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Editorial

The importance of the functional network between endothelial microparticles and late endothelial progenitor cells for understanding the physiological aspects of this new vascular repair system

See related article (assigned to December 2017 issue): N. Alexandru, E. Andrei, L. Niculescu, E. Dragan, V. Ristoiu and A. Georgescu. 2017. Microparticles of healthy origins improve endothelial progenitor cell dysfunction via microRNA transfer in an atherosclerotic hamster model. *Acta Physiol* **221**, 230–249.

The article by Alexandru *et al.*, entitled '*Microparticles of healthy origins improves endothelial progenitor cell dysfunction via microRNA transfer in an atherosclerotic hamster model*', represents an important advance in the physiology of the late endothelial progenitor cells (EPCs), documenting the complementary role played by endothelial microparticles (MPs) that play an active role in this repair system.[1]

The authors showed, in their experimental study on an animal model, two very important aspects: significant morphological and functional differences of late EPCs between control and hypertensive-dyslipidaemic animals and their functional improvement after co-incubation with MPs from control animals. This study showed that late EPCs obtained from an experimental model of atherosclerosis have altered morphological and functional characteristics, and altered immunophenotypic profile and lower levels of miRNAs involved in vascular repair processes (miR-10a; miR-21; miR-126; miR-146a; miR-223). Moreover, MPs of healthy animals improve atherosclerosis-associated late EPC dysfunction by their ability to transfer these miRNAs and to stimulate IGF-1 expression.[1]

Endothelial progenitor cells, firstly identified in 1997, are circulating cells in the adult organism that derive from bone marrow, expressing CD34 or KDR (kinase-insert domain receptor) surface antigens, able to proliferate and differentiate into mature endothelial cells (both *in vitro* and *in vivo*) and to participate in neo-angiogenesis processes *in vivo*.[2] The mechanism by which EPCs stimulate angiogenesis is the typical feedback circuit. Tissue ischaemia causes the production and release of growth factors and chemokines [e.g. vascular endothelial growth factor (VEGF), stromal cell derived factor (SDF)-1α], which, in turn, mobilize EPCs from the bone marrow to the peripheral circulation. Once in circulation, EPCs reach selectively ischaemic and/or damaged tissues through the chemokine/receptor interaction (especially the SDF-1α/CXCR4) and promote endothelial repair and/or compensatory angiogenesis. The most current accredited hypothesis is that an EPCs malfunction could prevent such mechanisms of protection and thus promote the onset and/or progression of cardiovascular disease.[3]

Many studies have been published on EPCs, but there is no univocal definition of EPCs in the literature. There are two different approaches for the identification and characterization of these cells: the cytofluorimetric technique and cell cultures. These methods respond to different research questions and both are not free from limitations. The cytofluorimetric EPCs characterization has the advantage of selecting a more homogeneous population of cells and identifying more subpopulations simultaneously, but it remains complex because there is no consensus on the antigenic phenotype to be used for their identification given the variety of surface antigen co-expression in the various differentiation stages of this complex system. In addition, given the low number of these cells in the peripheral blood and the difficulty in the simultaneous identification of surface antigens (e.g CD34, CD133, KDR), it becomes necessary to select subpopulations (e.g $CD34^+/KDR^+$ and $CD133^+/KDR^+$) with a less restrictive phenotype and then more easily identifiable but perhaps inevitably less accurate. Another important limitation of the cytofluorimetric method is the lack of information on functional aspect of EPCs.[4]

The *ex vivo* approach (peripheral blood cell cultures) allows not only the identification and numerical evaluation of EPCs, but also provides functional information. It is based on isolation of EPCs from the mononuclear population circulating in peripheral blood and on the evaluation of their ability to proliferate (ability to form colonies) and differentiate into mature endothelial cells. In addition, functional tests may be performed. These include evaluation of the ability to form similar vascular tubular structures (tube formation assay) and to migrate in response to chemotactic substances (migration assay). However, even this technique, apparently more complete, is not without limits. The main criticism lies in the fact that the cells are grown in medium enriched with growth factors and with cytokines, hence, not in physiological conditions. Therefore, paradoxically endothelial cells might differentiate in other cell lines.[5]

As EPCs have protective functions within the cardiovascular system, it is intuitive that a lower number or a dysfunction of these cells may promote the

development or the progression of cardiovascular disease. Indeed, many clinical trials have highlighted that the presence of any of the classic risk factors for cardiovascular disease (arterial hypertension, dyslipidaemia, diabetes mellitus, smoking, obesity) is associated with decreased number or dysfunction of circulating EPCs compared to controls without these factors. The molecular mechanisms behind these alterations have been partially clarified. For example, the excess of angiotensin II or aldosterone decreases the production of EPCs, smoke and excess of low-density lipoproteins act by decreasing EPCs through an inflammatory and pro-apoptotic mechanism, while hyperglycaemia reduces the mobilization and survival of EPCs in peripheral blood. An inverse correlation between the level of circulating EPCs and other surrogate biomarkers of cardiovascular risk, such as reactive protein C, homocysteine and oxidized LDL, has been shown.[6]

Drugs commonly used in internal medicine and in particular in patients with diabetes and metabolic syndrome have also been able to improve mobilization and function of EPCs. They include statins that result in an increase in the bioavailability of NO and have anti-inflammatory and antioxidant properties. Recent studies have shown that ACE inhibitors and sartans are able to increase the number and function of EPCs in animal models, independently of the antihypertensive action, suggesting another their potential beneficial action mechanism on the cardiovascular system.[6]

The discovery of EPCs has revolutionized the vision of the mechanisms involved in vascular damage, opening up new therapeutic perspectives. The use of EPCs, although conceptually considered 'rational' in ischaemic cardiovascular disease, presents numerous problems. The main critical points are represented by choosing the most appropriate dysfunctional moment in the continuum of the atherosclerotic process. This is probably in the final stages, characterized by severe and irreparable endothelial damage, as in the initial stages the correction of traditional risk factors could increase the release of EPCs from the bone marrow. Another problem is the low number of EPCs in the peripheral blood that requires the use of factors that can expand its number both *in vitro* and *in vivo*. One option might be the use of mobilizing agents from the bone marrow cells *in vivo*, such as erythropoietin (which has been found to increase the number, proliferation and migration of EPCs), granulocyte colony-stimulating factor (G-CSF) and VEGF, that have shown beneficial effects on the mobilization of the EPCs.[7]

Microparticles are membrane vesicles shed from the plasma membrane of endothelial cells generated in physiological and pathological conditions. Recent studies have shown that MPs may represent important transporters for cytokines, proteins and microRNA. The formation and release of MPs occur mainly during two different biological processes: cell activation induced by proinflammatory or prothrombotic stimulation and cell apoptosis. *In vitro*, the release has been identified in several types of cells. The major cells capable of releasing MPs are as follows: erythrocytes, globules, leucocytes, platelets and endothelial cells. MPs are identified and measured by antigens constitutively expressed by mature endothelial cells (e.g. CD31, CD51, CD105, CD144 and CD146). In particular, CD144 and CD146 represent specific endothelial markers as it seems that in humans, they are not expressed in any other blood cell.[8]

Endothelial cells generate MPs after exposure *in vitro* to thrombin, lipopolysaccharide and cytokines (interleukin-1 and TNF-α). Regardless of their antigenic characteristics, MPs values reflect the presence of endothelial dysfunction. For example, a strong association between high levels of MPs ($CD31^+$, $CD51^+$, $CD144^+$) and vascular abnormalities from a structural and functional point of view. MPs are coated with a double phospholipid layer. In cells, the asymmetric distribution of phospholipids is usually altered during the MPs formation, resulting in exposure to the outer surface of phospholipids negatively charged, such as phosphatidylserine and phosphatidylethanolamine. This aspect plays an important role in the effects in of the MPs as phosphatidylserine effectively binds coagulation factors.[9]

Although MPs were initially considered cellular debris, then numerous studies have described their role as vehicles for the intercellular exchange of biological signals and information. MPs modulates cellular properties and responses, exposing bioactive molecules able to bind and activate receptors present on the cell surface target, or transferring directly part of their content including proteins, bioactive lipids or RNA. This transfer can be facilitated by momentary interactions or may require a stable association, merger of membrane or MPs incorporation within the target cell.[8–10]

Microparticles are also able to transfer substantial amounts of mRNA and microRNA. The transfer of genetic information altered consequently the expression of genes in both nearby and distant cells. Later to target cell interactions and subsequent internalization, the MPs can reprogramme the target cell phenotype and give it specific features, under different conditions. MicroRNAs are a highly conserved and non-coding RNA family, ranging from 21 to 25 nucleotides, of which the fundamental role is to regulate negatively the gene expression at post-transcriptional level. MicroRNAs act by recognition of specific mRNA

targets and determine its degradation or repression of the translation.[8–11]

The role of MPs in the pathophysiology of EPC repair response has often been overlooked. Traditionally, to MPs has been attributed the significance of early marker of endothelial damage, without highlighting the significant role that they can exert on the function of EPCs and especially on late EPCs (closer to full maturation and therefore the cellular elements responsible for the final stages of vascular damage repair). The importance of the physiological role of MPs on EPCs function must necessarily be evaluated in traditional clinical models (e.g. diabetes mellitus, arterial hypertension, ischaemic heart disease), but also in pathological conditions where traditionally the assessment of endothelial damage is not a first level evaluation.[3,4]

The most important and common example is represented by patients with erectile dysfunction (ED). Erectile dysfunction is classified in organic and psychogenic forms. The organic causes include endocrine, neurological, iatrogenic and vascular (arterial insufficiency of the cavernous arteries and/or alterations of the veno-occlusive mechanism) forms. In the clinical practice, the most suitable diagnostic tool for assessing the presence of penile arterial insufficiency is represented by penile echo-colour Doppler. The importance of this diagnosis is given by the frequent association between penile arterial insufficiency and atherosclerotic plaques of carotid and coronary arteries.[3,4]

Arterial ED represents the first relevant event indicative of endothelial dysfunction (already present even when carotid or coronary plaques are not present), that without correction of traditional risk factors, may end-up in the years to come in a widespread atherosclerosis with the involvement of major vascular districts.[3,4]

One particular aspect concerns the phenotypic characterization of MPs in diabetic patients with ED. In particular, in these patients, Esposito *et al.*[12] showed that EMP62/EMP31 ratio, an index of endothelial activation (high ratio) or apoptosis (low ratio), was lower in diabetic men with ED compared to ED

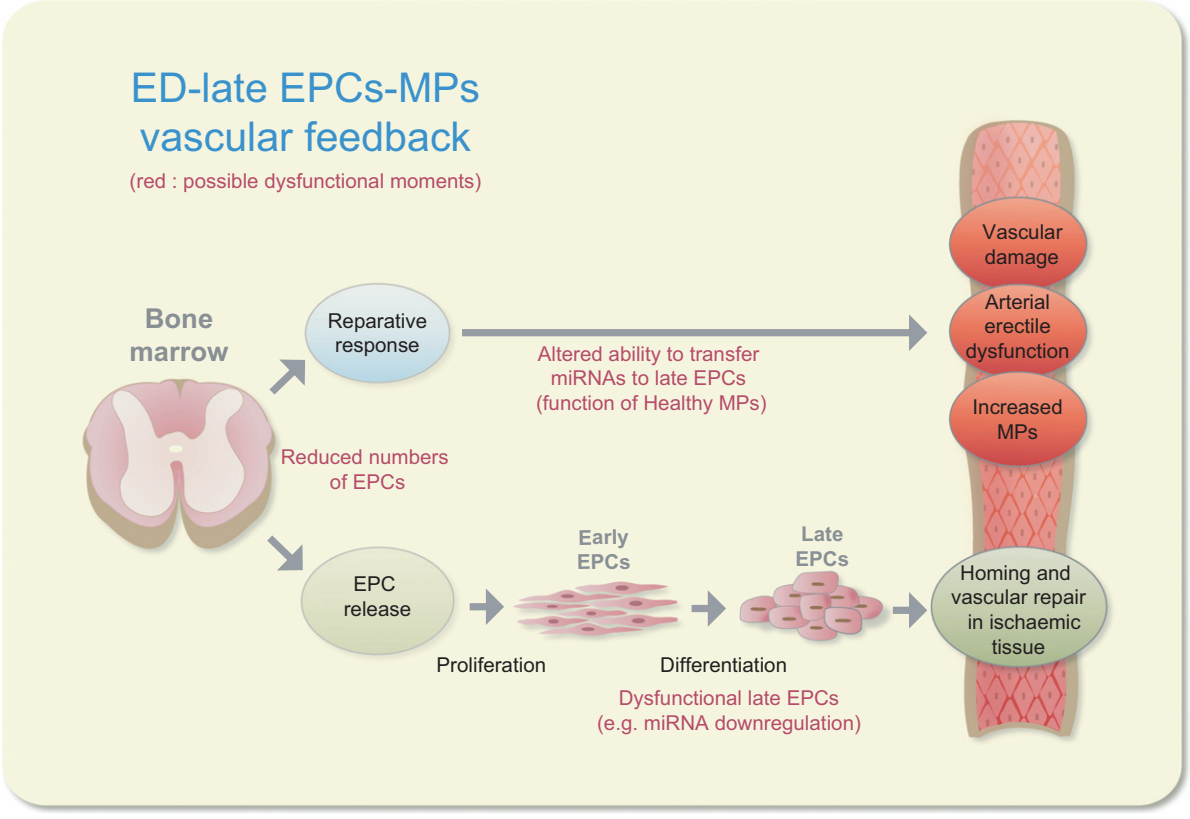

Figure 1 In patients with arterial erectile dysfunction, endothelial damage is associated with an increase in microparticles (MPs) in peripheral blood. Bone marrow endothelial progenitor cells (EPCs) are released, ensuring a vascular reparative system. Circulating EPCs mature from early to late phenotype (acquiring vascular repair ability). The possible dysfunctional moments of the system are shown in red: low bone marrow EPCs production, the presence of dysfunctional late EPCs or impaired MPs ability to ameliorate late EPCs function.

patients without diabetes, underlining that the phenotypic assessment of MPs in diabetic patients with ED is consistent with increased apoptotic activity.

In these patients, the number of MPs is significantly higher in the systemic circulation compared to controls, and concomitantly, they have an increased number of late EPCs. Therefore, arterial ED induces a feedback on EPCs release from the bone marrow. In the peripheral blood, EPCs undergo a maturation process from an early to a late phenotype, which can be evaluated by cytofluorimetric analysis by the immunophenotype change. Possible dysfunctional moments of this complex repair system could be schematized in: decreased mobilization of EPCs from the bone marrow,[13] decreased homing capacity of late EPCs,[14] lower ability to modulate positively the function of late EPCs by MPs[1] (Fig. 1).

Conflict of interest

I and my co-authors have no conflict of interest to declare.

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