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Forgetting to switch off *SMAD2* in aneurysmal disease

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Keywords

VSMC; aneurysm; Smad2; epigenetics

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 (CVDs) such as restenosis, hypertension, aneurysms and atherosclerosis. Investigating the signaling pathways in both normal and dysregulated VSMCs is important for understanding how these CVDs develop and for the development of more effective therapies for these potentially life threatening disorders.

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[2-6]. 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 due 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 made up of arrays of subunits called nucleosomes. Each nucleosome consists of an octamer of histones wrapped around DNA. Chromatin modifying enzymes such as histone acetyltransferases (HATs) 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]. Over thirty years

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ago, Weintraub et al suggested that post-translational modifications of chromatin could be heritable[13] and more recent investigations by many groups have shown that chromatin states can be maintained from mother to daughter cells, though the exact mechanism remains to be elucidated[14].

In this issue of *Circulation Research*, Gomez et al investigated the molecular mechanism associated with the constitutive transcription of *SMAD2* in human TAA VSMCs[15]. They had previously observed that TAA VSMCs retain constitutive transcription of *SMAD2* even after three 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 to controls[10]. The hyperacetylation of histones at the *SMAD2* promoter is dependent on PCAF and p300, two HATs that are specifically recruited to the *SMAD2* locus. With HAT inhibitors of p300 and PCAF, Gomez et al found that *SMAD2* transcription is attenuated suggesting that the 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 to 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 1**).

The findings of Gomez et al are very interesting because they discover 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 that is described in their earlier work. Building upon 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 co-activators and reactivation of target genes following mitosis[17]. It has also been shown that MLL, a metazoan transcription factor which has H3K4 methyltransferase activity, also binds to mitotic chromosomes and allows for the fast reactivation of target genes[18]. It is not clear whether the methyltransferase activity of MLL plays a role in the fast reactivation since 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 or not 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 to controls would be valuable to determine other dysregulated loci in addition to *SMAD2*. It may also lead to the identification of SMAD target genes that may contribute the phenotype of the aneurysms. Since, 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 HAT 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 highlight 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 non-diabetic 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 methyltranferase, Suv39h1[19]. 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.

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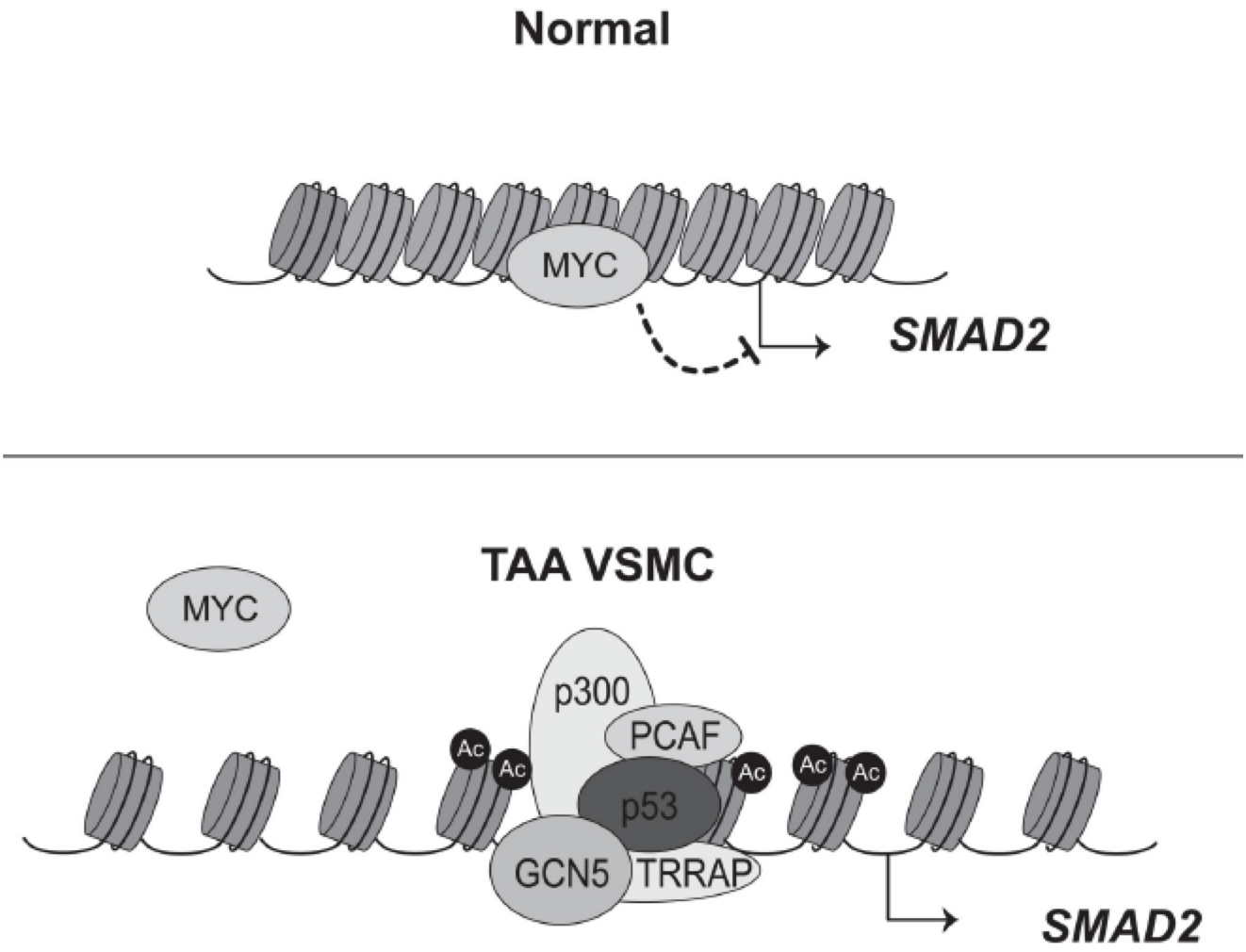

Figure 1.

Vascular smooth muscle cells (VSMCs) from thoracic aortic aneurysm (TAA) switch from a Myc-dependent repression to a p53- and histone acetylation-dependent activation of the *SMAD2* promoter. *Top:* In normal VSMCs, Myc binding prevents transcription of the *SMAD2* locus and the nearby chromatin is quite compact. *Bottom:* In TAA VSMCs, Myc is displaced and p53 binding and histone acetylation of the promoter driven by p300 and PCAF, two histone acetyltransferases, leads to chromatin relaxation and activates transcription of *SMAD2*. Nucleosomes are schematically represented as cyclinders wrapped with DNA.