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Dynamic Interplay Between Smooth Muscle Cells and Macrophages in Vascular Disease

Hiroshi Iwata and Masanori Aikawa

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Abstract

Vascular smooth muscle cells (SMCs) and monocytes/macrophages represent major players in atherosclerotic vascular diseases. In addition to physiological and pathological roles of each cell type in atherosclerosis, dynamic interplay between SMCs and monocytes/macrophages may contribute to the pathogenesis of atherosclerosis more critically than previously understood. Activated macrophages accelerate pro-atherogenic functions of SMCs in vascular lesions. Activated SMCs promote additional accumulation of pro-inflammatory macrophages through expression of chemoattractants. More recent evidence suggests the interchangeability between SMC and monocyte/macrophage lineages. Future efforts to understand such dynamic interactions between SMCs and macrophages may provide novel insight into the pathogenesis of vascular disease and the development of new classes of medical solutions.

Keywords: atherosclerosis, smooth muscle cells, macrophages, inflammation

1. Introduction

Atherosclerosis is a complex multifactorial disorder and involves various cell types, including vascular smooth muscle cells (SMCs), macrophages, lymphocytes, neutrophils, and endothelial cells [1]. Evidence has established that each cell type changes its phenotype in response to a microenvironmental cue. SMC phenotype ranges from the undifferentiated state to differentiated state during vascular development [2]. Vascular lesions in adults also exhibit such

phenotypic diversity of SMCs, which often mirrors their functions (e.g., contractile vs. synthetic SMCs). The role of lesional SMCs appears to vary depending on the disease context and stage of the disease. The production of extracellular matrix by SMCs often contributes to the lesion development [3, 4], but also may exert beneficial effects (e.g., stabilizing the fibrous cap of atherosclerotic plaques) [5, 6]. SMCs often reside in vascular lesions in close proximity to macrophage clusters, and appear to be influenced by factors released from inflammatory cells. Particularly, macrophages in the lesion may promote activation and pro-atherogenic functions of vascular SMCs.

In the middle of the nineteenth century, German pathologist Rudolf Virchow made significant contributions to cardiovascular medicine. He identified the formation of tunica intima as a key atherosclerotic change and suggested that the contents of the intima promote expansion of matrix components [4, 7]. He further indicated that infiltrated leukocytes may contribute to the pathogenesis of atherosclerosis. Numerous studies have subsequently unraveled the role of immune cells, leading to a widely accepted theory that atherosclerosis is a complex chronic inflammatory disorder [1, 4, 8].

Coronary and cerebrovascular atherosclerosis underlies life-threatening complications such as acute myocardial infarction and stroke. Major risk factors, including dyslipidemia, accelerate the development of atherosclerotic changes in arteries. Elevated levels of low-density lipoprotein (LDL) cholesterol in the circulating blood cause dysfunction of endothelial barriers and infiltration of circulating leukocytes (e.g., monocytes) into the artery wall. Monocytes differentiate into macrophages within the subendothelial space. Accumulating LDL undergoes oxidative modifications in the arterial wall (oxidized LDL), which is then recognized and taken up by macrophages, leading to the accumulation of lipid-laden foam cells. Foam cells secrete proinflammatory mediators that facilitate lipoprotein retention and maintain vascular inflammation [9, 10]. To minimize the risk of atherosclerotic complications, primary and secondary prevention strategies seek to control risk factors such as hyperlipidemia. LDL-lowering drugs (e.g., 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitors or statins) reduce the onset of acute complications of atherosclerosis.

Bone marrow-derived progenitor cells, including endothelial progenitor cells (EPCs) and smooth muscle progenitor cells (SMPCs), also may serve as a source of atherosclerosis-related cell lineages [11, 12]. Pioneering work suggested that these progenitors differentiate into mature and functional endothelial cells (ECs) and SMCs, respectively, in physiological and pathological settings [11, 13]. However, their functional contributions to atherogenesis remain unclear [14-17]. The evidence also indicated that some intimal SMC may originate from circulating monocytes or their subset [14, 18, 19]. In contrast, other lines of evidence have proposed an opposite direction of transdifferentiation of SMC into macrophage or macrophage-like cells [20-25].

In this chapter, we address the functions and interplay of SMCs and monocytes/macrophages present within the pathological arterial wall. We also discuss emerging concepts of the interchangeability of these two cell lineages. Better understanding of these complex biologies may provide important insight into the mechanisms and new therapeutic strategies for vascular diseases.

2. The many faces of SMCs in vascular disease

SMCs exerts various functions during development, in normal homeostasis in adults, and in the pathogenesis of vascular diseases [2]. While vascular tissues develop, SMC need to migrate, and produce selected proteins that contribute to these functions. After birth, SMC are highly specialized in contraction, which is their main role in normal homeostasis for regulation of vessel tone and diameter to control of blood pressure and flow. Adult SMCs thus express specific contractile proteins, ion channels, and signaling molecules that are unique to this muscle compared with other muscles, including the skeletal muscle and cardiac muscle [2, 3]. Such SMC-selective or -specific genes thus serve as markers of SMCs. These include the proteins that comprise the contractile apparatus including SM α-actin, SM myosin heavy chain (SM-MHC) isoforms SM1 and SM2, h1-calponin, SM22α, and smoothelin [3]. However, some of these SMC markers are expressed, at least transiently, in other cell types during development, tissue repair, or disease states, while SM-MHC isoforms are specific to SMCs [2]. Therefore, SM-MHC isoforms are the most definitive markers for SMC.

Accumulation of SMC in the subendothelial space contributes to the formation of the intima, as a precursor of the future atherosclerotic plaques. The process appears to involve migration and proliferation of activated SMCs. Where those SMCs originate from is an interesting question that we discuss later. The ability of SMC to migrate and proliferate in vascular lesions often associates with their phenotypic modulation from a contractile type to a synthetic one, as characterized by loss of contractile proteins (e.g., SM-MHC) and the increased synthesis of matrix proteins (e.g., collagen) [2, 26-29]. Reduced expression of SMC-specific proteins also indicates their decreased state of differentiation. Platelet-derived growth factor (PDGF), released by platelets on the surface of dysfunctional endothelium, and activated macrophages, may participate in the loss of SMC differentiation marker genes and promote their migration and proliferation [30, 31, 32].

Collagens are major products of SMCs. The balance of collagen production by SMCs and degradation by macrophage-derived matrix metalloproteinases (MMPs) may positively or negatively contribute to the mechanisms of vascular diseases. Depending on the context, the presence of vascular SMCs within atherosclerotic plaques may be beneficial. Particularly, accumulation of fibrillar collagen in the fibrous cap of atherosclerotic plaques may be protective. Macrophage expression of collagenases of the MMP family or reduced SMC due to cell death may impair the integrity of plaques, leading to physical disruption ("rupture") and thrombosis [5, 6, 33]. Libby et al. claimed in the early 1990s that the pro-inflammatory T cell cytokine IFNγ inhibits collagen I and III synthesis by human SMCs [34]. Interestingly, IFNγ has become known to promote pro-inflammatory macrophage activation (the so-called M1 polarization). This may indicate that a pro-inflammatory microenvironment promotes plaque instability, another example of SMC-macrophage crosstalk participating in the pathogenesis of acute cardiovascular complications. There are other examples for the beneficial or adverse role of collagen production by SMCs in vascular disease. After stent implantation, migrating SMCs cover the luminal surface of stent struts, as a healing process. But SMCs often overgrow, resulting in restenosis, which remains the most prevalent complication of percutaneous coronary intervention.

3. Existence of immature SMCs in vascular disease

SMCs exhibit the diversity of phenotypes in the vascular lesions. A panel of antibodies for SMC differentiation markers enables to identify a wide spectrum of their phenotypes in experimental and human vascular diseases. In the arterial lesions after acute mechanical injury and in chronic atherosclerotic plaques of experimental animals, intimal SMC often express lower levels of SM-MHC isoforms SM1 and SM2, whereas the medial SMC usually stain positively for SM-MHC and α-actin. While SM1 expression appears at the late stage of vascular development, SM2 expression increases later, particularly after birth [2, 35-37]. SM2 thus serves as a sensitive marker of mature SMC. Aikawa et al. demonstrated that intimal SMC of the rabbit aorta 4 months after mechanical injury and the initiation of a high-cholesterol diet showed an immature phenotype as gauged by decreased SM2 immunoreactivity (Figure 1, top panels) [38]. Interestingly, dietary cholesterol lowering for 8 or 16 months induced the increased expression of SM2 in intimal SMC to the levels similar to those of medial SMC (Figure 1, bottom panels). Such SMC plasticity may depend on microenvironmental cues. Alternatively, different microenvironment may affect the balance of SMC subpopulations. Thus, the recovery of SM2 expression by lipid lowering may result from either "redifferentiation" of the same group of intimal SMC, the expansion of a subset of SMC with a mature phenotype, or the repopulation of immature SMC by mature SMC.

In human coronary arteries, SMC begin to lose their SM2 expression even in the physiological intima of young adults with no obvious signs of atherosclerotic changes such as macrophage accumulation [28]. In more advanced plaques, immature SMCs often exist in areas where macrophages accumulate. In Figure 2, SM2 was undetectable in SMC identified by SMα-actin and SM1. Co-existence of intraplaque microvessels and macrophages may indicate active recruitment of circulating monocytes into this area. A pro-inflammatory microenvironment produced by activated macrophages via the release of IL-1β, PDGF-BB, and other factors and production of oxidative stress may have promoted phenotypic modulation of SMC in this region. As we discuss later, some of these immature SMC may have originated from a subset of monocytes. Alternatively, some SMCs may have transdifferentiated into macrophage-like cells. Understanding of such crosstalk and potential interchangeability between SMCs and macrophages may provide important insight into the identification of new therapeutic targets. Of note, "redifferentiation" or "repopulation" of intimal SMC after mechanical injury also occurs in human coronary arteries. The differentiation state of SMC reduces a first few months after percutaneous coronary intervention while it increases over time [27]. The key question is where those intimal SMCs originate from.

4. The role of SMC-derived foam cells

Pathological changes in the atherosclerotic intima include increased modification of lipoproteins (e.g., oxidized LDL) and SMC uptake of modified lipoproteins. Foam cell formation by SMCs, in addition to macrophage-derived foam cells, may represent a pivotal
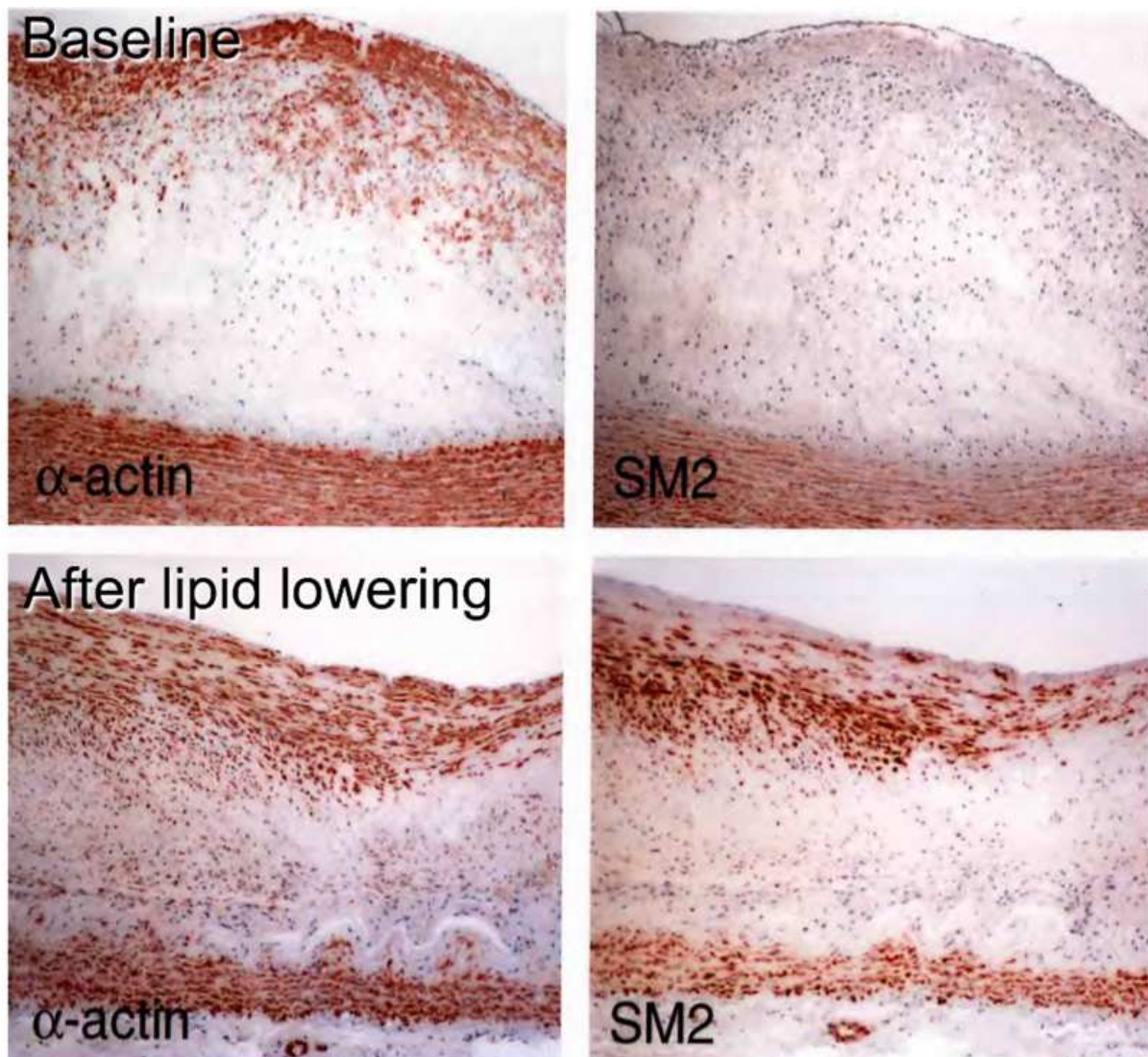
Figure 1. Accumulation of immature SMC in the arterial intima. Top panels: SM α-actin is expressed in a wide range of differentiated states of SMC, while mature SMCs produce SM2. In the atherosclerotic rabbit aorta 4 months after mechanical injury and the initiation of a high-cholesterol diet, medial SMC stained positively for SM α-actin and SM2. SM2 was undetectable in intima SMC identified by SM α-actin antibody, indicating their immature phenotype. Bottom panels: Dietary cholesterol lowering for 16 months increased SM2 immunoreactivity in the intima. The data indicate highly plastic nature of SMCs ("re-differentiation"). Alternatively, a repopulation of intimal cells by a more mature SMC subset may have resulted in increased SM2-positive cells, although their origin is unknown.

step in the transition of physiological intimal thickenings into nascent atherosclerotic lesions. [39]. Atherogenic lipoproteins also promote SMC growth by modulating calcium (Ca2+) signaling [40, 41]. SMC foam cell formation may, in part, result from the increased uptake and impaired clearance of lipids [42]. SMCs within intimal thickenings express increased levels of receptors regulating endocytosis of modified lipoproteins, including scavenger receptor A (SR-A), CD36, lectin-type oxidized LDL receptor 1, and low-density lipoprotein receptor-related protein 1 (LRP1) [43]. In parallel, the expression of ATP-binding cassette transporter A1 (ABCA1) and apolipoprotein A1, key molecules for reverse cholesterol transport, decreases in intimal SMCs [39].
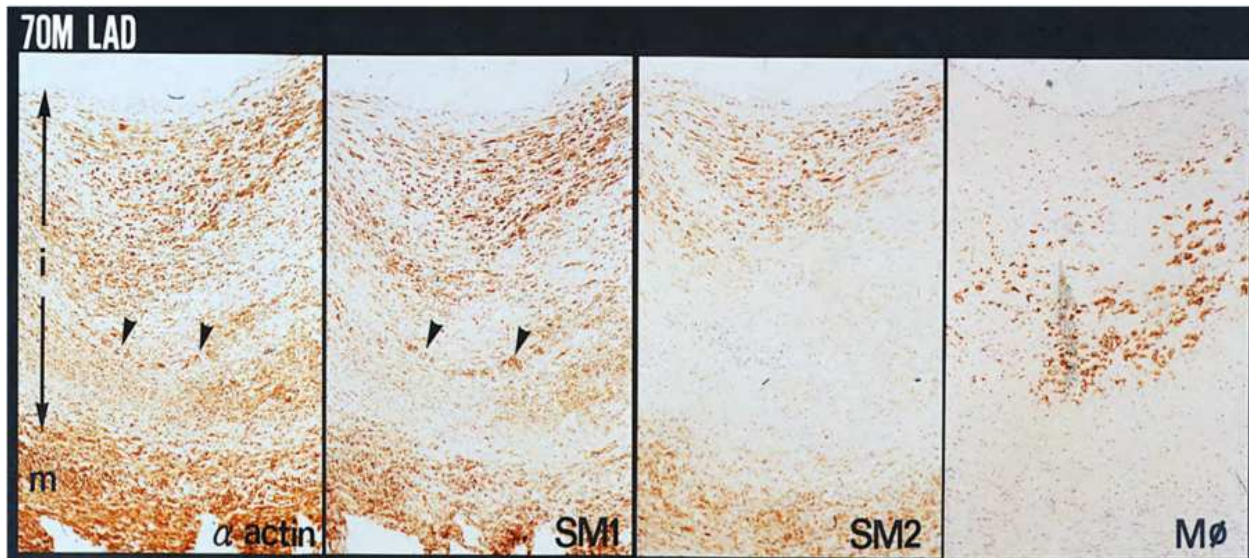
Figure 2. Decreased SMC differentiation in macrophage-rich regions of human coronary arteries. Immunohistochemistry of SMC differentiation markers in the left ascending coronary artery (LAD) of a 78-year-old male had developed the thickened intima. SM α-actin covers a wide spectrum of SMC differentiation. SMC-specific myosin heavy chains isoforms are definitive markers of differentiated SMC. In particular, SM2 identifies mature SMC. In this atherosclerotic human artery, medial SMC stained positively for all three markers, suggesting an apparently "normal" phenotype. Intima SMC exhibited the diversity of SMC phenotype. Some SM α-actin expressing cells were not immunoreactive for SM1 and SM2 antibodies. Especially, many SMC did not express the detectable level SM2 in the area where many macrophages (CD68) accumulated. They may have undergone phenotypic modulation ("de-differentiation") due to a pro-inflammatory microenvironment induced by neighboring macrophages. As an alternative possibility, some of these SM2-negative cells probably co-expressed SM α-actin, SM1, and CD68, an intermediate phenotype between SMC and macrophage lineages. Of note, intraplaque microvessels surrounded by macrophages may have recruited a subset of activated monocytes as precursor cells for smooth muscle–like cells of the bone marrow origin.

In lipid-laden intimal SMCs, cholesterol accumulation induces cell death. SMC death and subsequent necrosis promote a series of pro-inflammatory events: the release of pro-inflammatory cytokines including monocyte chemo-attractant protein-1 (MCP-1) and IL-1β from the dying and surrounding SMCs [44]; migration and proliferation of adjacent SMCs [45], MCP-1-mediated monocyte infiltration. MCP-1 and IL-1β modulate SMC phenotype, growth, and MMP production [46]. Such a cascade of events accelerates a positive feedback loop of vascular inflammation. To assess the anti-inflammatory and anti-atherosclerotic effects of a monoclonal anti-human IL-1β antibody, a randomized, placebo-controlled trial entitled CANTOS is currently ongoing in high-risk cardiovascular patients [47].

5. The origin of intimal SMC

As discussed, SMCs participate in the development of atherosclerotic plaques and the onset of acute thrombotic complications. Lesional SMCs show dynamic changes in their phenotypes depending on the disease context and the stage of each disease. Where do they come from? Many studies have led to the traditional theory that intimal SMC originate from the tunica media via proliferation and migration. More recently, several lines of evidence have indicated

that intimal SMC or intimal cells that possess phenotypes similar to SMC (often called "SM-like cells") may originate from sources other than the media, including circulating precursors, adventitial cells and local stem cells [15, 32]. The relative contribution of each of these sources, however, remains obscure, and may also depend on the disease context in humans, and models or species in experimental animals [48]. Figure 3 illustrates possible sources of intimal SMC or SM-like cells.
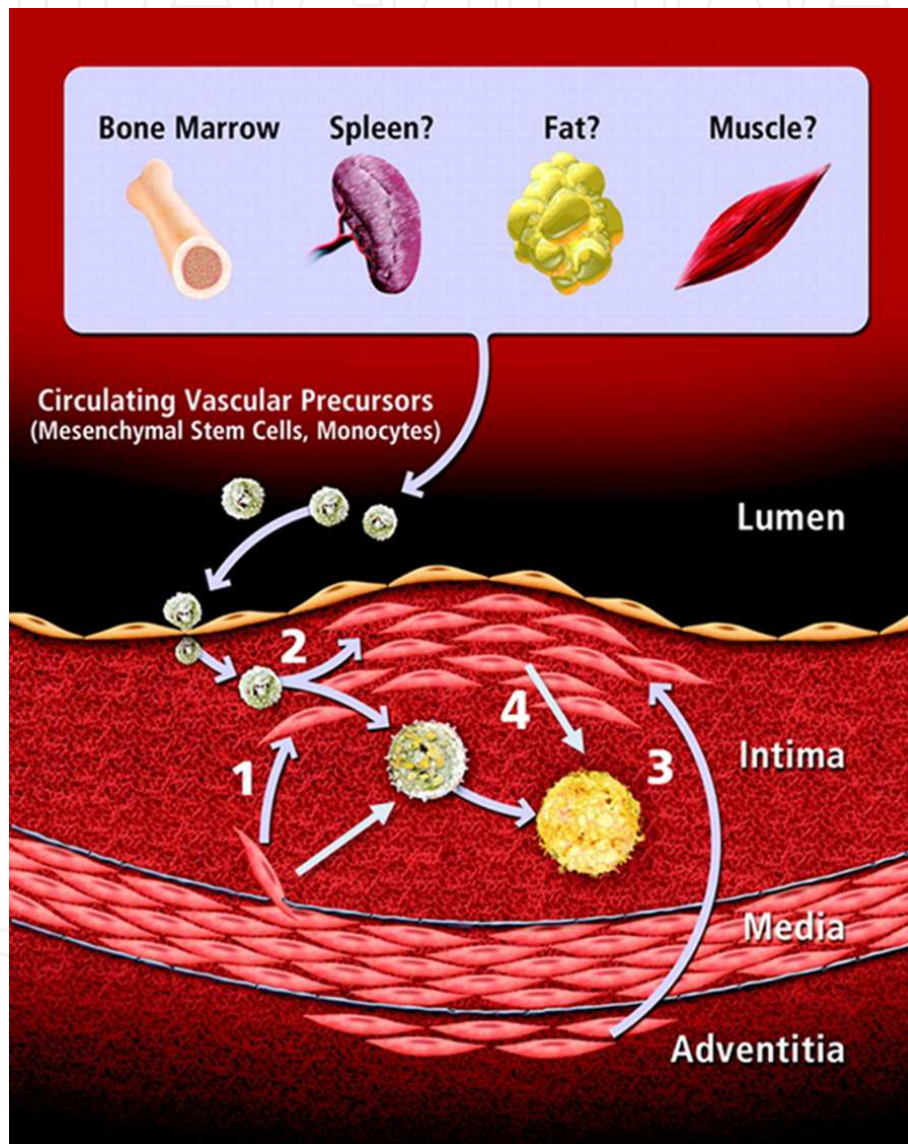

Figure 3. Potential sources of intimal smooth muscle cells (SMCs) 1) In response to injury or inflammatory stimuli, medial SMC undergo phenotypic modulation, migrate into the subendothelial space, and form the tunica intima. Resident progenitor cells in the media may contribute to intima formation. **2)** Circulating progenitor cells such as mesenchymal stem cells and activated monocytes may engraft in the intima and contribute to lesion development. Other organs, including the spleen, fat, and skeletal muscles may also release SMC progenitors. **3)** The adventitia may contain SMC precursors (resident stem cells), and contribute to intima formation, particularly after mechanical injury. **4)** Potential transdifferentiation of SMCs into macrophage- or macrophage-like cells may also contribute to atherogenesis. (Modified from Ref. 48 by Fukuda et al.)

Recent cell lineage studies have indicated that some bone marrow–derived cells express SMC-specific markers, while SMCs can display proteins associated with macrophages [15, 32]. For instance, a subpopulation of circulating monocytes may become SM-like cells in the intima [14], whereas SMC can transdifferentiate into macrophage-like cells. [25]. Therefore, the origin and fate of SMC in vascular lesions are not so clear as we traditionally thought [49]. We need to overcome several challenges to explore seemingly complex, intertwined mechanisms [32]. The limited availability of lineage-tracing methods that enable to identify the specific origin of SMC cells, particularly in disease contexts where cells tend to reduce the expression of differentiation markers. Therefore, some lesional SMCs may not be identified (false negative). As suggested by many studies, cell types other than SMCs in vascular lesions can express SMα-actin (false positive). According to a study by Caplice et al. up to 10% of cells in advanced atherosclerotic lesions of human coronary arteries expressing SMα-actin are of the myeloid lineage [24]. TGF-β or thrombin may induce macrophage expression of SMC markers including SMα-actin and SM22α [50, 51].

6. Monocytes and macrophages in vascular disease

In atherosclerosis research, monocytes and macrophages have called particular attention. Cardiovascular risk factors, such as dyslipidemia, hypertension, smoking, and diabetes mellitus, promote sustained activation of monocytes and macrophages. Activated monocytes and macrophages critically contribute to multiple processes of atherogenesis from the initiation to the acute onset of devastating complications [52-54]. Dyslipidemia provokes monocytosis by expanding the pro-inflammatory subset of circulating monocytes [55]. These inflammatory monocytes attach to vascular EC and invade the subendothelial space. Monocytes then differentiate into macrophages, although how recruited monocytes are retained and differentiate into macrophages remains incompletely understood [55, 56]. Monocytes recruitment to the subendothelial space mediated by chemokines, such as MCP-1 [57, 58] and C-C chemokine-5 (CCL5) [59], and adhesion molecules, including intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) on activated ECs, initiates the early processes of atherogenesis [60, 61]. The secretion of collagenolytic MMPs by atherosclerotic plaque macrophages may weaken the protective fibrous cap, causing plaque disruption and thrombosis [62-65].

7. Monocytes, subsets, and functions in atherosclerosis

Several subsets may exist in circulating monocyte in mice and humans. At least two distinct subsets of mouse monocytes are identified via differential expression levels of Ly-6C, CX3CR1, and CCR2 [66]. One subset exhibiting Ly-6C^{high}CX3CR1^{low}CCR2$^+$ is designated as pro-inflammatory monocytes, and the other subset with Ly-6C^{low}CX-3CR1^{high}CCR2$^-$ as resident or patrolling monocytes [67, 68]. Human monocytes are often classified into three subsets based on the expression levels of CD14 and CD16 [69]. The classical subset CD14^{++}CD16$^-$CCR2^{high} may correspond to mouse Ly-6C^{high} pro-inflammatory monocytes, whereas the nonclassical

subset $CD14^+CD16^{++}CCR2^{low}$ may be equivalent to mouse $Ly\text{-}6C^{low}$ patrolling monocytes [70-72]. The intermediate subset is identified as $CD14^{++}CD16^+$ cells [73]. $CD14^{++}CD16^-CCR2^{high}$ cells appear to preferentially enter sites of atherosclerosis [74, 75]. In mice, $Ly\text{-}6C^{high}$ monocytes in peripheral circulation were induced by hypercholesterolemia [76]. Similarly, in human, elevated $CD14^{++}CD16^-$ monocyte levels are associated with an increased risk of cardiovascular events [77]. Human monocyte subpopulations are also associated with the status of atherosclerosis [78, 79].

In addition to the influence of phenotypes of infiltrating monocytes, as macrophage precursors, microenvironmental cues in the vascular wall may determine macrophage phenotype. In vitro studies have established the paradigm of macrophage heterogeneity. IFNγ induces a pro-inflammatory phenotype of macrophages (M1). IL-4 promote non/anti-inflammatory macrophages (M2) [74, 80]. The in vivo significance of such macrophage polarization has driven many investigations in the contexts of cardiovascular and metabolic disorders [81-86]. More specific terms, M(IFNγ) and M(IL-4), rather than M1 and M2, have been recently proposed [87].

Hyperlipidemia causes the generation of monocytes in bone marrow through medullary hematopoiesis and thus induces monocytosis. Hematopoietic stem and progenitor cells, however, may relocate to the splenic red pulp and differentiate into $Ly\text{-}6C^{high}$ monocytes [88]. The spleen as a monocyte pool may participate in further accumulation of monocytes/macrophages in peripheral organs such as atherosclerotic plaques. The fate and function of $Ly\text{-}6C^{low}$ monocytes also remain incompletely understood. These cells may patrol the endothelium for injury and infection and also promote wound healing [89].

8. Roles of macrophages in the development and progression of atherosclerosis

The major functions of lesional macrophages include removal of excessive lipids such as oxidized LDL and glycolaldehyde-LDL. Macrophages internalize oxidized lipids through scavenger receptors and become lipid-laden foam cells. Activated macrophage foam cells produce potent chemoattractants, such as MCP-1, which recruit additional monocytes/macrophages from the circulating blood, accelerating the positive feedback loop of vascular inflammation. The imbalance between uptake and removal causes excessive cholesterol ester accumulation and apoptosis in lesional macrophages. Due to the lack of negative feedback mechanisms of scavenger receptor expression, macrophages cannot limit the uptake of lipids and largely depend on the cholesterol efflux to maintain cellular lipid homeostasis [42]. In early atheroma, neighboring macrophages take up apoptotic macrophages (efferocytosis) [90-92]. As atherosclerosis progresses, however, efferocytosis becomes impaired [93], leading to secondary necrosis. Due to the macrophage release of their cellular contents (e.g., debris, oxidized lipids, proinflammatory mediators), secondary necrosis amplifies pro-inflammatory response and develop the necrotic core within the lesions.

The balance between proinflammatory and anti-inflammatory populations of accumulating macrophages may determine the development of atherosclerotic plaques [53]. Active pro-

inflammatory responses of macrophages may destabilize atherosclerotic plaques. The production of MMPs by macrophages may degrade collagen in the fibrous cap and make plaques susceptible for plaque rupture and thrombosis. Fukumoto et al. and Deguchi et al. used genetically altered mouse strains to provide the first in vivo direct evidence for the role of collagenases of the MMP family for the loss of fibrillar collagen within the intima [94, 95].

As mentioned, emerging data have proposed the heterogeneity of macrophages. A subpopulation of T lymphocytes (Th1 cells) secretes such as IFNγ, IL-2, IL-12, and TNFα and promotes the activation of macrophages toward a pro-inflammatory phenotype (M1). Th2 cytokines (e.g., L-4 and IL-13) induce an alternative form of activation toward a non/anti-inflammatory (M2) phenotype. The balance of such macrophage polarization (M1/M2 balance) may affect plaque outcome [71]. The high M1/M2 ratio in atherosclerotic plaques may induce lesion formation and plaque vulnerability [80, 96]. The evidence has identified switching of macrophage phenotypes from M1 to M2 during the regression of atherosclerosis or in response to anti-inflammatory therapies [84, 97]. Proinflammatory M1 macrophages also induce SMC proliferation [98].

Alternatively activated M2 macrophages may generally exert anti-atherogenic effects. M2 cells suppress Th1 inflammatory responses. TGF-β released by M2 macrophages may inhibit the recruitment of inflammatory cells and the development of atherosclerosis [99]. M2 macrophages also release IL-10, which inhibits the production of inflammatory cytokines from T lymphocytes and other macrophages. M2 macrophages suppress inflammatory milieu by clearing apoptotic cells and tissue debris [100, 101]. During the repair process after tissue injury, M2 cell may promote fibrosis [102], [103]. This action may potentially be beneficial in plaque instability via thickening the fibrous cap [104].

While the M1/M2 paradigm has clear relationships between stimulators and downstream effects and has thus served as a useful mechanistic model, the evidence suggests that macrophages are more diverse. In particular, M2 may further contain various forms of macrophage activation, e.g, M2a to M2d [104]. Mox macrophages develop in response to atherogenic phospholipids and have lower phagocytotic and chemotactic capacity than do conventional M1 and M2 cells [104, 105]. Mhem cells, induced by intraplaque hemorrhage, often associate with atherothrombotic complications [105, 106]. CXC chemokine ligand 4 induces M4, a recently proposed subtype of atherogenic macrophages [107]. The heterogeneity of macrophages thus seems to be more complex than previously proposed. Furthermore, in vivo functional significance of each macrophage subpopulation remains incompletely understood. Recently, new terms more specific to each stimulator were proposed, e.g. M(IFNγ), M(LPS), M(IL-10), and M(IL-4) [87].

9. Smooth Muscle Progenitor Cells (SMPCs)

Studies have identified circulating SM progenitor cells (SMPCs) and EPCs that can acquire SMC-like or EC-like phenotypes in mouse and human: [11, 13, 108]. These cell types share similar surface markers and functions with myeloid cells [109, 110] and SMCs and ECs,

although their origin, identity, and physiological and pathological functions remain unclear. Many studies have identified BM-derived cells that express SMC or EC-specific genes within vascular lesions.

SMPCs, identified as circulating BM-derived cells, enter blood vessels and acquire phenotypes expressing SMC marker genes, particularly SMα-actin [13, 19, 111, 112]. Atherosclerotic vessels in patients who received sex-mismatched BM transplantation contain donor-derived smooth muscle-like cells, suggesting the possible involvement of circulating SMPCs in the lesion development [24]. Functions of SMPCs in the process of atherogenesis, however, remain obscure. SMPCs may promote inflammation and plaque instability by producing cytokines and MMPs [113]. SMPCs may participate in pathological angiogenesis [109]. In contrast, atheroprotective effects of SMPCs remain unknown. Further studies are needed to understand the role of SMPCs in the vascular disease.

Challenges in identification of SMC lineage are in part caused by their outstanding plasticity during the development and pathological processes. Non-SMCs also express SMC differen-tiation markers other than SM-MHC. For instance, ECs, fibroblasts/myofibroblasts, and macrophages express SMα-actin in certain conditions [2]. Iwata et al. used multiple SMC differentiation markers to analyze BM-derived smooth muscle-like cells [14]. BM-derived SMα-actin-immunopositive cells in vascular lesions in mice did not express the definitive SMC lineage marker, SM-MHC [14]. As mentioned, SM-MHC expression reduces in intimal SMCs after vascular injury and increases over time [27, 38] (Figure 1). Twelve months after vascular injury in mouse arteries, when resident SMCs had fully recovered SM-MHC expression, BM-derived cells did not express SM-MHC. Instead, the BM-derived SMα-actin-positive cells expressed markers of monocytes/macrophages. Moreover, we found that adoptively transfer-red CD11b+Ly-6C+ BM monocytes expressed SMα-actin in the injured artery (Figure 4). Interestingly, increased expression of inflammatory genes and MMPs in these BM-derived SMα-actin+ cells indicated their potential role in the remodeling processes [14]. These results suggest that an activated monocyte population can become SM-like cells in atherosclerotic lesions, which may promote plaque instability. Future investigations will further evaluate the origin and functionality of SMC and macrophage lineages in vascular lesions [114].

10. Circulating fibrocytes

Fibrocytes, BM-derived mesenchymal progenitors [115, 116], coexpress markers of hemato-poietic stem cells, the monocyte-lineage, and fibroblasts. Fibrocytes may participate in various diseases, including inflammatory bowel diseases, allergy, and pulmonary and liver fibrosis. They produce extracellular matrix components as well as matrix-degrading enzymes and further differentiate into myofibroblast-like cells [117, 118]. Human fibrocytes also express genes, including Tall-like receptor 4, IL-1β, CCL2, CCL3, CCL7, CCL22, and C5aR, suggesting that they mediate inflammatory responses [119]. They may originate from CD14+ BM–derived monocytes in humans [119] and from the Gr1+CD115+CD11b+ monocyte population in mice [120], suggesting that circulating fibrocytes are a transitional cell population between mono-
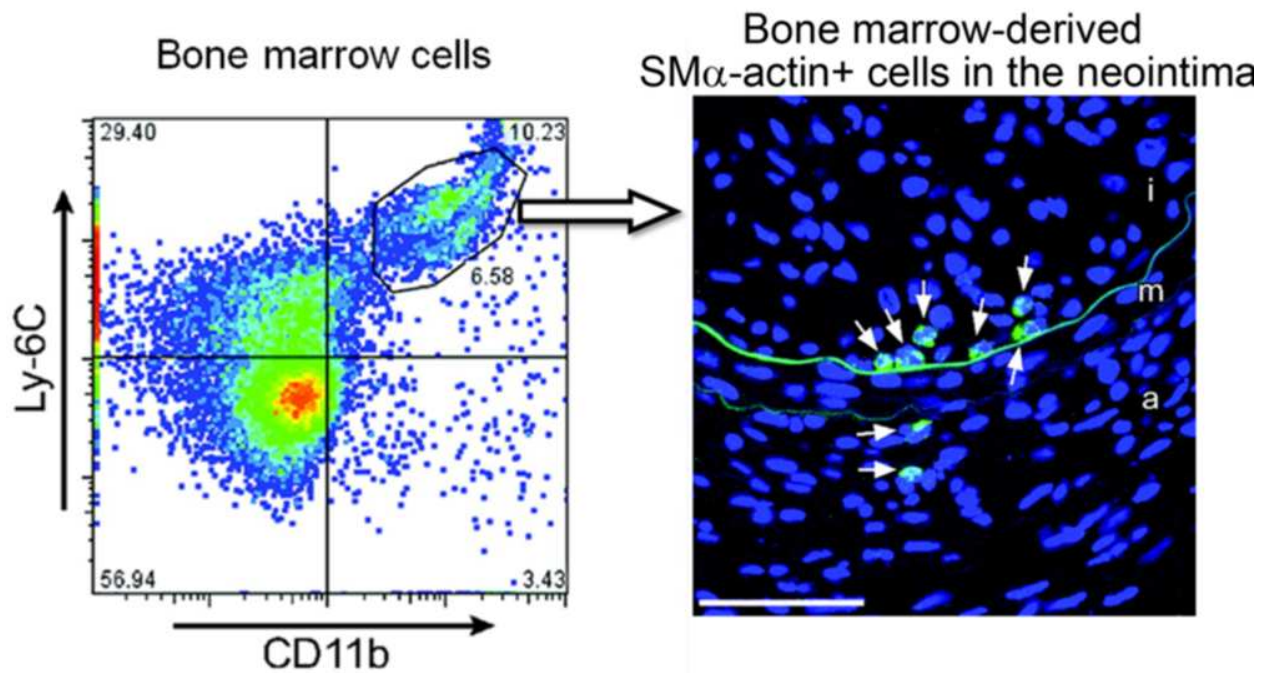
Figure 4. Bone marrow CD11b+Ly-6C+ cells migrate to the arterial wall and express SM α-actin. CD11b+Ly-6C+ cells within the bone marrow of SM α-actin-EGFP mice were sorted and adoptively transferred into wild-type mice with femoral arteries that had been subjected to wire-induced arterial injury. Four weeks after the injury, EGFP+ cells corresponding to CD11b+Ly-6C+-derived SM α-actin+ cells (arrows) were found in the walls of the injured vessel. Scale bar=50 μm. i Indicates intima; m, media; and a, adventitia (Modified from Ref 14 by Iwata et al.).

cytes and fibroblasts. Considerable overlaps exist in the gene expression profiles among human monocytes, macrophages, fibrocytes, and fibroblasts [121]. Human fibrocytes also may differentiate into cells with characteristics of adipocytes, chondrocytes, and osteoblasts [122, 123]. In human peripheral blood, 0.1% to 0.5% of nucleated cells are circulating fibrocytes that express type 1 and 2 collagens, vimentin, and SMα-actin [124]. Because no single marker can unequivocally identify fibrocytes, the combined use of collagen and other surface markers, including CD34, CD45, and CD68, is a common approach. More recent studies have used a combination of CD45RO, 25F9, and S100A8/A9 or CD49.

The fibrous cap of human atherosclerotic lesions contains fibrocytes expressing procollagen I and CD34 [125]. Subendothelial SMα-actin–positive myofibroblasts expressing the monocyte marker CD68 have been found in lipid-rich areas of the atherosclerotic intima in human aorta [126]. The overexpression of TGF-β1 resulted in the increased accumulation of fibrocytes in atherosclerotic plaques of Apoe-/- mice [127]. The pro-inflammatory monocyte subset $CD14^{++}CD16^-CCR2^{high}$ may be precursors of fibrocytes. The expression profile of marker genes indicates considerable overlaps between fibrocytes, SMPC, smooth muscle–like cells, and monocytes/macrophages, suggesting the importance to clarify the relationship in lineages and functions between these cell types to unfold intertwined mechanisms for atherosclerosis and provide insight into the development of new classes of therapeutics.

11. Macrophage influence over SMC activation

Macrophages and SMCs play pivotal roles in vascular diseases. The evidence suggests the interplay between these cell lineages. Macrophages promote SMC activation. In vitro co-culture studies showed macrophage-derived PDGF promotes SMC growth [128]. IL-6 released by macrophages promotes SMC MMP-1 production [129]. Several in vivo studies identified macrophage MCP-1 and its receptor CCR2 depletion as instigators of SMC activation [130-132]. Macrophage affects SMC differentiation through mechanisms of PDGF-BB, a major macrophage product. PDGF-BB suppresses SMC differentiation markers as gauged by the expression of SM α-actin, SM-MHC, and α-tropomyosin [30]. In a rabbit model of atherosclerosis, lipid lowering decreases the accumulation of macrophages expressing PDGF-B, and concomitantly increases the differentiated state of intimal SMCs, as gauged by increased expression of the SM-MHC isoform SM2 [26]. While these intimal SMCs regained SM2 expression, they decreased MMP expression. As discussed, more recent evidence indicates that BM-derived cells in the circulating blood (e.g., a subset of activated monocytes, SMPC) may differentiate into smooth muscle–like cells and contribute to the development of vascular lesions and the onset of their clinical complications [14].

12. SMC transdifferentiation into macrophages

In addition to the potential existence of intimal SMC or SMC-like cells of monocytes origin, accumulating evidence suggests transdifferentiation of SMC into macrophage lineage. Rong et al. demonstrated that cholesterol or oxidized LDL loading of SMCs suppresses SMC marker expression, but induces macrophage markers (e.g., CD68, Mac-2, and ABCA1) and phagocytic activity in cultured SMCs, raising the possibility that some macrophages within lesions may originate from SMC [20]. Bentzon et al. presented evidence that a significant fraction of lesional cells of Apoe-/- mice immunopositive for the macrophage marker Mac2 are not of the bone marrow origin [21]. Medial SMCs may undergo clonal expansion, loose classical SMC marker expression, and convert to macrophage-like cells in mouse atherosclerotic plaques [22]. Immunohistological analysis of human coronary atherosclerotic lesions demonstrated that a subpopulation (>40%) of macrophage foam cells (CD68 and oil-red O positive) co-express SMα-actin [23]. Caplice et al. showed that more than 90% of SM α-actin-expressing cells within lesions are not of the hematopoietic cell lineage [24]. More recently, Vengrenyuk et al. demonstrated that cholesterol loading converts vascular SMCs into cells similar to macrophage foam cells via the mechanisms dependent on micro RNA-143/145, and the transcription factor myocardin, and its co-activator serum response factor, responsible for SMC differentiation [25]. These studies indicate more dynamic crosstalk between SMC and monocytes/macrophage lineages than traditionally thought (Figure 5).
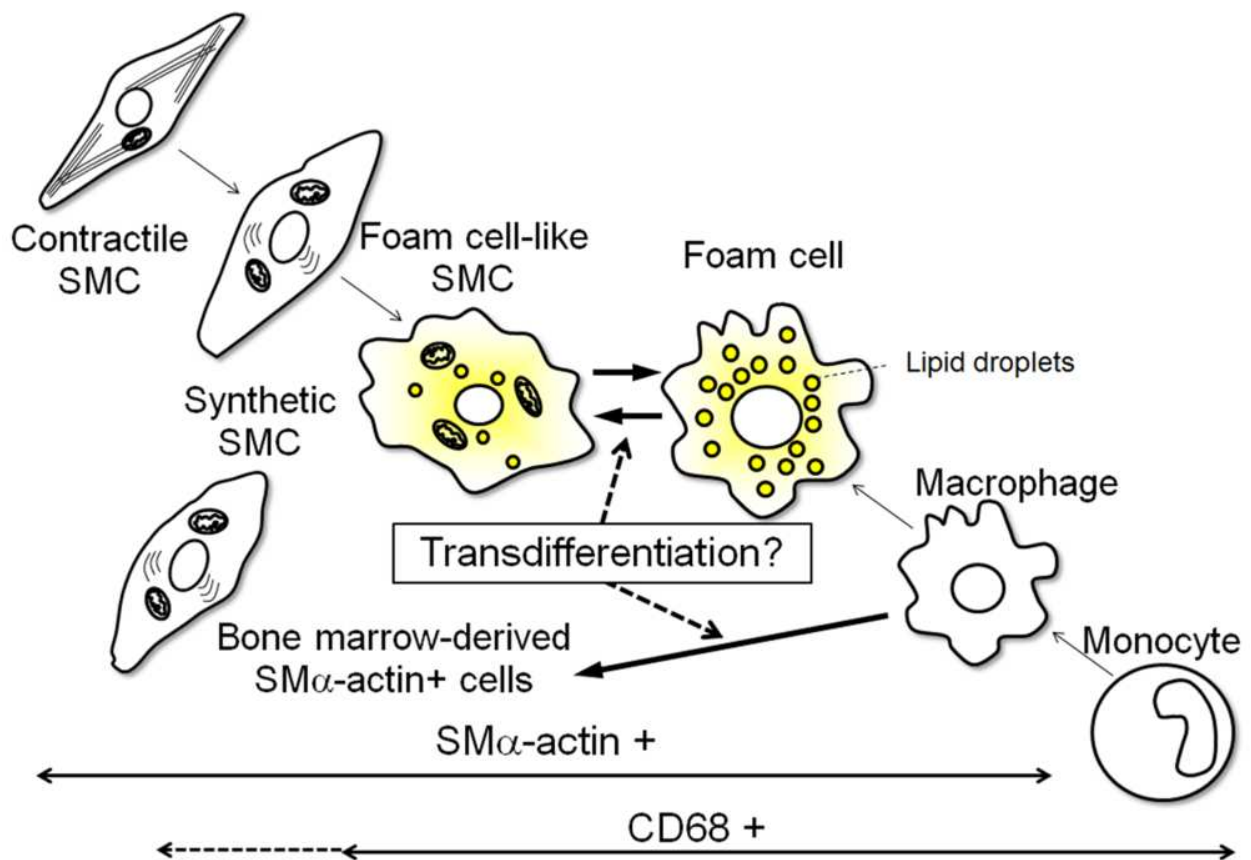
Figure 5. Possible transdifferentiation processes between SMC and macrophage lineages in the atherosclerotic plaques. The evidence suggests possible transdifferentiation between cells in the SMC and monocyte/macrophage lineages within the plaque. Various studies have demonstrated this concept in two directions: from SMC to macrophage foam cells (Ref. 20-25) and from monocytes to smooth muscle–like cells (Ref. 13, 14, 18, 19, 111,) and addressed its potential contribution to the pathogenesis of vascular disease.

13. Conclusions and future perspective

The evidence has used cutting-edge technologies, particularly in mouse models, to propose the substantial heterogeneity of SMCs and monocytes/macrophages. Due to technical difficulties in identifying SMC and monocyte/macrophage lineages in lesions, addressing the origin and the functionality of each cell type remains challenging. Lineage tracing of lesional cells in humans particularly requires highly sophisticated technologies. Gomez et al. recently reported a rigorous method with detection of histone modification at specific gene loci of SM-MHC gene [133]. Such specific cell lineage tracing methods will serve as powerful tools to provide insights into the crosstalk between SMCs and macrophages in human atherosclerosis. In addition, the use of multidisciplinary strategies, involving in vitro models, animal experiments, human samples, and more systemic approaches such as network analysis may help to unfold complex mechanisms for human atherogenesis. In addition, such strategies may identify new classes of therapeutic targets for atherosclerosis and its devastating complications

such as myocardial infarction and stroke, and may help to evaluate the effects of new therapeutics.

Author details

Hiroshi Iwata[1] and Masanori Aikawa[1,2*]

*Address all correspondence to: maikawa@rics.bwh.harvard.edu

1 The Center for Interdisciplinary Cardiovascular Sciences, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

2 The Center for Excellence in Vascular Biology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

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