The conversion of contractile vascular smooth muscle cells (SMCs) to a synthetic or proliferative phenotype is thought to play a major role in vascular diseases, such as atherosclerosis and restenosis.1-3 Our recent work presents evidence that challenges this widely accepted dogma. Our findings suggest that multipotent vascular stem cells (MVSCs) are a major contributor to vascular remodeling.4 The experimental results demonstrate that the major population of the traditionally defined proliferative/synthetic SMCs is derived from the differentiation of MVSCs rather than the dedifferentiation of mature SMCs. Both in vitro and in vivo results suggest that vascular disease is a stem cell disease, which raises the question on the previous dogma: Is vascular disease a SMC disease?

In this issue of Circulation Research, a group of leaders in the area of SMC biology wrote a commentary on this work.5 They present evidence in the literature that seems to support the SMC dedifferentiation hypothesis. However, in many previous studies, it was incorrectly assumed that the vascular cells in the primary culture and in injured blood vessels were mostly derived from SMCs. Thus, the previous experimental findings on vascular cells were often attributed to SMCs, which resulted in data misinterpretation and the overstatement on SMC functions. We agree that further investigations are needed to determine the relative contribution of MVSCs and SMCs to vascular remodeling in various animal models; however, we respectfully disagree on some of the arguments in the commentary.

Vascular SMC biology has been studied in vitro and in vivo extensively in the past 50 years since the method for SMC culture was established. The concept of phenotypic modulation of SMCs was originated from the ultrastructural characterization of SMC culture in 1960s to 1970s, which has been summarized in the comprehensive review by Chamley-Campbell et al.6 The contractile SMCs are characterized by the cytoplasm largely filled with thick and thin myofilaments, whereas synthetic SMCs have few myofilaments but have large amount of rough endoplasmic reticulum, free ribosomes, and Golgi complexes.6 A correlative conclusion suggests that the phenotypic modulated SMCs, which barely grow in the first week of the primary culture, account for a sudden cell expansion afterward and that these expanded cells can undergo redifferentiation under the same culture condition. These expanded cells are traditionally characterized as proliferative/synthetic SMCs, assuming that they are derived from mature SMCs. As shown later by numerous studies, this expanded cell population expresses the markers of immature SMCs, such as smooth muscle α-actin (SMA), calponin-1, and SM-22α, which seems to support the conclusion. However, to date, there is no direct evidence (eg, by using rigorous lineage tracing) to show that these fast growing cells are indeed derived from mature SMCs. Thus, it is not unreasonable to ask the following questions: Can the phenotypically modulated SMCs grow fast enough to account for the cell expansion in SMC primary culture? Is it possible that the fast growing cells are derived from a minority population of stem cells in SMC culture?

Indeed, our data provide a reasonable explanation: the fast expansion of SMCs after the first week of in vitro culture may be mainly attributed to the expansion of a minority population of cells (ie, MVSCs), and MVSCs can spontaneously differentiate into proliferative/synthetic SMCs. In addition, the lineage tracing experiments using smooth muscle myosin heavy chain (SM-MHC) as a marker suggest that these proliferative/synthetic SMCs indeed are not derived from mature SMCs.4 Therefore, although our data interpretation and conclusion are different from the previous theory, the experimental data in the classical studies do not contradict our findings. Our findings imply that SMCs in many previous in vitro studies may be derived from MVSCs. Indeed, as exemplified in Figure 1, a commercially available human aortic SMC line expresses low level of Sox10, an MVSC marker, suggesting that these cells are likely derived from MVSCs. In contrast, human MVSCs of primary culture have high level of Sox10 expression and low level of SMA that is not incorporated into stress fibers. There are >40 000 articles on vascular SMCs. If the proliferative/synthetic SMCs may be derived from MVSCs, many previous conclusions on SMCs may need to be revisited.

Do phenotypically modulated SMCs grow as fast as previously thought? In the primary culture of SMCs, mitotic bodies were observed in phenotypically modulated SMCs but were not commonly found in mature SMCs.6,7 Consistently, we did not find proliferative cells in SM-MHC+ population in the primary culture, but we did observe a small percentage of newly differentiated SM-MHC+ cells in the cell cycle.4 However, by using SM-MHC as a marker for lineage tracing, we showed that SMCs in the primary culture did not expand as much as expected and that MVSCs outgrew SMCs and became proliferative/synthetic SMCs,4 suggesting that...
MVSC expansion dominates over SMC multiplication. SMC multiplication, if any, may be a much slower process compared with MVSC expansion. Because our study is the only report on the tracing of SMC fate in culture, further studies are being performed in several laboratories with different transgenic mouse lines to verify the results.

As discussed in the commentary, there are numerous studies showing proliferative cells in neointima. However, these studies did not directly show that proliferative cells were derived from mature SMCs and therefore cannot exclude the involvement of MVSCs and other cell types. Nevertheless, SMCs do show changes in gene expression after injury. Figure 2 in the commentary provides evidence that SMCs can indeed replicate DNA and get into S phase in vivo after ligation or wire injury. Figure 2A is the only lineage tracing data in the literature showing that SMCs or SMC-derived cells can proliferate in vivo, although the diffused β-gal staining throughout the vascular wall (including matrix) is questionable. It is noted that the SMCs appear to be the only cell type in the injured arteries, which is contradictory to the previous reports that the cells from diverse origins, including bone marrow, circulation, and adventitia besides the medial layer of blood vessel wall, are also involved in the vascular remodeling after the injury. In contrast, in our animal model, after wire injury, extensive SMC death was observed within 2 days, and we did not observe proliferative SM-MHC+ cells. In some regions of injured vessel, all cells in the vessel wall were replaced by MVSC-derived cells within a week. One explanation for the discrepancy between the data shown in the commentary and ours is that the extent of injury in the arteries was different. As we pointed out, “the extent of MVSC activation, proliferation, and differentiation could be dependent on the extent of vascular injury and SMC damage.” We agree that a less severe injury could allow the investigation of how SMCs contribute to neointima formation. Regarding SMC lineage tracing model, the tamoxifen-inducible SM-MHC-Cre mouse line does have an advantage because it only traces existing SMCs but not newly differentiated SMCs.

Can SMCs dedifferentiate? Although the phenotype of SMCs could be modulated, there is no direct evidence suggesting that SMCs can dedifferentiate and return to a multipotent state, which was noted 30 years ago and is still the case to date. In many previous studies, the conclusion on the phenotypic modulation of SMCs in vivo was mostly based on the assumption that the cells in injured arteries were derived from mature SMCs, which is not necessarily true. As cited in the commentary, one of the classical studies showed that, after artery ligation, the cells in the neointima had synthetic phenotype and differed from SMCs in ultrastructure, which was assumed to be dedifferentiated SMCs without rigorous cell tracing. Similarly, the loss of the expression of SMC markers, such as SMA, SM-22α, and SM-MHC, in response to injury was attributed to the silence of these genes in SMCs in many previous studies. It is intriguing that SMA and SM22α, the 2 immature SMC markers downregulated but not completely silenced in proliferative/synthetic SMCs, are not expressed in the vascular cells after the injury. Is it possible that the cells in the neointima arise from other vascular
cells such as MVSCs? In another study, SM-22α was used as the lineage tracing marker to show that SMCs contribute to the chondrogenic and osteogenic remodeling of arteries. However, SM-22α is also expressed in many other types of cells in blood vessels, including adventitia myofibroblasts, activated macrophages, and MVSC-derived cells. Therefore, the lineage tracing using SM22α as a marker cannot specifically trace mature SMCs, thus compromising the conclusion on SMC dedifferentiation. As recently reviewed by Gomez and Owens, there is a lack of studies that directly trace the fate of mature SMCs.

Where are MVSCs located? Indeed it is impossible to isolate cells only from the medial or adventitial layer without the contamination of cell type from the other layer, especially in rat and mouse vascular tissues. Therefore, the cell isolation method is not accurate to define the origin and location of MVSCs in the rodent models and may lead to misinterpretation. Recently, we were able to isolate MVSCs from human blood vessels in which the medial and adventitial layers can be separated, and we found that MVSCs could be isolated from both media and adventitia. In addition, we have used Sox10 as a marker to identify and trace MVSCs in mouse blood vessels with a higher spatial resolution. As shown in Figure 2, MVSCs are present in both the medial layer and the adventitial layer of mouse thoracic aorta, and MVSC multiplication and activation (emergence of Sox10+ cells) can be observed with ex vivo 2-photon time-lapse microscopy. This ex vivo model provides a valuable tool to further investigate the dynamics of MVSCs and SMCs in injured blood vessels. In addition, the commentary also raises the question on the relationship of MVSCs with previously identified vascular progenitors. As we reported previously, MVSCs are negative for Sca-1, Sox2, c-kit, and CD146 and are different from other vascular progenitors, such as Sca-1- cells, CD44+/CD29+/ Sox2-/Oct4+ cells, and CD146+ pericytes, in the aspects of fate of mature SMCs.

In summary, we provided compelling evidence that MVSCs are a major contributor of proliferative/synthetic SMCs in vitro. The relative contribution of MVSCs and other vascular progenitors to vascular diseases needs further investigation. In summary, we provided compelling evidence that MVSCs can be activated on vascular injury and that the rapid expansion and the aberrant differentiation of MVSCs contribute to neointima formation. However, the relative contribution and importance of vascular stem cells and SMCs need further investigation. The findings on MVSCs provide a new perspective on vascular biology and may lead to the development of new therapies for vascular diseases. Nevertheless, besides the possibility of SMC de-differentiation, SMCs may play a role in mediating inflammatory responses and cell-cell signaling. We hope that this debate will promote the research to further elucidate the roles of MVSCs and SMCs in vascular remodeling and address this fundamental issue: Is vascular disease a stem cell disease or SMC disease, or both?

**Key Words:** differentiation ■ stem cell plasticity ■ stem cells ■ vascular biology ■ vascular smooth muscle
Smooth Muscle Cells: To Be or Not To Be?: Response to Nguyen et al
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