Diverse origin of vascular smooth muscle cells in the neointima

Kalınlaşan intima tabakasındaki vasküler düz kas hücrelerinin değişik kökenleri

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ABSTRACT

Vascular smooth muscle cells accumulate excessively in the formation of neointima and have a key role in the pathogenesis of vasculoproliferative disorders such as atherosclerosis, allograft vasculopathy, bypass graft occlusion, in-stent restenosis and restenosis after percutaneous balloon angioplasty. To date there is no clinically established treatment to prevent the accumulation of smooth muscle cells in the neointima. However, much attention has been devoted to experimentally targeting the inhibition of migration and proliferation of medial smooth muscle cells. The recent identification of circulating bone marrow-derived smooth muscle progenitor has challenged the classical concept of infiltration of solely medial smooth muscle cells in the neointima. In addition, other potential sources such as circulating smooth muscle cell precursors that may not be of direct bone marrow origin, adventitial stem cells or smooth muscle cell progenitors that are released from other organs into the circulation have been demonstrated to have the potential to differentiate into smooth muscle cells. These discoveries have motivated us to reconsider how neointima forms in pathological conditions in the adult human. This update discusses recent insights on smooth muscle progenitors both from a biological and therapeutic perspective. *(Anadolu Kardiyol Derg 2005; 5: 216-20)*

Key words: Neointima, vascular smooth muscle, proliferation

ÖZET

Damar duvarında intima tabakasının kalınlaşmasıyla ortaya çıkan neointima çok sayıda damar düz kas hücresi içerir. Bu hücreler damar sertliği, nakil edilen organların reddine neden olan damarsal değişiklikler, baypas ameliyatı, stent yerleştirilmesi, ve balon anjiyoplasti girişimi sonrası yeniden oluşan damar daralmalarının patojenezinde önemli rol oynarlar. Bugüne kadar bu hücrelerinin neointimada birikimini önleyecek klinik bir tedavi metodu bulunamamıştır. Bununla birlikte, bu hücrelerin damar media tabakasından neointimaya göçünü ve çoğalmasını durdurmaya yönelik birçok deneysel çalışma yapılmıştır. Son yıllarda, kemik iliğinden kökenli, kanda dolaşan ve damar düz kas hücresine dönüşebilen kök hücrelerinin saptanması, neointimadaki düz kas hücrelerinin sadece media tabakasından geldiğine dair klasik bilgimizi tartışmaya açmıştır. Kanda dolaşan bu hücrelerin yanı sıra, kemik iliğinden doğrudan kaynaklanmayan, adventitia tabakasında bekleyen ya da diğer organlardan dolaşıma katılan ve düz kas hücresine dönüşebilen kök hücrelerinin varlığı media tabakası dışında kaynaklarının bulunduğunu göstermektedir. Tüm bu yeni gözlemler bizleri patolojik damar hastalıklarında neointimanın nasıl geliştiğine dair bilgilerimizi yeniden yargılamaya zorlamaktadır. Biz bu kısa derlemede damar düz kas hücresine dönüşebilme yeteneğine sahip hücreler hakkındaki son bulguları ve bu hücrelerin biyolojik ve tedavi yönünden özelliklerini tartışmak istedik. *(Anadolu Kardiyol Derg 2005; 5: 216-20)*

Anahtar kelimeler: Neointima, vasküler düz kas, proliferasyon

Introduction

More than half of all deaths in industrialized countries are linked to the complications of atherosclerosis (1). Its pathogenesis remains to be further elucidated since no effective therapy has been established in human. Both inflammatory and noninflammatory cells are involved in vasculoproliferative responses. Monocytes/macrophages, T and B cells, neutrophils, mast cells, thrombocytes, endothelial cell (EC) and smooth muscle cells (SMC) are present in the neointima (Fig. 1), whereas the majority of neointimal cells are SMC (1). It was generally believed that neointimal SMC originate locally from the medial layer. Vascular SMC in this layer normally regulate vascular tone and blood flow. During the development of arterial remodelling, they migrate into the neointima and play a principal role in atherogenesis by proliferating and synthesizing a cascade of molecules (1). Once SMC are present in the neointima to limit the initial inflammatory response, they differ from their medial counterparts with regard to phenotype and

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gene expression profile. Neointimal SMC secrete and express extracellular matrix, chemotactic, and mitogenic proteins during arterial remodelling (1). In addition, it has been recently shown that they express a number of hematopoietic lineage markers (2).

A recent paradigm has been proposed in which the bone marrow constitutes the reservoir of hematopoietic (3) and mesenchymal (4) stem cells that produce not only the blood cells but also cardiovascular cells such as vascular SMC, EC, and cardiac myocytes. Various populations of hematopoietic stem cells (HSC) are being studied, exploiting cell surface marker expression, such as Sca-1, c-kit, and CD34. To identify the bone marrow cells with the potential of generating vascular SMC, a Sca-1$^+$/c-kit$^+$ population has been isolated from the bone marrow by fluorescence-activated cell sorting following removal of all mature cell types (lin$^-$) using a cocktail of monoclonal antibodies (5). Recent experimental studies indicated a potential role in atherosclerosis, transplant arteriopathy and angiogenesis for putative SMC progenitors in the circulation, adult tissues, and the perivascular adventitia (6). The mobilization, homing, differentiation, and proliferation of bone marrow-derived vascular SMC progenitors are currently poorly understood, but may provide a new platform for the re-visiting of SMC biology during atherogenesis with implications for diagnosis and therapy of vascular diseases.

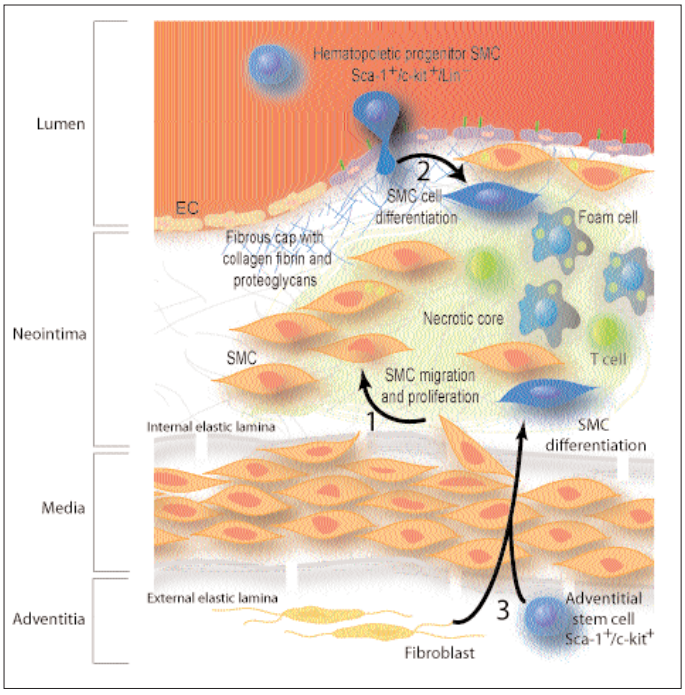

Figure 1. Vascular intima normally consists of a single layer of endothelial cells, however it thickens during the progression of arterial remodelling. Circulating hematopoietic and mesenchymal progenitors, medial SMC, and adventitial stem cells may contribute to the presence of neointimal SMC. In addition, adventitial fibroblasts and vascular endothelial cells have the potential to differentiate into SMC. These cells in the neointima secrete exaggerated levels of extracellular matrix proteins and express chemoattractants to exacerbate the inflammatory response. Inflammatory cells such as monocyte-derived macrophages and lymphocytes are also present in the neointima.
EC- endothelial cell, SMC - smooth muscle cells

Murine models to study the contribution of bone marrow-derived SMC progenitors

Involvement of SMC progenitors in the neointima formation has been recently demonstrated in several experimental models of vascular diseases, including post-balloon injury, graft vasculopathy and hyperlipidemic atherogenesis in mice (Fig. 2). However, significant divergence of opinion exists on the extent of bone marrow contribution to the neointima formation. Genetically-engineered mice expressing reporter genes such as LacZ or green fluorescent protein (GFP) have been used as experimental tools (5). These ectopic transgenes have been driven by either a SMC-specific promoter or the ROSA26 promoter that drives gene expression ubiquitously in all tissues and cells. Following bone-marrow transplantation from these mice into their wild-type counterparts, X-gal histochemical staining or visualization of GFP-positive cells by fluorescence microscopy are used to track the fate of bone-marrow-derived cells in the cardiovascular tissues. However, the simple detection of GFP-positive cells has the superiority over the β–gal staining that requires histochemically optimized reagents. These tracking methods are then combined with immunohistochemical detection of cell-specific surface markers to identify the differentiated state of SMC in tissues. The most commonly used identification marker for SMC is the SMC-specific α-actin. Other markers include myosin heavy chain, calponin, SM22α, and h-caldesmon.

A majority of murine models of allograft vasculopathy indicated a recipient origin for infiltrating vascular SMC within the neointima. Some investigators demonstrated that the majority of neointimal SMC within the atherosclerotic plaques are derived from the bone marrow (5, 7). However, there were technical concerns to detect individual neointimal SMC expres-

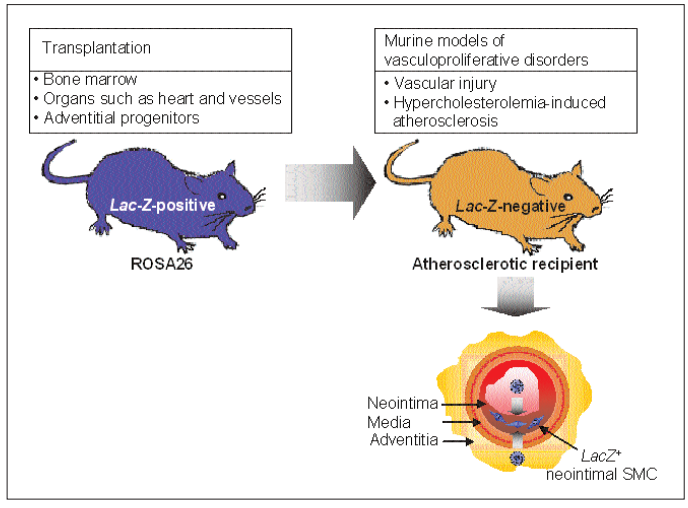

Figure 2. The origin of neointimal SMC can be studied in murine models of vascular disease where Lac-Z-labelled cells or tissues of ROSA26 mice are transplanted into LacZ-negative atherosclerotic counterparts. Vascular lesions in recipient mice can be induced either by an injury or hypercholesterolemic diet. Neointimal SMC can be detected in vascular sections stained with a combination of X-gal and immunohistochemistry using antibodies recognizing SMC surface markers.
SMC - smooth muscle cells

sing Lac-Z in these studies. Other investigators reported that most of the neointimal SMC are derived from the host and only 11% are of bone marrow origin in transplant atherosclerosis (8). In another study, Hu et al. demonstrated that 40% of the SMC in a vein graft model of atherosclerosis were host derived and the remaining 60% of the cells were of donor origin without the presence of any bone marrow-derived neointimal cells (9). In sex-mismatched human renal allografts using a combination of fluorescence *in situ* hybridization (FISH) detection of Y-chromosome and SMC-specific α-actin staining, it has been concluded that approximately 35% of neointimal SMC have recipient origin (10). A similar contribution has also been demonstrated in the coronary arteries of human cardiac transplants (11). Sata et al. showed that more than 60% of neo intimal cells during vascular remodelling after injury, more than 80% of neointimal cells during graft vasculopathy after heterotopic heart transplantation and more than 40% of neointimal SMC in hypercholesterolemia-induced atherosclerosis are derived from bone marrow origin (5). In summary, it is clearly evident that recipient-derived SMC infiltrate both transplant vasculopathy and atherosclerotic plaque, but the precise extent of bone marrow contribution of neointimal SMC remains to be further studied.

New potential sources of SMC within the neointima

During embryogenesis, SMC arise from at least two different lineages; one derived from the neuronal crest (ectomesenchymal SMC), the other originating from the mesoderm (mesenchymal SMC) (12, 13). Recent data suggest that other sources of SMC such as adult stem and progenitor cells either in circulation or in tissues, as well as transdifferentiation of other vascular cells may contribute to the presence of neointimal SMC (Table 1). Smooth muscle cells progenitors have been shown to exist in skeletal muscle cells with divergent differentiation fates during injury and regeneration (14). Majka et al. isolated highly purified HSC from skeletal muscle cells, divided them into side and non-side populations (non-SP), and showed that while SP cells contribute to EC replacement, non-SP cells within the skeletal muscle differentiate into SMC. Similarly, Sca-1$^+$/c-kit$^+$/ lin$^-$ resident progenitors within the heart have been described in mice (15). These cells have the potential to differentiate into vascular cells including SMC following myocardial infarction (16). It would be interesting to determine whether these tissue resident stem cells are released into the circulation either at physiological or pathological conditions.

The other potential sources of circulating SMC progenitors may be of non-bone marrow origin (17) or from the vascular adventitia (18). It is known that adventitial cells such as fibroblasts migrate to the neointima and differentiate to myofibroblasts (19). Recently, the existence of Sca-1$^+$/c-kit$^+$ precursors has been demonstrated in the adventitia (18). These cells differentiate into neointimal cells that express SMC surface markers in the presence of platelet-derived growth factor (PDGF). PDGF-BB is required for SMC recruitment to arteries since mice deficient for PDGF-BB lack microvascular pericytes and develop microaneurysms (20). Interestingly, human peripheral blood mononuclear cells cultured with collagen in the presence of PDGF-BB differentiate into SMC-like cells that express SMC α-actin and calponin (21). Moreover, KDR$^+$ murine embryonic stem cells can differentiate into SMC lineage when induced with PDGF-BB and reproduce their vascular organization *in vivo* (22). In another study, it has been shown that EC, when cultured with TGF-β, can express SMC-specific α-actin and concomitantly lose factor VIII-related antigen expression (23). This finding has now been supported by an in vivo developmental study which suggested that endothelium is the source of at least some of SMC in the arteries, although this event occurs only in a small percentage (24). Thus, these data raise the possibility that SMC and EC progenitors share a common precursor.

Cell fusion as a possible source of bone marrow-derived SMC

Many articles documented that adult stem cells transdifferentiate into other lineages including cardiovascular cells. However, recent studies challenge the existence of adult stem cells by transdifferentiation. To study whether HSC transdifferentiate into cardiovascular cells or fuse with the existing cells, different identification techniques ranging from major histocompatibility complex class immunohistochemistry to FISH of sex chromosomes between mismatched donor and recipient cells have been performed. Precise co-localization of these markers requires careful confocal imaging studies. It has now been reported that HSC adopt tissue-specific phenotype by cell fusion both *in vitro* and *in*

Table 1. Potential origins of neointimal SMC

Anatomical origin	Reference
• Medial SMC	Ross et al., 1993
• Circulating SMC precursors that may be of direct bone marrow origin	Saiura et al., 2001
	Sata et al., 2002
• Other tissue-resident SMC progenitors that may be released into the circulation	Majka et al., 2003
such as skeletal muscle and heart	Beltrami et al., 2003
• Adventitial SMC progenitors	Hu et al., 2004
• Other vascular cells with the potential of differentiating into SMC such as EC and fibroblasts	Frid et al., 2002
	Li et al., 2000

EC- endothelial cell, SMC - smooth muscle cells

vivo but not by transdifferentiation (25, 26). Earlier studies on the polyploidization of SMC in response to mechanical and humoral stimuli may support this notion (27). Thus, the presence of bone marrow-derived SMC-like cells in the neointima may be partly explained by the fusion phenomenon. In addition, some recent papers raised doubts on the differentiation of HSC into cardiovascular cells and suggested that HSC solely adopt mature hematopoietic fates in ischemic myocardium (28, 29).

Conclusions

Recent exciting data clearly point out that neointimal SMC have diverse origins and can be recruited from a variety of sources depending on the type, severity and duration of vascular injury. However, it is still unclear whether they have a reparative and protective function or whether they contribute significantly to the formation of neointima and disease progression.

Studies determining gene expression profile between diverse origins of vascular SMC would answer whether their contribution is harmful or necessary during arterial remodelling. Pharmacological agents such as statins have been shown to inhibit the migration and proliferation of vascular SMC (30), however, their inhibitory effect on different source of SMC remains to be studied. Environmental factors such as infections and aging aggravating SMC proliferation and migration may also affect diverse origin of SMC differently.

After the discovery of progenitor EC (31), great attention was given to HSC in the hope of treatment of acute myocardial infarction in human (32). In addition, the studies of circulating cardiac progenitor cell to repair murine myocardial infarction have excited many cardiologists (16). Despite the initial positive results, wide-scale clinical implantation is not warranted until fundamental questions regarding these novel cell populations have been answered. Nevertheless, these studies are prompting observers to speculate that SMC progenitors exist in the circulation and originate from either bone marrow or extramedullary sources. They may play a critical role, not only in maintaining the arterial wall, but also in the formation of neointima during vascular remodelling. The molecular mechanisms that regulate their mobilization from different tissues, recruitment signals in the vascular microenvironment, homing properties, and functional importance of the SMC precursors compared to other cell populations require further elucidation. If these data can be obtained, we will witness a rapid translation of progenitor cell biology to clinical application within the next decades.

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