Acta Pharmacologica Sinica (2014) 35: 433–443

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Review

Microvesicles and diabetic complications — novel mediators, potential biomarkers and therapeutic targets

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Microvesicles (MVs), also known as microparticles, are small membrane vesicles released from different cell types under different conditions. MVs have been detected in the circulation and in organs/tissues in various diseases, including diabetes. Patients with different types of diabetes and complications have different cellular MV patterns. Studies have shown that MVs may mediate vascular thrombosis, vascular inflammation, angiogenesis, and other pathological processes of the disease through their procoagulant, pro-inflammatory, pro-angiogenic, proteolytic, and other properties. Therefore, MVs contribute to the development of diabetic macrovascular and microvascular complications. In addition, clinical studies have indicated that changes in MV number and composition may reflect the pathophysiological conditions of disease, and therefore, may serve as potential biomarkers for diagnostic and prognostic use. Understanding MVs' involvement in the pathophysiological conditions may provide insight into disease mechanisms and would also be helpful for the development of novel therapeutic strategies in the future. Here, we review the latest publications from our group and other groups and focus on the involvement of MVs in diabetic complications.

Keywords: microvesicles; microparticles; diabetes; thrombosis; vascular inflammation; endothelial dysfunction; angiogenesis; diabetic nephropathy; diabetic retinopathy; cardiovascular disease

Acta Pharmacologica Sinica (2014) 35: 433–443; doi: 10.1038/aps.2013.188; published online 10 Mar 2014

Introduction

Microvesicles (MVs, also known as microparticles, 0.1 to 1 μm in diameter) bud directly from the cell membrane surface, resulting in widely heterogeneous vesicle morphology[1, 2]. Patients with both T1DM (type 1 diabetes mellitus) and T2DM (type 2 diabetes mellitus) and diabetic complications have different cellular MV patterns. Recent studies have shown that MVs may mediate vascular thrombosis, vascular inflammation, angiogenesis, plaque proteolytic, and other pathological processes, and contribute to the development of diabetic vascular complications. Moreover, MVs might also be a novel therapeutic target in diabetic complications. In this regard, MVs are believed to serve as potential biomarkers for the diagnosis and prognosis of related vascular complications and the assessment of treatment response. Here, we review recent advances in MV-based research on diabetic complications.

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Received 2013-09-10 Accepted 2013-12-12

General characteristics of MVs

In mammals, MVs are released from almost all cell types, including blood cells (such as platelets, erythrocytes, leukocytes, and monocytes/macrophages), vascular endothelial cells, and smooth muscle cells, as well as the cells of other tissues/organs under both physiological and pathological conditions. Therefore, MVs can be found in blood, urine, vitreous fluid, atherosclerotic plaques of the vascular wall, and extracellular spaces of solid organs[3, 4].

MVs are released during cell activation or apoptosis[2, 5]. Many chemical and physical stimuli, such as cytokines, unesterified cholesterol[6], thrombin, endotoxins, tobacco smoke extract[7], hypoxia, and shear stress[8] have been reported to trigger cellular MV release in studies from our group and other groups. All of the above-mentioned stimuli and factors are potentially involved in the development of diabetic vascular complications. Accordingly, circulating MVs are increased in various diseases, including diabetes, diabetic

complications, cardiovascular diseases, and abdominal aortic aneurysm[2, 5].

Heterogeneity is an important feature of MVs. The same cells treated with different stimuli release MVs carrying different components due to different responses to the stimuli. In contrast, different cell types treated by the same stimulus will release MVs carrying different components due to the intrinsic dissimilarity of the different cell types. Studies with liquid chromatography coupled to tandem mass spectrometry have characterized the human plasma MV proteome[9] and showed that the cellular composition of plasma MVs is altered in many diseases, including acute coronary syndrome, diabetes mellitus, sepsis, and sickle cell disease[9].

Recent studies showed that the levels of $CD31^+/CD41a^+$, annexin V^+, and $CD105^+$, and the ratio of $CD31^+/CD41a^+$ Dim to Bright expressed by MVs are significantly higher in diabetes patients than those in normal healthy controls[10]. T1DM and T2DM patients have circulating MVs with different cellular origins. Sabatier *et al* indicated that blood levels of platelet-derived MVs, endothelial-derived MVs, and total annexin V^+ MVs are significantly increased in T1DM, whereas the increases are not statistically significant in T2DM patients, except for total MVs when compared with age-matched control subjects[11]. $CD36^+$-MV levels are significantly higher in obese people with T2DM than those in lean and obese controls without T2DM, and the MVs in T2DM patients are primarily derived from erythrocytes[12]. In contrast, the main source of $CD36^+$-MVs in non-T2DM individuals is endothelial cells[12]. In addition, the blood levels of platelet-derived MVs and monocyte-derived MVs have been associated with diabetic microvascular damage[13–16]. However, elevated blood levels of endothelial-derived MVs have been suggested to predict the presence of coronary atherosclerotic lesions[17, 18].

On the surface, MVs carry membrane lipids and transmembrane proteins, including the anionic phospholipid phosphatidylserine (PS) and surface-specific membrane antigens, which are highly specific and reflect the membranous elements of the cell of origin. For instance, platelet-derived MVs expose platelet membrane glycoprotein Ib (GPIb; CD42b), platelet-endothelium adhesion molecule-1 (PECAM-1; CD31), P-selectin (CD62P), CD36[19], and the integrin αIIbβ3 (GPIIb-IIIa)[20]. The monocyte-derived MVs expose CD14, while the endothelial-derived MVs expose CD31, CD34, CD51, CD62E, CD144, and CD146[17, 21]. Thus, an MV's cellular origin can be determined by flow cytometry using antibodies against cell surface-specific markers. In addition, apoptotic membrane vesicles may also carry oxidatively modified membrane lipids that are generated by the release of reactive oxygen species (ROS) from damaged mitochondria[22].

Inside MVs, various intracellular organelle remnants[23] as well as DNA[24], RNA fragments, and microRNAs[25] can be found. Recent studies demonstrated that MVs represent protective transport vehicles for microRNAs that are specifically packaged by their parental cells[26]. As potential diagnostic and prognostic biomarkers, standardized methodologies for measuring MVs in human samples are still not available. However, several methods for MVs detection are commonly used, such as flow cytometry, the enzyme-linked immunosorbent assay, the thrombin generation test, and the procoagulant phospholipid-dependent clotting time assay[27, 28]. Flow cytometry is the primary method widely used to detect MVs. The advantage of this method is the ability to measure multiple markers and quantify MVs and their parental cells simultaneously. The major problem with this method is the limitation in detecting small MVs. However, fluorescent staining of MVs could be helpful for distinguishing signals from noise. In addition, a recent study indicated that high sensitivity flow cytometry can detect 8- to 20-fold increases in MVs counts compared with standard flow cytometry when applied to coronary patient samples by improving forward scatter resolution and lowering background noise[29].

Studies indicate that pre-analytical phases, including blood collecting and handling, plasma preparation and storage conditions, also affect the quantification of circulating MVs[30]. Increasing the time between venepuncture and centrifugation and a single freeze-thaw cycle of samples lead to increased levels of MVs[31]. Washing samples, double centrifugation of MVs prior to freezing, and long-term storage of MV samples at -80°C result in decreased MV blood levels[31]. The three major parameters are the delay before the first centrifugation, agitation of the tubes during transportation and the centrifugation protocol[27]. Therefore, standardization is essential for the development of technologies for MV studies.

Potential pathogenic effects of MVs in diabetic complications

Increased levels of circulating MVs from platelet, monocyte, lymphocyte, granulocyte, and endothelial cells have been reported in patients with T1DM, T2DM[32], diabetic retinopathy[33], diabetic nephropathy, cardiovascular disease, cerebral vascular ischemia[34], and diabetic foot disease[35]. These MVs may have pathogenic effects on vascular thrombosis, vascular inflammation, and angiogenesis, which are essential in the development of diabetic complications.

MVs and thrombosis

MVs were first described as 'platelet dust' with procoagulant properties in 1967[36]. The procoagulant effects of MVs are mainly due to the exposure of negatively charged PS[37] and tissue factor (TF)[2, 6, 7, 37, 38], which are involved in the coagulation cascade. The negatively charged membrane lipid PS enhances clot propagation. TF is a transmembrane molecule, which has been identified on monocyte-derived MVs and endothelial-derived MVs[38]. TF-containing MVs in the blood stream may bind to activated platelets through interaction between P-selectin glycoprotein ligand-1 (PSGL-1, on the MVs) and P-selectin (on the platelet surface)[39]. *In vivo* studies, conducting with mouse or monkey under intravital microscopy, have shown that the TF^+ MVs are incorporated into the thrombus formation[40]. The appearance of P-selectin[20], PSGL-1, glycoprotein (GP) IIb/IIIa[41], protein disulfide isomerase[42], and factor VIII and factor Va[43] receptors on MVs may also

be involved in thrombosis. The platelet-derived MVs expose more binding sites for factors Va, VIIIa, and IXa per unit of membrane surface area than activated platelets[44]. However, the lack of an association between the increased phospholipid composition of circulating MVs and coagulation has also been reported[45].

Studies have shown that levels of procoagulant MVs are increased in patients with diabetes and diabetic complications. Cimmino *et al* found that highly procoagulant TF-bearing MVs are increased in diabetes, which coincides with strongly elevated levels of coagulation activation markers[46]. Procoagulant platelet-derived MVs[47, 48], monocyte-derived MVs[14], and endothelial-derived MVs[49] are significantly increased in patients with diabetes, even in well-controlled DM without complications[50] and newly diagnosed T2DM[51]. In addition, patients with diabetes accompanied by hypertension[15, 16, 32, 52–57], hyperlipidemia[58–60], stable coronary disease[61], angina with[62] or without[17] symptomatic episodes, myocardial infarction[49, 63], diabetic retinopathy, and nephropathy[33, 64] have significantly increased levels of procoagulant MVs compared with those without diabetic complications. Therefore, elevated levels of MVs may be associated with the increased risk of thrombo-embolic diabetic complications.

It has been reported that procoagulant MVs can initiate and propagate coagulation in diabetes both *in vitro* and *ex vivo*[35, 46, 63, 65, 66]. Because thrombin is able to activate platelets, the activated platelets release platelet-derived MVs that lead to further thrombin formation. This positive feedback loop may explain the novel mechanisms of hypercoagulability in diabetes[67]. Sabatier *et al*[11] reported that the procoagulant potential of MVs is increased and correlated with the degree of glycemic control in T1DM. In contrast, although total MVs increase in T2DM, there is no associated increase in their procoagulant potential[11]. An *in vivo* study showed that artificially induced high levels of insulin can cause increased monocyte TF expression in healthy volunteers[68], suggesting the pro-thrombotic effect of hyperinsulinemia. Administration of antiplatelet drugs can significantly decrease circulating MVs and activated platelets in patients with diabetes[47, 59, 69–72].

MVs and vascular inflammation

Leukocyte-endothelial interaction and subsequent transendothelial migration of leukocytes are the early stage events in the development of atherosclerosis[73]. *In vitro* experiments have demonstrated that MVs derived from apoptotic endothelial cells or activated platelets act as cellular effectors, disseminating pro-inflammatory and pro-adhesive potentials in the vasculature[64, 74]. The activated platelets and the platelet-derived MVs isolated from T2DM patients promote the interaction between endothelial cells and monocytes[74]. Terrisse *et al* found that MVs promote the formation of platelet strings at the surface of human umbilical vein endothelial cells (HUVECs) *in vitro*, and the MVs are internalized into the HUVECs[64]. This uptake induces the production of ROS, which is essential for von Willebrand factor expression on the endothelial cell surface and subsequent interaction between platelets and endothelial cells[64]. We recently reported that the unesterified cholesterol-induced MVs (UCMVs) from human monocytes robustly enhanced leukocyte recruitment to microvascular endothelium *in vivo*, aortic endothelium *ex vivo*, and cultured human endothelial monolayers *in vitro*[22]. The malondialdehyde-like peroxidized epitopes on the UCMVs surface mediate monocyte recruitment to the endothelial cells through lectin-like oxidized low-density lipoprotein receptor-1 (LOX1) expressed on endothelial cells[22]. These studies indicate the potential importance of MVs in endothelial activation, leukocyte recruitment, and vascular inflammation, which may contribute to vascular diseases and diabetic cardiovascular complications.

MVs and endothelial dysfunction

Studies in diabetic patients indicate that MVs can affect endothelial function. Two consecutive fat-rich mixed meals to healthy young males[75] or two consecutive mixed meals to T2DM patients[76] yield similar results in which impaired endothelium-dependent vasodilatation is associated with elevated levels of circulating platelet-derived MVs and endothelial-derived MVs. Increased level of $CD31^+$/annexin V^+ MVs is positively correlated with the impairment of coronary endothelial function in patients with coronary artery disease (CAD)[62]. T2DM patients show decreased endothelium-dependent flow-mediated dilation (FMD) and increased brachial ankle pulse wave velocity (baPWV). Moreover, FMD is found to be negatively correlated with $CD31^+/CD42^-$ endothelial-derived MVs and $CD51^+$ endothelial-derived MVs, and baPWV is positively correlated with these two MV types[50].

In vitro and *ex vivo* experiments have shown that MVs might cause endothelial dysfunction by decreasing both nitric oxide (NO) and prostacyclin production in endothelial cells[73]. Coincubation of endothelial-derived MVs with aortic rings and cultured endothelial cells causes increased superoxide production, decreased NO production, and impairment of acetylcholine-induced vasorelaxation[77]. The T lymphocyte-derived MVs from diabetic patients impair shear stress-induced dilatation of mouse small mesenteric arteries by affecting NO and prostacyclin production[78]. In addition, a recent study reported that endothelial-derived MVs exposed to high glucose could significantly impair endothelial function and increase macrophage infiltration and adhesion molecule expression due to increased nicotinamide adenine dinucleotide phosphate oxidase (NOX) activity and higher ROS levels[79].

Due to the central importance of endothelial cells in vascular thrombotic and inflammatory conditions, using MVs as a biomarker of endothelial dysfunction is promising but still under investigation. The MVs from endothelial cell-based methodologies can be used to study vascular inflammation in diabetic vascular complications[2, 80].

MVs and angiogenic effects

In addition to coagulation and vascular inflammation, numerous studies indicate that MVs are also involved in angiogen-

esis. Diamant *et al* reported that high levels of TF^+ MVs in diabetic patients might be involved in transcellular signaling or angiogenic processes other than the classical procoagulant function of TF^+ MVs shown before[81]. Tsimerman *et al* found that incubation of HUVECs with MVs from patients with severe diabetic foot ulcers could induce the formation of stable and branched networks[35], suggesting the key role of MVs in angiogenesis and the skin wound healing process in diabetes. Similarly, incubation of HUVECs with MVs from diabetic retinopathy patients merely induces the formation of unstable tube networks, therefore partly reflecting the mechanisms of proliferative diabetic retinopathy[35]. Ettelaie *et al* showed that the TF-containing MVs released from advanced glycation end products (AGE) or glucose-treated renal mesangial cells induce capillary formation with human dermal microvascular endothelial cells *in vitro*[82].

In contrast to the pro-angiogenic effects discussed above, the anti-angiogenic role of MVs has also been reported. Taraboletti *et al* demonstrated that although the low concentrations of MVs isolated from HUVECs promote angiogenesis *in vitro*, the high concentrations of endothelial-derived MVs suppress angiogenesis[83]. In accordance with these results, Mezentsev and colleagues reported that isolated endothelial-derived MVs in pathophysiological concentrations impair angiogenesis *in vitro* by affecting all parameters of capillary-like network formation[84]. In addition, the MVs isolated from T2DM patients with CAD fail to induce angiogenesis, which may partially explain the lack of vascular regeneration characterization in T2DM with CAD[35].

As discussed above, MVs bear both pro- and anti-angiogenic features and thereby influence angiogenic activities. The underlying mechanisms, however, are not fully understood. It has been suggested that growth factors and cytokines derived from MVs are involved in endothelial angiogenesis[35]. Moreover, tissue factors on MVs may regulate vascular proliferation and angiogenesis[82]. Other studies also demonstrated that platelet-derived MVs induce angiogenesis *in vitro* by increasing cell proliferation and decreasing apoptosis, as mediated by the MVs' lipid components, notably sphingosine 1-phosphates[85]. In addition, matrix metal proteinase (MMP) is involved in the early stage of angiogenesis and destructs the extracellular matrix. We recently reported that exposure of human macrophages to tobacco smoke induces the release of proteolytic $MMP\text{-}14^+$ MVs through the activation of JNK and p38 MAPK[86]. Moreover, several microRNAs (miRs) have been identified as critical regulators of vascular development and angiogenesis; these miRs are called angiomiRs[87]. Li *et al* reported that monocyte-derived MVs have strong pro-angiogenic activity *in vitro* and *in vivo* due to monocyte-secreted miR-150, which is increased in ob/ob diabetic mice[88]. Mocharla *et al* reported that miR-126 levels in MVs/exosomes of the $CD34^+$ peripheral blood mononuclear cell (PBMC) subset are substantially higher compared with those of $CD34^-$ PBMC subsets[89]. Reduced miR-126 levels of $CD34^+$ PBMCs have been reported in T2DM and high-glucose conditions and therefore are associated with impaired pro-angiogenic properties[89]. Similarly, Jansen *et al* showed MVs derived from glucose-treated endothelial cells and diabetic patients contain significantly lower amounts of miR-126, which is involved in endothelial cell migration and proliferation *in vitro* and re-endothelialization *in vivo* via sprout-related EVH-1 domain-containing protein 1 (SPRED1)[90].

Therefore, MVs can transfer biological messages among cells with specific characteristics related to the type of vascular complications. Indeed, MVs act as key information vectors between elevated glucose and the development of diabetic vasculopathy in order to maintain cell homeostasis or favor cell repair and angiogenesis.

MVs as carriers of RNAs (mRNA and miRNA)

With the development of epigenetics, MVs have been drawing increasing attention due to their inclusion of specific miRNAs and RNAs derived from their parental cells. Several miRNAs are present in MVs involved in CAD and diabetes, such as miR-126, miR-21, and miR-29[91, 92]. The miRNA profiles of MVs differ significantly between patients with stable and unstable CAD and between stimulated and non-stimulated cultured cells *in vitro*[26]. MVs isolated from patients with atherosclerosis contain higher levels of miR-150 and can promote human microvascular endothelial cell-1 (HMEC-1) cell migration, compared with MVs from healthy controls[93]. These findings suggest selective transport of miRNA into MVs under certain pathophysiological conditions[26]. There is increasing evidence for the potential use of specific endothelial-derived MV-associated miRNA signatures in body fluids or particularly in peripheral blood as biomarkers to predict metabolic diseases[94].

In *in vitro* studies, several reports have demonstrated that miRNAs can be transferred to target cells by MVs. Sahler *et al* have used lentiviral technology to transfer transcription factors from platelet and megakaryocyte MVs to target cells[95], which affect the amount of mRNA that can be transcribed. A recent study showed that MVs released from large adipocytes stimulate lipid storage in small adipocytes by mediating the horizontal transfer of lipogenic information, which is encoded by the relevant (micro) RNA and glycosylphosphatidylinositol-anchored protein species[96].

These results demonstrate that MVs can deliver miRNAs into recipient cells, where the exogenous miRNAs can regulate target gene expression and the functions of the recipient cells. Instead of one single type of message, MVs may manage to deliver multiple messengers, including mRNA, specific subsets of the transcripts, miRNAs, and proteins, at one time. MVs may regulate the expression of functionally related genes and the activity of complex, hierarchical signaling and metabolic pathways between neighboring cells in a concerted and coordinated fashion[96].

MVs in diabetic macrovascular complications

Studies with human subjects indicate that circulating MVs, such as platelet-derived MVs[97], $CD31^+$/annexin V^+ endothelial-derived MVs[61, 62, 98], $CD31^+/CD42b^-$ endothelial-

derived MVs[98], and CD144$^+$ endothelial-derived MVs[17, 99], are increased in T2DM patients with macroangiopathy, including coronary, peripheral and cerebrovascular diseases. A recent study reported significantly higher levels of monomeric C-reactive protein (CRP)-positive MVs in patients with myocardial infarction compared with healthy controls[80]. In addition, elevated levels of circulating MVs have been associated with cardiovascular risk factors, including hypertension[15, 32, 52, 100], obesity[101, 102], cholesterol, and dyslipidemia[49, 103], as well as exposure to tobacco smoke[7] or air pollution[104, 105]. An *in vivo* study also demonstrated that the levels of circulating endothelial-derived MVs and endothelial progenitor cell (EPC)-derived MVs are increased in diabetic animal models with ischemic stroke[106].

Regression analysis demonstrated that the levels of CD31$^+$/annexin V$^+$ endothelial-derived MVs are independently associated with macroangiopathy in diabetic patients[98]. CD31$^+$/annexin V$^+$ endothelial-derived MVs have been identified as an independent predictor of cardiovascular events in patients with stable CAD and may be useful for risk stratification[61]. Bernard *et al* reported an association between circulating CD144$^+$ endothelial-derived MVs and unstable coronary plaques in T2DM patients[99]. In addition, compared with traditional cardiovascular biomarkers, Koga *et al* showed that elevated levels of CD144$^+$ MVs are the most significant risk factor for CAD relative to lipid levels, CRP, duration of diabetes, and presence of hypertension, especially in diabetic patients without typical angina symptoms[17]. Another study compared MVs' diagnostic value with traditional cardiovascular biomarkers in healthy Japanese people and concluded that platelet-derived MVs are more closely correlated with metabolic syndrome than CRP[107]. Curtis *et al* showed that the ratio of PS$^+$ MVs to CD34$^+$ progenitor cells is more informative than many standard individual biomarkers commonly used to stratify individuals at heightened cardiovascular risk[108]. Therefore, the elevated MVs may be potential biomarkers for endothelial dysfunction and cardiovascular risk.

MVs' pathological mechanism in macroangiopathy is still unclear. Chen *et al* reported that MVs isolated from *db/db* diabetic mice could induce the impairment of EPC function *in vitro* and reduce the vascular reparative ability of EPCs *ex vivo*[106]. The *in vitro* experiment demonstrated that MVs are capable of converting pentameric CRP into pro-inflammatory monomeric CRP[80]. MVs containing CRP monomers are able to bind to the surface of endothelial cells and generate pro-inflammatory signals *in vitro*, suggesting a potential role of MVs in transport and delivery of pro-inflammatory CRP monomers in vascular disease[80]. Jansen *et al* showed that the decreased miR-126 in MVs may be responsible for the reduced endothelial repair in patients with CAD and diabetes[90]. We recently reported that cholesterol enrichment of human monocytes/macrophages induces the generation of TF$^+$ MVs with high procoagulant activity, which may contribute to prothrombotic conditions and atherothrombosis in hypercholesterolemia[6]. In addition, exposure to tobacco smoke substantially increases cardiovascular risk. Our recent study showed that tobacco smoke exposure increases the release of procoagulant TF$^+$ MVs[7]. Our latest publication further showed that tobacco smoke exposure in human macrophages induces the generation of MMP-14$^+$ MVs with strong proteolytic activities; these MVs degrade native collagen, a major component of the arterial wall matrix, suggesting their potential contribution to atherosclerotic plaque instability in smokers with cardiovascular disease[86]. This study offers important insights and implications regarding tobacco smoke-induced damage to the extracellular matrix of the vascular wall and other tissues[109].

Moreover, measurement of MV levels and the characterization of MVs' cellular origin might also be helpful in assessing responses to treatment (Table 1). Several drugs, such as statins, anti-platelet agents, anti-oxidants, angiotensin II receptor blockers, and calcium-channel blockers, have been reported to attenuate both MV numbers and/or procoagulant factors[15, 16, 41, 49, 54, 56, 58–60, 72, 103, 110–113]. These observations suggest that the beneficial effects of these drugs might be partially due to their effects on MV attenuation. Several recent studies have reported that the cardiovascular benefits of anti-hyperglycemic treatment in T2DM patients with glibenclamide[114], acarbose[115], miglitol[116], and glyclazide[117] might be at least partially attributed to the medications' anti-atherothrombotic effects through the reduction of procoagulant MVs and platelet-activating factors. Although recent evidence showed that insulin may reduce TF expression in monocytes and monocyte-derived MVs *in vitro*[118], an *in vivo* study showed that high levels of insulin can cause increased monocyte TF expression in healthy volunteers[68], suggesting the pro-thrombotic effect of hyperinsulinemia. Pioglitazone treatment improves the imbalance between endothelial damage and repair capacity by both increasing the level of CD34$^+$/KDR$^+$ EPCs and decreasing the level of circulating endothelial-derived CD31$^+$ MVs and the endothelial-derived MVs/EPCs ratio[51]. Interestingly, the endothelial-derived MVs, platelet-derived MVs, and monocyte-derived MVs are successively reduced after bariatric surgery in T2DM patients in parallel with glycemic control normalization[119]. This result reflects an attenuation of the inflammatory response after weight loss, and this mechanism might contribute to glycemic control normalization[119].

Therefore, elevated levels of circulating MVs may be an indicator and a useful risk stratification tool for diabetic macrovascular complications. Circulating MVs may be potential pathogenic factors that impair endothelial cells and atherosclerotic plaque instability. MVs might also be novel therapeutic targets or biomarkers to monitor the therapeutic response to treatments in diabetic macrovascular complications.

MVs in diabetic microvascular complications

MVs and diabetic retinopathy

Ogata *et al* observed that the levels of circulating platelet-derived MVs and monocyte-derived MVs gradually increase with the progression of diabetic retinopathy, from the non-proliferative stage to the proliferative stage, and are significantly higher in diabetic retinopathy with areas of capillary

Table 1. Effects of therapeutic medicine on circulating microvesicles in diabetes.

Pharmacological effect	Medicine	Patients/materials	Microvesicles type	Therapeutic effects on microvesicles	References
Antiplatelet	Cilostazol	T2DM with high thrombomodulin levels	PDMVs	Decrease	69
	Ticlopidine	T2DM	PDMVs, MDMVs	Decrease	59, 71, 72
	Sarpogrelate	T2DM with diabetic neuropathy or STZ induced diabetic rats	PDMVs		70, 125
	Clopidogrel+aspirin	T2DM with TIA	PDMVs	Decrease	34
Antihypertensive	Efonidipine	T2DM with hypertension	PDMVs, MDMVs	Decrease	14
	Nifedipine	T2DM with hypertension	PDMVs, MDMVs, EDMVs	Decrease	15, 54
	Losartan	T2DM with hypertension	PDMVs, MDMVs, EDMVs	Decrease	55, 103
	Valsartan	T2DM with hypertension	MDMVs	Decrease	16
Antiatherosclerosis	Pitavastatin	T2DM with hyperlipidemia	PDMVs	Decrease	110
	Pravastatin	T2DM with hyperlipidemia	PDMVs	Decrease	41
	Simvastatin	HUVEC	EDMVs	Increase	112
	Simvastatin+losartan	T2DM with hyperlipidemia and hypertension	EDMVs, PDMVs	Decrease	103
	Atorvastatin	T1DM with hyperlipidemia	PDMVs	Decrease	113
	Bezafibrate	T2DM with hyperlipidemia	PDMVs	Decrease	60
	Probucol	T2DM with hyperlipidemia	MDMVs	Decrease	59
	Eicosapentaenoic acid	T2DM with hyperlipidemia	PDMVs, MDMVs, EDMVs	Decrease	58, 110, 111
	Vitamin C	T2DM with acute myocardial infarction	EDMVs	Decrease	49
Antihyperglycemic	Acarbose	T2DM	PDMVs	Decrease	115
	Miglitol	T2DM	PDMVs	Decrease	116
	Pioglitazone	T2DM	EDMVs	Decrease	51
	Glibenclamide	PBMC from healthy blood donors	MDMVs	Decrease	114
	Insulin	PBMC from healthy blood donors and monocytic THP-1 cells	MDMVs	Decrease	118

T2DM, type 2 diabetes mellitus; TIA, transient ischemic attack; HUVEC, human umbilical vein endothelial cells; PBMC, peripheral blood mononuclear cell; PDMVs, platelet-derived microvesicles; EDMVs, endothelial-derived microvesicles; MDMVs, monocyte-derived microvesicles.

occlusion than in patients without areas of capillary occlusion[13, 33]. The level of monocyte-derived MVs is positively correlated with platelet-derived MVs, activated platelets, and adhesion molecules in diabetic patients and could be a prognostic factor for diabetic retinopathy progression[13]. Another study found that MVs of endothelial, platelet, photoreceptor, and microglial origin can be identified in vitreous samples[3]. MVs of endothelial origin are the most abundant MV subpopulation in vitreous samples from diabetic patients[3]. Moreover, proliferative diabetic retinopathy is associated with a specific increase in the local shedding of endothelial MVs originating from new vessels[3].

It is controversial whether high levels of MVs contribute to the progression of diabetic retinopathy by stimulating the coagulation cascade. The high level of platelet-derived MVs may stimulate the coagulation cascade and increase adhesion of leukocyte and endothelial cells[33]. In contrast, another study reported that the MVs from diabetic retinopathy cohorts are less procoagulant than those from T2DM patients with CAD and diabetic foot ulcers[35]. These results suggest that T2DM patients with retinopathy have high levels of TF but a low TF/TF pathway inhibitor ratio, suggesting a low procoagulant state[35].

Vitreous MVs stimulate endothelial proliferation *in vitro* and new vessel formation in a Matrigel plug model *in vivo*, which suggests the vitreous MVs may contribute to the progression of diabetic retinopathy[3]. In *in vitro* experiments, TF directly promotes ocular angiogenesis by activating MAPK and protein kinase C-dependent signaling[120]. In addition, abnormal expression of miRNA in MVs may be involved in neoangiogenesis[121]. Decreased expression of miRNA-200b reduces vascular endothelial growth factor (VEGF) expression[122], while increased expression of miR-29b regulates certain apoptotic genes and increases VEGF expression[123]; these miRNAs may contribute to uncontrolled cell proliferation in diabetic retinopathy. However, the branched tube networks induced by MVs in diabetic retinopathy were unstable and collapsed over time[35]. Therefore, the underlying role of circulating MVs in diabetic retinopathy pathogenesis may based on their ability to convey angiogenic and inflammatory signals.

MVs and diabetic nephropathy

Nakajima and colleagues observed the presence of intraglomerular extracellular MVs with microspherical and thread-like structures under an electron microscope in renal biopsy tissue from patients with various renal diseases, including diabetic nephropathy[124]. A few studies have indicated that the levels of monocyte-derived MVs[14, 55], endothelial-derived MVs[74], and platelet-derived MVs[70, 125, 126] are increased in patients with diabetic nephropathy. The increase in monocyte-derived MVs is most significant in patients with diabetic nephropathy among the T2DM patients with diabetic microangiopathy, *ie*, nephropathy, retinopathy, or neuropathy[14]. Elevated levels of endothelial-derived MVs and monocyte-derived MVs may serve as biomarkers for nephropathy progression in T2DM[14, 55, 74]. A recent study showed that MV-associated dipeptidyl peptidase-IV (DPP-IV) is the major form of DPP-IV in urine, which is secreted from tubular epithelial cells and is related to early tubular impairment[127]. Moreover, urinary excretion of MV-associated DPP-IV is increased in T2DM patients, and the levels of MV-associated DPP-IV in urine are positively correlated with the urinary albumin/creatinine ratio[127]. Therefore, MV-associated DPP-IV in urine may be an early marker of renal damage before the onset of albuminuria[127].

The general consensus is that hyperglycemia, AGE and ROS/NO imbalance can cause glomerular endothelial dysfunction and microalbuminuria in the early stages of diabetic nephropathy. The circulating platelet-derived MVs[70, 125, 126], monocyte-derived MVs[14, 55], endothelial-derived MVs[74], and MVs derived from renal mesangial cells[82] may act as mediators and influence endothelial function by simulating the release of cytokines and the expression of various adhesion molecules by endothelial cells, inducing the morphological changes that lead to angiogenesis induction in microvascular endothelial cells.

Treatment with sarpogrelate, a serotonin (5-HT) 2A receptor antagonist, can reduce the level of albuminuria in streptozotocin-induced diabetic rats[125]. An experiment showed that sarpogrelate improves ROS/NO imbalance and suppresses platelet aggregation in glomeruli, which may be associated with reduced circulating platelet-derived MVs[125]. Administration of angiotensin II receptor blockers can decrease circulating monocyte-derived MVs, chemokines (monocyte chemoattractant protein 1 and RANTES), and soluble adhesion markers (soluble P-selectin and sVCAM-1)[55]. The ability of angiotensin II receptor blockers to inhibit monocyte-derived MV generation might be an additional protective mechanism beyond the blood pressure-lowering effects in T2DM.

MVs and diabetic neuropathy

A few studies have shown that neuropathy in both T1DM and T2DM is associated with increased levels of circulating MVs. T2DM patients with diabetic neuropathy have increased blood concentrations of monocyte-derived MVs compared to those without diabetic complications[14]. Similarly, T1DM patients suffering from one or more microvascular complications, including neuropathy, display higher levels of endothelial-derived MVs compared to those without diabetic complications[11].

Therefore, the blood levels of MVs are increased in diabetic patients with microvascular complications and may be a biomarker for the progression of microvascular complications in T2DM. The elevated circulating MVs may play a pathological role by stimulating coagulation, endothelial inflammation and dysfunction, as well as angiogenesis.

Conclusions

All in all, MVs may be involved in the pathophysiology of diabetic complications, inducing atherosclerosis, thrombosis, inflammation, endothelial dysfunction, and angiogenesis. However, the precise *in vivo* MV generation mechanisms in diabetes remain unclear. Moreover, it is still unclear whether increased MVs are the cause or the consequence of vascular diseases. The *in vitro* studies by our group and others have shown the different pathological effects of MVs. However, a lack of good animal models is still a limitation for studying the causal role of MVs under certain disease or pathologic conditions *in vivo*.

In addition, clinical studies have indicated that the MVs in blood, urine, and other body fluids may serve as a biomarker that reflects the pathological state of their parental cells and diseased organs/tissues in diabetic complications. Further research is necessary to establish standardized protocols for measuring MVs and comparing the predictive power of MVs with traditional biomarkers of cardiovascular diseases (*ie*, CRP and microalbuminuria). Therefore, measurement and characterization of changes in concentration, cellular origins and composition of MVs might be useful for disease diagnosis, prognosis, and monitoring therapeutic effects in the future.

Acknowledgements

Li-ming CHEN is supported by National Natural Science Foundation of China (NSFC) 81273915 and 81072922. Ming-lin LIU is supported by American Heart Association (AHA)-Great Rivers Affiliate Beginning Grant-In-Aid and Temple University Department of Medicine Career Development Award.

Abbreviations

AGE, advanced glycation end products; baPWV, brachial ankle pulse wave velocity; CAD, coronary artery disease; CRP, C-reactive protein; EPC, endothelial progenitor cell; FMD, flow-mediated dilation; HUVEC, human umbilical vein endothelial cell; LOX1, lectin-like oxidized low-density lipoprotein receptor-1; miRs, microRNAs; MMP, matrix metal proteinase; MV, microvesicle; NOX, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase; NO, nitric oxide; PBMC, peripheral blood mononuclear cell; PS, Phosphatidylserine; ROS, reactive oxygen species; SPRED1, sprout-related, EVH-1 domain-containing protein 1; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; TF, tissue factor; UCMV, unesterified cholesterol-induced microvesicle; VEGF, vascular endothelial growth factor.

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