 2016;59:1558-1568.
62. Wei J, Zhang Y, Luo Y, Wang Z, Bi S, Song D, Dai Y, Wang T, Qiu L, Wen L, Yuan L, Yang JY. Aldose reductase regulates miR-200a-3p/141-3p to coordinate Keap1–Nrf2, Tgfβ1/2, and Zeb1/2 signaling in renal mesangial cells and the renal cortex of diabetic mice. Free Radic Biol Med. 2014;67:91-102.
63. Marques-Rocha JL, Samblas M, Milagro FI, Bressan J, Martínez JA, Marti A. Noncoding RNAs, cytokines, and inflammation-related diseases. FASEB J. 2015;29(9):3595-3611.
64. Zhang L, He S, Wang Y, Zhu X, Shao W, Xu Q, Cui Z. miRNA-20a suppressed lipopolysaccharide-induced HK-2 cells injury via NFκB and ERK1/2 signaling by targeting CXCL12. Mol Immunol. 2020;118:117-123.
65. Rovira-Llopis S, Escribano-Lopez I, Diaz-Morales N, Iannantuoni F, Lopez-Domenech S, Andújar I, Jover A, Pantoja J, Pallardo LM, Bañuls C, Victor VM. Downregulation of miR-31 in diabetic nephropathy and its relationship with inflammation. Cell Physiol Biochem. 2018;50(3):1005-1014.
66. Liu Y, Xiao J, Zhao Y, Zhao C, Yang Q, Du X, Wang X. microRNA-216a protects against human retinal microvascular endothelial cell injury in diabetic retinopathy by suppressing the NOS2/JAK/STAT axis. Exp Mol Pathol. 2020;115:104445.
67. Chaurasiya V, Kumari S, Onteru SK, Singh D. Up-regulation of miR-326 regulates pro-inflammatory cytokines targeting TLR-4 in buffalo granulosa cells. Mol Immunol. 2020;119:154-158.
68. Yang M, Kan L, Zhu Y, Wu L, Bai S, Cha F. The effect of Baicalein on the NF-κB/P65 expression in the peripheral blood of patients with diabetic nephropathy and in vitro. Biomedical Research. (India) 2017;28:5540-5545.
69. Eissa S, Matboli M, Bekhet MM. Clinical verification of a novel urinary microRNA panal: 133b, -342 and -30 as biomarkers for diabetic nephropathy identified by bioinformatics analysis. Biomed Pharmacother. 2016;83:92-99.
70. Zhou Z, Wan J, Hou X, Geng J, Li X, Bai X. MicroRNA-27a promotes podocyte injury via PPARγ-mediated β-catenin activation in diabetic nephropathy. Cell Death Dis. 2017;8(3):e2658.