Angiogenesis and Cardiovascular Diseases: The Emerging Role of HDACs

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Abstract

Cardiovascular diseases (CVD) continue to be the leading cause of death in the world despite recent therapeutic advances. Although many CVDs remain incurable, enormous efforts have been placed in harnessing angiogenesis as therapeutics for these diseases. Epigenetics, the modification of gene expression post-transcriptionally and post-translationally, are important in regulating many biological processes. One of the main post-translational epigenetic modifications, modification of chromatin structure by the acetylation of histone tails within the chromatin by either histone deacetylases (HDACs) or histone acetyltransferases (HATs), is important in modulating gene transcription and has emerged as an important regulatory player from pathogenesis to therapeutics in CVDs. Particularly, HDACs, which are largely involved in promoting chromatin compaction and hence inhibitions of gene transcription, have been implicated in the pathogenic signalling underlying many aspects of CVDs. Recently, histone modifications have been demonstrated to play important roles in the angiogenesis process. Pharmacological inhibitions of HDACs have displayed promising therapeutic potentials in several pre-clinical models of CVDs where angiogenesis is of paramount importance. There are many evidences proving that pro- and anti-angiogenic therapies—and the impact of epigenetics in these processes—can help to artificially reconstruct the vasculature in patients with cardiovascular diseases. Conversely, utilising knowledge of HDACs in angiogenesis might help to develop anti-angiogenic therapies in tackling diseases that are characterised with excessive pathological angiogenesis, including cancer and age-related macular degeneration. Understanding the molecular mechanisms underlying HDACs in modulating angiogenesis will undoubtedly benefit future therapeutics development. This chapter focuses on the emerging role of HDACs in angiogenesis and discuss their potentials and challenges in utilising HDAC inhibitors as therapeutics in several major cardiovascular diseases.

Keywords: angiogenesis, cardiovascular disease, atherosclerosis, histone deacetylase, epigenetics
1. Introduction

Cardiovascular diseases (CVDs) are a worldwide epidemic that have serious implication in public health and constitute a huge amount of healthcare expenditure. Although there are a number of preventable controllable risk factors, such as hypertension, hypercholesterolemia, smoking, obesity, lack of physical activity and diabetes, and others such as age, gender and family history are unmodifiable [1]. Progress in genetic sequencing has allowed the identification of numerous genetic variants associated with specific CVDs [2], but their mechanisms remain unclear. The last few years of research have been a key in understanding how epigenetic mechanisms such as histone modifications are involved in the occurrence and progression of CVDs including atherosclerosis, heart failure, myocardial infarction and cardiac hypertrophy.

Epigenetics represent a phenomenon of altered heritable gene expression without changes to the underlying DNA sequences. The epigenetic alterations can be affected by exogenous stimuli such as diabetes milieu, diets and smoking, while at other times these alterations can subsequently trigger disease initiation [3]. Thus, the impact of epigenetics in CVD is now emerging as an important regulatory key player at different levels from pathophysiology to therapeutics. For instance, histone alterations have been implicated in ECs response to hypoxia and shear stress, in angiogenesis and in endogenous recovery following myocardial infarction (MI) [4]. On the other hand, HDAC inhibitors (HDACi) have been investigated for potential protective effects in heart muscles during acute MI [4, 5].

Tissue repair is one of the main therapeutic challenges facing the scientific community. There are various approaches in improving tissue recovery depending on the pathological conditions, but most of these conditions are initiated by local ischaemia and require a rich network of blood supply for tissue regeneration. Hence, angiogenesis plays a vital part in tissue regeneration in the treatment of CVDs. At present, the promising potentials of angiogenesis therapies are in full swing.

2. Vascular system

2.1. Cardiovascular system

The cardiovascular system consists of three main components: heart, blood vessels (arteries, veins and capillaries) and blood. There are three types of anatomically and functionally distinct blood vessels: arteries, veins and capillaries. The arteries are primarily involved in the delivery of oxygenated blood and nutrients from the heart to target organs and tissues. They have thicker and more elastic vessel walls to complement the higher blood pressure for blood delivery from the heart. The veins carry deoxygenated blood, together with waste products and other factors secreted by the tissues back to the heart. They tend to have larger luminal areas and thinner vessel walls compared to the arteries, and have valves to complement the pressure changes. Connecting these two vessel systems are the capillaries that allow the direct exchanges of oxygen and nutrients with carbon dioxide and waste.
products between the target tissues and the blood. The walls of all vessels are generally composed of three layers: the tunica intima, tunica media and tunica adventitia. The innermost layer is formed by the tunica intima, which is made up of a single layer of ECs and connective tissues, both of which overlie the internal elastic lamina. The tunica intima has an important function as a selective permeable barrier between the extravascular space, the vascular wall and the blood. The tunica media forms the muscular element of blood vessels that resides between the tunica intima and the tunica adventitia, and comprises circumferentially arranged smooth muscle cells (SMCs) enclosed by a layer of external elastic lamina. They provide supports to the vessels and regulate blood flow and pressure via controlling the luminal diameter. The outermost layer, the tunica adventitia, is made up of connective tissues and matrix-secreting fibroblasts. It is critical to maintaining vascular structure and helps to anchor vessels in place to fit into the surrounding tissues. Capillaries constitute non-muscular vessels and are only made up of an internal elastic lamina covered by a monolayer of ECs, and provide a huge surface area for exchanges of vital blood components and factors between vessels and tissues.

2.2. Endothelial cells and their impairments in CVDs

Vascular endothelial cells (ECs) have a crucial and diverse role, arraying the innermost layer of the entire circulatory system. They act as the semi-selective and non-adherent barrier between the lumen of the vessels and the underlying tissues, regulating tissue perfusion and movement of inflammatory cells between them [6]. They are involved in regulating vascular permeability, blood flow, vascular tone and blood coagulation and are essentially involved in vascular remodelling in responses to diverse physiological and pathological stimuli. Physiologically, ECs exert anti-coagulant and anti-thrombotic effects through the secretion of anti-coagulant factors such as prostacyclin, nitric oxide (NO) and prostaglandin-E_2 and inhibit inflammatory cell adhesion in order to maintain vascular homeostasis [7]. Under pathological states, ECs are activated by vascular insults or pro-inflammatory cytokines, leading to increased permeability, encouraging extravasations of immune cells, which are followed by a series of pathological events leading to eventual vascular remodelling [7]. Decreased EC secretion of the potent vasodilator NO as a result of repressed activity of endothelial NO synthase (eNOS) also contributes to the circus of vascular pathogenesis [8]. These endothelial dysfunctions, whether environmental, genetic or a combination of both, critically contribute to the pathophysiology of many CVDs such as hypertension and atherosclerosis, and represent the discernible therapeutic targets for drug development [9].

3. Angiogenesis

There are three main processes that contribute to the formation of new blood vessels that are termed globally as neovascularisation:

– Vasculogenesis is defined as the de novo formation of vascular structures by the migration of stem cells to the site of vascularisation and differentiation into ECs. Although it was originally thought to be exclusive to embryonic development, it is now widely accepted...
that the process can also take place in adults, which opens up a new avenue for clinical applications [10].

– Angiogenesis is the formation of new blood vessels by sprouting from pre-existing small vessels in embryonic and adult tissue or by intravascular subdivision process [11]. This process is believed to be induced by angiogenic factors including fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF).

– Arteriogenesis results from the hypertrophy and luminal distention of pre-existing collateral vessels, which involves specific remodelling of existing nascent EC tubules for greater size, elasticity and stability through the recruitment of and enclosure by SMCs and pericytes that secrete specific extracellular matrices. Therefore, these vessels have fully developed tunica media and tunica adventitia [11].

Angiogenesis is a very complex process that can be simplified into three categories: mechanical, chemical and molecular factors (see Ref. [12] for a more extensive review).

– **Cellular factors**: There are many molecules that can modulate angiogenesis. The most essential angiogenic growth factors are as follows: FGF, VEGF, placenta growth factor, angiopoietin-1 and angiopoietin-2. Several pathological conditions can also initiate angiogenesis. For example, hypoglycaemia increases the expression of critical angiogenic inducer VEGF [13]. It has also been extensively demonstrated that the presence of inflammatory cells, like macrophages and neutrophils, is sufficient to induce angiogenesis [14].

– **Environmental factors**: Angiogenesis can be induced by hypoxia and through increased EC production of NO. Hypoxia stimulates the release of several angiogenic factors including platelet-derived growth factor and FGF-1 and FGF-2 by macrophages. Hypoxia also upregulates VEGF production, which is known to induce the production and secretion of NO from ECs, while eNOS production is amplified during VEGF-induced angiogenesis [15].

– **Mechanical factors**: There are two main factors: haemodynamic and shear stress. Haemodynamic changes trigger an augmentation of blood flow and might therefore stimulate vascular sprouting, maintain patency of the newly formed collateral vessels and provide blood flow to the ischemic area [16]. Shear stress has an important influence on the development of collateral vessel networks in the ischaemic tissues.

### 4. Epigenetics

The nucleosome is the fundamental subunit of chromatin in eukaryotes. Each nucleosome consists of a 146-bp DNA segment wrapped around an octamer of core histone proteins that includes two molecules of histones H2A, H2B, H3 and H4 associated with a single copy of histone H1. Epigenetics is defined as the study of stable alterations of gene expression without alterations of DNA itself. These alterations include the post-translational addition or removal of methyl groups to DNA as well as methyl, acetyl, sumoyl and phospho groups to histones and other kind of proteins. These changes participate in remodelling chromatin and modifying its accessibility to transcription factors and cofactors [17]. Epigenetic control is one of the main
regulatory systems contributing to phenotypic differences between cell types in multicellular organisms. Epigenetic changes may explain why subjects with similar genetic backgrounds and risk factors for particular diseases can differ greatly in clinical manifestation and therapeutic response [18]. It has been reported that epigenetic mechanisms play a critical role in regulating endothelial gene expression [19]. Among these epigenetic changes are the methylation of DNA, RNA-based mechanisms and the posttranslational modification of histone proteins.

**DNA methylation**

The methylation of DNA involves the covalent modification of the 5-position of cytosine to define the ‘fifth base of DNA’, 5-methyl-cytosine [20]. In mammals, DNA methylation is almost exclusively restricted to CpG dinucleotides. DNA methylation is catalysed by DNA methyltransferases and regulates biological processes underlying CVD, such as atherosclerosis, inflammation, hypertension, and diabetes [21].

**RNA-based mechanisms**

1. **miRNA therapeutics**

   MicroRNAs (miRNA or miR) are short (20–22 nucleotides) non-coding RNAs modulating gene expression further by down-regulating the translation of target mRNAs through the inhibition of post-transcriptional events, through transcript degradation or through direct translational repression.

2. **Long non-coding RNAs (lncRNAs)**

   Long non-coding RNAs (lncRNAs) are gaining more prominence as regulators of gene expression. The central role that lncRNAs play in heart development is only slowly being recognised [18]. Besides, understanding the function of these molecules in CVD is even further away.

**Histone modification**

It is well established that histone residues can undergo a wide array of modifications. At least eight different types of modification have been characterised with a range of enzymes identified for each: acetylation, methylation, phosphorylation, ubiquitination, sumoylation, ADP-ribosylation, deimination, and proline isomerisation (Table 1).

Histone methylation is modulated by two enzymes: histone methyltransferases and histone demethylases. The acetylation status of histone is fine-tuned by histone acetyltransferases (HATs) and HDACs. HDACs are enzymes that remove acetyl groups from histone lysine residues thereby increasing their negative charges, which lead to chromatin condensation and gene repression [17].

4.1. **The HDAC family**

Deacetylation of histones in nucleosomes induces chromatin compaction, which represses transcription by preventing the binding of transcription factors and other components of the
transcriptional machinery onto the gene promoter and enhancer regions. HDACs are enzymes that remove acetyl groups from hyperacetylated histones, and modification by HDACs leads to a closed chromatin structure and suppression of genes. HDACs are recruited to gene promoters by DNA-binding proteins that recognise certain DNA sequences and in this way provide specific modulation on gene expression.

There are 18 characterised members of the HDAC family in mammals, which can be grouped into four classes depending on their functional similarities and their homology with yeast HDACs. The class I and class II HDACs are considered as the ‘classical’ HDACs [23].

Class I HDACs comprise nuclear, ubiquitously expressed HDACs 1, 2, 3, and 8. HDAC1, 2, and 8 reside nearly exclusively in the nucleus. HDAC3 is found to shuttle between nucleus and cytoplasm. Because these are ubiquitously expressed and involved in cell proliferation and survival, aberrations in their gene expression have been implicated in a wide range of cancers [24, 25].

Class II HDACs shuttle between the cytoplasm and the nucleus depending on specific cellular signals; they share a tissue-specific expression pattern and are divided into two subgroups:

| Modification type | Amino acid modification | Examples of modifying enzymes | Role |
|-------------------|-------------------------|--------------------------------|------|
| Acetylation       | Lysine                  | Histone acetyl transferases (HATs) | Transcription Repair |
|                   |                         | Histone deacetylases (HDACs)     | Replication Condensation |
| Methylolation      | Lysine                  | Lysine methyltransferases        | Transcription |
|                   |                         | Lysine demethylases              | Repair |
|                   | Arginine                | Arginine methyltransferases      | Transcription |
|                   |                         | Arginine demethylases            | |
| Phosphorylation    | Serine                  | Serine/threonine kinases         | Transcription |
|                   | Threonine               | Dephosphorylated by phosphatases | Repair Condensation |
| Ubiquitination     | Lysine                  | Ubiquinases (ubiquitin ligases)  | Transcription |
|                   |                         | Deubiquinating enzymes           | Repair |
| SUMOylation        | Lysine                  | Small ubiquitin-like modifier    | Transcription |
|                   |                         | SUMO (Small ubiquitin-like modifier) | |
|                   |                         | De-SUMOylating enzymes: sentrin-specific proteases | |
| ADP ribosylation   | Glutamate               | ADP-ribosyltransferases          | Transcription |
| Deimination        | Arginine (to Citrulline)| Peptidylarginine deiminases      | Transcription |
| Proline isomerisation | Proline             | Proline isomerases               | Transcription |

Table 1. Types of histone modifications and the enzymes responsible (modified from Ref. [22]).
class IIa (HDACs 4, 5, 7, and 9) and class IIb (HDACs 6 and 10). Class IIa HDACs distinguish themselves with their extended N-terminal regulatory domain, whereas class IIb HDACs contain two catalytic domains. Class IIa HDACs appear to have tissue-specific roles and can shuttle between the cytosol and the nucleus. In fact, the phosphorylation status is a critical event to determine their localisation in the nucleus or cytoplasm and the ability to act as transcriptional co-repressors in the nuclear region. Conversely, class IIb is mostly found in the cytosol [26].

Class III HDACs regroup the ubiquitously expressed 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. Interestingly, these have a critical role in a wide range of cellular processes such as ageing, transcription, cell survival, DNA repair, apoptosis, and inflammation. Sirtuins appear to have contradictory roles in disease. On the one hand, they control many vital functions involved in cellular protection, while on the other hand, they are also involved in several disease pathologies such as metabolic diseases, neurodegenerative disorders, and cancer [27].

Finally, class IV HDAC is the newly discovered HDAC11. HDAC11 is most closely related to class I HDACs. However, since the overall sequence similarities are low, it cannot be grouped into any of the three existing classes. HDAC11 is primarily expressed in heart, smooth muscle, kidney, and brain tissues.

Recent reports suggest that HDACs can deacetylate non-histone proteins as additional functions of HDACs (Figure 1). The roles of HDACs in cancer and neurological diseases have

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**Figure 1.** Schematic illustration of HDACi downstream effects. Inhibition of HDACs by HDACi induces acetylation of histone proteins as well as non-histone proteins, which leads to the alteration in various physiological and pathological processes (modified from Refs. [28–30]).
been extensively examined. However, the functions of HDACs in cardiovascular diseases and arteriosclerosis are less explored [23].

4.2. HDAC inhibitors

There has been a breakthrough in the development of HDACi. These HDACi induce acetylation of histone proteins, as well as non-histone proteins, which leads to the alteration and regulation of biological events including angiogenesis, apoptosis/autophagy, cell cycle, fibrogenesis, immune response, inflammation, and metabolism (Figure 1). As a result, HDAC inhibitor-based therapies have gained substantial attention as treatments for cardiovascular diseases and cancer.

In the following sections, we will describe the different exerted functions of HDACi in different physiological and pathological conditions.

5. The role of HDACs in angiogenesis: HDAC-regulated ECs functions in vitro

During development of the embryo and the physiological repairs of any tissue damages, the formation of new blood vessels plays a major role. The process can either involve vasculogenesis where ECs may be derived from the differentiation of different kinds of stem cells such as embryonic stem (ES) cells, while angiogenesis requires the proliferation, migration, and sprouting of ECs. Some of these new blood vessel formations are normal and beneficial as seen in wound healing after trauma and ischemic tissue restoration. However, pathological neovascularisation leads to many diseases such as diabetic retinopathy, tumour, and inflammation. Over the past decade, investigations into the role of HDACs in the regulations of these processes have gained some tractions. We have previously shown that the stabilisation of the class I HDAC3 plays an essential role in VEGF receptor 2 (VEGFR2)-mediated endothelial differentiation of mouse ES cells (mESC)-derived Sca-1+ progenitors [31], while these events can also signal through VEGFR2-HDAC3 stabilisation in a ligand-independent manner through exposure to laminar shear flow [32]. These derived EC-like cells display increased angiogenic potential by significantly enhancing re-endothelialisation with the host vessels upon their transplantation into a mouse wire injury model and substantially attenuated the injury-induced neointimal hyperplasia [32]. In addition, HDAC3 is also essential for the survival of ECs under atherogenic disturbed flow, and knock-down of HDAC3 increases neointima formation in the atheroprone ApoE−/− mice [33]. Overall, these show HDAC3 plays a role in the angiogenic processes.

Angiogenic activation of ECs to migrate and to form sprouts is associated with characteristic changes in gene expression profiles [34], which can be modulated by the inhibition of HDAC. HDAC inhibitions by pan-HDAC inhibitors such as suberoylanilide hydroxamic acid (SAHA) and trichostatin A (TSA) can suppress VEGF-induced capillary-like structures formation in human umbilical vein endothelial cells (HUVEC) by suppressing angiogenic factors such as hypoxia-inducible factor 1 alpha (HIF-1α), VEGF, and eNOS while both HDACi also prevent
the sprouting of capillaries from rat aorta [35]. Discrepant reports exist however, as another study has shown that treatment with SAHA or a more class I selective inhibitor valporic acid (VPA) in combination with VEGF indeed resulted in enhanced EC sprouting [36]. These discrepancies could be due to the contrasting roles that other HDACs within the same class and/or from the other classes might play in regulating angiogenesis.

In fact, it is common that completely opposite role has been reported for other HDACs in the regulation of angiogenesis. HDAC4, a class IIa HDAC, was reported to negatively regulate angiogenesis by reducing VEGF expression [37], while others have reported HDAC4 induces angiogenesis through an increase in stability of HIF-1γ [38]. In addition, Zhang et al. showed that HDAC4 inhibition facilitated c-kit+ cardiac stem cells (CSCs) into the differentiation of cardiac lineage commitments with EC potential in vitro [4].

Diverse role has also been reported in another class IIa HDAC HDAC5. On the one hand, HDAC5 has been shown to repress KLF2 an important regulator of EC homeostasis that is normally expressed in the laminar flow-exposed (therefore atheroprotective) segments of the vessels, which results in repressed eNOS expression in ECs [39]. This anti-angiogenic role of HDAC5 was validated by siRNA-mediated knock-down of HDAC5 that promoted migration and sprouting of ECs [40]. Conversely, phosphorylation-dependent nuclear exports of HDAC5 [40] and HDAC7 [41], thereby the de-repression of target genes, are crucial for the expression of VEGF or metalloproteinase-10 in ECs that lead to increased angiogenesis. Moreover, blockade of HDAC7 phosphorylation with a signal-resistant HDAC7 mutant represses EC proliferation and migration in response to VEGF, confirming the important role of both class IIa HDACs plays in VEGF-mediated angiogenesis [42]. In addition, HDAC7 has also been identified as a key modulator of EC migration at least in part by regulating PDGF-B/PDGF-beta gene expression [43].

Evidence from our laboratory demonstrated that mouse HDAC7 undergoes alternative translation during mouse ESCs differentiation, resulting in the production of a 7-amino acid peptide (Data not publish yet). This peptide was shown to enhance mouse vascular progenitor cell migration and VEGF-induced differentiation towards the EC lineage in vitro. Overall, HDAC7 appears to be pro-angiogenic, while the mediating role of HDAC5 in angiogenesis could be largely dependent on its translocation within the nucleus.

There is a limited research into the role of class IIb HDAC in angiogenesis, but nevertheless HDAC6 can be classified as a pro-angiogenic factor as it induces cell migration by the deacetylation of cytoskeletal proteins [44, 45]. Class III HDAC SIRT1 is also highly expressed in the vasculature during blood vessel growth where it controls the angiogenic activity of ECs. Loss of SIRT1 function leads to blockage of sprouting angiogenesis [46]. Furthermore, SIRT1 associates with and deacetylates transcription factor Foxo1 and hence restricts its anti-angiogenic activity [46].

6. The role of HDAC in therapeutic angiogenesis

Different strategies for therapeutic angiogenesis, including the direct delivery of angiogenic growth factors and the delivery of cells to ischemic tissues, have been developed. Moreover,
there is a recent progress on therapeutic angiogenesis by utilising polymeric biomaterials, combined with stem cell and gene therapy as well as stimulation of endogenous stem cell homing (see Ref. [47] for a more comprehensive review).

Owing to the disadvantage of invasiveness, limited drug diffusion, and lack of selectivity towards targeted tissues, treatments with traditional drugs and surgery are becoming less commonly used [48]. An emerging technique, ultrasound-targeted microbubble destruction (UTMD), has been proposed as a non-invasive and specific targeting approach in angiogenic therapy of CVDs. UTMD might create a series of biological effects, including improving recovery of local tissue damages, improving transient membrane permeability, and extravasation to facilitate the entering of targeted genes or drugs into the tissues or cells of interest.

There are several approaches indicating that inhibition of HDAC protects the heart against injury in different cardiovascular-related diseases, including myocardial infarction, myocardial hypertrophy, and diabetic cardiomyopathy. In addition, HDACi also seem to play a therapeutic role in other CVDs with vascular remodelling as one of their main manifestations. Here, we review the role of HDACs in these diseases one by one in order to better understand the context-dependent effects of HDACs in angiogenesis regulation in these diseases.

6.1. Atherosclerosis

Atherosclerosis of the arteries is a main causative pathogenesis of various CVDs including coronary artery disease (CAD), peripheral vascular disease (PVD), and stroke. It is a chronic pathological condition of the arteries that is characterised by the accumulation of lipids, chronic inflammation, generation of a fibrous cap, proliferation of SMCs, calcification in vascular smooth muscle layer, with the resultant loss of elasticity of arteries. In addition, disturbed shear stress (the tangential force of the flowing blood on the endothelial surface of the blood vessel) contributes to several elements of atherosclerotic disease. As a result of the growth of atheroma, the lumen of the artery is gradually narrowed, which changes the local environment. Activated ECs within the injury lesions produce adhesion molecules [intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule-1 (VCAM-1)], chemotactic proteins [monocyte chemotactic protein-1 (MCP-1)], E- and P-selectin, and growth factors [macrophage colony-stimulating factor (M-CSF)] that create a pro-inflammatory environment. The inflammatory molecules then recruit monocytes to the vessel wall and promote their transmigration across the endothelial monolayer into the intima, where they proliferate, differentiate into macrophages, and foam cells by taking up the lipoproteins, leading to neointimal formation. With time, the foam cells and macrophages die and release lipid-filled contents and tissue factors, contributing to the formation of the lipid-rich necrotic core, which is a key component of unstable plaques. Meanwhile, SMCs migrate from the medial layer and accumulate within the intima, where they synthesise and secrete interstitial collagen and elastin and form the fibrous cap over the lesion. Ultimately, the thin fibrous caps rupture, then expose, and release procoagulant materials into the blood, triggering the thrombosis that impedes blood flow and results in acute stenosis of the arteries, leading to clinical manifestations [49–51].

Because many patients are not candidates for the standard treatments such as angioplasty or bypass surgery, a great enthusiasm has emerged for the utilisation of angiogenesis as a
therapeutic modality for atherosclerotic arterial disease. It must be taken into account that angiogenesis plays a vital part in the pathogenesis and treatment of CVDs and has become one of the hotspots that are being discussed in the past decades. Therapeutic angiogenesis provides a valuable tool for treating cardiovascular diseases by stimulating the growth of new blood vessels from pre-existing vessels.

This avenue needs to be explored with caution however, as the role of angiogenesis in atherosclerosis remains a very contentious topic, and currently, there is no consensus as to whether angiogenesis is a way to treat coronary heart disease or in fact is a key causative factor in the pathogenesis of atherosclerotic plaque formation. The controversy surrounding the role of angiogenesis in ischemic heart disease reflects, in part, the complexity of the underlying disease process. There are lot of studies supporting the therapeutic role of angiogenesis in atherosclerosis since a key therapeutic objective has been to use the angiogenic cytokines such as VEGF or FGF to stimulate collateral blood vessel formation in the ischemic heart and limb [52]. But, on the other hand, the pathogenic role of angiogenesis has been suggested as VEGF, and other angiogenic growth factors can promote atherosclerosis in certain animal models and potentially destabilise coronary plaques by promoting intralesion angiogenesis [53].

Apolipoprotein E-deficient (ApoE−/−) mice, created by homologous recombination in ES cells, was first described in 1992 [54, 55]. Since then, this model becomes the most commonly used mouse model of atherosclerosis that is able to develop severe hypercholesterolemia and lesions of atherosclerosis highly similar to those observed in humans. Endogenous SIRT1 has been shown to decrease macrophage foam cell formation and atherogenesis in ApoE−/−mice [56], while endothelial-specific overexpression of human SIRT1 reduces atherogenesis in ApoE−/− mice and improves vascular function [57]. So, in the vasculature, SIRT1 gain-of-function using SIRT1 overexpression has been shown to improve endothelial function in mice. Subsequently, it was described that SIRT1 does not directly influence endothelium-dependent vascular function in ApoE−/− mice, but it improves vascular function by preventing superoxide production in ECs and reduces the expression of inflammatory adhesion molecules by suppressing NF-κB signalling [58].

HDACi TSA has been shown to exert contradictory role in the formation of atherosclerotic lesion. TSA successfully prevents neointima formation after injury [59, 60]. In contrast, however, several reports have elucidated the proatherogenic effects of TSA [61]. Another example of the discrepancies in TSA roles is the reduction of angiogenesis through the decrease of NO level (a key second messenger in angiogenesis signalling) through downregulation of eNOS [62]. These contrasting findings reinforce the theory of the contesting role angiogenesis plays in atherosclerosis. In addition, it was reported that TSA can reduce the cholesterol biosynthesis by repressing the genes involved in the cholesterol, fatty acids, and glycolysis pathways [63]. These evidences suggest that TSA could be used as a potential therapeutic agent for the control of cholesterol levels as high cholesterol level is one of the main triggers of atherosclerosis.

6.2. Myocardial infarction

Myocardial infarction (MI) occurs when blood flow stops to part of the heart causing damage to the cardiomyocytes. In physiological conditions, oxygen and nutrients are supplied to the
ventricular myocytes by the coronary arteries. Under pathological condition, the coronary artery is often occluded by various pathological condition such as the growth of atheroma in the coronary artery, rupture of vulnerable plaque, thrombi from proximal lesions, emboli secondary to atrial fibrillation, or vegetation after endocarditis.

Several gene or protein therapies to deliver angiogenic factors such as VEGF, FGF2, or FGF4, as well as cell therapy using endothelial progenitor cells (EPCs), mesenchymal stem cells (MSCs), or induced pluripotent stem cells (iPSCs), have been developed as potential pro-angiogenic therapeutics for ischemic heart disease and peripheral vascular disease [64, 65]. HDAC4 inhibition has been demonstrated to promote cardiac stem cells mediated cardiac regeneration and improve the restoration of cardiac function in mice [4]. Granger et al. observed that ischemia induces HDAC activity in the heart resulting in increased deacetylation of histones H3/4 in vitro and in vivo that leads to injured cardiomyocytes [66]. Furthermore, HDACi exert direct antifibrotic activities that alter the response to ischemic cardiac injury and reduce infarct size, which are accompanied by improvement in cardiac functions in the mouse infarcted heart. However, it is unclear whether these therapeutic effects have any links with angiogenesis in these earlier studies.

TSA have exerted an increased angiogenic response in vivo in the mouse infarcted hearts. This indicates that TSA preserves cardiac performance and mitigates myocardial remodelling through stimulating cardiac endogenous regeneration that could be dependent on enhanced angiogenesis within the infarcted heart tissues [67]. The repression of ischemia-induced gene expression such as HIF-1α and VEGF has been suggested as possible mechanisms mediated by HDACi to stabilise vascular permeability [66]. Recruitment of stem cells has also been suggested as another main mechanism that TSA mediates through. After 8 weeks of TSA treatment in MI mice with or without c-kit deficiency, significantly improved neovascularisation and cardiac repair accompanied by cardiac functions and reduced cardiac remodelling can be observed in the wildtype infarcted heart compared to the c-kit-deficient mice [68]. It is also important to distinguish between the timing of the HDACi effects. Many reports show that long-term (8 weeks) administration of HDACi induces neovascularisation [67], while acute treatments (12 h) with HDACi inhibit angiogenesis [66].

6.3. Cardiac hypertrophy

Cardiac hypertrophy is a form of remodelling and is an adaptive response to the request for high workload from peripheral tissue or from intrinsic underlying disease conditions such as valvular dysfunction, hypertension, and MI. The heart responds to stresses by undergoing a remodelling process that is associated with myocyte hypertrophy, myocyte death, inflammation, and fibrosis, which often result in impaired cardiac function and heart failure. These are accompanied by activation of the myocyte enhancer factor-2 (MEF2) transcription factor and reprogramming of cardiac gene expression. Recent studies have revealed key roles for HDACs as both positive and negative regulators of pathological cardiac remodelling (Figure 2).

Members of MEF2 transcription factors family are some of the key regulators of myocardial hypertrophy. The first connection between HDACs and the regulation of pathological cardiac remodelling was provided by the discovery that class IIa HDACs interact with members of
MEF2 transcription factor family [69]. The transcriptional activity of MEF2 factors is upregulated in response to pathological stress in the heart, and ectopic overexpression of constitutively active forms of MEF2 in mouse heart causes dilated cardiomyopathy. It was reported that class II HDACs are substrates for a stress-responsive kinase specific for conserved serines that regulate MEF2-HDAC interactions. Those kinases phosphorylate the signal-responsive sites in class II HDACs, and mutant proteins lacking these phosphorylation sites can act as signal-resistant repressors of cardiomyocyte hypertrophy and fetal cardiac gene expression in vitro [70]. These studies support a role for class IIa HDACs as endogenous repressors of cardiac hypertrophy. Conversely, the function of class IIb HDACs in the heart remains largely unknown in heart hypertrophy.

Nevertheless, administration of HDACi TSA 2 weeks after the induction of pressure overload can reverse cardiac hypertrophy in mice [71]. The class I selective HDACi MPT0E014 also improves cardiac contractibility and attenuates structural remodelling in isoproterenol-induced dilated cardiomyopathy [72]. As there is an intrinsic relationship between decreased
capillary density and the transition of cardiac hypertrophy to cardiac failure [73], it remains to be investigated whether the cardioprotective effects exerted by HDACi are related to increased angiogenesis within the hypertrophic heart.

6.4. Peripheral artery disease

Peripheral artery disease (PAD) can be defined as the narrowing of the peripheral arteries that are not directly linked to the supply to the heart or the brain. PAD development is a multifactorial process with many different forms [74].

Different action mechanisms have been proposed for different HDACi in terms of regulating angiogenesis in the case of vascular diseases. It was reported in a mouse model of hindlimb ischaemia that the inhibition of class IIa HDACs is pro-angiogenic while class I HDAC inhibition is anti-angiogenic in mouse models of hindlimb ischemia [75].

6.5. Stroke

Stroke is a devastating illness and the second cause of death and disability worldwide after cardiac ischemia. A stroke occurs when a blood vessel that carries oxygen and nutrients to the brain is either blocked by a clot or bursts. As a consequence, part of the brain can die. Post-mortem studies have revealed that angiogenesis can be observed several days after cerebral ischemic stroke; it is noteworthy that higher microvessel density correlates with longer patient survival [76]. Enhanced angiogenesis facilities neurovascular remodelling processes and promotes brain functional recovery after stroke.

There are several studies testing the effects of HDACi in neurovascular remodelling processes and in brain functional recovery after stroke. Sun et al. showed that VPA treatment enhanced post-ischemic angiogenesis by increasing microvessel density, facilitating EC proliferation, and up-regulating rate of cerebral blood flow in the ipsilateral cortex. These events may be associated with up-regulation of HIF-1α and its downstream proangiogenic target VEGF as well as extracellular MMP2/9 [77]. Similar results were obtained by treating rats with VPA during permanent middle cerebral artery occlusion (pMCAO). They exhibit reduced infarct volume, promote functional recovery, enhance angiogenesis by upregulating VEGF [78], and reduce monocytes infiltration [79]. SIRT1 is proangiogenic and increases EC tube formation, especially in post-natal angiogenesis [46]. So loss of SIRT1 reduces angiogenesis and increases brain infarction, while SIRT1 was also demonstrated to play an important role in neuroprotection against brain ischemia by deacetylation and subsequent inhibition of p53-induced and nuclear factor κB-induced inflammatory and apoptotic pathways [80].

After pMCAO, sodium butyrate and TSA induce neurogenesis via HDACi in multiple ischemic brain regions in rats. Sodium butyrate also strongly upregulated VEGF, increasing angiogenesis and functional recovery after stroke. It was also described that sodium butyrate exhibits neuroprotective/neurogenic effects in rat model of neonatal hypoxia-ischemia [81]. All these results highlight that the inhibition of HDAC in brain after stroke enhances angiogenesis, and this may contribute to the long-term functional recovery after stroke.
6.6. HDACs role in angiogenesis in diabetes

Diabetes mellitus is a chronic disease where the lack of insulin leads to anomalies in the substrate metabolism, causing a range of acute and long-term complications. One of the main complication is the loss of small blood vessels. Another related secondary disease is diabetic glomerulomegaly or kidney disease. One of the predominant feature of diabetic glomerulomegaly is an increase in glomerular capillary volume [82], which can be controlled by anti-angiogenic therapies. As there is evidence of genetic association between diabetes and HDACs, treatment with HDACi exerts a reduction in glomerular endothelial markers expression, which demonstrates the anti-angiogenic benefit [83]. This effect seems to be opposite when it applies to the diabetic heart failure model, as another HDACi sodium butyrate exerts improved cardiac functions and increased microvessel density within the diabetic myocardium [84]. Moreover, HDACi also modulates cardiac peroxisome proliferator-activated receptors (PPARs) and fatty acid metabolism in diabetic cardiomyopathy [85].

7. Pathogenic role of angiogenesis

7.1. Cancer

There are more than 200 different kinds of cancers, and each type behaves and responds to treatments in different ways. Epigenetic enzymes are dysregulated in tumours through mutation or altered expression. More importantly, tumourigenesis is largely due to overexpression of oncogenes or the loss of function of tumour suppressor genes. The identification of these proteins has driven the rapid development of small-molecule inhibitors.

As we mention above, the function of HDACs is not solely on modifying histones, but they can also target many different cellular substrates and proteins, including those that are involved in tumour progression. Currently, many HDACi are in clinical trials for cancer therapeutics as HDACi result in hyperacetylation (and therefore repression) of genes related to tumour cell apoptosis, growth arrest, senescence, differentiation, cell invasion, and metastasis [86].

An exemplary role of HDACi play in modulating the tumour cells directly is its action on vasculogenic mimicry (VM). VM refers to the process by which highly aggressive tumour cells mimic ECs to form vessel-like structures that aid in supplying enough nutrients to rapidly growing tumours [87]. HDAC3 has demonstrated an important facilitative role on VM in gliomas, as HDAC3 expression is directly correlated with the number of VM in tumours with worsen tumour grade [88]. HDACi such as SAHA exert significant anti-VM effect in the progressive pancreatic cancer cells through its inhibition of AKT and ERK signalling pathways [89].

The role of HDACs play in tumour angiogenesis has also been studied. It is widely known that hypoxia induces tumour angiogenesis and cell survival through the up-regulation of VEGF expression in tumour cells [90]. Different studies have reported that inhibition of HDAC activity by TSA blocks hypoxia-induced tumour angiogenesis [91]. Other HDACi also exert similar effects, as exemplified by MPT0G157, a potent inhibitor of HDAC1, 2, 3, and 6,
which was found to promote HIF-1α degradation followed by the downregulation of VEGF expression [92]. There are also reports of the anti-tumoural effects of other HDACi (TSA, sodium butyrate, and VPA) that are also partly mediated by the reduction of VEGFR-2 expression that might be related to repressing tumour angiogenesis [93].

SIRT1, a class III HDAC, also plays an important role in tumour initiation, progression, and development of drug resistance by hindering senescence, stress-induced apoptosis [94, 95], and activating cell growth and angiogenesis. MiR-34a, whose expression level was found to be reduced in various tumour cell lines [96, 97], was reported to exert its tumour suppression effect via direct binding onto SIRT1 mRNA and regulate cell apoptosis via SIRT1-p53 pathway [98]. MiR-34a also exerts its anti-tumoural effect through inhibiting SIRT1 to induce the senescence of EPCs to suppress EPC-mediated tumour angiogenesis [99].

There are emerging HDACi for cancer therapy. HDACi-targeting class I, II, and IV HDACs to be used as anticancer agents are currently under development. One of them, vorinostat, has been approved by FDA for treating cutaneous T-cell lymphoma for patients with persistent or recurrent disease or following two systemic therapies. Other inhibitors, for example, FK228, PXD101, PCI-24781, ITF2357, MGCD0103, MS-275, valproic acid, and LBH589 have also demonstrated therapeutic potential as monotherapy or combination with other anti-tumour drugs [86, 100].

7.2. Age-related macular degeneration

Age-related macular degeneration (AMD) is the leading cause of blindness worldwide. AMD is characterised by the deposition of drusen aggregates under the retinal epithelium. Clusterin is one of the major proteins in drusens [101], and during aging, the expression of clusterin increases [102]. The impact of epigenetic modifications on the pathogenesis of AMD has been reported. It is known that aging affects histone acetylation status, so it is reasonable to presume that the epigenetic regulation might have a role in clusterin expression. It was reported that the treatment with HDACi induces prominent increases in the expression levels of clusterin mRNA and the secretion of clusterin protein. This result indicates that epigenetic factors regulate clusterin expression which could be affecting the pathogenesis of AMD via the inhibition of angiogenesis and inflammation [103].

7.3. Pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) is a condition characterised by increased pulmonary vascular resistance and pulmonary artery pressure leading to right heart failure and premature death [104]. During the process, there is a vascular remodelling caused by dysregulated cell proliferation, migration, and survival. The cause of PAH is complex, but the excessive proliferation of SMCs and ECs within the pulmonary artery is thought to play an essential role in its pathogenesis.

Elevated levels of HDAC1 and HDAC5 have been observed in the PAH lungs, and treatments with HDACi such as SAHA and VPA reduce disease worsening in rat models of pulmonary hypertension [105]. In addition, MEF2 might have a protective role in PAH progression as the expression of MEF2 and its transcriptional targets are significantly decreased in pulmo-
nary artery ECs from patients with PAH. The impaired MEF2 activity in ECs from PAH was associated with increased nuclear accumulation of HDAC4 and HDAC5. So, increasing MEF2 activity by the selective inhibition of class Iia HDACs by MC1568 seems to suppress excessive EC migration and proliferation by PAH-ECs and can rescue experimental PAH model [106]. Although the increased migration and proliferation of pulmonary artery ECs in PAH are also hallmarks of angiogenesis, it is still contentious to link excessive angiogenesis with the pathogenesis of PAH [107], and any potential anti-angiogenic therapy for PAH should be proceeded with caution.

8. Concluding remarks and future perspectives

The past 15 years of research have significantly advanced our understanding of the functions and modes of regulation of HDACs in CVD. With all the studies discussed above, we can get an idea about how complex it is to translate HDACi as clinical therapeutics as they exert contradictory functions in many occasions. Extensive evidence for HDAC involvement in multi-protein complexes and cell-specific signalling indicates that a deeper understanding of these pathways will be crucial to effective pharmacological targeting in future.

Although angiogenesis seems to be a very promising therapeutic possibility for the majority of CVDs where patients are not responding to conventional treatments, there are also times that angiogenesis participates in the pathological processes. So in some diseases such as MI and diabetic cardiomyopathy, enhancement of angiogenesis is beneficial by improving recovery of injured myocardium. In the other circumstances where aberrant neoangiogenesis is one of the main disease manifestations (such as cancer and AMD), potentiation of anti-angiogenic signalling could be beneficial. Thus, the crucial role that angiogenesis can play as a therapy can only be achieved by thoroughly understanding the underlying mechanisms.

In addition, the diverse and contrasting effects that the current available HDACi exert might be due to their low specificity to a particular HDAC. Class Iia HDACs are expressed in limited organs such as the muscles, brain, or bone, whereas class I HDACs exist ubiquitously. Thus, one may question the specificity and adverse effects of unspecific HDACi for therapeutic uses. Therefore in the future, creation of more specific HDACi, armed with better understanding of the underlying mechanisms of specific HDAC in angiogenesis within each pathological condition, could help the development of more targeted treatments to improve vascularisation and tissue repairs with higher efficiency and efficacy.

Alternative methods where HDAC modulation can be utilised in therapeutic angiogenesis are to modulate endothelial differentiation of stem or progenitor cells, which can be applied as cell therapy to enhance angiogenesis within the ischemic tissues. Next-generation gene-editing tool, such as CRISPR-Cas9, can also be extremely useful in accurately targetting specific gene responsible for suppressing or exacerbating angiogenesis depending on the diseases. Moreover, with diseases such as PAH that are characterised by both lack of angiogenesis (within the right ventricles) and excessive angiogenesis (within the pulmonary vasculature), the development of nanoparticles to deliver drugs to specific target tissues can be highly ben-
ecial. This approach will also be extremely useful for patients that manifest both CVD and cancer.

We are currently in an exciting era for translational research with a lot of new inspiring technologies that can truly transform therapeutic approaches. With diligent efforts to devise the role of HDACs underlying angiogenesis robustly in various CVDs, in conjunction with the creation of more selective HDAC inhibitors, advanced engineering solutions, and gene-editing tools to correct genes responsible for repressing angiogenesis, and a commitment in rigorous placebo-controlled clinical trials, superior therapies for CVDs are on the horizon.

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