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Review

Epigenetic regulation of smooth muscle cell plasticity☆

Renjing Liu [a,b], Kristen L. Leslie [c], Kathleen A. Martin [c,*]

[a] Agnes Ginges Laboratory for Diseases of the Aorta, Centre for the Endothelium, Vascular Biology Program, Centenary Institute, Sydney, Australia
[b] Sydney Medical School, University of Sydney, Australia
[c] Departments of Internal Medicine and Pharmacology, Yale Cardiovascular Research Center, Section of Cardiovascular Medicine, Yale University, New Haven, CT 06511, USA

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ABSTRACT

Smooth muscle cells (SMC) are the major cell type in blood vessels. Their principal function in the body is to regulate blood flow and pressure through vessel wall contraction and relaxation. Unlike many other mature cell types in the adult body, SMC do not terminally differentiate but retain a remarkable plasticity. They have the unique ability to toggle between a differentiated and quiescent "contractile" state and a highly proliferative and migratory "synthetic" phenotype in response to environmental stresses.

While there have been major advances in our understanding of SMC plasticity through the identification of growth factors and signals that can influence the SMC phenotype, *how* these regulate SMC plasticity remains unknown. To date, several key transcription factors and regulatory *cis* elements have been identified that play a role in modulating SMC state. The frontier in understanding the molecular mechanisms underlying SMC plasticity has now advanced to the level of epigenetics. This review will summarize the epigenetic regulation of SMC, highlighting the role of histone modification, DNA methylation, and our most recent identification of a DNA demethylation pathway in SMC that is pivotal in the regulation of the SMC phenotypic state.

Many disorders are associated with smooth muscle dysfunction, including atherosclerosis, the major underlying cause of stroke and coronary heart disease, as well as transplant vasculopathy, aneurysm, asthma, hypertension, and cancer. An increased understanding of the major regulators of SMC plasticity will lead to the identification of novel target molecules that may, in turn, lead to novel drug discoveries for the treatment of these diseases. This article is part of a Special Issue entitled: Stress as a fundamental theme in cell plasticity.

1. SMC plasticity in health and disease

SMC are a unique cell type in that they are remarkably plastic. They can readily switch between two phenotypic states – contractile and synthetic – depending on environmental cues. Within adult blood vessels, SMC exhibit the contractile phenotype, characterized by low proliferation rates, high levels of cytoplasmic myofilaments, low rates of protein synthesis, and a unique repertoire of contractile proteins including smooth muscle alpha actin (ACTA2), smoothelin, h-caldesmon, calponin, transgelin (TALGN), and smooth muscle myosin heavy chain (MYH11) [1,2]. SMC can undergo phenotypic modulation in response to extracellular signals and de-differentiate to the synthetic phenotype. In the synthetic state, SMC express relatively few contractile proteins, re-enter the cell cycle, and become highly proliferative and migratory, and have high rates of protein synthesis and extracellular matrix secretion [3,4]. SMC can readily switch between these two states when remodeling is required in response to changes in blood flow or when repair is needed following vascular injury. A disruption of this balance, such that the synthetic phenotype predominates, is a major underlying cause of many vascular diseases such as atherosclerosis and aneurysms.

Atherosclerosis is the major underlying cause of myocardial infarction, heart failure, stroke, and peripheral vascular disease. Lipid deposition in the vascular wall alters the integrity of the endothelium, permitting the transmigration of circulating monocytes into the SMC layer where they further mature into macrophages and engulf cholesterol. Growth factors and inflammatory mediators released by activated macrophages promote the de-differentiation of resident medial SMC from a contractile phenotype to the synthetic state. Migratory SMC populate the intima and participate in fibrous cap formation through heightened proliferation and extracellular matrix synthesis [5]. As it is difficult to identify de-differentiated SMC due to loss of their contractile markers, it has been hypothesized that phenotypically modulated SMC may play other critical roles in atherosclerotic lesions as well [5–8].

Severe atherosclerosis can require revascularization procedures, including angioplasty, stenting, or bypass grafts to restore blood flow. Restenosis is the re-narrowing of blood vessels following these procedures that leads to restricted blood flow. This is a major vascular complication caused by SMC phenotypic modulation [9]. Following injury to

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* Corresponding author at: Yale Cardiovascular Research Center, 300 George St., Room 759, New Haven, CT 06511, USA. Tel.: +1 203 737 5079; fax: +1 203 737 6118.
E-mail address: kathleen.martin@yale.edu (K.A. Martin).

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the vessel wall and the release of multiple growth factors into the microenvironment by platelets and infiltrating inflammatory cells, SMC de-differentiate from a contractile to the synthetic phenotype. These synthetic SMC proliferate and migrate to the intimal space where they actively secrete extracellular matrix proteins, leading to the formation of intimal hyperplastic lesions and the narrowing of the blood vessels [10].

The profound changes in SMC during phenotypic modulation also contribute to aortic aneurysms, which can arise from genetic or environmental causes, including smoking [11]. Aneurysms develop as the SMC undergo phenotypic modulation that is characterized by a decrease in contractile markers such as ACTA2 and MYH11 with increases in the secretion of MMPs, in particular MMP2 and MMP9 [12]. Apoptosis of SMC then ensues; this loss of the primary cells responsible for extracellular matrix synthesis results in the further weakening of the vessel wall and can ultimately lead to vessel rupture.

Treatment strategies for these diseases of SMC phenotypic modulation are limited at present. Strategies to prevent progression of atherosclerosis include lifestyle modifications and lipid lowering agents such as statins. Statins also appear to reduce systemic inflammation [13] and, interestingly, can promote SMC differentiation *in vitro* [14]. Ultimately, revascularization procedures are often required for advanced atherosclerosis. Drug-eluting stents (DES) including analogs of rapamycin have helped to prevent restenosis in the coronary arteries for many, but not all patients, with diabetic patients being at particularly high risk for restenosis [10]. DES have proven less effective for peripheral vascular disease, although new studies with drug-eluting balloons have shown promise [15]. Local delivery of rapamycin and its analogs may be particularly effective therapeutic agents due to their potent ability to promote SMC differentiation, in addition to inhibiting proliferation and matrix synthesis [16,17]. Systemic treatment with rapamycin inhibits transplant vasculopathy in rodent models [18,19], but systemic rapamycin is not an option for transplant vasculopathy or to maintain patency following bypass grafts in humans due to the very high doses required. For aneurysm, surgery is currently the only treatment, and many aneurysms go undetected until a critical rupture occurs. For these reasons, better treatments for these diseases associated with dysfunctional SMC phenotypic modulation are urgently warranted.

2. Role of epigenetics in regulating SMC plasticity

Many of the genes that characterize SMC phenotypic switching, including the contractile proteins, are regulated at the level of transcription. As such, most research has focused on identifying the transcription factors and promoter elements that drive such responses. Smooth muscle-specific genes are generally regulated by dual CArG elements in their promoter or intronic regions that bind serum response factor (SRF). SRF can also regulate pro-proliferative genes when bound to growth factor-regulated co-factors such as Elk-1 [20]. Smooth muscle-specific gene expression is governed by the smooth muscle- and cardiomyocyte-specific transcriptional co-activator myocardin (MYOCD) [21], which binds to SRF at CArG elements and is sufficient to promote the SMC contractile phenotype [22]. Many studies have focused on other factors that can oppose the actions of MYCOD to promote de-differentiation, including KLF4 [23], FoxO4 [24], and others. Micro RNAs (miRNAs) can also regulate SMC phenotype, including miR-143 and miR-145 which inhibit expression of KLF4 and KLF5 [25–27]. New studies have now emerged that are aimed at understanding the epigenetic influences on transcriptional control of SMC phenotype.

Epigenetics refers to heritable changes in gene expression that occur without any changes in the genomic sequence, often as a result of environmental influences. It is becoming increasingly clear that epigenetic mechanisms play an important role in the regulation of chromatin structure and remodelling, and are key mediators of cell type-specific gene expression during development and disease [28]. Chromatin is a dynamic complex composed primarily of genomic DNA and protein. The nucleosome is the fundamental unit of chromatin encompassing 146 base pairs of DNA wrapped around an octamer of histone proteins. This octamer contains two copies each of histones H2A, H2B, H3, and H4. The histone N-terminal tails are not bound to the nucleosome core [29] and frequently undergo modifications including acetylation, phosphorylation, ubiquitination, and ADP-ribosylation [30]. Epigenetic regulation of histones alters chromatin conformation and the accessibility of transcription factors to DNA, resulting in the activation or silencing of gene transcription. Two of the most extensively studied epigenetic changes are histone modification (which alter the packaging of the chromatin) and DNA methylation (occurring at the 5′-cytosine in CpG dinucleotides).

In recent years, multiple *in vitro* and *in vivo* studies have provided evidence of a key role of epigenetic modification, particularly histone modification, in controlling SMC gene expression during normal cell differentiation versus in disease. A 2012 review elegantly addressed the epigenetics of SMC phenotypic modulation [31]. Herein, we summarize some early pioneering studies, as well as the most recent literature on histone modifications and DNA methylation in regulation of SMC phenotype, with an additional focus on our recent study of DNA methylation in this area.

2.1. Histone modification

The first report describing epigenetic regulation of SMC differentiation arose from a retinoic acid (RA)-inducible A404 cell model of early SMC differentiation [32]. Despite its high expression, SRF was unable to bind to CArG boxes of SMC genes within intact chromatin. However, RA-induced differentiation led to an enrichment of SRF and the hyperacetylation of histones H3 and H4 in SMC CArG-containing regions [32]. Consistent with these results, increasing histone acetyltransferase (HAT) activity with tricostatin A (TSA) treatment or overexpression of CBP/p300 increased the promoter activity and gene expression of SMC genes [33,34]. In contrast, inhibition of histone acetylation via Twist1, E1A, or histone deacetylase (HDAC) overexpression resulted in the suppression of SMC markers [34–36].

Chromatin is more open at CArG-containing regions of the *ACTA2* promoter in SMC where contractile proteins are highly expressed, compared to in endothelial cells, where *ACTA2* and other smooth muscle-specific markers are not expressed [37]. These active regions exhibit increased histone methylation and acetylation as demarcated by increased H3K4me2, H3K79me2, H3K9Ac, and H4Ac [37]. The methylation of H3K4 is partially attributed to the recruitment of WDR5 and the associated histone lysine methyltransferase SET/MLL by Pitx2 to SMC promoters in early stages of differentiation [38]. Although the acetylation of histones is diminished during the de-differentiation process, H3K4me2 persists through SMC phenotypic modulation [6,37]. A novel *in vivo* assay combining *in situ* hybridization and the proximity ligation assay provided concrete evidence that H3K4me2 at the *MYH11* locus is restricted to the SMC lineage in tissue sections [6]. Together, these results suggest that there are cell type-specific epigenetic mechanisms that govern the expression of cellular markers during differentiation.

Other stimuli of SMC differentiation have also been shown to induce epigenetic changes that alter chromatin accessibility and hence gene transcription. Overexpression of MYOCD in 10T1/2 cells increased H3Ac at CArG elements at the *ACTA2* and *TAGLN* promoters [35]. TGFβ, which stimulates smooth muscle gene transcription, [39,40] also increased H3Ac and H4Ac at the *TAGLN* locus [34]. Conversely, inducers of SMC de-differentiation, such as PDGF-BB, facilitated the compaction of chromatin at SMC contractile gene loci. PDGF-BB-induced KLF4 recruited HDAC2, HDAC4, or HDAC5 to CArG regions on the *ACTA2* and *MYH11* promoters, reducing histone acetylation and inhibiting the accessibility of this region to the transcription factors MYOCD, SRF, and MRTF [36,37,41] (Fig. 1). In cerebral SMC, cigarette smoke extract increased the binding of HDAC2 to *ACTA2* and *MYH11* promoters,