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Mini review

Vascular complications in diabetes: Microparticles and microparticle associated microRNAs as active players

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ABSTRACT

The recognition of the importance of diabetes in vascular disease has greatly increased lately. Common risk factors for diabetes-related vascular disease include hyperglycemia, insulin resistance, dyslipidemia, inflammation, hypercoagulability, hypertension, and atherosclerosis. All of these factors contribute to the endothelial dysfunction which generates the diabetic complications, both macro and microvascular. Knowledge of diabetes-related vascular complications and of associated mechanisms it is becoming increasingly important for therapists. The discovery of microparticles (MPs) and their associated microRNAs (miRNAs) have opened new perspectives capturing the attention of basic and clinical scientists for their potential to become new therapeutic targets and clinical biomarkers. MPs known as submicron vesicles generated from membranes of apoptotic or activated cells into circulation have the ability to act as autocrine and paracrine effectors in cell-to-cell communication. They operate as biological vectors modulating the endothelial dysfunction, inflammation, coagulation, angiogenesis, thrombosis, subsequently contributing to the progression of macro and microvascular complications in diabetes. More recently, miRNAs have started to be actively investigated, leading to first exciting reports, which suggest their significant role in vascular physiology and disease. The contribution of MPs and also of their associated miRNAs to the development of vascular complications in diabetes was largely unexplored and undiscussed.

In essence, with this review we bring light upon the understanding of impact diabetes has on vascular biology, and the significant role of MPs and MPs associated miRNAs as novel mediators, potential biomarkers and therapeutic targets in vascular complications in diabetes.

1. Diabetes as a major risk factor for vascular disease

The number of people with diabetes mellitus is alarmingly increasing due to the growing prevalence of obesity, genetic susceptibility, urbanization, and aging [1]. Epidemiological and pathological data show that diabetes is an independent risk factor for cardiovascular disease (CVD) in both men and women [2–5]. CVD is increasing dramatically throughout the world being a common cause of morbidity and mortality. This is a complex and multifactorial disease and is usually related to a combination of both macrovascular and microvascular dysfunction [6]. Also, the complications of diabetes are divided into macrovascular (due to damage to larger blood vessels) and microvascular (due to damage to small blood vessels). *Macrovascular complications* are mainly represented by atherosclerotic disease and its manifestations, such as peripheral vascular disease (PVD), stroke, and coronary artery

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disease (CAD). *Microvascular diseases* are related to diabetes and include retinopathy and nephropathy, the major causes of blindness and renal insufficiency [1]. Diabetes also affects the heart muscle, causing both systolic and diastolic heart failure.

1.1. Endothelial dysfunction a link between diabetes and vascular disease

Normal function of endothelial cells is important for the homeostasis of the vascular wall, while endothelial dysfunction is a systemic pathological state of the endothelium, associated with several pathophysiological conditions including diabetes, hypertension, and atherosclerosis [7].

Endothelial dysfunction is one of the most important features in type 2 diabetes that contributes to the increased cardiovascular risk in this group of patients. Endothelial dysfunction refers to the inability of the endothelium to regulate vascular homeostasis, and the abnormalities in endothelial function are detected early in the development of CVD, often before symptoms are clinically evident [8].

Endothelial dysfunction in diabetes can be induced either by one of or by the combination of the following: hyperglycemia, insulin resistance, dyslipidemia, inflammation, and hypercoagulability [9]. Fig. 1 outlines the common risk factors for vascular disease in diabetes.

Endothelial dysfunction is a consequence of these factors and a cause of vascular diseases and diabetes. Diabetes itself fosters vascular complications.

1.2. Mechanisms for vascular disease in diabetes

1.2.1. Hyperglycemia - associated mechanisms

Macro- and microvascular diabetic complications are mainly due to prolonged exposure to hyperglycemia, the hallmark of diabetes mellitus, clustering with other risk factors such as arterial hypertension, dyslipidemia as well as genetic susceptibility [1].

Early dysglycemia caused by obesity-related insulin resistance or impaired insulin secretion is responsible for functional and structural alterations of the vessel wall culminating with diabetic vascular complications.

The alterations in vascular homeostasis due to endothelial and smooth muscle cell dysfunction are the main features of diabetic vasculopathy favoring a pro-inflammatory/thrombotic state which ultimately leads to atherothrombosis.

One of the mechanisms for vascular disease in diabetes is the imbalance between nitric oxide (NO) bioavailability and accumulation of reactive oxygen species (ROS) that lead to endothelial dysfunction [10].

Hypergycemia-induced ROS overproduction, most notably increased levels of $O_2^{\bullet -}$, is believed to be mediated by four main pathways: protein kinase C (PKC) activation, activation of the hexosamine and polyol pathways, and formation of advanced

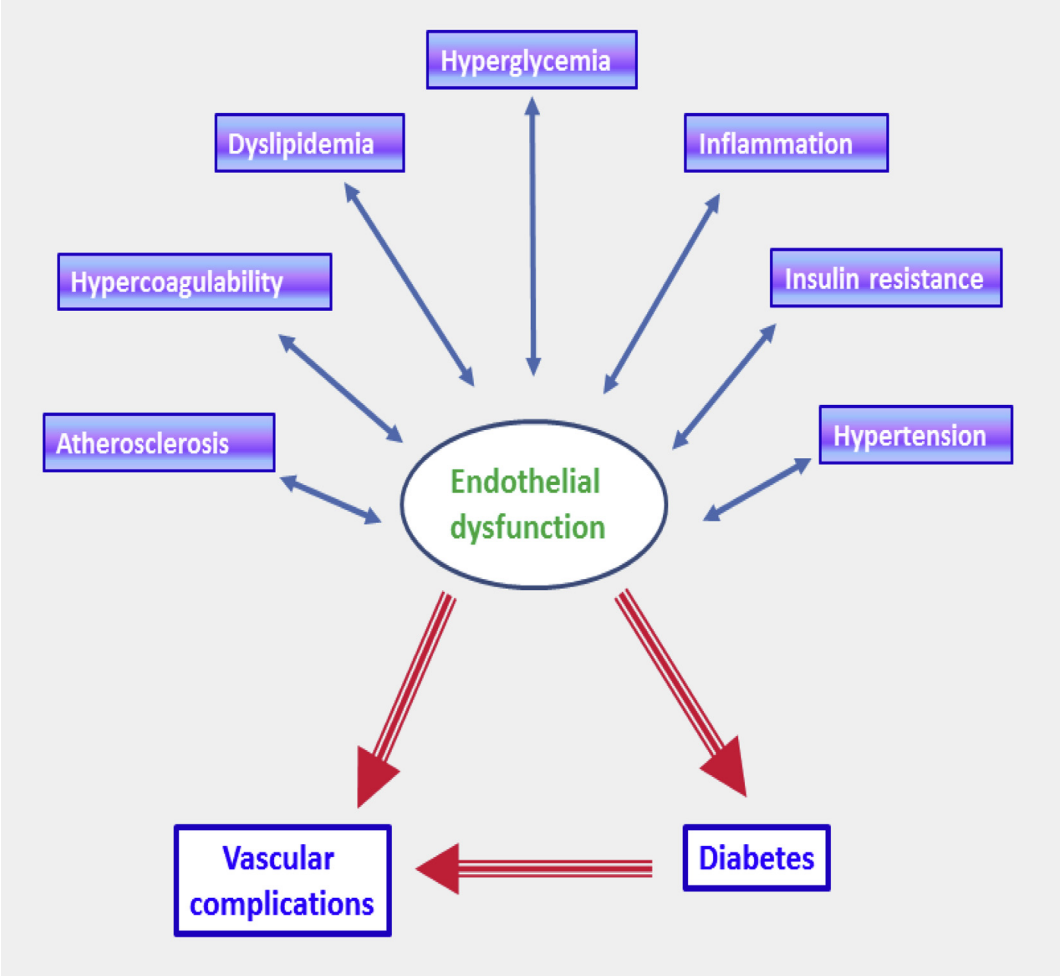

Fig. 1. Causes and consequences of endothelial dysfunction: diabetes and its vascular complications.

glycation end-products (AGEs) [11,12].

Additionally, activation of the receptor for advanced glycation end products (RAGE) on endothelial cells (ECs) influences ROS production and leads to nuclear factor kappaB (NFκB) activation, which induces atherogenic gene expression [13,14]. Collectively, these mechanisms have been shown to increase the proton gradient across the inner mitochondrial membrane, consequently increasing $O_2^{\bullet -}$. Superoxide dismutase-enabled inhibition of mitochondrial ROS is capable of inhibiting increases in all these mechanisms [12].

1.2.2. Insulin resistance - associated mechanisms

Insulin resistance, another major symptom of diabetes, is characterized by specific impairment in phosphoinositide 3-kinase (PI3K)-dependent signaling pathways, whereas other insulin-signaling branches, including Ras/mitogen-activated protein kinase (MAPK)—dependent pathways, are unaffected [15,16,9]. Also, insulin resistance inhibits eNOS phosphorylation by down-regulating the PI3K/Akt pathway in the vascular endothelium, decreasing NO production and thus its bioavailability [17]. In addition, insulin resistance leads to increased secretion of vasoconstrictor endothelin-1 (ET-1), a characteristic of endothelial dysfunction [9]. Moreover, it has been shown that the association of obesity in type 2 diabetic patients alters structure and function of periumbilical adipose tissue arterioles by decreasing eNOS expression and the levels of insulin receptor substrate 1 and 2, PI3K and Akt in the insulin signaling pathway [18].

1.2.3. Dyslipidemia - associated mechanisms

Atherogenic dyslipidemia is a reliable predictor of cardiovascular risk and its pharmacological modulation reduces vascular events in subjects with type 2 diabetes and metabolic syndrome [19-21]. Atherogenic dyslipidemia is characterized by 3 lipoprotein abnormalities: elevated levels of both very-low-density lipoproteins (VLDL) and small and dense low-density lipoprotein (LDL) particles, with low levels of high-density lipoprotein (HDL) cholesterol [22].

In patients with diabetes, LDL particles can also become glycated, have a lengthened half-life which therefore increases their ability to promote atherogenesis [23].

Moreover, oxidized LDL is pro-atherogenic and produces several abnormal biological responses, such as attracting leukocytes to the intima of vessels, improving the ability of leukocytes to ingest lipids and differentiate into foam cells, and stimulating the proliferation of ECs, leukocytes, and smooth muscle cells. All of these are important steps in the formation of atherosclerotic plaque [6]. It has been found that the lipid composition in the coronary atherosclerotic lesions of patients with diabetes mellitus is greater than in patients without diabetes suggesting increased vulnerability in such plaque with higher risk for rupture [24].

1.2.4. Inflammation – associated mechanisms

Diabetes is associated with a systemic inflammatory state that impairs the endothelial function and contributes to atherosclerosis [25]. Elevated levels of circulating inflammatory markers, including C-reactive protein (CRP), tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), and intercellular adhesion molecule 1 (ICAM-1), are observed in patients with diabetes and obesity and are believed to confer a pathological EC phenotype [26-28]. Furthermore, increased levels of inflammatory markers predict cardiovascular risk in diabetic patients [29] and also relate to the incidence of new diabetes [30-33]. Currently, a diverse range of harmful stimuli have been shown to contribute to vascular complications in diabetes: pro-inflammatory cytokines (IL-1, -6, -18, TNF-α, CRP), chemokines [monocyte chemoattractant protein-1 (MCP-1), fractalkine, regulated on activation, normal T cell expressed and secreted (RANTES)], adhesion molecules [ICAM-1, vascular cell adhesion molecule-1 (VCAM1), E-selectin] and transcription factors (e.g. NFκB) [34,35]. It has been shown that TNF-α stimulates the expression of CRP which down-regulates eNOS and increases the production of adhesion molecules and ET-1 [36,37]. Also, studies in cultured ECs and experimental animals support links between activation of NFκB, development of an inflammatory phenotype, insulin resistance, and impaired NO bioactivity [38,39].

1.2.5. Hypercoagulability - associated mechanisms

Diabetes is also related to a hypercoagulable state. Blood coagulability is crucially important in ischemic cardiovascular events because the majority of myocardial infarction (MI) and stroke events are caused by the rupture of atherosclerotic plaque and the resulting occlusion of a major artery by a blood clot (thrombus) [6].

Up to 80% of patients with diabetes die by a thrombotic disorder. Seventy-five percent of these deaths are the result of an MI, and the remainder is the result of cerebrovascular events and complications related to PVD [40]. The first line of defense against a thrombotic event is the vascular endothelium. The endothelium and the blood components are intricately linked, such that clotting signals initiated in the ECs can activate platelets and other blood components, and vice versa [41]. Patients with diabetes exhibit enhanced activation, adhesion, and aggregation of platelets, increased numbers of circulating platelet aggregates, and high levels of clotting factors (including fibrinogen, factor VII, factor VIII, factor XI, factor XII, kallikrein, and von Willebrand factor) and platelet activation products (such as beta-thromboglobulin, platelet factor 4, and thromboxane B_2) [42,43,44]. Moreover, platelets in type 2 diabetes mellitus adhere to vascular endothelium and aggregate more readily than those in healthy people. The major defect in platelet function is its loss of sensitivity to prostacyclin and NO released by the vascular endothelium [45]. Coagulation activation markers, such as prothrombin activation fragment $1+2$ and thrombin—antithrombin complexes, are also elevated in diabetes [6]. The levels of tissue factor (TF), the key initiator of the coagulation cascade, are elevated under diabetic conditions [46]. Conversely, anticoagulant mechanisms are diminished in diabetes. The fibrinolytic system, the primary means of removing clots, is relatively inhibited in diabetes both as a consequence of previous formation of abnormal clot structures that are more resistant to degradation, and also because of the increased levels of plasminogen activator inhibitor (PAI)-1 [47]. In addition, increased levels of tissue plasminogen activator (t-PA) have been observed in patients with diabetes [48,49]. Given that t-PA activates plasminogen and is thus responsible for the fibrinolysis of fibrin clots, elevated t-PA levels could have a beneficial effect. However, several studies have demonstrated an association between elevated t-PA levels and cardiovascular disease [50-52].

Consequently, the discovery and understanding of mechanisms of endothelial dysfunction in diabetes may lead to new management strategies for the prevention of cardiovascular disease in diabetes. Thus, treating endothelial dysfunction is an important step in preventing the vascular complications associated with all forms of diabetes mellitus.

2. Microparticles as novel effectors of the pathogenesis of vascular complications in diabetes

Microparticles (MPs) known as small vesicles (between 0.1 and 1 μm in diameter) are distinguished from other groups of cell-derived vesicles (such as exosomes and apoptotic bodies), released into body fluids from various cell types including platelets, endothelial cells, erythrocytes, monocytes, lymphocytes, and

leucocytes upon apoptosis or activation. In their lifetime, the cells subjected to different stimuli as physiological agonists, shear stress or apoptotic stimulation, release MPs, but this phenomenon is aggravated under pathological conditions such as inflammation or other cellular stress, which enhance MP production. MPs were shown to differ in composition between samples and between MP size classes [53,54]. MPs contain biological materials selectively assimilated from their parental cell, which includes bioactive lipids, integrins, cytokines, enzymes, mRNA, micro-RNAs (miRNAs), and transcription factors (e.g. peroxisome proliferator-activated receptor gamma, PPARγ) [55,56]. Packaging mechanisms for MPs have not yet been completely identified and such studies are necessary for the understanding of MPs' influences on their environment [56].

Differences in circulating levels of MPs, resulting from the active balance between MP generation and clearance, have been detected in the plasma of healthy subjects and patients with cardiovascular disorders, providing important clinical information [57]. Elevated levels of circulating MPs are associated with a number of cardiovascular and inflammatory pathologies (e.g. atherosclerosis, heart failure, thrombosis, acute myocardial infarction, diabetes, hypertension, and metabolic syndrome) making them useful biomarkers for monitoring disease activity [58-62]. MPs' potential as biomarkers has gained the interest for detection of MP subpopulations in human plasma during the past two decades [63]. Considered as remnants of parental cell injury, MP levels are easily measurable in body fluids using different techniques such as protein concentration, ELISA assays, or flow cytometry associated with their procoagulant activity [64-66]. Their identification relies on the presence of externalized phosphatidylserine and specific markers from the parental cell membrane. In patients with diabetes mellitus it was found that elevated circulating levels of various MPs predict cardiovascular outcome [58,67–69].

Once MPs are released into the circulation, they can interact with other blood cells they encounter, such as lymphocytes, leukocytes, platelets, and with ECs in three specific modes: (1) surface receptor signaling, (2) plasma membrane fusion, or (3) internalization [70]. Membrane fusion or internalization of MPs provides the MP internal composition transfer (such as lipids, miRNAs, and protein) into the recipient cell cytoplasm that can reprogram these cells [71,72,56]. Thus, MPs have a key role in intercellular communication and represent a delivery mechanism for efficient and effective transfer of biological information in target cells. They act as biological vectors modulating coagulation, endothelial dysfunction, inflammation, and angiogenesis, which are essential in the development of diabetic vascular complications [73].

There is many experimental evidence which confirms these complex functions of MPs. Original data has shown that MPs from patients with type 2 diabetes increase the coagulation activity in ECs, and have a key role in angiogenesis and skin wound healing [68]. Ettelaie and collaborators have established that the TF-containing MPs released from AGEs or glucose-treated renal mesangial cells induce capillary formation in human dermal microvascular ECs *in vitro* [74]. Moreover, analyses of MPs from those patients compared with healthy controls, revealed elevated numbers of TF-bearing MPs. These, correlated with parameters of the metabolic syndrome, suggest a role for MPs in the genesis of the prothrombotic profile in diabetes [69,75]. Additionally, MPs are postulated to influence their environment by scavenging NO, generating ROS, cleaving cellular surface proteins via metalloproteinases, signaling cells via surface proteins, and sometimes exacerbating inflammation [76]. Also, impairing NO release by circulating MPs could amplify the endothelial dysfunction in diabetes [77]. Interestingly, the elevated endothelial-derived microparticle (EMP) levels are predictive in identifying a subpopulation of diabetic patients without typical anginal symptoms who have angiographic evidence of CAD [78]. Moreover, another study has suggested that EMPs could be used as a surrogate marker of unstable plaques and might help to improve cardiovascular prediction in patients with type 2 diabetes at intermediate risk [79]. Besides EMPs, endothelial progenitor cell (EPCs) - derived MPs (EPCs-MPs) could be used as predictive biomarkers for ischemic stroke complications in diabetes [80].

However, the potential mechanisms involved in the increased MP release in diabetes remain unknown and there is no currently available information regarding the effect of insulin or AGE-proteins [77]. A study on obese leptin receptor deficient *db/db* diabetic mice showed that increased EMPs and EPCs-MPs are positively correlated with blood glucose concentration and infarct volume after ischemic cerebral injury [80]. Also, elevated levels of EMPs and platelet-derived-MPs (PMPs) have been correlated with increased levels of oxidized LDL in plasma of type 2 diabetic patients, suggesting that oxidized LDL contributes to endothelial membrane vesiculation [81]. Moreover, high levels of remnant lipoproteins in type 2 diabetic patients were associated with high plasma PMPs, suggesting that reducing elevated lipoproteins with lipid-lowering therapy may be an effective strategy to prevent MPs related-thrombogenic vascular complications in type 2 diabetes [77].

Subsequently, MPs are involved in the progression of macro and microvascular complications in diabetes, and the differential risk of MPs on macroangiopathy (cerebrovascular disease, coronary artery disease, peripheral artery disease) and microangiopathy (retinopathy, nephropathy, peripheral neuropathy) in patients with diabetes has been evaluated in several scientific studies.

Fig. 2 indicates the pathological role of MPs in the development of vascular complications in diabetes.

2.1. MPs and macro and microvascular complications in diabetes

2.1.1. MPs and macrovascular complications

In type 2 diabetic patients with *macrovascular complications* including *coronary, peripheral and cerebrovascular disease,* the increased levels of circulating MPs were reported, such as PMPs [82], $CD31^+$/annexin V^+ EMPs [69,83,84], $CD31^+/CD42b^-$ EMPs [84], and $CD144^+$ EMPs [85,79]. An *in vivo* study also demonstrated that the levels of circulating EMPs and EPCs-MPs are increased in diabetic animal models with ischemic stroke [80]. Moreover, MPs 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*.

Notably, it has been demonstrated that EMP ($CD31^+/CD42b^-$, $CD31^+/annexinV^+$) levels are higher in patients with macroangiopathy than in patients with microangiopathy and no complications [84]. Thus, the EMP quantification could differentiate the risk of diabetic vascular complications. The authors of this study concluded that, the EMP levels could be independently associated with macroangiopathy in diabetic patients [84]. Moreover, EMPs have been identified as an independent predictor of cardiovascular events in patients with stable CAD and may be useful for risk stratification [69]. Bernard and collaborators reported an association between circulating $CD144^+$ EMPs and unstable coronary plaques in type 2 diabetic patients [79]. In addition, Koga and collaborators showed that elevated levels of $CD144^+$ EMPs are predictive for the presence of coronary artery lesions [85]. Compared with traditional cardiovascular biomarkers, EMPs are the most significant independent risk factor, relative to lipid levels, CRP, duration of diabetes, and the occurrence of hypertension, especially in diabetic patients without typical angina symptoms [85]. Another study compared MPs' diagnostic value with traditional cardiovascular biomarkers and concluded that PMPs are more closely

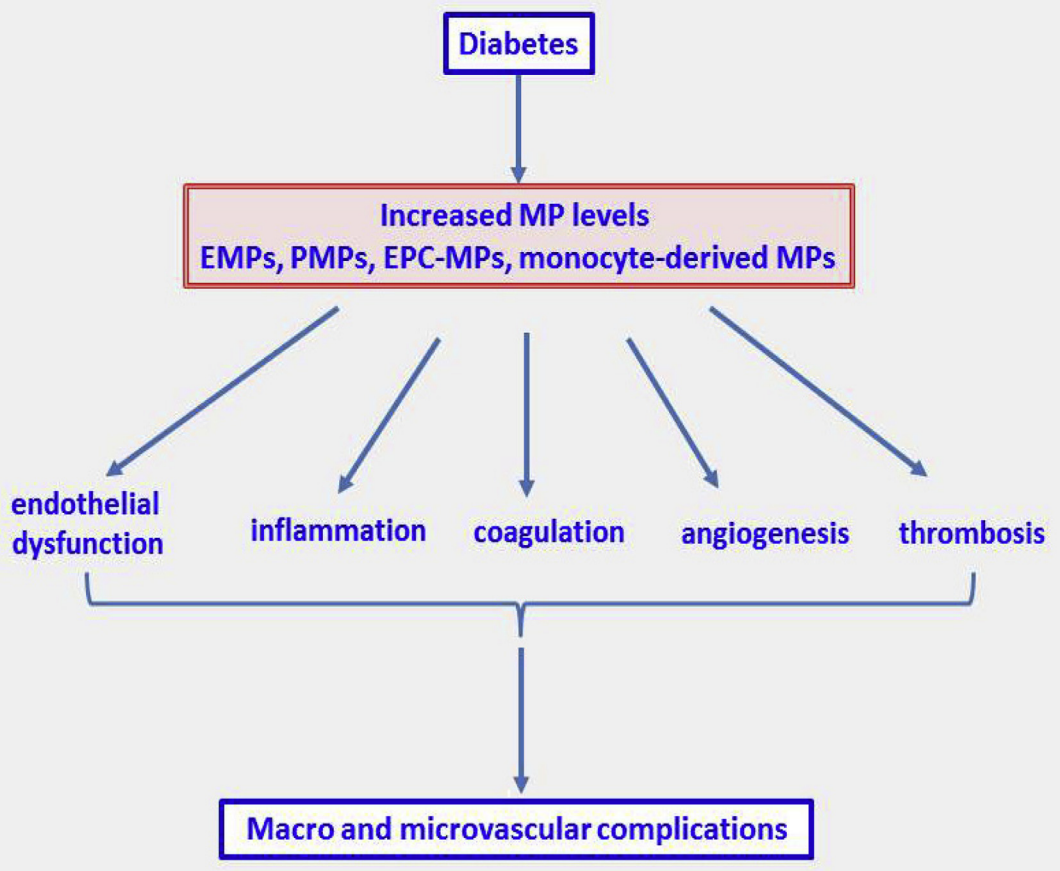

Fig. 2. Complex functions of MPs in diabetes: enhanced levels of MPs contribute to development of micro and macrovascular complications.

correlated with metabolic syndrome than CRP [86]. Curtis and collaborators showed that the ratio of MPs to EPCs is more informative than many standard individual biomarkers commonly used to stratify individuals at heightened cardiovascular risk [87]. The *in vitro* experiments demonstrated that MPs are capable of converting pentameric CRP into pro-inflammatory monomeric CRP [88]. MPs containing CRP monomers are able to bind to the surface of ECs and generate pro-inflammatory signals *in vitro*, suggesting a potential role of MPs in transport and delivery of pro-inflammatory CRP monomers in vascular disease [88].

Therefore, elevated levels of circulating MPs may be an indicator and a useful risk stratification tool for diabetic macrovascular complications. Moreover, circulating MPs may be potential pathogenic factors that impair ECs and increase atherosclerotic plaque instability [89].

2.1.2. *MPs and microvascular complications*

In both type 1 and 2 diabetes, the *microvascular complications including retinopathy, nephropathy, peripheral neuropathy,* have been associated with increased levels of circulating MPs.

Accordingly, the levels of PMPs and monocyte-derived MPs have been shown to correlate with the presence of diabetic complications, gradually increasing 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 occlusion than in patients without areas of capillary occlusion [90,91]. The levels of monocyte-derived MPs have been positively correlated with PMPs, activated platelets, and adhesion molecules in diabetic patients, and consequently they have been proposed as a prognostic factor for diabetic retinopathy progression [91]. Also, it has been found that the high levels of PMPs stimulate the coagulation cascade and increase the adhesion of leukocyte and ECs [90]. In contrast, another study reported that MPs from diabetic retinopathy cohorts are less pro-coagulant than those from type 2 diabetic patients with CAD and diabetic foot ulcers [68]. Another study has identified MPs of endothelial, platelet, photoreceptor, and microglial origin in vitreous samples from diabetic patients, and showed that EMPs are the most abundant MP subpopulation [92]. Also, this study indicates that vitreous MPs stimulate the endothelial proliferation *in vitro* and new vessel formation in a matrigel plug model *in vivo*, which suggests that vitreous MPs may contribute to the progression of diabetic retinopathy. Moreover, proliferative diabetic retinopathy is associated with a specific increase in local shedding of EMPs originating from new vessels [92]. Also, the abnormal expression of miRNA in MPs has been associated with neoangiogenesis [93]. Thus, the underlying role of circulating MPs in diabetic retinopathy pathogenesis may be based on their ability to convey angiogenic and inflammatory signals.

In addition, high levels of MPs have been detected in the renal biopsy tissue obtained from patients with various renal diseases, including diabetic nephropathy [94]. A few studies have found increased levels of monocyte-derived MPs [95,96], EMPs [81], and PMPs [97-99] in patients with diabetic nephropathy and suggested that they may serve as biomarkers for nephropathy progression in type 2 diabetes [95,96,81]. Moreover, several studies have reported that circulating PMPs [97-99], monocyte-derived MPs [95,96], EMPs [81], and MPs derived from renal mesangial cells [74] may act as mediators and influence the glomerular endothelial function by stimulating the release of cytokines and the expression of various

adhesion molecules by ECs, inducing the morphological changes that lead to angiogenesis induction in microvascular ECs.

Furthermore, it was found that type 2 diabetic patients with neuropathy have increased plasma concentrations of monocyte-derived MP [95], while type 1 diabetic patients suffering from one or more microvascular complications, including neuropathy, display higher levels of EMPs, compared to those without diabetic complications [100]. Therefore, elevated levels of circulating MPs may play a pathological role in the progression of microvascular complications in type 2 diabetes, by stimulating coagulation, endothelial inflammation and dysfunction, as well as angiogenesis [89].

Accordingly, MPs may become novel therapeutic targets or biomarkers used to monitor the progression of macro, microvascular complications, and also the therapeutic response to their treatments.

3. Microparticle associated microRNAs as modulators in the vascular complications in diabetes

MicroRNAs (miRNAs) are a newly identified class of small non-coding RNAs (21–24 nt) which can be released by cells and taken up by vascular cells, modulating their cellular biology.

MiRNAs may exit in two ways: (1) by passive leakage from necrotic or apoptotic cells [101]; (2) by active secretion from living cells within microvesicles (that are exosomes, microparticles, and apoptotic bodies) or in RNA-lipid/protein complexes [102].

The extracellular miRNAs could be degraded by ribonuclease [103], but encapsulation into microvesicles or complexing with transporting proteins (Ago2)protect them from degradation and hence make them stable in blood circulation [104,105].

There are studies revealing that miRNAs serve as messengers between cells [106-108] and are key players in the pathogenesis of hyperglycemia-induced vascular damage [109,110]. These small non-coding RNAs orchestrate the different aspects of diabetic vascular disease by regulating gene expression at the post-transcriptional level. In patients with type 2 diabetes, microarray studies have described an altered profile of miRNAs expression playing a role in angiogenesis, vascular repair, and endothelial homeostasis [111-113]. Circulating miRNAs may thus have the potential to reflect endothelial function or provide non-invasive insights into plaque vulnerability. However, less is known about the use of circulating miRNAs as biomarkers to detect early stages of atherosclerosis or atherosclerotic plaque characteristics [63].

The presence of miRNAs in MPs has led to the intriguing idea that circulating miRNAs could play a role in cell-to-cell communication. Currently, this is an intense area of investigation, and there are studies revealing that miRNAs may indeed function as mediators of cell-to-cell communication linking disparate cell types, diverse biological mechanisms, and homeostatic pathways [108,107,114]. Furthermore, some miRNAs have been found to be selectively accumulated inside the released MPs suggesting an organized package of miRNAs derived from their parental cells.

Thus, with the development of epigenetics, MPs have been drawing increasing attention. It has been shown that miRNAs from MPs are transferred and accumulated into recipient cells, where they may downregulate specific targets [115]. Also, the miRNA profiles of MPs differ significantly between patients with stable and unstable CAD and between stimulated and non-stimulated cultured cells *in vitro* [116].

Currently, only one miRNA, microRNA-126 (miR-126), present in MPs has been reported to be involved in diabetes [111]. Recently, it has been shown that EMPs released from apoptotic ECs promote vascular endothelial cell repair by transferring functional miR-126 to target ECs [117]. In pathological hyperglycemic conditions, EMPs show reduced regenerative capacity, suggesting that hyperglycemia not only directly harms the endothelium, but also indirectly promotes vascular damage by altering endogenous vascular regeneration mechanisms. The explanation for this phenomenon is that, in diabetic conditions, the amount of miR-126 in EMPs is significantly lower, and the capacity of endothelial repair is reduced under *in vivo* and *in vitro* approaches. Moreover, the analysis of miR-126 expression in circulating MPs from patients with stable coronary artery disease with and without diabetes mellitus has exposed a diminished miR-126 expression in MPs from diabetic patients [117]. The endothelial cell–derived miR-126 found in EMPs has been most consistently associated with diabetes mellitus, which is interesting because miR-126 has also been one of the identified downregulated miRNAs in atherosclerotic CAD [118,119]. In addition, it has been shown that miR-126 plays an important role in maintaining endothelial cell homeostasis and vascular integrity by facilitating the vascular endothelial growth factor signaling, regulating the sprouty-related protein (SPRED1) and phosphoinositol-3 kinase regulatory subunit 2 (PIK3R2/p85-β) [120,121]. Besides, there is evidence suggesting that reduced miR-126 expression is partially responsible for the impaired vascular repair capacity in diabetes [122,123]. Moreover, a reduction in the miR-126 expression, as well as in the expression of miR-15a and miR-223, was proven to be already present many years before clinical manifestation of diabetes, which therefore suggests these miRNAs may become useful for risk prediction [124,125]. The role of other miRNAs in the endothelial dysfunction related to diabetes and in atherosclerotic plaque development has been discussed in detail in recent reviews [109,110,125-127].

These results demonstrate that MPs can deliver miRNAs into recipient cells, where the exogenous miRNAs can regulate target gene expression and the functions of recipient cells. Instead of one single type of message, MPs may manage to deliver multiple messengers, including mRNA, specific subsets of the transcripts, miRNAs, and proteins, all at one time. MPs 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 [89].

3.1. Microparticle associated microRNAs as diagnostic biomarkers and therapeutic strategies

Because of their high stability in the circulation, the ease by which miRNAs can be detected in a quantitative manner by common methods (such as real-time PCR and microarrays), the reproducibility of the results along with the consistency among individuals of same species, miRNAs are gaining a lot of interest as diagnostic biomarkers of several chronic diseases such as diabetes and cardiovascular diseases [128,125,124]. An ideal biomarker fulfills a number of criteria, such as: accessibility through noninvasive methods; a high degree of specificity and sensitivity; the ability to differentiate pathologies, allowing early detection; sensitivity to relevant changes in the disease; long half-life within the sample; and the capability for rapid and accurate detection. Circulating miRNAs are able to fulfill a number of these criteria [129]. There is increasing evidence for the potential use of specific EMP-associated miRNA in body fluids or particularly in peripheral blood as biomarkers to predict metabolic diseases [130].

The disregulation of miRNA function has been linked to diabetes, although it is not yet fully certain whether this is a cause or effect of this pathology. If miRNAs, by modifying their expression, are indeed active in the pathogenesis of diabetes and its related complications, in the restoration of normal function, they may be a therapeutic target for managing this disease. Chemically modified siRNA-like oligonucleotides have been used to decrease the miRNA

expression (antagomirs) *in vivo* [131,132]. However, due to the hypothetical transient nature of their effects, it is likely that frequent doses may be required to sustain benefit. Given the chronic nature of diabetes, this would require the need for repeated injections, with not negligible associated costs. Adeno-associated virus (AAV) vectors containing miRNA mimics have been found to promote miRNA expression *in vivo* [133]. However, for their short transgene expression, adenovirus may not be the best approach to treat diabetes and its chronic complications in the clinical arena. Another therapeutic strategy involves 'miRNA sponges'. These artificial miRNA decoys bind native miRNA to create a loss of function of a particular miRNA. These sponges contain multiple binding sites directed against a particular miRNA or against a miRNA seed sequence family [134]. The use of miRNA sponges in an AAV vector delivery system may be a potential novel strategy for miRNA therapeutics. While the first reports on miRNA therapeutics are encouraging, the fact that a typical miRNA targets several genes suggests that clinical intervention may be very complex. Moreover, the functional delivery of miRNAs to distant cells also may make MPs ideal candidates as vehicles for such therapies.

4. Future directions and conclusions

Although the field of vascular complications in diabetes research has advanced considerably in the past years, this research area is still fraught with a number of challenges. Many of the challenges are to find out the mechanisms linking between diabetes and cardiovascular disease, new mediators and biomarkers to generate new management strategies for prevention and therapy.

There is now accumulating evidence on the multiple faces of MPs as conveyors of cell information with major role in inflammation, thrombosis and angiogenesis. They can exert functional effects on target cells through a number of currently known mechanisms. In addition, miRNA content within MPs elicited many questions concerning their pathological effects and whether they are friends or foes. The miRNAs transferred by MPs also play an important role in the regulation of biological processes involved in the vascular complications in diabetes.

These putative abilities of MPs may give scientists ideas to develop new research strategies to demonstrate and consolidate their clinical relevance. In the near future, MPs may serve as clinical diagnostic biomarkers and delivery vehicles for new therapy in the vascular complications in diabetes and other pathological states. Thus, MPs can become pharmacological targets to prevent and treat diabetes-related vascular complications. Therapeutic agents could be developed from naturally released or artificial MPs obtained by engineering their specific components from their original cells, such as under-expressed or over-expressed miRNAs (depending on their expression in a certain pathological state). It is currently hoped that addressing MPs as targets for diagnostic and therapy in diabetes will favorably modify the risk for vascular complications and survival.

In conclusion, a better understanding of the regulatory role of MPs and their associated miRNAs in the pathogenesis of vascular complications in diabetes may unveil new avenues and targets for therapeutic control.

This review will undoubtedly uncover new research directions and provide novel insights into the world of MPs with a role in both the intracellular communication and the modulation of vascular disorders in diabetes.

Conflict of interest

The authors confirm that this article content has no conflicts of interest.

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References

[1] F. Paneni, J.A. Beckman, M.A. Creager, F. Cosentino, Diabetes and vascular disease: pathophysiology, clinical consequences, and medicaltherapy: part I, Eur. Heart J. 34 (2013) 2436–2443.
[2] P.W. Wilson, R.B. D'Agostino, D. Levy, A.M. Belanger, H. Silbershatz, W.B. Kannel, Prediction of coronary heart disease using risk factor categories, Circulation 97 (1998) 1837–1847.
[3] P.W. Wilson, Diabetes mellitus and coronary heart disease, Am. J. Kidney Dis. 32 (1998) S89–S100.
[4] H.C. McGill Jr., C.A. McMahan, Determinants of atherosclerosis in the young: pathobiological determinants of atherosclerosis in youth (PDAY) research group, Am. J. Cardiol. 82 (1998) 30T–36T.
[5] V. Brezinka, I. Padmos, Coronary heart disease risk factors in women, Eur. Heart J. 15 (1994) 1571–1584.
[6] B.B. Dokken, The pathophysiology of cardiovascular disease and diabetes: beyond blood pressure and lipids, Diabetes Spectr. 21 (2008) 160–165.
[7] C.M. Sena, A.M. Pereira, R. Seiça, Endothelial dysfunction - a major mediator of diabetic vascular disease, Biochim. Biophys. Acta 1832 (2013) 2216–2231.
[8] C. Tabit, W. Chung, N. Hamburg, J.A. Vita, Endothelial dysfunction in diabetes mellitus: molecular mechanisms and clinical implications, Rev. Endocr. Metab. Disord. 11 (2010) 61–74.
[9] J. Xu, M.-H. Zou, Molecular insights and therapeutic targets for diabetic endothelial dysfunction, Circulation 120 (2009) 1266–1286.
[10] M.A. Creager, T.F. Luscher, F. Cosentino, J.A. Beckman, Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I, Circulation 108 (2003) 1527–1532.
[11] T. Nishikawa, D. Edelstein, X.L. Du, S. Yamagishi, T. Matsumura, Y. Kaneda, M.A. Yorek, D. Beebe, P.J. Oates, H.P. Hammes, I. Giardino, M. Brownlee, Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage, Nature 404 (2002) 787–790.
[12] M. Brownlee, Biochemistry and molecular cell biology of diabetic complications, Nature 414 (2001) 813–820.
[13] M.-P. Wautier, O. Chappey, S. Corda, D.M. Stern, A.M. Schmidt, J.L. Wautier, Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression via RAGE, Am. J. Physiol. Endocrinol. Metab. 280 (2001) E685–E694.
[14] A.M. Schmidt, O. Hori, J. Brett, S.D. Yan, J.L. Waitier, D. Stern, Cellular receptors for advanced glycation end products. Implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions, Arterioscler. Thromb. 14 (1994) 1521–1528.
[15] Z.Y. Jiang, Y.-W. Lin, A. Clemont, E.P. Feener, K.D. Hein, M. Igarashi, T. Yamauchi, M.F. White, G.L. King, Characterization of selective resistance to insulin signaling in the vasculature of obese Zucker (fa/fa) rats, J. Clin. Invest. 104 (1999) 447–457.
[16] K. Cusi, K. Maezono, A. Osman, M. Pendergrass, M.E. Patti, T. Pratipanawatr, R.A. DeFronzo, C.R. Kahn, L.J. Mandarino, Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle, J. Clin. Invest. 105 (2000) 311–320.
[17] G. Zeng, F.H. Nystrom, L.V. Ravichandran, L.N. Cong, M. Kirby, H. Mostowski, M.J. Quon, Roles for insulin receptor, PI3-kinase, and Akt in insulin-signaling pathways related to production of nitric oxide in human vascular endothelial cells, Circulation 101 (2000) 1539–1545.
[18] A. Georgescu, D. Popov, A. Constantin, M. Nemecz, N. Alexandru, D. Cochior, A. Tudor, Dysfunction of human subcutaneous fat arterioles in obesity alone or obesity associated with Type 2 diabetes, Clin. Sci. 120 (2011) 463–472.
[19] M. Lee, J.L. Saver, A. Towfighi, J. Chow, B. Ovbiagele, Efficacy of fibrates for cardiovascular risk reduction in persons with atherogenic dyslipidemia: a meta-analysis, Atherosclerosis 217 (2011) 492–498.
[20] J.C. Fruchart, F. Sacks, M.P. Hermans, G. Assmann, W.V. Brown, R. Ceska, M.J. Chapman, P.M. Dodson, P. Fioretto, H.N. Ginsberg, T. Kadowaki, J.M. Lablanche, N. Marx, J. Plutzky, Z. Reiner, R.S. Rosenson, B. Staels, J.K. Stock, R. Sy, C. Wanner, A. Zambon, P. Zimmet, The residual risk reduction initiative: a call to action to reduce residual vascular risk in patients with dyslipidemia, Am. J. Cardiol. 102 (2008) 1K–34K.
[21] M. Arca, A. Montali, S. Valiante, F. Campagna, G. Pigna, V. Paoletti, R. Antonini, F. Barilla, G. Tanzilli, A. Vestri, C. Gaudio, Usefulness of atherogenic dyslipidemia for predicting cardiovascular risk in patients with angiographically defined coronary artery disease, Am. J. Cardiol. 100 (2007) 1511–1516.
[22] S.M. Grundy, I.J. Benjamin, G.L. Burke, A. Chait, R.H. Eckel, B.V. Howard, W. Mitch, S.C. Smith Jr., J.R. Sowers, Diabetes and cardiovascular disease: a statement for healthcare professionals from the American Heart Association, Circulation 100 (1999) 1134–1146.

[23] C. Napoli, M. Triggiani, G. Palumbo, M. Condorelli, M. Chiariello, G. Ambrosio, Glycosylation enhances oxygen radical-induced modifications and decreases acetylhydrolase activity of human low density lipoprotein, Basic Res. Cardiol. 92 (1997) 96–105.
[24] P.R. Moreno, A.M. Murcia, I.F. Palacios, M.N. Leon, V.H. Bernardi, V. Fuster, J.T. Fallon, Coronary composition and macrophage infiltration in atherectomy specimens from patients with diabetes mellitus, Circulation 102 (2000) 2180–2184.
[25] J.A. Beckman, M.A. Creager, P. Libby, Diabetes and atherosclerosis: epidemiology, pathophysiology, and management, J. Am. Med. Assoc. 287 (2002) 2570–2581.
[26] J.F. Keaney Jr., J.M. Massaro, M.G. Larson, R.S. Vasan, P.W. Wilson, I. Lipinska, D. Corey, P. Sutherland, J.A. Vita, E.J. Benjamin, Heritability and correlates of intercellular adhesion molecule-1 in the Framingham offspring study, J. Am. Coll. Cardiol. 44 (2004) 168–173.
[27] B. Vozarova, C. Weyer, K. Hanson, P.A. Tataranni, C. Bogardus, R.E. Pratley, Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion, Obes. Res. 9 (2001) 414–417.
[28] A. Festa, R. D'Agostino Jr., G. Howard, L. Mykkanen, R.P. Tracy, S.M. Haffner, Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS), Circulation 102 (2000) 42–47.
[29] M.B. Schulze, E.B. Rimm, T. Li, N. Rifai, M.J. Stampfer, F.B. Hu, C-reactive protein and incident cardiovascular events among men with diabetes, Diabetes Care 27 (2004) 889–894.
[30] J.B. Meigs, F.B. Hu, N. Rifai, J.E. Manson, Biomarkers of endothelial dysfunction and risk of type 2 diabetes mellitus, J. Am. Med. Assoc. 291 (2004) 1978–1986.
[31] A.D. Pradhan, J.E. Manson, N. Rifai, J.E. Buring, P.M. Ridker, C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus, J. Am. Med. Assoc. 286 (2001) 327–334.
[32] M.I. Schmidt, B.B. Duncan, A.R. Sharrett, G. Lindberg, P.J. Savage, S. Offenbacher, M.I. Azambuja, R.P. Tracy, G. Heiss, Markers of inflammation and prediction of diabetes mellitus in adults (atherosclerosis risk in communities study): a cohort study, Lancet 353 (1999) 1649–1652.
[33] B.B. Duncan, M.I. Schmidt, J.S. Pankow, C.M. Ballantyne, D. Couper, A. Vigo, R. Hoogeveen, A.R. Folsom, G. Heiss, Atherosclerosis Risk in Communities Study, Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study, Diabetes 52 (2003) 1799–1805.
[34] J.M. Forbes, M.E. Cooper, Mechanisms of diabetic complications, Physiol. Rev. 93 (2013) 137–188.
[35] A.C. Roberts, K.E. Porter, Cellular and molecular mechanisms of endothelial dysfunction in diabetes, Diab. Vasc. Dis. Res. 10 (2013) 472–482.
[36] J.A. Kim, M. Montagnani, K.K. Koh, M.J. Quon, Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms, Circulation 113 (2006) 1888–1904.
[37] C. Cardillo, U. Campia, M.B. Bryant, J.A. Panza, Increased activity of endogenous endothelin in patients with type II diabetes mellitus, Circulation 106 (2002) 1783–1787.
[38] F. Kim, K.A. Tysseling, J. Rice, M. Pham, L. Haji, B.M. Gallis, A.S. Baas, P. Paramsothy, C.M. Giachelli, M.A. Corson, E.W. Raines, Free fatty acid impairment of nitric oxide production in endothelial cells is mediated by IKKbeta, Arterioscler. Thromb. Vasc. Biol. 25 (2005) 894–989.
[39] F. Kim, M. Pham, E. Maloney, N.O. Rizzo, G.J. Morton, B.E. Wisse, E.A. Kirk, A. Chait, M.W. Schwartz, Vascular inflammation, insulin resistance, and reduced nitric oxide production precede the onset of peripheral insulin resistance, Arterioscler. Thromb. Vasc. Biol. 28 (2008) 1982–1988.
[40] K. Gu, C.C. Cowie, M.L. Harris, Mortality in adults with and without diabetes in a national cohort of the US population, 1971–1993, Diabetes Care 21 (1998) 1138–1145.
[41] A.S. Weyrich, S.M. Prescott, G.A. Zimmerman, Platelets, endothelial cells, inflammatory chemokines, and restenosis: signaling in the vascular play book, Circulation 106 (2002) 1433–1435.
[42] N. Alexandru, D. Popov, A. Sbarcea, M. Amuzescu, Platelet free cytosolic calcium concentration during ageing of type 2 diabetic patients, Platelets 18 (2007), 280–473.
[43] N. Alexandru, I. Jardín, D. Popov, M. Simionescu, J. García-Estañ, G.M. Salido, J.A. Rosado, Effect of homocysteine on calcium mobilisation and platelet function in type 2 diabetes mellitus, J. Cell. Mol. Med. 12 (2008) 2015–2026.
[44] J.L. Ferreiro, J.A. Gómez-Hospital, D.J. Angiolillo, Platelet abnormalities in diabetes mellitus, Diabetes Vasc. Dis. Res. 7 (2010) 251–259.
[45] R. Maiti, N.K. Agrawal, Atherosclerosis in diabetes mellitus: role of inflammation, Indian J. Med. Sci. 61 (2007) 292–306.
[46] G. Boden, V.R. Vaidyula, C. Homko, P. Cheung, A.K. Rao, Circulating tissue factor procoagulant activity and thrombin generation in patients with type 2 diabetes: effects of insulin and glucose, J. Clin. Endocrinol. Metab. 92 (2007) 4352–4358.
[47] M.E. Carr, Diabetes: a hypercoagulable state, J. Diabetes Complicat. 15 (2001) 44–54.
[48] K. Takanashi, T. Inukai, Insulin resistance and changes in the blood coagulation-fibrinolysis system after a glucose clamp technique in patients with type 2 diabetes mellitus, J. Med. 31 (2000) 45–62.
[49] M.C. Eliasson, J.H. Jansson, B. Lindahl, B. Stegmayr, High levels of tissue plasminogen activator (TPA) antigen precede the development of type 2 diabetes in a longitudinal population study. The northern Sweden MONICA study, Cardiovasc. Diabetol. 2 (2003) 19.
[50] D. Tousoulis, C. Antoniades, E. Bosinakou, M. Kotsopoulou, C. Tsoufis, K. Marinou, M. Charakida, E. Stefanadi, M. Vavuranakis, G. Latsios, C. Stefanadis, Differences in inflammatory and thrombotic markers between unstable angina and acute myocardial infarction, Int. J. Cardiol. 115 (2007) 203–207.
[51] A. Nordenhem, K. Leander, J. Hallqvist, U. de Faire, M. Sten-Linder, B. Wiman, The complex between tPA and PAI-1: risk factor for myocardial infarction as studied in the sheep project, Thromb. Res. 116 (2005) 223–232.
[52] P.M. Mannucci, L. Bernardinelli, L. Foco, M. Galli, F. Ribichini, M. Tubaro, F. Peyvandi, Tissue plasminogen activator antigen is strongly associated with myocardial infarction in young women, J. Thromb. Haemost. 3 (2005) 280–286.
[53] W.L. Dean, M.J. Lee, T.D. Cummins, D.J. Schultz, D.W. Powell, Proteomic and functional characterization of platelet microparticle size classes, Thromb. Haemost. 102 (4) (2009) 711–718.
[54] P. Bastos-Amador, F. Royo, E. Gonzalez, J. Conde-Vancells, L. Palomo-Diez, F.E. Borras, et al., Proteomic analysis of microvesicles from plasma of healthy donors reveals high individual variability, J. Proteom. 75 (12) (2012) 3574–3584.
[55] L.V. Norling, J. Dalli, Microparticles are novel effectors of immunity, Curr. Opin. Pharmacol. 13 (2013) 570–575.
[56] K.L. Lannan, J. Sahler, N. Kim, S.L. Spinelli, S.B. Maggirwar, O. Garraud, F. Cognasse, N. Blumberg, R.P. Phipps, Breaking the mold: transcription factors in the anucleate platelet and platelet-derived microparticles, Front. Immunol. 6 (2015) 48–64.
[57] A. Piccin, W.G. Murphy, O.P. Smith, Circulating microparticles: pathophysiology and clinical implications, Blood Rev. 21 (2007) 157–171.
[58] B. Feng, Y. Chen, Y. Luo, M. Chen, X. Li, Y. Ni, Circulating level of microparticles and their correlation with arterial elasticity and endothelium dependent dilation in patients with type 2 diabetes mellitus, Atherosclerosis 208 (2010) 264–269.
[59] N. Amabile, P.E. Rautou, A. Tedgui, C.M. Boulanger, Microparticles: key protagonists in cardiovascular disorders, Semin. Thromb. Hemost. 36 (2010) 907–916.
[60] A. Georgescu, N. Alexandru, E. Andrei, I. Titorencu, E. Dragan, C. Tarziu, S. Ghiorghe, E. Badila, D. Bartos, D. Popov, Circulating microparticles and endothelial progenitor cells in atherosclerosis; pharmacological effects of irbesartan, J. Thromb. Haemost. 10 (2012) 680–691.
[61] A. Georgescu, N. Alexandru, M. Nemecz, I. Titorencu, D. Popov, Irbesartan administration therapeutically influences circulating endothelial progenitor cell and microparticle mobilization by involvement of pro-inflammatory cytokines, Eur. J. Pharmacol. 711 (2013) 27–35.
[62] M. Baron, C.M. Boulanger, B. Staels, A. Tailleux, Cell-derived microparticles in atherosclerosis: biomarkers and targets for pharmacological modulation? J. Cell. Mol. Med. 16 (2012) 1365–1376.
[63] I.E. Hoefer, S. Steffens, M. Ala-Korpela, M. Bäck, L. Badimon, M.L. Bochaton-Piallat, C.M. Boulanger, G. Caligiuri, S. Dimmeler, J. Egido, P.C. Evans, T. Guzik, B.R. Kwak, U. Landmesser, M. Mayr, C. Monaco, G. Pasterkamp, J. Tuñón, C. Weber, On behalf of the ESC Working Group Atherosclerosis and Vascular Biology, Novel methodologies for biomarker discovery in atherosclerosis, Eur. Heart J. 36 (2015) 2635–2642.
[64] A. Georgescu, N. Alexandru, D. Popov, M. Amuzescu, E. Andrei, C. Zamfir, H. Maniu, A. Badila, Chronic venous insufficiency is associated with elevated level of circulating microparticles, J. Thromb. Haemost. 7 (2009) 1566–1575.
[65] E. Bădila, A.M. Daraban, S. Ghiorghe, A. Georgescu, N. Alexandru, D. Bartoş, C. Tîrziu, Rethinking cardiovascular therapy - the effect of irbesartan on circulating microparticles and endothelial progenitor cells in patients with hypertension and dyslipidemia, Farmacia 62 (2014) 93–106.
[66] E. Andrei, N. Alexandru, E. Dragan, A. Georgescu, Flow cytometric analysis of circulating microparticles and endothelial progenitor cells in plasma; a research tool for atherosclerosis and therapy, Exp. Clin. Cardiol. 20 (2014) 1554–1563.
[67] A. Tripodi, A. Branchi, V. Chantarangkul, M. Clerici, G. Merati, A. Artoni, P.M. Mannucci, Hypercoagulability in patients with type 2 diabetes mellitus detected by a thrombin generation assay, J. Thromb. Thrombolysis 31 (2011) 165–172.
[68] G. Tsimerman, A. Roguin, A. Bachar, E. Melamed, B. Brenner, A. Aharon, Involvement of microparticles in diabetic vascular complications, Thromb. Haemost. 106 (2011) 310–321.
[69] J.M. Sinning, J. Losch, K. Walenta, M. Bohm, G. Nickenig, N. Werner, Circulating $CD31^{+}$/Annexin V^{+} microparticles correlate with cardiovascular outcomes, Eur. Heart. J. 32 (2011) 2034–2041.
[70] G. Camussi, M.C. Deregibus, S. Bruno, V. Cantaluppi, L. Biancone, Exosomes/microvesicles as a mechanism of cell-to-cell communication, Kidney Int. 78 (9) (2010) 838–848.
[71] O.P. Barry, D. Pratico, J.A. Lawson, G.A. FitzGerald, Transcellular activation of platelets and endothelial cells by bioactive lipids in platelet microparticles, J. Clin. Invest. 99 (9) (1997) 2118–2127.
[72] S.F. Mause, C. Weber, Microparticles: protagonists of a novel communication network for intercellular information exchange, Circ. Res. 107 (9) (2010) 1047–1057.
[73] M. Diamant, M.E. Tushuizen, A. Sturk, R. Nieuwland, Cellular microparticles: new players in the field of vascular disease? Eur. J. Clin. Invest. 34 (2004)

392–401.
[74] C. Ettelaie, S. Su, C. Li, M.E. Collier, Tissue factor-containing microparticles released from mesangial cells in response to high glucose and AGE induce tube formation in microvascular cells, Microvasc. Res. 76 (2008) 152–160.
[75] M. Diamant, R. Nieuwland, R. Pablo, A. Sturk, J.W. Smit, J.K. Radder, Elevated numbers of tissue-factor exposing microparticles correlate with components of the metabolic syndrome in uncomplicated type 2 diabetes mellitus, Circulation 106 (2002) 2442–2447.
[76] D. Burger, S. Schock, C.S. Thompson, A.C. Montezano, A.M. Hakim, R.M. Touyz, Microparticles: biomarkers and beyond, Clin. Sci. (Lond) 124 (7) (2013) 423–441.
[77] A.S. Leroyer, A. Tedgui, C.M. Boulanger, Microparticles and type 2 diabetes, Diabetes Metab. 34 (2008) S27–S31.
[78] S. Nomura, Dynamic role of microparticles in type 2 diabetes mellitus, Curr. Diabetes Rev. 5 (2009) 245–251.
[79] S. Bernard, R. Loffroy, A. Serusclat, L. Boussel, E. Bonnefoy, C. Thevenon, M. Rabilloud, D. Revel, P. Moulin, P. Douek, Increased levels of endothelial microparticles CD144 (VE-Cadherin) positives in type 2 diabetic patients with coronary noncalcified plaques evaluated by multidetector computed tomography (MDCT), Atherosclerosis 203 (2009) 429–435.
[80] J. Chen, S. Chen, Y. Chen, C. Zhang, J. Wang, W. Zhang, G. Liu, B. Zhao, Y. Chen, Circulating endothelial progenitor cells and cellular membrane microparticles in *db/db* diabetic mouse: possible implications in cerebral ischemic damage, Am. J. Physiol. Endocrinol. Metab. 301 (2011) E62–E71.
[81] S. Nomura, A. Shouzu, S. Omoto, M. Nishikawa, T. Iwasaka, S. Fukuhara, Activated platelet and oxidized LDL induce endothelial membrane vesiculation: clinical significance of endothelial cell-derived microparticles in patients with type 2 diabetes, Clin. Appl. Thromb. Hemost. 10 (2004b) 205–215.
[82] K.T. Tan, M.H. Tayebjee, H.S. Lim, G.Y. Lip, Clinically apparent atherosclerotic disease in diabetes is associated with an increase in platelet microparticle levels, Diabet. Med. 22 (2005) 1657–1662.
[83] N. Werner, S. Wassmann, P. Ahlers, S. Kosiol, G. Nickenig, Circulating $CD31^{+}$/annexin V^{+} apoptotic microparticles correlate with coronary endothelial function in patients with coronary artery disease, Arterioscler. Thromb. Vasc. Biol. 26 (2006) 112–116.
[84] K.H. Jung, K. Chu, S.T. Lee, J.J. Bahn, J.H. Kim, M. Kim, S.K. Lee, J.K. Roh, Risk of macrovascular complications in type 2 diabetes mellitus: endothelial microparticle profiles, Cerebrovasc. Dis. 31 (2011) 485–493.
[85] H. Koga, S. Sugiyama, K. Kugiyama, K. Watanabe, H. Fukushima, T. Tanaka, T. Sakamoto, M. Yoshimura, H. Jinnouchi, H. Ogawa, Elevated levels of VE-cadherin-positive endothelial microparticles in patients with type 2 diabetes mellitus and coronary artery disease, J. Am. Coll. Cardiol. 45 (2005) 1622–1630.
[86] T. Ueba, T. Haze, M. Sugiyama, M. Higuchi, H. Asayama, Y. Karitani, T. Nishikawa, K. Yamashita, S. Nagami, T. Nakayama, K. Kanatani, S. Nomura, Level, distribution and correlates of platelet-derived microparticles in healthy individuals with special reference to the metabolic syndrome, Thromb. Haemost. 100 (2008) 280–285.
[87] A.M. Curtis, L. Zhang, E. Medenilla, M. Gui, P.F. Wilkinson, E. Hu, J. Giri, V. Doraiswamy, S. Gunda, M.E. Burgert, J.S. Moore, J.M. Edelberg, E.R. Mohler, Relationship of microparticles to progenitor cells as a measure of vascular health in a diabetic population, Cytom. B Clin. Cytom. 78 (2010) 329–337.
[88] J. Habersberger, F. Strang, A. Scheichl, N. Htun, N. Bassler, R.M. Merivirta, P. Diehl, G. Krippner, P. Meikle, S.U. Eisenhardt, I. Meredith, K. Peter, Circulating microparticles generate and transport monomeric C-reactive protein in patients with myocardial infarction, Cardiovasc. Res. 96 (2012) 64–72.
[89] Y. Wang, L. Chen, M. Liu, Microvesicles and diabetic complications - novel mediators, potential biomarkers and therapeutic targets, Acta Pharmacol. Sin. 35 (2014) 433–443.
[90] N. Ogata, M. Imaizumi, S. Nomura, A. Shozu, M. Arichi, M. Matsuoka, M. Matsumura, Increased levels of platelet-derived microparticles in patients with diabetic retinopathy, Diabetes Res. Clin. Pract. 68 (2005) 193–201.
[91] N. Ogata, S. Nomura, A. Shouzu, M. Imaizumi, M. Arichi, M. Matsumura, Elevation of monocyte-derived microparticles in patients with diabetic retinopathy, Diabetes Res. Clin. Pract. 73 (2006) 241–248.
[92] S. Chahed, A.S. Leroyer, M. Benzerroug, D. Gaucher, A. Georgescu, S. Picaud, J.S. Silvestre, A. Gaudric, A. Tedgui, P. Massin, C.M. Boulanger, Increased vitreous shedding of microparticles in proliferative diabetic retinopathy stimulates endothelial proliferation, Diabetes 59 (2010) 694–701.
[93] E. Stepien, A. Kablak-Ziembicka, J. Czyz, T. Przewlocki, M. Malecki, Microparticles, not only markers but also a therapeutic target in the early stage of diabetic retinopathy and vascular aging, Expert Opin. Ther. Targets 16 (2012) 677–688.
[94] M. Nakajima, T.D. Hewitson, D.C. Mathews, P. Kincaid-Smith, Localisation of complement components in association with glomerular extracellular particles in various renal diseases, Virchows Arch. A Pathol. Anat. Histopathol. 419 (1991) 267–272.
[95] S. Omoto, S. Nomura, A. Shouzu, M. Nishikawa, S. Fukuhara, T. Iwasaka, Detection of monocyte-derived microparticles in patients with Type II diabetes mellitus, Diabetologia 45 (2002) 550–555.
[96] S. Nomura, A. Shouzu, S. Omoto, M. Nishikawa, T. Iwasaka, Effects of losartan and simvastatin on monocyte-derived microparticles in hypertensive patients with and without type 2 diabetes mellitus, Clin. Appl. Thromb. Hemost. 10 (2004a) 133–141.
[97] A. Shouzu, S. Nomura, T. Hayakawa, S. Omoto, H. Shimizu, Y. Miyake, T. Yonemoto, S. Fukuhara, T. Iwasaka, M. Nishikawa, M. Inada, Effect of sarpogrelate hydrochloride on platelet-derived microparticles and various soluble adhesion molecules in diabetes mellitus, Clin. Appl. Thromb. Hemost. 6 (2000) 139–143.
[98] S. Kobayashi, M. Satoh, T. Namikoshi, Y. Haruna, S. Fujimoto, S. Arakawa, N. Komai, N. Tomita, T. Sasaki, N. Kashihara, Blockade of serotonin 2A receptor improves glomerular endothelial function in rats with streptozotocin-induced diabetic nephropathy, Clin. Exp. Nephrol. 12 (2008) 119–125.
[99] S. Omoto, S. Nomura, A. Shouzu, T. Hayakawa, H. Shimizu, Y. Miyake, T. Yonemoto, M. Nishikawa, S. Fukuhara, M. Inada, Significance of platelet-derived microparticles and activated platelets in diabetic nephropathy, Nephron 81 (1999) 271–277.
[100] F. Sabatier, P. Darmon, B. Hugel, V. Combes, M. Sanmarco, J.G. Velut, D. Arnoux, P. Charpiot, J.M. Freyssinet, C. Oliver, J. Sampol, F. Dignat-George, Type 1 and type 2 diabetic patients display different patterns of cellular microparticles, Diabetes 51 (2002) 2840–2845.
[101] M.H. Hoofnagle, B.R. Wamhoff, G.K. Owens, Lost in transdifferentiation, J. Clin. Invest. 113 (2004) 1249–1251.
[102] S. Ramachandran, V. Palanisamy, Horizontal transfer of RNAs: exosomes as mediators of intercellular communication, Wiley Interdiscip. Rev. 3 (2012) 286–293.
[103] P.S. Mitchell, R.K. Parkin, E.M. Kroh, B.R. Fritz, S.K. Wyman, E.L. Pogosova-Agadjanyan, A. Peterson, J. Noteboom, K.C. O'Briant, A. Allen, D.W. Lin, N. Urban, C.W. Drescher, B.S. Knudsen, D.L. Stirewalt, R. Gentleman, R.L. Vessella, P.S. Nelson, D.B. Martin, M. Tewari, Circulating microRNAs as stable blood-based markers for cancer detection, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 10513–10518.
[104] M. Busch, A. Zernecke, MicroRNAs in the regulation of dendritic cell functions in inflammation and atherosclerosis, J. Mol. Med. 90 (2012) 877–885.
[105] C. Urbich, A. Kuehbacher, S. Dimmeler, Role of microRNAs in vascular diseases, inflammation, and angiogenesis, Cardiovasc. Res. 79 (2008) 581–588.
[106] E. Hergenreider, S. Heydt, K. Treguer, T. Boettger, A.J. Horrevoets, A.M. Zeiher, M.P. Scheffer, A.S. Frangakis, X. Yin, M. Mayr, T. Braun, C. Urbich, R.A. Boon, S. Dimmeler, Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs, Nat. Cell Biol. 14 (2012) 249–256.
[107] Y. Zhang, D. Liu, X. Chen, J. Li, L. Li, Z. Bian, F. Sun, J. Lu, Y. Yin, X. Cai, Q. Sun, K. Wang, Y. Ba, Q. Wang, D. Wang, J. Yang, P. Liu, T. Xu, Q. Yan, J. Zhang, K. Zen, C.Y. Zhang, Secreted monocytic miR-150 enhances targeted endothelial cell migration, Mol. Cell 39 (2010) 133–144.
[108] A. Zernecke, K. Bidzhekov, H. Noels, E. Shagdarsuren, L. Gan, B. Denecke, M. Hristov, T. Koppel, M.N. Jahantigh, E. Lutgens, S. Wang, E.N. Olson, A. Schober, C. Weber, Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection, Sci. Signal 2 (2009) ra81.
[109] S. Shantikumar, A. Caporali, C. Emanueli, Role of microRNAs in diabetes and its cardiovascular complications, Cardiovasc. Res. 93 (2012) 583–593.
[110] A. Zampetaki, M. Mayr, MicroRNAs in vascular and metabolic disease, Circ. Res. 110 (2012) 508–522.
[111] A. Zampetaki, S. Kiechl, I. Drozdov, P. Willeit, U. Mayr, M. Prokopi, A. Mayr, S. Weger, F. Oberhollenzer, E. Bonora, A. Shah, J. Willeit, M. Mayr, Plasma microrna profiling reveals loss of endothelial mir-126 and other microRNAs in type 2 diabetes, Circ. Res. 107 (2010) 810–817.
[112] D.S. Karolina, A. Armugam, S. Tavintharan, M.T. Wong, S.C. Lim, C.F. Sum, K. Jeyaseelan, MicroRNA 144 impairs insulin signaling by inhibiting the expression of insulin receptor substrate 1 in type 2 diabetes mellitus, PLoS One 6 (2011) e22839.
[113] M.A. Dehwah, A. Xu, Q. Huang, MicroRNAs and type 2 diabetes/obesity, J. Genet. Genom. 39 (1) (2012) 11–18.
[114] R.A. Boon, K.C. Vickers, Intercellular transport of microRNAs, Arterioscler. Thromb. Vasc. Biol. 33 (2013) 186–192.
[115] F. Collino, M.C. Deregibus, S. Bruno, L. Sterpone, G. Aghemo, L. Viltono, C. Tetta, G. Camussi, Microvesicles derived from adult human bone marrow and tissue specific mesenchymal stem cells shuttle selected pattern of miRNAs, PLoS One 5 (2010) e11803.
[116] P. Diehl, A. Fricke, L. Sander, J. Stamm, N. Bassler, N. Htun, M. Ziemann, T. Helbing, A. El-Osta, J.B. Jowett, K. Peter, Microparticles: major transport vehicles for distinct microRNAs in circulation, Cardiovasc. Res. 93 (2012) 633–644.
[117] F. Jansen, X. Yang, M. Hoelscher, A. Cattelan, T. Schmitz, S. Proebsting, D. Wenzel, S. Vosen, B.S. Franklin, B.K. Fleischmann, G. Nickenig, N. Werner, Endothelial microparticle-mediated transfer of microRNA-126 promotes vascular endothelial cell repair via SPRED1 and is abrogated in glucose-damaged endothelial microparticles, Circulation 128 (2013) 2026–2038.
[118] S. Fichtlscherer, S. de Rosa, H. Fox, T. Schwietz, A. Fischer, C. Liebetrau, M. Weber, C.W. Hamm, T. Röxe, M. Müller-Ardogan, A. Bonauer, A.M. Zeiher, S. Dimmeler, Circulating microRNAs in patients with coronary artery disease, Circ. Res. 107 (5) (2010) 677–684.
[119] A.J. Tijsen, E.E. Creemers, P.D. Moerland, L.J. de Windt, A.C. van der Wal, W.E. Kok, Y.M. Pinto, MiR423–5p as a circulating biomarker for heart failure, Circ. Res. 106 (2010) 1035–1039.
[120] S. Wang, A.B. Aurora, B.A. Johnson, X. Qi, J. McAnally, J.A. Hill, J.A. Richardson, R. Bassel-Duby, E.N. Olson, The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis, Dev. Cell 15 (2008) 261–271.
[121] J.E. Fish, M.M. Santoro, S.U. Morton, S. Yu, R.F. Yeh, J.D. Wythe, K.N. Ivey, B.G. Bruneau, D.Y. Stainier, D. Srivastava, miR-126 regulates angiogenic

signaling and vascular integrity, Dev. Cell 15 (2008) 272–284.
[122] S. Meng, J.T. Cao, B. Zhang, Q. Zhou, C.X. Shen, C.Q. Wang, Downregulation of microRNA-126 in endothelial progenitor cells from diabetes patients, impairs their functional properties, via target gene SPRED-1, J. Mol. Cell. Cardiol. 53 (2012) 64–72.
[123] D.E. Wang, MicroRNA regulation and its biological significance in personalized medicine and aging, Curr. Genom. 10 (2009), 143–143.
[124] E.E. Creemers, A.J. Tijsen, Y.M. Pinto, Circulating microRNAs: novel biomarkers and extracellular communicators in cardiovascular disease? Circ. Res. 110 (2012) 483–495.
[125] A.J. Tijsen, Y.M. Pinto, E.E. Creemers, Circulating microRNAs as diagnostic biomarkers for cardiovascular diseases, Am. J. Physiol. Heart Circ. Physiol. 303 (2012) H1085–H1095.
[126] V.P. van Empel, L.J. De Windt, P.A. da Costa Martins, Circulating miRNAs: reflecting or affecting cardiovascular disease? Curr. Hypertens. Rep. 14 (6) (2012) 498–509.
[127] T. Nishiguchi, T. Imanishi, T. Akasaka, MicroRNAs and cardiovascular diseases, Biomed. Res. Int. 2015 (2015) 682857.
[128] J.A. Jansen, M. Noorman, H. Musa, M. Stein, S. de Jong, R. van der Nagel, T.J. Hund, P.J. Mohler, M.A. Vos, T.A. van Veen, J.M. de Bakker, M. Delmar, H.V. van Rijen, Reduced heterogeneous expression of Cx43 results in decreased Nav1.5 expression and reduced sodium current that accounts for arrhythmia vulnerability in conditional Cx43 knockout mice, Heart Rhythm. 9 (2012) 600–607.
[129] A. Etheridge, I. Lee, L. Hood, D. Galas, K. Wang, Extracellular microRNA: a new source of biomarkers, Mutat. Res. 717 (2011) 85–90.
[130] G. Muller, Microvesicles/exosomes as potential novel biomarkers of metabolic diseases, Diabetes Metab. Syndr. Obes. 5 (2012) 247–282.
[131] I.G. Kolfschoten, E. Roggli, V. Nesca, R. Regazzi, Role and therapeutic potential of microRNAs in diabetes, Diabetes Obes. Metab. 11 (2009) 118–129.
[132] J. Krutzfeldt, N. Rajewsky, R. Braich, K.G. Rajeev, T. Tuschl, M. Manoharan, M. Stoffel, Silencing of microRNAs in vivo with 'antagomirs', Nature 438 (2005) 685–689.
[133] O. Snove Jr., J.J. Rossi, Expressing short hairpin RNAs in vivo, Nat. Methods 3 (2006) 689–695.
[134] M.S. Ebert, J.R. Neilson, P.A. Sharp, MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells, Nat. Methods 4 (2007) 721–726.