1. Introduction

Arteriosclerosis is a progressive process characterized by the hardening and loss of elasticity of arteries, which plays a major part in the pathogenesis of cardiovascular diseases. Importantly, during the formation of arteriosclerosis, vascular cell homeostasis plays a significant role. Endothelial cells (ECs) and smooth muscle cells (SMCs) are the two most important types of vascular cells, the proliferation, migration, and apoptosis of which have been proved indispensable in atherosclerosis. Although the detailed mechanism of atherosclerosis is yet unclear, it is generally believed to be a multi-step inflammatory disease.1,2 The initial step is endothelial dysfunction involving EC proliferation, migration, and apoptosis, which leads to increased endothelial permeability to lipoproteins, increased leucocyte migration and adhesion, and decreased nitric oxide production, and results in fatty streak formation.3 The migration as well as proliferation of SMCs plays a critical role in the formation of a fatty streak. Then, as fatty streaks progress to advanced lesions, fibrous caps are formed.4 In most patients with myocardial infarction, the final step is thinning and rupture of fibrous caps, in which the apoptosis of SMCs is considered a possible cause.5 Also, other cell types such as macrophages have an indispensable function in this process.6 Recent reports have shown that stem cells possess a crucial role in the pathogenesis of atherosclerosis. For instance, endothelial progenitor cell-derived cells could be protective in terms of atherosclerosis.5,6 while stem cell-derived SMCs may contribute to atherosclerotic lesion formation.6

Epigenetic modifications can modulate gene expression without changing the DNA sequence, and thus provide rapid and reversible regulation of the repertoire of expressed genes.7 The epigenetic process includes the modification of DNA as well as histone proteins, a modification that results in epigenetic modulation of gene expression. Although originally shown to be involved in cancer and neurological disease, HDACs are also found to play crucial roles in atherosclerosis. This review summarizes the effects of HDACs and HDAC inhibitors on proliferation, migration, and apoptosis of endothelial and smooth muscle cells. In addition, an updated discussion of HDACs' recently discovered effects on stem cell differentiation and atherosclerosis is provided. Overall, HDACs appear to be promising therapeutic targets for the treatment of arteriosclerosis and other cardiovascular diseases.

2. Histone deacetylases

HATs hyperacetylate histones and result in an open structure of the DNA, which facilitate the binding of transcription factors and promote gene expression. HDACs on the other hand remove acetyl groups from hyperacetylated histones and counteract with HATs. Modification by HDACs leads to a closed chromatin structure.
and suppression of genes. HATs and HDACs are recruited to gene promoters by DNA binding proteins that recognize certain DNA sequences, and in this way provide specific modulation on gene expression.

There are 18 characterized members of HDACs in human, which can be grouped into four classes based on function and DNA sequence similarity (Table 1). The class I and class II HDACs are considered as the ‘classical’ HDACs, whose activities could be inhibited by trichostatin A. Class III HDACs represent the silent information regulator 2 (Sir2) family of nicotinamide adenine dinucleotide (NAD(+) dependent HDACs (SIRT1–7), which share structural and functional similarities with the yeast Sir2 protein. Borradaile and Picketing have summarized the role of class III HDACs in cardiovascular disease. For more information about class III HDACs, please refer to reviews. Finally, class IV HDAC is the newly discovered HDAC11. HDAC11 is most closely related to class I HDACs, but since the overall sequence similarities are low, it cannot be grouped into any of the three existing classes.

Class I HDACs (HDAC1, 2, 3, 8) are widely expressed, with HDAC1, 2, and 8 reside nearly exclusively in the nucleus. HDAC3 is found to shuttle between nucleus and cytoplasm. The members of this class are shown to be involved in cell proliferation and cell survival.

Class II HDACs (HDAC4, 5, 6, 7, 9, 10) can shuttle between the nucleus and cytoplasm. They can be further divided into subclasses IIa (HDAC4, 5, 7, 9) and IIb (HDAC6, 10). Class IIa HDACs distinguish themselves due to their extended N-terminal regulatory domain, whereas class IIb HDACs contain two catalytic domains.

Class II HDACs have been shown to possess limited HDAC activity comparing with class I HDACs, and they are found to form a complex with co-repressors such as SMRT (Silencing Mediator for Retinoid and Thyroid receptors) and N-CoR (Nuclear receptor co-Repressor), and with class I HDACs such as HDAC3.

Since it is generally believed that HDACs could suppress gene expression only when they are in the nucleus, modulation of cellular localization is considered to be critical in defining the functions of class II HDACs. McKinsey et al. reported that calcium/calmodulin-dependent protein kinase (CaMK) signalling plays a central role in regulating class II HDACs nuclear export. They found that CaMK could phosphorylate class II HDACs and promote their nuclear exportation, and thus provide de-repression of HDAC responsive

genes. de Ruijter et al. summarized that after phosphorylation by CaMK, class II HDACs are exported to the cytoplasm through the cellular export factor CRM-1. 14-3-3 proteins (a cytoplasmic anchor protein family) then bind to and retain phosphorylated class II HDACs in the cytoplasm.

Except their roles in gene repression via histone deacetylation, HDACs can also deacetylate non-histone substances. For example, HDAC3 is known to deacetylate the p65 subunit of NFκB, which promotes its association with IκBα, enhancing NFκB nuclear exportation. Moreover, transcription factors such as p53, E2F1, and STAT1 could also be directly deacetylated by HDACs, and thus provide extra-modulation of gene transcription. More information about non-histone substrate of HDACs could be found here. Such novel functions refine our knowledge of HDACs, and could provide new therapeutic target in cardiovascular medicine.

The interruption of HDACs during embryonic development is a powerful way to understand their functions. Olson and colleagues have contributed significantly to this field. Among all the studied HDACs, HDAC2, HDAC3, HDAC7, and HDAC9 are related to cardiovascular system: deficiency of HDAC2 attenuated cardiac hypertrophy in hearts exposed to hypertrophic stimuli. While HDAC5 or HDAC9 deficiency led to cardiac hypertrophy (cardiomyocytes). Disruption of the HDAC7 gene in mice resulted in embryonic lethality due to a failure in endothelial cell–cell adhesion and rupture of blood vessels. These findings indicate the importance of HDACs in cardiovascular diseases.

### 3. Histone deacetylase inhibitors

As HDACs have broad functions in the cell, various HDAC inhibitors are designed targeting on the catalytic sites of HDACs. According to the structural diversity of the inhibitors, they could be grouped into four classes, e.g. hydroxamic acids, short chain fatty acids, cyclic tetrapeptides, and benzamides. The potency of HDAC inhibitors can be assessed by assays using purified recombinant HDACs to test their inhibitory activities, or assays using transformed cells to test their anti-proliferative activities. Moreover, inhibition of HDACs could also lead to the proteasomal degradation of HDACs, which provides extra-regulation of HDAC functions.

Some inhibitors are shown to be able to inhibit the HDAC activity of both class I and class II HDACs, such as TSA. However, specific inhibitors that could only inhibit the activity of one specific HDAC have been developed. An example is tubacin, a small molecule which selectively inhibits HDAC6 activity. More information about the selective HDAC inhibitors could be found in this review.

### 4. HDAC and EC proliferation

EC proliferation has a major influence on EC turnover. Reports have shown that high EC turnover correlates with high EC permeability. Caplan and Schwartz showed that increased Evans Blue uptake was observed in the area of increased EC turnover in vivo. Also elevated Evans Blue uptake was observed in the areas with higher proliferation marker [H3]-thymidine index. HDAC is a key modulator of cell proliferation, and its role on EC proliferation has not been fully understood.

<table>
<thead>
<tr>
<th>Table 1 Classes of HDACs</th>
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<tbody>
<tr>
<td>Class</td>
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<tr>
<td>I</td>
</tr>
<tr>
<td>HDAC2</td>
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<tr>
<td>HDAC8</td>
</tr>
<tr>
<td>IIa</td>
</tr>
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<td>III</td>
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<td>IV</td>
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Our laboratory has found that HDAC7 controls EC growth through modulation of β-catenin translocation. We found overexpression of HDAC7 suppresses human umbilical vein EC proliferation by preventing nuclear translocation of β-catenin and downregulating T-cell factor-1/d2 (inhibitor of DNA binding 2) and cyclin D1, leading to G1-phase elongation. Knockdown of HDAC7 by shRNA induced β-catenin nuclear translocation but down-regulated cyclin D1, cyclin E1, and E2F2, causing EC hypertrophy. Further experiments showed that HDAC7 could retain β-catenin in the cytoplasm by direct binding. We also found that VEGF could induce HDAC7 degradation via PLCγ1/PI3K signal pathway and partially rescue HDAC7-mediated suppression of proliferation. Previously, it was generally believed that class II HDACs, which could shuttle between the nucleus and cytoplasm, are only functional when they are in the nucleus. Our findings demonstrate a novel function of HDAC7 in the cytoplasm, which interacts with β-catenin in a low proliferation stage (Figure 1). Other researchers have also investigated HDAC7 in EC function. Importantly, Chang et al. have shown that disruption of the HDAC7 gene in mice resulted in failure of endothelial cell—cell adhesion and enlarge of the branchial arteries. The enlargement of the branchial arteries may imply an increase in EC number or cell size, in agreement with our findings that knockdown of HDAC7 by shRNA increased EC size with a concomitant increase in cellular metabolism. These findings suggest that HDAC7 regulates EC cycle and growth. However, Mottet et al. reported that siRNA-mediated knockdown of HDAC7 does not influence EC proliferation. This discrepancy may result from different culture systems, different effects of gene-knockdown assays, or different methods of measuring cell proliferation and growth.

5. HDAC and EC migration

EC migration helps maintain the seamless barrier between circulating blood in the lumen and the rest of vessel wall, especially after endothelial denudation. Endothelial denudation is usually caused by injury of the lumen of vessel, e.g., percutaneous coronary intervention or percutaneous transluminal coronary angioplasty. Such injury induces local inflammation, followed by the proliferation and migration of SMC towards the lesion, leading to neointima formation and restenosis. Re-endothelialization, which is promoted by EC migration and proliferation, can rescue this restenosis process.

Several researches have been performed to investigate the potential effect of HDACs on EC migration. Urbich et al. revealed that siRNA-mediated knockdown of HDAC5 could promote EC migration and sprouting, while knockdown of HDAC7 and HDAC9 decreased EC migration. Deletion and mutation study of HDAC5 revealed that the nuclear localization of HDAC5 is crucial for its function in EC migration, while its binding with myocyte enhancer factor 2 (MEF2) and deacetylase activity are dispensable. It seems that the HDAC location is more important than HDAC activity. However, we could not exclude the role of deacetylation in this case, because class II HDACs can recruit other HDACs. Microarray analysis indicated that HDAC5 silencing increased the expression of secretory protein fibroblast growth factor 2 (FGF2). In addition, the conditional medium from ECs transfected with HDAC5 siRNA attracted more migrated cells comparing with scramble siRNA. However, addition of the FGF2 protein in the medium failed to further augment the increase of sprouting induced by HDAC5 siRNA, suggesting the involvement of other effectors. Chromatin immunoprecipitation assay showed that HDAC5 bound to the promoter region of FGF2, indicating HDAC5 functions as a repressor for FGF2 gene transcription. Their work suggests that HDAC5 is a repressor of EC migration and angiogenesis partially through modulation of FGF2 expression.

HDAC7 has also been reported to modulate EC migration. Mottet et al. showed that siRNA-mediated knockdown of HDAC7 inhibited EC tube formation and migration. Moreover, platelet-derived growth factor-B (PDGF-B) and its receptor (PDGFR-β) were the most up-regulated genes following HDAC7 silencing. The increased expression of PDGF-B and PDGFR-β are partially responsible for the inhibition of EC migration. Furthermore, treatment of ECs with phorbol 12-myristate 13-acetate resulted in the translocation of HDAC7 out of the nucleus through a protein kinase C/protein kinase D pathway and induced, similarly to HDAC7 silencing, an increase in PDGF-B expression, as well as a partial inhibition of EC migration. Collectively, these data identify HDAC7 as a key modulator of EC migration and hence angiogenesis, at least in part by regulating PDGF-B/PDGFR-β gene expression.

Ha et al. demonstrated another way that the VEGF could modulate HDAC7 and EC migration. They found that VEGF stimulated phosphorylation of HDAC7 at the sites of Ser178, Ser344, and Ser479 in a dose- and time-dependent manner, leading to the cytoplasmic accumulation of HDAC7. The phosphorylation of HDAC7 has been proved to be mediated by phospholipase Cγ/protein kinase C/protein kinase D1 (PKD1)-dependent signal pathway. Infection of ECs with adenoviruses encoding a mutant of HDAC7 specifically deficient in PKD1-dependent phosphorylation inhibited VEGF-induced primary aortic EC migration.

Figure 1 A schematic illustration shows the role of HDAC7 in controlling EC growth. HDAC7 acts as a bridge between 14-3-3 proteins and β-catenin, which stabilizes β-catenin in the cytoplasm, resulting in inhibition of EC growth and leading to G1-phase elongation. VEGF treatment increases HDAC7 degradation, releasing β-catenin from the HDAC7–β-catenin–14-3-3 complex, leading to β-catenin nuclear translocation. The overall effect is an increase in β-catenin target gene expression and EC growth (adapted from reference 12).
6. HDAC and EC apoptosis

EC apoptosis has a similar role with endothelial dysfunction in atherosclerosis. Moreover, recent researches have shown that luminal EC apoptosis may be responsible for thrombus formation on eroded plaques without rupture.\(^3,37\) So, the importance of EC apoptosis in atherosclerosis could not be neglected. HDACs and HDAC inhibitors have been shown to be involved in EC apoptosis.

Our group has previously found that HDAC3 plays a crucial role in the differentiation of ECs from embryonic stem cells.\(^3,39\) Zamptaki et al.\(^40\) found that shRNA-mediated knockdown of HDAC3 resulted in an increase of cells showing extensive membrane blebs, reduced cell number and survival, enhanced presence of nucleosomes in cytosol, and more Annexin V staining. Ex vivo experiments showed loss of ECs in the aortic segments treated with ShRNA of HDAC3. Co-immunoprecipitation experiments revolved that HDAC3 forms a complex with Akt. Overexpression of HDAC3 resulted in increased phosphorylation of Akt and up-regulation of its kinase activity. Taken together, our findings demonstrated that HDAC3 plays a critical role in maintaining EC survival and prevents arteriosclerosis via Akt activation.

A class III HDAC SIRT1 has a direct role in EC apoptosis and senescence. Hou et al.\(^41\) showed that EC SIRT1 is vital for the prevention of early membrane apoptotic phosphatidylserine externalization and subsequent DNA degradation, through a pathway involving Akt1 and FoxO3a. Zu et al.\(^42\) demonstrated that SIRT1 could promote EC proliferation and prevent senescence by regulating a serine/threonine kinase and tumour suppressor LKB1.

HDAC inhibitor valproic acid can also affect EC apoptosis. Michaelis et al.\(^43\) showed that valproic acid could increase extracellular signal-regulated kinase1/2 (ERK 1/2) phosphorylation in ECs. ERK 1/2 phosphorylation causes phosphorylation of the anti-apoptotic protein Bcl-2 and inhibits serum starvation-induced EC apoptosis and cytochrome c release from the mitochondria. Collectively, their results provide an explanation how valproic acid can prevent EC apoptosis through the phosphorylation of ERK 1/2 (Table 2).

7. HDAC and SMC proliferation

SMC proliferation has an indispensable contribution to the neointima formation or arteriosclerosis. After EC injury and activation, various growth factors (e.g., PDGF, TGF-β) and cytokines (interferon-γ) are released by different cell types, including EC, platelets, and monocytes. These cytokines and growth factors promote SMC proliferation, which exacerbate the formation of advanced lesion in arteriosclerosis.

Okamoto et al.\(^44\) have found that TSA at the concentrations of 0.1, 1, 10 \(\mu\)mol/L could time-dependently suppress proliferation of primary SMCs isolated from rat thoracic aorta. They have used both cell count and \([H^3]-\)thymidine incorporation methods to measure proliferation. Further experiments revealed that TSA reduced the phosphorylation of Rb protein, and induced the expression of p21/WAF1 but not of p16INK4a, p27KIP1, or p53. Finally, TSA inhibited HDAC activity of SMCs from p21/WAF1 knockout mice but did not influence the proliferation of these cells. Their work suggests that TSA inhibits SMC proliferation via the induction of p21/WAF1 and subsequent cell-cycle arrest with the reduction in the phosphorylation of Rb.

Song et al.\(^45\) argued that TSA at the concentration of 0.5 \(\mu\)mol/L could increase PDGF-BB stimulated proliferation of primary SMC isolated from thoracic and abdominal aorta of rats. They pre-treated SMC with TSA for 48 h, and then stimulated with PDGF-BB for 24 h, cell viability was measured and demonstrated that TSA treatment time-dependently decreased thioredoxin 1 expression in rat SMCs at both the mRNA and protein levels. Moreover, siRNA-mediated knockdown of thioredoxin 1 could potentiate Akt phosphorylation and enhance SMC proliferation in response to PDGF and serum. Collectively, these results indicate that TSA could enhance SMC proliferation by down-regulating thioredoxin 1, thus activating an Akt-dependent pathway.

The controversy may be attributed to different methods. Okamoto et al.\(^44\) measured the proliferation of SMC in response to serum when treated with TSA, while Song et al.\(^46\) measured the proliferation of SMC in response to PDGF-BB. Therefore, there may be a possibility that TSA activates or inhibits a certain pathway which is specifically involved in the PDGF stimulation. Also, based on the fact that TSA can inhibit both class I and class II HDACs, it may have different functions in the cell, especially when it is applied in different concentrations.

Very recently, we sought to examine the effect of two HDAC7 isoforms on SMC proliferation and the function in neointima formation (Zhou et al., submitted in revision).\(^46\) We found that overexpression of the unspliced HDAC7 isoform (HDAC7u) could suppress SMC proliferation through down-regulation of cyclin D1 and cell cycle arrest, while the spliced HDAC7 isoform (HDAC7s) did not have the same effect. siRNA-mediated knockdown of HDAC7 increased SMC proliferation and induced β-catenin nuclear translocation. Further experiments showed that only HDAC7u could bind with β-catenin and retain it in the cytoplasm. Reporter gene assay and reverse transcription PCR revealed less β-catenin activity in the cells overexpressing HDAC7u, but not HDAC7s. Deletion studies indicate that the C-terminal of HDAC7u

### Table 2  EC, SMC, and HDAC

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Nature</th>
<th>Impact</th>
<th>Interaction</th>
<th>References</th>
</tr>
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<td>β-Catenin</td>
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<tr>
<td>Migration</td>
<td>SIRT1†</td>
<td>LKB1</td>
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<tr>
<td></td>
<td>Knockdown HDAC5 †</td>
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<tr>
<td></td>
<td>Knockdown HDAC7 †</td>
<td>Not specified</td>
<td>35</td>
<td></td>
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<tr>
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<td>Knockdown HDAC9 †</td>
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<td>35</td>
<td></td>
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<tr>
<td></td>
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<td>PDGF-B/ PDGFR-ββ</td>
<td>33</td>
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<tr>
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<td>Knockdown HDAC3 †</td>
<td>Akt</td>
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<tr>
<td>SMC</td>
<td>Proliferation</td>
<td>TSA †</td>
<td>P21 WAF1</td>
<td>44</td>
</tr>
<tr>
<td>Migration</td>
<td>TSA †</td>
<td>Trx1</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Apoptosis</td>
<td>TSA †</td>
<td>Trx1</td>
<td>45</td>
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<tr>
<td></td>
<td>HDAC1 †</td>
<td>IGF1R</td>
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<tr>
<td></td>
<td>(indirect)</td>
<td>NAD+ HDAC activity †</td>
<td>Not specified</td>
<td>51</td>
</tr>
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is responsible for the binding with β-catenin. However, N-terminal additional amino acids disrupted the binding, which gives more strength to the fact that HDAC7s did not bind with β-catenin. Growth factor PDGF-BB increased the splicing of HDAC7, decreasing the expression of HDAC7u. Importantly, in an animal model of femoral artery wire injury, we demonstrated that knockdown of HDAC7 by siRNA aggravates neointimal formation in comparison with control siRNA. Our findings demonstrate that splicing of HDAC7 modulates SMC proliferation and neointima formation through β-catenin translocation, which provide a potential therapeutic target in vascular disease.

8. HDAC, SMC migration, and apoptosis

Under physiological conditions, SMCs are surrounded by extracellular matrix, which keeps SMCs in a low migratory activity. However, during the development of arteriosclerosis, matrix metalloproteinase could be released from various cell sources (e.g., activated macrophages) to degrade matrix proteins, thus promoting the migration of SMCs. SMC migrating from the media to the intima is an important step during atherogenesis, which turns fatty streak into advanced, complicated lesion.1

SMCs respond to mechanical strain, but the role of HDACs in modulating SMC migration induced by mechanical strain is poorly understood. Yan et al.37 stated that cyclic strain could significantly inhibit the migration of cultured SMCs. The cyclic strain up-regulated the levels of acetylated histone H3 and HDAC7, while down-regulated the level of HDAC3/4 in SMCs. Furthermore, the mechanically induced SMC migration was diminished by treatment with tributyrin, an HDAC inhibitor. They also observed hyperacetylation of histone H3 and reduced expression of HDAC7 upon tributyrin treatment. These results provide evidence that HDACs are involved in the migration of SMCs induced by mechanical strain. Similarly, Song et al.45 found that TSA could enhance SMC migration in response to PDGF-BB.

By comparing the SMC from normal human coronary arteries and from coronary plaques, Bennett et al.48 observed higher rates of SMC apoptosis in plaque, which may ultimately contribute to plaque rupture.

Patel et al.49 have previously demonstrated that plaque-derived SMCs have reduced insulin-like growth factor 1 (IGF1) signalling, resulting from a decrease in the expression of IGF1 receptor compared with normal aortic SMCs. Further, they have found that over-expression of IGF1 receptor could abolish oxidative-stress-induced apoptosis in SMCs, and oxidative stress repressed IGF1 receptor gene expression in turn, which needed HDAC1.50 Oxidative stress first phosphorylated p53, and thus promoted p53 binding with TATA box-binding protein. P53 negatively regulated IGF1 receptor promoter activity via TATA box-binding protein. Oxidative stress also increased the association of p53 with HDAC1, and in this way HDAC1 provided extra repression to IGF1 receptor. These findings suggest that HDAC1 is a critical molecule in the signalling of oxidative-stress-induced SMC apoptosis.

Class III HDACs have also been involved in SMC apoptosis. Veer et al.51 reported that pre-B-cell colony-enhancing factor could reduce SMC apoptosis as revealed by TdT-mediated dUTP Nick-End Labeling (TUNEL) analysis. They found that NAD+-dependent protein deacetylase activity was required for SMC maturation and that NAD+-dependent HDAC activity was augmented by pre-B-cell colony-enhancing factor. These results provide a novel pathway that class III HDACs can influence SMC apoptosis and phenotype switch (Table 2).

9. HDAC and stem cell differentiation into ECs

As mentioned earlier, the migration and proliferation of flanking ECs have been considered a major source of the repair of denuded endothelium. However, recent discoveries have emphasized the role of stem cell repair in atherosclerosis.45 Accumulated evidence suggests that stem cells, under certain circumstances, can differentiate into ECs, which are capable of repairing the damaged endothelium.

In an animal model of VEGF-stimulated EC differentiation from embryonic stem cells. These stem cell-derived ECs (so called esECs) were expressing full range of EC lineage-specific markers. During the VEGF-induced differentiation, HDAC3 was shown to be up-regulated. Moreover, TSA treatment or siRNA-mediated knockdown of HDAC3 abolished VEGF-induced EC lineage marker expression. Overexpression of HDAC3 enhanced the expression of these markers. Taken together, these results emphasize the function of HDAC3 in mediating VEGF-induced ECs differentiation into ECs.

We further characterized the pathway of stem cell differentiation into ECs.39 Laminar flow stabilized and activated HDAC3 through the Flik-1–PI3K–Akt pathway, which in turn deacetylated p53, leading to p21 activation. Overexpression of p53 could enhance EC marker expression, and up-regulate p21. A similar signal pathway was detected in VEGF-induced EC differentiation as well. Local transfer of stem cell-derived progenitors (differentiated by laminar flow) incorporated into injured femoral artery and reduced neointima formation in a mouse model. These data suggest that shear stress is a key regulator for stem cell differentiation into ECs, especially in progenitor differentiation, which can be used for vascular repair, and that the Flik-1–PI3K–Akt–HDAC3–p53–p21 pathway is crucial in this process.

10. HDAC and stem cell differentiation into SMCs

Traditionally, SMCs in the neointima of atherosclerosis were believed to be originated from the media of injured arteries. These media-originated SMCs then proliferate and migrate towards the intima in response to signals from inflammatory cells and ECs, and form neointima together with inflammatory cells, lipid, and other components.1 However, recent findings from different groups emphasized the importance of stem/progenitor cell-derived SMCs in neointima formation and atherosclerosis.5,52–57

With the help of transgenic animal and transplant atherosclerosis animal model, researchers provided extensive evidence suggesting the involvement of recipient progenitor-like cells in the transplant arteriosclerosis.5,52–57 These data also indicated that the recipient SMC-like cells come from bone marrow5,52–57 or non-bone marrow5,52–57 stem cells. Our group has found that SMCs in transplant atherosclerotic lesions were originated from recipients, but
11. HDAC and atherosclerosis in animal models

Atherosclerosis develops at specific sites of the vasculature that experience disturbed blood flow. Zampetaki et al. found that HDAC3 expression was up-regulated in areas in close vicinity to branch openings where disturbed flow occurs. In aortic isografts of apolipoprotein E-deficient mice, beta-gal(+) cells were found in atherosclerotic lesions of the intima, and these cells enhanced the development of the lesions. In summary, works on adult stem cells provide solid evidence that a large population of vascular progenitor cells existing in the adventitia can differentiate into SMCs that contribute to atherosclerosis.

In order to elucidate the mechanism involved in stem cell differentiation into SMCs, further researches were performed. Data from our group have shown that HDAC7 splicing is a key event in stem cell differentiation into SMCs. First, HDAC7 has a parallel expression pattern with SMC marker genes during stem cell differentiation. In addition, knockdown of HDAC7 reduced SMC marker expression. Finally, HDAC7 was found to undergo alternative splicing during stem cell differentiation. Spliced HDAC7 increased SMC marker expression, and could bind with SMC differentiation-related transcription factor, such as serum response factor. Unspliced HDAC7 decreased SMC marker expression by binding to SMC differentiation transcription factor myocyte enhancer factor 2C and degrading it. These findings suggest that HDAC7 splicing is important in inducing stem cell differentiation towards SMCs.

Figure 2 Signal pathways leading to stem cell differentiation towards endothelial cell (EC) and smooth muscle cell (SMC). Laminar shear stress directly stimulates VEGFR2 (Flk-1) on stem cells, thus activate the P13K-Akt pathway, which stabilizes the HDAC3 protein, leading to p53 deacetylation, p21 activation, and EC marker expression. On the other hand, the presence of collagen IV triggers cell differentiation into SMCs. During this process, HDAC7 is upregulated and undergoes alternative splicing, which can be enhanced by PDGF-BB. The spliced HDAC7 binds with SRF and promotes SMC marker expression.

12. Summary and future perspectives

Atherosclerosis is characterized by its complexity and multi-gene changes during pathogenesis. Various signal pathways are actively involved in modulating vascular cell homeostasis, in which HDACs play a central part. HDACs are a family of enzymes that remove acetyl groups from lysine amino acid, which can suppress the expression of certain gene without changing the DNA sequence. Such epigenetic modifications by HDACs attracted more and more attention of cardiovascular researchers in recent years.
HDACs have been found to be able to modulate most steps of atherosclerosis in the traditional view, e.g. proliferation, migration, and apoptosis of both ECs and SMCs (Table 2). For the function of individual HDACs, HDAC7 is involved in EC proliferation, migration, and SMC proliferation, while HDAC3 modulates EC apoptosis. Moreover, in the stem cell theory of atherosclerosis, HDAC3 and HDAC7 have been proved to direct stem cell differentiation towards ECs and SMCs, separately (Figure 2). Finally, in various animal models that mimic the pathogenesis of atherosclerosis, HDACs have also been identified as potent modulators.

Recent studies on inflammatory diseases indicate HDACs are also involved in endothelial adhesion molecule expression, which is also an important event in atherosclerosis. HDAC inhibitor TSA has been shown to suppress vascular cell adhesion molecule-1 expression and monocyte adhesion in HUVEC. Other reports also revealed that HDAC inhibition has a critical function in modulating intercellular adhesion molecule-1 and leucocyte-endothelial adhesion, E-cadherin expression, and tissue factor expression.

Despite the outstanding progress in understanding the role of HDACs in atherosclerosis, there are still several questions that need to be answered. One question is whether the effects of HDACs are tissue- or cell type-specific, e.g. whether the HDACs have the same effect on migration of SMCs and ECs. This is crucial because if we want to suppress the migration of SMC during atherogenesis, the impact on other cell types needs to be determined. Otherwise, suppression of SMC migration with HDACs (or HDACs) could lead to suppression of EC migration as well. To answer this question, more experiments need to be performed on the functions of HDACs in various vascular cells.

As to a clinical prospect, several clinical trials of HDAC inhibitors have been done in cancer and neurological patients. Among these clinical trials, HDAC inhibitors have shown potent inhibition of HDAC activities in patients, and only limited side effects have been observed. Marks and Xu reviewed that HDAC inhibition tends to have effect on apoptosis of cancer cells vs. normal human cells. This emphasizes the possibility of a tissue-specific effect of HDAC inhibition, which could explain the fact that HDAC inhibition had limited side effects in humans. Therefore, it seems HDAC inhibition could be a promising target in treating cardiovascular diseases. However, detailed attention should be paid to the application of such drugs, which may have unexpected effects due to the fact that the majority of HDAC inhibitors are non-specific. And since different HDACs are responsible for different functions, non-selective inhibition of HDACs could trigger side effect responses. Also, as the epigenetic modifier of gene expression, single HDAC is usually involved in several signal pathways. Thus, development of highly selective and cell type-specific HDAC inhibitors could be a promising field in future cardiovascular research. Along with the progress of HDACs’ functions in cancer and neurological disease, our researches on HDACs will benefit more and more arteriosclerosis patients.

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