Forgetting to Switch Off SMAD2 in Aneurysmal Disease

Amy Leung, Rama Natarajan

Vascular smooth muscle cells (VSMCs) are key component cells of the vascular wall that are critical for contractility of the aorta.1 They respond to biochemical and mechanical signals and play an important role in the regulation of blood pressure. Dysregulation of VSMCs is a hallmark of cardiovascular diseases such as restenosis, hypertension, aneurysms, and atherosclerosis. Investigating the signaling pathways in both normal and dysregulated VSMCs is important for understanding how these cardiovascular diseases develop and for the development of more effective therapies for these potentially life-threatening disorders.

**Article, see p 881**

Aneurysms are dangerous vascular diseases characterized by aortic dilatation with thinning of the medial VSMC layer. Several mechanisms have been implicated in the development of human aortic aneurysms, including the role of transforming growth factor-β1 (TGF-β1), although there are some controversies in the field.2,4 Evidence shows that VSMCs from thoracic aortic aneurysms (TAA) exhibit enhanced activity of SMAD2, a transcription factor that is part of the canonical TGF-β1 pathway and a key effector of the actions of TGF-β1.7 TGF-β1 signaling results in the phosphorylation and translocation of cytoplasmic SMAD2 into the nucleus and consequent transcriptional activation of its target genes.8,9 However, the activation of SMAD2 in TAA has previously been shown to be dissociated from the TGF-β1 pathway. Instead, the SMAD2 hyperactivity is suggested to be attributed to the constitutive overexpression of SMAD2 at the transcriptional level.10 Furthermore, SMAD2 overexpression was associated with key chromatin histone modifications at the SMAD2 promoter, suggesting an element of epigenetic control.10

In the nucleus of mammalian cells, chromosomal DNA is tightly packaged into chromatin, a higher-order structure comprised of subunits called nucleosomes. Each nucleosome consists of an octamer of histones wrapped around DNA. Chromatin-modifying enzymes, such as histone acetyltransferases, can be recruited to specific chromatin sites to modify histone tails. These so-called epigenetic modifications, including DNA methylation and histone post-translational modifications, can affect the dynamics of DNA–nucleosome interactions and gene expression.11 For example, acetylation of histone tails has been shown to influence DNA–nucleosome contacts, allowing for more accessibility of the DNA for proteins such as transcription factors and the transcriptional machinery.12 More than 30 years ago, Weintaub et al13 suggested that post translational modifications of chromatin could be heritable, and more recent investigations by many groups have shown that chromatin states can be maintained from mother to daughter cells, although the exact mechanism remains to be elucidated.14

In this issue of *Circulation Research*, Gomez et al15 investigated the molecular mechanism associated with the constitutive transcription of SMAD2 in human TAA VSMCs. They had previously observed that TAA VSMCs retain constitutive transcription of SMAD2 even after 3 passages in vitro, suggesting a heritable, cell-autonomous mechanism of cellular memory.10 Interestingly, histones of the SMAD2 promoter in TAA VSMCs are hyperacetylated compared with controls.10 The hyperacetylation of histones at the SMAD2 promoter is dependent on p300/CREB-associated factor (PCAF) and p300, 2 histone acetyltransferases that are specifically recruited to the SMAD2 locus. With histone acetyltransferase inhibitors of p300 and PCAF, Gomez et al found that SMAD2 transcription is attenuated, suggesting that histone acetylation is an important step for transcription of SMAD2. Furthermore, they observed a decrease in Myc binding and an increase in p53 binding at the SMAD2 promoter of TAA VSMCs compared with controls. The results demonstrated a switch in recruitment between Myc and p53 at the SMAD2 promoter, with Myc acting as a transcriptional repressor and p53 functioning as a transcriptional activator. Overall, these observations lead to a model in which loss of Myc binding, increased p300/PCAF-dependent histone acetylation, and p53 activation are necessary components for the sustained transcription of SMAD2 in TAA VSMCs (Figure).

The findings of Gomez et al are interesting because they discovered that in TAA VSMCs, p53 binding and histone acetylation at the SMAD2 promoter drive constitutive SMAD2 transcription. Also, these data suggest that p53 binding and histone acetylation are key components to the heritability of the phenotype in the in vitro cultured TAA VSMCs described in their previous work. Building on their findings, further work to elucidate the molecular mechanism(s) that allows TAA VSMCs in culture to remember their previous states in vivo is an interesting avenue of research. Are p53 binding and histone acetylation maintained through mitosis or reestablished after mitosis is complete in daughter cells?

In recent years, there has been increasing evidence that transcription factors, once thought to be completely excluded from mitotic chromosomes, can be at least partially bound to mitotic chromosomes, leading to the phenomenon termed mitotic bookmarking.16 For example, GATA1, a zinc finger protein that is important for erythroid development, has been
shown to bind to specific sites on the mitotic chromosomes that allow for the rapid recruitment of its coactivators and reactivation of target genes after mitosis. It has also been shown that mixed-lineage leukemia gene (MLL), a metazoan transcription factor that has H3K4 methyltransferase activity, also binds to mitotic chromosomes and allows for the fast reactivation of target genes. It is not clear whether the methyltransferase activity of MLL plays a role in the fast reactivation because local H3K4 methylation does not depend on MLL. Together, these studies indicate that transcription factors, including chromatin modifiers, may contribute to persistent transcriptional changes that are inherited through mitosis. To date, there is no evidence to conclude whether p300, PCAF, and p53 bind to mitotic chromosomes. These studies, especially at the SMAD2 promoter in TAA VSMCs, would certainly be worth exploring.

How TAA VSMCs initially establish histone acetylation or p53 binding at the SMAD2 promoter is another avenue of interest. Is there an intrinsic propensity such as genetic predisposition for these human aneurysmal VSMCs to constitutively transcribe SMAD2, or is the overexpression the result of an environmental signal that is maintained in the cells? Of note, Gomez et al observed that the global level of p300/PCAF-dependent histone acetylation is increased in TAA VSMCs. This suggests that there may be additional loci that are also dysregulated in addition to the promoter of SMAD2. Epigenome-wide analysis to determine differentially histone acetylated regions of the genome in TAA VSMCs compared with controls would be valuable to determine other disregulated loci in addition to SMAD2. It may also lead to the identification of SMAD target genes that may contribute to the phenotype of the aneurysms. Unlike genetic changes, the reversibility of epigenetic changes presents an additional window of opportunity for therapeutic intervention alone or in combination with traditional therapies. The observation that histone acetyltransferase inhibitors can reduce the expression of SMAD2 suggests that specific p300/PCAF inhibitors might be effective in the clinical setting for TAAs, which are not always easy to diagnose and treat.

The study presented by Gomez et al also highlights the importance of chromatin dynamics in modulating VSMC gene expression, which is associated with physiological and pathological states of the vessel wall and progression of vascular diseases such as aneurysms. It provides additional evidence of a role for epigenetic mechanisms to facilitate cellular memory. Interestingly, cellular memory is not limited to human TAA VSMCs. In another report, it was shown that, relative to those from nondiabetic mice, VSMCs isolated from the aorta of diabetic mice displayed increased migration, adhesion, and inflammatory gene expression even after several passages in vitro, and this was associated with decreased expression and promoter occupancy of the repressive histone methyltransferase Suv39h1. Clearly, both human and mouse VSMCs may remember their past disease states, and for human VSMCs, forgetting to switch off SMAD2 can lead to aneurysmal diseases.

Disclosures
This study was supported by grants to R. Natarajan from the National Institutes of Health (R01 HL106089, R01 HL087864, and R01 DK 065073) and the Juvenile Diabetes Research Foundation, and by National Institutes of Health T32 fellowship (T32 DK007571-24) to A. Leung.

References

Key Words: Editorials • aneurysm • epigenomics • Smad2 protein • vascular smooth muscle cell
Forgetting to Switch Off *SMAD2* in Aneurysmal Disease
Amy Leung and Rama Natarajan

*Circ Res*. 2013;113:843-845
doi: 10.1161/CIRCRESAHA.113.302138

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/113/7/843

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation Research* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation Research* is online at:
http://circres.ahajournals.org/subscriptions/