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Natural Products as Regulators of Progenitor Cells: Role in Cardiovascular Regenerative Medicine

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ABSTRACT

The cellular network in vasculature is predominantly composed of two distinct cell types i.e., endothelial and smooth muscle cells, which along with the connective tissue and extracellular matrix govern the tone and function of the blood vessels. In response to vascular injury, progenitor cells are mobilized from local niece, peripheral circulation, or bone marrow in order to contain the inflammatory process in progress and/or repair the damaged vasculature. The progenitor cells are reported to play a significant role in the repair of vascular disorders. A variety of cytokine-chemokine interplay are shown to precisely regulate the mobilization, homing, and differentiation of progenitor cells as a consequence to the vascular injury and enable restoration of vascular structure and function. The cytokine-chemokine interplay and factors leading to impairment of the progenitor cell dynamics are complex cascades, which govern the regenerative capacity of vascular progenitors, thus negatively modulating the development of atherosclerosis or other vascular diseases. These complex cascades provide an opportunity for identifying novel diagnostic markers and therapeutic targets for combating vascular diseases. However, many challenges remain in understanding dynamics of progenitor cells origin, mobilization, homing, and differentiation. Recent studies have reported the potential of natural products to mimic the role of cytokines in modulating the vascular progenitor function. Furthermore, some natural products have been shown to restore the defective progenitor cell function, thus promoting the vascular repair process. In this review, we provide a basic background on vascular progenitor biology and highlight the untapped potential of natural products to positively modulate the progenitor biology.

Key words: Endothelial cells, natural products, smooth muscle cells, vascular progenitors

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INTRODUCTION

In recent years, there has been tremendous focus on understanding the role of vascular progenitor cells in the therapy of several cardiac and vascular disorders [Figure 1]. Several adult and embryonic precursor cell populations have reported to differentiate the vascular cell phenotype[1] and participate in a range of biological repair functions within the cardiovascular system.[2,3] The cell therapy era as moved from the preclinical arena to the clinical phase wherein potential benefits are reported with the use of progenitor cells in the context of cardiovascular disorders, especially myocardial infarction.[2,4-9] However, considerable preclinical work is needed to further refine the progenitor cell therapy based medicine.

Diseases associated with vascular complications such as atherosclerosis, stroke, hypertension, diabetes, and myocardial infarction are a major cause of morbidity and mortality globally despite advancements in nutritional,

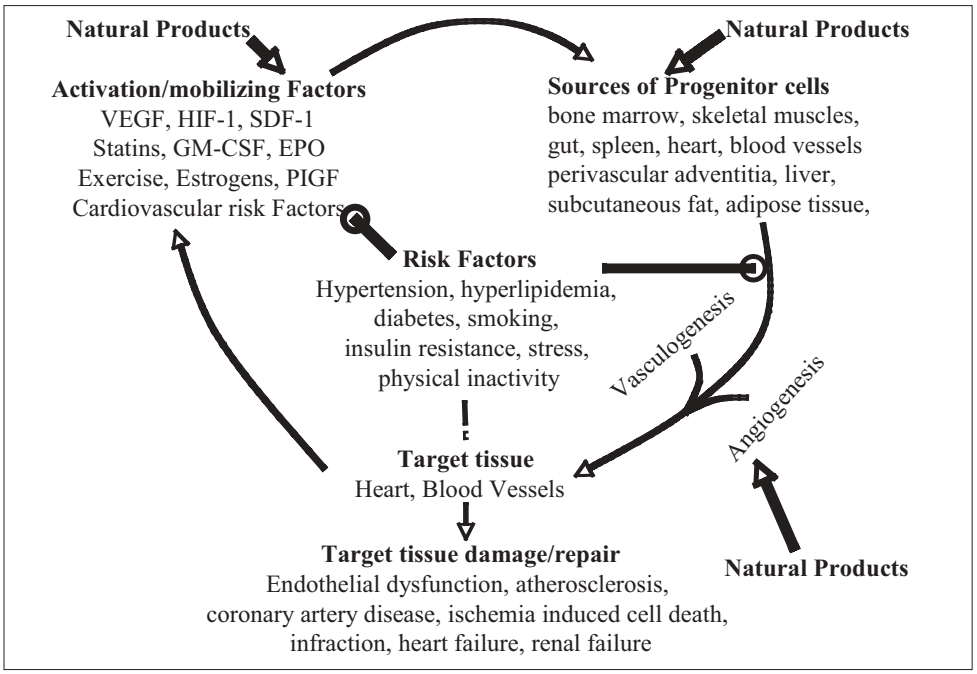

Figure 1: Schematic representation of the progenitor cell role in physiology and pathophysiology. Under a physiological state, the routine tissue repair process is carried out by the endogenous progenitor cells. However, this function is compromised under a pathological state. Interestingly, the progenitor function can be revoked using pharmacological or possibly natural product based approaches

pharmacological, and interventional approaches.[1,10–24] Regeneration medicine is a new addition to the current therapeutic approaches to strengthen the global fight against combating the devastation of a cardiovascular disease pandemic[12,25–32]. The focus on regenerative medicine has increased in the last decade with the identification of several types of stem cells with the potential to be differentiated into functional endothelium and/or smooth muscle cells i.e., the so-called vascular progenitors.[33–36] In general, two types of stem cell i.e., embryonic and adult stem cells have been explored for potential use in regenerative medicine. While embryonic stem cells have potential to differentiate into a variety of cell types, adult stem cells (progenitor cells) are limited in their plasticity.[37–40] Due to the ethical issues and technical challenges involved in the isolation and use of embryonic stem cells, increasing interest is focused on progenitor cells. In the exploration of progenitor cells, several possible sources of these cells have been documented that can be classified into at least three distinct groups i.e., marrow-derived, the circulating pool, and tissue-resident progenitor cells. It's debatable as well as likely that the circulating pool and the tissue resident progenitor cells are at some point derived from the marrow.[37–39,41] Vascular progenitor cells are a broad category of cells that include precursor cells in the bone marrow, circulation, and in several local tissues and have the capacity to differentiate into endothelial cells and/or smooth muscle cells under appropriate conditions.[1,12,14,42] However, the pathway for differentiation of these cells from a common, separate, or multiple precursors is poorly characterized/understood, hence leading to confusion in the lineage tracing of these cells and wide variations in the results observed by different working groups across the globe.

ENDOTHELIAL PROGENITOR CELLS

Endothelial progenitor cells (EPCs) are cells with the potential to differentiate into functional endothelial cells. EPCs were initially identified and isolated in 1997 by Asahara, *et al.*[43] on the basis of vascular endothelial growth factor receptor-2 (VEGFR2) and CD34 coexpression. Since the initial report, several groups have attempted to better define the EPC population, and are reported to express fibroblast growth factor receptor, CD38, c-kit, CD31, CD146, CXCR4, von Willebrand factor (vWF), vascular endothelial cadherin (VE-cadherin), Tie-2/TEK (angiopoietin-1 receptor precursor or tunica intima EC kinase), flk1, and CD133.[6,44–46] Possibly, subsets of $CD34^+$ cells that express CD133 and Flk-1 are the phenotypical and functional markers of EPCs that play a role in postnatal angiogenesis.[47,48] However, expression of the surface markers described above does not include stem cells from other sources (mesenchymal or even in the vessel wall) or their progenitor cells.[49–53] Despite this controversy over the EPC surface marker, these are widely studied cells both in the preclinical and clinical context[8,27,47,54–64] and have been

reported to considerably contribute to the angiogenesis and vascular repair process. [58,60,65-71]

Smooth muscle progenitor cells or smooth muscle out growth cells

Smooth muscle progenitor cells (SPCs) are cells with the potential to differentiate into functional smooth muscle cells. CD14/CD105 double positive populations from human peripheral blood mononuclear cells (PBMC) are reported to be a precursor of SMC, and indeed atherosclerotic patients contain significantly higher circulating levels of these cells.[33,37,72-75] These precursors, when maintained under specific growth factor-supplemented media, appear spindle-shaped and express α-smooth muscle actin in addition to $CD34^-$, $CD45^+$, $CD14^+$, and $CD105^+$ markers.[44] Human mononuclear cells isolated from buffy coat seeded on collagen type 1 matrix yield outgrowth cells in endothelial growth medium (EGM-2) and platelet-derived growth factor media, which have SPCs like phenotype. Selection in platelet-derived growth factor-enriched medium results in rapid outgrowth and expansion of smooth muscle outgrowth cells (SOC). These SOCs are positive for smooth muscle cell-specific alpha actin (alphaSMA), myosin heavy chain, and calponin and are also positive for CD34, Flt1, and Flk1 receptor but negative for Tie-2 receptor expression, suggesting a potential smooth muscle progenitor phenotype. Furthermore, integrin alpha5beta1 expression is significantly increased in SOCs compared with EOCs. SOCs show a significantly greater *in vitro* proliferative potential compared with EOCs.[54,72] Recent studies have reported an expansion of primary high proliferative potential smooth muscle outgrowth cells (HPP-SOC) from human peripheral blood mononuclear cells (PBMC) that participate in vasculogenesis and the formation of neointima and adventitial microvasculature of diseased arteries.[37–39] However, currently the knowledge on the SPC is very limited and considerable controversy exits over their lineage and phenotype.

PROGENITOR CELL FUNCTION AND VASCULAR DISEASE

An obvious question arising in the study of progenitor cells is, "With the in-house availability of progenitor cells, why do various vascular disorders develop? Why is it that the system can not self-restore its function utilizing the progenitor cells?' A logical answer is that under disease conditions, functions of the progenitor cells are compromised. Indeed it is postulated that oxidative stress may modulate progenitor cells at the site of vascular injury,[1,42] thus hampering progenitor cell functions.[76] It is still unclear whether such modulations occur at the bone marrow, in the peripheral circulation, or only at the site of vascular repair/remodelling. Reactive oxygen species are a family of highly reactive molecules that are formed within eukaryotic cells both enzymatically and non enzymatically by the one electron reduction of molecular oxygen, yielding superoxide anions ($O_2^{\bullet}$).[77,78] By maintaining the concentrations of superoxide within physiological limits through tight regulation of their production and removal, cells are able to utilise these molecules in diverse signalling pathways ranging from acute vasodilation, maintaining vascular tone, vessel growth, and remodelling.[77,78] However, an imbalance between ROS production and removal leads to oxidative stress, a hallmark of virtually all vascular pathophysiological states.[79] In a recent study, enhanced Nox2 expression was observed in EPCs derived from diabetic mice and this was associated with diminished EPC ability to differentiate endothelial cells.[80] Similarly, gene deletion of glutathione peroxides (intracellular antioxidant enzyme) resulted in an oxidative stress-associated decline in EPC function.[80,81] In both these studies, oxidative stress attenuated EPC function without affecting cell number. Of interest, gene deletion of Nox2 improved EPC function when these cells were treated (*ex vivo/in vivo*) with antioxidants. Contrary to this, treatment of EPCs from wild type mice with antioxidant diminished EPC differentiation and recruitment.[80,81] These results indicate that although reactive oxygen species hamper EPC function, they may also be necessary for their physiological function. However, further research is needed in this field to critically answer these vital questions.

PHARMACOLOGICAL APPROACHES TO MODULATE PROGENITOR CELLS FUNCTION

As we now know that progenitor cell function is compromised under disease scenario, the obvious question would be, could we develop pharmacological approaches to improve the compromised function? Indeed, several pharmacological moieties that also have antioxidant potential mediate beneficial effects via progenitor cell recruitment. For instance, in response to ischemic stress, enalapril (angiotensin-converting enzyme inhibitor) treated mice displayed a six-fold increase in the contribution of bone marrow-derived EPCs to the ischemia-induced neovascularisation.[82] Statins also increased EPC recruitment in patients with chronic kidney disease[83] and heart failure.[58] This may be caused by their ability to regulate redox signalling. Erythropoietin, which does have antioxidant potential, plays an important role in the mobilization of functionally active EPCs.[67,84,85]

Exogenous erythropoietin treatment inhibits the neointimal hyperplasia after arterial injury by mobilizing EPC to the neo-endothelium.[86,87] C reactive protein, which is a hallmark of oxidative stress, is shown to attenuate endothelial progenitor cell survival, differentiation, and function via inhibiting nitric oxide.[88–91] A low molecular weight fucan (LMWF) compound, a sulfated polysaccharide with antioxidant effect, has been demonstrated to increase plasma levels of stromal cell-derived factor 1 (SDF-1) and consequently to mobilize bone marrow-derived vascular progenitor cells (BMVPC).[92–94] Considering the nature of the pharmacological effects, it is likely that these pharmacological approaches can be mimicked by natural product-based compounds as well. Indeed, recent studies have shown that certain natural products have the potential to improve the progenitor cell (especially EPC) function.[94–100]

CYTOKINES INFLUENCING PROGENITOR CELL DYNAMICS

A number of blood borne and marrow derived cells are capable of differentiating into an endothelial phenotype; however, the differentiation pathways are likely to be complex and currently are poorly understood. While the cytokines responsible for the activation of EPCs are largely known, the exact sequence of events leading to EPC differentiation, migration, and recruitment into the site of injury is intensively investigated. It is well known that the redox status of cells is a crucial determinant in the regulation of the cytokine/chemokine system.[101]

Granulocyte-macrophage colony stimulating factor (GMCSF) and stromal cell-derived factor-1alpha (SDF) improves cardiomyocyte viability and function following ischemic cardiomyopathy.[102,103] GMCSF is reported to enhance reactive oxygen species formation in hematopoietic cells, which is vital to their differentiation into functional adult cells.[104] Transforming growth factor beta 1 (TGFbeta1) is yet another cytokine activated in cells under oxidative stress,[105] whose expression on smooth muscle cells increase endothelial progenitor cells adhesion and differentiation.[106] Reactive oxygen species (ROS) participate in the regulation of platelet activation in an autocrine manner[107,108] and activated platelets secrete the chemokine SDF-1alpha, which facilitate primary adhesion and migration of progenitor cells.[109,110] Preferential engraftment of rapidly self-renewing marrow stromal cells into adult mice was due to SDF-1-mediated mobilization and peripheral homing of progenitor cells in response to ischemia and associated oxidative stress.[79] Similarly, several other factors such as 8bromo-cAMP or adrenomedullin,[111] VEGF, Notch, Urokinase-type plasminogen activator (uPA),[112] ICAM-1,[113,114] vascular endothelial cadherin,[115] and beta-catenin are associated with promoting angiogenic progenitor cell mobilization, recruitment and differentiation[96,116-118] and it is indeed interesting to note that all these factors are directly or indirectly under tight redox regulation.[71,106,119,120]

WHY SHOULD WE LOOK AT NATURAL PRODUCTS?

Contrary to affordability of scientifically tested pharmaceutical products by rich nations, many in developing and underdeveloped nations have relied upon the naturally available and time-tested products of nature. Among these, the most popular are the ones used for a broad range of disorders, by virtue of their general tonic nature. These herbal general tonics enhance the overall ability of the body's immune system to fight against infections and several pathological agents. Such tonics or herbal preparations are extensively used in developing and underdeveloped nations as a supplement to first-line treatment. These supplements are extremely useful in improving the poor health associated with environmental pollution induced lung and micro vascular diseases and compromised immune system. Indeed, to many people's surprise/satisfaction, some of these herbal products have shown promising benefits for health and general well-being. However, the scientific rationale for such benefits remains unanswered and the lack of proper documentations and scientific evaluations have often led to some adverse effects due to their improper use. When appropriately tested and documented, these herbal products, which may be locally grown, will prove to be of immense aid in improving the general health status of the population in the less privileged nations of the world. It is also interesting to note that in the last couple of years natural products are gaining increasing demand in the western world as well.

Flavonoids (flavonols, flavones, isoflavones, and flavanones) belong to a group of natural compounds with variable phenolic structures and are present in plants used for preparing herbal general tonics.[95,99,100] Various hypotheses are suggested for beneficial effects of these compounds in improving the poor health associated with micro and macro vascular diseases. Over 4000 structurally unique flavonoids have been identified in plant sources. The flavonoids have long been recognized to possess anti-inflammatory, antioxidant, anti-allergic, hepatoprotective, antithrombogeneic, and anti-viral properties. Hence, a

resurgence of interest in traditional medicine together with an expanded effort in pharmacognosy has rekindled interest in flavonoids.[95,100,121] Most of the flavonoids reduce macrophage M-CSF induced proliferation without affecting cellular viability and also inhibit TNFalpha production, iNOS expression, and NO production, which is associated with the inhibition of the NF-kB pathway and stimulation of anti-inflammatory cytokine IL-10. These features are likely to modulate the vascular progenitor function as M-CSF is reported to mobilize progenitor cells to the site of vascular injury.[99,100] Recently, prenylflavonoids were reported to significantly promote the cardiac differentiation that was partly mediated via ROS signaling cascades. Furthermore, the anti-oxidative activity of the prenylflavonoids influenced the cardiomyogenesis process.[95] Hence, it is envisaged that the antioxidant nature of the natural products may play a critical role in the differentiation of the vascular progenitor cells as well. In recent years, the identification of progenitor cells in the adult organ-systems has opened an altogether new approach to therapeutics. These progenitor cells are capable of repairing the damaged cells/tissues and because many of them are derived from macrophages, they can combat pathogens to a certain extent. Moreover, a continued effort is on to use the circulating levels of the progenitor cells as a marker of general health and capacity of the body's reparative process. Although there are several markers identified on the progenitor cells, as a general rule, the expression of CD133 and CD34 are accepted as markers representing circulating progenitor cells, which may be derived from bone marrow and these cells have the potential to be involved in several tissue repair processes.

A few recently published studies show the potential beneficial effects of natural products (prenylflavonoids, icariin, berberine, green tea, fucodian, and icaritin) [95,100] on EPCs; however, it is likely that such beneficial effects also exist with other vascular or non vascular progenitor cells, which may have several health benefits. For instance, it was shown that low-molecular-weight fucodian enhances the circulating levels of EPCs.[94] A similar effect on bone marrow derived progenitor cells is observed in chronic smokers consuming green tea.[96] Such effects may be attributed to the antioxidant nature of the natural products enriched with flavonoids.[99,100] Thus, it is likely that flavonoid constituents of the natural products contribute to the beneficial effects on progenitor cells. Moreover, it can be hypothesized that the flavonoids in herbal general tonics enhance the levels of circulating progenitor cells ($CD133^{+ve}$ or $CD34^{+ve}$ cells), which in turn may contribute to general well being. Hence, it's very likely that several natural products exist that can mimic the effects of pharmacological agents/cytokines in positively modulating the vascular progenitors function. It's highly envisaged to discover such natural products and employ them in the main stream of vascular regenerative medicine.

CURRENT PITFALLS AND CHALLENGES AHEAD

While we now know that several progenitors exist with the system that contribute significantly to the vascular repair process, the interesting fact that needs to be explored is identifying the factors that govern the dynamics of the progenitor cells. What are the factors that precisely regulate or trigger progenitor cell mobilization, recruitment, and differentiation events? Can natural products mimic and/or influence these functions? Despite considerable advancement in the progenitor cell biology, there are several challenges that remain unanswered and research towards addressing these issue is envisaged. For instance, the precise panel of cell surface markers that define EPCs, SPCs, or vascular progenitors as well as the different mechanisms that precisely regulate their dynamics are unknown. It would be interesting to know if natural products have the tendency to regulate the progenitor's cell surface markers and precisely guide their differentiation into endothelial and/or smooth muscle cells. As the progenitor cell distribution is diffused, it would be interesting and therapeutically vital to understand the behavior of progenitor cells isolated from different regions and sources and it would be even more interesting to know if natural products can selectively influence their function. Also, understanding the mechanism by which stem/progenitor cells achieve a functional improvement is the need of the hour. Although the current benefits of natural products are limited to their antioxidant functions, it will be therapeutically valuable to know if the benefits are beyond the antioxidant effects and are rather due to a direct or indirect effect on the cell surface markers and cell migration and differentiation events. Unrevealing the mystery of differentiated progenitors working in such close concert with the vasculature will be a paradigm shift in the vascular pathophysiology and will redefine the way vascular diseases are currently treated.

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