

Review

Stem cells, vascular smooth muscle cells and atherosclerosis

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Summary. Stem cells have the ability to differentiate into a variety of cells to replace dead cells or to repair tissue. Recently, accumulating evidence indicates that mechanical forces, cytokines and other factors can influence stem cell differentiation into vascular smooth muscle cells (SMCs). In developmental process, SMCs originate from several sources, which show a great heterogeneity in different vessel walls. In adult vessels, SMCs display a less proliferative nature, but are altered in response to risk factors for atherosclerosis. Traditional view on SMC origins in atherosclerotic lesions is challenged by the recent findings that stem cells and smooth muscle progenitors contribute to the development of atherosclerotic lesions. Vascular progenitor cells circulating in human blood and the presence of adventitia in animals are recent discoveries, but the source of these cells is still unknown. The present review gives an update on the progress of stem cell and SMC research in atherosclerosis, and discusses possible mechanisms of stem/progenitor cell differentiation that contribute to the disease process.

Key words: Stem cells, Vascular progenitors, Smooth muscle cells, Atherosclerosis

Introduction

The unique capability of stem cells to transform and replenish different tissue types has made research within this field incredibly promising. In general, stem cells can be divided into "embryonic stem cells", "embryonic germ stem cells" and "tissue-resident or "adult" stem cells" (Mathur and Martin, 2004). Embryonic stem cells are obtained from hollow sphere shaped embryos of 200

to 250 cells known as blastocysts, while, embryonic germ stem cells come from 5 to 9 week old fetus that can develop into ovaries or testes. Adult stem cells are derived from the umbilical cord and placenta or from blood, bone marrow, skin, and other tissues. Aside from their origin, the major distinction between different stem cells lies in their capacity to develop into every cell type of the body (pluripotent). Although a number of experiments have suggested that certain adult stem cells are pluripotent, this characteristic is solely attributed to embryonic stem cells.

Arteriosclerosis is an overlying term covering all pathologies in which arteries become harder and less elastic. Atherosclerosis, the most common form of arteriosclerosis, is a disease responsible for over 55% of all deaths in Western civilization (Ross, 1999). Traditionally, a widely accepted theory for the pathogenesis of arteriosclerosis is that proposed by Ross (Ross and Glomset, 1976a,b). He stated that one of the main contributors to the pathogenesis of arteriosclerosis is smooth muscle cells (SMCs). More specifically, the theory suggested that in response to vascular injury (Ross and Glomset, 1976a,b), SMCs migrate from the media into the intima, where they contribute to neointimal formation by turning into foam cells and producing extracellular matrix. Today, this theory is being challenged by the growing evidence that stem cells and smooth muscle progenitor cells contribute to arteriosclerosis by differentiating into SMCs in the intima (Han et al., 2001; Hillebrands et al., 2001; Li et al., 2001; Saiura et al., 2001; Shimizu et al., 2001; Hu et al., 2002a,b; Sata et al., 2002). This means that stem/progenitor cells may play an important role in the pathogenesis of atherosclerosis.

This paper reviews the current state of research in stem cells, SMCs and atherosclerosis, with a strong focus on 1) the mechanisms of stem cell differentiation into SMCs during development, 2) the role of smooth muscle progenitors in keeping vessel wall integrity, and

3) the role of stem cells and progenitors in the pathogenesis of atherosclerosis.

Embryonic stem cells

In the last several years, a major accomplishment has been the ability to differentiate embryonic stem cells into vascular endothelial cells, SMCs and cardiomyocytes *in vitro*, representing not only an understanding on the developmental process, but also a potential cell source for cardiovascular tissue repair (Gepstein, 2002). In addition, immense progress has been made towards understanding the underlying mechanisms for these specific differentiation processes. Yamamoto et al. (2005) studied the effect of shear stress on differentiation and reported that mechanical forces generated by fluid flow, could induce embryonic stem cell differentiation into endothelial cell. Accordingly, Wang et al. (2005) reported that shear stress significantly upregulated angiogenic growth factors while down-regulated growth factors associated with SMC differentiation. Aside from mechanical forces, a number of growth factors and cytokines have been reported to be involved in stem cell differentiation. The expression levels of cytokines and growth factors were altered during differentiation of the mesenchymal stem cells (Kim et al., 2005). Further support for the role of cytokines in stem cell differentiation comes from the fact that co-culture of mouse neural stem cells with human endothelial cells results in neural stem cells converted to endothelial-like cells that have the capacity to form capillary networks (Wurmser et al., 2004). These findings indicate that the use of different cytokines can direct differentiation to specific pathways. Thus, the differentiation of embryonic stem cell into specific cell lineage, e.g. vascular cells will depend on its microenvironment, including mechanic forces, cytokines or growth factors, extracellular matrix, and communication with adjacent cells.

Adult stem cells

Adult stem cells reside in specific tissues, giving rise to replacement of damaged cells and other demands for the specific tissue or organ. With reference to the cardiovascular pathologies, two significant adult stem cell sources have been identified in heart and bone marrow tissues. It has been demonstrated that, bone marrow stem cells could grow into many different tissue types, including muscle, cartilage, bone, liver and different types of neurons and brain cells (Eglitis and Mezey, 1997; Jiang et al., 2002), although controversial results exist (Balsam et al., 2004). It was also reported that blood vessel formation could be derived from hematopoietic stem cells, while adult peripheral blood CD34+ (Haemopoietic progenitors) cells were shown to transdifferentiate not only into endothelial cells, but also SMCs *in vivo* (Yeh et al., 2003). The second major finding is that residential stem cells existing in the heart

have an ability to differentiate into cardiomyocytes, endothelial cells and SMCs (Anversa and Nadal-Ginard, 2002). In the last several years, a major effort has been made in an attempt to identify stem cells capable of differentiating into cardiovascular cells for the regeneration of the damaged heart (Losordo and Dimmeler, 2004a,b). The recognition that the adult heart possesses a stem cell compartment that can regenerate myocytes and coronary vessels has raised a unique possibility to rebuild dead myocardium after infarction, to repopulate the hypertrophic decompensated heart with new better functioning myocytes and vascular structures, and, perhaps, to reverse ventricular dilation and wall thinning (Beltrami et al., 2003; Leri et al., 2005). Cardiac stem cells may become the most important cells for cardiac repair. The above data suggest that adult stem cell differentiation can be a potential target for clinical applications.

SMCs

Since different types of stem cells are capable of differentiating into SMC, it is important to discuss the fate of the later cells especially with reference to atherosclerosis. An important feature of SMC biology that makes their study important is the considerable heterogeneity in their origins, both during development and in the adult vasculature (Drab et al., 1997). In embryo, vascular SMCs have a complex origin, with the first mural cells investing endothelial tubes being derived from transdifferentiated endothelium during nascent vascular and cardiac valve development (Nakajima et al., 1997). Subsequently, mural cells within the developing embryo derive from a number of sources. For instance, vascular SMCs in coronary arteries derive from the epicardial lining and are therefore of mesodermal origin (Gittenberger-de Groot et al., 1999), whereas coronary vein SMCs originate from the atrial myocardium (Dettman et al., 1998). In contrast, vascular SMCs of the aortic arch are likely of neuroectodermal origin, and those cells constituting the descending aorta originate predominantly from the local mesenchyme (Gittenberger-de Groot et al., 1999).

By moving to late embryogenesis and postnatal development, SMCs are known to have an extremely high rate of proliferation (Cook et al., 1994), yet at this time they undergo the most rapid rate of induction of expression of multiple SMC differentiation marker genes (Owens and Thompson, 1986). During vascular development, SMCs also exhibit high rates of synthesis of extracellular matrix components including collagen, elastin, proteoglycans, cadherins, and integrins that comprise a major portion of the blood vessel mass. Thus, according to Hungerford during this stage of development, SMCs form abundant gap junctions with endothelial cells on one hand, and the process of investment of endothelial tubes with SMCs or pericytes on the other, is critical for vascular maturation and vessel remodeling (Hungerford and Little, 1999).

In adult blood vessels, SMCs show a different behavior, characterized by an exceedingly low rate of proliferation/turnover, largely non-migratory, and an extremely low rate of synthesis of extracellular matrix components. More specifically, SMCs in mature animals are highly specialized cells whose major function is the contraction and regulation of blood vessel tone-diameter, blood pressure, and blood flow distribution. Indeed, Owens (1995b) supports that the fully differentiated mature SMC expresses a repertoire of appropriate receptors, ion channels, signal transduction molecules, calcium regulatory proteins, and contractile proteins necessary for the unique contractile properties of the SMC. The study suggests that the role of SMCs appears to vary depending on the stage of the disease, which is playing a maladaptive role in lesion development and progression (Ross and Glomset, 1976a; Owens, 1995b). In response to vascular injury, SMCs dramatically increase their rate of cell proliferation, migration, and synthetic capacity and play a critical role in vascular repair. On the other hand, recent evidence suggested that stem cells and smooth muscle progenitors participate in vessel wall biology in several settings of experimental vascular disease (Han et al., 2001; Hillebrands et al., 2001; Li et al., 2001; Saiura et al., 2001; Shimizu et al., 2001; Hu et al., 2002a,b; Sata et al., 2002). These observations may provide a platform in order to start a re-evaluation of SMC heterogeneity and progenitor biology in atherosclerosis, with main implications including the diagnosis and therapy of vascular disease.

SMCs and atherogenesis

In atherosclerotic lesions, three major cell components are SMCs, which dominate the fibrous cap, macrophages (inflammatory cells), which are the most abundant cell type around the necrotic core, and the lymphocytes (intracellular and extracellular lipid) (Xu et al., 1990; Hansson et al., 2002), which have been mainly ascribed to the fibrous cap. A complex and still incompletely understood interplay exists between these cells and the development of an atherosclerotic lesion (Ross, 1999). Therefore, in an attempt to define the complex and interrelated biological processes that contribute to the clinical manifestations of the disease, a number of risk factors should be considered such as the abnormal vasomotor function, the thrombogenicity of the blood vessel wall, the state of activation of the coagulation cascade, the fibrinolytic system, SMC migration and proliferation, and inflammation process (Libby, 2002; Wick et al., 2004; Hansson, 2005). However, the exact cause that bears the development of the atherosclerosis is not completely understood and is still under ambiguous discussion. In 1976, Ross and Glomset phrased the "response to injury" hypothesis suggesting that all risk factors, e.g. hyperlipidemia, smoking (toxins), and altered mechanical stress, damage the endothelium and alter the nature of the endothelial barrier to the passage of blood constituents (Ross and

Glomset, 1976a,b). This results in the increase of the permeability for lipoproteins and other plasma constituents-including growth factors, oxidised lipids, which result in medial SMC activation, migration and proliferation (Ross, 1999). In this hypothesis, a key issue is that all SMCs accumulated in lesions are derived from medial mature SMCs. Based on this hypothesis, many studies trying to clarify the mechanisms of SMC de-differentiation or different phenotypes have been carried out.

Phenotypes differ between medial and neointimal SMCs

In the last two decades, a large number of studies demonstrated that the phenotypes of SMCs differ between media and atherosclerotic lesions, and a noticeable attempt has occurred in order to approach the phenotypic switching of SMCs, which indicates to play a central role in atherosclerosis according to Ross's hypothesis (Ross and Glomset, 1976a,b). It is believed that before SMCs can migrate from the media into intima, a transition in their phenotype is required (Campbell and Campbell, 1994). Medial non-proliferating SMCs have a contractile phenotype that enables them to regulate vascular tone. When SMCs proliferate, they acquire a synthetic phenotype. According to Hiltunen (Hiltunen et al., 2002), the proliferative state of the SMC requires profound changes in gene expression and protein synthesis.

Obviously, a major challenge in understanding the differentiation of vascular SMCs is their ability to appear as a wide range of different phenotypes at different stages of development. Nevertheless, the investigation of the role of SMC phenotypic switching in vascular disease has to face a number of difficulties and deficiencies. The major deficiency is the failure of most studies to adequately distinguish "differentiation markers" that serve as indices of the relative state of differentiation of SMCs versus "lineage markers" that can serve to identify SMCs to the exclusion of all other cell types. Another limitation is the reduced information of known regarding factors that regulate SMC differentiation and the different phenotypes in vivo. On the other hand, the phenotypes of cultured SMCs can be markedly influenced by a large number of factors, including mechanical forces (Reusch et al., 1996; Li et al., 1999; Li and Xu, 2000), contractile agonists (Hautmann and Owens, 1997; Garat et al., 2000), reactive oxygen species, endothelial-SMC interactions, thrombin, neuronal factors, TGF- β 1, and extracellular matrix components such as laminin and type I and IV collagens (Hirschi, 1999; Owens, 1995a; Pickering, 2001; Su et al., 2001). Although it is widely acceptable that the phenotypic modulation of SMCs is a critical process in atherogenesis and vascular injury repair, no direct evidence indicates that such phenotypic modulation is essential for atherosclerosis development. Therefore, it is difficult to conclude that lesional SMCs

are derived from the medial mature cells after de-differentiation.

Stem cells, SMC and atherosclerosis

As mentioned above, the traditional hypothesis that SMCs in atherosclerotic lesions are derived from the media of the affected vessel and migrate into the neointimal space is now shrouded in doubt. There is accumulating evidence from animal models and humans that it is impossible for all the SMCs in an atherosclerotic lesion to have originated from a single source (Han et al., 2001; Hillebrands et al., 2001; Li et al., 2001; Saiura et al., 2001; Shimizu et al., 2001; Hu et al., 2002a,b; Sata et al., 2002; Torsney et al., 2005). Importantly, there is evidence that smooth muscle progenitor cells circulate in human blood (Simper et al., 2002). However, there is still uncertainty about the origin and residency sites of smooth muscle progenitors *in vivo*, and given the undoubted innate heterogeneity of SMCs, it is not surprising that there is currently conflicting data and it is likely that multiple origins and residency sites will be identified.

There is a number of evidences suggesting that SMCs or SMC-like cells within an injured blood vessel in animal models, or human atherosclerotic lesion may be derived from a variety of sources including circulating blood, medial stem cells, transdifferentiation of endothelial cells (Han et al., 2001; Hillebrands et al., 2001; Li et al., 2001; Saiura et al., 2001; Shimizu et al., 2001; Frid et al., 2002; Hu et al., 2002a,b; Sata et al., 2002; Simper et al., 2002; Tintut et al., 2003) and adventitial progenitor cells (Hu et al., 2004). Earlier studies by two groups showed that the majority of neointimal SMCs within plaques of experimental atherosclerosis in sex-mismatched chimeric scenarios and transgenic bone marrow transplant settings are derived from the bone marrow (Shimizu et al., 2001; Sata et al., 2002). Other investigators have shown that whereas most of SMCs in transplant atherosclerosis were derived from the host, only approximately 11% were of bone marrow origin, with the remainder being of non-bone marrow origin (Hu et al., 2002a; Zhang et al., 2004). On the other hand, Hu et al. (2002b) have showed that 40% of the vascular SMCs in a vein graft model of atherosclerosis were host derived, and 60% were of donor origin, while no bone marrow-derived SMCs were found in this allograft model (Hu et al., 2002a). These findings indicate that recipient-derived SMCs take part in transplant arteriopathy and plaque development, however the extent of bone marrow contribution and the link with the neointimal smooth muscle accumulation remains under consideration.

Hillebrands et al. (2003) recently discussed that other possible sources for recipient-derived progenitor cells exist, including circulating smooth muscle precursors that may not be of direct bone marrow origin, some tissue-resident smooth muscle progenitors that are released into the circulation (Hirschi and Majesky,

2004), or other locally derived perivascular sources of smooth muscle progenitors (Hu et al., 2004). Hu et al. (2004) reported that there are abundant progenitor cells within the adventitia of the arterial wall in apoE-deficient mice that were stained positive for a number of stem cell markers, including sca-1 and c-kit. By moving forward, Mayr et al. (2000) approached an aspect and tried to speculate whether adventitia alone can act as a reservoir for vascular progenitor cells on one hand, and whether the degree of medial cell apoptosis or necrosis after vein graft implantation leads to the extensive recruitment of these cells to the neointima on the other hand. Taken together, these findings support the hypothesis that stem/progenitor cells in the blood and adventitia could contribute to the accumulation of SMCs in atherosclerotic lesions via direct migration across the media via vasa vasorum and via circulating blood as well (Fig. 1).

Studies on the molecular mechanisms of smooth muscle progenitor recruitment and differentiation have investigated the presence of integrins on smooth muscle outgrowth cells and their adhesion to extracellular matrix proteins. Simper et al. investigated the integrins found on smooth muscle progenitors (Caplice et al., 2003). These cells showed greater quantity of $\alpha 5/\beta 1$ on their surface in comparison to endothelial cells. The smooth muscle progenitors were more adherent to fibronectin. Similarly, Deb et al. (2004) used a similar experimental setup to produce a profile of smooth muscle progenitor integrins. They demonstrated that the larger quantity of α and β integrin expression by smooth muscle progenitors that bound to fibronectin. However, further studies are still needed to clearly understand recruitment and differentiation processes.

Conclusions and perspectives

Stem cell research provides a unique opportunity for our understanding the molecular mechanisms of cell differentiation toward SMCs *in vitro* and *in vivo*. Traditional view on SMC origins is challenged by the recent findings that stem cells and smooth muscle progenitors contribute to the development of atherosclerotic lesions and as these cells may have close developmental links with vascular progenitors, they may express similar phenotypes in their immature forms. Smooth muscle progenitor cells circulating in human blood and the presence of adventitial smooth muscle progenitor cells in animals are recent discoveries (Fig. 1), however, the source of these cells is still unknown.

Based on the recent discovery that stem cells have a role in vascular repair, stem cell research could be important not only for understanding the pathogenesis of the disease, but also for development of cell-based therapies and tissue-engineering. A better understanding of the genetic and molecular controls of stem cells may yield information to scientists to fully understand the signals that turn specific genes on and off in order to

increase their number and to influence the differentiation of the stem cell. In fact, we are just beginning to understand those factors and molecular mechanisms that might control stem cell differentiation in the phenotypic state of the SMC associated with vascular injury. Furthermore, our knowledge regarding what controls differentiation during different stages of development of atherosclerosis in human vessels is still limited. Therefore, the next step should be an accumulated attempt to understand the biology of the smooth muscle progenitor cells, which may provide novel perspicacity into the atherogenesis process and at the same time provide insights for new therapeutic strategies in the

prevention and treatment of atherosclerosis.

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Figure

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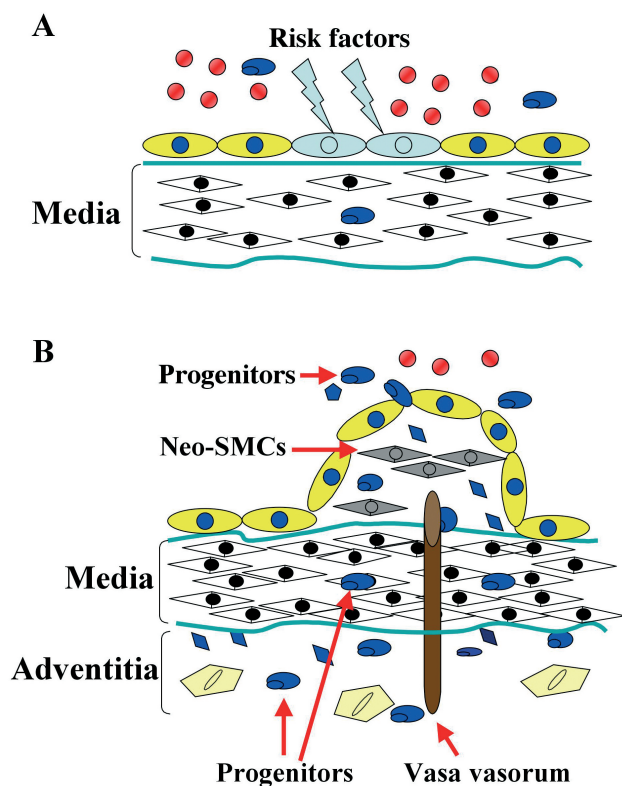


Fig. 1. Schematic representation for smooth muscle cells to participate in the formation of atherosclerosis. **A.** Endothelial cells may be derived from progenitor cells, which can be damaged by stimulation of locally generated cytokines and free radicals and risk factors such as disturbed blood flow, hyperlipidemia and toxins. **B.** Stem cells and smooth muscle progenitors existing in blood deposit into the intima. Meanwhile, angiogenesis within atherosclerotic lesions occur because lesions become enlarged, creating a hypoxic environment. These microvessels or vasa vasorum play a part in transport vascular stem or progenitor cells from the media and adventitia into the intima. These cells differentiate into neo-smooth-muscle cells within atherosclerotic lesions, which differ from medial smooth-muscle cells. This process repeats many times, leading to the formation of atheroma.

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