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Potential contribution of bone marrow-derived precursors to vascular repair and lesion formation: lessons from animal models of vascular diseases

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1. ABSTRACT

Atherosclerosis is responsible for more than half of all deaths in western countries. Numerous studies have reported that exuberant accumulation of smooth muscle cells (SMCs) plays a principal role in the pathogenesis of occlusive vascular diseases. It has been assumed that SMCs derived from the adjacent medial layer migrate towards the atherosclerotic lesion, proliferate and synthesize extracellular matrix, thus contributing to atheroma growth. Although much effort has been devoted to targeting the migration and proliferation of medial SMCs, no effective therapy to prevent occlusive vascular remodeling has been established. By taking advantage of genetically-modified mice, we recently reported that bone marrow cells substantially contribute to the pathogenesis of vascular diseases. It was suggested that bone marrow cells may have the potential to give rise to vascular progenitor cells that home in the damaged vessels and differentiate into smooth muscle cells or endothelial cells, thereby contributing to vascular repair, remodeling, and lesion formation. This article summarizes what we learned from genetically-modified animals regarding the origins and the fates of vascular cells that contribute to lesion formation.

2. CONTRIBUTION OF SMOOTH MUSCLE CELLS TO VASCULAR LESIONS

Exuberant accumulation of SMCs plays a principal role in the pathogenesis of vascular diseases (1-6). In atherosclerotic plaques, SMCs proliferate and synthesize extracellular matrix, thereby contributing to lesion formation. Percutaneous coronary interventions (PCIs) have been widely adopted for treatment of coronary atherosclerosis. Although the increasing use of new devices, such as drug-eluting stents for dilatation of stenosed arteries has lowered the incidence of restenosis, it still limits the long-term outcome of PCI (7). Furthermore, SMC hyperplasia is also a major cause of postcoronary bypass surgery occlusion (8, 9) and graft vasculopathy after cardiac transplantation, a leading cause of graft failure and retransplantation after the first postoperative year results (10). Therefore, much effort has been devoted to understanding the molecular pathways that regulate SMCs hyperplasia in order to prevent vascular diseases (1-4). However, the pathogenesis remains largely unknown and, consequently, effective therapy has not yet been established (6). There is a widely accepted view that atherosclerotic lesions result from an excessive, inflammatory–

fibroproliferative response to various forms of insult to the endothelium and smooth muscle of the artery wall (1-4, 11-13). In brief, after endothelial injury, inflammatory cells infiltrate and secrete various cytokines, such as tumor necrosis factor alpha, interleukin-1β, and interferon-γ, which trigger dedifferentiation of the medial contractile-SMCs that regulate vascular tone and blood flow under normal physiological conditions (4, 5). Dedifferentiated synthetic-SMCs are characterized by a large cell body containing synthetic and secretary organelles (4, 5, 14-23). Medial synthetic-SMCs are believed to migrate into the subendothelial space, proliferate, and synthesize extracellular matrix (2). It was hypothesized that all of the neointimal cells in post-PCI restenosis and graft vasculopathy derive from medical SMCs (2). Thus, numerous pharmacological and gene therapies have been proposed to target the dedifferentiation, migration and proliferation of medial SMCs (24-35).

However, there are several phenomena that could not be explained in accordance with the aforementioned hypothesis. First, very few papers documented that SMCs were migrating across the internal elastic lamina from the media into the subendothelial layer, as often illustrated in cartoon (2, 4). On the other hand, many studies have shown that blood cells attach to the luminal side of mechanically-injured arteries prior to the development of neointimal hyperplasia that is composed exclusively of SMCs (36, 37). Second, it was observed that neointima could be formed in the absence of medial cells after they underwent apoptosis induced by severe injury (36). In this study, it was noted that neointimal cells were negative for SMCs markers and appeared to be hematopoietic rather than vascular cells at one week after injury. Third, many studies reported that SMC hyperplasia could be prevented by blocking chemokines or adhesion molecules (38, 39), which play a crucial role in recruiting blood cells but have no effect on migration and proliferation of differentiated SMCs (38). Fourth, neointimal SMCs are quite distinct from medial SMCs in phenotype and gene expression patterns (4, 40, 41). For example, neointimal SMCs have been shown to express a number of hematopoietic lineage markers, including FK506-binding protein 12, interferon regulatory factor, and proinflammatory proteins (40, 41). These findings suggest that some of the neointimal SMCs might derive from blood cells rather than from medial SMCs. Evidence in support of this hypothesis was provided in several models of vascular diseases (42-45).

3. BONE MARROW-DERIVED SMOOTH MUSCLE LIKE CELLS IN VASCULAR LESIONS

By taking advantage of genetically-modified mice, we and others recently suggested that some of the SMCs in vascular lesions may derive from circulating cells. Particularly, transgenic or knock-in mice that express marker genes enabled us to investigate the origin and the fate of vascular cells *in vivo*.

3.1. Bone marrow-derived smooth muscle like cells in graft vasculopathy

The contribution of bone marrow cells (BMCs) was firstly investigated in graft vasculopathy (Figure 1), a robust form of atherosclerosis that develops rapidly in transplanted organs, leading to failure of the allograft (3, 10). Heterotopic cardiac transplantation was carried out between wild-type mice and ROSA26 mice (42, 43), which are transgenic mice expressing LacZ ubiquitously (LacZ-mice) (46, 47). Four weeks after transplantation of wild-type hearts into ROSA26 mice, the allografts were stained with 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside (X-gal) to identify LacZ-expressing cells. Recipient-derived SMCs were defined as positive against both of X-gal and anti-SMalpha-actin immunostaining. Similarly, ECs and macrophages were identified by immunostaining with anti-CD31 and MOMA2 antibodies, respectively. The lumens of the large epicardial coronary arteries, their smaller branches and arterioles were found to have narrowed because of concentric hyperplastic growth of neointimal cells, the majority of which were recipient cells expressing LacZ (42, 43). It was also observed that some of the medial SMCs, as well as endothelial cells (ECs), had been replaced by recipient cells. Immunofluorescence studies revealed that the LacZ-positive cells in the neointima expressed smooth muscle alpha- actin (SMalpha-actin) (43). Conversely, when LacZ-positive hearts were transplanted into wild-type mice, LacZ-negative neointima developed on the LacZ-positive coronary arteries. These results indicated that, in graft vasculopathy, the vast majority of the neointimal cells were derived from the recipient cells, but not from the medial cells of donor origin. To identify the potential source of recipient cells, bone marrow transplantation (BMT) was performed from LacZ-mice to wild type mice ($BMT^{LacZ\rightarrow wild}$ mice). After 4–8 weeks, wild type hearts were transplanted into the $BMT^{LacZ\rightarrow wild}$ mice, and 4 weeks later, most of the neointimal cells were found to be LacZ-positive. Similarly, in studies of BMT followed by cardiac transplant using transgenic mice that ubiquitously expressed enhanced green fluorescent protein (GFP mice) ($BMT^{GFP\rightarrow wild}$ mice) (43), it was observed that GFP positive cells accumulated on the luminal side of the graft coronary arteries. The immunofluorescence study revealed that some of the GFP-positive neointimal cells in graft vasculopathy also expressed SMalpha-actin. These results indicate that recipient BMCs may substantially contribute to neointimal formation in transplanted grafts.

Consistent with these observations, others independently reported that recipient cells are a major source of graft vasculopathy in the aortic transplantation model (48-51). Moreover, it has been reported that in human transplant-associated arteriosclerosis after renal transplantation most of the neointimal cells and ECs are derived from the recipient (52, 53).

3.2. Contribution of bone marrow-derived cells to neointima hyperplasia after mechanical injury

We previously reported that BMCs can also contribute to the pathogenesis of lesion formation after mechanical vascular injury. (42, 43) (Figure 2). The bone marrow of wild-type mice were replaced with that of LacZ-mice ($BMT^{LacZ\rightarrow wild}$ mice) and it was found that transplanted LacZ BMCs had settled in bone marrow, spleen, and thymus, whereas LacZ-positive cells were not

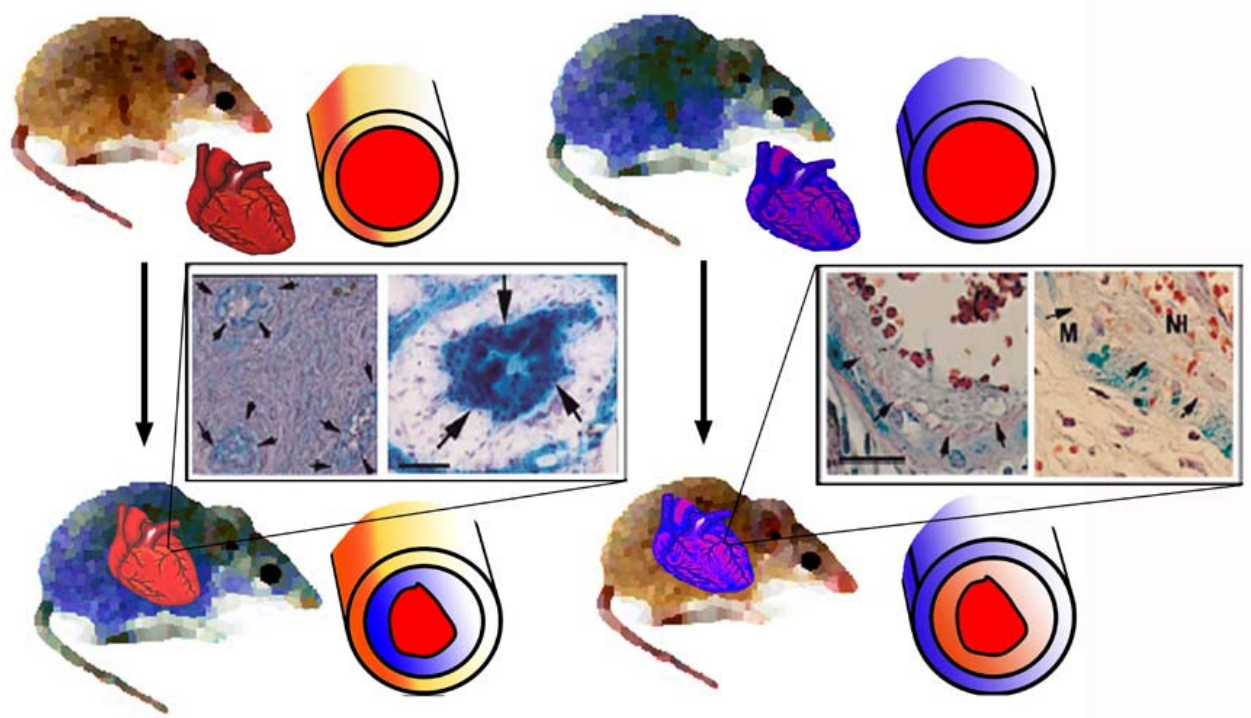

Figure 1. Recipient cells contribute to graft-vasculopathy. When wild-type hearts were transplanted into LacZ-expressing ROSA26 mice, 90% of neointimal cells were LacZ-positive and thus originating form the recipient (Left; Bar, 50 micrometer). Conversely, LacZ-negative recipient cells formed neointima on the LacZ-positive coronary arteries after cardiac transplantation from LacZ-mice to wild-type mice (Right; Bar, 25 micrometer). Bone marrow-derived SMCs were defined as positive against both of X-gal and anti-SMalpha-actin staining. Similarly, ECs and inflammatory cells were identified by immunostaining with anti-CD31 and MOMA2 antibodies, respectively. Arrows in histological panels indicate internal lamina. Reproduced with permission from Nature Publishing Company.

detected in the uninjured femoral arteries of the $BMT^{LacZ\rightarrow wild}$ mice. Four to 8 weeks after BMT, the femoral artery of the $BMT^{LacZ\rightarrow wild}$ mice was injured by inserting a large wire, an excellent model of vascular injury that resembles balloon angioplasty (36). This injury led to complete denudation of the endothelium and marked enlargement of the lumen (36, 45, 54) followed by a decrease in cellularity in the medial layer caused by acute onset of SMC apoptosis. One week after the injury, the artery remained dilated and X-gal staining revealed that LacZ-positive cells were attached to the luminal side of the injured vessels. The LacZ-positive cells did not express markers of neither SMCs (SMalpha-actin) nor ECs (CD31). The dilated lumen gradually narrowed because of the formation of neointimal lesions, which were primarily composed of SMCs. A significant number of the neointimal and medial cells were LacZ-positive on X-gal staining (43). Immunofluorescence double-staining documented that some bone marrow-derived LacZ positive cells in the neointimal lesions expressed SMalpha-actin or CD31 (45). These results indicate that BMCs may give rise to vascular cells, thereby contributing to arterial remodeling after wire-mediated endovascular injury.

Most of the bone marrow derived cells expressed alpha-SMA, but not markers for highly differentiated SMCs. Some CD45-positive cells also expressed alpha-SMA. These results indicate that bone marrow-derived cells present in the neointima easily express alpha-SMA even when they remain positive for hematopoietic markers. In contrast, it seems a rare event, if not at all, for bone marrow-derived cells to express markers of highly differentiated SMCs, at least within a few months after a wire injury. Consistently, it was reported that most of the bone marrow-derived cells detected in human atherosclerotic plaques expressed alpha-SMA-positive cells, but not calponin, a marker for differentiated SMCs (55).

3.3. Potential mechanism by which bone marrow-derived cells contribute to vascular lesion formation

Coronary angioplasty causes vessel wall injury and induces SMC proliferation with subsequent abundant production of extracellular matrix. Transplant-associated arteriosclerosis is also considered a consequence of an immunological attack against the allograft by the recipient. (3, 10) Various atherogenic substances, such as oxidized low density lipoprotein (56-58), homocysteine (59), angiotensin II (60) and lipopolysaccharides (61), have been reported to induce vascular cell apoptosis, presumably initiating the earliest phase of lesion development in atherosclerosis (62). Therefore, neointima formation appears to be similar to the healing process in response to vascular injuries (2). In addition to the conventional assumption that damaged tissues are repaired by individual parenchymal cells, an accumulating body of evidence suggests the occurrence of somatic stem cell mobilization

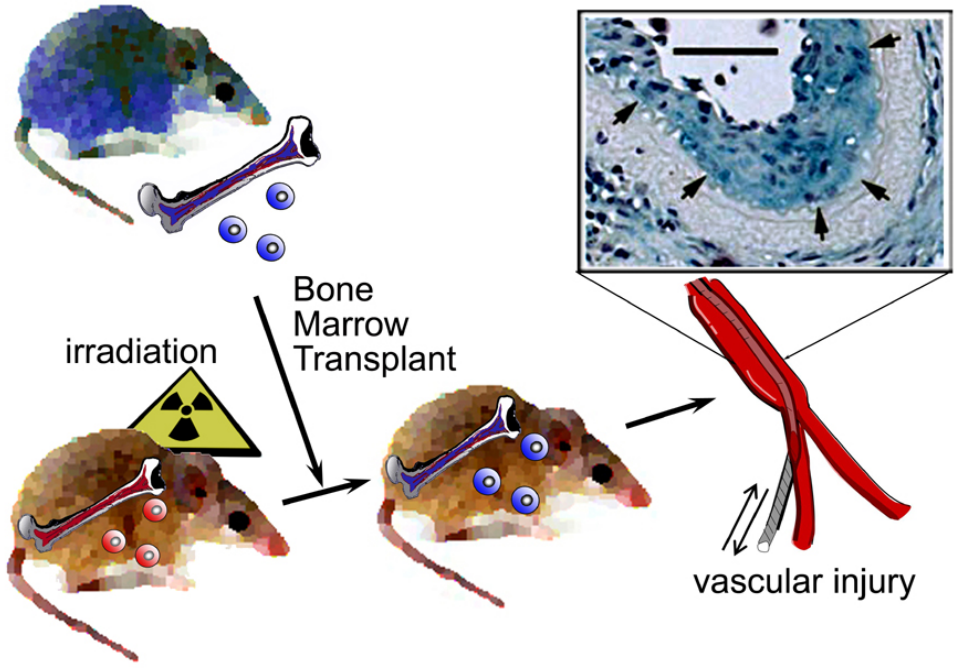

Figure 2. Contribution of bone marrow cells to healing and lesion formation after mechanical injury. A large wire was inserted into the femoral artery of irradiated wild-type mice whose bone marrow had been reconstituted with that of LacZ-expressing ROSA26 mice. Bone marrow-derived SMCs were defined as positive against both of X-gal and anti-SMalpha-actin staining. Similarly, ECs and inflammatory cells were identified by immunostaining with anti-CD31 and MOMA2 antibodies, respectively. At four weeks post-injury, we observed that about 60% of neointimal cells and 40% of medial cells were LacZ-positive, and thus derived from the transplanted bone marrow. Arrows and arrowheads in histological panel indicate internal and external elastic lamina, respectively. Scale bar, 50 micrometer. Reproduced with permission from Nature Publishing Company.

to remote organs, where they differentiate into required lineages and participate in organ repair and regeneration (63-67). Consistent with this notion, Minami *et al.* reported that extracardiac progenitor cells repopulated into various types of cells, including endothelial cells and VSMCs by evaluating human specimen of sex-mismatched cardiac transplantation (68). Bone marrow might be an additional source of vascular cells that contribute to both vascular repair and the pathological remodeling at least in models of post-angioplasty restenosis and transplant-associated arteriosclerosis.

3.4. Fractions of bone marrow cells that contribute to vascular remodeling

Pluripotent cells in bone marrow are classified as hematopoietic stem cells (HSCs) and mesenchymal stem cells. Although it was assumed that HSCs give rise only to blood cells of hematopoietic lineage (69), recent reports suggest that they may have the broader potential to differentiate into various cell types, including epithelial cells (70), hepatocytes (71), and cardiomyocytes (72). To identify the bone marrow cells that have the potential to generate vascular cells, a HSC-enriched fraction (c-Kit$^+$, Sca-1$^+$, Lin$^-$) was isolated from the bone marrow of LacZ-mice by fluorescence-activated cell sorting (43) and 3000 cells were injected into lethally irradiated wild-type mice. Four weeks after bone marrow reconstitution, the femoral artery of the recipient mice was mechanically injured with a large wire (36). At four weeks after the injury, both the neointima and the media contained many LacZ-positive cells (43), some of which expressed SMalpha-actin. LacZ-positive cells were also found to contribute to endothelial regeneration (43). These findings suggest that the c-Kit$^+$, Sca-1$^+$, Lin$^-$ fraction (KSL fraction) of bone marrow cells may have the potential to differentiate into either SMCs or ECs that participate in vascular remodeling.

In contrast, Wagers *et al.* extensively analyzed organs of wild-type mice whose bone marrow had been reconstituted with a single HSC (c-kit$^+$Thy1.1^{lo}Lin$^-$Sca-1$^+$). The authors concluded that transdifferentiation of HSCs into other lineages is an extremely rare event (73). This apparent discrepancy could merely derive from the analysis of non-injured vs. injured tissues in the two studies, or from the contribution of other cell types in the c-Kit$^+$, Sca-1$^+$, Lin$^-$ fraction. However, a recent study on ischemic myocardium failed to detect a contribution of hematopoietic cells to cardiac, smooth or endothelial phenotype (74). Thus, we investigated the vascular lesion induced by wire after the bone marrow was reconstituted by a single HSC ("Tip"-SP CD34$^-$KSL cell, a bone marrow cell that had the strongest dye-efflux activity ["Tip"-side population (SP) cells] with a phenotype of CD34$^-$ c-Kit$^+$ Sca-1$^+$ Lin$^-$ (CD34$^-$KSL))(75, 76). Although we noted appreciable level of hematopoietic engraftment activity, very few cells in the lesion were derived from this single HSC fraction. Our result suggests that it is a rare property for a highly-purified HSC to transdifferentiate into vascular

cells, whereas the KSL fraction of bone marrow cells contained a distinct population that could substantially contribute to lesion formation. Although the KSL fraction is considered to be enriched in HSCs (71), mesenchymal stem cells or multipotent cells that are more primitive than HSCs (77) could be included in this fraction. It is therefore plausible that those non-hematopoietic cells in the KSL fraction might be responsible for the KSL-derived endothelial-like cells or smooth muscle-like cells observed in the vascular lesion (43).

3.5. Controversy on the methods to detect "bone marrow-derived smooth muscle-like cells"

Recently, there are great controversy on the method to detect trans-differentiation of ectopic cells (73, 74, 78-80). In most of the studies, double immunofluorescent method is used to detect bone marrow-derived cells that express a marker of smooth muscle cells after transplantation of bone marrow cells that had been genetically labeled with LacZ or EGFP (43, 45, 76). However, other investigators made a caution regarding the specificity of co-localizing staining for the cell types and the markers to detect the origin of the cells in bone marrow chimeric animals and humans (80). Use of conventional microscopies potentially increases false co-localization signal by the overlap of two different cells. For example, when a "GFP-positive alpha-SMA-negative" inflammatory cell locates adjacent to a "GFP-negative alpha-SMA-positive" media-derived SMC, a "GFP-positive alpha-SMA-positive" pseudo-"bone marrow-derived SMCs" could be imaged artificially. Thus, higher 3-dimensional resolution with confocal/deconvolution microscopy is required to identify colocalization of signal in tissue sections (80). In this regard, we employed a high resolution confocal microscopy to convincingly demonstrate that bone marrow-derived cells did express alpha-SMA in neointima after wire-mediated vascular injury (45, 76). To rigorously identify GFP-positive cells, we used plastic embedding to preserve GFP-fluorescence signal and avoided use of anti-GFP antibodies that potential increase the risk of false signalling by non-specific binding of the antibody.

Moreover, there is criticism for the markers to be used to identify the SMC-like cells. In most of the studies, alpha-smooth muscle actin (alpha-SMA) is used as a marker of SMCs, because anti-alpha-SMA antibodies with high specificity and sensitivity are commercially available (81). It is well established that alpha-SMA is not a definitive lineage marker to identify differentiated SMCs, since alpha-SMA is reported to be expressed in a wide variety of non-SMC cell types under certain circumstances including 1) skeletal and cardiac muscle during normal development, 2) in adult cardiomyocytes in association with various cardiomyopathies, 3) in fibroblasts (or so-called myofibroblasts) in a wide range of circumstances including wound repair , in endothelial cells during vascular remodeling and/or in response to transforming growth factor (TGF)-beta stimulation, and 5) in numerous tumor cells (82). In addition, it is known that some macrophages are positive for alpha-SMA (82). It is important to identify SMCs expressing only this differential marker. Therefore, detailed lineage-tracing study of SMCs in atherosclerotic lesions comparing SMalpha-actin expression with more specific SMC marker including smooth muscle myosin heavy chain (SM-MHC), caponin, SM-22 caldesmon and smoothelin (83) would be required to further characterize bone marrow-derived SMC-like cells that are positive for alpha-SMA. Moreover, transgenic animals that express a marker gene only in SMC-lineage cells under the transcriptional control of SMC-specific promoter would be useful to avoid potential artificial co-localizing signaling by overlapping of bone marrow-derived cells and local residual cells (84, 85).

4. DIVERSE ORIGINS OF NEOINTIMAL CELLS IN VASCULAR LESIONS

It is certain that bone marrow could not be the only source of neointimal cells (44). Numerous reports have shown that neointimal cells are heterogeneous and that the SMCs in vascular lesions are composed of cells of diverse origins (19, 45, 86). We also reported that the cellular constituents in neointimal lesions differ according to the type of vascular injury (45). Three distinct types of mechanical injuries were induced in the same mouse whose bone marrow had been reconstituted with that of GFP- or LacZ-mice. After wire-mediated endovascular injury (36), a significant number of the neointimal and medial cells were found to be derived from bone marrow. In contrast, marker-positive cells were seldom detected in the lesion induced by perivascular cuff placement. There were only a few bone marrow-derived cells in the neointima following ligation of the common carotid artery. These findings suggest that the mode of injury is crucial for the recruitment of bone marrow-derived cells to tissue remodeling.

There might be several reasons by which different forms of vascular injuriy led to different contribution of bone marrow-derived cells. After perivascular cuff-placement or flow-restriction by ligation, endothelial cells and medial cells remained relatively intact with mild expression of MCP-1, SDF-1, and VEGF. Those minimal changes in vessel wall were associated with little contribution of bone marrow cells to neointimal hyperplasia. In contrast, wire injury induced complete endothelial denudation and medial cell loss due to apoptosis (87). In this model, the cellularity of the injured media remains very low until one or two weeks after the injury (88). The injury induces expression of MCP-1, SDF-1, and VEGF that may be important for homing of bone marrow-derived cells. It was observed that neointimal hyperplasia developed when the media remained acellular (88). It is most likely that bone marrow-derived cells must be recruited to repair the injured artery, when there are not enough local mesenchymal cells for the process.

Recent advances in gene-manipulating techniques have produced various genetically modified mice to determine the role of specific molecules in vascular remodeling, such as post-PCI restenosis. However, mouse arteries, unlike those of larger animals, are too small for transluminal injury with a balloon. Alternatively, several

models of vascular injury (89-91) have been shown to produce neointima-like hyperplasia and are used to evaluate the susceptibility of transgenic/knock-out mice to vascular lesion formation. Our findings suggest that we should be cautious about the difference in the mechanisms of neointimal hyperplasia when we compare findings obtained in different experimental systems.

Given the complexity of human atherosclerotic lesions, none of the aforementioned experimental vascular injury models would represent the exact human pathogenesis. It has been suggested that BMCs would substantially contribute to lesion formation when arteries are subjected to severe forms of injury (45). Advanced human atherosclerotic lesions exhibit a higher incidence of internal elastic rupture and intimomedial interface damage (92), both of which are associated with focal intra-plaque micro-hemorrhage (93). Angioplasty markedly denudes the endothelium and mechanically dilates atherosclerotic lesions with a tear in the luminal surface (94). It is likely that circulating progenitors substantially contribute to vascular remodeling in humans when arteries are subjected to severe injuries, such as PCI, transplantation and plaque rupture (43, 45, 54, 95, 96). Consistent with this notion, an analysis of sex-mismatched bone marrow transplant subjects revealed that SMCs throughout the atherosclerotic vessel wall are derived from the donor bone marrow (97). Of interest is the finding that recruitment of bone marrow-derived SMC-like cells was more extensive in diseased compared with undiseased arterial segments.

5. CELL FUSION AS A POSSIBLE MECHANISM OF BMC 'DIFFERENTIATION'

Recent evidence suggests that somatic stem cells or adult stem cells remain in the adult organism (98). Many animal studies have documented that adult stem cells can transdifferentiate into other lineages (65, 70, 99, 100). In gender-mismatched human BMT, it has been reported that donor-derived HSCs participated in organ regeneration (70, 71). On the other hand, the results of recent studies suggest that adult stem cells adopt a tissue-specific phenotype by cell fusion, in vitro (101, 102) and in vivo (103, 104), but not by transdifferentiation. Alvarez-Dolado et al. demonstrated that bone marrow-derived cells fuse spontaneously with neural progenitors in vitro using a simple method based on Cre/lox recombination to detect cell fusion events (105). Furthermore, bone marrow transplantation demonstrated that bone marrow-derived cells fuse in vivo with hepatocyte in liver, Purkinje neurons in the brain and cardiac muscle in the heart, resulting in the formation of multinucleated cells (105). The authors observed no evidence of transdifferentiation of bone marrow-derived cells without fusion in these tissues (105, 106). Consistent with this notion, polyploidization of vascular SMCs in response to mechanical and humoral stimuli has been documented (107). Thus, it is possible that fusion between BMCs and SMCs can account for the presence of at least some of the cells identified as bone marrow-derived SMC-like cells in vascular lesions (43).

On the other hand, we seldom detected neointimal cells that were positive for both LacZ and GFP, when we performed heterotopic heart transplantation using genetically modified mice that express LacZ or green fluorescent protein (GFP) ubiquitously and constitutively (108). It was suggested that spontaneous cell fusion between recipient and donor-derived cells seems to be a rare event, if it occurs at all, in a murine model of cardiac transplantation (42). Furthermore, culture of mononuclear cells can give rise to smooth muscle-like cells in the absence of SMCs (109, 110), suggesting that circulating cells can differentiate into adherent cells that express some SMC-lineage markers. Consistently, it was reported that bone marrow cells, when properly administrated in the infarcted heart, efficiently differentiated into myocytes and coronary vessels with no detectable differentiation into hemopoietic lineages independently of cell fusion (111). Here we show that vascular endothelial cells can differentiate from common myeloid progenitors and granulocyte/macrophage progenitors. Here we show that vascular endothelial cells can differentiate from common myeloid progenitors and granulocyte/macrophage progenitors (112, 113). Thus, it is unlikely that cell fusion accounts for all types of bone marrow-derived smooth muscle-like cells.

6. THERAPEUTIC STRATEGIES TARGETING CIRCULATING VASCULAR PROGENITOR CELLS

Although a lot of effort has been devoted to targeting the migration and proliferation of medial SMCs (5), effective therapies based on such strategies have not yet been established to prevent vascular lesion formation. Our findings indicate that bone marrow-derived vascular progenitor cells might become additional targets for this purpose (43). Consistent with this notion is a previous observation that transient myelosuppression inhibited SMC hyperplasia in balloon-injured coronary arteries (114). Similar effects could be obtained by inhibition of a chemokine (38) or an adhesion molecule (39), which may play a crucial role in the recruitment and homing of putative SMC progenitors. Recently, the sirolimus-eluting stent has emerged as a promising strategy to inhibit post-PCI restenosis (115, 116). In spite of clinical enthusiasm, very little is known as yet about the mechanism by which sirolimus-eluting stent effectively prevents neointimal hyperplasia. It is possible that sirolimus effectively inhibits accumulation of bone marrow-derived vascular precursors when delivered locally at the site of progenitor cell accumulation. We recently reported sirolimus potently inhibits differentiation of human vascular progenitor cells (110). The potent inhibitory effects of sirolimus on circulating smooth muscle progenitor cells may, at least in part, mediate the clinical efficacy of SES. On the other hand, sirolimus potentially inhibited differentiation of endothelial progenitor cells and retarded reendothelialization. Influence on re-endothelialization might be, at least in part, responsible for late thrombosis, which could be observed occasionally in some patients

treated with SES after interruption of anti-platelet therapy (117).

7. PERSPECTIVE

Further studies are needed to identify and characterize putative vascular progenitor cells. It has been observed that bone marrow-derived cells were negative for the markers of SMCs and ECs when they homed on the luminal side of the artery at 1 week after mechanical injury (43), so it is likely that plastic, immature cells may be mobilized to the injured vessels, where they differentiate in response to mechanical and humoral stimuli. Consistent with this notion, it has been reported that blood cells contain progenitors that have the potential to differentiate into either ECs or SMCs *in vitro* according to the composition of the culture medium under certain conditions (95, 97, 109, 118). Experiments that dissect the molecular mechanisms by which progenitors are recruited and differentiate at the site of injury are required.

8. ACKNOWLEDGEMENTS

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