Acta Pharmacologica Sinica (2018) 39: 1–11
 1671-4083/18
www.nature.com/aps

Review article

Pathogenic roles of microvesicles in diabetic retinopathy

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Abstract

Diabetic retinopathy (DR) is a common complication of diabetes and has been recognized as the leading cause of blindness in adults. Several interrelated molecular pathways are involved in the development of DR. Microvesicles (MVs) are cell membrane vesicles, which carry many biologic molecules, such as mRNAs, microRNAs, transcription factors, membrane lipids, membrane receptors, and other proteins. They may be involved in intercellular communication that can promote inflammation, angiogenesis, and coagulation. Recent studies have indicated that changes in the number and composition of MVs may reflect the pathologic conditions of DR. At present, MVs are well recognized as being involved in the pathophysiological conditions of tumors and cardio-metabolic diseases. However, the roles of MVs in DR have yet to be investigated. In this review, we provide an overview of DR-induced microvascular injury that is caused by MVs derived from endothelial and circulating cells, and discuss the possible mechanisms by which MVs can lead to endothelial dysfunction, coagulation and inflammation. In addition, the protective effects of preconditioned MVs and stem cell-derived MVs are also described . Understanding the involvement of MVs in the pathophysiological conditions of DR may provide insight into the disease mechanisms and may suggest novel therapeutic strategies for DR in the future.

Keywords: microvesicles; diabetic retinopathy; stem cells; vascular inflammation; miRNAs

Acta Pharmacologica Sinica (2018) 39: 1–11; doi: 10.1038/aps.2017.77; published online 17 Jul 2017

Introduction

Diabetic retinopathy (DR) is a common complication of diabetes and is considered a microcirculatory disease of the retina that is caused by the deleterious metabolic effects of hyperglycemia[1]. The number of people at the risk of vision loss is predicted to double and the prevalence of DR is expected to increase by the year 2030[2]. DR is staged into several levels of severity, including mild, moderate, and severe nonproliferative DR (NPDR), followed by advanced proliferative DR (PDR), which is defined by the presence of retinal neovascularization[3]. The early pathogenesis of DR may be due to the chronic degeneration of retinal nerve tissue, including reactive glial cell hyperplasia and neuronal apoptosis. In PDR, the proliferative neovasculature causes severe complications, such as vitreous hemorrhage and tractional retinal detachment, which may lead to irreversible vision loss[4]. The pathological mechanism of DR is still unclear.

The pathophysiology of DR development is highly complicated due to the involvement of multiple interlinked mechanisms that result in adaptive changes and cellular damage in the retina[5]. Thus, the underlying causes of DR have not been fully elucidated. In the past, retinopathy was largely characterized by its microvascular abnormalities, including vessel leakage, endothelial cell dysfunction, and vascular occlusion and degeneration[6]. However, recent studies have indicated that the retinal complications of diabetes are a composite of the structural and functional alterations in both the neuroglial and the microvascular compartments[7]. The exact mechanisms by which diabetic hyperglycemia induces neuronal and vascular alterations have not been completely defined in retinopathy. Retinal damage may be caused by a variety of mechanisms (Figure 1), including an increase in polyol pathway flux, the production of advanced glycation end products

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Received 2016-12-11 Accepted 2017-03-23

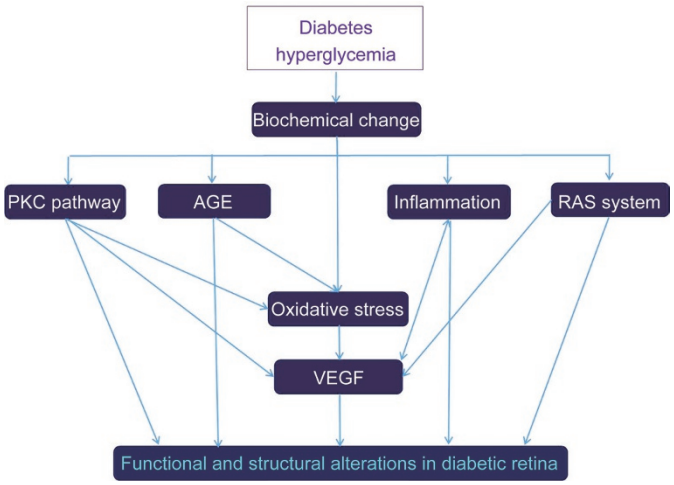

Figure 1. General pathological mechanism of DR. Hyperglycemia-induced biochemical pathways that contribute to DR pathophysiology. PKC: protein kinase C; AGE: advanced glycation end-product; DR: diabetic retinopathy; RAS: renin-angiotensin system; VEGF: vascular endothelial growth factor.

(AGEs), an increase in oxidative stress and protein kinase C (PKC) activation, but many of these hypotheses have not yet been verified in human studies[8]. DR also has similarities to chronic inflammatory diseases, namely: it can lead to increased vascular permeability, inflammatory cell infiltration, edema, angiogenesis, tissue destruction, and the expression of pro-inflammatory cytokines and chemokines in the retina. The increased expression of vasoactive factors and cytokines may play an important role in mediating functional and structural changes in the retina[9]. Recent studies have suggested that inflammation is also very important in the early onset of experimental DR, although human studies have not shown a consistent association between systemic inflammation markers and retinopathy[10]. Therefore, it remains uncertain whether inflammation also plays a crucial role in the occurrence and progression of DR in humans.

We recently reported that impaired proliferation, adhesion, and migratory activities were observed in cultured EPCs from the bone marrow of rats with DR, and this effect may contribute to prothrombotic conditions and atherothrombosis in the pathogenesis of DR[11]. Our previous publication further showed that EPCs may serve as a marker for DR progression and that simvastatin may be a promising candidate for the clinical management of DR; these EPCs decreased their mRNA expression levels of iNOS, Ang-1, and Ang-2 while increasing their eNOS mRNA expression in retinal tissue, suggesting a potential contribution to repairing endothelial dysfunction in rats with DR[12]. This study offers important insights and implications regarding the simvastatin increase in circulating EPCs, which consequently suppresses the formation and progression of DR[12]. In addition, the relationship between the C-reactive protein (CRP) level and DR remains controversial. Our recent meta-analysis study showed that the CRP level might be used as a biomarker for determining the severity of DR[13]. The *in vitro* studies by our group have shown that DR can be improved by an intravitreal injection of human umbilical mesenchymal stem cells[14].

Microvesicles (MVs) are submicron membrane vesicles between 100 and 1000 nm that shed from the cell surface of both healthy and damaged cells[15]. By contrast, exosomes are smaller in diameter (between 40 and 100 nm) and are released during facultative or exocytosis[16]. The shedding of membrane MVs is a strictly regulated, cytoskeleton-dependent process that is enhanced by cytokines, the activation of apoptotic pathways, and reactive oxygen species[17]. Numerous studies now indicate that the transfer of information from MV-releasing cells to target cells via MVs that are circulating in the blood may be an important mechanism of intercellular communication[18]. MVs have biological activities and may be involved in thrombosis, inflammation, and angiogenesis, according to studies from our group and others[19-23].

For example, MVs may communicate local inflammation, thus playing a key role in vascular diseases, such as DR[24]. DR is associated with increased local apoptosis or the activation of the retinal, vascular endothelial, and neural cells in the eye, both in humans and in animal models[25]. Reports indicate that MVs of different cellular origins might be locally generated in the eye of diabetic patients by the transfer of pro-inflammatory factors from the cells of origin[26]. Alternatively, the presence of MVs in the eye could also result from an increased vascular permeability that is associated with DR[27]. Thus, MVs are believed to serve as potential biomarkers for the diagnosis and prognosis of related vascular complications and the assessment of treatment response[28]. In the following sections, we summarize current knowledge of the role of MVs in the pathogenesis of DR, along with a projection of their importance in the screening and therapy of disease.

Biological properties of MVs

MVs are shed directly from the cell membrane by a "budding" process and typically range in size from approximately 100 to 1000 nm, although these values are somewhat arbitrary and subclass overlap may exist[29]. MVs are identified by the expression of phosphatidylserine (PS) on their surface, which is indicative of their release from apoptotic or activated cells[30]. In these cells, PS is externalized, whereas the membrane PS has a cytosolic orientation in quiescent cells[31]. As Annexin V binds preferentially to PS, it can be used to detect the exposed PS on the surface of apoptotic cells and the PS-positive MV subclass[32]. In addition, some researchers have found that a portion of circulating MVs are PS-negative, and it was suggested that the measurement of lactadherin may be a more sensitive alternative to Annexin V[33, 34]. However, the number of other MV receptors is unknown; some MVs work in combination with Annexin I or are independent of Annexin I.

MVs are released from almost all cell types, including blood cells (such as erythrocytes, leukocytes, platelets, and macrophages/monocytes), vascular endothelial cells, smooth muscle cells, and the cells of other tissues/organs, under both pathological and physiological conditions[35]. Thus, MVs can be found in the urine, blood, vitreous fluid, atherosclerotic

plaques of the vascular wall, and the extracellular spaces of organs. There are two well-known cellular processes that can lead to the formation of MVs: chemical and physical cell activations and apoptosis[36]. Many chemical and physical stimuli, such as thrombin, endotoxins, cytokines, tobacco smoke extract, hypoxia, unesterified cholesterol, and shear stress, have been reported to trigger cellular MV release in studies from several groups[36]. Other chemical stimuli of MV shedding include LDL and pro-inflammatory cytokines, such as IL-1β and TNFα. All of the abovementioned stimuli and factors may be involved in the development of diabetic vascular complications. Accordingly, there is an increase in circulating MVs in various diseases, including diabetes, cardiovascular diseases, diabetic complications, and abdominal aortic aneurysm[37].

MVs display a versatile spectrum of membrane-anchored receptors and adhesion molecules on their surface, allowing specific interactions and crosstalk among various target cells[38]. When they are incorporated into the membranes of target cells, these receptors regulate cellular adhesive functions, such as promoting immune cell adhesion to the vascular endothelium. In addition to a common series of proteins, MVs have been shown to possess a specific lipid organization and composition[39]. A recent finding indicates that MVs contain double-stranded DNA, mRNA and noncoding RNA (microRNA and lncRNA)[40]. It is important that the mRNAs and microRNAs of the MVs are functional in the recipient cells. This suggests that the exchange of MV medium contents between cells may represent an efficient and effective form of inter-cellular communication. In addition, it is now known that RNA and protein sorting into MVs is highly regulated by a variety of patho-physiological stress stimuli and diseases[41]. This allows the cells to produce different functional characteristics that are tailored to the MVs and are reflective of their parent cell status. In this regard, any stress or disease conditions may be mirrored in the contents of the MVs, which can be used to develop future biomarkers for the diagnosis and prognosis of DR[42].

At present, MVs have been implicated in mediating cellular communication through different mechanisms of interaction with the recipient cells, including: 1) direct ligand receptor interaction, leading to the activation of downstream signaling pathways, 2) extracellular protease cleavage of the membrane proteins of the MV, releasing soluble ligands to bind the target receptors of the recipient cells, 3) direct membrane fusion, leading to release of the contents of the MV into the recipient cells, and 4) internalization of the MVs by endocytic mechanisms (phagocytosis, macropinocytosis or receptor-mediated endocytosis)[43]. Considering the multiple attributes of the MVs and the secretion of MVs from a variety of different cell types within the retina, it is reasonable to expect that MVs may be involved in DR[44]. Although there is clinical evidence of the involvement of MVs in physiological and pathophysiological processes, the role of MVs is not well established and the mechanisms regulating MV formation are still unclear; these topics are still extensively studied *in vivo* and *in vitro*.

Involvement of MVs in the initiation and development of DR

The MVs released by endothelial cells, platelets, leukocytes and tumor cells have been shown to be pro-angiogenic both *in vivo* and *in vitro*[45]. Studies with human subjects indicate that the circulating levels of endothelial-derived MVs increase in retinopathy, obesity, physical inactivity, type 2 diabetes mellitus, end-stage renal disease and ischemic left ventricular dysfunction[46]. Importantly, the plasma concentrations of endothelial-derived MVs in these studies correlated with the degree of impaired vasodilation in these individuals[47]. Similarly, studies of diabetic patients with DR have indicated that the plasma concentrations of erythrocyte-derived, endothelial-derived, or platelet-derived MVs correlate with the circulating endothelial markers of inflammation (vascular cell adhesion molecule [VCAM]) and a prothrombotic state (von Willebrand factor)[40]. The composition, formation, catabolism, and general functions of MVs have been the subject of recent studies. Here, we focus on the latest works that implicate MVs in the initiation and development of DR and suggest MVs as markers of retinopathy.

The clinical works discussed above suggest that the levels of circulating MVs may serve as a new biomarker for endothelial dysfunction in neovascular retinopathy[48]. Although there is extensive literature demonstrating the involvement of MVs in various diseases, little is known about their roles in retinopathy. Analyses of vitreous fluids from patients with proliferative retinopathy have revealed remarkably increased levels of MVs, suggesting their involvement in retinopathy[49]. In support of this concept, studies have shown that MV antagonists block both choroidal and retinal angiogenesis. MVs from several abnormal clinical states of retinopathy have been shown to adversely affect endothelial function. Circulating MVs isolated from patients with proliferative retinopathy, myocardial infarction, or metabolic syndrome impair endothelium-dependent vasodilation *ex vivo*[50]. Moreover, MVs isolated from vitreous fluids provoke the recruitment of leukocytes in cultured endothelial cells. In addition to their role in promoting abnormal cell growth, alterations in MV generation have been demonstrated to play a key role in the atrophy of the choroid and retina[51]. MVs have also been found to play a key role in retinal microvascular function[37]. Basic studies have shown that MVs obtained from rats with proliferative retinopathy, or MVs generated *in vitro* using different stimuli on endothelial cells or retinal capillaries, can inhibit nitric oxide production in cultured retinal endothelial cells[37]. Moreover, these MVs also induce endothelial dysfunction *in vivo*[38]. MVs produced by the stimulation of several cell types are able to induce retinal endothelial cells to recruit monocytes *in vitro*.

The potential pathogenic mechanisms of MVs in the functional and structural alterations in DR are summarized in Figure 2. Under hyperglycemia conditions, the platelet-derived MVs (pMVs) released from platelets can lead to the elevation of MMPs/ADAMs, while the activated MMPs/ADAMs damage the mitochondria and cause the release of cytochrome *c* from the damaged mitochondria. This process accelerates capillary cell apoptosis, ultimately resulting in acellular capil-

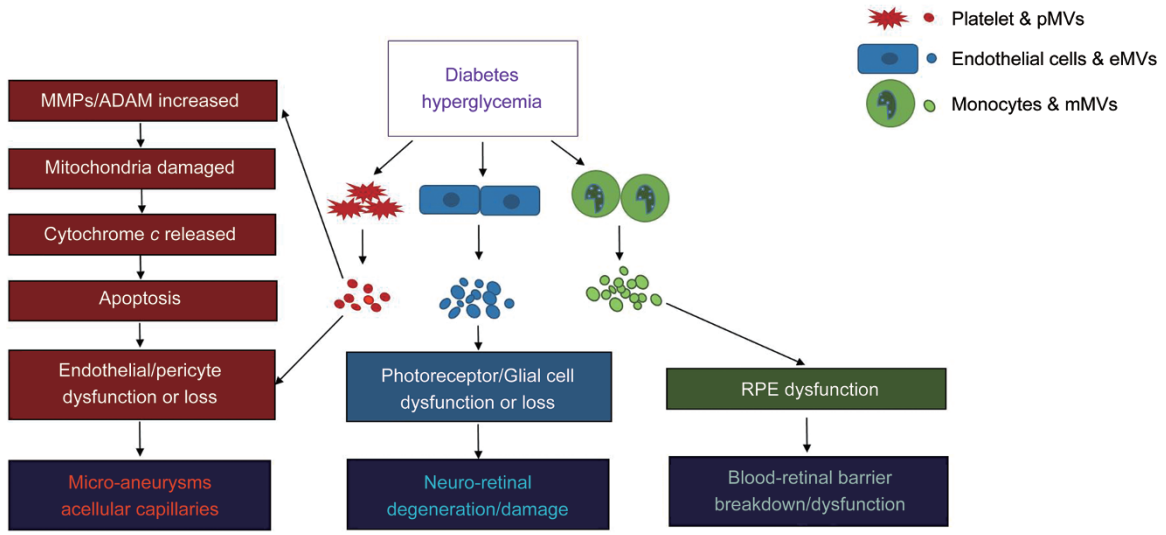

Figure 2. Pathogenic mechanisms of MVs in functional and structural alterations in DR. Under hyperglycemic conditions, platelets secrete pMVs, which leads to the elevation of MMPs/ADAMs, the activation of MMP/ADAM damage to the mitochondria, and the release of cytochrome *c* from the mitochondria. This accelerates capillary cell apoptosis, ultimately resulting in acellular capillaries and pericyte ghosts. With time, the capillaries become hypoxic, ultimately leading to neovascularization. Endothelial cells secrete eMVs, which leads to photoreceptor/glial cell dysfunction or loss, ultimately resulting in neuro-retinal degeneration or damage. Monocytes secrete mMVs, which leads to retinal pigment epithelium (RPE) dysfunction, ultimately resulting in blood-retinal barrier breakdown.

laries and pericyte ghosts. Over time, the capillaries become hypoxic, a condition that ultimately leads to neovascularization. The endothelial cell-derived MVs (eMVs) released from endothelial cells may lead to photoreceptor/glial cell dysfunction or even loss and may ultimately result in neuro-retinal degeneration or damage. The monocyte-derived MVs (mMVs) released from monocytes may cause RPE dysfunction, ultimately resulting in blood-retinal barrier breakdown. In addition, the pMVs, eMVs and mMVs released from their parental cells under hyperglycemic conditions may lead to angiogenesis, vascular dysfunction, an inflammatory response and a coagulation cascade in the retina (Figure 3). Evidence from the literature suggests an important role of the MVs in retinal vascular dysfunction, retinal inflammation, and pathological angiogenesis during the development of DR[43]. The following sections will present additional evidence for the spe-

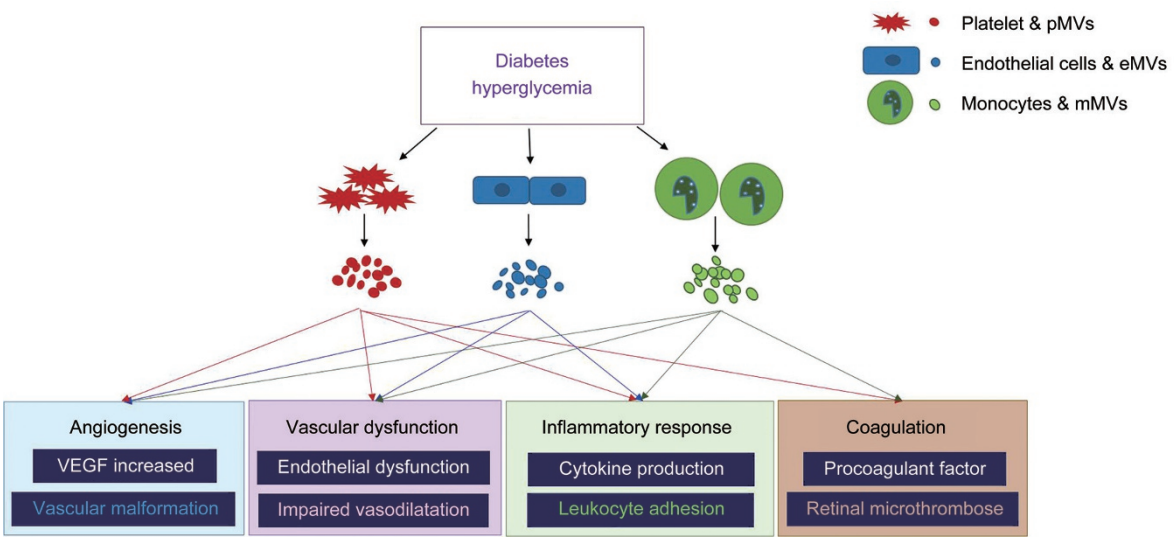

Figure 3. Pathogenic mechanisms of MVs contributing to DR pathophysiology. Under hyperglycemic conditions, platelets secrete pMVs, which leads to angiogenesis, vascular dysfunction, an inflammatory response and coagulation in the retina. Endothelial cells secrete eMVs, which leads to angiogenesis, vascular dysfunction, and an inflammatory response in the retina. Monocytes secrete mMVs, which leads to angiogenesis, vascular dysfunction, an inflammatory response and coagulation in retina.

cific involvement of MVs in models of DR.

Role of MVs in angiogenesis during DR

Proliferative DR is associated with a specific increase in endothelial MVs derived from new vessels[52]. Some studies suggest that the levels of endothelial-derived MVs, monocyte-derived MVs, and platelet-derived MVs are increased in patients with DR[47]. The increase in monocyte-derived MVs is most significant with the progression of DR from the nonproliferative stage to the proliferative stage, and monocyte-derived MVs are significantly higher in DR with areas of retinal neovascularization[27]. A recent study showed that MVs of endothelial, platelet, photoreceptor, and microglial origins can be identified in vitreous samples. Moreover, MVs of endothelial origin are the most abundant MV subpopulation in the vitreous samples from diabetic patients[53].

The general consensus is that advanced glycation end products (AGE), hyperglycemia and ROS/NO imbalance can lead to retinal endothelial dysfunction and inflammation in the stage of DR. The circulating endothelial-derived MVs, monocyte-derived MVs, platelet-derived MVs, and MVs derived from retinal pigment epithelium cells may act as mediators and may influence endothelial function by simulating the expression of various adhesion molecules and the release of cytokines by endothelial cells, leading to morphological changes and angiogenesis induction in the microvascular endothelial cells[51]. The functional link between the endothelial-related pro-angiogenic response and erroneous local inflammation is proven by the recruitment of endogenous coagulation factor (FXII), which is observed on endothelial cell populations that present externalized PS by MV-delivery[54].

Vitreous MVs stimulate endothelial cell proliferation *in vitro* and new vessel formation by a Matrigel plug model *in vivo*, which suggests that vitreous MVs may contribute to the progression of DR[52]. One of the suggested mechanisms of DR is increased MV release from endothelial cells and platelets, triggering TF in patients. In *in vitro* experiments, TF directly promotes ocular angiogenesis through MAPK activation and protein kinase C-dependent signaling[55]. Another mechanism is proposed for the transfer of secondary messenger molecules (lipids, receptors), mainly from immune cells (T lymphocytes), regulating vascular function. Moreover, the abnormal expression of miRNA in MVs may be involved in angiogenesis. Reduced expression of miRNA-200b decreases vascular endothelial growth factor (VEGF) expression, and increased expression of miR-29b regulates certain apoptotic genes and increases the expression of VEGF[51]; these miRNAs may be involved in uncontrolled cell proliferation in DR. However, the branch tube networks induced by MVs in DR are unstable and collapse over time[31]. Thus, elevated levels of monocyte-derived MVs and endothelial-derived MVs may serve as biomarkers of DR progression[54, 56].

Role of MVs with protease activities in DR

The diabetic environment stimulates the secretion of several MMPs that may participate in many diabetic complications, especially in DR[57]. Patients with DR and DR animal models have shown increased levels of MMP-9 and MMP-14 in their retina and vitreous fluid[58]. Retinal mRNA levels of MMP-2, MMP-9 and MMP-14 are elevated in diabetes, and the proforms of MMP-9 and MMP-14 are significantly elevated in the neovascular retinal membranes[59]. Our group recently reported that the production of MVs relies on a series of regulated steps that result in the induction and maturation of cellular MMP-14, leading to a remarkable accumulation of MMP-14 in nascent plasma membrane blebs and, finally, to caspase- and MAPK-dependent apoptosis and apoptotic MV generation[60]. This indicates that the proteolytically active MVs induced by tobacco smoke may be novel mediators of clinically important matrix destruction in smokers[60]. Therefore, MMPs could contribute to the disease process via MV-dependent pathways. MMPs play important roles in maintaining the integrity of the blood–retinal barrier (BRB) and in the development of DR[61]. The increased level of retinal MMPs in DR facilitates an increase in the BRB via the proteolytic degradation of tight junction proteins (ICAM, occluding) and the disruption of overall tight junction complexes[62, 63]. We recently demonstrated persistent, enzymatically active a disintegrin and metalloprotease (ADAMs) on MVs in the intraluminal thrombus, adjacent to the aneurysmal wall[64]. The production of ADAM10- and ADAM17-positive MVs from smoke-exposed neutrophils provides a novel molecular mechanism for the vastly accelerated risk of abdominal aortic aneurysm[64]. Increased expression of ADAMs is observed in retinal pericytes incubated in high glucose and in the retina in DR, and increased ADAM-9 activity is considered to compromise the survival of retinal pericytes[65]. Glycated low-density lipoprotein (LDL) and heavily oxidized LDL, which is elevated in DR, increases ADAM-10 in retinal pericytes[66]. ADAM-15 is also up-regulated in retinal vascular cells cultured under high glucose conditions and in the human retina showing active neovascularization[65, 66].

Role of MVs in endothelial dysfunction and vascular inflammation during DR development

The function of endothelial cells is key to the formation and propagation of atherosclerotic plaques, and MVs are involved in the intercellular transfer of both pro-apoptotic and pro-angiogenic signals[67]. Studies in diabetic patients show that MVs can affect vascular endothelial function. When two consecutive fat-rich mixed meals were given to T2DM patients, the results showed that impaired endothelium-dependent vasodilatation is associated with increased levels of circulating endothelial-derived MVs and platelet-derived MVs[68]. An increased level of endothelial-derived MVs is positively correlated with the impairment of coronary endothelial function in patients with T2DM[69]. T2DM patients show reduced endothelium-dependent flow-mediated dilation (FMD) and increased brachial ankle pulse wave velocity. We previously reported that cholesterol enrichment of human macrophages/monocytes causes the generation of TF^+ MVs with high procoagulant activity, which may contribute to endothelial

dysfunction in hyperglucose conditions[23]. In addition, we further reported that exposure of human macrophages to tobacco smoke causes the release of proteolytic MMP-14$^+$ MVs through the activation of JNK and p38 MAPK[60]. Moreover, MPs derived from human atherosclerotic plaques incorporate the ICAM-1 of endothelial cells to recruit inflammatory cells, which suggests that plaque-derived MPs promote inflammation progression[70, 71]. MVs isolated from diabetic patients express more CD40L and were more potent in inducing endothelial proliferation when compared with non-diabetic MVs or circulating MVs[72].

In vitro and *in vivo* experiments have indicated that MVs might contribute to endothelial dysfunction by decreasing the production of nitric oxide (NO) and prostacyclin in endothelial cells[73]. Co-culture of endothelial-derived MVs with aortic rings and cultured endothelial cells leads to increased superoxide generation, reduced NO production, and impaired vascular relaxation induced by acetylcholine[74]. The T lymphocytes derived from the MVs of diabetic patients damage the shear stress-induced dilatation of mouse mesenteric arteries by affecting the production of prostacyclin and NO[75]. Our recent study showed that tobacco smoke exposure increases the release of procoagulant MVs[22]. Our latest publication further showed that tobacco smoke exposure in human macrophages causes the generation of MMP-14$^+$ MVs with strong procoagulant activities; these MVs degrade a major component of the arterial wall matrix, suggesting their potential contribution to endothelial dysfunction in patients with cardiovascular disease[76]. In addition, a recent study reported that endothelial-derived MVs exposed to high glucose could significantly increase macrophage infiltration and could impair endothelial function and adhesion molecule expression, causing higher ROS levels and increased nicotinamide adenine dinucleotide phosphate oxidase (NOX) activity[77]. Therefore, MVs derived from endothelial cell-based methodologies could be used to study vascular inflammation in diabetic vascular diseases.

Leukocyte-endothelial interactions and the subsequent endothelial migration of leukocytes are important early stage events in the development of DR[78]. *In vitro* experiments have shown that MVs derived from activated platelets or apoptotic endothelial cells act as cellular effectors, disseminating pro-adhesive and pro-inflammatory potentials in the vasculature[55]. Activated platelets and the platelets derived from MVs that were isolated from DR patients facilitate the interaction between monocytes and endothelial cells[74]. Lee *et al* showed that MVs facilitate platelet string formation at the surface of human umbilical vein endothelial cells (HUVECs) before the MVs are internalized into the HUVECs *in vitro*[79]. This uptake induces ROS production, which is necessary for the expression of von Willebrand factor on the endothelial cell surface and the subsequent interaction between endothelial cells and platelets[80]. We recently demonstrated that unesterified cholesterol-induced MVs (UCMVs) from human endothelial cells robustly increased leukocyte recruitment to cultured human endothelial monolayers *in vitro*, aortic endothelium *ex vivo*, and microvascular endothelium *in vivo*[20]. The malondialdehyde-like epitopes on the UCMV surface intercede monocyte recruitment to the endothelial cells through lectin-like oxidized low-density lipoprotein receptor-1 (LOX1)[81]. As reviewed above, MVs derived from different conditions may contain different functional factors, which decide their harmful properties (Table 1). These studies indicate the potential importance of MVs in vascular inflammation, endothelial activation, and leukocyte recruitment, which may lead to vascular diseases and DR.

Prothrombotic MVs in DR

Diabetes is a procoagulant state and retinal microthromboses occur in DR. An increased prevalence of platelet-fibrin microthrombi has been observed in the retinal microvasculature in both experimental rodent models of diabetes and in human eyes from diabetic patients[82, 83]. It is well established that diabetes induces endothelial dysfunction and injury, which can lead to increased leukocyte and platelet adhesion in the

Table 1. Pathologic effects of MVs.

MV sources	Key findings	Cargo responsible for effect	Ref
MSC cultured in physiological and diabetic-like conditions	Influence vessel remodelling during angiogenesis	miR-146a	54
T2DM patients with retinopathy	Inhibit endothelial cell coagulability and tube formation	miR-320	50
EDMV from T2DM patients with retinopathy	Reduce endothelial activity in retinas	miR-146a	51
PDMV from patients with DR	Stimulate the coagulation cascade and increase leukocyte adhesions	NADPH, NOS, PDI	46
Vitreous samples from patients with DR	MV of endothelial, platelet, and retinal origin contribute to DR progression	miR-21	70
MV released from human macrophages	Caspase-and MAPK-dependent endothelial apoptosis	MMP14	49
MV from human aortic aneurysm	Activate microvascular thrombus	ADAM10, ADAM17	53
MV released from human platelet	Lead to further thrombin formation	TGF-β protein and mRNA	89
MV from human macrophages/monocytes	Contribute to endothelial dysfunction	TF	81
MV from T lymphocyte	Damage shear stress induced dilatation of mesenteric arteries	AGE, miR-222	75

retinal microvasculature, suggesting that microthromboses are concomitant with or secondary to capillary damage[84]. Studies have found that the levels of procoagulant MVs are increased in patients with diabetes and DR; increases in the highly procoagulant TF-bearing MVs coincide with strongly elevated levels of coagulation activation markers[85]. Another study also showed that procoagulant endothelial-derived MVs, platelet-derived MVs, and monocyte-derived MVs are significantly increased in patients with diabetes, even in well-controlled DM without DR[86]. Blood platelet-derived MVs are more likely to adhere to the diabetic endothelium than healthy vessels, which may contribute to the occlusion of injured retinal capillaries[87]. In a cross-sectional study of patients with type II diabetes mellitus and nondiabetic subjects, it was demonstrated that procoagulant endothelial-derived MVs are an independent risk factor for retinopathy[88]. Therefore, elevated levels of MVs may be associated with an increased risk of thrombo-embolic DR.

Procoagulant MVs can initiate and propagate coagulation in diabetes, and the activated platelets release platelet-derived MVs that lead to further thrombin formation, which may explain the novel mechanisms of hypercoagulability in DR[89]. Razmara *et al*[90] reported an increase in procoagulant MVs in DR that correlated with the degree of diabetic duration. In addition to the roles of MVs in thrombosis, these coagulation and fibrinolytic factors increase the inflammatory responses that have been implicated in DR. The administration of anti-platelet drugs can significantly decrease circulating procoagulant MVs and platelets in patients with DR[91]. Taken together, elevated levels of prothrombotic MVs adhered to the injured diabetic endothelium may contribute to both ischemia and inflammation in DR.

Therapeutic potential of MVs in DR

As reviewed above, MVs derived from disease conditions elicit adverse effects on DR. Recent research has also found that the MVs collected from various stem cells or ischemic preconditioning provide vascular-protection, which may have therapeutic potential in the treatment of DR[92]. We will summarize the recent reports of the role of stem cell-derived, gene-modified cell-derived, and stress-preconditioned MVs in retinal protection, including inhibited endothelial dysfunction, reduced oxidative stress, and limited inflammatory response.

Various types of stem cells (adult stem cells, embryonic stem cells, and induced pluripotent stem cells) have been extensively studied for therapeutic potential in microvascular disease[93]. It was previously thought that stem cell therapy appeared to regenerate tissue through reproduction and then differentiation, but recent studies have proven that the advantageous effects of stem cells in the repair of ischemic tissue is through the release of autocrine and paracrine factors[94]. Stem cells can secrete numerous types of molecules/factors, including microRNAs, proteins, growth factors, proteasomes, antioxidants and MVs. In particular, MVs have gained specific attention in cell-free-based stem cell therapy for microvascular disease. He *et al* reported that human ESC-derived MSCs secreted MVs. By using an *ex vivo* model of ischemia/reperfusion injury, they observed that these purified MVs were able to decrease injury size in mouse retinas. A recent study showed that MV treatment restored the redox state and energy depletion in mouse retinas within 30 min after I/R, which was evidenced by a reduction in oxidative stress and an elevation in ATP and NADH levels[95]. Moreover, phosphorylated PFKFB3 and active CD73 are also wrapped in MSC-derived MVs. In addition, MV treatment could decrease systemic inflammation in diabetic mice[96]. Thus, MSC-derived MVs may have significant therapeutic potential for patients suffering from DR.

Recently, a study by Bauchl *et al*[97] found that human $CD34^+$ stem cells had the ability to secrete MVs. *In vitro*, it was shown that MVs replicated angiogenic activity by increasing endothelial cell proliferation, viability and tube-like formation on Matrigel. *In vivo*, it was shown that both stem cells and MVs decreased the formation of endothelial vessel-like structures, which was accompanied by a greatly raised proportion of endothelial cells in the Matrigel plug. In the angiogenesis assay, pellets containing stem cell-derived MVs were associated with greater vessel formation[98]. Thus, these findings demonstrate that MVs are the key paracrine component of $CD34^+$-cell-induced vessel growth. However, the mechanisms underlying $CD34^+$-MV-mediated angiogenesis could be ascribed to the overall transfer of exosomal contents (proteins/RNAs) into the cytosol of endothelial cells or MV receptor-induced activation of angiogenic signaling cascades[54]. Indeed, Ju *et al*[99] have shown data demonstrating that $CD34^+$-MVs are significantly enriched with pro-angiogenic miR-130a and miR-126 compared with CD34-MVs. Therefore, the MV-mediated delivery of miR-130a and miR-126 to an ischemic area may represent a major mechanism that explains the preservation of vascular function in DR mice treated with $CD34^+$ cells.

Recently, Choi *et al*[100] performed a microRNA array for profiling the miRNAs in MSC-derived MVs. These MSCs were isolated from mouse bone marrow and subjected to cycles of anoxia-reoxygenation as ischemic preconditioning (IPC). Lozito *et al*[101] showed that the levels of miR-22 were higher and were accompanied by miR-210, miR-21, miR-24, and miR-199a-3p, in MVs collected from IPC-MSCs than in control MVs. In particular, an *in vivo* treatment of mice with MVs from IPC-MSCs resulted in a significant reduction of ischemia and apoptosis by direct injection into the retina[102]. In addition, these authors identified that MV-IPC-mediated protective effects are highly associated with the transfer of miR-22 to the surrounding cells. Nonetheless, whether these preconditioned MVs contain ligands/receptors or other beneficial proteins remains unclear. As reviewed above, stem cell-derived, gene-modified cell-derived, and stress-preconditioned MVs may contain different functional factors that may decide their beneficial properties (Table 2).

Conclusion

Circulating MVs show great promise not only as biomarkers in vascular disease diagnosis but also as potential targets for

Table 2. Protective effects of MVs.

MV sources	Key findings	Cargo responsible for effect	Ref
Mouse mesenchymal stromal cells	Inhibits vascular remodeling	miR-126, miR-22	72
Mouse mesenchymal stromal cells	Enhance the migration of microvascular endothelial cells	miR-146a, miR-22	78
Human Embryonic Stem Cell	Attenuate infarction size in non-preconditioned recipient hearts	miR-204	94
Mouse mesenchymal stem cells subjected to IPC	Delivery of IPC MVs ameliorates fibrosis and reduces infarction sizes	miR-210	100
Endothelial progenitor cell	Promote angiogenesis and reduce infarct size	miR-130a	96

treatment in DR in the future. The evaluation of MVs could provide value in the clinical setting of DR patients. Moreover, monitoring of the process leading to MV formation may be relevant in controlling DR progression. Despite the fact that the mechanisms that regulate MV formation are barely understood, it has been documented that the cellular machinery leading to MV shedding involves plasma membrane remodeling and PS externalization. From the research data, it seems reasonable to assume that the variable expression and profiles of MVs and microRNAs might be involved in a variety of inflammatory and neoangiogenic processes. This clearly suggests that the pharmacological regulation and control of MV shedding remains a challenge in DR.

Acknowledgements

This work was supported by the Tianjin Science and Technology Project of China (No 13ZCZDSY01500) and the National Natural Science Foundation of China (No 81370422 and No 81700846).

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