Endothelial Progenitor Cells as Biomarkers of Cardiovascular Pathologies

Subjects: Cardiac & Cardiovascular Systems

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Endothelial progenitor cells (EPC) may influence the integrity and stability of the vascular endothelium. Endothelial dysfunction is one of the key mechanisms in CVD. The assessment of endothelial dysfunction in vivo remains a major challenge, especially for a clinical evaluation of the need for therapeutic interventions or for primary prevention of CVD. Endothelial cells (EC) can become senescent, and the majority of circulating endothelial cells (CEC) show evidence of apoptosis or necrosis. There are a few viable CECs that have properties similar to those of an endothelial progenitor cell. To use EPC levels as a biomarker for vascular function and cumulative cardiovascular risk, a correct definition of their phenotype, as well as an update on the clinical application and practicability of current isolation methods, are an urgent priority.

Keywords: endothelial cells ; progenitors ; cardiovascular disease ; biomarker

1. Introduction

Cardiovascular diseases (CVD) such as myocardial infarction (MI), cerebral and peripheral arterial disease (PAD) and arterial hypertension are the leading causes of globalmortality [1][2].

Coronary artery disease (CAD), leading to narrowing or complete blockage of arterial blood supply to the myocardium, is the most prevalent heart disease [2]. Pharmacological agents, interventional and surgical procedures, as well as diet and lifestyle-related concepts to better control established and newly discovered cardiovascular risk factors, are still not sufficient to prevent the millions of disease-related deaths worldwide every year [1][2][3][4].

Known cardiovascular risk factors (CVF) contribute to the cascade of atherogenesis especially by inducing injury and dysfunction in endothelial cells. Endothelial integrity is highly reliable due to repair and renewal by endothelial progenitor cells (EPC) derived from different sources, e.g., bone marrow (BM), circulating endothelial progenitor cells (CEPC) or adventitial residents [5]. The impaired mobilization or depletion of these cells contributes to endothelial dysfunction and CVD progression [6].

Ross' classic paradigm already stated that endothelial cells (EC) injury is one of the most important stimuli for the development of atherosclerotic plaque [7]. In 1997, Asahara et al. reported the isolation of putative EPC from human peripheral blood, based on the cell-surface expression of CD34 and other endothelial markers and introduced the novel concept of CEPCs. These specific cells were reported to further differentiate, at least in vitro, into endothelial cells [8]. They could be identified at sites of active angiogenesis as well as in various animal models of ischemia [8]. CEPCs contribute to on-going endothelial repair through their ability to form layers of neo-endothelium at the site of injury or to serve as a cellular reservoir to replace dysfunctional endothelium [9].

2. Characterisation and Various Origins of Ecs/ Epcs in Humans

A consensus on the ideal marker for the identification of EPC cell types is lacking. This marker may originate from multiple precursors, such as a haemangioblast (HPC), BM progenitors, tissue-resident mesenchymal stem cells (MSC), and especially, from adipose tissue [10], impeding fast and simple isolation [5].

The use of density barrier centrifugation is one method that can be utilised in order to separate mononuclear cells from peripheral blood mononuclear cells (PBMNCs). In most cases, cells are sown onto plates that have been coated with fibronectin and then grown using endothelial growth factors. The remaining spindle-shaped cells not only endocytose acetylated low-density lipoprotein (LDL) but also express EC markers and possess other characteristics of ECs. This is, in

addition to the fact that EC indicators are present in these cells [11], acquired via antigen transfer from platelets that contaminate isolates of PBMNCs [12]. Platelets on mononuclear cell cultures degrade into micro-particles (vesicles that retain specific antigens from the cell of origin) within 7 days, and CD31 expression (along with platelet-specific markers) was present at that time on EPCs, now called putative EPC'-aggregates', whereas EPCs were CD31-negative on day 1 [12]. Attempts to further purify the cell cultures led to the establishment of new protocols.

Using markers such as CD133, combined with CD34 and VEGFR2, ensured that only progenitor cells with vasculogenic properties were identified [13][14]. Cell labelling with antigen-specific antibodies and fluorescence-activated cell sorting (FACS) to select EPCs was applied [8], while still culturing the cells on fibronectin [12]. The CD34+ cells were surrounded by spindle-shaped cells expressing increased EC markers. Through the use of this marker combination, this cell type was successfully isolated from adult peripheral blood, umbilical cord blood, and foetal liver [15]. Recent research shows that human CD34+/CD133+/VEGFR2-positive cells are separate primitive haematopoietic progenitors lacking vessel formation ability and expressing CD45, a haemangioblast marker [16][17]

Thereafter, in vitro colony-forming cell assays allowed for the isolation of two cell types: colony-forming unit (CFU)-Hill cells and endothelial colony-forming cells (ECFC). In the 'Hill assay' and the ECFC, or 'Ingram protocol', monocytic cells isolated from blood samples were cultured for two days on fibronectin-coated dishes and then replated for further cultivation [6][14]. CFU-Hill cells are phagocytic and express EC-like markers (CD14, CD45, and CD115) but lack proliferative and vasculogenic activity. While lacking CD14, CD45, and CD115, ECFCs express EC markers and have the ability to form capillary-like structures in vitro and vessels in vivo [18]. ECFCs have been shown to reside in the arterial wall suggesting that this may be the main origin of these cells [13].

In summary, EPCs seem to represent two distinct populations with overlapping antigen expressions (e.g., CD34/VEGFR2): hematopoietic-derived spindle-shaped cells from isolation method I/II, also referred to as circulating angiogenic cells or early EPCs (CFU-Hill colonies), and ECFCs, or late EPCs [19]. Late EPCs have a vasculogenic ability in vitro and are well-integrated into membranes, whereas early EPCs act via a paracrine mechanisms [20] and might even protect late EPCs from oxidative stress [21].

3. Influence on Vascular Pathologies and Role as a Biomarker

At present, there is a lack of information or knowledge regarding cell phenotypes in different diseases as the cells originate from different vascular beds and sources [22]. Half of the CECs from healthy controls express CD36, a marker for cells of microvascular origin, whereas in sickle cell anaemia, this percentage increases to 80% [23]. Contrastingly, no CD36 could be stained in CECs from patients with acute coronary syndrome, consistent with the macro-vascular origin of these cells [24].

Investigating the role of CECs in endothelial injury with regard to plasma markers of endothelial injury (vWf, tissue plasminogen activator, soluble E-selectin) led to a correlation between CECs and vWf in heart failure [25].

Almost all types of CVD were associated with hypertension, diabetes, smoking and high cholesterol. These CVFs can contribute to endothelial dysfunction [26][27][28][29][30][31][32][33][34][35][36]. High homocysteine and ADMA values also showed a negative effect on EPC count [37][38]. On the other hand, high HDL cholesterol and TG levels correlated with CFU but not with CD34/133+ cell count [39]. Statin [40] and Angiotensin receptor II inhibitors [41], as well as oestrogen levels (high oestrogen levels in women were associated with an increased EPC count [42] in animal carotid injury oestrogen-enhanced EPC function [43]), glitazones [44], erythropoietin [41][45][46][47], and PDE5 inhibitors [48] all showed beneficial effects. EPC count was also dependent on SDF-1 [49][50], VEGF [51][52], NO [53], GCS-F and GM-CSF [54][55] levels.

Physical activity at a moderate level was identified to be potentially beneficial for preventing CVD. The increase in EPC count was found to be mediated by eNOS and VEGF, and apoptosis was reduced in the cells [56]. Physical activity lead to a higher amount of circulating CD34-positive EPCs in CVD patients [57]. Furthermore, this identified an association of CD34-positive cell count with lower all-cause and cardiovascular mortality [57]. Vascular damage progression correlated with EPC count [58] just as a decreased amount of CD34-positive but increased amount of $CD34^+CD133^+CD309^+$ and $CD34^+CD133^+$ cells suggested the progression of cerebral small vessel disease [59]. EPC count could, therefore, serve as a biomarker for CVD course. A correlation with CAD progression was also found for osteocalcin, a regulator of early EPC. A higher number of CVRFs was associated with a decreased total osteocalcin count. Osteocalcin positivity in EPCs was related to LDL, total cholesterol and TGs in both early and, significantly, in late CAD [60]. EPC count could also be used as a marker in treatment monitoring, such as in chronic total coronary artery occlusion since an association with Rentrop grade at baseline and 1 year post operation was discovered [61].

Some data still suggest that those “monitoring effects” are mostly seen in the young population. Aging by itself is another depriving factor of EPC [6][53]. Age directly limits EPC mobilisation but also via VEGF depletion and physiologically lowered NO levels, which contribute to the bad survival and proliferation of EPCs [53][62]. Moreover, several mechanisms, including the co-existence of CVF, lead to the impaired maintenance of endothelial integrity [63]. In elderly CAD patients with stable disease, EPC count was significantly reduced compared to younger patients [64]. The mobilisation of EPCs was also lower after a coronary bypass grafting in advanced age [65][66]. The severity of stable CAD shows an inverse correlation with the total/early EPC number [50][67]. Additionally, chronic vascular disease appears to have opposite effects on early and late EPC numbers and does not influence their functional capacity [50][67].

Using EPC as a therapeutic target for CVD may therefore underlay an age-related effect. Early EPC implantation increased neovascularisation in young mice, but not in older mice with elevated cholesterol or other CVD risk factors [68].

4. The Role of Epc in Congenital Heart Disease Heart Failure

Mechanisms of heart failure in adult heart disease recently received a lot of research attention, but its pathogenic and prognostic significance in single-ventricle physiology is still unknown [69][70][71]. Congenital cardiac malformations with a single ventricle have a high risk of mortality in the first year of life in such patients and frequently result in late complications developing during this stage of palliative repair [72]. Even though single-ventricle reconstruction trials have sought to identify predictors of poor outcomes at three years in patients with single-ventricle physiology based on the types of initial shunt (Norwood procedure with ventriculo-pulmonary Sano shunt or with modified subclavio-pulmonary Blalock shunt) and the timing of stage 2 palliation, a 12-year longitudinal cohort study in patients with Fontan (stage 3 procedure) circulation found that the risk of death or cardiac transplantation was closely associated with poorer ventricular function [73][74]. No definitive therapy was shown to improve heart function with a chronic volume or pressure overload, which may worsen prognosis for single-ventricle patients [75].

However, early phase 1/2 clinical trials utilizing the intracoronary delivery of derived progenitor cells demonstrated dependable and safe outcomes in patients with single ventricle physiology. Except for all-cause mortality after staged procedures, derived cardiac progenitors cells administration improved ventricular function and was linked with fewer late problems in patients with single ventricles. Patients treated with cardiac progenitors cells and those who suffered from heart failure with reduced EF but not heart failure with intact EF may experience a substantial improvement in clinical outcomes [76][77].

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