mCAT Got Your TEF?

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TEF and RTEF-1 Binding to the Smooth Muscle α-Actin Gene Distinguishes Myofibroblasts and Adult Smooth Muscle Cells

Adult smooth muscle cells are highly plastic and can exhibit a range of phenotypes in response to different environmental and developmental cues. Their phenotypes can range from quiescent highly contractile cells with high levels of characteristic contractile protein isoforms, to highly proliferative cells that secrete large amounts of extracellular matrix and express only low levels of smooth muscle-specific isoforms of contractile proteins. These two states are often referred to as differentiated and dedifferentiated or phenotypically modulated states.1 In reality the situation is more complex, with smooth muscle cells likely existing in a continuum of phenotypes between these two extremes. This smooth muscle cell plasticity often makes the unequivocal identification of smooth muscle cells challenging, particularly for the more dedifferentiated smooth muscle cells that are very similar to fibroblasts. The situation is further complicated by the existence of cell types with phenotypes part way between a fibroblast and a fully differentiated adult smooth muscle cell, namely myoepithelial and myofibroblast cells, and pericytes.2–4 A major challenge to developmental biologists is determining how these cells relate to each other, determining whether they are derived from common or distinct precursors, and whether myofibroblasts or pericytes can become smooth muscle cells. One approach to begin to answer these questions is to determine the molecular mechanisms that control the phenotype of each of these cell types. A new study by Gan and colleagues,3 described in this issue of Circulation Research, provides definitive molecular evidence that distinguishes myofibroblasts from adult smooth muscle cells by the distinct transcriptional mechanisms that they use to direct expression of a shared molecular marker, smooth muscle α-actin. In this elegant study, the authors demonstrate that the binding of RTEF-1 to MCAT elements within the smooth muscle α-actin promoter is required for transcription in myofibroblasts but not in adult smooth muscle cells (Figure). These results reveal that although both adult smooth muscle cells and myofibroblasts express smooth muscle α-actin, its expression in these cell types is regulated by distinct cis- and trans-acting factors. That myofibroblasts and adult smooth muscle cells use distinct cis-regulatory elements to control expression of a single gene should not, however, be too surprising given previous studies showing that a single gene often uses distinct elements for expression even among adult smooth muscle cells in different tissues. This phenomenon is perhaps best illustrated by earlier studies by the Owens group which showed that distinct regions of the smooth muscle myosin heavy chain gene direct expression in different vascular beds and among different visceral smooth muscle tissues.6 Perhaps the most intriguing finding of this study is that the MCAT elements required for expression of smooth muscle α-actin in myofibroblasts are also required for expression in smooth muscle cells during early embryonic development. This provides unequivocal evidence that smooth muscle cells also use distinct regulatory elements to drive expression of a gene in embryonic and adult cells. This finding certainly begs the question as to what distinguishes an embryonic smooth muscle cell from a myofibroblast and raises the possibility that myofibroblasts could represent a resident population of embryonic smooth muscle cells that persist, undifferentiated, in an adult. Careful lineage mapping using temporally and spatially restricted lineage markers will be required to answer this important question.

What Controls the Switch From MCAT-Dependent to MCAT-Independent Transcription Regulation During Development of Embryonic Smooth Muscle?

Mutation of the MCAT elements within the smooth muscle α-actin promoter completely abolished expression of a transgene in early embryonic smooth muscle, before E13.5, but did not affect expression later in development or in adult tissues. The smooth muscle α-actin promoter thus undergoes a dramatic switch between embryonic day 12.5 and day 13.5 from being dependent on MCAT elements and presumably RTEF-1 for activation, to a gene that is totally independent of these elements. Most surprising is the finding that this switch seems to occur in all smooth muscle tissues, both vascular and visceral. This raises the intriguing questions as to what happens at this stage of development to initiate this switch. Is this specific for smooth muscle α-actin or are other genes coordinately regulated? Although we obviously do not yet have answers to these questions, it seems reasonable to postulate that this switch occurs in response to changes in the microenvironment, such as a change in hormone signaling. One potential mechanism accounting for this switch would be a change in dependence of the differentiating smooth muscle cells on TGFβ signaling. Embryonic smooth muscle cells may switch from being dependent on TGFβ signaling before E13.5 to being largely independent of TGFβ after this time. This would be analogous to the switch that endothelial cells...
Myofibroblasts

Adult Smooth Muscle Cells

**Figure.** Transcriptional regulation of smooth muscle α-actin in myofibroblasts and adult smooth muscle cells. A number of cis-acting regulatory elements have been shown to be required for regulation of the smooth muscle α-actin promoter in myofibroblasts (left panel) and in smooth muscle cells (right panel) in vivo (for simplicity only the proximal promoter region is shown). In myofibroblasts TGFβ has been shown to increase expression of SRF, RGC-32, δEF1,12,16, Gan and colleagues also show that TGFβ promotes binding of RTEF-1 to the MCAT elements that are required for TGFβ-mediated increases in smooth muscle α-actin in myofibroblasts. Binding of TEF family members to the MCAT1 element can be antagonized by the single stranded DNA binding proteins Purα/β.17 In addition, phosphorylation of SMAD3 facilitates its nuclear accumulation where it can interact with SRF or δEF1.12,16 In contrast, in adult smooth muscle cells MCAT elements bind to TEF, which does not stimulate promoter activity. A number of signaling pathways have been shown to be important for expression of smooth muscle-specific proteins in adult smooth muscle cells including increases in intracellular calcium via voltage gated calcium channels, activation of RhoA and ROK, Gβγ subunits of G heterotrimeric proteins, and PKG.19–23 Together these molecules increase the activity of SRF complexes to activate transcription of the smooth muscle α-actin gene. Complexes containing myocardin related transcription factors are shown, although other positive-acting SRF complexes likely play important roles in specific smooth muscle cell types.

Signaling pathways may also induce covalent modification of TEF or RTEF-1 or they may cause changes in chromatin structure to facilitate binding of TEF as compared with RTEF-1 to the MACT elements in the smooth muscle α-actin promoter. Signaling-induced changes in chromatin structure, regulated by either ATP-dependent remodeling complexes or by changes in HAT or HDAC activity, would have the advantage that these changes could simultaneously alter the binding of a number of transcription factors, thereby facilitating a coordinated switch between different transcription regulatory complexes. This may allow an embryonic smooth muscle cell to switch from a myofibroblast type mode of regulation of smooth muscle α-actin to a more adult smooth muscle type of mode (Figure). An exciting challenge of future investigations will be to capitalize on these exciting findings to elucidate the mechanisms controlling the switch from MCAT-dependent to MCAT-independent transcription during smooth muscle development.

**The MCAT Mutated Smooth Muscle α-Actin Promoter Provides a Valuable Tool for Smooth Muscle-Specific Gene Targeting**

Gan and colleagues report that the MCAT mutated smooth muscle α-actin promoter failed to be expressed in embryonic skeletal or cardiac muscle. Besides indicating the importance of this element for smooth muscle α-actin expression in these tissues, mutation of the MCAT element, resulted in the restriction of the expression of the altered promoter to smooth muscle cells throughout development. This finding has important implications for the use of the mutant promoter to target expression of proteins, such as cre recombinase, to undergo from being dependent on VEGF during early development to becoming VEGF independent in the adult. In support of this proposal, RTEF-1 binding to MCAT elements is required for TGFβ-mediated induction of α-actin in myofibroblasts and for α-actin expression in embryonic smooth muscle cells. TGFβ stimulation of myofibroblasts has also been shown to increase expression of serum response factor (SRF),7 which is critically required both for smooth muscle α-actin expression in smooth muscle cells and for TGFβ-mediated activation of smooth muscle α-actin in myofibroblasts.8,9 In addition, TGFβ is known to be important for induction of smooth muscle differentiation both in vitro and in vivo.10,11 However, although SRF is required to maintain the differentiated phenotype of smooth muscle cells throughout development, it is not clear whether TGFβ is required, under normal conditions, in adult smooth muscle cells. We speculate that the switch from MCAT-dependent transcription of the smooth muscle α-actin gene to MCAT-independent transcription may also reflect a switch from TGFβ-dependent SRF complexes, such as SRF-SMAD3-δEF1, to TGFβ-independent complexes, such as SRF-myocardin (Figure).12,13 As discussed by Gan and colleagues, a switch to SRF-myocardin complexes likely does not simply reflect an induction of myocardin expression, as myocardin mRNA is readily detectable in the gut by E12.5.14 A caveat to this, however, is that studies on myocardin protein expression have not been performed because of the low specificity of most available antibodies, hence it is not known whether myocardin protein expression directly parallels its mRNA levels.
smooth muscle tissues. To date all of the currently available smooth muscle-restricted promoters, used to target gene expression in vivo, have ectopic expression in other tissues, either in the heart and skeletal muscle during embryonic development (eg, SM22α and wild-type smooth muscle α-actin promoters) or in eggs and sperm of transgenic mice (smooth muscle myosin heavy chain promoter). The MCAT mutated smooth muscle α-actin promoter will thus provide a unique and very valuable tool for targeting gene expression to adult smooth muscle tissues.

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Disclosures

None.

References


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