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Invited Review

Myocardin and smooth muscle differentiation

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ABSTRACT

Myocardin (MYOCD), a co-transcriptional activator of serum response factor (SRF), stimulates the expression of smooth muscle (SM) genes and inhibits the cell cycle. In addition to its roles in the development, MYOCD may be critically involved in the pathogenesis of proliferative vascular diseases. This review mainly focuses on how MYOCD activity is regulated and how it inhibits cell proliferation.

Introduction

SM contractility is critical to maintaining the physiological functions of the internal organs, such as the blood vessel wall, gastrointestinal tract, airway, uterus and bladder. Notably, deregulation of vascular contractility, for example, contributes to the pathogenesis of hypertension. In addition, abnormal proliferation of SMCs is involved in neointima formation in arteries, uterine leiomyoma (a benign tumor within the uterus), renal leiomyolipoma in tuberous sclerosis complex (TSC)[1] patients, and lung lymphangioleiomyomatosis (LAM) in female patients.

The methodology for the culture of smooth muscle cells (SMCs) was established about 30 years ago, but it remained unknown as to why SMCs could be cultured and what controlled SM differentiation until MYOCD was discovered in 2001.

In order to proliferate, SMCs are believed to undergo phenotypic conversion from contractile differentiation to synthetic dedifferentiation status. This phenotypic conversion model has been challenged by a recent study showing proliferating SMCs in culture resulting from vascular stem cells, instead of dedifferentiated SMCs [1].

Nevertheless, the critical roles of MYOCD have well been established in SMC differentiation. This review will focus on the following questions: how is MYOCD activity regulated, and how does MYOCD regulate differentiation and the cell cycle of SMCs?

MYOCD and its expression

MYOCD (935 aa) was discovered in a research screening for novel cardiac-specific genes *in silico* through a BLAST search using ESTs from mouse embryonic heart cDNA libraries in the database [2]. Two other family members, MYOCD-related transcription factor-A (MRTF-A, 929 aa, also called MAL, MKL-1, and BSAC), and MRTF-B (1080 aa, also called MKL-2), were identified through searching the NCBI databases using the mouse *Myocd* cDNA sequence [3]. None of the members bind to DNA, but rather they initiate SRF-dependent gene transcription [2,3]. MYOCD forms a stable ternary complex with SRF and is defined as a co-transcriptional activator [2]. SRF binds to CArG [CC(A/T)$_6$GG] boxes and transactivates transcription of many genes, including SM-specific ones (Fig. 1). MYOCD does not have transcriptional activity in Srf−/− cells [3]. Notably, MYOCD may exert its effects independently of SRF resulting from its binding to other signaling proteins.

MYOCD is expressed in the heart and in most developing and adult SMC compartments including the dorsal aorta, bladder, stomach, intestine, and uterus [3,4]. Evidence suggests that MYOCD may be expressed in other cells or tissues. For example, lineage tracing studies have shown that MYOCD is transiently expressed in skeletal muscle during development and plays a suppressor role in the skeletal muscle differentiation program [5]. In endothelial cells,

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[1] *Abbreviations used*: TSC, tuberous sclerosis complex; LAM, lymphangioleiomyomatosis; SMCs, smooth muscle cells; MRTF-A, MYOCD-related transcription factor-A; TADs, transcription activation domains; Foxo, forkhead transcription factor; CRM, chromosomal region maintenance protein; PECAM-1, platelet endothelial cell adhesion molecule 1; MHC, myosin heavy chain; MLCK, myosin light chain kinase; SBE, Smad-binding element; HRT, hairy-related transcription factor; uPAR, urokinase-type plasminogen activator receptor; TDG, thymine DNA glycosylase; KLF, Kruppel-like transcription factor; ER, estrogen receptor; SRC3; steroid receptor coactivator 3; STRAP, SRF-dependent transcription regulation-associated protein; Cdc7, cell division cycle 7; Ang II, Angiotensin II; IGF, insulin-like growth factor; NKE, Nkx2.5 responsive element; HSC, hepatic stellate cells; ATV, atorvastatin; CBP, CREB-binding protein; CHIP, C-terminus of Hsc70-interacting protein; ERα, estrogen receptor α; TFs, transcription factors; PDGF, platelet-derived growth factor.

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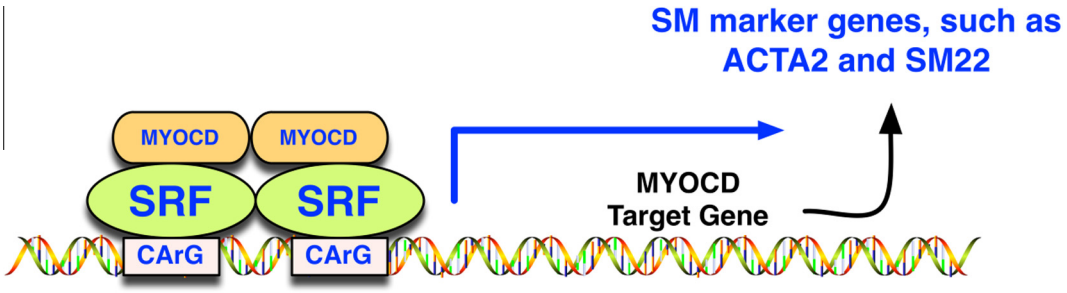

Fig. 1. Myocardin (MYOCD) stimulation of gene expression through serum response factor (SRF). MYOCD activity is indicated by the expression levels of MYOCD target genes, such as SM α-actin and SM22.

MYOCD mediates hypoxia-induced transdifferentiation into SM-like cells [6]. In peripheral blood mononuclear cells, thrombin stimulates MYOCD and SM myosin heavy chain expression [7].

In addition, distinct MYOCD splice variants have been reported [8,9], which may confer differential tissue expression and functions. In contrast, MRTF-A and -B are expressed in a broad range of embryonic and adult tissues, including ES cells [10].

The features of domain structures

First, there has been no DNA binding domain revealed in any member of the MYOCD family. Their C-terminal regions contain transcription activation domains (TADs, Fig. 2), which are required for transcriptional activity of MRTFs, but have no target gene specificity [3]. Deletion of this region results in a dominant-negative mutant.

Second, their interaction with SRF is through a short peptide sequence containing a basic and glutamine-rich region (Fig. 2) [2,3]. The binding region of Smad1 includes a basic domain and a stretch of glutamine residues [11]. The forkhead transcription factor (Foxo) 4 (amino acid residues 89–325) directly interacts with the 129–510 aa fragment of myocardin [12]. GATA4 directly binds two regions (326–438 and 439–713 aa) of myocardin [13].

In addition, several other domain structures have been identified in the MYOCD family. (1) RPEL motifs: Two are present in the MYOCD N-terminal region (Fig. 2). Three are in MRTF-A/B, which mediate their association with G-actin [14]. (2) SAP domain (SAF-A/B, Acinus, and PIAS): It contains 35 amino acids (residues 380–414) and may participate in chromosomal dynamics, nuclear breakdown, and apoptotic DNA fragmentation as described for other SAP-containing proteins [15]. (3) Coiled-coil motif: It resembles a leucine zipper and mediates homo- and heterodimerization of MYOCD members, which stabilizes their binding with SRF on CArG boxes of target genes (Fig. 2) [2,3,10,14].

Subcellular localization

MYOCD is in the nucleus, but MRTF-A/B are in the cytosol. This is possibly because MRTF-A/B have three RPEL motifs, but there are only two in MYOCD. The three RPEL motifs can mediate MRTF-A/B binding to cytosolic G-actin [16], but the two RPEL domains in MYOCD have relatively weak binding to actin [16]. It is also possibly due to: (1) the higher affinity of MYOCD to importin/1 than MRTF-A/B, and (2) G-actin inhibition of importin/1 binding with MRTF-A/B [17]. In addition to importin, recent studies have shown that difference in subcellular localization is also determined by their differential interaction with chromosomal region maintenance protein (CRM)1, SRF, and G-actin [18]. Differential localization may be explained by MYOCD's weak binding to CRM1, but strong binding to SRF, which are opposite to MRTF-A [18].

Notably, the subcellular localization of all members may change in response to various stresses. Hyperosmotic stress, for example, was reported to regulate the distribution and stability of MRTF in kidney tubular cells [19].

The phenotypes of knockout mice

Myocd knockout

Mice with a homozygous null mutation of *Myocd* died at embryonic day 10.5 (E10.5) [20]. This study showed no staining for either SM α-actin or SM22 in the dorsal aortae in transverse section of *Myocd*−/− embryos, suggesting the lack of SMC differentiation. However, the staining for PECAM-1 (Platelet Endothelial Cell Adhesion Molecule 1), an endothelial marker, at E8.5 suggested a normal vascular patterning in mutant embryos [20]. It is unclear what causes the embryonic death, but the lethality may result from abnormal vasculature of the yolk sac and the pericardial effusion observed by E10.5 [20]. Selective knockout of *Myocd* in neural crest-derived SMCs results in the lack of the contractile phenotype

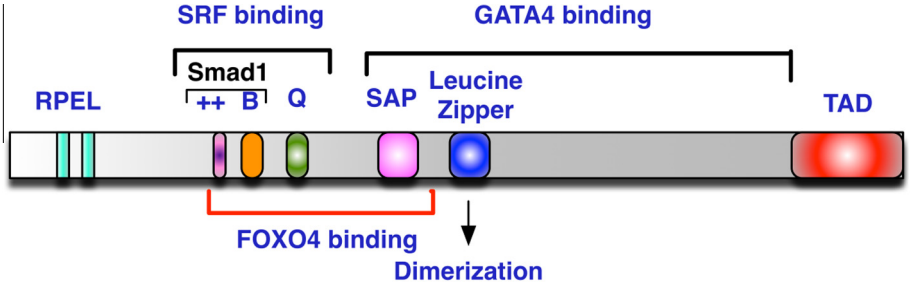

Fig. 2. Domain structure of myocardin (MYOCD). The functions of the various domains have been described in the text. SAP: SAF-A/B, Acinus, and PIAS; TAD: transactivation domain; SRF: serum response factor; ++: basic domain.