UltraRapid Communication

The Histone Demethylase, Jmjd1a, Interacts With the Myocardin Factors to Regulate SMC Differentiation Marker Gene Expression

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Abstract—We and others have previously shown that the myocardin transcription factors play critical roles in the regulation of smooth muscle cell (SMC) differentiation marker gene expression. In a yeast 2-hybrid screen for proteins that interact with myocardin-related transcription factor-A (MRTF-A), we identified the histone 3 lysine 9 (H3K9)-specific demethylase, Jmjd1a. GST pull-down assays demonstrated that Jmjd1a bound all 3 myocardin family members, and further mapping studies showed that the jumonjiC domain of Jmjd1a was sufficient to mediate this interaction. Overexpression of Jmjd1a in multipotential 10T1/2 cells decreased global levels of di-methyl H3K9, stimulated the SM α-actin and SM22 promoters, and synergistically enhanced MRTF-A– and myocardin-dependent transactivation. Using chromatin immunoprecipitation assays, we also demonstrated that TGF-β–mediated upregulation of SMC differentiation marker gene expression in 10T1/2 cells was associated with decreased H3K9 dimethylation at the CArG-containing regions of the SMC differentiation marker gene promoters. Importantly, knockdown of Jmjd1a in 10T1/2 cells and primary rat aortic SMCs by retroviral delivery of siRNA attenuated TGF-β–induced upregulation of endogenous SM myosin heavy chain expression. These effects were concomitant with increased H3K9 dimethylation at the SMC differentiation marker gene promoters and with inhibition of MRTF-A–dependent transactivation of the SMC-specific transcription. These results suggest, for the first time, that SMC differentiation marker gene expression is regulated by H3K9 methylation and that the effects of the myocardin factors on SMC-specific transcription may involve the recruitment of Jmjd1a to the SMC-specific promoters. (Circ Res. 2007;101:e115-e123.)

Key Words: SRF ■ myocardin factors ■ histone methylation ■ jumonjiC domain ■ smooth muscle

Vascular smooth muscle cell (SMC) differentiation is a very important process during vasculogenesis and angiogenesis, and it is well recognized that alterations in SMC phenotype play a role in the progression of several prominent cardiovascular disease states including atherosclerosis, hypertension, and restenosis (see1 for review). The transcription mechanisms that direct SMC lineage determination are not completely clear and are complicated by the lack of terminal differentiation in this cell-type and the fact that SMCs derive from multiple locations including local mesoderm, cardiac neural crest, the proepicardial organ, somites, gut mesothelium, and possibly circulating stem cells (see2 for review).

We and others have shown that nearly all of the SMC differentiation marker genes are regulated by serum response factor (SRF), a ubiquitously expressed transcription factor that binds to conserved CArG boxes found in their promoters.3–8 The discovery of the cardiac and SMC-selective SRF cofactor, myocardin, was an extremely important advance because myocardin was shown to powerfully upregulate SMC-specific promoter activity and to be required for SMC differentiation in vivo.9–11 Interestingly, 2 Myocardin-Related Transcription Factors (MRTF-A and MRTF-B) were also described that have similar activities to myocardin.12,13 Although these factors are more widely expressed, recent evidence from various in vitro and in vivo model systems suggests that the MRTFs play important roles in regulating SMC differentiation marker gene expression in at least some SMC-types.14–17 and may be critical for the control SMC-specific gene expression in response to environmental cues.18–20

Given the absence of SMC differentiation marker gene expression in many tissues that express high levels of myocardin or the MRTFs (especially the adult heart), it is clear that additional mechanisms are critical for the overall pattern of SMC-specific gene expression observed in vivo. One mechanism that is likely to be important is modification of chromatin structure by posttranslational modification of histones (see21,22 for reviews). Several lines of evidence suggest that SRF-dependent SMC-specific gene expression is coregulated by histone acetylation. First, recruitment of SRF to the CArG-containing regions of the SMC-specific promoters correlates directly with H3 and H4 acetylation at the SMC-specific promoter regions.23–28 Second, expression of...
myocardin has been shown to increase histone acetylation of the SMC-specific promoters most likely through a direct interaction with the histone acetyl transferase (HAT), p300.25,27 These findings have particular importance because they suggest that myocardin may help bring about the changes in chromatin structure that are required for SMC differentiation marker gene expression. Interestingly, SRF itself has also been shown to physically associate with p300.28 Finally, histone deacetylases (HDACs) have been shown to regulate SRF-dependent skeletal and cardiac muscle-specific gene expression during development and under hypertrophic conditions (see29 and30 for reviews). Although this paradigm has not been as well developed in SMCs, Qiu et al demonstrated that overexpression of several different HDACs decreased SM22 promoter activity, whereas inhibition of HDAC activity with trichostatin A increased endogenous SM22 expression in SMCs.23

Histones lysines can also be mono-, di-, or trimethylated by several families of histone methyltransferases (HMTs) (see31,32 for reviews). Unlike acetylation, methylation exerts both positive and negative effects on gene expression through the recruitment of additional transacting factors. One of the most well-characterized histone methylation events occurs on histone 3 at lysine 9 (H3K9). The trimethylated form of H3K9 is strongly associated with transcriptional silencing and heterochromatinization, and these effects are thought to be attributable, at least in part, to recruitment of heterochromatin protein-1 (HP-1).33–35 H3K9 can also be acetylated suggesting that it plays a critical role in epigenetic regulation of gene expression. Because no specific histone demethylases had been described, it was originally thought that histone lysine methylation was a fairly permanent modification that could maintain expression patterns for extended periods. However, very recent studies have identified the jumonji family of proteins as histone demethylases that catalyze the removal of specific methyl groups from specific histone lysines.32–35 These findings were extremely important because they were the first to demonstrate that histone methylation, like histone acetylation, was reversible, specifically regulated by a group of enzymes, and likely to play a dynamic role in the regulation of gene expression.

In a yeast 2-hybrid screen for proteins that modulate MRTF-A activity we identified the H3K9 histone demethylase, Jmjd1a/JHDM2a, as an MRTF-A interacting protein. The studies described herein demonstrate that Jmjd1a interacts with the myocardin factors and enhances SMC differentiation marker gene expression by demethylating H3K9 near the CArG-containing regions of the SMC-specific promoters.

Materials and Methods

Yeast 2-Hybrid Screen and GST Fusion Pull-Downs

The 2-hybrid screen was performed using the Matchmaker GAL4 system (Clontech). In brief, the N-terminus of MRTF-A (AA 1 to 260) was subcloned into the GAL4 binding domain vector, pGBK7, and used to screen a GAL4 activation domain cDNA library derived from E16.5 mouse embryos (Clontech). Yeast colonies that grew on 2-hybrid screen were performed using the Matchmaker GAL4 system (Clonetech). In brief, the N-terminus of MRTF-A (AA 1 to 260) was subcloned into the Gal4 binding domain vector, pGBKT7, and used to screen a GAL4 activation domain cDNA library derived from E16.5 mouse embryos (Clontech). Yeast colonies that grew on -galactosidase, and positive clones were PCR amplified and identified by direct sequencing. To facilitate further analysis of Jmjd1a, a full-length clone was obtained form the IMAGE consortium and subcloned into a flag-tagged pcDNA3 expression vector (Invitrogen). GST pull down assays of proteins expressed in Cos cells or in vitro (TriT kit, Promega) were performed as previously described.36

Cell Culture, Transient Transfections, and Reporter Assays

Multipotent 10T1/2 cells and SMCs from rat thoracic aorta were cultured as described previously.39,40 For transfection of promoter-luciferase and expression constructs, cells were maintained in 10% serum media and transfected 24 hours after plating at 70% to 80% confluence using LT-1 transfection reagent (Mirus) per protocol. Luciferase activity was measured 24 hours posttransfection and is expressed relative to plus empty expression vector. In some experiments, cells were serum-starved overnight and then treated with TGF-β (1 ng/mL) for 24 hours. Luciferase results are presented as means and standard errors from at least 3 independent transfections. Statistical comparisons between groups were made using the 2-tailed Student t test with statistical significance accepted at P<0.05. We did not cotransfect a viral promoter/Lac Z construct as a control for transfection efficiency because such constructs exhibit unknown and variable squelching effects on the SM-specific promoters presumably attributable to competition for common transcription factors.38 Moreover, transfection efficiency between independent experimental samples is routinely very small (<10%). For harvesting of cell protein, cells were lysed in RIP4A buffer plus inhibitors 48 hours posttransfection as previously described.37

Generation of Jmjd1a Knockdown Cells

The MSCVneo-Jmjd1a siRNA was kindly provided by Yi Zhang (University of North Carolina, Chapel Hill) and has been previously described.44 The 293GPG packaging cell-line was kindly provided by Richard Mulligan, and these cells were cultured as previously described.40 293GPG cells were transfected with MSCVneo-Jmjd1a using Trans-IT LT1 (Mirus) according to the manufacturer’s protocol. Virus was harvested by collecting supernatants on days 3, 4, 6, and 7. After centrifugation, virus was resuspended in 250 μL of 50 mmol/L Tris pH 7.8, 130 mmol/L NaCl, 1 mmol/L EDTA. 10T1/2, and rat aortic SMCs were transduced at 70% confluence in serum-free DMEM by adding 50 μL of virus containing 4 μg/mL polybrene. After incubation for 4 hours, FBS was added to a concentration of 5%. Cells were split into a 150-mm dish at 24 hours posttransduction and selected in 600 μg/mL Geneticin (Invitrogen) for 5 days before selection. Used cells were selected for promoter-luciferase assays, Western Blots, and ChIP analysis. The antibody for Jmjd1a was kindly provided by Yi Zhang (University of North Carolina, Chapel Hill, NC). The antibody for SM MHC has been previously described40 and was used at 1:10 000.

Semi-Quantitative PCR

RNA was prepared from tissues and cell lines using Trizol Reagent (Invitrogen) per manufacturer’s protocol, and RNA was quantified by Ribogreen Assay (Molecular Probes). cDNA was generated using 1 μg of RNA in the iScript cDNA synthesis kit (Biorad) per manufacturer’s protocol. Exon spanning primers were designed and used to amplify PCR product was visualized by running on 1% agarose.

ChIP Assays

ChIP assays were performed according to the abcam X-ChIP protocol, with the following modifications. Cells were grown to 90% confluence in 150-mm dishes. After crosslinking, cells were lysed in 10 mmol/L HEPES pH 7.9, 0.5% NP-40, 1.5 mmol/L MgCl2, 10 mmol/L KCl, 0.5 mmol/L DTT on ice for 10 minutes. Nuclei were pelleted at 4000 RPM for 5 minutes, and were resuspended in 1% SDS, 10 mmol/L EDTA, 50 mmol/L Tris-Cl pH 8.0. Chromatin was sheared into 500 bp to 1000 bp fragments by sonication. Twenty micrograms of total chromatin was used for immunoprecipitation of histones and 200 μg for immunoprecipitation of transcription fac-
tors. Two micrograms of the following antibodies were used for IPs: anti-dimethyl H3K9 (Abcam), anti-SRF (Santa Cruz), and anti-flag (Invitrogen). Protein A Sepharose (Sigma) was preabsorbed for 30 minutes with 1.5 μg herring sperm DNA (Clontech) before immunoprecipitation overnight at 4°C. Precipitants were washed according to the X-ChIP protocol, with the addition of 1 LiCl buffer wash (250 mmol/L LiCl, 1% NP-40, 1% Na deoxycholate, 1 mmol/L EDTA, 10 mmol/L Tris-Cl pH 8.0). ChIP PCRs were performed using Red Taq ReadyMix (Sigma) using primers that were described previously.25

Immunohistochemistry
For immunohistochemical visualization of H3K9 di-methylation, cells fixed in 3.7% paraformaldehyde were permeabilized in 0.5% Triton X-100 for 3 minutes, blocked in 20% goat serum/3% BSA for 2 hours, then exposed to mouse anti-dimethyl H3K9 and rabbit anti-flag antibody (Sigma) for 2 hours at a dilution of 1:500. Texas Red and FITC-conjugated secondary antibodies were used at 1:1000.

Results
Identification of Jmjd1a as an MRTF-A Binding Partner
To identify proteins that regulate MRTF-A activity, we performed a yeast 2-hybrid screen using the N-terminal region of MRTF-A as bait. This region contains the RPEL motifs that mediate MRTF-A cytoplasmic trapping as well as the 2 basic domains that are important for nuclear import and SRF binding. Two clones were isolated from a mouse E16.5 cDNA library that coded for the jumonjiC domain of the jumonji protein, Jmjd1a. Secondary screens were performed to rule out potential false-positives because of an interaction of Jmjd1a with the Gal4 DNA binding domain, direct activation of yeast selection markers by the Jmjd1a/Gal4 activation domain fusion protein, or spontaneous yeast mutations that were somehow permissive to colony growth in the absence of a specific protein-protein interaction (data not shown).

To confirm the MRTF-A–Jmjd1a interaction, we used a GST–MRTF-A fusion protein (AA 1 to 260) to pulldown full-length flag-tagged Jmjd1a from Cos cell lysates (top). The Jmjd1a deletions shown were expressed and 35S labeled in vitro and were precipitated with GST–MRTF-A (bottom). A GST fusion protein containing the Jmjd1a jumonjiC domain was used to precipitate full-length flag-tagged myocardin factors expressed in vitro. C, Semiquantitative RT-PCR was used to measure Jmjd1a mRNA levels in various adult mouse tissues and cell lines. Band intensities were quantified using imageJ software and relative Jmjd1a expression is depicted in the bottom panel.

Although Jmjd1a was originally described as a testis specific gene expressed in rat male germ cells, a recent Northern analysis in Swiss Webster mice demonstrated a more ubiquitous expression pattern. To better examine Jmjd1a expression in SMCs and in SM-containing tissues, we performed semi-quantitative RT-PCR. As shown in Figure 1c, Jmjd1a expression in adult C57/Bl6 mice was very high in...
testis, relatively strong in aorta, brain, and lung, and less strong in bladder, esophagus, intestine, kidney, and stomach. Jmjd1a was also expressed relatively strongly in primary aortic SMCs, the A7r5 SMC line, and multipotential 10T1/2 cells, but not in Cos-7 epithelial cells.

**Jmjd1a Stimulated CArG-Dependent Transcription**

To test the effects of Jmjd1a on transcription, we transfected Jmjd1a and several different promoter luciferase constructs into multipotential 10T1/2 cells. As shown in Figure 2A, Jmjd1a stimulated the activities of the SMC-specific promoters, SM α-actin and SM22, by 3.3- and 4.4-fold, respectively, and the SRF-dependent c-fos promoter by 2.7-fold. Jmjd1a had only minimal effects on thymidine kinase promoters or on luciferase constructs driven by CRE, NFκB, or AP1 response elements. Cotransfection experiments demonstrated that Jmjd1a also strongly and synergistically enhanced MRTF-A–dependent transactivation of the SM α-actin and SM22 promoters (Figure 2B). In agreement with our previous studies, the effects of MRTF-A on c-fos promoter activity were much less dramatic, and coexpression of Jmjd1a enhanced these effects only modestly. Because Jmjd1a associates with myocardin and MRTF-B, we also tested its effects on SM22 transactivation by full-length myocardin (and myocardin 128 to 935, data not shown) but this effect was less robust than that seen with MRTF-A (Figure 2C). The relatively low level of transactivation stimulated by MRTF-B was not significantly affected.

**Jmjd1a Associated With the SMC-Specific Promoters**

The results presented so far demonstrate that Jmjd1a interacts with the myocardin factors and activates the transcription of the SMC-specific differentiation marker genes. Based on the recent demonstration that Jmjd1a specifically demethylates H3K9, we hypothesized that recruitment of Jmjd1a to the SMC differentiation marker gene promoters by the myocardin factors would promote local chromatin remodeling to activate SMC-specific gene expression. To begin to test this, we used chromatin immunoprecipitation assays (ChIP) assays to determine whether Jmjd1a associates with the SMC differentiation marker gene promoters. If Jmjd1a is present at the CArG-containing regions of the endogenous SMC-specific promoters on fixation, then the DNA fragment containing that promoter region will be present after immunoprecipitation and will be detected by PCR. Importantly, this assay has already been used extensively to monitor the association of SRF with the SMC-specific genes. As shown in Figure 3A, Jmjd1a and MRTF-A associated with the CArG-containing regions of both the SM α-actin and SM MHC promoters. In addition, using an Ab specific for dimethyl H3K9, we used immunohistochemistry to demonstrate that H3K9 dimethylation was dramatically reduced in virtually all 10T1/2 cells overexpressing Jmjd1a (Figure 3B). It is also important to note that there were no observable differences in the morphology of Jmjd1a expressing cells suggesting that Jmjd1a overexpression was not overtly toxic. Taken together, these results suggest that the effects of Jmjd1a on SMC-specific transcription were likely attributable to decreased H3K9 methylation near the CArG-containing regions of the SMC-specific promoters.

**Knockdown of Jmjd1a Inhibited SMC Differentiation Marker Gene Activation by Increasing H3K9 Methylation**

Virtually nothing is known about the role of H3K9 methylation in the regulation of the SMC differentiation marker
gene expression. Thus, we performed chromatin immunoprecipitation assays for dimethyl H3K9 in a 10T1/2 cell model of SMC differentiation in which multiple SMC differentiation marker genes are upregulated by treatment with TGF-β.\(^{20,43,44}\) As shown in the left panel of Figure 4B, measurable levels of dimethyl H3K9 were detected at the CArG-containing regions of the SM α-actin, SM22, and SM MHC promoters in serum-starved 10T1/2 cells. Importantly, treatment of cells with TGF-β resulted in a significant reduction in dimethyl H3K9 levels at each of the SMC-specific promoters (Figure 4B, compare lanes 2 and 4) suggesting that demethylation of H3K9 near the SMC differentiation marker gene promoters contributes to the activation of these genes. TGF-β had no effect on H3K9 dimethylation at the non–CArG-containing region of the proximal GAPDH promoter.

To determine whether Jmjd1a was important for the effects of TGF-β on H3K9 demethylation at the SMC differentiation marker gene promoters, we used retroviral siRNA strategy previously described by Yamane et al\(^{44}\) that resulted in an approximately 70% reduction in Jmjd1a expression in 10T1/2 cells (Figure 4A). Importantly, knockdown of Jmjd1a resulted in an increase in the level of dimethyl H3K9 at all of the SMC-specific promoters under control conditions (Figure 4B, compare lanes 2 and 6). Furthermore, knockdown of Jmjd1a completely prevented the reduction in H3K9 dimethylation that was associated with TGF-β treatment (compare lanes 2 versus 4 with lanes 6 versus 8). To better quantify these results, 3 independent experiments were analyzed using imageJ software. Importantly, the summary graph shown in Figure 4C confirms that TGF-β treatment significantly decreased H3K9 dimethylation at all of the SMC-specific promoters while knockdown of Jmjd1a significantly prevented this effect.

We next examined the requirement of Jmjd1a for SMC-specific gene expression in 10T1/2 cells and primary rat aortic SMCs. As shown in Figure 5A, we found that MRTF-A-dependent transactivation of the SM22 promoter was inhibited by 50% after knockdown of Jmjd1a in 10T1/2 cells. In addition, Western analysis demonstrated that knockdown of Jmjd1a also completely prevented the activation of endogenous SM myosin heavy chain expression by TGF-β and 10% serum. Importantly, knockdown of Jmjd1a in primary rat aortic SMCs also prevented TGF-β-induced upregulation of endogenous SM MHCs (Figure 5B). Collectively, these data indicate that Jmjd1a is required for the activation of SMC differentiation marker gene expression in these model systems. Importantly, TGF-β did not significantly upregulate Jmjd1a protein expression in SMCs (data not shown), indicating that TGF-β–dependent signaling mechanisms are important for the effects of Jmjd1a on SMC-specific transcription.

**Discussion**

Condensation of DNA into higher order chromatin is essential for normal cell function but inhibits gene expression by preventing transcription factor access to regulatory cis ele-
ments. Although the role of histone acetylation in the regulation of SMC-specific transcription is starting to become clear, we know very little about the specific molecular mechanisms involved and even less about the role of other histone modifications in this process. Our examination of the effects of Jmjd1a on myocardin factor activity suggest, for the first time, that dynamic regulation of H3K9 methylation regulates SMC-specific gene expression. Several lines of evidence from the current studies indicate that Jmjd1a interacts with 1 or more of the myocardin factors including a yeast-2 hybrid interaction, reciprocal GST pull down assays, the presence of Jmjd1a at the SMC differentiation marker gene promoters, and synergistic functional interactions between Jmjd1a and MRTF-A and myocardin. We also demonstrated that H3K9 demethylation at the CArG-containing regions of the SMC-specific promoters correlates with TGF-β-induced activation of SMC-specific transcription in 10T1/2 cells. Importantly, knockdown of Jmjd1a in both 10T1/2 and primary SMCs inhibited TGF-β-induced upregulation of endogenous SMC differentiation marker gene expression, and these effects were concomitant with increased H3K9 dimethylation at the SMC-specific promoters. Given our demonstration that Jmjd1a is expressed in SMCs, we postulate that Jmjd1a acts as a myocardin factor coregulator and enhances SMC differentiation marker gene expression by demethylating H3K9.

The recent characterization of the jumonji family of histone demethylases was a major advance in our understanding of control of chromatin structure and provides support for the dynamic regulation of gene expression by histone methylation (see for review). By coupling a biochemical assay for measuring formaldehyde (a product of the demethylation reaction) with classical chromatography, Tsukada et al identified FBX11 as an H3K36-specific demethylase and isolated this demethylase activity to the conserved jumonjiC domain of FBX11. Using a similar approach, this same group identified Jmjd1a as a histone demethylase that was specific for the mono and dimethylated forms of H3K9. These authors went on to demonstrate that Jmjd1a interacted with the androgen receptor and that knockdown of Jmjd1a in a prostate cancer cell line decreased expression of several androgen-dependent genes.

In the current studies, Jmjd1a selectively activated the SMC-specific promoters and this specificity was probably mediated by its interaction with myocardin and the MRTFs. Jmjd1a also activated the CArG-containing c-fos promoter. However, because previous studies have demonstrated that c-fos expression is regulated, to at least some extent, by the MRTFs, these effects were likely mediated by an interaction between Jmjd1a and MRTF-A at the c-fos promoter. It is also important to note that we know very little about basal levels of H3K9 methylation and that the lack of an effect of Jmjd1a overexpression on a particular promoter could also reflect low basal levels of H3K9 methylation. Given the general importance of histone methylation in the regulation of gene expression, it is likely that future studies will identify additional Jmjd1a targets, and it will be critical to identify the mechanisms that regulate Jmjd1a recruitment to specific genes.

Approximately 30 jumonjiC-containing proteins are present in the mouse and human genomes, and it is thought that differences in jumonjiC domain homology, perhaps in combination with differences in other domains, determine substrate lysine specificity (ie, H3K9 versus H3K36) as well as substrate methylation state specificity (ie, mono-, di-, or trimethylated H3K9). For example, members of the Jmjd2 subfamily (Jmjd2 a-d) were shown to have histone demethylase activity that is specific for the trimethylated forms of H3K9 and H3K36, and the recently reported crystal structure of Jmjd2a bound to methylated H3K9 and H3K36 supports the idea that structural features determine this specificity. Two Jmjd1a-related factors (Jmjd1 b and c) have been identified that are homologous to Jmjd1a mainly in...
their jumonjiC domains. We are currently trying to determine whether these demethylases also modulate myocardin-factor-dependent gene expression.

Jmjd1 family members, although relatively large (>150 Kd), contain only 1 other recognized domain, a zinc finger in the central portion of the molecule. While deletion of this motif inhibited the demethylase activity of Jmjd1a,34 the molecular mechanisms for this requirement have not been fully addressed. Our deletion analyses indicated that regions outside of the jumonjiC domain were also required for the effect of Jmjd1a on myocardin factor transactivation (Lockman and Mack, unpublished observations, 2007). Interestingly, Knebel et al identified Jmjd1a as a binding partner of the ETS protein, ER71.42 These authors demonstrated that several Jmjd1a domains contained repressor activity and that Jmjd1a inhibited ER71-mediated activation of the MMP-1 promoter. Thus, taken together, the effects of Jmjd1a on gene expression are probably modulated by interactions with additional positive or negative transacting factors and may involve demethylase-dependent and independent functions. Furthermore, our studies also indicate that such interactions regulate the gene specificity of Jmjd1a.

Another implication of our studies is that H3K9 methylation may have a previously unrecognized role in the regulation of SMC-specific transcription. Trimethylated H3K9 is known to associate with HP-1 through the conserved chromodomain of HP-1.52,53 By mechanisms that are still not completely understood, HP-1 mediates the conversion of euchromatin into heterochromatin resulting in prolonged gene inactivation. Whereas Jmjd1a is thought to demethylate only the mono- and dimethyl forms of H3K9,34 it is likely that the presence of Jmjd1a at the SMC-specific promoters would ultimately decrease trimethylated H3K9 by reducing the availability of the dimethyl substrate. Interestingly, the mono- and dimethylated forms of H3K9 have been associated with silent domains within euchromatin54,55 suggesting that Jmjd1a could have positive effects on transcription that are independent of heterochromatinization. Future studies on trimethylation of H3K9 and HP-1 association at the SMC-specific promoters coupled with a more detailed ChIP analysis of transcription factor binding in these regions will be required to distinguish between these possibilities. In addition, because acetylated H3K9 has been associated with active SMC-specific promoters,29 it will also be critical to determine whether the presence of Jmjd1a increases this open chromatin mark.

A critical but difficult question that remains is whether chromatin-modifying enzymes such as Jmjd1a help to maintain the SMC-specific genes in an open chromatin state in SMCs (thus allowing transcription factors access) or whether the chromatin modifiers are recruited to the SMC-specific gene promoters subsequent to the binding of SMC-specific selective factors. A recent study by MacDonald et al has demonstrated that active SMC-specific promoter regions were associated with methylation of H3K4 and H3K79.25 These authors demonstrated that methylated H3K4 (but not unmethylated H3K4) interacted with the myocardin/SRF complex indicating that this histone modification may help recruit critical transcription factors to the SMC differentiation marker gene promoters. We are currently trying to address these questions by performing ChIP assays for the myocardin factors and SRF in Jmjd1a knockdown cells. If binding at the SMC-specific promoters is attenuated in Jmjd1a-deficient cells, this would suggest that Jmjd1a loosens chromatin structure to allow complex formation. It will also be interesting to analyze H3K9 methylation at the few SMC selective/promoter proteins that have been shown to be non-CArG dependent such as FRNK and ACLP.56–58

H3K9 methylation is catalyzed by several HMTs of the SET family (Suv(var)3 to 9, Enhancer of Zeste, Trithorax) including G9a, G9a-related protein, ESET, Suv39h1, and Suv39h2 (see45 for review). Based on studies of G9a-deficient mice, MEFs and ES cells, this HMT is likely responsible for mono- and dimethylation of H3K9 whereas Suv39h1 and Suv39h2 (see45 for review). Based on studies of G9a-deficient mice, MEFs and ES cells, this HMT is likely responsible for mono- and dimethylation of H3K9 whereas Suv39h1 and Suv39h2, and ESET are thought to direct H3K9 trimethylation.52,60 The current results support a closer examination of the expression and activity of the various SET proteins in SMCs.

In summary, the present studies indicate that Jmjd1a acts as a coregulator of MRTF-A– and myocardin-dependent tran-
scription and that the dynamic regulation of H3K9 methylation may be a novel mechanism in the regulation of SMC-specific gene expression. As presented in the model in Figure 6, recruitment of Jmjd1a to the CArG-containing regions of the SMC-specific gene promoters leads to demethylation of H3K9. This histone modification, most likely in combination with a variety of others, loosens chromatin structure facilitating SMC differentiation marker gene expression. Further studies will address the mechanisms that regulate H3K9 methylation in SMCs and whether this pathway plays an important role in the differentiation of SMCs in developing vessels or in the modulation of SMC phenotype that occurs after vascular injury.

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Disclosures

None.

References


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