Diagnostic and Therapeutic Application of Exosomal microRNAs Inducing Inflammation in Type 2 Diabetes Mellitus

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ABSTRACT: Diabetes mellitus is a class of noncommunicable chronic metabolic disorders marked by hyperglycemia due to insulin production, insulin action or both and has reached epidemic levels around the world. The two most frequent types of diabetes are type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). Despite substantial improvements in the knowledge and treatment of DM, the associated incidence and mortality rates remain steadily increased. Reliable markers for the early detection, monitoring and focused treatment of DM are desperately required. Conversely, microRNAs (miRNAs) have received much significance due to their regulatory involvement in gene expression. Fascinatingly, exosomes can be enclosed into miRNAs to transport or distribute them into the target cells or tissues in which they have a physiological regulatory action. Thus, exosomal miRNAs are proving to be important regulators in the establishment and maintenance of DM, however, further mode of action will be needed to investigate in order to fully comprehend the pathophysiological process. Hereby, this review outlines the recent findings on the role of exosomal miRNAs intending to understand the precise function in diagnostic and therapeutic aspects in T2DM disease.

KEY WORDS: type 2 diabetes mellitus, miRNAs, exosomal miRNAs, diagnostic, prognostic, therapeutic target

I. INTRODUCTION

Diabetes mellitus (DM) is a class of noncommunicable chronic metabolic disorders marked by hyperglycemia due to insulin production, insulin action or both[1] and has now increased exponentially around the world.[2] Diabetes is the most frequent endocrine disorder, with a prediction that by 2025, 300 million people will be affected with DM.[3] Diabetes hyperglycemia is linked to long-term dysfunction, and organ damage, particularly the heart, kidney, blood vessels, nerves and eyes. The two most common types of diabetes are type 1 diabetes mellitus (T1DM), in which a complete lack of insulin results from the loss of pancreatic beta cells and type 2 diabetes mellitus (T2DM), wherein insulin deficiency can lead to hyperglycemia.[2] Diabetes can be caused by a genetic component or by a variety of unhealthy choices such as sedentary lifestyle, lack of physical exercise and regular smoking and alcohol intake.[4] Moreover, T2DM is the most common type of diabetes in developing nation which allows insulin level fluctuation and also alters blood glucose concentration, this is caused by genetics and combination of environmental and behavioural risk factors.[1,4] Despite substantial improvements in the knowledge and treatment of DM, the associated incidence and mortality rates remain steadily increased. Reliable markers for the early detection, monitoring and focused treatment of DM are desperately required.

Extracellular vesicles (EVs), which are secreted by most of the cell types, may be found in blood circulation and other body fluids have proven to be operated as promising biomarkers for metabolic aliments.[5] However, EVs are classified as small EVs and large EVs based on their size,[5] and also classified broadly as apoptotic bodies, microvesicles, and exosomes according to their biogenesis.[6] Interestingly, exosomes are a form of small EVs with a diameter of 40–160 nm and are found in all types of human cells.[7] Exosomes can initiate cellular activity via cargo molecules and are found across a wide range of biological body fluids like saliva, serum, urine, breast milk, cerebral spinal fluid and semen.[8] Some of the

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cargoes carried by exosomes are DNA, RNAs (miRNAs, mRNA, tRNA and rRNA), lipids, proteins, and metabolites are used as communication among cells.[9] MicroRNAs (miRNAs) are short, noncoding RNAs with 17–24 nucleotides that influence post-transcriptional gene silencing by connecting to target mRNAs' 3′-untranslated region (UTR).[10] According to the recent findings, it was proved that miRNAs are more stable due to their short sequence and are found in human body fluids like blood, saliva, breast milk and urine. Fascinatingly, exosomes can be enclosed into miRNAs to transport or distribute them into the target cells or tissues in which they have a physiological regulatory action. Moreover, exosomes carrying miRNAs can be released in human body cells like endothelial cells, lymphocytes, epithelial cells, platelets and neurons.[11] Exosomal miRNAs are proving to be important regulators in the establishment and maintenance of DM, however, in order to completely appreciate the pathophysiological process, further mode of action will be need to be investigated. This review summarises latest findings on the involvement of exosomal miRNAs in T2DM disease, with the goal of better understanding their specific function in prognostic, diagnostic, and therapeutic aspects.

II. OVERVIEW OF EXOSOMAL MIRNAS

EVs are minute spherical bundles released into the extracellular environment by a number of cells. They play an essential role in transfer of information between donor and recipient cells.[12] Exosomes and microvesicles (MVs) are two primary EVs that differ in size, biomarkers expressed and biological function.[5] The promising use of EVs as therapeutic and diagnostic tools for a number of aliments, such as cancer, cardiovascular dysfunction including DM, has sparked research in this sector and in addition focused on EV classification and separation methods.[7] Moreover, exosomes biological activity is the topic of interest to the researchers in the current days. Interestingly, exosomes are lipid bilayers with a proportion of 40–100 nm that carry a variety of bioactive molecules such as functional proteins, nucleic acids and single lipids. They can be observed in bodily fluids like serum, urine, saliva, plasma, bile, breast milk, etc.[13] Cargo molecules like DNA, RNA (mRNA, tRNA, miRNAs), protein and lipids which are released by exosomes are used to facilitate intercellular communication.[9] However, exosomes and their recipient cells interact in three ways. Firstly, exosomes' transmembrane proteins come into direct contact with the target cell signalling receptors.[14] Secondly, exosomes bind to the recipient cells' plasma membrane and transfer their components into the cytoplasm.[14] Thirdly, the exosomes are then absorbed by the recipient cells and have two possible outcomes. In one way, some absorbed exosomes may combine with endosomes and undergo transcytosis, which allows exosomes to travel across recipient cells and into neighboring cells. On the contrary, endosomes fused from absorbed exosomes and mature into lysosomes and are degraded.[14] As stated above, exosomes contain a large number of compounds. Of these compounds, microRNAs (miRNAs) have secured much significance due to their regulatory involvement in gene expression. miRNAs are the most common type of short non-coding RNAs, ranging in length from 19–25 nucleotides, and are synthesized by two RNase III proteins, Drosha and dicer, which functions as RNA silencing guide molecules.[15] Exosomes carrying miRNAs can move with their respective vehicle, all owing them to reach nearby and distant cells. After being delivered into recipient cells, exosomal miRNAs execute a critical role.[16] The sorting of miRNAs into exosomes takes place in four ways namely the neural sphingomyelinase 2 (nSMase2)–dependent pathway, the heterogenous nuclear ribonucleoprotein (hnRNP)–dependent pathway, the 3' end of the miRNA sequence-dependent pathway and the miRNA induced silencing complex (miRISC)-dependent pathway.[14] As a result, miRNAs have a unique sequence that may direct exosome insertion. Figure 1 represents the biogenesis of exosomal miRNAs.

III. ROLE OF EXOSOMAL MIRNAS IN T2DM PROGRESSION

A. Exosomal miRNAs Role in Insulin Resistance

Insulin resistance is the inability of a known amount of exogenous or endogenous insulin to boost glucose absorption and utilization in an individual to the

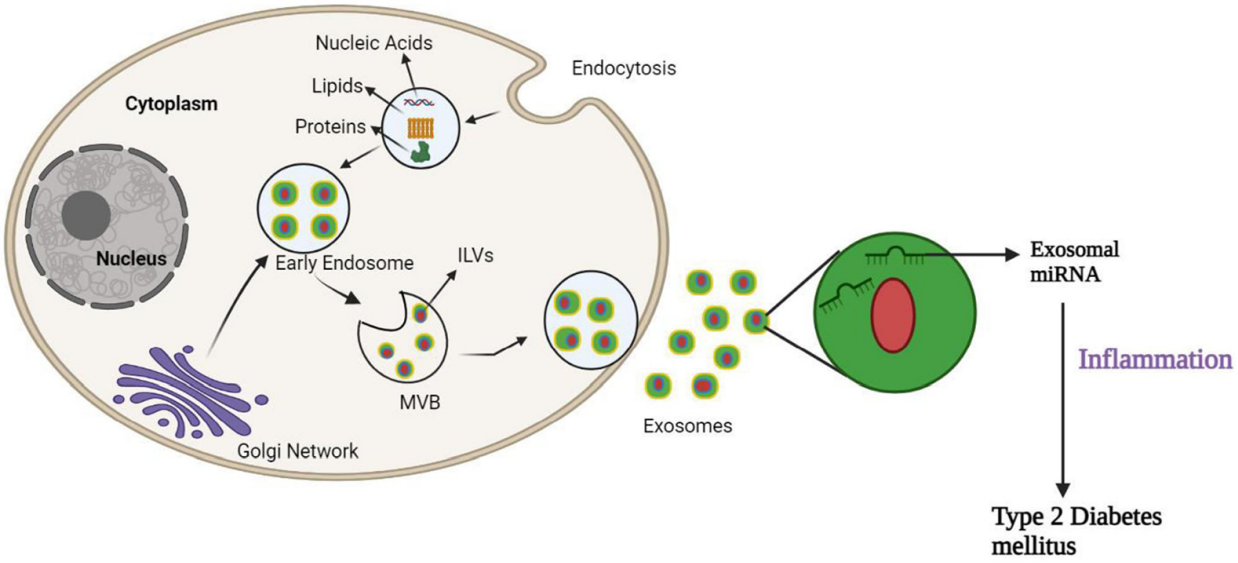

FIG. 1: Biogenesis of exosomal miRNA. Vesicles in the intraluminal lumen (ILVs) create exosomes. The endocytotic pathway is used to transport the proteins, lipids and nucleic acids to early endosomes. Early endosome maturation results in the formation of multivesicular bodies (MVBs). During the ILV manufacturing process, lipids like ceramides and cholesterol, proteins like cytoplasmic proteins, tetraspanins, and membrane receptors, and nucleic acids like miRNAs, DNAs, and RNAs are all integrated into exosomes. These exosomal miRNAs can be found in excess in case of inflammation in type 2 diabetes mellitus (T2DM). Hence, they could be found in circulation in bodily fluids and could be used as a potential biomarker for the diagnosis of T2DM.

same extent as it does in the regular population and it is a hallmark of T2DM.[17] Insulin resistance primarily affects cell tissue such as fat tissue, muscle tissue and hepatocytes that reliant on insulin to absorb glucose.[18] Recent research has discovered that exosomal miRNAs have an underlying role in the insulin resistance mechanism.[19] In 2019, Su et al. revealed that exosomal miR-29b-3p produced from bone marrow mesenchymal stem cells might alter aging-related insulin resistance, suggesting that it could be exploited as a therapeutic target for aging-related insulin resistance.[20] The *in vitro* and *in vivo* studies of Liu et al. stated that macrophage reside within adipose tissue (ATM)-derived exosomal miR-29a reduced insulin sensitivity and the effect of miR-29a on obesity-induced insulin resistance are mediated by PPAR-δ, which is a target gene. Thus, suggesting that it could be utilized as a therapeutic target for obesity related T2DM.[21] In 2020, Li et al. demonstrated that increased levels of gonadal white adipose tissue (gWAT)-derived exosomal miR-222 in the serum of obese model mice induced insulin resistance in the liver and skeletal muscle tissue via inhibiting insulin receptor substrate 1 (IRS1) and thus exosomal miR-222 can be applied as a possible target for the treatment of obesity-induced metabolic syndrome and T2DM.[22] In 2021, Ying et al. stated that treatment with M2 exosomes improved insulin sensitivity both *in vivo* and *in vitro*, but deletion of exosomal miRNA blocked these benefits. Ying and his team discovered that miR-690 is a critical insulin-sensitizing miRNA that is overexpressed within M2 Exos, implying that miR-690 could be used to treat metabolic illnesses as a new insulin-sensitizing drug.[23] Li et al. confirmed that obesity in high-fat diet-fed animals caused bone marrow-derived macrophages to polarize to the M1 type and produce exosomes that delivered miR-143-5p to hepatocytes. Thus, by inhibiting MKP5 expression miR-143-5p altered the insulin signalling pathway in hepatocytes.[24] In 2022, Byun et al. studied on exosomal miR-25-3p using saliva of T2DM patients and their findings even provide evidence on how exosomal miR-25-3p regulates interleukin-17 (IL-17)–mediated local inflammation throughout

the progression of periodontitis. Their findings also demonstrated that inhibiting miR-25-3p stopped periodontal inflammation and bone loss from progressing by deactivating IL-17–producing T cells. However, more mechanistic research is required to determine whether miR-25-3p inhibitors are useful in treating diabetes-related periodontitis in patients.[25] Inclusively, all these findings provided that exosomal miRNAs play an important function in the pathogenic phase of insulin resistance and more validated research are required in finding out the novel exosomal miRNAs to treat insulin resistance. Table 1 shows the role of exosomal miRNA in insulin resistance.

IV. EXOSOMAL MIRNA ROLE IN T2DM COMPLICATIONS

A. Exosomal miRNA Role in Diabetic Cardiomyopathy

Diabetic cardiomyopathy is a complication of T2DM that causes structural, physiological and metabolic abnormalities in the heart. Diabetic cardiomyopathy affects 12% of T2DM patients, is now recognized as a crucial complication of the disease caused by long-term hyperglycemia and hyperlipidemia, which causes an increase in cardiac oxidative stress, inflammation, and myocardial fibrosis, as well as unfortunate events in Ca^{2+} handling and mitochondrial function.[26] Exosomal miRNAs have been linked to a variety of diabetic cardiomyopathy risk factors in recent researches, suggesting that they could be used as therapeutic targets. Wang et al., studies stated that in the recipient mouse cardiac endothelial cells (MCECs), exosomal miR-320 significantly inhibited its target genes IGF-1, Hsp20 and Ets2 and upregulation of miR-320 prevented MCEC migration and tube formation. In Goto-Kakizaki (GK) rat model exosome-mediated angiogenesis inhibitory effects were eliminated by knocking out miR-320. As a result, the study revealed a unique mechanism underlying diabetes-induced cardiac vascular deprivation, which may be mediated by cardiomyocytic release of anti-angiogenic exosomes.[27] In 2017, De Gonzalo-Calvo et al. examined the cardiomyocyte-enriched miRNA profile in serum from patients with well-controlled T2DM and no mechanical heart disease or inducible ischemia (N = 86), in serum from a high-fat diet-fed mouse model, and in exosomes from lipid-loaded HL-1 cardiomyocytes. It was noted that exosomes released from lipid-loaded HL-1 cardiomyocytes have greater levels of miR-1 and miR-133a. Thus, the findings demonstrated that miR-1 and miR-133a can be applied as a diagnostic tool for diabetic cardiomyopathy in the subclinical stage.[28] However, more research into molecular pathways is needed in order to improve diabetic cardiomyopathy treatment options. Table 2 shows the role of exosomal miRNAs in T2DM complications.

B. Exosomal miRNAs Role in Diabetic Nephropathy

Diabetic nephropathy is a microvascular condition linked with poor glycemic control in people with

TABLE 1: Role of exosomal miRNAs in insulin resistance

Exosomal miRNA	Target	Mechanism of action	Ref.
miR-29b-3p	SIRT1	Modulate aging-related insulin resistance	Su et al.[20]
miR-29a	PPAR-δ	Reduced insulin sensitivity	Liu et al.[21]
miR-222	IRS1	Promoted insulin resistance in the liver and skeletal muscle of HFD-fed obese mice	Li et al.[22]
miR-690	NADK	Role in modulating macrophage inflammation and insulin signaling	Ying et al.[23]
miR-143-5p	MKP5	Induced insulin resistance in hepatocytes	Li et al.[24]
miR-25-3p	CD69	Contributed to development and progression of diabetes-associated periodontitis	Byun et al.[25]

TABLE 2: Role of exosomal miRNAs in T2DM complications

Complications	Exosomal miRNA	Target	Mechanism of action	Ref.
Diabetic cardiomyopathy	miR-320	IGF-1, Hsp20 and Ets2	Prevents mouse cardiac endothelial cells migration and tube formation	Wang et al.[27]
	miR-1 and miR-133a	SNORD48	Reflects myocardial steatosis in uncomplicated T2DM	de Gonzalo-Calvo et al.[28]
Diabetic nephropathy	miR-15a-5p, miR-362-3p, miR-150-5p, and miR-877-3p	SESN1	Prevents fibrosis of DM function and act as biomarkers	Xie et al.[31]
	let-7c-5p	CD9, Alix, and TSG101	Correlated with renal function and progression of diabetic nephropathy	Li et al.[32]
	miR-21-5p and miR-30b-5p	EGFR	Potential markers of renal function	Zang et al.[33]
	miR-4534	BNIP3	Role in the progression of type 2 diabetic kidney disease	Zhao et al.[34]
	miR-483-5p	HNRNPA1	Extracellular matrix deposition and the progression of DN-induced renal interstitial fibrosis	Liu et al.[35]
Diabetic retinopathy	miR-17-3p	STAT1	Ameliorates inflammatory reaction and antioxidant injury	Li et al.[39]
	miR-377-3p	VEGF	Suppresses retinal pigment epithelium proliferation	Jiang et al.[40]
	miR-486-3p	TLR4	Inhibits oxidative stress, inflammation, and apoptosis, and promotes proliferation of HG-treated Muller cells	Li et al.[41]
	miR-9-3p	S1P1	Promotes angiogenesis	Liu et al.[42]
	miR-133b-3p	FBN1	Inhibits angiogenesis and oxidative stress	Liang et al.[43]
Diabetic neuropathy	miR-146a	IRAK1, TRAF6	Promotes neurite outgrowth of diabetic DRG neurons and migration of Schwann cells	Wang et al.[49]
	miR-146a	TLR4	Suppresses peripheral blood inflammatory monocytes and the activation of endothelial cells	Fan et al.[51]
Diabetic foot ulcers	miR-20b-5p	Wnt9b	Reverses diabetes-associated impaired wound healing	Xiong et al.[54]
	miR-15a-3p	NOX5	Accelerates diabetic wound repair	Xiong et al.[55]
	miR-24-3p	PIK3R3	Restores angiogenesis and facilitates wound repair	Xu et al.[56]
	miR-128-3p	SIRT1	Increases angiopoiesis and suppresses apoptosis by autophagy activation	Shi et al.[57]
	miR-21-5p	VEGFR	Promotes ischemic repairment and angiogenesis of diabetic foot	Huang et al.[58]
	miR-31-5p	HIF1AN	Promotes angiogenesis and enhances diabetic wound healing	Yan et al.[60]

T1DM and T2DM. It eventually leads to end-stage renal failure, which affects 40% of individuals who require renal replacement treatment.[29] Glomerular hypertrophy, proteinuria, reduced glomerular filtration, and renal fibrosis with loss of kidney function are all symptoms of diabetic nephropathy.[30]

Interestingly, in 2017, Xie et al. demonstrated that many investigations have used microarrays to explore urinary exosome derived miRNAs. Xie et al. discovered a number of new diabetic kidney disease related miRNAs, including miR-15a-5p, miR-362-3p, miR-150-5p and miR-877-3p, that could be potential candidate biomarkers as well as a potential therapeutic target for incipient diabetic kidney disease, particularly miR-877-3p.[31] Moreover, in 2018, Li et al. conducted research using urine sample which were taken from fifteen healthy volunteers, twenty each T2DM patients without diabetic nephropathy and with diabetic nephropathy who had their kidney biopsy. Exosomal miRNAs such as let-7c-5p, miR-15b-5p, miR-29c-5p, and RNU6 were determined using RT-PCR. In diabetic nephropathy patients' urinary exosomes, let-7c-5p was considerably elevated compared with control, while miR-15b-5p and miR-29c-5p were substantially downregulated relative to control. Thus, Let-7c-5p generated from urinary exosomes is linked to renal function as well as the course of diabetic nephropathy, implying that it could be used as a biomarker for the disease.[32] In 2019, Zang et al. showed that unlike T2DM individuals with good kidney function, urinary exosomal miR-21-5p was shown to be elevated and whereas miR-30b-5p expression was decreased in T2DM and diabetic kidney disease and chronic kidney disease. However, both the miRNAs have a strong relationship with serum creatinine levels. Although more research is needed to identify the degree of this correlation more broadly among participants with various renal diseases. Thus, urinary exosomal miR-21-5p and miR-30b-5p may indicate potential markers of renal function.[33] In 2020, Zhao et al. showed that 14 urinary exosomal miRNAs (miR-4491, miR-2117, miR-4507, miR-5088-5p, miR-1587, miR-219a-3p, miR-5091, miR-498, miR-4687-3p, miR-4534, miR-1275, miR-5007-3p, and miR-4516) were up-regulated in diabetic kidney disease patients when compared with healthy individuals and DM patients. However, urinary exosomal miR-4534 may influence the FoxO signaling pathway by targeting BNIP3, and it is likely to develop as a new diagnostic marker for the advancement of type 2 diabetic kidney disease, which allowing for more research into the disease pathophysiology.[34] In 2021, Liu et al. confirmed that, under the disease state of diabetes HNRNPA1- mediated exosomal sorting transferred cellular miR-483-5p out of tubular epithelial cells (TEC) into the urine. The restriction of cellular miR-483-5p on TIMP2 mRNAs and MAPK1, ultimately enhanced extracellular matrix accumulation and the development of diabetic nephropathy-induced renal interstitial fibrosis. Significantly, the expression of miR-483-5p in exosomes originating from urine was strongly associated with urinary albumin to creatinine ratio (ACR) in diabetic patients' urine samples, suggesting that miR-483-5p may be linked to the development of renal disease in medical care. This research findings could lead to new insights into the management and treatment of diabetic nephropathy.[35] Cho et al. investigated the effect of dipeptidyl peptidase-4 (DPP-4) inhibitors on the renal microenvironment and its kidney-protective in T2DM patients. The expression pattern of urine exosome-derived miRNAs (let-7c-5p, miR-200a, miR-23a-3p, miR-205, miR-30d, and miR-26a-3p) in patients using a DPP-4 inhibitor versus patients on a sulfonylurea as a second-line antihyperglycemic treatment was examined and compared. It was observed that there were no notable changes in urine exosomal miRNA expression amongst diabetic on a DPP-4 inhibitor and those receiving sulfonylurea, but, however, it was noted that diabetic individuals had possessed high levels of miR-23a-3p than nondiabetic individuals. Thus, there is a requirement for more research on the impact of diabetes treatment on exosomal miRNAs expression.[36] All of the above-mentioned exosomal miRNAs are critical for the evaluation and management of T2DM-related kidney disorders.

C. Exosomal miRNA Role in Diabetic Retinopathy

Diabetic retinopathy is the key reason of vision impairment and blindness, a sight-threatening neurovasculopathy.[37] Angiogenesis, leaky retinal vasculature,

retinal inflammation, and retinal ischemia are all symptoms of diabetic retinopathy.[38]

In 2021, Li et al. revealed that administration of up-regulated exosomal miR-17-3p lessen blood glucose and glycosylated hemoglobin (HbAlc), increased weight, hemoglobin content and glutamine synthetase level, decreased inflammatory factors and vascular endothelial growth factor (VEGF), reduced oxidative stress, and suppressed retinal cell death in diabetic retinopathy mice.[39] Interestingly, Jiang et al. showed that when ARPE-19 cells were treated with exosomes from patients with T2DM and diabetic macular edema (DME), vascular endothelial growth factor (VEGF) was considerably increased compared with exosomes from patients with T2DM alone. Luciferase assay stated that miR-377-3p can directly influence VEGF expression. Thus, the serum exosomal miR-377-3p was discovered to be a potential biomarker for DME.[40] Moreover, Li et al. showed that bone marrow mesenchymal stem cells (BMSC)-derived exosome induced upregulation of miR-486-3p protected diabetic retinopathy mice via suppression of the TLR4/NF-κB axis.[41] Li et al. showed that exosomal miR-9-3p in proliferative diabetic retinopathy (PDR) released by hMGs had a role in retinal angiogenesis control by targeting S1P1/AKT/VEGFR2 pathway. This, overall research contributed to a deeper knowledge of the precise cellular and molecular mechanisms that underpin diabetic retinopathy, as well as novel potential treatment targets in the future.[42] In 2022, Liang et al. performed an experiment to figure out how miR-133b-3p works in diabetic retinopathy. Mice retinal microvascular endothelial cells (mRMECs) were co-cultured with or transfected with BMSC-derived exosomes after being treated with high glucose. It was observed that upregulating miR-133b-3p or downregulating FBN1 or BMSC-derived exosomes in HG-treated mRMECs reduced proliferation, angiogenesis, oxidative stress, migration, and accelerated apoptosis. These findings stated that exosomal miR-133b-3p from BMSCs inhibited angiogenesis and oxidative stress in diabetic retinopathy via FBN1 regulation.[43] For proper diagnosis, prognosis, and therapeutic aim, further validation of the above-mentioned discovered exosomal miRNAs in diabetic retinopathy is required.

D. Exosomal miRNA Role in Diabetic Neuropathy

Diabetic neuropathy is a rare peripheral nerve system neurodegenerative condition that primarily affects sensory axons, autonomics axons, and to a lesser extent motor axons.[44] It affects 30–50% of persons with diabetes and results in a decreased quality of life and physical functioning.[45] Diabetic amyotrophy, radiculopathy, and mononeuropathy, among other less common but more painful symptoms, are more common in patients with T2DM.[46] According to the finding it was stated that up to 50% of diabetic peripheral neuropathy cases are asymptomatic.[47] As a result, more sensitive and practical biomarkers that discovered the intensity or stage development are needed in addition to clinical signs and neurological findings for the early diagnosis of diabetic neuropathy.

In 2019, Venkat et al. studied the neurorestorative effects of exosomes generated from mouse brain endothelial cells (EC-Exo) as a stroke treatment in T2DM mice, as well as the involvement of miR-126 in EC-Exo derived therapeutic benefits in T2DM-stroke animals. The study concluded that EC-Exo therapy promoted endothelial capillary tube formation and primary cortical neuron axonal outgrowth in invitro condition, whereas miR-126/EC-Exo inhibits EC-Exo induced capillary tube formation and axonal outgrowth. Thus, in T2DM mice, miR-126 may regulate the neurorestorative effects of EC-Exo.[48] In 2020, Wang et al. demonstrated that Schwann cell-derived exosomes (SC-Exo) treatment reversed diabetes by reducing mature form of miRNAs (miR-21, miR-27a and miR-146a) and increased semaphorin 6A (SEMA6A), Ras homolog gene family, member A (RhoA), nuclear factor-κB (NF-κB) and phosphatase and tensin homolog (PTEN) expression in sciatic nerve tissues. Overall, these new findings show that SC-Exos have a therapeutic impact on diabetic peripheral neuropathy (DPN) in mice and that SC-Exo regulation of miRs plays a role in this therapy.[49] Fan et al. suggested that mesenchymal stroma cells (MSC)-exosome

(miR-17, miR-23a and miR-125b) administration decreased inflammatory response and alleviated DPN neurovascular reconstruction and enhanced functional recovery in diabetic mice. Thus, these data expand the range of applications for which they can be used as MSC-exosomes as a possible therapy for DPN patients.[50] Furthermore, another study conducted by Fan et al. showed that exosomes, which act as biologic carriers for miR-146a, can efficiently mediate and improved MSC therapeutic efficacy in diabetic mice.[51] These findings demonstrated that exosomal miRNAs can be used to treat diabetic neuropathy.

E. Exosomal miRNA Role in Diabetic Foot Ulcers

Diabetic foot ulcers are a frequent consequence of DM that cause a lot of morbidity, fatality and medical costs. It is evaluated that 19–34% of diabetic patients are expected to acquire a diabetic foot ulcer, and the International Diabetes Federation estimates that 9.1–26.1 million people will develop diabetic foot ulcers each year.[52] Ulcers can develop for a multitude of factors, and once established, they often heal slowly and with a high risk of recurrence, putting a massive economic strain on those who are affected.[53] One of the most common causes of diabetic foot ulcer is peripheral artery disease, which leads to foot ulcers and nonhealing ulcers.

According to new data, exosomal miRNAs have been demonstrated to have a significant part in the progression and improvement of diabetic wound healing in T2DM patients. In 2020, Xiong et al. demonstrated that diabetic circulating exosomes in plasma hindered cutaneous wound healing in both *in vitro* and *in vivo* by altering the angiogenic activity of vascular endothelial cells. Exosomal accumulation of miR-20b-5p performed a pivotal function in preventing wound healing by inhibiting the Wnt9b/-catenin signalling pathway. This results also indicated that using nanomaterials in combination with miR-20b-5p inhibitors to improve diabetic wound healing could be a potential therapeutic method in the future.[54] Another study by Xiong et al. examined the impact of circulating exosomal miRNA (miR-15a-3p) on diabetic wound repair. Exosomes from diabetic patients had higher levels of miR-15a-3p, which hindered wound healing. In diabetic exosomes, knocking down miR-15a-3p partially corrected their adverse impact *in vitro* and *in vivo*. NADPH oxidase 5 (NOX5) was discovered to be a viable target of miR-15a-3p, and inhibiting NOX5 lowered the reactive oxygen species (ROS) production, compromising the efficiency of human umbilical vein endothelial cells (HUVEC). Thus, this study concluded that inhibiting circulating exosomal miR-15a-3p enhanced diabetic wound repair by promoting NOX5, suggesting a new therapeutic target for diabetic foot ulcer treatment.[55] Xu et al. have observed that circulating exosomal miR-24-3p was found to be abundant in diabetic patients. As a result, inhibiting exosomal miR-24-3p and binding of miR-24-3p to the 3' UTR of phosphatidylinositol 3-kinase regulatory subunit gamma (PIK3R3) mRNA and PIK3R3 expression improved the functioning of HUVEC *in vitro*. Hence, the result suggested that inhibition of exosomal miR-24-3p via targeting PIK3R3 can act as a therapeutic target in treating diabetic foot ulcers.[56] Shi et al. found that absorption of miR-128-3p and activation of SIRT1 by mmu-circ-0000250 improved the therapeutic impact of adipose derived mesenchymal stem cells (ADSCs)-exosomes to promote wound healing in diabetes. Thus, targeting the mmu-circ-0000250/miR-128-3p/SIRT1 axis as a potential treatment strategy for diabetic ulcers is being considered.[57]

Recently, in 2021, Huang et al. discovered that exosomal miR-21-5p was found to increase angiogenesis by upregulating vascular endothelial growth factor receptor (VEGFR) and activating serine/threonine kinase (AKT) and mitogen-activated protein kinase (MAPK) (MAPK). Their studies also implied that exosomes improved ischemic tissue healing and angiogenesis through a new mechanism involving miR-21-5p. Thus, exosomal miR-21-5p might be used as a novel biomarker for MSC in the treatment of diabetic foot.[58] In 2022, Wang et al. explained that, when compared with fibroblast exosomes (FB-Exo) and PBS control, treatment with both epidermal stem cells (ESCs) and ESCs-derived exosomes (ESCs-Exo) increased wound healing in db/db mice by reducing inflammation, increasing wound cell

proliferation, boosting angiogenesis, and inducing M2 macrophage polarization. According to their *in silico* functional analysis, ESCs-Exo-miRNAs target genes were predominantly engaged in homeostatic processes and cell differentiation, which also revealed regulatory control of the PI3K/AKT and TGF-β signaling pathways.[59] Thus, the findings suggest that ESCs and ESCs-Exo are similarly successful in improving diabetic wound healing, and that ESCs-Exo treatment could be a viable and technically efficient substitute to stem cell therapies. Interestingly, in 2022, Yan et al. established a new method for improving diabetic wound healing by loading miR-31-5p into milk-derived exosomes. Their study showed that raw milk can be used to capture large numbers of exosomes in a safe and cost-effective manner, and milk exosomes offer significant potential as a miRNA delivery vehicle, enhancing the stability and cell uptake of miRNA. By suppressing the expression of HIF1AN, exosomal formulation of miR-31-5p greatly improved endothelial cell activities and promoted the recovery process of the diabetic lesion. These properties of miR-31-5p exosomes give information on their role in treating a diabetic wound, and also milk exosome use in nucleic acid medication delivery has a great potentiality.[60] This research suggests that exosomal miRNAs could be useful in the treatment of diabetic foot ulcers.

V. FUTURE PERSPECTIVES

Exosomal miRNAs play a prominent role in T2DM onset and development. Exosomes, as endogenous nanocarriers, can be employed in target therapy by carrying drug cargos to disease-associated targeted cells.

Some of the challenges that are to be rectified in using exosomal miRNAs are presently, there is no best purification procedure for isolating high-purity exosomes. Exosomal miRNAs need to be studied further to see if they are linked to one or more diseases, as well as to learn more about the underlying molecular mechanisms in diseases. Despite the fact that exosomal miRNAs appear to play an impact on the prevalence of diabetes and its complications in numerous animal models, clinical trials are needed to establish the reliability and feasibility of these findings.

VI. CONCLUSION

Exosomal miRNAs are linked to the advancement of DM and its consequences. Exosomal miRNAs have been recognized as unique and possibly helpful molecules in the field of diabetes research. It will be critical to explore miRNAs as a new real therapeutic alternative that improves T2DM patients by designing and conducting safe clinical studies in which they are employed as therapeutic instruments. Thus, exosomal miRNAs are being proved to be exploited as a diagnostic, prognostic and therapeutic targets in the treatment of T2DM progression and its complications.

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