Review

Integrative biology of extracellular vesicles in diabetes mellitus and diabetic complications

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Received: 2021.08.06; Accepted: 2021.12.11; Published: 2022.01.01

Abstract

Diabetes mellitus (DM) is a chronic systemic disease with increasing prevalence globally. An important aspect of diabetic pathogenesis is cellular crosstalk and information exchange between multiple metabolic organs and tissues. In the past decade, increasing evidence suggested that extracellular vesicles (EVs), a class of cell-derived membrane vesicles that transmit information and perform inter-cellular and inter-organ communication, are involved in the pathologic changes of insulin resistance (IR), inflammation, and endothelial injury, and implicated in the development of DM and its complications. The biogenesis and cargo sorting machinery dysregulation of EVs may mediate their pathogenic roles under diabetic conditions. Moreover, the biogenesis of EVs, their ubiquitous production by different cells, their function as mediators of inter-organ communication, and their biological features in body fluids have generated great promise as biomarkers and clinical treatments.

In this review, we summarize the components of EV generation and sorting machinery and highlight their role in the pathogenesis of DM and associated complications. Furthermore, we discuss the emerging clinical implications of EVs as potential biomarkers and therapeutic strategies for DM and diabetic complications. A better understanding of EVs will deepen our knowledge of the pathophysiology of DM and its complications and offer attractive approaches to improve the prevention, diagnosis, treatment, and prognosis of these disorders.

Key words: diabetes mellitus, extracellular vesicles, biogenesis, sorting, adipocytes, macrophages, islet, endothelial cells

Introduction

Diabetes mellitus (DM), a systemic disease with an alarming increase in incidence worldwide, is characterized by chronic hyperglycemia resulting from insulin resistance (IR) and/or insulin secretion deficiency. The International Diabetes Federation estimates that 9.3% of adults aged 20–79 years are currently living with DM and this prevalence is projected to rise to 10.2% by 2030 and to 10.9% by 2045 [1]. DM and associated complications account for 11.3% of global deaths from all causes [1]. Moreover, DM is an independent risk factor for various diseases, including coronary heart disease, stroke, cancer, chronic kidney disease, blindness, and lower limb amputation, placing a heavy burden on global health [2-6]. There are no efficacious pharmacological treatments for DM, primarily due to its complicated pathophysiologic mechanisms and unclear etiopathogenesis.

Generally, DM can be mainly divided into type 1 diabetes (T1D) and type 2 diabetes (T2D). T2D is the most common type of DM, accounting for about 90% of cases worldwide, whereas T1D constitutes more than 10% [7, 8]. Although the etiologies of T1D and T2D are distinct, the progression of both diseases is
primarily due to dysregulated intercellular and inter-organ communication. In T1D, the interaction between immune cells and pancreatic islets contributes to the dysfunction and death of β cells. More recently, the involvement of gut microbiota in the auto-immune response against β cells has added another layer of complexity to T1D pathogenesis [9]. In T2D, intricate inter-organ communication among the pancreas, adipose tissue (AT), liver, muscle, intestine, hypothalamus, and other tissues plays an essential role in hyperglycemia and IR, two hallmarks of T2D [10]. For example, increased inflammatory cytokines and free fatty acids (FFAs) derived from obese AT can induce lipotoxicity and IR in the liver and skeletal muscle, and also impair the glucoregulatory function of the central nervous system (CNS) and gut, in turn, perturbing the AT secretome and reinforce its IR [11]. Furthermore, systemic IR triggers a rise in insulin demand, overstressing β cells and eventually resulting in islet dysfunction and relative insulin insufficiency. Therefore, dysregulated inter-organ communication plays a key role in initiating and amplifying the deleterious vicious cycle of IR and hyperglycemia in T2D. Under these circumstances, signaling molecules mediating inter-organ conversation are likely key pathogenic factors for T1D and T2D. Indeed, several classes of signaling mediators, including adipokines, hepatokines, peptides from CNS, and hormones from the pancreas and intestine, are crucial in the initiation and progression of both T1D and T2D [12-18], and therapeutic strategies targeting these molecules have been partially applied in the clinic benefiting patients.

Recently, extracellular vesicles (EVs) have emerged as a novel class of signaling molecules mediating intercellular and inter-organ communication. Released by various cell types, EVs are widely distributed in diverse tissues and body fluids. Moreover, bioactive contents loaded in EVs including proteins, DNAs, RNAs, lipids, and metabolites, are protected by the lipid bilayer membrane against harsh environments and prevented from degradation and digestion. The size, quantity, morphology, cargoes, and other characteristics of EVs are highly variable and influenced by the parental cell type. Because of these fundamental features, EVs are well-suited to serve as versatile carriers and transporters transmitting signals from parental cells to recipient cells. Correspondingly, EVs have been shown to regulate various biological and physiological processes and are implicated in multiple human diseases, such as cancer, cardiovascular diseases, metabolic disorders, and neurodegenerative diseases [19]. In particular, understanding the role of EVs in the crosstalk among multiple metabolic tissues would provide a new perspective to understand the pathogenesis of DM and diabetic complications and develop therapeutic strategies.

Here we outline the current knowledge of diabetic pathogenesis, focusing on the potential mechanisms underlying the altered EV biogenesis in DM and the role of EVs originating from different cells in regulating systemic metabolism. Finally, we summarize studies of EV-RNAs as markers and discuss potential applications of EVs derived from native cells to treat DM and diabetic complications.

Pathology of DM and its complications

IR, also known as low insulin sensitivity, is an essential mechanism underlying T2D occurrence and a critical driver of associated complications [20]. Although there is no consensus on the molecular mechanism(s) triggering IR, inter-organ communication has been widely recognized as a key contributor [21]. During obesity, massive expansion of AT, often accompanied by inadequate vascularization, induces hypoxic response and inflammation, leading to increased infiltration of pro-inflammatory macrophages and inflammatory cytokine release [22]. Inflammation can further disturb insulin signaling in AT, resulting in enhanced lipolysis and increased release of FFAs and adipokines into circulation. Subsequently, elevated circulating FFAs elicit lipotoxicity and impair insulin action in the liver and skeletal muscle [23, 24]. Also, downstream pathways aroused by IR cooperatively induce reactive oxygen species (ROS) production and systemic inflammation, further worsening IR. Consequently, IR suppresses plasma membrane translocation of the glucose transporter (GLUT) and glucose uptake, leading to elevated blood glucose levels and systemic energy metabolism disturbance. In addition to classic metabolic tissues, other organs such as the gut, vascular endothelium, and brain, have recently been shown to participate in the development of IR and T2D [25-29]. For example, vascular endothelium can function as an adjustable barrier to control the transport of metabolic macromolecules such as FFAs, lipoproteins, and glucose to metabolic organs, including the skeletal muscle and AT. Bioactive molecules secreted by endothelial cells (ECs), for e.g., nitric oxide and growth factors, may modulate systemic metabolism by modulating insulin sensitivity, maintaining pancreatic islet structure, and insulin secretion [30, 31].

Pancreatic β cell failure, another hallmark of T2D, is also associated with inter-organ communication. Increased demand for insulin, typically due to peripheral IR, leads to excess insulin sensitivity, maintaining pancreatic islet structure, and insulin secretion [30, 31].
secretion and elevated islet amyloid polypeptide (IAPP) production. Simultaneously expressed and secreted with insulin, IAPP is a membrane-permeant toxic agent, and its accumulation forms amyloid deposits, causing pancreatic damage [32]. Meanwhile, chronic elevated FFAs and glucose elicit endoplasmic reticulum (ER) stress and inflammatory response in islets, which aggravate the pancreatic injury and compromise insulin secretion [33, 34]. In this circumstance, the combination of IR and β cell decompensation contributes to overt T2D.

Insulin deficiency, primarily resulting from reduced β cell function and mass, is the major driver of T1D. Insulitis induced by autoimmune response results in β cell death and chronic autoantigen exposure, reamplifying the immune attack [35]. The uncontrollable autoimmune response against β cells accounts for T1D pathology. Besides the crosstalk between the islet and immune cells, gut and immune system communication has also been implicated in T1D pathogenesis. For example, loss of gut integrity and changes in metabolites caused by enteric dysbacteriosis can significantly promote innate and adaptive autoimmune response against islet cells, thereby participating in T1D development [36-39].

The development of diabetic complications shares multiple pathological processes with DM, such as glucose variability, lipotoxicity, and activation of advanced glycation end products (AGEs) and receptors for AGE (RAGEs) signaling, and consequent mitochondrial dysfunction, oxidative stress, epigenetic changes, and inflammation response [40-47]. Besides the in-depth understanding of underlying molecular mechanisms, the significance of inter-organ crosstalk in the pathogenesis of diabetic complications has recently been emphasized. For instance, there is increased lipoprotein secretion by insulin-resistant hepatocytes that can be glycated and oxidated, leading to renal lipid metabolism disorder and promoting the development of diabetic nephropathy (DN) [48-51].

Furthermore, inter-organ and intercellular communication are crucial in the pathogenesis of DM and diabetic complications, and our current understanding can only be considered as the tip of the iceberg. In this context, EVs, an emerging mediator of intra- and inter-organ crosstalk, have been shown to play a critical role in various pathological pathways of DM and its complications (Figure 1), providing a novel paradigm in pathological mechanisms and therapeutic interventions. Future investigation is required to delineate the molecular mechanisms of EV-mediated signaling under diabetic conditions and further explore their implications in treating these disorders.

**EVs**

Based on their biogenesis, EVs can be divided into two major groups, exosomes (30-100 nm) and microvesicles (MVs, 50-1000 nm) (also known as ectosomes, microparticles) [52]. MVs are generated directly by budding and shedding from the plasma membrane, while exosome generation involves intraluminal vesicle (ILV) budding and shedding, intracellular multivesicular endosome (MVE) trafficking, and ILV release [53, 54]. In this section, we mainly introduce EVs from two perspectives: (i) generative processes and (ii) mechanisms of cargo sorting.

**EV generation**

Both exosomes and MVs are vesicles formed by membrane budding away from the cytosol, and their generation requires an integrative cytoskeleton and membrane reorganization (Figure 2).

**Exosome generation**

The generation of exosomes principally consists of biogenesis, transport, and release. ILV formation is the first step of exosome generation, which depends on the endosomal sorting complex required for transport (ESCRT). Several ESCRT-independent ILV formation pathways mediated by ceramide-, CD63-, Rab31, and others have been detected [55-58]. As the best known ESCRT-independent mode, cone-shaped lipid ceramide enriched in specific microdomains of the endosomal membrane can effectively lead to membrane curvature alteration and budding of ILVs [55]. Tetraspanin CD63, the specific surface marker of exosomes, can favor the budding of ILVs by interacting with a cluster of other tetraspanins and proteins [56, 57]. Although several distinct exosome generation cellular pathways have been reported, the regulatory mechanisms within cells have not yet been elucidated.

The formation of ILVs follows the transport of MVEs toward the plasma membrane. Various intracellular trafficking molecules have been shown to participate in this process, including the cytoskeleton, molecular motors, and Rab GTPases. The final step is the fusion of MVEs with the plasma membrane for which the soluble N-ethylmaleimide-sensitive fusion attachment protein receptor (SNARE) complex is believed to be essential [59-63]. The interaction between vesicle-membrane SNAREs (v-SNAREs) and target-membrane SNAREs (t-SNAREs) initiates the SNARE complex assembly, presumably allowing the fusion of MVEs with the plasma membrane and leading to exosome secretion.
Figure 1. Inter-organ crosstalk mediated by EVs in the pathogenesis of DM and diabetic complications. EVs contain different proteins, RNAs, DNAs and lipids (inner circle). EVs participate in the development of DM and its complications via multiple ways. EVs derived from various tissues, including adipose, liver, pancreas, skeletal muscle, immunocytes, vascular endothelium and gut microbiota, play a role in the development and progression of DM (inner ring). Moreover, these EVs are involved in the pathogenesis of diabetic complications including diabetic foot, cardiomyopathy, nephropathy, retinopathy, neuropathy and atherosclerosis (outer ring). Abbreviations: circRNAs: circular RNAs; DM: diabetes mellitus; EV: extracellular vesicle; lncRNAs: long noncoding RNAs; miRNAs: microRNAs.

MV generation

MV generation consists of two crucial steps: plasma membrane blebbing and scissoring. Plasma membrane rearrangement involving lipid and protein composition remodeling is the first essential step for membrane budding, and is believed to be a calcium (Ca²⁺)-dependent process [64]. A group of Ca²⁺-dependent enzymes, including flippases, floppases, and lipid scramblases, are involved in the rearrangement of membrane phospholipids [65]. Mechanistically, phospholipid redistribution and maintenance can induce membrane lipid asymmetry and alter membranous curvature [66, 67]. Besides lipid redistribution, unlocking the plasma membrane-cytoskeletal anchorage is necessary for membrane blebbing and vesiculation. In this respect, calpain, a Ca²⁺-activated cysteine protease, can disrupt the attachment between the plasma membrane and cytoskeleton by cleaving several cytoskeletal components under the plasma membrane, such as actin and filamin [68, 69]. However, our understanding of MV generation is limited and further mechanistic investigation is required.

In addition to the EV biogenesis machinery, recent studies suggest that several types of cell death, such as apoptosis, necroptosis, pyroptosis, and neutrophil extracellular trap formation (NETosis), are associated with EV generation, indicating the involvement of additional sophisticated mechanisms modulating EV biogenesis [70-76]. EVs secreted by necroptotic cells mediate MLKL release, which can, in turn, serve as a self-control mechanism of necroptosis [77]. These findings collectively indicate that EVs can function as a specialized intra- and inter-cellular messaging system, highlighting the importance of illustrating EV generation mechanisms.
Figure 2. **EV biogenesis and cargo sorting.** Microvesicles and exosomes are two major categories of EVs. Microvesicles are released directly from plasma membrane budding and shedding. Exosomes are generated by inward budding of endosomes, known as MVEs, which fuse to plasma membrane, and are followed by the release of exosomes. Multiple molecules are implicated in the biogenesis of microvesicles and exosomes, such as ESCRT complexes and related proteins, ceramide, SMase, syntenin, syndecan, calpain, Rab GTPases, and so on [see text]. Exosomes contain different types of proteins and RNAs, whose sorting are modulated by several molecules, including ESCRT complexes, syntenin, tetraspanins, and RBPs. PTMs on certain proteins also have a role in the sorting of exosomal cargos. **Abbreviations:** aSMase: acid sphingomyelinase; ESCRT: endosomal sorting complex required for transport; EVs: extracellular vesicles; ILV: intraluminal vesicle; MVE: multivesicular endosome; nSMase: neutral sphingomyelinase; PTMs: post-translational modifications; RBP: RNA binding protein; SMase: sphingomyelinase, SNARE: soluble N-ethylmaleimide-sensitive fusion attachment protein receptor; t-SNAREs: target-membrane SNAREs; v-SNAREs: vesicle-membrane SNAREs.

**Sorting mechanism of EVs**

Bioactive molecules, including proteins, DNAs, mRNAs, non-coding RNAs (ncRNAs), and metabolites, are encapsulated in EVs. Accumulating evidence suggests that cargoes are not randomly packaged into EVs or simply replicate the composition of their parental cells [78]. Because of the significance of cargoes in signal communication, the mechanisms of cargo sorting of EVs are central to shed light on the physiological and pathological functions of EVs and their therapeutic implications. Although these mechanisms are far from being fully elucidated, recent advances provide exciting insights into this topic.

Recent studies have provided some clues on the sorting of proteins. First, ESCRT components and their related proteins can recruit exosomal cargoes through direct molecular interactions. For example, the ESCRT-I component TSG101 can recruit BAG6 into EVs, possibly playing a key role in directing EV proteins [79]. In addition, the noncanonical ESCRT-dependent syntenin pathway also contributes to the sorting of specific exosomal cargoes, including LMP1 and KRS [80, 81]. Second, common protein markers of EVs, particularly tetraspanins, have been suggested to account for sorting a great proportion of the exosomal proteins [82-85]. High-throughput proteomic analysis of potential proteins interacting with tetraspanin-enriched microdomains revealed a significant overlap between the tetraspanin interactome and exosomal proteome, highlighting tetraspanins as important sorting machinery for protein inclusion into exosomes [82]. Third, specific post-translational modifications (PTMs) seem to be emerging determinants for protein sorting in EVs, such as ubiquitylation [86-91], sumoylation [92], palmitoylation [93-96], farnesylation [97], phosphorylation [98-100], glycosylation [101-103], and lipidation [103]. For instance, there was a 60% reduction of total protein levels in EVs derived from ubiquitin-like 3 (UBL3)-knockout mice, and UBL3 could function as a PTM factor by directly interacting with more than 1,200 proteins [86]. Also, ESCRT components HRS, STAM, and TSG101 with their ubiquitin-binding domains might participate in
ubiquitination [104]. These observations emphasize the significance of ubiquitination, one of the most common PTMs, in sorting EV protein cargoes. Interestingly, ubiquitinated proteins have also been detected in the EVs secreted by insulin-secreting β cells, indicating a potential involvement in EV-associated islet cell dysfunction and T2D pathogenesis [105].

In addition to protein cargo, EVs carry a rich diverse RNA cargo, involved in many EV functions. The enrichment of distinct RNAs in EVs responsive to different cellular statuses relies on the sophisticated RNA sorting system. Recent studies suggest that the selective sorting of RNA in EVs is attributed mainly to RNA binding proteins (RBPs), accounting for about 25% of the protein content in EVs [106,107]. RBPs usually recognize RNAs with specific “tags”, such as certain motifs, modifications, structures, or sequences, and sort them into EVs [107,108]. For instance, hnRNPA2B1, a well-known regulator of RNA metabolism, can package specific miRNAs (miR-198, miR-30b-3p) and long noncoding RNAs (lncRNAs) (AFAP1-ASI, LNMAT2) into EVs by the direct interaction between its RNA-binding domains and the GGAG motif of the RNAs [109-113]. Besides hnRNPA2B1, other members of the hnRNP family, including hnRNPA1, hnRNPC1, hnRNP1H1, hnRNPK, hnRNPO, and hnRNPCU, have been implicated in RNA sorting and enrichment in EVs [114-120]. Additionally, YBX1 (miR-223 in HEK293T cells), human antigen R (HuR) (miR -122 in human hepatic cells), and other RBPs have been reported to play a role in the selective miRNA enrichment in EVs [121-123].

The exploration of EV cargo sorting machinery is not restricted to EVs per se. An exciting correlation of autophagy with EV biogenesis and content loading has been recently reported, in which the LC3-conjugation machinery is proposed to govern the RBP capture and thus specify RNAs in secreted EVs [124-126], adding another layer of complexity to EV cargo sorting. In summary, EVs are emerging as an important mediator of intercellular communication. Elucidation of the mechanism underlying EV generation and content packaging has been an active area of research.

EV biogenesis machinery in DM and diabetic complications

EVs are crucial information transmitters between original and recipient cells, and their abnormalities contribute to the development of DM and diabetic complications. Exploring the mechanisms underlying EV biogenesis and cargo sorting is critical in developing novel therapies for various diseases. So far, the role of EV biogenesis and sorting machinery in diabetic pathology has not been systemically reviewed. Here we summarize current knowledge about the EV machinery involved in the pathogenic process of DM and its complications (Table 1 and Figure 3).

**EV generation**

**ESCRTs**

ESCRT complexes participate in the generation of the majority of EVs. Multiple components of ESCRT complexes have been shown to play a role in various metabolic processes, especially glucose and lipid metabolism, indicating their potential involvement in DM and diabetic complications. Therefore, it is a reasonable assumption that EVs may partially mediate ESCRT functions in metabolism and metabolic diseases, albeit direct evidence is currently limited.

ESCRT complexes are involved in the transportation of lipid droplets and translocation of GLUT4 and glycogen synthase kinase 3β (GSK3β) in adipocytes, thereby mediating the regulation of neutral lipids micro-autophagy consumption, adipogenesis, and insulin-stimulated glucose uptake [213-215]. Disruption of these cellular biological processes is involved in the pathogenesis of DM. Lipotoxicity, a common risk factor for IR and T2D, can induce TSG101 expression in adipocytes and thus promote the biogenesis of exosomes [216]. Subsequently, TSG101 upregulation triggers the sorting of CD36 into EVs, which then are delivered into hepatocytes and evoke hepatic lipid accumulation [216]. Furthermore, several factors interacting with ESCRT components may regulate EV formation, such as MLKL and HSP20 [217-219]. MLKL, a critical factor involved in plasma membrane disruption and necroptosis, is upregulated in multiple tissues, including the adipose, liver, muscle, kidney, and cardiomyocytes under diabetic conditions [220-224]. MLKL can engage in the biogenesis of both exosomes and MVs by binding ESCRT proteins (TSG101, MVB128, VPS28, VPS37A, VPS25, CHMP3, CHMP4B, and CHMP2A) [218,219]. Interestingly, MLKL can also regulate insulin sensitivity in diabetic mice independent of its proinflammatory and necroptotic roles [220]. These observations indicate that non-necroptotic functions of MLKL might be mediated by its effect on EV formation.

In contrast to MLKL, HSP20 is downregulated in T1D and T2D and its reduction is considered a primary driver for DM-induced organ damage. HSP20 function, at least partially, is attributed to its regulatory activity on exosomes. Specific overexpression of HSP20 in cardiomyocytes can
increase the generation/secretion of exosomes enriched in HSP20, p-AKT, survivin, and SOD1 through interacting with TSG101, thereby attenuating cardiac dysfunction, hypertrophy, and microvascular rarefaction under diabetic conditions [217,225]. Besides the role of ESCRT components in metabolism and metabolic disease, it is anticipated that the crosstalk between ESCRTs and EVs may be involved in the pathogenesis of DM and its complications.

Table 1. Expressions and implications of EV biogenesis and sorting machinery under diabetic conditions.

| Genes  | Level/activity [Reference] | Sample/resource | Function |
|--------|----------------------------|-----------------|----------|
| aSMase | 1 | AT: T2D patients with FLD, ob/ob mice | Promoting thrombosis and inflammation |
|        | 1 | Serum: T2D patients, db/db mice | Promoting endothelial dysfunction |
|        | 1 | Plasma, REC, CD34+ CACs: T2D patients | Promoting inflammation and CACs migration |
|        | 1 | RPECs: STZ rats | Impairing mitochondrial function |
|        | 1 | Liver and brain: HFD mice | Promoting hepatic IR and neurodegeneration |
|        | 1 | Kidney: GK rats | Promoting ER stress |
| nSMase | 1 | AT: T2D patients with FLD, ob/ob mice | Promoting thrombosis and inflammation |
|        | 1 | Liver, brain: HFD mice | Promoting hepatic IR and neurodegeneration |
|        | 1 | Skeletal muscle: Wistar fatty rats | Promoting IR in the muscle |
|        | 1 | Vastus lateralis muscle: obese IGT patients | UD |
|        | 1 | Islet β cells: Akita mice | Promoting β cell apoptosis |
|        | 1 | Atrial appendage: obese T2D patients | UD |
| Sdc1   | 1 | Liver: obese Zucker fa/fa rats | Promoting hepatic IR |
|        | 1 | Neutrophils, serum: T2D patients | UD |
|        | 1 | Serum, small intestine: STZ mice | Promoting epithelial barrier damage |
|        | 1 | Plasma, serum: TID DN patients | Promoting inflammation and microalbuminuria |
|        | 1 | Vitreous fluid: PDR Patients | Promoting angiogenesis |
|        | 1 | Skeletal muscle, heart: HDF ob/ob mice | UD |
|        | 1 | Heart, skeletal muscle: STZ rats | Promoting cardiac dysfunction |
|        | 1 | Kidney: KK/Ta mice | UD |
|        | 1 | Skeletal muscle, heart: HDF ob/ob mice | Promoting growth factor resistance |
| HPSE   | 1 | Islet: NOD/Lt mice, T1D patients, STZ mice | Promoting β cell death |
|        | 1 | Serum: obese patients with diabetes | Promoting endotheial injury and inflammation |
|        | 1 | Urine, plasma: T2D patients | UD |
|        | 1 | Islet: STZ mice and rats, DN patients | Promoting renal damage, protein excretion |
|        | 1 | Vitreous fluid, serum, retina: PDR patients, STZ rats | Promoting inflammation, angiogenesis, and subendothelial barrier damage |
|        | 1 | Serum, small intestine: STZ mice | Promoting epithelial barrier damage |
|        | 1 | Carotid artery: DM patients, STZ rats | Promoting atherosclerosis |
| Calpain | 1 | Heart: STZ rats | Promoting apoptosis |
|        | 1 | Heart: HFD, STZ, OVE26 mice | Promoting myocardial hypertrophy, and fibrosis |
|        | 1 | Aorta: STZ and OVE26 mice | Promoting ROS and peroxynitrite production |
|        | 1 | Platelet: T2D patients, STZ mice | Promoting platelet hyperaggregability |
|        | 1 | Plasma: T2D patients | Promoting platelet activation and inflammation |
|        | 1 | Platelet: T2D patients | Promoting MVs release and inflammation |
|        | 1 | Dorsal root ganglion: STZ rats | Promoting oxidative stress and inflammation |
|        | 1 | Penis: STZ mice | Promoting erectile dysfunction |
|        | 1 | Lens epithelial cells: DR patients | UD |
| Calpain-1 | 1 | Heart: STZ rats | Promoting oxidative stress and apoptosis |
|        | 1 | Vascular mesentery: STZ and ZDF rats | Promoting endotheial inflammation |
|        | 1 | Retina: STZ rats, HDF rats | Promoting retinal ganglion cell death |
| Calpain-10 | 1 | Islet: T2D patients | Biomarker for islet dysfunction |
|        | 1 | Kidney: STZ rats, HDF rats | Promoting apoptosis and renal failure |
|        | 1 | Kidney: STZ rats, ob/ob mice | Promoting apoptosis and renal failure |
| SNARE  | 1 | Islet: T2D patients, GK rats, ZDF rats | Impairing insulin secretion |
|        | 1 | AT: STZ-NA rats | Promoting IR |
|        | 1 | Islet: skeletal muscle: Zucker rats, STZ rats | Promoting IR |
|        | 1 | Hippocampus: STZ rats | UD |
|        | 1 | Serum: TID patients | Promoting insulin as autoantigen |
| CD63   | 1 | Platelets: T2D patients | UD |
| CD82   | 1 | Kidney: DN patients | Promoting renal cell apoptosis |
| HuR    | 1 | Skin: DM patients | Promoting chronic inflammation |
|        | 1 | Kidney: DN patients, db/db mice, STZ rats | Promoting pyropptosis, inflammation, and EMT |
|        | 1 | Retina: STZ rats | Promoting angiogenesis |
|        | 1 | BMH/M03, heart: db/db mice | Promoting cardiac fibrosis and dysfunction |
|        | 1 | Heart: diabetic cardiomyopathy patients | Promoting pyropptosis and inflammation |
| hnRNPK | 1 | Islet: db/db mice | Promoting oxidative stress and apoptosis |
|        | 1 | Kidney: Akita mice | Promoting RAS activation and hypertension |

AT: adipose tissue; BM-MØ: bone marrow-derived macrophage; CACs: circulating angiogenic cells; DN: diabetic nephropathy; FLD: fatty liver disease; GK rats: Goto-Kakizaki rats; HFD: high-fat diet; IGT: impaired glucose tolerance; HPSE: heparanase; IGT: impaired glucose tolerance; IGT: insulin-dependent diabetes; LDL: low-density lipoprotein; LDLr: low-density lipoprotein receptor; N-ethylmaleimide-sensitive fusion attachment protein receptor; PDI: prolamin proteinase inhibitor; PPI: parvalbumin protein; PPAR: peroxisome proliferator-activated receptor; PTM: posttranslational modification; RNase: renin-angiotensin system; Sdc1: syndecan 1; Sdc4: syndecan 4; Sdc10: soluble N-ethylmaleimide-sensitive fusion attachment protein receptor; STZ+NA: streptozotocin+ nicotinamide; UD: undetermined; ZDF: Zucker fat diabetic; ↓: synaptotagmin, VAMP-2, syntaxin-1A and -2 and SNAP-25; ↑: SNAP23, syntaxin-4 and VAMP-2; •: syntaxin-4; ◊: syntaxin-1; ◊: VAMP2; ◊: phosphorylation.
Figure 3. Involvement of the EV biogenesis and cargo sorting machineries in DM and diabetic complications. Diabetic conditions trigger the alteration in the expression and activity of the molecules involved in the process of EV biogenesis and cargo sorting. 1. Lipotoxicity induces TSG101 expression and influences its interaction with CD36 and HSP20, leading to their exosomal sorting dysregulation; 2. Elevated syndecans and heparinase in DM animals and patients can potentially activate the syntenin-syndecan-ALIX pathway and promote exosomes biogenesis; 3. Elevated ceramide levels and nSMase/aSMase expression and activity may induce EV generation; 4. High glucose may impact the expression and activity of calpain 1 and 2, leading to elevated microvesicle generation; 5. Reduced SNARE components in diabetic conditions may influence exosomes release; 6. Altered expression of some regulators associated with EV cargo sorting, as well as certain PTMs of specific proteins, may also affect EVs proteome and RNA profile under DM conditions. Abbreviations: aSMase: acid sphingomyelinase; DM: diabetes mellitus; ESCRT: endosomal sorting complex required for transport; EVs: extracellular vesicles; HuR: human antigen R; MV: microvesicle; nSMase: neutral sphingomyelinase; SNARE: soluble N-ethylmaleimide-sensitive fusion attachment protein receptor; t-SNARE: target-membrane SNARE; v-SNARE: vesicle-membrane SNAREs.

Ceramide and SMases

EVs are enriched in cholesterol and sphingolipids, such as sphingomyelin and hexosylceramide, and have a remarkable ceramide enrichment. Neutral sphingomylinase (nSMase) and acid SMase (aSMase) potentially mediate the budding of vesicles into MVEs and plasma membrane, respectively, and thus promote the generation of exosomes and MVs [55,226]. Accumulating evidence suggests a role of the SMase-ceramide pathway in the pathogenesis of DM and its complications, although direct experimental data supporting EV contribution are lacking [127-141].

An elevated level of circulating ceramide is associated with the severity of IR in obesity [227]. Specifically, membranous ceramide can influence the structural organization of plasma membrane and insulin receptor translocation, impairing insulin signaling [228, 229]. In parallel, ceramide metabolism is over-represented in the plasma and markedly associated with the progression of T1D, consistent with its crucial role in immune regulation [230].
Ceramide also serves as a critical lipotoxic mediator and drives the development of vascular dysfunction and damage [231-233]. Similarly, abnormality and dysfunction of both aSMase and nSMase have been reported in DM and its complications (Table 1) [127-141]. The pathogenic roles of these enzymes have generally been attributed to mediating sphingomyelin hydrolysis and ceramide in the AT, retina, liver, kidney, and other tissues. For example, aSMase and nSMase are increased in obese epididymal fat, along with altered levels of sphingomyelin, ceramide, and downstream ceramide metabolites in AT and plasma, promoting the expression of prothrombotic and proinflammatory genes and subsequently contributing to obesity-associated metabolic and cardiovascular diseases, such as atherosclerosis [127-129]. Thus, inhibition of SMase-ceramide is considered an effective therapy for IR and DM by inhibiting inflammatory responses [131,132,234]. However, the contribution of EVs in SMase-ceramide-mediated functions remains unknown and awaits future investigation.

**Syndecan-syntenin pathway**

The syndecan-syntenin-ALIX axis has been shown to regulate the formation of ILVs and exosomes [235]. Syndecan, syntenin, and ALIX co-exist in a subset of exosomes. The PDZ domains of syntenin have a high affinity to syndecan, which recruits syntenin to membranes, while the N-terminal domain of syntenin directly interacts with ALIX. Heparanase, the only catalytic enzyme of syndecan, trims its heparan sulfate and significantly promotes exosome budding and generation [236]. Syntenin can also recognize ligands with PDZ-binding motifs, which are specifically sorted into the exosomes. For example, syntenin directly binds the exposed PDZ-binding motif of KRS and targets it into exosomes, thereby contributing to caspase-8-triggered inflammation [80]. These recent findings collectively suggest an important role of the syndecan-syntenin pathway in the biogenesis and function of exosomes.

Syndecan is a ubiquitous transmembrane protein and plays important physiological and pathological roles in development, differentiation, and human diseases, including DM and its complications (Table 1) [142-154]. Generally, syndecan, particularly syndecan-1 and syndecan-4, are upregulated in diabetic humans and animals compared with euglycemic controls. Syndecan-1 is induced in the liver of obese Zucker fa/fa rats and potentially promotes lipid uptake, resulting in hepatic IR and dyslipidemia [142]. Moreover, elevated syndecan-1 expression is associated with body mass index (BMI) and serum apoA1 in T2D, suggesting its involvement in vascular inflammation and injury [143-145]. In T1D, syndecan-1 expression is positively correlated with microalbuminuria and inflammatory indicators, implying a role in DN pathogenesis [147, 148]. Syndecan-4 is also increased in the heart and kidney of diabetic mice and rats and has a role in diabetic cardiomyopathy and DN [152-154].

The expression and activity of heparanase, a unique endoglycosidase known to degrade heparan sulfate chains, including those of syndecan-1, are increased under diabetic conditions [155-170]. Notably, heparanase derived from insulitis leukocytes can degrade heparan sulfate of β cells and thus promote islet cell death in T1D. In mice, inhibition of heparanase can effectively delay the onset of T1D induced by STZ and NOD [155-158]. Also, the level of heparanase in the circulation and urine is positively correlated with glucose and HbA1c [159-161] and is also closely associated with albuminuria in DM, indicating its crucial role in diabetic renal injury [162-167]. Specifically, heparanase can potentially lead to the loss of heparan sulfate in the glomerular basement membrane, induce glomerular inflammation, and promote renal fibrosis in DN [150,162-167]. Similarly, elevated heparanase has also been implicated in diabetic microangiopathies, such as diabetic retinopathy (DR) [150,168,169], and carotid artery atherosclerosis [170].

Together, these findings highlight the key roles of syndecan and heparanase in the pathogenesis of DM and its complications. Given the importance of the syndecans-syndecan-ALIX pathway in exosome biogenesis and cargo sorting, it is conceivable that exosomes could, at least partially, mediate syndecans and heparanase functions under diabetic conditions despite a lack of direct evidence.

**Calpain**

Calpains are a superfamily of Ca\(^{2+}\)-dependent intracellular cysteine proteases and have a role in generating MVs via remodeling the cytoskeleton and facilitating the budding of the plasma membrane. Emerging data suggest that calpains, particularly calpain 1, 2, and 10, contribute to the genetic causes and biochemical defects of T2D, albeit a clear involvement of EVs in calpain-modulated T2D phenotypes remains elusive [171-191].

**CAPN10** encoding calpain 10 is the first positionally cloned gene for T2D [237-244]. Its polymorphisms are closely associated with chronic diabetic vascular complications, such as DN, DR, diabetic neuropathy, and cardiovascular diseases [245]. By utilizing multiple calpain inhibitors, recent studies have uncovered the function of calpains in IAPP-mediated cell dysfunction, insulin secretion in
islet cells, insulin-stimulated glucose uptake, and
glycogen synthesis in adipocytes and skeletal muscle
cells [246,247]. Notably, O-GlcNAcylation modifi-
cation may facilitate the exosomal release of calpain 2
in hepatocytes under the high glucose (HG) condition
[248]. Exosomal calpain 2 can cleave the ectodomain
of the insulin receptor and thus impair insulin action,
providing a credible link between calpain 2, exosomes, and T2D etiology [248]. Moreover, activation of calpain 1 and 2 contributes to accelerated
atherothrombosis development in T2D by regulating
different substrates in platelets and ECs [177-181].
Since MVs loaded with elevated calpain 1 can be
delivered to ECs and induce vascular inflammation
[180,181], MVs might contribute to the phenotypes
mentioned above.

SNARE proteins

The assembly of the SNARE complex mediates
MVE fusion with the plasma membrane and allows
exosome secretion into extracellular space. The role of
SNAREs in glucose metabolism and T2D pathology
has been extensively reported, although the
involvement of EVs in the SNAREs-mediated effects
remains unclear and awaits further investigation
[192-198].

SNAREs fundamentally maintain glucose
homeostasis via participating in insulin and
The concentration of insulin-like peptide 1 (GLP-1) secretion
and GLUT4-mediated glucose uptake [249-251]. Many
SNARE components, including VAMP2, syntaxin-1A,
-2 and -4, SNAP-23 and -25, and synaptotagmin, are
deleted in human and rodent T2D islets [192-195],
and are associated with β cell hypertrophy and
defective insulin secretion. Abnormal expression of
SNARE proteins is implicated in IR in insulin-responsive tissues like the AT and muscle,
probably due to impaired GLUT4 intracellular
translocation [196, 197]. In addition to dysregulated
expression, abnormal location of SNAREs may have a
role in systemic metabolism and T2D development.
For example, abnormal sorting of VAMP2 into lipid
droplets leads to inadequate trafficking of GLUT4 on
the plasma membrane and IR in adipocytes [252].
Additionally, VAMP2 is elevated in serum and
possibly induces autoimmune response and consequent insulitis, suggesting it as a potential autoantigen of T1D [199].

EV cargo sorting

Protein cargo sorting

Proteomic analysis has uncovered that diabetic
carbohydrate status alters the protein composition of EVs of
different origins [253-257]. Therefore, the cellular
expression and function of EV protein sorting
machinery in response to diabetic stimulations could
be attributed to proteomic alterations of EVs in DM.
In addition to ESCRTs mentioned above, tetraspanins
CD63 and CD82 that participate in EV protein cargo
sorting [83-85], have also been implicated in developing DM and its complications [200-203, 258].

Under glucolipotoxic conditions, CD63 mediates
stress-induced nascent granule degradation of insulin in
β cells, thereby mitigating insulin secretion and
accelerating T2D [258]. Moreover, AGEs can induce
the expression of CD63, the marker of platelet activation, and the CD63+ platelet level is elevated in
T2D patients with progression of carotid wall
thickness [200, 201]. CD63 is also upregulated in
diabetic patients with DN and contributes to renal cell
death by inhibiting the Wnt/β-catenin signaling
pathway [202]. CD82 is highly expressed in diabetic
skin tissue and possibly associated with diabetic
chronic inflammatory and hypoxic state [203], albeit
its precise role and mechanism in diabetic
dermopathy remain elusive. Taken together, altered
expression and function of CD63 and CD82 under
diabetic conditions may contribute to selective
enrichment of cargoes in EVs and consequently
induce changes in the EV proteome profile.

Additionally, several PTMs of proteins are
required for their sorting in EVs, possibly involved
in ubiquitination of PTEN and DMT1, phosphorylation
of caveolin 1, and other diabetic pathological changes
[259-265]. Specifically, the concentration of
polyubiquitinated PTEN, which plays an important
role in regulating renal fibrosis, is increased in the
serum and urine of DN patients [260]. It has been
reported that ubiquitination at lysine 13 of PTEN is
required for the selective enrichment of PTEN in
exosomes [90], which may partially mediate the
pathological role of PTEN in DN. Similarly,
the release of DMT1 from MVs is mediated by Nedd4-2
ubiquitin ligase, suggesting a role of ubiquitination in
the cargo sorting of EV proteins in the gut explant
[91]. Moreover, in vivo and in vitro studies have found
that HG leads to elevated DMT1 levels in intestinal
epithelial cells partially by inhibiting DMT1
ubiquitination and promoting DMT1 membrane
translocation, resulting in increased iron uptake and
iron loading [259]. Together, it is reasonable to
hypothesize that the ubiquitinated DMT1 located at
the plasma membrane is sorted into the budding vesicles and secreted into the extracellular
environment. In contrast, the deubiquitinated DMT1
is trapped within cells, leading to elevated expression
of DMT1 in the diabetic intestine. Also,
phosphorylation of caveolin-1, a scaffolding protein
involved in protein sorting of MVs [100], has been
shown to be important in DN development [261-265].
Hypoxia induces the phosphorylation of caveolin-1 that can directly interact with hnRNPA2B1, facilitating the sorting of hnRNPA2B1 and its-associated miRNAs into MVs [100]. Under diabetic conditions, HG promotes caveolin-1 phosphorylation in podocytes and glomerular mesangial cells (GMCs), resulting in renal cell apoptosis, inflammation, EMT, and glomerular matrix accumulation [261-265]. Moreover, circulating MVs derived from diabetic rats can be delivered into vascular ECs and lead to elevated caveolin-1 levels in recipient cells [266].

RNA cargo sorting

RNA cargo affects many EV functions in various diseases, including DM and diabetic complications. Diabetic conditions induce alterations of mRNAs, miRNAs, IncRNAs, and circular RNAs (circRNAs) in EVs [267-270], primarily due to the dysregulation of numerous RBPs. HuR, an extensively studied RBP, is involved in EV RNA sorting by directly recognizing and binding RNAs bearing AU-rich elements, such as miR-122 and miR-21 [121]. Both HuR and its associated miRNAs have been implicated in the diabetic heart, DN, and DR developing [204-210].

In the context of DM, target proteins post-transcriptionally modified by HuR have been shown to play a role in the pathogenesis of diabetic complications like DN, DR, and diabetic cardiomyopathy [204-210]. For instance, HuR can post-transcriptionally modulate the expression of several regulators involved in renal injury, such as claudin-1, IL-17, NOD2, NLRP3, CTGF, TGF-β1, and Snail [204-207]. Similarly, pyroptosis, inflammation, oxidative stress, and EMT have been mechanically involved in HuR-mediated DN development and progression. Moreover, it has recently been shown that HuR can be delivered into cardiomyocytes and thus elicit inflammatory and profibrogenic responses, highlighting its importance in the diabetic heart [209].

It has been reported that miR-122 and miR-21, two miRNAs sorted by HuR into EVs [121], have a role in the diabetic heart [271-273]. MiR-21 is significantly decreased in cardiomyocytes of diabetic mice and contributes to diastolic cardiac dysfunction by directly targeting gelsolin and consequent oxidative stress. In contrast, circulating levels of miR-21 and miR-122 are increased in T2D patients with heart failure [272,273], probably resulting from increased EV secretion triggered by HuR upregulation. Thus, miR-21 and miR-122 may be selectively encapsulated in the EVs via HuR and secreted extracellularly, leading to an increase in their extracellular levels while causing a decrease in their intracellular levels, possibly mediating the pathogenic effect of HuR in diabetic cardiomyopathy. Similarly, these two miRNAs could play a role in DN and DR [274-277]. For instance, it has been shown that miR-21 encapsulated in EVs exerts a pro-angiogenic effect on ECs and promotes DR development [278, 279]. Further research is required to clarify whether the HuR function in the pathogenesis of diabetic complications depends on EV-miRNA sorting.

HnRNPK is another RBP involved in the RNA sorting of EV and its phosphorylation can be induced by glucolipotoxicity, a classic metabolic abnormality associated with T2D [211,212]. Phosphorylated hnRNPK can significantly modulate the expression of oxidative and inflammatory genes in β cells [211]. HnRNPK expression is decreased in the kidney of T1D mice and can potentially mediate RAS activation and hypertension in T1D [212]. The altered expression and post-translational modification of hnRNPK might lead to different RNA selection in EVs, possibly contributing to hnRNPK function in DM.

Collectively, it is reasonable to conclude that EVs may mediate specific pathogenic roles of EV generation machinery in the initiation and progression of DM and diabetic complications. Thus, elucidating the association between EV biogenesis and diabetic pathogenesis represents an attractive direction for future investigation, which would pave the way for developing novel targeted therapeutics for DM and diabetic complications.

Roles of EVs in DM

EVs are emerging as novel effectors of intercellular and interorgan communication and play active roles in multiple pathophysiological situations of metabolic modulation like metabolic homeostasis, maintenance, and disturbance. Of note, EV-induced phenotypic and molecular alterations in target cells are often associated with the composition and origin of these microstructures. In this section, we summarize the diverse EV functions in DM pathology and diabetic complications in the context of the cellular origin of EVs.

Adipocytes

AT is central in regulating systemic insulin sensitivity, hypertrophic adipocyte-induced elevated FFA release, inflammation, and adipokine alterations that are the drivers of the whole-body IR in T2D. Lipogenic stimulus and excess fat expansion promote EV generation in obese adipocytes, which, in turn, contribute to IR and islet cell dysfunction via paracrine effect and/or distant action (Figure 4) [280-285]. These EVs induce lipid droplets deposit by directly delivering neutral fatty acids [286], and promote lipid synthesis by transmitting the key lipid synthesis enzyme FASN, lipogenic-related miRNAs
and mRNAs, and CD73 [281-284, 287]. In vitro experiments showed that EVs can impair insulin response and glucose uptake in recipient adipocytes [280]. Besides the paracrine effect on local adipocytes, EVs secreted by adipocytes can result in peripheral IR and metabolic disorder by functioning as adipokine carriers [288]. Hypertrophic adipocyte-derived exosomes loaded with resistin, a canonical obesity-related adipokine, triggered hepatic ER stress and liver steatosis [289]. Several studies have described functional lncRNA (MALAT1), miRNAs (miR-27a, miR-141-3p) and proteins (CD36, and Akr1b7) encapsulated in adipocyte-derived EVs as novel adipokines that exert metabolic modulatory effects on distant organs [216, 290-293]. These newly discovered adipokines are sufficient to induce hepatic lipid accumulation and IR in the liver and skeletal muscle [216, 292, 293]. In addition, adipocyte-derived EVs have a modulatory effect on the survival and function of distant islets by delivering specific miRNAs [285].

Macrophage infiltration in AT is a hallmark of obesity and contributes to chronic inflammation and subsequent IR [294]. Obese adipocyte-derived EVs have been demonstrated to play a role in recruiting and activating circulating monocytes and polarizing resident macrophages toward the proinflammatory phenotype [287, 295-300]. Interestingly, based on the specific interactions between surface proteins of EVs and recipient cells, EVs are preferentially taken up by circulating monocytes in vivo and promote macrophage activation and IR [52, 295]. In addition, obese adipocyte-derived EVs can shuttle bioactive molecules, such as miR-34a and miR-155, into recipient macrophages, and thus promote pro-inflammatory M1 polarization and inhibit anti-inflammatory M2 polarization [296-298].

EVs can also function as a mode of communication between adipocytes and vascular ECs, which may be dynamically influenced by metabolic status [301], and have a role in cardiovascular complications. EVs derived from obese AT often exert detrimental effects on vascular cells, including ECs, vascular smooth muscle cells (VSMCs), and cardiomyocytes [302-308]. It has been shown that miR-221-3p, miR-130b-3p, IncRNA SNHG9, and VACM-1 within the EVs can result in endothelium inflammation, vascular stenosis, unstable atherosclerotic plaque formation, and impaired cardiac recovery [304-307].

Macrophages

Inflammatory macrophages infiltrated in AT lead to low-grade tissue inflammation, which is the key cause of IR in T2D [309]. EVs derived from obese AT macrophages (ATMs) can serve as systemic inflammation factors and impair insulin signaling in distal organs (Figure 4). Metabolic regulatory miRNAs, such as miR-29a, miR-155, and miR-210, can be carried by EVs and delivered into insulin-responsive cells and organs via paracrine or endocrine routes [310-313]. These miRNAs robustly regulate insulin action on AT, liver, and skeletal muscle and cooperatively modulate systemic glucose homeostasis [310-313].

Macrophage-derived EVs also play a role in DM complications. It has been reported that HG and RAGEs induce EVs production in macrophages [314-318]. Biomolecular cargoes within these EVs, such as IL-1β, iNOS, HuR, miR-21-5p, miR-486-5p, and TGF-β mRNA can be transferred to target cells and subsequently induce renal and cardiac injury and dysfunction [314-319]. In particular, two miRNAs closely related to cardiac fibrosis and diastolic dysfunction, miR-122 and miR-1246 [320, 321], have been shown to be specifically sorted into EVs by the RBP HuR [121, 322], raising the possibility that some pathogenic effects of HuR may be mediated by miRNAs enclosed in macrophage-derived exosomes. The oxidized low-density lipoprotein (oxLDL) is known to induce M1 polarization of macrophages and foam cell formation in the arterial wall, two crucial atherogenic events in DM. Interestingly, recent studies suggest an important role of miRNAs carried by activated macrophage-derived EVs in atherosclerosis [323-332]. Thus, EVs can effectively transmit pathogenic miRNAs to target cells, including VSMCs, ECs, neutrophils, and macrophages, leading to vascular stenosis, dysfunction, and inflammation that promote atherosclerosis and thrombosis (Figure 4) [323-329]. Besides miRNAs, functional factors, including IncRNA GAS5, integrin β1A, and α5, loaded in EVs, also participate in the progression of vascular injury and cardiovascular diseases [331,332].

Macrophage-derived EVs have been implicated in multiple immune response processes [333-335] and can present dead cell-associated auto-antigens to dendritic cells, and activate an autoimmune response [333,336]. Exosomes derived from M1 macrophages can also act on T cells, amplifying Th1 response and aggravating neuritis in Guillain–Barré syndrome [337]. Notably, macrophages infiltrated in islets are the main source of free radicals and pro-inflammatory cytokines, inducing β cell death in T1D [338,339]; however, the potential contribution of EVs in this process awaits further exploration.
Figure 4. Involvement of adipocyte- and macrophage-derived EVs in DM-related pathological changes. Adipocyte-derived EVs play a distinct role at multiple processes in the development of DM-related pathology. These EVs with specific cargoes (FASN, neutral fatty acids, CD73, resistin, Akr1b7, CD36 and miR-27a) can circulate throughout the body and reach their destination for IR development and metabolic disturbance in the adipose, liver and skeletal muscle. Islet inflammation, damage and dysfunction can also be induced by adipocyte-derived EVs. Upon uptake by recipient cells, these EVs can deliver several pathogenic mediators to ECs, hypothalamus and heart (increased miR-221-3p and VCAM-1, reduced SNHG9 to ECs, and increased MALAT1 to hypothalamus), resulting in vascular injury, elevated appetite, and myocardial damage, respectively. SHH-, RBP4-, MIP1-α-, miR-34a- and miR-155-containing EVs taken up by macrophages can promote M1 polarization and foam cell differentiation, while inhibit M2 polarization, leading to localized adipose and systemic inflammation, and accelerated atherosclerosis. Reciprocally, inflamed macrophage-derived EVs carrying elevated miR-210 and miR-27a can be transferred to adipocytes, causing IR in the adipose tissues. EVs containing miR-29a originated from macrophages can also be delivered to the liver and skeletal muscle, leading to IR in target organs. Elevated HuR, integrin β1 and α5, IL-1β, iNOS, TGF-β mRNA, miR-21-5p, miR-185-3p, miR-146a, miR-503-3p, miR-486-5p, miR-106-3p, miR-430, miR-150, and IncRNA GAS5 in these EVs ultimately result in cardiac fibrosis and dysfunction, atherosclerosis, renal inflammation, and glomerular mesangial matrix accumulation. **Abbreviations:** AT: adipose tissue; ECs: endothelial cells; ER: endoplasmic reticulum; EVs: extracellular vesicles; FAs: fatty acids; HuR: human antigen R; IR: insulin resistance; SHH: sonic hedgehog; TG: triglyceride.
Hepatocytes

Lipid stress under obese conditions leads to abnormal fat accumulation and inflammation in the liver in T2D, contributing to localized and systemic IR and inflammation. EVs derived from hepatocytes with overnutrition participate in this process via paracrine and endocrine actions. For instance, increased geranylgeranylation of Rab27a in hepatocytes promotes vesicle docking toward the plasma membrane and the subsequent EV release into circulation [340]. Specifically, let-7e-5p, with the greatest increase in EVs under a high-fat diet (HFD), can be transferred to adipocytes and increase lipogenesis and adipose expansion through targeting Pgc1α [340]. When taken up by the pancreas, EVs can promote islet cell proliferation and participate in the compensatory response in the early onset of T2D [341]. In addition, these EVs are enriched in proinflammatory molecules, including S1P, TRAIL, integrin β1, ceramide, miR-122, and miR-192-5p, which can induce inflammatory cell infiltration and inflammation by attracting circulating monocytes and polarizing macrophages toward pro-inflammatory differentiation in the liver [342-348].

Moreover, EVs derived from lipid-stressed hepatocytes can mediate the crosstalk between the liver and cardiovascular system and contribute to related complications. EVs shed by steatotic hepatic cells contain elevated miR-1 and miR-122, which can induce expression of adhesion molecules and diminish mitochondrial activity in target ECs and cardiomyocytes, resulting in atherosclerosis aggravation and cardiac function impairment [349, 350].

Islet cells

Insulin-releasing cells are considered the main effectors of autoimmune response, and their destruction is the main cause of T1D. In the past few years, EVs derived from islet cells under inflammatory stress have underscored their pathogenic function in autoimmune insulinitis of T1D. Inflammatory cytokines induce islet autoantigen enclosure [351-354] and RNA profile alteration [270,355] in these EVs. Several known canonical diabetic antigens, for e.g., GAD65, IA-2, ZnT8, GLUT2, and proinsulin, as well as the newly identified Gag antigen, can be effectively delivered to antigen-presenting cells (APCs), leading to T cell activation and autoimmune response [351-354]. In addition, these EVs can transfer bioactive RNAs and proinflammatory molecules, such as MCP1 and IL-27, to immune cells [355-359], and thus might account for the activation of recipient immune cells, such as dendritic cells, macrophages, B lymphocytes, and T lymphocytes. EVs derived from inflamed islet cells may also impose a pro-apoptotic effect on neighboring β cells by paracrine action and horizontal transmission of pathogenic miRNAs (e.g., miR-375-3p and miR-21-5p) associated with pancreas injuries [360].

Bioactive miRNAs loaded in pancreatic β cell-derived exosomes can function as endocrine factors, whose level changes influence glucose homeostasis and T2D development. HFD, the common risk factor for obesity and T2D, can affect specific miRNA levels in β cell-derived EVs, such as an increase in miR-29 and a decrease in miR-26a [361-363]. These exosomal miRNAs can be transferred to peripheral tissues and impair insulin signaling in recipient cells, and also be transmitted to circulating monocytes and macrophages and induce chronic low-grade inflammation [361-363]. MiR-26a is widely expressed in human tissues and involved in the pathogenesis of various human diseases, including DM and its associated disorders [364-369]. Under T2D conditions, miR-26a expression is decreased in β cells, subsequently reducing circulating exosomal miR-26a, impairing insulin sensitivity and metabolic homeostasis in the liver and AT, thereby promoting the development of T2D [363]. In contrast, exosomal miR-29s and miR-29a derived from islet cells are induced by FFAs stimulation and inflammation [361,362]. These two exosomal miRNAs are delivered to the liver and inflammatory cells, resulting in hepatic IR and systemic metabolic dysregulation and inflammation [361,362]. Moreover, islet cell-derived EVs seemingly contribute to pancreatic failure in T2D and thus promote disease progression. Mechanistically, EVs may potentially facilitate IAPP aggregation and amyloid formation in pancreatic cells, resulting in cell death [370]. Additionally, pancreatic cell-derived EVs have a role in DM complications. HG stimulation significantly increases miR-15a levels in exosomes isolated from pancreatic β cells that can be readily absorbed by retinal cells and induce ROS production and apoptosis in recipient cells, leading to DR [371].

To sum up, the pancreas is the target organ of diabetic injury and also serves as the pathogenic tissue releasing damaging EVs that can effectively mediate the crosstalk among the pancreas, distant organs, and immune system (Figure 5). Given that the pancreas is an active and potent endocrine and exocrine tissue and plays a central role in systemic metabolic homeostasis and multiple diseases, its EVs are expected to be involved in diverse physiological and pathological processes.
Figure 5. Islet cell-derived EVs promote the development of T1D, T2D and diabetic retinopathy. Islet cell-derived EVs carry various molecular effectors that can trigger multiple signaling cascades, and may regulate the development of T1D, T2D and diabetic complications. In T2D, reduced miR-26a and NCDase in these EVs can exert a paracrine effect on ambient islet cells, resulting in cell death, dysfunction and IAPP accumulation. Distant delivery of EVs derived from islet cells with reduced miR-26a and elevated miR-29s to the liver, adipose and macrophages can promote IR and lipid accumulation in the liver, and cell expansion and systemic inflammation in the adipose, ultimately leading to T2D development. EVs with increased miR-15a are also be transmitted to retina and cause oxidative stress and cell apoptosis, promoting the occurrence of diabetic retinopathy. In T1D, islets cell-derived EVs are encapsulated with islet autoantigens and facilitate autoantigen presentation and autoimmune activation, along with activating phagocytes and promoting cytokines and chemokines release. Inflammatory islet cell-derived EVs are loaded with increased miR-375-3p and miR-21-5p, exerting a pro-apoptotic effect on surrounding β cells via paracrine action.

Abbreviations: APC: antigen presenting cell; AT: adipose tissue; EVs: extracellular vesicles; IAPP: islet amyloid polypeptide; IR: insulin resistance; ROS: reactive oxygen species; T1D: type 1 diabetes; T2D: type 2 diabetes.

ECs

ECs are centrally involved in the microvascular pathology and complications in DM [372]. Specifically, diabetic vascular complications are characterized by EC dysfunction and death, and endothelium inflammation. Accumulating evidence indicates that EC-derived EVs are involved in these processes via paracrine action (Figure 6). HG and AGEs have been shown to induce MV generation and alter EV cargo sorting in ECs [253, 373, 374]. These MVs can promote apoptosis and dysfunction of recipient ECs [375-378]. For example, reduced EV miR-126 and miR-222 are sufficient to decrease endothelium repair capacity, partially accounting for the loss of protective function of EC-derived EVs [376-378]. Moreover, MVs are rich in membrane tight-junction proteins, occludin and claudin-5, resulting in a reduction of these molecules on the surface of parental ECs and impaired vessel walls [373]. Additionally, these MVs can induce the expression of adhesion molecules in target ECs and facilitate inflammatory cells to attach and infiltrate into the endothelium [379, 380].

Capillary basement membrane thickening of the glomerular, retinal, cardiac, and cutaneous arterioles is the most common microvascular structural modification in DM, resulting in organ malperfusion and classic diabetic microangiopathy [372]. In the diabetic setting, EVs derived from HG-treated ECs encapsulate elevated Notch3, versican, PDGF-BB, and circRNA-0077930, which can be taken up by surrounding VSMCs [381-386]. Consequently, recipient VSMCs acquire an anti-apoptotic, osteoblast-like and senescent phenotype, leading to intimal hyperplasia and vascular calcification [381-386]. Furthermore, ECs from different tissues can exert paracrine actions on ambient cells and promote the development of diabetic cardiomyopathy, DN, and diabetic foot. Exosomes derived from HG-treated ECs can suppress autophagy, increase apoptosis, and interfere with energy metabolism in target cardiomyocytes [387]. Exosomes derived from diabetic glomerular ECs (GECs) transmit TGF-β1
mRNA to GMCs and podocytes then induce elevated proliferation and matrix production of GMCs and fibrosis of podocytes [388, 389]. More recently, it has been shown that specific circRNAs in these exosomes, such as circRNF169 and circSTRN3, may also contribute to the dysregulation of GMCs and mesentery proliferation in DN [269]. Similarly, AGEs can boost miR-106b-5p in EVs derived from ECs that can be efficiently transported to recipient fibroblasts, leading to fibroblast autophagy and subsequent delayed wound healing [390].

Furthermore, generation and abnormal miRNAs sorting of EVs induced by oxLDL are also considered important atherogenic events in DM. Elevated EV miRNAs, including miR-155, miR-4306, miR-505, and miR-92a-3p, are delivered into macrophages, neutrophils and surrounding ECs, leading to endothelial inflammation, dysfunction, and damage, and promoting atherosclerosis [328, 391-393]. Consequently, recipient inflammatory cells are aberrantly activated and exhibit a pro-inflammatory phenotype, while target ECs display decreased migration, proliferation, and angiogenic capacity [328,391-393]. Besides, other bioactive molecules with an atherogenic role, such as LINC01005, MALAT1, HSP70, and ICAM-1, have also been detected in EVs and may play a role in DM pathogenesis [394-397].

**Other cells**

Skeletal muscle is the major organ for glucose uptake, whose IR is one of the primary defects of T2D [398]. During lipid-induced IR, exosomes derived from skeletal muscle cells are enriched in saturated fatty acid palmitate, which can be taken up by insulin-sensitive tissues, particularly the pancreas and liver, representing a new paradigm of inter-organ

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**Figure 6. Role of EC-derived EVs in the pathogenesis of diabetic complications.** EVs derived from ECs are critically involved in the occurrence and progression of diabetic complications, including endothelial damage and inflammation, vascular sclerosis, diabetic cardiomyopathy, diabetic nephropathy and diabetic foot, by transferring functional biomolecules. On the one hand, by secreting occludin and claudin-5 via EVs, original ECs lose tight junctions. On the other hand, EC-derived EVs can promote apoptosis, induce the expression of adhesion molecules, and impair repairment capacity of recipient ECs, resulting in endothelial injury and inflammatory cell attachment and infiltration in endothelium. The protective function of EC-derived EVs on endothelium (ECs and VSMCs) is potentially mediated by miR-126 and miR-222, which is decreased under diabetic conditions. Notch 3, versican, PDGF-BB, LINC01005, circRNA-0077930 are delivered to VSMCs by EVs from ECs in a paracrine manner, resulting in apoptosis resistance and osteoblast-like differentiation in recipient VSMCs. EVs derived from ECs under oxLDL stress can transmit HSP70, ICAM-1, MALAT1, miR-155, miR-4306, miR-505 and miR-92a-3p into circulating system and local inflammatory cells including monocytes, macrophages and neutrophils, leading to endothelial inflammation and atherosclerosis. Glomerular EC-derived EVs are involved in the development of diabetic nephropathy via transferring TGF-β1 mRNA, circRNF16 and circSTRN3, thereby promoting renal cell proliferation, fibrosis and ECM production. EVs derived from ECs can disturb energy metabolism and induce cardiomyocyte apoptosis, facilitating the development of diabetic cardiomyopathy. MiR-106b-5p is increased in the EVs from ECs, and is subsequently transmitted into dermal fibroblasts and contributes to a refractory wound in diabetic foot.

**Abbreviations:** AGEs: advanced glycation end products; ECs: endothelial cells; ECM: extracellular matrix; EVs: extracellular vesicles; NET formation: neutrophil extracellular trap formation; oxLDL: oxidized low-density lipoprotein; VEC: vascular endothelial cells; VSMC: vascular smooth muscular cell.
communication and metabolic homeostasis [399]. MiR-16 encapsulated in these lipid toxic exosomes can promote the proliferation of target islet cells, acting as a compensatory IR mechanism during the onset of T2D [400]. Nevertheless, after exercise training, skeletal muscle-derived EVs of healthy individuals carry specific protein and miRNA signatures and display liver tropism [401, 402]. Bioactive miRNAs, including miR-133b, are transmitted to hepatic cells, inhibiting FoxO1 expression and leading to improved systemic metabolism [402]. The target specificity is thought to be mediated by interactions between the proteins distributed on the surface of exosomes and recipient cells [401, 402].

Gut microbiota dysbiosis has a driving role in T2D by inducing abnormal intestinal metabolites and intestinal permeability dysfunction [403]. Recent studies indicate that EVs derived from Akkermansia muciniphila, a beneficial bacterium preventing IR, contribute to the HFD-induced gut permeability elevation due to decreased intestinal tight junction function [404]. In general, intestinal barrier disruption causes an increase in EVs derived from gut microbes in the circulation and whole body [405-408]. The gut dysbiosis-related EVs appear to promote IR by transferring deleterious cargoes to recipient cells, such as HMGB1 and phosphatidylcholine [404-407].

In T1D, β cell death is primarily mediated by T cells, triggering diabeticogenic insults [409]. In addition to inflammatory cytokines that are traditionally viewed as inducers of islet mass loss, EVs loaded with pro-inflammatory miRNAs, such as miR-142-3p, miR-142-5p, and miR-155, have been shown to specifically target pancreatic β cells and function as a novel pathogenic factor mediating autoimmune attack of β cells in T1D [410].

In T2D, platelets are considered a mediator of cellular crosstalk and a driver of inflammation [411]. EVs shed by platelets carrying soluble inflammatory cytokines have been recently implicated in these processes [412-414]. In a diabetic setting, platelets can release more EVs containing increased CXCL7 and CXCL10 that could be targeted to ECs in the aorta, kidney, and retina, resulting in increased expression of adhesion molecules, ROS production, oxidative stress, and inflammation-induced endothelial injury, thereby promoting the development of DR, DN, and atherosclerosis [412-414].

EVs derived from the kidney also have a role in mediating intercellular crosstalk in diabetic conditions. On the one hand, HG and AGEs induce shedding of MVs from podocytes, potentially via activation of NOX4/ROS and the Smad3 pathway [415, 416]. These EVs mediate proximal tubular epithelial cell (PTECs) injury and apoptosis and proximal tubule fibrosis, partially due to transportation of miR-221 to target cells and subsequent regulation of Wnt/β-catenin signaling [415-419]. On the other hand, HG-treated GMC-derived exosomes can be delivered to podocytes, which induce apoptosis and inhibit cell adhesion, leading to impairment of the last line of defense of the glomerular filtration barrier [420]. These exosomes also potentially trigger an autocrine response in GMCs by delivering circ-DLGAP4 and miR-15b-5p that induce fibrosis and apoptosis [421,422]. Interestingly, HG seems to have a distinct effect on the generation of MVs and exosomes in PTECs. The MV release is increased under HG stimulation, which has a paracrine function on surrounding PTECs, promoting their fibrosis and impairing their adaptive responses combating hypoxia [423, 424]. In contrast, exosome biogenesis is decreased by HG treatment, which then exhibits a pro-proliferative effect on target fibroblasts and promotes extracellular matrix production [425].

Exosomes derived from HG-treated retinal pigmented epithelial cells can promote angiogenesis by directly delivering the pro-angiogenic factor VEGF into retinal ECs [426]. Exosomes released by limbal stromal cells from non-diabetic individuals, but not from diabetic patients, can improve proliferation and migration of recipient limbal epithelial cells and maintain the integrity of cornea limbal epithelium [427]. Additionally, diabetic condition disrupts the metabolism of Schwann cells (SCs), the most abundant cells in the peripheral nervous system, and results in their neurotrophic molecules production compromise, contributing to diabetic peripheral neuropathy [428]. SC-derived exosomes act as an important neuronal support factor, nurturing peripheral axons and maintaining neuronal structure and function [429]. Conversely, diabetic SC-derived exosomes likely function as carriers of pathogenic content, reducing the nerve conduction velocity and aggravating mechanical and thermal hyp aesthesia in diabetic mice [430].

Clinical applications of EVs in DM and diabetic complications

EVs as a biomarker for DM

As described previously, EVs function as paracrine and endocrine factors and facilitate the crosstalk between metabolic organs and tissues. In addition, EVs have promising potential as biomarkers due to their good stability in body fluids and the ease of isolation and detection by fast-evolving technologies. Indeed, accumulating data have demonstrated the promise of EVs for clinical
applications as biomarkers in DM. Several recent reviews, extensively summarizing EVs as potential biomarkers for the early detection of DM and diabetic complications, stratification of patients, and response monitoring of treatment from different perspectives, are highly recommended [431-433]. Given the emerging role of EV RNAs in DM, here we briefly summarize the application of EV RNAs, including mRNAs and ncRNAs, as clinical biomarkers for the identification of diabetic patients and disease management (Table 2) [434-451].

For example, urinary exosomal miR-424 is robustly associated with islet autoimmunity and could efficiently discriminate patients with T1D with an area under the receiver operating characteristic (ROC) curve (AUC) of 0.803. However, serum miR-424 showed a relatively low diagnostic accuracy and sensitivity of 43% [452], suggesting urinary exosomal miR-424 as a more efficient biomarker for early detection of T1D. Another cohort study found that the combination of miR-10b and miR223-3p in serum MVs can effectively predict the occurrence of T2D in individuals with pre-diabetes with an AUC of 0.884 [451]. Importantly, this correlation has been further confirmed in the validation set with an AUC of 0.807 [451]. It has recently been pointed out that during the serum sampling process, apoptotic MVs with surface membrane phosphatidylserine could be consumed and new populations of MVs generated [453]. The authors indicated that these possible major changes in serum MVs might raise controversy over the results [453]. Compared to serum, the sampling process for plasma is simple with relatively stable contents. In this regard, it has been proposed that plasma might be a better source of MVs for biomarker investigation.

### Native EVs for DM therapy

EVs have been used as carriers of therapeutic substances and the administration of exogenous EVs has great promise in diabetic treatment. The therapeutic potential of EVs in treating DM and its complications in animal trials have been summarized and discussed in recent reviews [432,454]. Here, we briefly discuss recent advances and the prospect of native EV-based therapeutics in DM and its complications.

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**Table 2. Diagnostic index of EV RNAs in DM and diabetic complications.**

| RNAs         | Types               | Source     | Number (ND/DM) | AUC   | SEN (%) | SPE (%) | 95% CI     |
|--------------|---------------------|------------|----------------|-------|---------|---------|------------|
| let-7c-5p    | T2DN [444]          | Urine      | 15/28          | 0.818 | 96      | 53.4    | 0.718-0.919 |
| miR-21-5p    | T2D [442]           | Plasma     | 60/57          | 0.859 | -       | -       | -          |
| miR-23a      | T2D [434]           | Plasma     | 36/42          | 0.828 | -       | -       | -          |
| miR-29c-5p   | T2D [444]           | Urine      | 15/28          | 0.747 | -       | -       | -          |
| miR-30a-5p   | T2D-DN [436]        | Urine      | 80/40          | 0.912 | -       | -       | -          |
| miR-30a      | T2D [443]           | Urine      | 56/110         | 0.897 | 76.4    | 90.9    | 0.858-0.936 |
| miR-34a      | Dyslipidemia [439]  | Serum      | 78/42          | 0.730 | -       | -       | 0.630-0.830 |
| miR-133b     | T2D [441]           | Urine      | 44/136         | 0.917 | 93.3    | 86.7    | 0.874-0.946 |
| miR-146a-5p  | T2D [442]           | Plasma     | 60/57          | 0.911 | -       | -       | -          |
| miR-156      | T2D [444]           | Urine      | 44/136         | 0.863 | 97.8    | 82.2    | 0.824-0.942 |
| miR-192      | T2D [434]           | Plasma     | 36/42          | 0.717 | -       | -       | 0.607-0.828 |
| miR-194      | MIC [440]           | Urine      | 30/30          | 0.802 | -       | -       | 0.696-0.907 |
| miR-215      | MIC [440]           | Urine      | 30/30          | 0.703 | -       | -       | 0.581-0.826 |
| miR-218      | T1D [437]           | Urine      | 30/30          | 0.797 | -       | -       | 0.545-0.869 |
| miR-342      | T2D [443]           | Urine      | 44/136         | 0.910 | 81.8    | 80.9    | 0.873-0.948 |
| miR-424      | T1D [437]           | Urine      | 30/30          | 0.803 | -       | -       | -          |
| miR-636      | T2D [441]           | Urine      | 44/136         | 0.984 | 97.8    | 93.3    | 0.971-0.997 |
| miR-4534     | DN [435]            | Urine      | 14/14          | 0.786 | 85.7    | 78.6    | 0.607-0.965 |
| miR-10b and miR223-3p | T2D [451] | Serum | 8/9 | 0.884 | - | - | - |
| circ_0009097 | DU [445]            | Serum      | 20/20          | 0.878 | 80      | 80.85   | -          |
| circ_0057362 | DU [445]            | Serum      | 20/20          | 0.848 | 86.05   | 70.22   | -          |
| Ace          | Overt DN [448]      | Plasma     | 100/37         | 0.75  | 73      | 72      | 0.66-0.83  |
| Incipient DN [448] | Plasma | 100/37 | 0.75 | 73 | 72 | 0.66-0.83 |
| Aebp1        | T2D [446]           | Plasma     | 15/15          | 0.742 | 53.3    | 86.7    | -          |
| Ccl21        | T2D [447]           | Urine      | 15/28          | 0.888 | -       | -       | 0.737-0.997 |
| Umod         | T2D [450]           | Urine      | 15/88          | 0.90  | 93      | 73      | -          |
| Wt1          | Incipient DN [448]  | Plasma     | 37/66          | 0.63  | 50      | 74      | 0.55-0.72  |
| Overt DN [448] | Plasma | 100/37 | 0.85 | 67.6 | 93 | 0.74-0.92 |

AUC: area under the ROC curve; CI: confidence interval; DFU: diabetic foot ulcer; DM: diabetes mellitus; DN: diabetic nephropathy; ESRD: end-stage renal disease; MIC: microalbuminuria; ND: non diabetes; SEN: sensitivity; SPE: specificity; T2DN: T2D with DN; T2D-C: T2D with complications.
Figure 7. Potential clinical applications of native cell-derived EVs in treating DM and its complications. EVs of native cells (such as pancreatic pathfinder cells, adipocytes, stem cells, retinal pigment epithelial cells, keratinocytes, endothelial progenitor cells, amniotic epithelial cells, endothelial cells, fibrocytes, and macrophages) show potent promise as novel therapies for T1D (via inhibiting autoimmune response) and T2D (via promoting islet cell function and survival, and/or improving peripheral insulin sensitivity). These EVs also have the potential to treat diabetic complications including atherosclerosis, diabetic retinopathy, diabetic heart, diabetic nephropathy, diabetic erectile dysfunction, diabetic neuropathy, and diabetic foot ulcer. **Abbreviations:** APC: antigen presenting cells; DM: diabetes mellitus; EMT: epithelial-mesenchymal transition; ECM: extracellular matrix; EVs: extracellular vesicles; GLUT4: glucose transporter 4.

Anti-diabetic EVs have been isolated from various native cells, such as pancreatic pathfinder cells [455,456], adipocytes [457], and stem cells [458-462] (Figure 7). The preclinical data collected so far indicate that these EVs can improve peripheral insulin sensitivity and pancreatic islet function, alleviate inflammation, and/or attenuate obesity, regardless of their origin. The therapeutic roles of EVs
in recipient cells have been ascribed to the delivery of bioactive proteins. For example, the anti-inflammatory and anti-apoptotic roles of exosomes have been attributed to active STAT3 and VEGF [459, 460]. Depending on the source and content of EVs, they can trigger various therapeutic effects, such as inhibiting β cell apoptosis, restoring the phosphorylation of the insulin receptor substrate 1 and protein kinase B, increasing hepatic glycogen storage, polarizing M2 macrophages, and inhibiting the auto-immune response [458-462]. Moreover, numerous examples of EV-mediated functional transfer of ncRNAs have been demonstrated for various diseases and the therapeutic applications of EV ncRNAs in treating DM offer a fertile field for study.

Another important clinical application of EVs from different origins is in treating diabetic complications, such as DN [463-466], DR [467-471], diabetic erectile dysfunction [472-475], diabetic foot [476-490], diabetic cardiomyopathy [217], atherosclerosis [491-500], and diabetic peripheral neuropathy [429] (Figure 7). Currently, the therapeutic roles of EVs in treating diabetic complications are mostly attributed to the delivery of ncRNAs, especially miRNAs. For example, the angiogenic role of EVs has been ascribed to miR-21, let-7, miR-10, miR-30, miR-148a-3p, miR-126, miR-130a, and miR-132 [474-478], whereas their anti-fibrotic function has been attributed to let-7b and let-7c [474]. Some of these miRNAs have been identified in previous studies as anti-diabetes therapies, such as miR-21 [271,501,502], let-7 [503], miR-126 [504], and miR-132 [505, 506], further highlighting their great potential in DM treatment. In addition to ncRNAs, some proteins like TGF-β1, angiogenin, BMP-7, Nrf2, and DMBT1 within EVs can also elicit biological therapeutic effects [463, 482, 484].

However, important limitations in eliciting functional responses must be overcome for EVs to be used as an effective clinical therapeutic tool. Efforts have been made to address the challenges of harnessing the full potential of native EVs in the treatment of DM and diabetic complications. Because the EV composition is dependent on features of their donor cells, transfecting the original cells with exogenous compounds might modulate EVs and realize the goal of improving their bioactivity and augmenting their therapeutic efficacy. For example, overexpression of sFas and anti-miR-375 in human bone marrow mesenchymal stem cells can increase their levels in exosomes, effectively inhibiting Fas and miR-375 in recipient pancreatic islet cells and thus improve islet viability and function against inflammation [462]. Similarly, overexpression of functional proangiogenic components, such as Nrf2 [482], miR-221-3p [507], mmu_circ_0000250 [480], and miR-126 [476], in parental stem cells is accompanied by upregulation of these genes in the secreted exosomes, thereby improving the therapeutic effect against diabetic foot ulcer.

Furthermore, biomaterials, such as the thermosensitive and/or antibacterial hydrogel, have been developed to prolong the half-life of EVs and can serve as the controlled drug delivery system of EVs for treating chronic wounds [483, 508-510]. Additionally, taking advantage of the high-yield EV-mimetic nanovesicles (EMNVs) as a novel drug delivery system, the nanocarriers loaded with lncRNA-H19 have been applied to treat diabetic wounds [511]. These EMNVs function effectively by restoring lncRNA-H19 expression in dermal microvascular ECs and remarkably increase vascular formation [511] to treat DM and diabetic complications in the future.

Conclusion and Perspective

EVs are major regulators of DM and diabetic complications and play an important role in IR, inflammation, and islet dysfunction. Significantly, EVs have shown promising efficacy in animal models to deliver bioactive proteins and RNAs and can be harnessed as effective therapies for DM and diabetic complications. Despite these tremendous advances, the basic and clinical research of EVs in DM and diabetic complications is still at an infant stage.

Although it is generally recognized that EVs communicate between cells and organs by delivering messages and exchanging information, many questions remain to be resolved. First, it is still challenging to categorize and characterize EV subclasses with high heterogeneity [512], mainly due to technological limitations in separating and analysing vesicles. Second, due to the complexity of EV contents, their functions, individually or collectively, are far from being fully elucidated. Many attempts have been made to address this issue, for e.g., by developing a single-vesicle array and imaging method to track EV uptake [513,514]. Third, limited information is available about the molecular mechanisms underlying the target specificity of EVs with different origins so far. This process is believed to be largely mediated by membranous interactions between EVs originating from different cell types and target cells. Finally, it is important to monitor the fate of EVs after docking at recipient cells and determine the mechanisms underlying the usage of their cargoes.

From the clinical perspective, therapeutic applications of EVs have multiple challenges that need to be addressed. Biological detection of EVs requires adequate enrichment together with high
sensitivity. Nanomaterials, such as magnetic nanoparticles, have been used to improve the sensitivity of EV detection [515, 516] by effectively increasing the interface between biological molecules and nanomaterials to facilitate the capture of target EVs, significantly raising the efficiency of EV isolation. Also, improving the specificity of EV separation required for the high specificity of biomarkers represents another challenge. Appropriate modifications of magnetic nanoparticles by attaching biological probes, such as antibodies targeting EV surface markers, can efficiently improve specificity [517,518].

However, there are several outstanding issues regarding the use of EVs as effective therapies for DM and diabetic complications, including scale-up of the production, shelf stability, prolonging the half-life of therapeutic EVs, toxicity, off-target effects, and the delivery specificity. Despite these problems, EV-based biomarker discovery and clinic application are feasible and promising with constantly developing technologies. Due to their unique biological characteristics, EVs still have a great potential for accurate early diagnosis of DM and overcoming diabetic complications.

Abbreviation

aSMase: acid sphingomyelinase; AGEs: advanced glycation end products; APCs: antigen-presenting cells; AT: adipose tissue; AUC: area under the receiver operating characteristic curve; BMI: body mass index; circRNAs: circular RNAs; CNS: central nervous system; DM: diabetes mellitus; DN: diabetic nephropathy; DR: diabetic retinopathy; ECs: endothelial cells; EMNVs: EV-mimetic nanovesicles; ER: endoplasmic reticulum; ESCRIT: endosomal sorting complex required for transport; EVs: extracellular vesicles; FFAs: free fatty acids; GECs: glomerular ECs; GLP-1: glucagon-like peptide 1; GLUT: glucose transporter; GMCs: glomerular mesangial cells; GSK3β: glycogen synthase kinase 3β; HG: high glucose; HFD: high-fat diet; HuR: human antigen R; IAPP: islet amyloid polypeptide; ILVs: intraluminal vesicles; IR: insulin resistance; IncRNAs: long noncoding RNAs; MVs: microvesicles; MVEs: multivesicular endosomes; ncRNAs: non-coding RNAs; NETosis: neutrophil extracellular traps formation; nSMase: neutral sphingomyelinase; oxLDL: oxidized low-density lipoprotein; PTECs: proximal tubular epithelial cells; PTMs: posttranslational modifications; RAGEs: receptors for AGE; RBPs: RNA binding proteins; ROC: receiver operating characteristic; ROS: reactive oxygen species; SCs: Schwann cells; SNAREs: soluble N-ethylmaleimide-sensitive fusion attachment protein receptor; t-SNAREs: target-membrane SNAREs; T1D: type 1 diabetes; T2D: type 2 diabetes; UBL3: ubiquitin-like 3; v-SNAREs: vesicle-membrane SNAREs; VSMCs: vascular smooth muscle cells.

Acknowledgements

We thank members of the laboratory of X.F. for helpful discussion.

Funding

This work was supported by the National Natural Science Foundation of China (92157205, 81970561, 82172986), the Ministry of Science and Technology of China (2018ZX092001018-005), National Clinical Research Center for Geriatrics, West China Hospital, Sichuan University (Z20191005 and Z20201003), and the 1.3.5 Project for Disciplines of Excellence, West China Hospital, Sichuan University (ZYJC18049 and ZYGD18017).

Author Contributions

X.F. and Y.T. conceived the idea; J.L. and Y.Z. performed the literature search and draft the manuscript; X.F., Y.T., N.T. and W.H. supervised and revised the manuscript.

Competing Interests

The authors have declared that no competing interest exists.

References

1. Williams R, Colagiuri S, Chan J, Gregg EW, Yang X. IDF Atlas 9th Edition 2019: IDF Atlas 9th Edition. 2019;2019.
2. Sarvar N, Cao P, Seshasai SR, Cobin R, Kaptoge S, Di Angelantonio E, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. Lancet. 2010; 375: 2215-22.
3. Tsilidis KK, Kasimis JC, Lopez DS, Nizami IE, Ioannidis JP. Type 2 diabetes and cancer: umbrella review of meta-analyses of observational studies. BMJ. 2015; 350: g7607.
4. Sarah R, Robinson B, Abbott KC, Agodoa L, Bragg-Gresham J, Balkrishnan R, et al. US Renal Data System 2018 Annual Data Report: Epidemiology of Kidney Disease in the United States. Am J Kidney Dis. 2019; 73: A7-8.
5. Flaxman SR, Bourne R, Resnikoff S, Ackland P, Braithwaite T, Cicinelli MV, et al. Global causes of blindness and distance vision impairment 1990-2020: a systematic review and meta-analysis. Lancet Glob Health. 2017; 5: e1221-34.
6. Mosey PW, Gogalnicnaru P, Hinslishf RJ, Loftus JM, Jones KJ, Thompson MM, et al. Lower extremity amputations–a review of global variability in incidence. Diabet Med. 2011; 28: 1144-53.
7. Katsarou A, GudbjornsSottti S, Rawshani A, Dabelea D, Bonifacio E, Anderson BJ, et al. Type 1 diabetes mellitus. Nat Rev Dis Primers. 2017; 3: 17016.
8. Zheng Y, Lei SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat Rev Endocrinol. 2018; 14: 88-98.
9. de Goffau MC, Luopaiarvi K, Knip M, Ionen J, Ruohola T, Harkonen T, et al. Fecal microbiota composition differs between children with beta-cell autoimmunity and those without. Diabetes. 2013; 62: 1238-44.
10. Kolb H, Eizirik DL. Resistance to type 2 diabetes mellitus: a matter of hormesis? Nat Rev Endocrinol. 2011; 8: 183-92.
11. Austerholm IW, Scherer PE. Enhanced metabolic flexibility associated with elevated adiponectin levels. Am J Pathol. 2010; 176: 1364-76.
12. Frohman LA. CNS peptides and glucoregulation. Annu Rev Physiol. 1983; 45: 95-107.
13. Sandoval DA, Obici S, Sreely RJ. Targeting the CNS to treat type 2 diabetes. Nat Rev Drug Discov. 2009; 8: 386-98.
14. Tups A, Benzler J, Sergi D, Ladyman SR, Williams LM. Central Regulation of Glucose Homeostasis. Compr Physiol. 2017; 7: 741-64.
98. Roche JV, Surveyor S, Kreida S, Nesverova V, Ampah-Korsah H, Gourdon M, et al.

93. Mariscal J, Vagner T, Kim M, Zhou B, Chin A, Zandian M, et al.

92. Kunadt M, Eckermann K, Stuendl A, Gong J, Russo B, Strauss K, et al.

95. Itoh S, Mizuno K, Aikawa M, Aikawa E. Dimerization of sortilin regulates its

94. Romancino DP, Buffa V, Caruso S, Ferrara I, Raccosta S, Notaro A, et al.

90. Putz U, Howitt J, Doan A, Goh CP, Low LH, Silke J, et al. The tumor

87. Ageta H, Tsuchida K. Post-translational modification and protein sorting to

86. Ageta H, Ageta-Ishihara N, Hitachi K, Karayel O, Onouchi T, Yamaguchi H, et al.

78. Pathan M, Fonseka P, Chitti SV, Kang T, Sanwlani R, Van Deun J, et al.

76. Liu ML, Lyu X, Werth VP. Recent progress in the mechanistic understanding

73. Theranostics. 2019; 216: 2202-20.

309. 11-25.

146. 36-48.

220. 16-17.

220. 2-16.

220. 2-16.

220. 2-16.
characterize subjects with high liver fat content independent of obesity. Diabetes. 2007; 56: 1960-8.

128. Blachnio-Zabielska AU, Pulka M, Baranowski M, Nikolajuk A, Zabielski P, Grabiec MA, et al. Tumor necrosis factor-alpha and metabolic syndrome: a role in obesity and diabetes in human adipose tissue. J Cell Physiol. 2012; 227: 550-7.

129. Samad F, Hester KD, Yang G, Hannun YA. Altered adipose and plasma sphingolipid metabolism in obesity: a potential mechanism for metabolic and metabolic risk. Diabetes. 2006; 55: 2579-87.

130. Gorska M, Baranczuk E, Dobrzyń Zn2+-dependent sphingomyelinase activity in the serum of patients with type 2 diabetes is elevated. Horm Metab Res. 2003; 35: 506-7.

131. Kolyath IB, Reine TM, Parker K, Sudworth A, Witczak BJ, Jenssen TG, et al. Sodium-dependent neutral amino acid transport NRT1 is associated with endothelial dysfunction in type 2 diabetes. J Am Coll Cardiol. 2012; 60: 75-81.

132. Baranowski M, Blachnio-Zabielska A, Hirdie T, Harasiuk D, Matlak K, Knapp AU, et al. Unexpected new roles for heparanase in Type 1 diabetes and immune gene regulation. Matrix Biol. 2013; 32: 228-33.

133. Simeonovic CJ, Popp SK, Stari MM, van den Hoven DJ, Zabielski P, et al. Mitochondrial Ceramide Effects on the Retinal Pigment Epithelium in Diabetes. Int J Mol Sci. 2020; 21: 3830.

134. Levitsky Y, Hammer SS, Fisher KP, Huang C, Gentles TL, Pegouske DJ, et al. Urinary heparanase activity in patients with Type 1 and Type 2 diabetes. BMC Proc. 2011; 5: C8.

135. Jiang M, Huang S, Duan W, Liu Q, Lei M. Inhibition of acid sphingomyelinase activity ameliorates endoplasmic reticulum dysfunction in db/db mice. Biosci Rep. 2019; 39: 1-10.

136. Chakravarthy H, Navitskaya S, O'Reilly S, Gallimore J, Mize H, Bell E, et al. Role of Acid Sphingomyelinase in the Shifting Between Proinflammatory and Reparative Bone Marrow Cells in Diabetic Retinopathy. Stem Cells. 2016; 34: 972-83.

137. Kady N, Yan Y, Salazar T, Wang Q, Chakravarty H, Huang C, et al. Increased expression of acid sphingomyelinase activity mediates retinal cell death in type 2 diabetes. J Biol Chem. 2018; 293: 2550-61.

138. Levitsky Y, Steinherz PM, Zhao X, O'Keefe S, Kulp K, et al. Phospholipase A2 and lipid metabolism in type 1 diabetes. Diabetes. 2013; 62: 394-400.

139. Murase K, Okada H, Suzuki M, Iki E, Itoh H. Proglutathione time-dependently reduces tumour necrosis factor alpha in muscle and improves metabolic abnormalities in Wistar fatty rats. Diabetologia. 1998; 41: 257-64.

140. Straczkowska K, Kowalska I, Baranowski M, Nikolajuk A, Otziomek E, Zabielski P, et al. Increased skeletal muscle sphingolipid content in men at risk of developing type 2 diabetes. J Appl Physiol. 2007; 103: 2206-13.

141. Lei X, Zhang S, Barbour SE, Bohrer A, Ford EL, Koizumi A, et al. Spontaneous development of endoplasmic reticulum stress that can lead to diabetes mellitus is associated with higher calcium-independent phospholipase A2 expression: a role for regulation by SREBP-1. J Biol Chem. 2010; 285: 6693-705.

142. Baranowski M, Blachnio-Zabielska A, Hirrde T, Harasiuk D, Matlak K, Knapp AU, et al. Myocardium of type 2 diabetic and obese patients is characterized by alterations in sphingolipid metabolic enzymes but not by accumulation of ceramide. J Lipid Res. 2010; 51: 74-80.

143. Gil N, Goldberg R, Neuman T, Garsen M, Zcharia E, Rubinstein AM, et al. Mitochondrial Ceramide Effects on the Retinal Pigment Epithelium in Diabetes. Int J Mol Sci. 2020; 21: 3830.

144. Chen B, Zhao Q, Ni R, Tang F, Shan L, Cepinskas I, et al. Inhibition of calpain improves metabolic abnormalities in Wistar fatty rats. Diabetologia. 1998; 41: 698-701.

145. Ni R, Zheng D, Xiong S, Hill DJ, Sun T, Gardiner RB, et al. Mitochondrial Ceramide Effects on the Retinal Pigment Epithelium in Diabetes. Int J Mol Sci. 2020; 21: 3830.

146. Shafat I, Dan N, Zois S, Vladysky I, Nakhoul F. Heparanase levels are elevated in the urine and plasma of type 2 diabetes patients and associate with blood glucose levels. Plos One. 2011; 6: e17312.

147. Zhao Y, Liu J, ten S, Zhou Y, Yu J, et al. Plasma heparanase is associated with blood glucose levels but not urinary microalbumin excretion in type 2 diabetic nephropathy at the early stage. Renal Failure. 2017; 39: 698-701.

148. Parish CR, Freeman C, Ziolkowski AF, He YQ, Sutcliffe EL, Zafar A, et al. Plasma heparanase activity is negatively correlated with BMI: a potential mechanism for improved metabolic abnormalities in type 2 diabetes patients. Diabetes. 2015; 64: 2126-32.

149. Rao G, Ding HG, Huang W, Le D, Maxhimer JB, Oosterhof A, et al. Reactive oxygen species mediate high glucose-induced heparanase-1 production and heparan sulphate proteoglycan degradation in human and rat endothelial cells: a potential role in the pathogenesis of proteinuria in diabetic patients. Diabetes. 2005; 54: 2172-7.

150. Wijnhoven TJ, van den Hoven MJ, Ding H, van Kuppevelt TH, van der Vlag J, Berden JH, et al. Heparanase induces a differential loss of heparan sulphate domains in overt diabetic nephropathy. Diabetologia. 2008; 51: 372-82.

151. Das S, Singh G, Baker AB. Overcoming disease-induced growth factor resistance in therapeutic angiogenesis using recombinant co-receptors delivered by a liposomal system. Biomaterials. 2014; 35: 196-205.

152. Sczepnicka A, Roggerio A, Cravero CL, Facanaro AP, Salerno V, Benvenuti LA, et al. Down-regulation of fibroblast growth factor 2 and its co-receptors heparan sulfate proteoglycans by resveratrol underlies the improvement of cardiac dysfunction in experimental diabetes. J Nutr Biochem. 2017; 40: 219-27.

153. Strunz CM, Matsuda M, Salesmi VM, Nogueira A, Mansur AP, Cestari IN, et al. Mitochondrial Ceramide Effects on the Retinal Pigment Epithelium in Diabetes. Int J Mol Sci. 2020; 21: 3830.

154. Parish CR, Freeman C, Ziolkowski AF, He YQ, Sutcliffe EL, Zafar A, et al. Unexpected new roles for heparanase in Type 1 diabetes and immune gene regulation. Matrix Biol. 2013; 32: 228-33.
177. Randriambovony J, Piotrowski F, Bolck B, Schwingner RH, Dixit M, Badenkoop H, et al. Platelet sarcoplasmic endoplasmic reticulum Ca2+-ATPase and mu-calpain activity are altered in type 2 diabetes mellitus and type 2 diabetes mellitus with retinopathy. Circulation. 2011; 123: 2137-45.

178. Elghazawy A, Shi L, Hu J, Wittig I, Laban H, Fischer J, et al. Dicer cleavage by calpain determines platelet microRNA levels and function in diabetes. Circ Res. 2013; 113: 157-67.

179. Randriambovony J, Isak J, Elghazawy A, Piotrowski F, Fromel T, Yin X, et al. Calpain inhibition stabilizes the platelet proteome and reactivity in diabetes. Blood. 2012; 120: 415-23.

180. Kyseleva A, Elghazawy A, Wittig I, Heidler J, Mann AW, Ruf W, et al. Platelet-stored response genes cleaves the endothelial proteome to induce vascular inflammation in diabetes. Basic Res Cardiol. 2020; 115: 75.

181. Giannella A, Ceiolotto G, Radu CM, Cattelan A, Iori E, Benetti A, et al. PARK-4/Calpain pathway activation stimulates platelet-derived microparticles in hyperglycemic type 2 diabetes. Cardiovasc Diabetol. 2021; 20: 57.

182. Khramatil SB, Singh NJ, Sharma SS. Calpain inhibitor, MDL 28170 confer electrophysiological, nociceptive and biochemical improvement in diabetic cardiomyopathy. J Cardiol Myocardiol. 2015; 97: 113-21.

183. Li H, Chen LP, Wang T, Wang SG, Liu JH. Calpain inhibition improves erectile function in diabetic mice via upregulating endothelial nitric oxide synthase expression and reducing apoptosis. Asian J Androl. 2018; 20: 342-8.

184. Ahn YJ, Kim MS, Chung SK. Calpain and Caspase-12 Expression in Lens Cells. Indian J Ophthalmol. 2016; 64: 255-68.

185. Smolock AR, Mishra G, Egochi K, Eguchi S, Scalali R. Protein kinase R upregulates interleukin cell molecule-1 and leukocyte-endothelium interactions in hyperglycemia via activation of endothelial expressed calpain. Am J Physiol Endocrinol Metab. 2015; 309: E544-50.

186. Stalker TJ, Gong Y, Scalari L. The calcium-dependent protease calpain causes endothelial dysfunction in type 2 diabetes. Diabetes. 2005; 54: 1132-40.

187. Shanab AY, Nakazawa T, Ryu M, Tanaka Y, Himori N, Taguchi K, et al. Metallothionein response implicated in diabetic cardiomyopathy: the role of calpain, and the therapeutic impact of calpain inhibitor. Neurobiol Dis. 2012; 48: 556-67.

188. Ling C, Group L, Guerra SD, Lupi R. Calpain-10 expression is elevated in pancreatic islet cells in patients with type 2 diabetes. Plos One. 2009; 4: e7393.

189. Wang T, Gao Y, Wang X, Shi Y, Xu J, Wu B, et al. Calpain-10 drives podocyte apoptosis and renal injury in diabetic nephropathy. Diabetes Metab Syndr Obes. 2019; 12: 1811-20.

190. Covington MD, Schnellmann RG. Chronic high glucose dose regulates mitochondrial calpain 10 and contributes to renal cell death and diabetes-induced renal injury. Kidney Int. 2012; 81: 391-400.

191. Zhang W, Khan A, Ostenson CG, Berggren PO, Elenis S, Meister B. Down-regulated expression of exocytotic proteins in pancreatic islets of diabetic GK rats. Biochim Biophys Acta. 2002; 1563: 1038-44.

192. Nagamatsu S, Nakamichi Y, Yamamura C, Matsushima S, Watanabe T, Ozawa M, et al. Downregulated expression of exocytotic proteins in pancreatic islets of diabetic GK rats. Biochem Biophys Res Commun. 2002; 291: 13753-61.

193. Xu H, Du X, Liu G, Huang S, Du W, Zou S, et al. The pseudokinase MLKL regulates hepatic insulin sensitivity independently of inflammation. Mol Metab. 2019; 23: 14-28.

194. Kang F, Wang J, Fang D, Tang Y, Yu Z, Wang W, et al. Activation of ALDH2 attenuates high glucose induced rat cardiomyocyte fibrosis and necrosis. Free Radic Biol Med. 2020; 146: 198-210.

195. Xu Y, Gao H, Hu Y, Yang F, Qi C, Huang J, et al. High glucose-induced necrosis and apoptosis in podocytes is regulated by UCHL1 via RIPK1/RIPK3 pathway. Exp Cell Res. 2019; 382: 111463.

196. LaRocca TJ, Sosunov SA, Haller JD, Wallach D, MLKL, the Protein That Makes Necroptotic Cell Death and Its Extracellular Vesicle Generation. Immunity. 2017; 47: 51-65.

197. Koumanov F, Pereira VJ, Whitley PR, Holman GD. GLUT4 traffic through an ESCRT-III-dependent sorting compartment in adipocytes. Plos One. 2012; 7: e44141.

198. Yan C, Tian X, Li J, Liu D, Ye D, Xie Z, et al. A High-Fat Diet Attenuates AMPK alpha in Adipocytes to Induce Exocrine Shedding and Nonalcoholic Fatty Liver Development In Vivo. Diabetes. 2021; 70: 577-88.

199. Wang X, Gu H, Huang W, Peng J, Li Y, Yang L, et al. Hsp20-Mediated Activation of Exosome Biogenesis in Cardiomyocytes Improves Cardiac Function and Angiogenesis in Diabetic Mice. Diabetes. 2016; 65: 3111-28.

200. Yoon S, Kovalenko A, Bogdanov K, Wallach D, MLKL, the Protein That Makes Necroptotic Cell Death and Its Extracellular Vesicle Generation. Immunology. 2017; 47: 51-65.

201. Romero M, Sabate-Perez A, Francis VA, Castrillon-Rodriguez I, Diaz-Ramos A, Sanchez-Feutrie M, et al. TPS3NP2 regulates adiposity by activating beta-catenin through autophagy-dependent sequestration of GSK3beta. Nat Cell Biol. 2018; 20: 443-54.

202. Koumanov F, Pereira VJ, Whiteley PR, Holman GD. GLUT4 traffic through an ESCRT-III-dependent sorting compartment in adipocytes. Plos One. 2012; 7: e44141.
anchored microdomain and lipid droplet signaling proteins. Cell Signal. 2009; 21: 324-38.

283. Muller G, Schneider B, Miemer-Daub G, Wied S. Microvesicles released from rat adipose tissue containing pro-inflammatory CD14(high)CD16(-)cells regulate macrophage activation via TNF and IL-1beta: RNA transfer by RNA transfer RNA stimulating lipid synthesis. Cell Signal. 2011; 23: 1207-23.

284. Muller G, Schneider B, Miemer-Daub G, Wied S. Upregulation of lipid synthesis in small rat adipocytes by microvesicle-associated CD73 from large adipocytes. Cell Physiol Biochem. April 2011; 28(1):115-21.

285. Gesmundo I, Pardini B, Gargantini E, Gamba G, Birolo G, Fanciulli A, et al. Adipocyte-derived extracellular vesicles regulate survival and function of pancreatic beta cells. JCI Insight. 2021; e114962.

286. Naherty SR, Gao A, Xu X, Ailes E, Noman A, Ferrante AJ. A lipase-independent pathway of lipid release and immune modulation by adipocytes. Science. 2019; 363: 989-93.

287. Camino T, Lago-Baamonte N, Bravo SB, Suarez A, Couto I, Santos F, et al. Vessels Shed by Pathological Murine Adipocytes Spread Pathology: Characterization and Functional Role of Insulin Resistant/Hyperglycemic Adiposites. J Mol Med. 2020; 21: 2252.

288. Zhang B, Yang Y, Xiang L, Zhao Z, Ye R. Adipocyte-derived exosomal resistin is essential for melanin ameliorating hepatic steatosis in mice. J Pineal Res. 2019; 66:e12561.

289. Yu Y, Du H, Wei S, Feng L, Li J, Yao F, et al. Adipocyte-Derived Exosomal MicroRNA-141-3p Induces Insulin Resistance in Sertoli Cells Through Repression of PPARGamma. Thrombosis. 2018; 8: 2177-88.

290. Gao J, Li X, Wang Y, Cao Y, Yao D, Sun L, et al. Adipocyte-Derived Extracellular Vesicles Contain Peroxiredoxin 4 to Inhibit Lipid Oxidation via ERK1/2-Smad3 Pathway in Diabetic Monocytes. Biochim Biophys Acta Mol Basis Dis. 2020; 1866:e163399.

291. Gu H, Yang K, Shen Z, Jia K, Liu P, Pan M, et al. ER stress-induced adipocyte-exerted microRNA-30c-5p represses adipogenesis by repressing miR-122 expression. Front Physiol. 2020; 11: 694864.

292. Deng ZB, Poliakov A, Hardy RW, Clements R, Liu C, Liu Y, et al. Adipose microparticles release by adipocytes act as “find-me” signals to promote M1 macrophage polarization through PRR48 and CD73. JCI Insight. 2017; 2: e94422.

293. Dang SY, Leng Y, Wang ZX, Xiao X, Zhang X, Wen T, et al. Adipocytokine-Exerted Proatherogenic Effects by Regulating Macrophage Foam Cell Formation. Atherosclerosis. 2019; 283: 19-27.

294. Zhang Y, Shi L, Mei H, Zhang J, Zhu Y, Han X, et al. Inflamed macrophage microvesicles induce insulin resistance in human adipocytes. Nutr Metab (Lond). 2015; 12: 21.

295. Zhang B, Yang Y, Xiang L, Zhao Z, Ye R. Adipocyte-Derived Exosomes Mediate Adipose Stroma-Derived MicroRNAs to Promote Intravascular Macrophage Migration by Exerting Proatherogenic Effects. J Am Heart Assoc. 2018; 7: e7442.

296. Zhang Y, Mei H, Chang X, Chen F, Zhu Y, Han X, et al. Adipocyte-Derived Exosomal microvesicles from obese mice induce M1 macrophage phenotype through secreted miR-155. J Mol Cell Biol. 2016; 8: 505-17.

297. Zhang Y, Mei H, Zhang J, Zhu Y, Han X, et al. Inflamed macrophage-derived exosomal microvesicles from obese mice induce M1 macrophage phenotype through secreted miR-155. J Mol Cell Biol. 2016; 8: 505-17.

298. Zhang Y, Sun X, Qi X, Xia L, Wu Y. Exosomes from high glucose-treated diabetes adipocytes promote leukocyte attachment to vascular endothelial cells. Atherosclerosis. 2019; 283: 19-27.

299. Rong B, Feng R, Liu C, Wu Q, Sun C. Reduced delivery of epididymal fat adipocyte-derived exosomal resistin is essential for melanin ameliorating hepatic steatosis in mice. J Pineal Res. 2019; 66:e12561.

300. Wang F, Chen FF, Shang YY, Li Y, Wang ZH, Han L, et al. Insulin resistance mechanisms of formation, action, and detoxification. Arterioscler Thromb Vasc Biol. 2020; 40: 1838-53.

301. Ding X, Jing N, Shen A, Guo F, Song Y, Pan M, et al. MiR-21-5p in macrophage-derived extracellular vesicles affects podocyte pyroptosis in diabetic nephropathy. Biochim Biophys Acta Mol Basis Dis. 2020; 1866:e163399.

302. Bouchareychas L, Duong P, Phu TA, Alosp E, Meechoovet B, Reiman R, et al. High glucose macrophage exosomes enhance atherosclerosis by driving cellular proliferation & hematopoiesis. Science. 2021; 324: 102847.

303. Goeppapura FR, Paul M, Zim, Girikapiti V, Verma SK, Sahaera S, Narasimhan G, et al. Targeting exosome-associated human antigen R attenuates fibrosis and inflammation in diabetic heart. Faseb J. 2020; 34: 2238-51.

304. Nair N, Kumar S, Gonga R, Gupta S. Circulating miRNA as novel markers for diabetic dysfunction. Mol Cell Biochem. 2013; 376: 33-40.

305. Liu Y, Song J, Lin JY, Miao R, Zhong JC. Roles of MicroRNA-122 in Cardiovascular Fibrosis and Related Diseases. Cardiovasc Toxicol. 2020; 20: 463-73.

306. Shi Y, Wang Z, Zhu X, Chen L, Ma Y, Wang J, et al. Exosomal miR-1246 in serum as a potential biomarker for early diagnosis of gastric cancer. Int J Clin Oncol. 2020; 26: 899-909.

307. Li C, Liu M, Zhang K, Wang G, Zhang S. MiR-1246-3p regulates adipoocyte extracellular vesicle release and promotes diaphragm atrophy in lipodystrophic mice. JCI Insight. 2020; 2019; 3: e133160.

308. Wu J, Wang C, Hu X, Wang X, Zeng J, Cao T, et al. Exosomal extracellular vesicles secrete aldo-keto reductase 1B7-containing exosomes that cause nonalcoholic steatohepatitis in mice. Free Radiol Med Biol. 2021; 163: 220-33.

309. Bouchareychas L, Meechoovet B, Alosp E, Meechoovet B, Reiman R, et al. High glucose macrophage exosomes enhance atherosclerosis by driving cellular proliferation & hematopoiesis. Science. 2021; 324: 102847.

310. Gu H, Yang K, Shen Z, Jia K, Liu P, Pan M, et al. ER stress-induced adipocyte-exerted microRNA-30c-5p represses adipogenesis by repressing miR-122 expression. Front Physiol. 2020; 11: 694864.

311. Ying W, Riopel M, Bandyopadhyay G, Dong Y, Birmingham A, Seo JB, et al. Targeting exosome-associated human antigen R attenuates fibrosis and inflammation in diabetic heart. Faseb J. 2020; 34: 2238-51.

312. Nair N, Kumar S, Gonga R, Gupta S. Circulating miRNA as novel markers for diabetic dysfunction. Mol Cell Biochem. 2013; 376: 33-40.

313. Zhang Y, Shi L, Mei H, Zhang J, Zhu Y, Han X, et al. Inflamed macrophage microvesicles induce insulin resistance in human adipocytes. Nutr Metab (Lond). 2015; 12: 21.

314. Zhu QJ, Zhu M, Xu XG, Meng XM, Wu YG. Exosomes from high glucose-treated diabetes adipocytes promote leukocyte attachment to vascular endothelial cells. Atherosclerosis. 2019; 283: 19-27.

315. Zhu M, Sun X, Qi X, Xia L, Wu Y. Exosomes from high glucose-treated diabetes adipocytes activate macrophages and induce inflammatory responses via NF-kappaB signaling pathway in vitro and in vivo. Int Immunopharmacol. 2020; 84: 106531.
335. Zhang Y, Liu F, Yuan Y, Jin C, Chang C, Zhu Y, et al. Inflammamso-Derived Exosomes Activate NF-kappaB Signaling in Macrophages. J Proteome Res. 2017; 16: 170-8.

336. Lindemans MJ, Stoorvogel W. Antigen Presentation by Extracellular Vesicles from Professional Antigen-Presenting Cells. Annu Rev Immunol. 2018; 36: 433-59.

337. Du T, Yang CL, Ge MR, Liu Y, Zhang P, Li H, et al. MiMammograph Derived Exosomes Agonize Human Experimental Autoimmune Neuritis via Modulating Th2 Response. Front Immunol. 2020; 11: 1603.

338. Burg AR, Tse HM. Redox-Sensitive In innate Immune Pathways During Macrophage Activation in Type 1 Diabetes. Antioxid Redox Signal. 2018; 29: 197-213.

339. Fediusma JK, Tse HM. The proinflammatory effects of macrophage-derived NADPH oxidase function in autoimmune diabetes. Free Radic Biol Med. 2018; 125: 81-93.

340. Zhao Y, Zhao ME, Jiang S, Wu J, Liu J, Yuan NW, et al. Liver governs adipose remodelling via extracellular vesicles in response to lipid overload. Nat Commun. 2020; 11: 719.

341. Fu Q, Li Y, Jiang H, Shen Z, Gao R, He Y, et al. Hepatocytes derived extracellular vesicles from high-fat diet induced obese mice modulate genes expression and proliferation of islet beta cells. Biochem Biophys Res Commun. 2019; 516: 1159-66.

342. Liu XL, Pan Q, Cao XJ, Xin FZ, Zhao ZH, Yang RX, et al. Lipotoxic Hepatocyte-Derived Exosomal MicroRNA-192-5p Activates Macrophages Through RhoA/ ROCK/ Rac1 Signaling in Nonalcoholic Fatty Liver Disease. Hepatology. 2020; 72: 454-69.

343. Hirsova P, Ibrahim SH, Krishnan A, Verma VK, Bronk SF, Werneburg NW, et al. Lipid-Induced Signaling Causes Release of Inflammatory Extracellular Vesicles. J Biol Chem. 2016; 165: 956-67.

344. Zhao Z, Zhong L, Li P, He K, Qiu C, Zhao L, et al. Cholesterol impairs hepatocyte lysosomal function causing M1 polarization of macrophages via exosomal miR-122-5p. Exp Cell Res. 2020; 387: 111738.

345. Kukuzaki Y, Mizumoto Y, Yin M, Malhi H. Hepatocytes release ceramide-enriched pro-inflammatory extracellular vesicles in an IRE1alpha-dependent manner. J Lipid Res. 2016; 57: 233-45.

346. Guo Q, Purata K, Lucien F, Gutierrez SL, Hirsova P, Krishnan A, et al. Integrin beta-3-enriched extracellular vesicles mediate monocytic adhesion and promote liver inflammation in murine NASH. J Hepatol. 2019; 11: 2193-1205.

347. Liao CY, Song MJ, Gao Y, Mauer AS, Revzin A, Malhi H. Hepatocyte-Derived Exosomes from Non-Alcoholic Steatohepatitis Model Cause Hepatic Insulin Resistance. J Hepatol. 2020; 62: 1901-14.

348. Zhao Z, Zhong L, Li P, He K, Qiu C, Zhao L, et al. Cholesterol impairs hepatocyte lysosomal function causing M1 polarization of macrophages via extracellular vesicles. Exp Cell Res. 2020; 387: 111738.

349. Ramos S, Heldt NA, Gaigahate S, Seliga A, Reichenbach NL, Persiody S. High glucose induces inflammation via IRF1 and IL12 in high glucose condition increases NADPH oxidase activity in endothelial cells. Cardiovasc Res. 2020; 125: 2497-509.

350. Jansen F, Stumpf T, Proebsting S, Franklin BS, Wenzel D, Pfeifer P, et al. High glucose derived endothelial microparticles increase active caspase-3 and reduce microRNA-Let-7a expression in endothelial cells. Biochim Biophys Acta. 2017; 493: 1026-39.

351. Schober A, Nazari-Jahantigh M, Wei Y, Bidzhekovek K, Greemse F, Grommes J, et al. MicroRNA-126-5p promotes endothelial proliferation and limits atherosclerosis by suppressing DR1. Nat Med. 2014; 20: 368-76.

352. Jansen F, Yang X, Hoelscher M, Cattelan A, Schmitz T, Proebsting S, et al. Endothelial microparticle-mediated transfer of MicroRNA-126 promotes vascular endothelial cell proliferation via SPRED1 and is abrogated in glucose-damaged endothelial microparticles. Circulation. 2013; 128: 2026-38.

353. Jansen F, Yang X, Baumann K, Przybilla D, Schmitz T, Flender A, et al. Endothelial microparticles reduce RAM-1 expression in a microRNA-22-dependent mechanism. J Cell Mol Med. 2015; 19: 2202-14.

354. Hjijmans JG, Bammert TD, Stockelmann KA, Reikavik WR, Greiner JF, DeSouza CA. High glucose-induced endothelial microparticles increase adhesion molecule expression on endothelial cells. Diabetol Int. 2019; 10: 145-7.

355. Jansen F, Yang X, Franklin BS, Hoelscher M, Schmitz T, Bedorf J, et al. High glucose condition increases NADPH oxidase activity in endothelial microparticles that promote vascular inflammation. Cardiovasc Res. 2013; 98: 94-106.

356. Jansen F, Stumpf T, Proebsting S, Franklin BS, Werndel D, Pfeifer P, et al. Intercellular transfer of miR-126-3p by endothelial microparticles reduces vascular smooth muscle cell proliferation and limits neointima formation by inhibiting ERK1. J Mol Cell Cardiol. 2017; 104: 43-52.

357. Jansen F, Zietzer A, Stumpf T, Flender A, Schmitz T, Nickenig G, et al. Endothelial microparticle-promoted inhibition of vascular remodeling is abrogated under hyperglycemic conditions. J Mol Cell Cardiol. 2017; 112: 91-4.

358. Lin X, Li S, Wang YJ, Wang Y, Zhong JY, He JY, et al. Extracellular Notch3 from high glucose-stimulated endothelial cells regulates vascular smooth muscle cells calcification/aging. Life Sci. 2019; 232: 116582.

359. Li S, Zhan JK, Wang YJ, Lin X, Zhong JY, Wang Y, et al. Exosomes from hyperglycemia-stimulated vascular endothelial cells contain vesicles that trigger calcification/senescence in vascular smooth muscle cells. Cell Biosci. 2019; 9: 1.

360. Togliatto G, Dentelli P, Rosso A, Lombardo G, Gilli M, Gallo S, et al. PDGF-BB Carried by Endothelial Cell-Derived Extracellular Vesicles Reduces Vascular Smooth Muscle Cell Apoptosis in Diabetes. Diabetes. 2018; 67: 758-76.

361. Wang S, Zhan J, Lin X, Wang Y, Wang Y, Liu L. CircRNA-007790 from hyperglycemia-stimulated vascular endothelial cell exosomes regulates
senescence in vascular smooth muscle cells. Cell Biochem Funct. 2020; 38: 1056-68.

387. Hu J, Wang S, Xiong Z, Zheng Y, Zhang L, J et al. Exosomal Mfn2 transfer from cardiomyocytes to vascular endothelial cells to cardiomyocytes deteriorates diabetic cardiomyopathy. Biochim Biophys Acta Mol Basis Dis. 2018; 1864: 3639-49.

388. Wu XM, Gao YB, Cui FQ, Zhang N. Exosomes from high glucose-treated glomerular endothelial cells activate mesangial cells to promote renal fibrosis. Biol Open. 2016; 5: 484-91.

389. Wu X, Gao Y, Xu L, Dang W, Yan H, Zou D, et al. Exosomes from high glucose-treated glomerular endothelial cells trigger the epithelial-mesenchymal transition and dysfunction of podocytes. Sci Rep. 2017; 7: 9371.

390. Zeng T, Wang X, Wang W, Feng Q, Lao G, Liang Y, et al. Endothelial cell-derived small extracellular vesicles suppress cutaneous wound healing through regulating fibroblasts autophagy. Cell Sci (Lond). 2019; 133: 2034-46.

391. He S, Wu C, Xiao J, Li D, Sun Z, Li M. Endothelial extracellular vesicles modulate the macrophage phenotype: Potential implications in microRNA-driven extracellular vesicles influence gut permeability through the regulation of tight junctions. Exp Mol Med. 2018; 50: e430.

392. Chen L, Hu L, Li Q, Li M, Li H. Exosome-encapsulated miR-505 from ox-LDL-treated vascular endothelial cells aggravates atherosclerosis by inducing NET formation. Acta Biochim Sin (Shanghai). 2019; 51: 70-81.

393. Liu Y, Li Q, Hosen MR, Zietzer A, Fledner A, Levermann P, et al. Atherosclerotic Conditions Promote the Packaging of Functional MicroRNA-92a-3p Into Endothelial Microvesicles. Circ Res. 2019; 124: 575-87.

394. Zhan R, Leng X, Liu X, Wang X, Gong J, Lan Y, et al. Heat shock protein 70 is sequestered from endothelial cells by microvesicles and involved in extracellular vesicles. Biochem Biophys Res Commun. 2009; 387: 229-33.

395. Zhang Z, Yi D, Zhou J, Zheng Y, Gao Z, Hu X, et al. Exosomal LINC00103 derived from oxidized low-density lipoprotein-treated endothelial cells regulates vascular smooth muscle cell phenotypic switch. Biofactors. 2020; 56: 743-53.

396. Gao H, Wang X, Lin C, An Z, Yu J, Gao H, et al. Exosomal MALAT1 derived from high glucose-treated glomerular endothelial cells trigger the epithelial-mesenchymal transition and contribute to diabetic kidney disease. Biochim Biophys Acta Mol Basis Dis. 2018; 1864: 1056-68.

397. Liu Y, Li Q, Hosen MR, Zietzer A, Fledner A, Levermann P, et al. Atherosclerotic Conditions Promote the Packaging of Functional MicroRNA-92a-3p Into Endothelial Microvesicles. Circ Res. 2019; 124: 575-87.

398. Prabu P, Rome S, Sathis kumar C, Gastebois C, Meugnier E, Mohan V, et al. Spatial Variation of Microbiome Based on Extracellular Vesicles from Gut Microbiota from Normal and Diabetic Individuals. Microbiome. 2020; 8: 15173.

399. Aswad H, Forterre A, Wiklander OP, Vial G, Danty-Berger E, Jalabert A, et al. Extracellular Vesicles Provide a Means for Tissue Crosstalk during Exercise. Cell Metab. 2018; 27: 237-51.

400. Jabakkot C, Choi Y, Kim DK, Park HT, Ghim J, Kwon Y, et al. Akkermansia muciniphila-derived extracellular vesicles mediate renal proximal tubule cells dedifferentiation via microRNA-221 in diabetic nephropathy. Mol Cell Endocrinol. 2020; 518: 110568.

401. Pratichizzo F, Matacchione G, Giuliani A, Sabbatinelli J, Olivieri F, de Candia Lucito HA, et al. Extracellular Vesicles as Diagnostic Biomarkers in Type 2 Diabetes Mellitus. Front Endocrinol (Lausanne). 2020; 11: 596011.

402. Rathnam S, Panda M, Agouri A, Manuamniet S. Microvesicles as Potential Mediators of High Glucose-Induced Renal Cell Injury. Biomolecules. 2019; 9: 348.

403. Wen J, Ma Z, Livingston MJ, Zhang W, Yuan Y, Gao C, et al. Decreased secretion and profibrotic activity of tubular exosomes in diabetic kidney disease. Am J Physiol Renal Physiol. 2020; 319: F664-73.

404. Maisto R, Orla M, Vital-Gil L, Martinez-Gil N, Sancho-Pelizzi F, Filippo CD, et al. ARF6-19-derived VEGC-containing exosomes promote neovascularization in HUVEC: the role of the melanocortin receptor 5. Cell Cycle. 2019; 18: 413-24.

405. Leszczynska A, Kulkarni M, Ljubimov AV, Saghihzadeh M. Exosomes from normal and diabetic human corneal keratocytes differentially regulate migration, proliferation and marker expression of limbal epithelial cells. Sci Rep. 2018; 8: 15173.

406. Goncalves NP, Vaegter CB, Andersen H, Ostergaard L, Calculc NA, Jensen TS. Schwann cell interactions with axons and microvesicles in diabetic neuropathy. Nat Rev Neurol. 2017; 13: 135-47.

407. Wang L, Chopp M, Szalad A, Lu X, Zhang Y, Wang X, et al. Exosomes Derived From Schwann Cells Ameliorate Peripheral Neuropathy in Type II Diabetic Mice. Diabetes. 2020; 70: 649-74.

408. Jia L, Chopp M, Wang L, Lu X, Szalad A, Zhang ZG. Exosomes derived from high-glucose-stimulated Schwann cells promote development of peripheral diabetic neuropathy. Faseb J. 2018; 32: [201900597R].

409. Liu J, Sun X, Zhang FL, Jin H, Yan XL, Huang S, et al. Clinical Potential of Extracellular Vesicles in Type 2 Diabetes. Front Endocrinol (Lausanne). 2020; 11: 596011.

410. Pratichizzo F, Matacchione G, Giuliani A, Sabbatinelli J, Olivieri F, de Candia Lucito HA, et al. Extracellular Vesicles as Diagnostic Biomarkers in Type 2 Diabetes Mellitus. Front Endocrinol (Lausanne). 2020; 11: 596011.

411. He X, Kuang G, Wu Y, Ou C. Emerging roles of exosomal miRNAs in diabetes mellitus. Clin Transl Med. 2021; 11: e468.

412. Liu C, Gao Y, Wu Z, Zou J. Exosomal miR-25a and miR-192, Potential Diagnostic Biomarkers for Type 2 Diabetes. Clin Lab. 2021; 67.

413. Zhao Y, Shen A, Gao F, Song Y, Jing N, Ding X, et al. Urinary Exosomal MiRNA-4534 as a Novel Diagnostic Biomarker for Diabetic Kidney Disease. Front Endocrinol (Lausanne). 2020; 11: 590.

414. Prabu P, Rome S, Sathis kumar C, Gastebois C, Meugnier E, Mohan V, et al. MicroRNAs from urinary extracellular vesicles are non-invasive early biomarkers of diabetic nephropathy in type 2 diabetes patients with the ‘Asian Indian phenotype’. Diabetologia. 2019; 4: 276-85.

415. Kong Q, Guo X, Guo Z, Su T. Urinary Exosome miR-424 and miR-218 as Biomarkers for Type 1 Diabetes in Children. Clin Lab. 2019; 65: doj10775/ClinLab.2020.206612.

416. Zang J, Maxwell AP, Simpson DA, McKay GJ. Differential Expression of Urinary Exosomal MicroRNAs miR-21-5p and miR-30b-5p in Individuals with Diabetic Kidney Disease. Sci Rep. 2019; 9: 10000.

417. Ibrahim AA, Wahby AA, Ashamawy I, Saleh RM, Soliman H. Association of Exosomal miR-34a with Markers of Dyslipidemia and Endothelial
Dysfunction in Children and Adolescents with T1DM. J Clin Res Pediatr Endocrinol. 2020; 12: 401-9.

440. Jia Y, Guan M, Zheng Z, Zang Q, Tang C, Xu W, et al. miRNAs in Urine Extracellular Vesicles as Predictors of Early-Stage Diabetic Nephropathy. J Diabetes Res. 2016; 2016: 7902765.

441. Eissa S, Matzboli M, Aboushahra R, Bekht M M, Soliman Y. Urinary exosomal miRNA panel unravels novel biomarkers for diagnosis of type 2 diabetic kidney disease. Diabetes Complications and Complications. 2018; 30: 1595-92.

442. Prattichizzo F, De Nigris V, Sabbatini J, Giuliani A, Castano C, Parrizas M, et al. CD31 (+) Extracellular Vesicles From Patients With Type 2 Diabetes Shuttles a miRNA Signature Associated With Cardiovascular Complications. Diabetes Care. 2021; 70: 240-54.

443. Eissa S, Matzboli M, Bekht M M. Clinical verification of a novel urinary microRNA panel: 133b, -342 and -390 as biomarkers for diabetic nephropathy identified by bioinformatics analysis. Biomed Pharmacother. 2016; 83: 92-9.

444. Li W, Yang S, Qiao R, Zhang J. Potential Value of Urinary Exosome-Derived miR-126-5p in the Diagnosis and Progression of Type II Diabetic Nephropathy. Clin Lab. 2018; 64: 799-708.

445. Chen ZJ, Shi XJ, Fu LJ, Liu J, Shi K, Zhang WB, et al. Serum and exosomal lncRNA SNHG7 suppresses endothelial-mesenchymal transition and tube formation in diabetic retinopathy via miR-34a-5p/BMAL1 axis. Life Sci. 2021; 272: 112932.

446. Gu C, Zhang H, Gao Y. Adipose mesenchymal stem cells-secreted extracellular vesicles containing microRNA-192 delayed diabetic retinopathy by targeting FGF1. J Cell Physiol. 2021; 236: 5036-51.

447. Gu S, Liu Y, Zou J, Wang W, Wei T, Wang X, et al. Retinal pigment epithelial cells secrete miR-202-5p-containing exosomes to protect against proliferative diabetic retinopathy. Exp Eye Res. 2020; 201: 108271.

448. Li W, Jin LY, Cui YB, Xie X. Human umbilical cord mesenchymal stem cells-derived exosomal microRNA-17-5p ameliorates inflammatory reaction and antioxidant injury of mice with diabetic retinopathy via targeting STAT1. Int Immunopharmacol. 2020; 107: 107510.

449. Chen F, Zhang H, Wang Z, Zeng T, Zeng Q, Liu W, et al. Adipose-Derived Stem Cell-Derived Exosomes Ameliorate Erectile Dysfunction in a Rat Model of Type 2 Diabetes. J Sex Med. 2017; 14: 1084-94.

450. Huo W, Li Y, Zhang Y, Li H. Mesenchymal stem cells-derived exosomal microRNA-21-5p downregulated and ameliorated ischemic retinal damage in a rat model of diabetes mellitus. Faseb J. 2020; 34: 13345-60.

451. Zhu LL, Huang X, Yu W, Chen H, Chen Y, Dai YT. Transplantation of adipose tissue-derived stem cell-derived exosomes ameliorates erectile function in diabetic rats. Andrology. 2018; 10: 250-62.

452. Ouyang B, Xie Y, Zhang C, Deng C, Lv L, Yao J, et al. Extracellular Vesicles From Human Urine-Derived Stem Cells Ameliorate Erectile Dysfunction in a Diabetic Rat Model by Delivering Proangiogenic MicroRNA. Sex Med. 2019; 7: 241-50.

453. Tao SC, Guo SC, Li M, Ke QF, Guo YP, Zhang CQ. Chitosan Wound Dressings Incorporating Exosomes Derived from MicroRNA-126-Overexpressing Synovium Mesenchymal Stem Cells Provide Sustained Release of Exosomes and TGF-β1 Full/Thick/Fast In Vivo Defects in a Diabetic Rat Model. Stem Cells Dev. 2016; 25: 736-747.

454. Li Q, Zhao H, Chen W, Huang P, Bi J. Human keratinocyte-derived microvesicle miRNA-21 promotes skin wound healing in diabetic rats through facilitating fibroblast function and angiogenesis. Int J Biochem Cell Biol. 2019; 114: 105570.

455. Huang C, Luo W, Wang Q, Ye Y, Fan J, Lin L, et al. Human mesenchymal stem cells promote ischemic repairment and angiogenesis of diabetic foot through exosome miRNA-21-5p. Stem Cell Res. 2021; 52: 102235.

456. Li X, Jiang C, Zhao J. Human endothelial progenitor cells-derived exosomes accelerate cutaneous wound healing in diabetic rats by promoting endotelly dysfunction. J Diabetes Complications. 2016; 30: 986-92.

457. Guo S, Liu Y, Han W, Li X. Exosomes derived from mmu_circ_0000250-modified adipose-derived mesenchymal stem cells promote wound healing in diabetic mice by inhibiting PI3K-AKT-mTOR-mediated proliferation in angiogenesis and fibroblast function. Burns Trauma. 2020; 8: a20.

458. Tao SC, Guo SC, Li M, Ke QF, Guo YP, Zhang CQ. Chitosan Wound Dressings Incorporating Exosomes Derived from MicroRNA-126-Overexpressing Synovium Mesenchymal Stem Cells Provide Sustained Release of Exosomes and TGF-β1 Full/Thick/Fast In Vivo Defects in a Diabetic Rat Model. Stem Cells Dev. 2016; 25: 736-747.

459. Li Q, Zhao H, Chen W, Huang P, Bi J. Human keratinocyte-derived microvesicle miRNA-21 promotes skin wound healing in diabetic rats through facilitating fibroblast function and angiogenesis. Int J Biochem Cell Biol. 2019; 114: 105570.

460. Huang C, Luo W, Wang Q, Ye Y, Fan J, Lin L, et al. Human mesenchymal stem cells promote ischemic repairment and angiogenesis of diabetic foot through exosome miRNA-21-5p. Stem Cell Res. 2021; 52: 102235.

461. Li X, Jiang C, Zhao J. Human endothelial progenitor cells-derived exosomes accelerate cutaneous wound healing in diabetic rats by promoting endotelly dysfunction. J Diabetes Complications. 2016; 30: 986-92.

462. Guo S, Liu Y, Han W, Li X. Exosomes derived from mmu_circ_0000250-modified adipose-derived mesenchymal stem cells promote wound healing in diabetic mice by inhibiting PI3K-AKT-mTOR-mediated angiogenesis and fibroblast function. Burns Trauma. 2020; 8: a20.
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488. Pomatto M, Gai C, Negro F, Cederino M, Grange C, Ceccotti E, et al. Differential Therapeutic Effect of Extracellular Vesicles Derived by Bone Marrow and Adipose Mesenchymal Stem Cells on Wound Healing of Diabetic Ulcers and Correlation to Their Cargos. Int J Mol Sci. 2021; 22: 3851.

489. Geiger A, Walker A, Nissen E. Human fibrocyte-derived exosomes accelerate wound healing in genetically diabetic mice. Biochem Biophys Res Commun. 2015; 467: 303-9.

490. Li M, Wang T, Tian H, Wei G, Zhao L, Shi Y. Macrophage-derived exosomes accelerate wound healing through their anti-inflammation effects in a diabetic rat model. Artif Cells Nanomed Biotechnol. 2019; 47: 3793-803.

491. Bouchareychas L, Duong P, Covarrubias S, Alspoh F, Puth TA, Chung A, et al. Macrophage Exosomes Resolve Atherosclerosis by Regulating Hematopoiesis and Inflammation via MicroRNA Cargo. Cell Rep. 2020; 32: 107881.

492. Lin B, Xie W, Zeng C, Wu X, Chen A, Li H, et al. Transfer of exosomal microRNA-203–3p from dendritic cells to bone marrow-derived macrophages reduces development of atherosclerosis by downregulating Cx3 in mice. Aging (Albany NY). 2021; 13: 15638-58.

493. Guo Z, Zhao Z, Yang C, Song C. Transfer of microRNA-221 from mesenchymal stem cell-derived extracellular vesicles inhibits atherosclerotic plaque formation. Trans Res. 2020; 226: 83-95.

494. Li L, Wang H, Zhang J, Chen X, Zhang Z, Li Q. Effect of endothelial progenitor cell-derived extracellular vesicles on endothelial cell ferroptosis and atherosclerotic vascular endothelial injury. Cell Death Discov. 2021; 7: 235.

495. Lin Y, Liu M, Chen E, Jiang W, Shi W, Wang Z. Bone marrow-derived mesenchymal stem cells microvesicles stabilize atherosclerotic plaques by inhibiting NLRP3-mediated macrophage pyroptosis. Cell Biol Int. 2021; 45: 820-30.

496. Lin F, Zhang S, Liu X, Wu M. Mouse bone marrow derived mesenchymal stem cells-secreted exosomal microRNA-125b-5p suppresses atherosclerotic plaque formation via inhibiting Map4k4. Life Sci. 2021; 274: 119249.

497. Li J, Xue H, Li T, Chu X, Xin D, Xiong Y, et al. Exosomes derived from mesenchymal stem cells attenuate the progression of atherosclerosis in Apolipoprotein-E-/- mice via miR-let7 mediated infiltration and polarization of M2 macrophage. Biochem Biophys Res Commun. 2019; 510: 565-72.

498. Hergenreider E, Heydt S, Treguer K, Boettger T, Horrovoets AJ, Zeiher AM, et al. Angiogenesis-Based Diabetic Wound Healing/Skin Reconstruction through Self-Healing Antibacterial Exosomes Hydrogel for Promoting Chronic Wound Healing. Stem Cell Res Ther. 2020; 11: 350.

499. Wu G, Zhang J, Zhao Q, Zhuang W, Ding J, Zhang C, et al. Moleculary Engineered Macrophage-Derived Exosomes with Inflammation Tropism and Intrinsically Heme Biosynthesis for Atherosclerosis Treatment. Angew Chem Int Ed Engl. 2020; 59: 2096-74.

500. Ma Q, Fan Q, Han X, Dong Z, Xu J, Bai J, et al. Platelet-derived extracellular vesicles to target plaque inflammation for effective anti-atherosclerotic therapy. J Control Release. 2021; 329: 445-53.

501. Zhang M, Chung AC, Chen HY, Dong Y, Mene XM, Li R, et al. miR-21 is a key therapeutic target for renal injury in a mouse model of type 2 diabetes. Diabetologia. 2013; 56: 663-74.

502. Kolling M, Kaucsar T, Schauerte C, Hubner A, Detting A, Park J, et al. Therapeutic miR-21 Silencing Ameliorates Diabetic Kidney Disease in Mice. Mol Ther. 2017; 25: 165-80.

503. Brennan E, Wang B, McClelland A, Mohan M, Marai M, Bruscatt O, et al. Protective Effect of let-7 miRNA Family in Regulating Inflammation in Diabetic-Affected Atherosclerosis. Diabetes. 2017; 66: 2266-77.

504. Pishavar E, Behravan J. miR-126 as a Therapeutic Agent for Diabetes Mellitus. Curr Pharm Des. 2017; 23: 3309-14.

505. Bijkkerk R, Esguerra J, Ellenbrook JH, Au YW, Hanegeaaf M, de Koning EJ, et al. In Vivo Silencing of MicroRNA-132 Reduces Blood Glucose and Improves Insulin Secretion. Nucleic Acid Ther. 2019; 29: 67-72.

506. Li X, Li D, Wang A, Chu T, Lohcharoenkal W, Zheng X, et al. MicroRNA-132 with Therapeutic Potential in Chronic Wounds. J Invest Dermatol. 2017; 137: 2630-8.

507. Yu M, Liu W, Li J, Lu H, Jia W, et al. Exosomes derived from atorvastatin-pertreated MSC accelerate diabetic wound repair by enhancing angiogenesis via AKT/ENOS pathway. Stem Cell Res Ther. 2020; 11: 350.

508. Wang C, Wang M, Xu T, Zhang X, Lin C, Gao W, et al. Engineering Bioactive Self-Healing Antibacterial Exosomes Hydrogel for Promoting Chronic Diabetic Wound Healing and Complete Skin Regeneration. Theranostics. 2019; 9: 65-76.

509. Zhang Y, Zhang P, Gao X, Chang L, Chen Z, Mei X. Preparation of exosomes encapsulated nanohydrogel for accelerating wound healing of diabetic rats by promoting angiogenesis. Mater Sci Eng C Mater Biol Appl. 2021; 120: 111671.

510. Wang M, Wang C, Chen M, Xi Y, Cheng W, Mao C, et al. Efficient Angiogenesis-Based Diabetic Wound Healing/Skin Reconstruction through Bioactive Antibacterial Adhesive Ultraviolet Shielding Nanodressing with Exosome Release. Acn Naro. 2019; 13: 10279-93.

511. Tao SC, Rui BY, Wang QY, Zhou D, Zhang Y, Guo SC. Extracellular vesicle-mimetic nanovesicles transport LncRNA-H19 as competing endogenous RNA for the treatment of diabetic wounds. Drug Deliv. 2018; 25: 241-55.

512. Zabeo D, Cvjetkovic A, Lasser C, Schorb M, Lotvall J, Hoog JL. Exosomes purified from a single cell type have diverse morphology. J Extracell Vesicles. 2017; 6: 1329476.

513. Pick H, Alves AC, Vogel H. Single-Vesicle Assays Using Liposomes and Cell-Derived Vesicles: From Modeling Complex Membrane Processes to Synthetic Biology and Biomedical Applications. Chem Rev. 2018; 118: 8588-654.

514. Han Z, Liu S, Pei Y, Ding Z, Li Y, Wang X, et al. Highly efficient magnetic labelling allows MRI tracking of the homing of stem cell-derived extracellular vesicles following systemic delivery. J Extracell Vesicles. 2021; 10: e21054.

515. Zhang W, Yu ZL, Wu M, Ren JG, Xia HF, Sa GL, et al. Magnetic and Folate Functionalization Enables Rapid Isolation and Enhanced Tumor-Targeting of Cell-Derived Microvesicles. Acn Nano. 2017; 11: 277-90.

516. Masud MK, Na J, Younus M, Hossain M, Bando Y, Shiddiky M, et al. Superparamagnetic nanotechnologies for disease-specific biomarker detection. Chem Soc Rev. 2019; 48: 5717-51.

517. Sancho-Albero M, Sebastian V, Sese J, Paxo-Gid B, Mendoza G, Arruebo M, et al. Isolation of exosomes from whole blood by a new microfluidic device: proof of concept application in the diagnosis and monitoring of pancreatic cancer. J Nanobiotechnology. 2020; 18: 150.

518. Chen H, Luo D, Shang B, Cao J, Wei J, Chen Q, et al. Immunoassay-type biosensor based on magnetic nanoparticle capture and the fluorescence signal formed by horseradish peroxidase catalysis for tumor-related exosome determination. Microchim Acta. 2020; 187: 282.