Drugs Targeting Epigenetic Histone Acetylation in Vascular Smooth Muscle Cells for Restenosis and Atherosclerosis

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It is well known that atherosclerosis and restenosis are common cardiovascular diseases and major health care problems. Vascular remodeling and migration and proliferation of vascular smooth muscle cells (VSMCs) are key features of these pathologies. There has been enormous progress in drug development and clinical management of these disorders, with angioplasty and drug eluting stents being standard procedures to treat vascular obstruction. However, despite their benefits, these current treatment modalities are not always efficacious. Furthermore, they can also be associated with postoperative complications and graft failures, some of which can be life threatening. Evaluation of newer mechanisms involved in VSMC proliferation aimed at uncovering additional therapeutic approaches to curb VSMC dysfunction in cardiovascular diseases is clearly warranted.

Accumulating evidence suggests that several common diseases, including cardiovascular disorders, diabetes, and the vascular complications of diabetes, are governed by a combination of genetic and environmental factors and that epigenetic mechanisms, such as DNA methylation and histone modifications in chromatin, form a key link between them.1–3 Epigenetics is the added layer of gene regulation that occurs in chromatin without changes in the actual underlying DNA sequence and plays a major role in dictating cell-specific gene expression patterns and transcriptional outcomes.4,5 Along with DNA methylation, key posttranslational modifications of histone N-terminal tails can alter chromatin structure to form an added layer of gene regulation and modulate gene transcription.6 Therefore, gene transcription depends on chromatin structure, which is very dynamic, depending on a multitude of histone posttranslational modifications that allow for the conversion of inaccessible, compact, or repressive heterochromatin to the accessible or active euchromatin state of DNA. Posttranslational modifications that occur on histone tails include acetylation, methylation, phosphorylation, and ubiquitylation.5,6 Because these epigenetic mechanisms can regulate the expression of genes involved in VSMC proliferation, migration, inflammation, and matrix protein synthesis,7–15 they present an exciting window of opportunity for therapeutic intervention.

One of the best-studied chromatin histone posttranslational modifications is lysine acetylation mediated by histone acetyltransferases (HATs), generally associated with gene activation. Histone deacetylases (HDACs), on the other hand, mediate the removal of lysine acetylation.8,16 Although histone lysine acetylation enables a more relaxed or open chromatin structure, allowing for transcription factor and RNA polymerase II recruitment permissible for transcription, HDACs are found to be components of repressor complexes or to be involved in various signaling pathways.5,6 Overall, histone acetylation can occur quite rapidly and is amenable to modulation by HDAC inhibitors. This is demonstrated by the dynamic balance between HATs and HDACs in regulating or fine-tuning cellular gene expression. This property of HDACs has been greatly exploited in the field of cancer, where HDAC inhibitors have been successfully used to reactivate tumor suppressor genes and thereby inhibit the proliferation of cancer cells.8,17 This has triggered a flurry of research in other proliferative diseases, such as atherosclerosis and restenosis.8 It is therefore important to determine how HATs and HDACs regulate the transcription of VSMC genes related to proliferation, migration, and matrix deposition; which are the specific HDACs involved; and whether inhibitors of these HDACs can have protective effects. In this connection, there have been several reports demonstrating that key HATs and HDACs can regulate the expression of inflammatory and other genes involved in VSMC contractility, VSMC differentiation, migration, proliferation, inflammation, matrix deposition, and angiotsensin II effects (Figure).3,8–15 However, there is a clear paucity of in vivo data. Hence, evaluation of HDAC inhibitors in models of VSM injury is clearly needed.

In this issue of ATVB, Findeisen et al18 used a nonselective HDAC inhibitor, Scriptaid, to elegantly demonstrate its protective effects in vitro and in vivo in a mouse model of neointimal thickening. They demonstrate that mitogens induce the transcriptional upregulation of HDACs 1 to 3 in rat VSMCs. Knockdown of these 3 HDACs with short interfering RNAs or pharmacological inhibition of HDAC could prevent platelet-derived growth factor–induced VSMC proliferation. Additional mechanistic studies demonstrated that HDAC inhibition could lead to cell cycle arrest because of an inhibition of retinoblastoma protein phosphorylation, although the upregulation of the cyclin-dependent kinase inhibitors p21Cip1 and p27Kip was relatively modest compared with the profound inhibition of VSMC proliferation. Importantly, they demonstrate that mitogen-induced cyclin D1 expression was downregulated by HDAC inhibition despite

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an increase in cyclin D1 promoter histone lysine-9 acetylation. In vivo relevance was obtained by demonstrating that HDAC inhibitors decrease neointimal thickening and cyclin D1 expression in a mouse model. These data confirm that HDAC is a critical component of the transcriptional machinery controlling the expression of key genes regulating VSMC proliferation (Figure) and provide new insights into the cellular mechanisms involved. They also demonstrate the therapeutic efficacy of Scriptaid, an HDAC inhibitor that seems to work without toxicity at the doses used.

These studies are important for several reasons. Given that cyclin D1 overexpression is well known to promote VSMC proliferation, the observation that Scriptaid blocks its expression provides mechanistic information. Although HDAC inhibitors intuitively are useful in cancer to reactivate protective tumor suppressor genes, a parallel mechanism in VSMCs is not fully clear. Hence, the fact that HDAC inhibitors decreased the expression of genes such as cyclin D1 in this study is interesting, given that an increase in cellular acetylation by HDAC inhibition would be expected to increase gene expression in general. However, as is well discussed by the authors, it is now clear that HDACs have multiple cellular effects and also affect signal transduction parameters to interrupt the expression of specific genes. Moreover, HDACs exist in 2 different classes, with HDACs 1 to 3 and 8 belonging to class I and HDACs 4 to 7, 9, and 10 to class II, and they have subtle differences in cellular actions because of their interactions with specific chromatin factors and repressor molecules. The authors used short interfering RNAs to demonstrate that class I HDACs 1 to 3 may be mediating VSMC proliferation, albeit via as yet unknown mechanisms. However, they have not shown whether Scriptaid specifically blocks HDACs 1 to 3 only or other HDACs also, and they have not shown whether Scriptaid actions can be attributed to more pleiotrophic effects. It should be noted that HATs/HDACs also have nonnuclear substrates. For example, HDAC8 has been shown to associate with smooth muscle α-actin to regulate smooth muscle cell contractility, and HDAC6, in class II, is a tubulin deacetylase. HDACs can also deacetylate nonhistone proteins, such as p53 and nuclear factor-κB, and hence not all of their actions are at the level of chromatin. HDACs may also cooperate with other epigenetic chromatin factors such as histone methylases and demethylases. In addition, because HATs have been shown to enhance inflammatory gene expression in VSMCs, it is unclear whether HDAC inhibitors may augment inflammation in vivo. Nevertheless, the studies by Findeisen et al provide additional impetus to explore the effect of HDAC inhibitors, including isoform-specific ones, for cardiovascular diseases associated with VSMC proliferation and migration.
given the multiple functions of histone acetylation/deacetylation in VSMCs (Figure). Specific HDAC inhibitors are already showing promise in the treatment of malignant disorders. Unlike the DNA code, the epigenetic code is reversible and therefore amenable to therapeutic intervention. The hope is that such targeted epigenetic therapies can be used alone or in combination with other currently used drugs as more efficient treatment options for various cardiovascular diseases.

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References

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