

Review

Integrative biology of extracellular vesicles in diabetes mellitus and diabetic complications

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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 pathological 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 pathophysiological 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

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. Insulinitis 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 glycosylated and oxidized, 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.

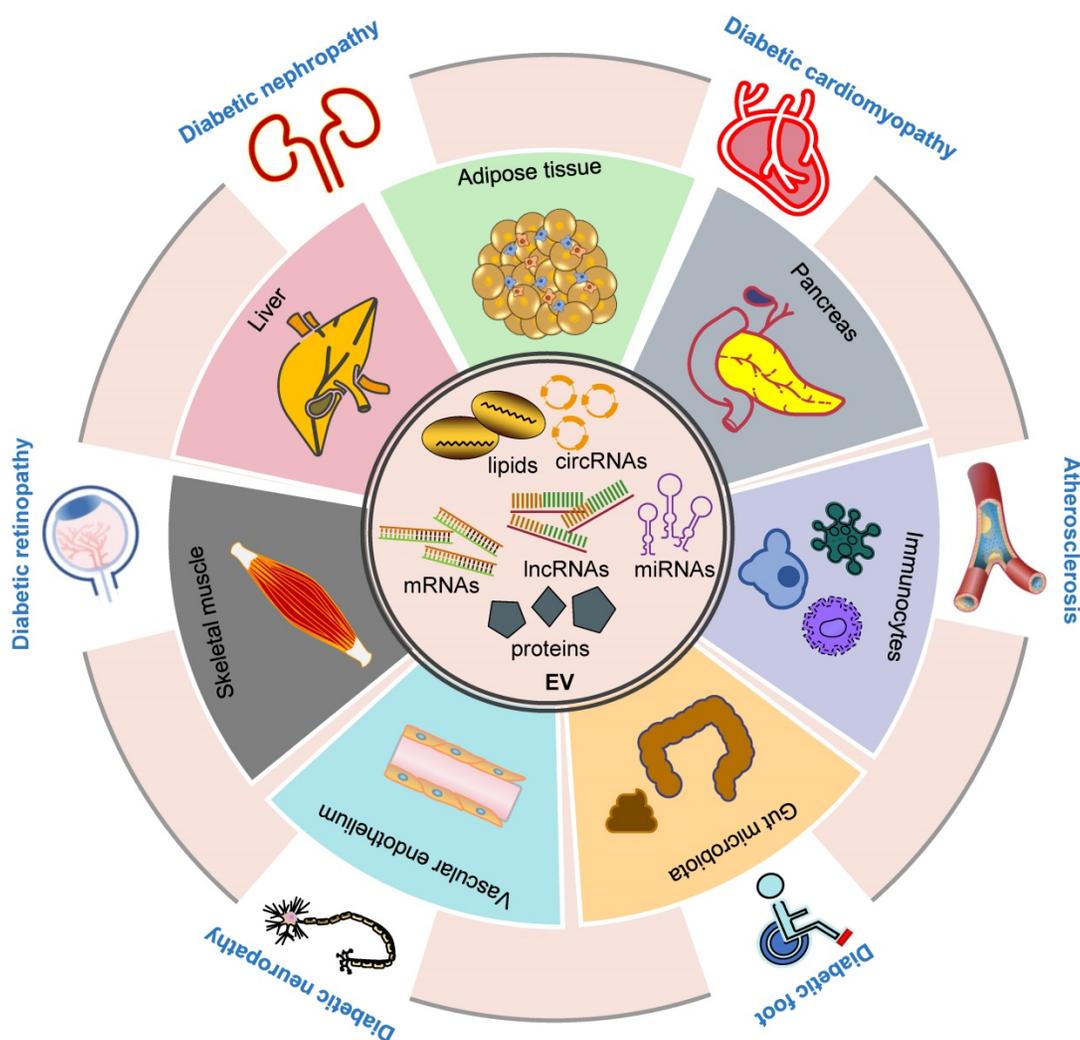


Figure 1. Inter-organ crosstalk mediated by EVs in the pathogenesis of DM and diabetic complications. EVs contain different proteins, RNAs, DNAs and lipids (inner circle). EVs participate in the development of DM and its complications via multiple ways. EVs derived from various tissues, including adipose, 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 (Ca^{2+})-dependent process [64]. A group of Ca^{2+} -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 Ca^{2+} -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.

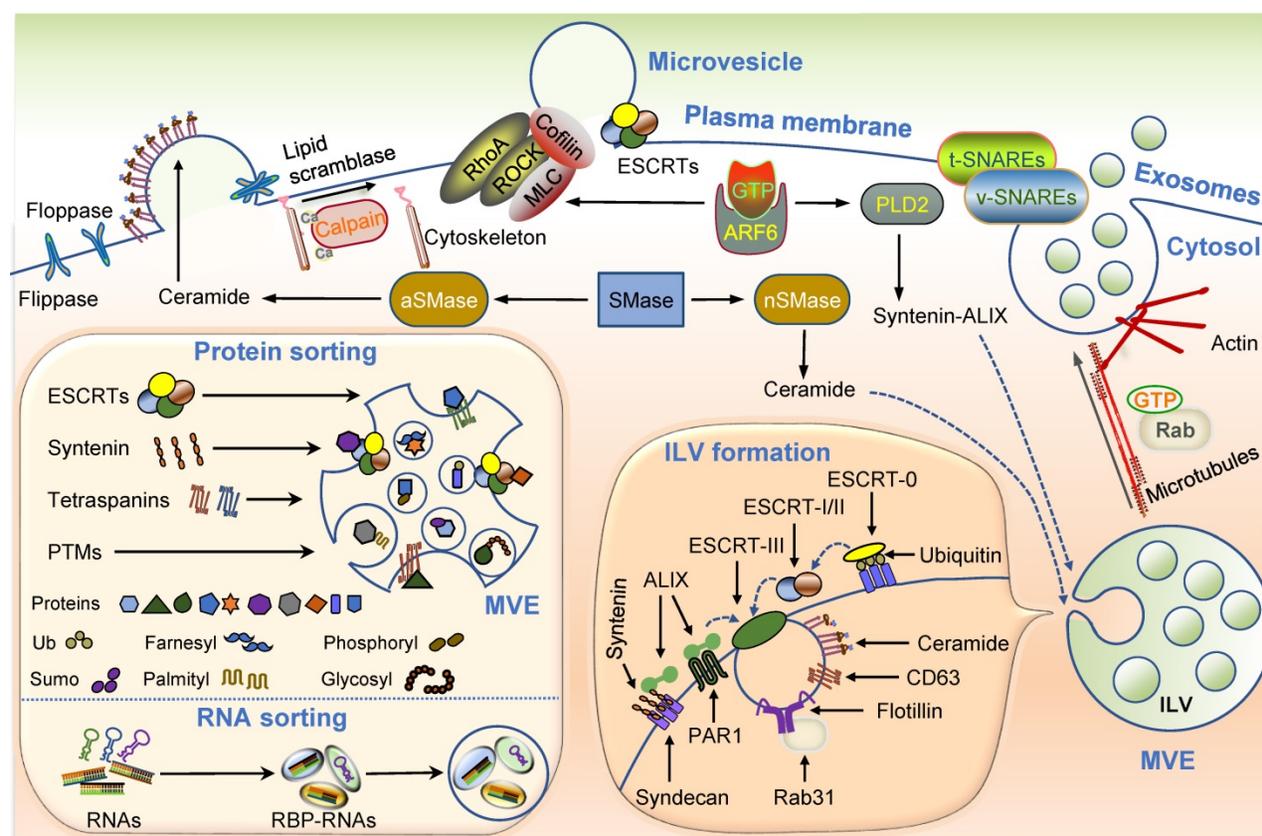


Figure 2. EV biogenesis and cargo sorting. Microvesicles and exosomes are two major categories of EVs. Microvesicles are released directly from plasma membrane budding and shedding. Exosomes are generated by inward budding of endosomes, known as MVEs, which fuse to plasma membrane, and are followed by 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 cargoes. **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-AS1*, *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, hnRNPH1, hnRNPK, hnRNPQ, and hnRNPU, 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 3 β (GSK3 β) 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	↑ [127-129]	AT: T2D patients with FLD, ob/ob mice	Promoting thrombosis and inflammation
	↑ [130,131]	Serum: T2D patients, db/db mice	Promoting endothelial dysfunction
	↑ [132,133]	Plasma, RECs, CD34 ⁺ CACs: T2D patients	Promoting inflammation and CACs migration
	↑ [134]	RPECs: STZ rats	Impairing mitochondrial function
	↑ [135]	Liver and brain: HFD mice	Promoting hepatic IR and neurodegeneration
	↑ [136]	Kidney: GK rats	Promoting ER stress
nSMase	↑ [127-129]	AT: T2D patients with FLD, ob/ob mice	Promoting thrombosis and inflammation
	↑ [135]	Liver, brain: HFD mice	Promoting hepatic IR and neurodegeneration
	↑ [137]	Skeletal muscle: Wistar fatty rats	Promoting IR in the muscle
	↑ [138]	Vastus lateralis muscle: obese IGT patients	UD
	↑ [139,140]	Islet β cells: Akita mice	Promoting β cell apoptosis
Sdc1	↑ [141]	Atrial appendage: obese T2D patients	UD
	↑ [142]	Liver: obese Zucker fa/fa rats	Promoting hepatic IR
	↑ [143-145]	Neutrophils, serum: T2D patients	UD
	↓ [146]	Serum, small intestine: STZ mice	Promoting epithelial barrier damage
	↑ [147,148]	Plasma, serum: T1D DN patients	Promoting inflammation and microalbuminuria
Sdc4	↑ [149,150]	Vitreous fluid: PDR Patients	Promoting angiogenesis
	↓ [151]	Skeletal muscle, heart: HFD ob/ob mice	UD
	↑ [152,153]	Heart, skeletal muscle: STZ rats	Promoting cardiac dysfunction
	↑ [154]	Kidney: KK/Ta mice	UD
	↓ [151]	Skeletal muscle, heart: HFD ob/ob mice	Promoting growth factor resistance
HPSE	↑ [155-158]	Islet: NOD/Lt mice, T1D patients, STZ mice	Promoting β cell death
	↑ [159]	Serum: obese patients with prediabetes	Promoting endothelial injury and inflammation
	↑ [160,161]	Urine, plasma: T2D patients	UD
	↑ [162-167]	Kidney: STZ mice and rats, DN patients	Promoting renal damage, protein excretion
	↑ [150,168,169]	Vitreous fluid, serum, retina: PDR patients, STZ rats	Promoting inflammation, angiogenesis, and subendothelial barrier damage
	↑ [146]	Serum, small intestine: STZ mice	Promoting epithelial barrier damage
Calpain	↑ [170]	Carotid artery: DM patients, STZ rats	Promoting atherosclerosis
	↑ [171]	Heart: STZ rats	Promoting apoptosis
	↑ [172-175]	Heart: HFD, STZ, OVE26 mice	Promoting myocardial hypertrophy, and fibrosis
	↑ [176]	Aortas: STZ and OVE26 mice	Promoting ROS and peroxynitrite production
	↑ [177,178]	Platelet: T2D patients, STZ mice	Promoting platelets hyperaggregability
	↑ [179,180]	Plasma: T2D patients	Promoting platelet activation and inflammation
	↑ [181]	Platelet: T2D patients	Promoting MVs release and inflammation
	↑ [182]	Dorsal root ganglion: STZ rats	Promoting oxidative stress and inflammation
	↑ [183]	Penis: STZ mice	Promoting erectile dysfunction
	↑ [184]	Lens epithelial cells: DR patients	UD
Calpain-1	↑ [185]	Heart: STZ rats	Promoting oxidative stress and apoptosis
	↑ [186,187]	Vascular mesentery: STZ and ZDF rats	Promoting endothelial inflammation
Calpain-10	↑ [188]	Retina: STZ rats, HFD rats	Promoting retinal ganglion cell death
	↑ [189]	Islet: T2D patients	Biomarker for islet dysfunction
	↑ [190]	Kidney: STZ rats, HFD rats	Promoting apoptosis and renal dysfunction
SNARE	↓ [191]	Kidney: STZ rats, ob/ob mice	Promoting apoptosis and renal dysfunction
	^a ↓ [192-195]	Islet: T2D patients, GK rats, ZDF rats	Impairing insulin secretion
	^b ↓ [196]	AT: STZ-NA rats	Promoting IR
	^c ↑ [197]	Skeletal muscle: Zucker rats, STZ rats	Promoting IR
	^d ↓ [198]	Hippocampus: STZ rats	UD
CD63	^e ↑ [199]	Serum: T1D patients	Promoting insulinitis as autoantigen
	↑ [200,201]	Platelets: T2D patients	UD
	↑ [202]	Kidney: DN patients	Promoting renal cell apoptosis
CD82	↑ [203]	Skin: DM patients	Promoting chronic inflammation
HuR	↑ [204-207]	Kidney: DN patients, db/db mice, STZ rats	Promoting pyroptosis, inflammation, and EMT
	↑ [208]	Retina: STZ rats	Promoting angiogenesis
	↑ [209]	BMMØ, heart: db/db mice	Promoting cardiac fibrosis and dysfunction
	↑ [210]	Heart: diabetic cardiomyopathy patients	Promoting pyroptosis and inflammation
hnRNPk	PTM [†] ↑ [211]	Islet: db/db mice	Promoting oxidative stress and apoptosis
	↓ [212]	Kidney: Akita mice	Promoting RAS activation and hypertension

AT: adipose tissue; BMMØ: 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; NOD/Lt mice: nonobese diabetic mice harboring a hybrid rat insulin-promoter/SV40 large T-antigen gene spontaneously develop β-cell adenomas; OVE26: FVB(Cg)-Tg(Ins2-CALM)26OveTg(Cryaa-Tag)1Ove/PneJ transgenic mice; insulin-promoter/SV40 large T-antigen gene spontaneously develop β-cell adenomas; OVE26: FVB(Cg)-Tg(Ins2-CALM)26OveTg(Cryaa-Tag)1Ove/PneJ transgenic mice; PDR: proliferative diabetic retinopathy; PTM: posttranslational modification; RAS: renin-angiotensin system; Sdc1: syndecan 1; Sdc4: syndecan 4; SNARE: soluble N-ethylmaleimide-sensitive fusion attachment protein receptor; STZ+NA: streptozotocin+ nicotinamide; UD: undetermined; ZDF: Zucker fat diabetic. ^a: synaptotagmin, VAMP-2, syntaxin-1A and -2 and SNAP-25; ^b: SNAP23, syntaxin-4 and VAMP-2; ^c: VAMP-2, syntaxin-4; ^d: syntaxin-1; ^e: VAMP2; [†]: phosphorylation.

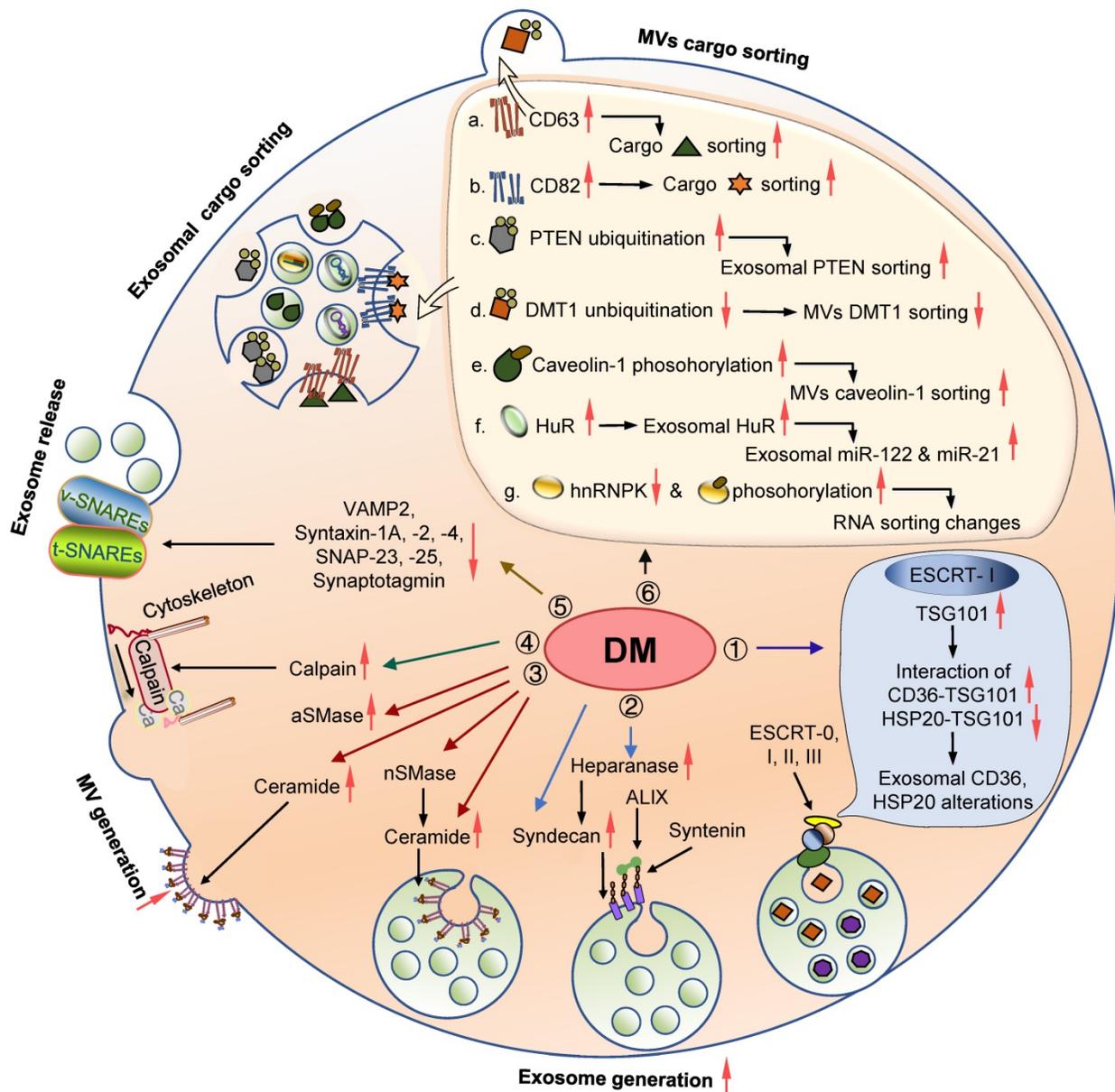


Figure 3. Involvement of the EV biogenesis and cargo sorting machineries in DM and diabetic complications. Diabetic conditions trigger the alteration in the expression and activity of the molecules involved in the process of EV biogenesis and cargo sorting. 1. Lipotoxicity induces TSG101 expression and 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 of 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 I 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 EVs 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-SNAREs: target-membrane SNAREs; v-SNAREs: 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 sphingomyelinase (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 insulinitis 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 modification 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 glucagon-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 decreased 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 insulinitis, suggesting it as a potential autoantigen of T1D [199].

EV cargo sorting

Protein cargo sorting

Proteomic analysis has uncovered that diabetic condition 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, lncRNAs, 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- β 1, 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 glucolipototoxicity, 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 to peripheral tissues, EV-encapsulated *MALAT1* can be transported to pro-opiomelanocortin neurons, increasing appetite and body weight [291]. Additionally, 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, lncRNA *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-1 β , 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 lncRNA *GAS5*, integrin β 1A, and α 5, 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.

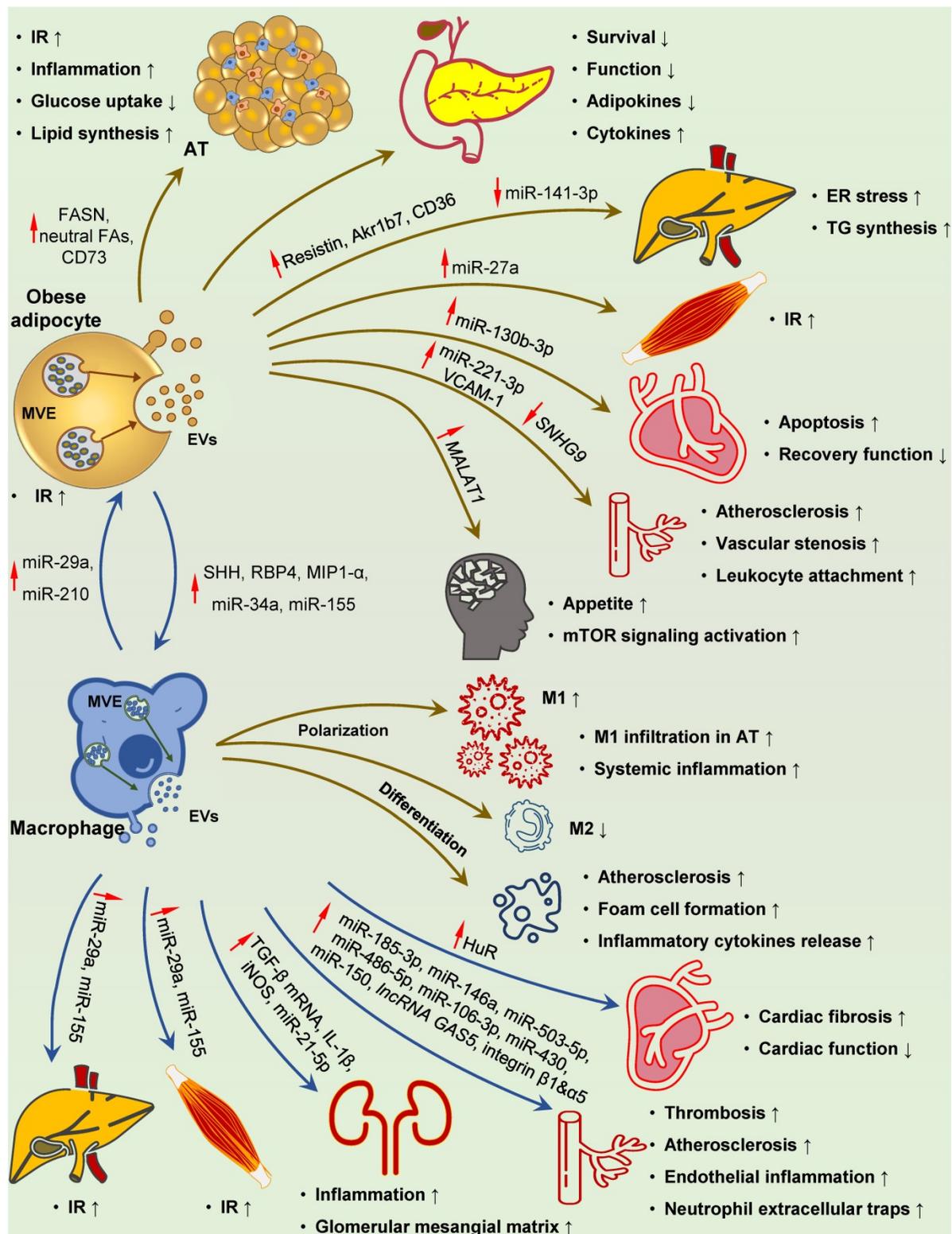


Figure 4. Involvement of adipocyte- and macrophage-derived EVs in DM-related pathological changes. Adipocyte-derived EVs play a distinct role at multiple processes in the development of DM-related pathology. These EVs with specific cargoes (FASN, neutral fatty acids, CD73, resistin, Akrlb7, CD36 and 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-29a 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-503-5p, miR-486-5p, miR-106-3p, miR-430, miR-150, and lncRNA GASS 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 Pgc1 α [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 β 1, 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.

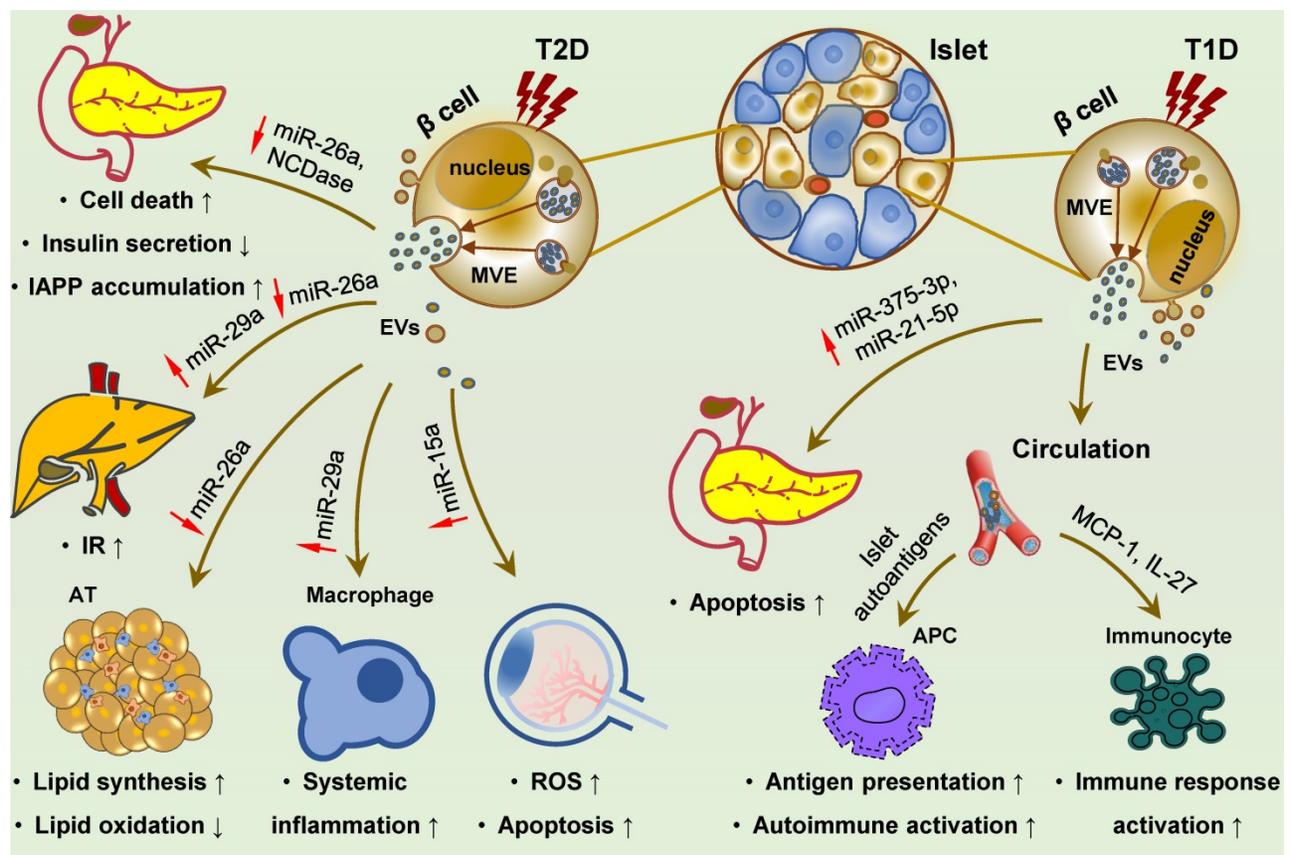


Figure 5. Islet cell-derived EVs promote the development of T1D, T2D and diabetic retinopathy. Islet cell-derived EVs carry various molecular effectors that can trigger multiple signaling cascades, and may regulate the development of T1D, T2D and diabetic complications. In T2D, reduced miR-26a and NCDase 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 islets 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 being 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 membranous 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- β 1

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

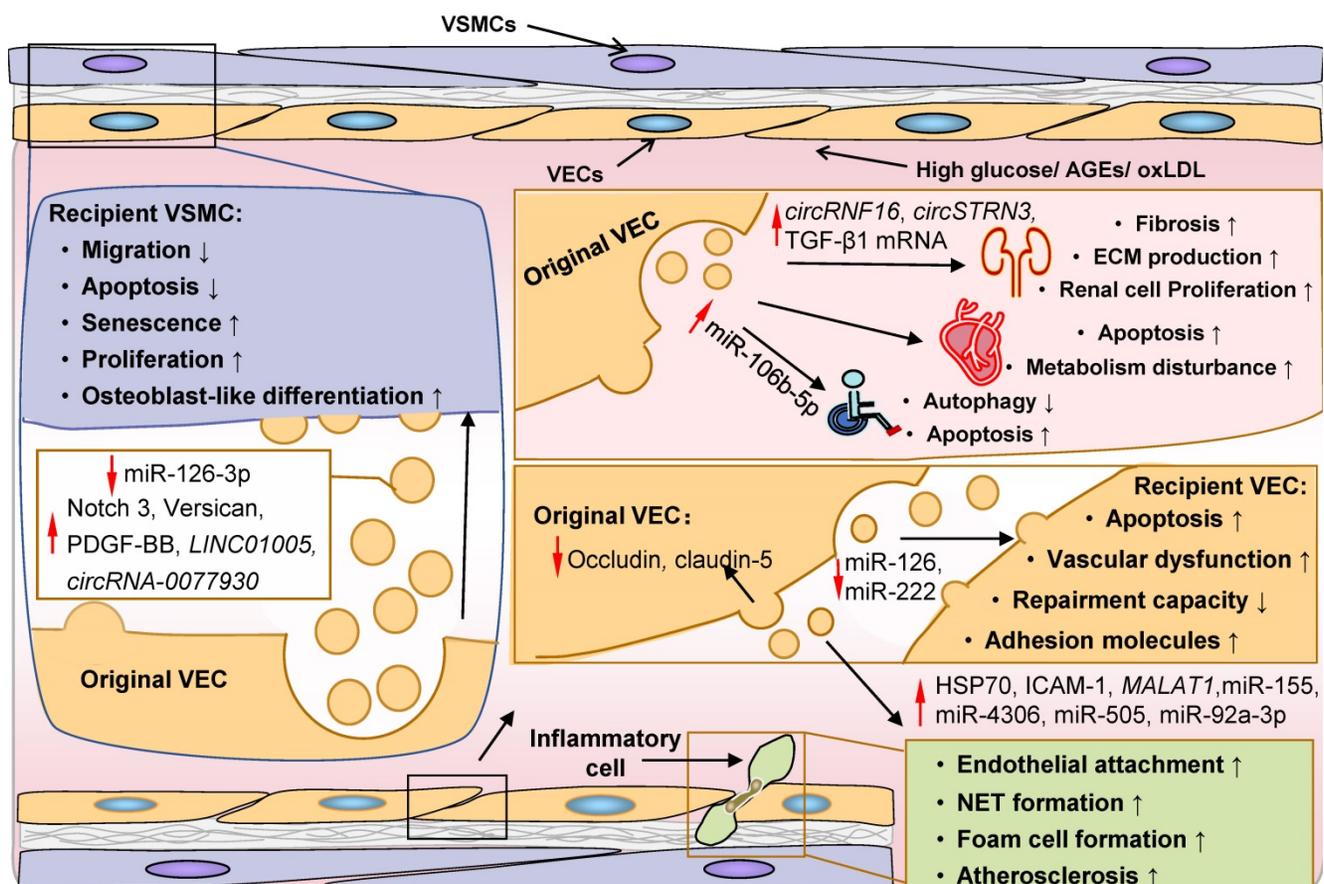


Figure 6. Role of EC-derived EVs in the pathogenesis of diabetic complications. EVs derived from ECs are critically involved in the occurrence and progression of diabetic complications, including endothelial damage and inflammation, vascular sclerosis, diabetic cardiomyopathy, diabetic nephropathy and 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-106-5p 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: oxidated 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 diabetogenic insulinitis [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 pigment 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 hypoesthesia 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.

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

RNAs	Types [Reference]	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	-	-	-
	T2D-C [442]	Plasma	57/101	0.744	-	-	-
	T2DN [438]	Urine	15/14	0.830	-	-	0.673-0.986
miR-23a	T2D [434]	Plasma	36/42	0.828	-	-	0.735-0.920
miR-29c-5p	T2DN [444]	Urine	15/28	0.774	-	-	-
miR-30a-5p	T2D-ESRD [436]	Urine	80/40	0.912	-	-	-
miR-30a	T2DN [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
	T2DN [441]	Urine	44/136	0.917	93.3	86.7	0.874-0.96
miR-133b	T2DN [443]	Urine	56/110	0.867	86.4	72.7	0.820-0.914
miR-146a-5p	T2D [442]	Plasma	60/57	0.911	-	-	-
	T2D-C [442]	Plasma	57/101	0.673	-	-	-
miR-156	T2DN [441]	Urine	44/136	0.883	97.8	82.2	0.824-0.942
miR-156-5p	T2DN [444]	Urine	15/28	0.818	-	-	-
miR-192	T2D [434]	Plasma	36/42	0.717	-	-	0.607-0.828
	MIC [440]	Urine	30/30	0.802	-	-	0.696-0.907
miR-194	MIC [440]	Urine	30/30	0.703	-	-	0.581-0.826
miR-215	MIC [440]	Urine	30/30	0.757	-	-	0.545-0.869
miR-218	T1D [437]	Urine	30/30	0.817	-	-	-
miR-342	T2DN [443]	Urine	56/110	0.910	81.8	80.9	0.873-0.948
miR-424	T1D [437]	Urine	30/30	0.803	-	-	-
miR-636	T2DN [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_0000907	DFU [445]	Serum	20/20	0.878	80	80.85
circ_0057362	DFU [445]	Serum	20/20	0.848	86.005	70.22	-
Ace	Overt DN [448]	Plasma	100/37	0.75	73	72	0.66-0.83
	Incipient DN [448]	Plasma	37/66	0.62	65.2	61	0.54-0.71
Aebp1	T2DN [446]	Plasma	15/15	0.742	53.3	86.7	-
Ccl21	T2DN [447]	Urine	15/28	0.888	-	-	0.737-0.997
Umod	T2DN [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	0.83	67.6	93	0.74-0.92
	DN [449]	Urine	10/10	0.705	-	-	-

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.

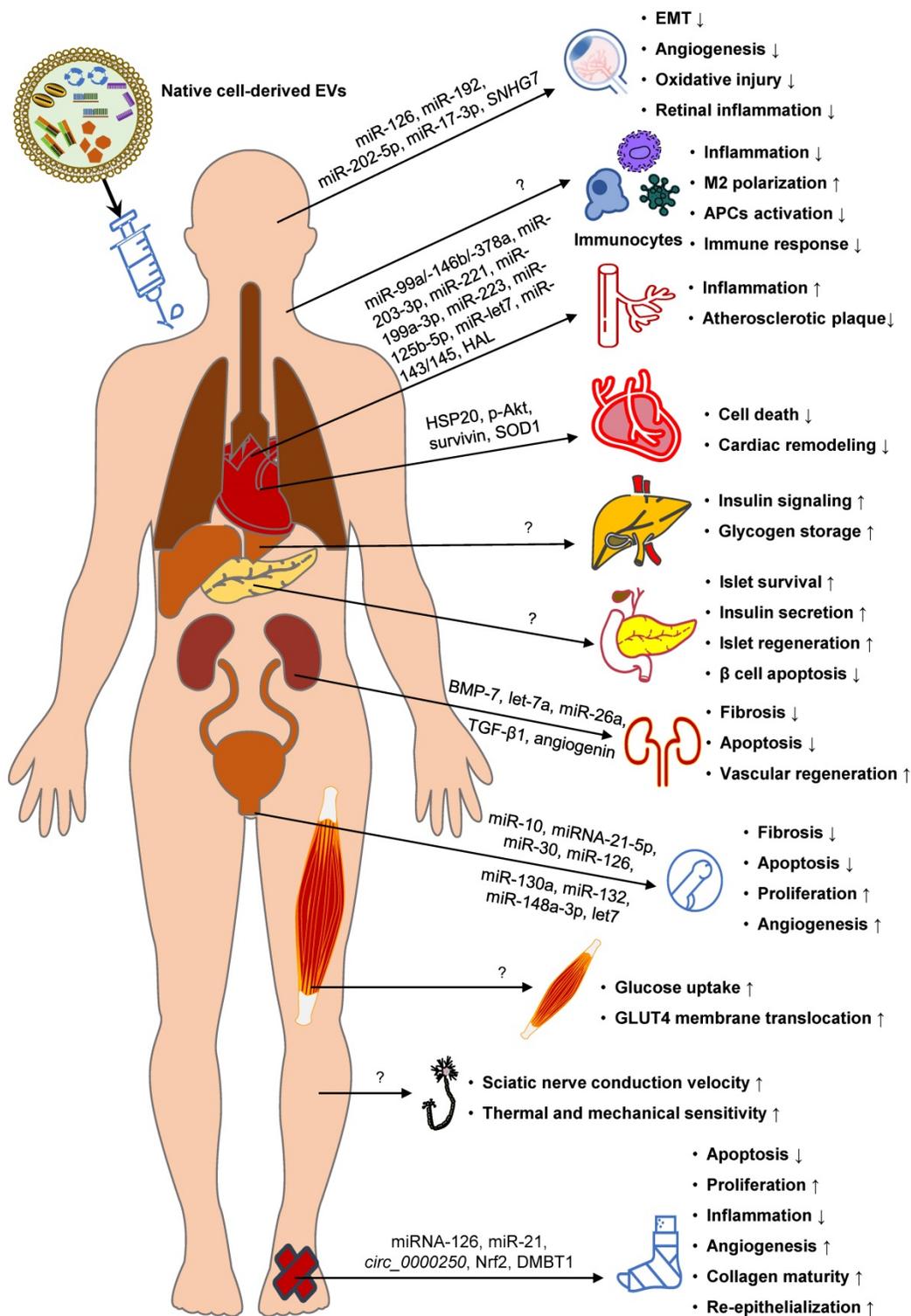


Figure 7. Potential clinical applications of native cell-derived EVs in treating DM and its complications. EVs of native cells (such as pancreatic pathfinder cells, adipocytes, stem cells, retinal pigment epithelial cells, keratinocytes, endothelial progenitor cells, amniotic epithelial cells, endothelial 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- β 1, 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 siFas 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; ESCRT: 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; GSK3 β : glycogen synthase kinase 3 β ; HG: high glucose; HFD: high-fat diet; HuR: human antigen R; IAPP: islet amyloid polypeptide; ILVs: intraluminal vesicles; IR: insulin resistance; lncRNAs: long noncoding RNAs; MVs: microvesicles; MVEs: multivesicular endosomes; ncRNAs: non-coding RNAs; NETosis: neutrophil extracellular traps formation; nSMase: neutral sphingomyelinase; oxLDL: oxidated 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; SNARE: 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.

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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.

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