Title:
The relevance of epigenetics to occlusive cerebral and peripheral arterial disease.

Authors:
Smriti Murali Krishna[1*], Alexandra Fay Trollope[1*], Jonathan Golledge[1, 2].

* First two authors contributed equally.

Affiliations:

[1]Queensland Research Centre for Peripheral Vascular Disease, College of Medicine and Dentistry, James Cook University, Townsville, QLD, Australia.

[2]Department of Vascular and Endovascular Surgery, The Townsville Hospital, Townsville, QLD, Australia.

Corresponding Author:

Professor Jonathan Golledge,
The Vascular Biology Unit,
Queensland Research Centre for Peripheral Vascular Disease,
College of Medicine and Dentistry,
James Cook University Townsville,
QLD, Australia 4811.
Fax +61 7 4433 1401
Telephone +61 7 4433 1417
Email: jonathan.golledge@jcu.edu.au

Key words: Peripheral arterial disease, atherosclerosis, ischemia, epigenetics

Words in abstract : 197
Number of Figures : 3
Number of Tables **:** 5

Running title: Epigenetics of occlusive peripheral arterial diseases

Table of Contents

Abstract

Athero-thrombosis of the arteries supplying the brain and lower limb are the main causes of stroke and limb loss. New therapies are needed to improve the outcomes of athero-thrombosis. Recent evidence suggests a role for epigenetic changes in the development and progression of ischemic injury due to atherosclerotic occlusion of peripheral arteries. DNA hypermethylation has been associated with cardiovascular diseases. Histone post-translational modifications have also been implicated in atherosclerosis. Oxidised low density lipoprotein regulated pro-inflammatory gene expression within endothelial cells is controlled by phosphorylation/acetylation of histone H3 and acetylation of H4 for example. There are a number of challenges in translating the growing evidence implicating epigenetics in atherosclerosis to improved therapies for patients. These include the small therapeutic window in conditions such as acute stroke and critical limb ischemia, since interventions introduced in such patients need to act rapidly and be safe in elderly patients with many co-morbidities. Preclinical animal experiments has also reported conflicting effects of some novel epigenetic drugs, which suggest that further in-depth studies are required to better understand their efficacy in resolving ischemic injury. Effective ways of dealing with these challenges are needed before epigenetic approaches to therapy can be introduced into practice.

Abbreviations

CAD	Coronary artery disease
CVD	Cerebrovascular disease
TIA	Transient ischemic attack
PAD	Peripheral arterial disease
CLI	Critical limb ischemia
ABI	Ankle-brachial systolic pressure index
5meC	5-methylcytosine
DNMTs	DNA methyl-transferases
MBD2	Methyl-CpG-binding domain protein 2
PTMs	Post translational modifications
HAT	Histone acetlytraferases
HDACs	Histone deacetylases
MCAO	Middle cerebral artery occlusion
TSA	Trichostatin A

1. Introduction

Atherosclerosis commonly affects the arteries supplying the heart, brain and peripheries which are commonly referred to as coronary artery disease (CAD), cerebro-vascular disease (CVD) and peripheral arterial disease (PAD), respectively. Most patients have atherosclerosis involving more than one site. Common causes of death listed for patients with PAD are CAD (40-60%) and CVD (10-20%) for example [1].

Clinical presentations of CVD include transient ischemic attack (TIA) and stroke. Stroke is a clinical syndrome characterized by an acute loss of neurological function, with symptoms lasting greater than 24 hours [2]. TIA is a more transient (lasting <24 hours) neurological dysfunction [2]. Stroke is the second leading cause of death and long-term disability world-wide according to the World Health Organisation statistics [3]. Ischemic stroke and TIA are usually secondary to thrombosis or embolization of the arteries supplying the brain. The impact of such athero-thrombo-embolism depends in part on the site of the arterial occlusion and the collateral blood supply to the part of the brain affected. The patency of the circle of Willis plays an important role in determining the effect of occlusions of the basilar and carotid arteries for example. The main current therapy for stroke is clot busting medicine or thrombolysis however this has to be administered within a few hours of the stroke to have any value and in some patients has limited benefit.

Common clinical presentations of PAD include pain in the leg on walking, known as intermittent claudication (IC), and rest pain or gangrene, often referred to collectively as critical limb ischemia (CLI), when the viability of the limb is at risk [4]. The main therapies used to treat IC and CLI are endovascular or surgical revascularization, however, these interventions are frequently ineffective at achieving long term functional improvement in the limb. Amputation is the only option for those patients with severe progression of limb ischemia where revascularisation is not possible [5, 6]. The incidence of lower limb amputation varies from approximately 120 to 500/million/year [7].

There is an urgent need for alternative therapies for patients with CVD and PAD. The complications of CVD and PAD largely result from tissue ischemia. Therapies that are successful in resolving this ischemic insult could potentially benefit both these diseases. Improved outcomes of arterial occlusions could result from growth of pre-existing collateral networks and also increased number and quality of newly formed smaller collateral vessels [8]. Factors contributing to the development of arteriolar collateral networks and other responses to ischemia are poorly understood. Improved understanding of the responses to ischemic injury is needed in order to design novel therapies for CVD and PAD.

Heritability estimates suggest that inherited genetic factors accounts for approximately 30% of the variation in the incidence of most cardiovascular diseases [9]. The remaining variation is believed to be explained by life style factors such as dietary and exercise habits, environmental exposure to toxins, and drug usage. The role of epigenetics in determining a range of processes that are believed to be critical in the development and outcome of CVD and PAD is being increasingly appreciated [10]. Epigenetics has been implicated in influencing atherosclerosis, angiogenesis, ischemia-reperfusion damage, and the cardiovascular response to hypoxia and fluid shear stress. In this review, we first briefly describe epigenetic control of gene expression, then outline evidence linking epigenetics with CVD and PAD and describe how epigenetics may influence processes important in complications and outcomes of CVD and PAD.

2. Epigenetic regulation of gene expression

Epigenetic variation can be acquired or inherited and constitute a means by which interactions between genes and environment can occur. Three important epigenetic regulators of gene expression are controlled by: (i) methylation of CpG islands, mediated by DNA methyltransferases (DNMTs), (ii) histone post translational modifications (PTMs) and (iii) microRNAs (miRNAs) **(Fig 1)**. These epigenetic regulators can control expression of various genes ultimately leading to gene activation or gene silencing **(Table 1)**.

A key element of such epigenetic control is that these regulators are mostly dynamic and therefore can potentially be manipulated to prevent or reverse the effects of aberrant expression of genes. The expression of a range of specific genes has been shown to be under epigenetic control and can potentially influence a variety of processes important in CVD and PAD complications and outcomes **(Table 2)**.

(i) DNA methylation

The most studied aspect of epigenetics is methylation of DNA at CpG islands. Methylation within the promoter is generally considered to represent an inactive or silenced gene. As a result, the DNA structure becomes more compact which is thought to prevent the necessary transcription factors accessing their binding sites on the DNA required for gene activation. DNA methylation involves the covalent addition of a methyl group to the 5th position in the 6-atom ring of the nucleotide cytosine (5-methylcytosine; 5meC). A group of enzymes known as DNMTs add the methyl group to the cytosine while the process of removing the methyl group (often referred to as a mark) is more complex. *Bird et al* demonstrated for the first time that the methyl group on DNA can be removed enzymatically by Methyl-CpG-binding domain protein 2 (MBD2) [40]. Alternatively the methyl group can be removed by DNA excision and repair where the methyl cytosine is excised and replaced by an unmodified cytosine [41]. This demonstrates that DNA methylation is not static and can be manipulated during gene regulation [42]. DNMTs can be subdivided into 2 groups. During maintenance methylation DNMT1 copies the methyl group onto newly synthesized DNA (hemi-methylated) after replication, while during *de novo* methylation DNMT 3a and 3b add novel methylation marks to the DNA. DNA methylation can result in gene repression by physically compacting the chromatin and excluding transcription factors or by methyl binding proteins (MBPs) that bind methylated DNA and subsequently recruit histone deacetylases (HDACs) and co-repressors.

(ii) Histone post-translational modifications

Histone regulation of gene transcription is more complex due to the different types of PTMs. The main ones are acetylation, phosphorylation and methylation [43] (**Table 1**). The effect that these modifications have on gene regulation can depend on which residue has been modified and to what extent. Combinations of the various histone marks can provide binding sites for the effector proteins that generate folding of the chromatin which ultimately lead to gene regulation generally referred to as the 'histone code'[44].

(a) Histone acetylation

Histone acetylation is by far the most studied PTM and is currently the best understood. Histone acetylation occurs at lysine residues and it changes the charge of the histone from

positive to neutral and therefore weakens the interaction between the histones along the chromatin to 'relax' and become more open. The acetylation is regulated by histone acetyl transferases (HATs) and deacetylation is regulated by HDACs. The recruitment of HDACs to specific regions of genes leads to a decrease in acetylation, chromatin condensing, and gene silencing. HDACs exist in two different classes. Class I comprises of HDACs 1-3 and 8, and Class II comprises of HDACs 4–7, 9 and 10. Each class of HDACs interacts with specific chromatin factors and repressor molecules and thus have slight differences in their actions [45].

(b) Histone methylation

Histone methylation occurs on lysine or arginine residues and unlike acetylation and phosphorylation, it does not change the charge of the histones. The degree of methylation varies. Lysine residues can be mono-, di- and tri-methylated whereas arginine residues can be mono- or di-methylated symmetrically or asymmetrically. Histone methylation is associated with both transcriptional activation and repression. H3K4me2/3 are marks that are generally associated with gene activation [46] while H3K9me3 is a mark of inactivation, these marks can also be tissue dependent. For example increased α-myosin heavy chain gene (α-MHC) expression requires H3K4me2 in the cardiac cells of the left ventricle in mice however, this histone mark is not required in the cardiac cells of the right ventricle demonstrating the specificity of the histone marks within tissues [47]. Smooth muscle cells (SMCs) cultured from db/db mice have an atherogenic and inflammatory phenotype associated with low levels of H3K9me3 at key inflammatory gene promoters [48].

(c) Histone Phosphorylation

Histone phosphorylation occurs on serine and threonine and like acetylation, the phosphorylation marks change the charge of the histone proteins from positive to negative. This mark is mostly associated with transcriptional repression however it can also be found on active chromatin alongside activation marks such as histone H3 acetylation/ phosphorylation (H3K4ac/S10p).

(iii) microRNAs

Non-coding RNAs (ncRNAs) are functional RNAs that are not translated into proteins. ncRNAs has been implicated in the epigenetic regulation of gene expression. Micro RNAs (miRNA) are small trans-acting ncRNAs that are first transcribed from the genome by RNA polymerase II as pri-miRNAs. These pri-mRNAs are cleaved by an enzyme complex comprised of Drosha (RNase III endonuclease) and the dsRNA binding protein Pasha. This results in a 70-100 nucleotide long hair-pin shaped precursor miRNA which is transported to the cytoplasm from the nucleus. In the cytoplasm precursor miRNAs are then cleaved by endoribonuclease III enzyme Dicer into around 18-22 nucleotide long fragments to form shorter transient, non-coding strands known as miRNAs. These small interfering RNA (siRNA) then assemble with the RNA-induced silencing (RISC) complex. The RISC complex degrades specific mRNA resulting in the suppression of target genes [49]. A single miRNA is capable of regulating a large number of mRNAs simultaneously [50], mainly by destabilising the target mRNAs and subsequently resulting in their destruction or by translation inhibition [51]. miRNAs control gene expression by directly targeting the gene promoters. A good example of this is miRNA-373 that was shown to be required for E-cadherin induction in cell culture [52].

Recent evidence suggests that miRNAs can also directly interact with chromatin and chromatin modifying complexes [53]. Thus, overlap and cross-talk exist between the various epigenetic pathways (**Fig 1**). For example, it has been shown that administration of a HDAC inhibitor (HDACi) not only increases histone acetylation but also effects histone methylation levels and miRNA pathways [54-56]. miRNAs have been shown to regulate epigenetic factors such as methyl binding CpG protein 2 (MECP2), which binds to methylated DNA and represses transcription of genes, dysregulation of this protein can lead to pathologies such as Rett syndrome [57]. Thus, it is well appreciated that the epigenetic system is a self-organising network that regulates the context dependent expression of myriads of genes by the co-ordinated effects of the entirety of epigenetic control mechanisms.

3. Evidence for the involvement of epigenetics in the pathology of CVD and PAD

(a) Atherosclerosis and laminar flow

Atherosclerotic occlusions usually occur in regions of disturbed blood flow near arterial branches. Endothelial cells (ECs) and SMCs are constantly exposed to haemodynamic forces, including blood flow-induced fluid shear stress and cyclic stretch. These forces modulate vascular cell gene expression as well as function and are implicated in vascular pathology [58]. Disturbed blood flow is associated with atherosclerosis development.

An important anti-inflammatory athero-protective transcription factor, Kruppel-Like Factor 4 (KLF4), was recently reported to be down regulated in regions of disturbed flow (**Table 3**). *In vitro* investigations suggested that disturbed flow leads to increased CpG island methylation in KLF4 leading to increased expression of downstream KLF4 transcription target genes such as endothelial nitric oxide (eNOS), thrombomodulin, and monocyte chemotactic protein-1 (MCP-1) [62]. Arterial tissue isolated from regions of disturbed flow in pigs had significantly lower expression of KLF4 and eNOS, and a hypermethylated myocyte enhancer factor-2 binding site in the KLF4 promoter [62]. Recently, it was also shown that disturbed flow regulates genome-wide DNA methylation patterns in a DNMT-dependent manner in studies using a partial carotid ligation murine model [19]. The reduction of DNMT1 activity by 5Azacytidine (5-Aza) or siRNA markedly attenuated endothelial inflammation. Furthermore, disturbed flow resulted in hypermethylation of several crucial mechano-sensitive genes, including HoxA5 and KLF3 whose expression was subsequently restored by 5-Aza administration [19]. These data suggest that disturbed flow controls the epigenetic phenotype of the vascular endothelium and thereby promotes atherosclerosis.

Research examining epigenetic mechanisms that may contribute to vascular remodelling in response to fluid sheer stress is limited [67] (**Table 3**). Laminar shear stress is an important modulating factor of eNOS expression in ECs and stimulates activating histone (H3 and H4) acetylation marks [68]. The activation of these histone acetylation marks is mediated via the p300 HAT complex, at a shear stress reporter element in the human eNOS promoter in the EC [68]. The sheer stress induced-epigenetic activation marks relaxes the chromatin complex keeping it open to allow constitutive expression of the eNOS gene. HDAC activity is believed to play a significant role in determining the severity of ischemia reperfusion damage, and Tricostatin A (TSA) mediated inhibition of HDAC activity in cultured embryonic stem cells was found to stimulate myogenesis and formation of collaterals [69].

These reports show that both elevated fluid sheer stress and disturbed flow, which are associated with atherosclerosis development and progression, induce epigenetic changes in the vasculature.

(b) Hypoxia

Hypoxia secondary to arterial occlusion plays a central role in initiating collateral formation by altering cell metabolism, changing cell growth, inducing angiogenesis, and increasing cell motility. Hypoxia modulates an array of genes mainly through the activation of a transcriptional complex named hypoxia-inducible factor (HIF)-1α [70] (**Table 3**). Hypoxia also induces the expression of HDAC leading to down regulation of the expression of numerous genes [71]. Recent findings indicate that several types of HDACis disrupt the function of HIF [72]. For example, hypoxia-induced histone modifications have been reported in both hypoxia-activated and hypoxia-repressed genes [73]. Certain histone demethylases and HMTs have been identified as HIF-1-regulated genes, including JMJD1A, JMJD2B, JARID1B, and G9a histone methyltransferase [74-79]. Additionally, it was found that hypoxia promotes removal of repressive histone marks such as H3K4 demethylation, H3K9 methylation, and H3K9 deacetylation, at the promoter region of vascular endothelial growth factor (VEGF) which is a crucial hypoxia-inducible gene [80]. Further studies using clinical samples from ischemic tissues are necessary to assess the role of hypoxia in CVD and PAD. Recent evidence suggests the direct role of HIF-1α in the upregulation of placental growth factor (PlGF) gene expression in cultured human and mouse ECs exposed to hypoxia through chromatin remodelling [81]. Brahma-related gene 1 (Brg1) is another gene implicated in the epigenetic response to hypoxia. Hypoxia induced Brg1 upregulation promotes cell adhesion molecule expression promoting leukocyte recruitment, endothelial dysfunction and inflammation in mice [82]. Brg1 has been suggested as a potential therapeutic target in CVD and PAD [83].

4. Evidence for the role of epigenetics in CVD

The main model of ischemic stroke used in pre-clinical animal studies relies on middle cerebral artery occlusion (MCAO). MCAO can be achieved by inserting a silicon-coated microfilament into the common carotid artery and advancing it along the internal carotid artery into the circle of Willis. Within the circle of Willis the filament blocks the origin of the middle cerebral artery and the resulting change in the blood flow can be monitored using laser doppler. MCAO can be either temporary (e.g. 30 minutes to 2 hours) or permanent. Permanent MCAO can be achieved by leaving the microfilament in place. Studies in this animal model suggest that a number of epigenetic drugs have benefits when used before and after ischemia injury. HDACi such as TSA, Valproic acid (VPA) or Sodium butyrate (SB) have been shown to reduce inflammation, apoptosis, and blood brain barrier (BBB) disruption, all of which are involved in the pathological progression of CVD. It has been shown that ischemia induces aberrant expression of genes [104] in part due to epigenetic changes. For example changes in DNA methylation have been associated with stroke in experimental models. Mice undergoing MCAO demonstrate increased genome-wide DNA methylation within their brain tissue. This change is believed to render the tissue more vulnerable to injury after ischemia [105]. Pharmacological inhibition of DNA methylation using the agent 5-Aza or a deficiency in DNMT1 gene expression significantly reduced ischemic injury in post-mitotic neurons resulting in neuronal protection after MCAO in mice [105]. A later study supported this finding by showing that DNMT1 conditional knockdown

mice which had decreased levels of DNMT1 in post-mitotic neurons were protected from cerebral infarction [106].

It has been shown that mouse forebrain ischemia-reperfusion can lead to genetic instability leading to mutations in the brain thought to be due to oxidative stress. After DNA damage has occurred, the DNA is eventually repaired through the cells base-excision repair (BER) pathway and then re-methylated by DNMT1 [107]. These observations suggest that a balance of DNA methylation, controlled by DNMT1, is essential in limiting cell death during cerebral ischemia.

Pharmacological manipulation of gene expression is a potential way to reduce post-ischemic brain damage and inhibition of HDACs has been suggested as a potential neuroprotective approach [89, 96]. A number of HDACis are being currently assessed as possible neuroprotective agents. The main suggested benefits of these drugs are reduction in inflammation, promotion of stem cell migration, reduction in neuron apoptosis, an increase in neurogenesis and angiogenesis, and preservation of the BBB. **Table 4** shows a list of HDACis currently used in the clinical setting and their effects. For example, VPA is widely used for a variety of seizure disorders and in certain cases for depression and anxiety and is a potent HDACi. It has been shown that one of the major consequences of cerebral ischemia is the reduction in histone H3 acetylation within the cortex and striatum in mice [93]. Administration of VPA after MCAO significantly reduced the infarct volume and resulted in an increase in H3K9 acetylation in a time-dependent manner. VPA administration also provided neuroprotection by inhibiting the expression of caspase-3, an enzyme that has been implicated in neuronal damage after focal cerebral ischemia, and increasing the expression of heat shock protein 70 (HSP70) [93], a protein that has been associated with neuroprotection [108].

5. Evidence for the role of epigenetics in PAD

Studies exploring the role of epigenetic mechanisms in the PAD are limited. There are many reports of successful revascularisation in CLI by novel regenerative cell therapies [109-111]. Pre-clinical studies using the most commonly used ischemic mouse hind limb model (which requires unilateral ligation and excision of the femoral artery) showed increased vascularity and blood flow after local gene transfer of VEGF-A. Thus, altering gene expression through epigenetic manipulation was proposed as a novel approach for the treatment of CLI. It was previously shown that lentivirus-mediated delivery of short hairpin RNAs (shRNA) targeted to specific regions in the VEGF-A promoter induced or repressed gene expression via histone modification [112]. Recently, *Palii et al,* showed that transcription factor T-cell acute leukemia 1 (TAL1) is a key mediator of the vascular repair function of primary human endothelial colony-forming cells (ECFCs) and that TAL1 upregulates the expression of genes promoting migration and adhesion through recruitment of the HAT p300 [113]. *Ex vivo* priming of ECFCs with TSA improved the revascularization efficiency of ECFCs showing that modifying the epigenome of stem/progenitor cells using small molecule epigenetic drugs is a potential way of enhancing their therapeutic potential before transplantation. Recently, a novel protein, prothymosin α (ProTα), was shown to be released extracellularly upon ischemia induced necrosis [114]. ProTα stimulates cell proliferation and differentiation through chromatin remodelling and was shown to be protective against ischemic stress. This observation suggests the possibility of involvement of other unknown proteins in epigenetic control of ischemic stress insults.

Even though HDACis are promising drugs for the treatment of several pathologies adverse side effects and cardiotoxicity have been reported in experimental models of PAD [115]. In a recent report, mice subjected to lower limb ischemia were injected with selective class I (DIs MS275) and IIa (MC1568) HDACis [115]. Between 14 and 21 days after ischemia, the MC1568 compound increased the number of newly formed muscle fibres but delayed their terminal differentiation. On the other hand MS275 abolished the early onset of the regeneration process resulting in atrophy and fibrosis [115]. Furthermore, the class selective HDACis had different effects. MC1568 promoted arteriogenesis whereas MS275 had an inhibitory effect. The HDACis had no effect on capillarogenesis, suggesting that class-selective HDACis interfere with normal mouse ischemic hind limb regeneration and suggesting caution in the application of these drugs. The HDACis were also reported to have contradictory effects on atherosclerosis and neointimal hyperplasia. For example, it was demonstrated previously that administration of TSA to low density lipoprotein receptor deficient (*Ldlr*$^{-/-}$) mice exacerbates atherosclerosis [58]. However, in a recent promising report a novel HDACi, MCT-3, was shown to attenuate neointimal hyperplasia in male FVB/N mice [116]. These reports emphasize the need for better understanding of epigenetic pathways involved in PAD and more comprehensive studies on the actions of different HDACis.

6. Epigenetic effects of risk factors for CVD and PAD

A number of risk factors for both CVD and PAD have been identified to have epigenetic effects. Examples are shown in **Table 5**.

Diabetes is an important risk factor for athero-thrombosis [117] and a number of studies suggest that dysglycaemia promotes epigenetic changes [118]. Elevated levels of homocysteine (Hcy) are also thought to cause epigenetic changes [119]. Hyperhomocysteinemia (HHcy) is associated with CVD and PAD [120-123] and a deficiency of folic acid has been previously linked to endothelial dysfunction, atherosclerosis, stroke and CLI [124]. HHcy is associated with decreased levels of global genome wide CpG methylation and promoter-specific gene methylation, promoting altered expression levels of a number of genes relevant to vascular damage [125-129]. Furthermore, HHcy also suppresses angiogenesis, promotes SMC proliferation, promotes dyslipidemia, stimulates vascular oxidative stress, and leads to impairment of EC regeneration and function [127, 128]. Advanced age is a major risk factor for CVD and PAD and aging is known to cause altered gene expression patterns due to epigenetic changes [130, 131]. It has been demonstrated that miRNAs play a role in cell senescence and that they can exert either positive or negative effects on cell growth depending on the genes they are regulating [132, 133]. Advanced age and ischemic injury have been related to aberrant miRNA transcription and altered expression of genes such as silent information regulator-1 (SiRT-1)[134, 135] (**Table 5**).

Hypertension has a strong association with CVD and PAD. Overexpression of the connective tissue growth factor (CTGF) has been found in a number of pathologies such as atherosclerotic lesions and can be induced by angiotensin II in models of hypertensive nephropathy and vascular fibrosis [136]. It has also been shown that hypermethylation of H3K79 at the CTGF promoter by the enzyme disruptor of teleomeric silencing-1 (Dot-1) results in gene silencing in the collecting ducts of the nephron [137] and the inhibition of CTGF has been shown to reverse the process of fibrosis [138].

Smoking is a major risk factor for CVD and PAD and has been implicated in inducing a number of epigenetic modifications [139]. Smoking induced epigenetic changes have been mainly associated with DNA methylation [140-142]. Prolonged smoking has been shown to increased gene promoter specific DNA methylation in the peripheral blood [143] as well as in the aero digestive tract and oral epithelium [144, 145] suggesting the profound impact of smoking on the epigenome. Tobacco abuse in the mother has also been shown to result in altered placental gene expression in addition to increased global DNA methylation levels [146, 147] suggesting the transgenerational effects of smoking.

7. The relevance of epigenetics to the effects of ischemia

The reduced blood flow associated with CVD and PAD can lead to progressive cell death and induce an inflammatory response which may exacerbate initial levels of tissue injury. Therapeutic revascularisation aims to increase collateral capacity (arteriogenesis) and new capillary formation (neo-angiogenesis) [8].

(a) Arteriogenesis

The formation of mature arteries from pre-existent interconnecting arterioles following an arterial occlusion is known as arteriogenesis. Even though arteriogenesis has many common features with angiogenesis, they follow different pathways. Arteriogenesis is potentially more able to compensate for an occluded artery than angiogenesis. Furthermore, arteriogenesis is induced by physical forces such as fluid shear stress and occurs in an environment of normoxia whereas angiogenesis is induced by hypoxia leading to formation of new capillaries [148]. Elevated fluid shear stress activates ion channels, releasing nitric oxide (NO) and initiates two major signalling pathways. One pathway originating as a result of shear stress, attracts bone marrow-derived stem cells and endothelial progenitor cells (EPCs) which are essential for structural remodelling and the other pathway results in the cell cycle entry of ECs and SMCs, leading to proliferation and differentiation [149].

(b) Neoangiogenesis

The process of formation of new blood vessels is called neoangiogenesis. It consists of several successive steps of vascular destabilization, angiogenic sprouting, formation of lumen and vascular stabilization. A number of growth factors and signalling pathways orchestrate angiogenesis through the direct or indirect regulation of quiescence, migration and proliferation of ECs [150, 151]. The majority of studies have focused on tumour angiogenesis, however additional insight is urgently required to improve our understanding about the role of genetic and epigenetic factors in angiogenesis in other diseases [152]. Gene or protein therapy delivering various growth factors such as VEGF, as well as cell therapy using EPCs, mesenchymal stem cells (MSCs) or induced pluripotent stem cells (iPSCs) have been developed as pro-angiogenic therapeutics for ischemic heart disease and PADs [151].

8. The relevance of epigenetics to the cellular response to ischemia

Epigenetic modifiers can influence various aspects of the results and response to ischemia by altering transcriptional regulation of cellular responses. The alteration in gene regulation may lead to a number of effects such as proliferation and differentiation of SMCs and ECs, attenuation of apoptosis and inflammation, improvement in blood flow and increased

plasticity of newly formed vessels. Thus, identifying the epigenetic contribution to ischemic injury and its resolution may influence drug development (**Fig 2**).

(a) SMC proliferation

SMC proliferation constitutes a central component of atherosclerosis formation and neointimal vascular remodelling. Histone acetylation constitutes a major epigenetic modification for the transcriptional cascade controlling proliferative gene expression in SMCs [153]. Pharmacological HDACis have been shown to arrest cell proliferation [154]. A novel non-selective HDACi, Scriptaid, has been shown to inhibit neointimal thickening in a mouse model [153]. Butyrate, a dietary HDACi, has been shown to stimulate PTMs of histone H3 and differently alters G1-specific cell cycle proteins leading to inhibition of SMC proliferation [155]. A recent study suggested the *in vitro* and *in vivo* potential of a novel HDACi, MCT-3, to modulate SMC migration, proliferation and neointimal hyperplasia [116]. MCT-3 administration induced histone H3 and H4 acetylation and regulation of expression of orphan nuclear receptor NUR77, plasminogen activator inhibitor type-1 (PAI-1) and cyclin dependent kinase inhibitors (CDKI) $p21^{CIP1/WAF1}$ and $p27^{KIP1}$. However, additional pre-clinical studies are necessary to determine the potential clinical utility of this novel compound [116] due to previous contradictory reports about the nonspecific effects of HDACis. Previously, the specific HDACi TSA was shown to inhibit SMC proliferation via induction of $p21^{CIP1/WAF1}$ [60]. However, in a subsequent study using $Ldlr^{-/-}$ mice, TSA exacerbated atherosclerosis and modulated expression of oxLDL receptors and some proatherogenic genes [59].

(b) SMC differentiation

Many of the pathways involved in SMC differentiation leading to vascular remodelling in adult are similar to those involved in embryogenesis [156]. A recent study assessed the role of neural crest-derived SMC lineage in formation of the cardiac outflow tract in mice [157]. The HDAC3 (class I HDAC) blocks SMC differentiation and is associated with downregulation of the Notch ligand Jagged1, a key driver of SMC differentiation. In a new *in vitro* organotypic model system for rat limb bud development the effect of the epigenetic drug 5-Aza was assessed [158]. Initially the limb buds were covered by immature epithelium and contained mesenchyme, and capillaries. However, following administration of 5-Aza, a decrease in cell proliferation was noted with disappearance of structures typical for vasculogenesis. This negative effect of an epigenetic modifying drug suggests that specific drugs have to be identified for each cell phenotype involved in the disease process. Recently a study compared the remodelling process in the femoral and internal carotid arteries within a novel mouse vascular injury model [159]. Compared to the remodelling in the femoral artery, intracranial internal carotid arteries showed a unique remodelling pattern. The formation of neointima was delayed and showed continuous macrophage accumulation, loss of SMCs followed by medial thinning and continuous expansion of the adventitia [159]. These findings suggest that pathophysiological differences exist in different peripheral arteries which are likely controlled by different genetic and epigenetic pathways.

(c) EC differentiation

Angiogenesis involves complex and highly dynamic interactions between ECs and their environment that promote endothelial motility, filopodia extension, proliferation and the formation of new cell-cell junctions. Lysine acetylation and cytosine methylation are

important transcriptional regulators of angiogenic genes in ECs such as VEGF and eNOS [160]. Angiopoietin-2 induces vessel destabilization leading to formation of new vessels or to regression of existing vessels. Angiopoietin-2 promoter methylation has been associated with decreased gene expression [161]. Studies suggest that laminar shear stress is an important modulator of eNOS expression in ECs and stimulates activating H3 and H4 acetylation marks via the HAT p300 complex [68]. The shear stress induced epigenetic activation marks result in chromatin remodelling promoting constitutive eNOS expression. Recent studies have shown that protein kinase D (PKD) stimulates phosphorylation and nuclear export of HDAC5 in ECs and regulates cell migration [162]. Studies in human umbilical vein ECs (HUVECs) showed that PKD also stimulates pro-angiogenic Fibroblast growth factor bFGF expression through the HDAC5 signalling pathway. Age-related impairment of angiogenesis is likely to play a central role in the outcome of CVD and PAD. Aging-induced dysregulation of Dicer1 dependent miRNA expression impairs angiogenic capacity of rat cerebral microvascular ECs [163].

(d) Fibroblasts

Studies of fibroblasts suggest that epigenetic factors including DNA methylation and histone acetylation regulate wound healing [164-166]. MeCP2 plays a critical role in epithelial to myofibroblast transformation [164] and in many fibrotic disorders there is an imbalance in the histone acetylation and deacetylation [165]. Proliferation of fibroblast induced by platelet-derived growth factor (PDGF) is reduced by TSA [166].

9. The influence of exercise on epigenetics as relevant to CVD and PAD

Increased physical activity is associated with reduced risk of developing CVD and PAD. Sedentary lifestyle has been associated with an unfavourable epigenome [167]. A study by *Alibegovic, et al*. demonstrated that 9 days of bed rest lead to increased peroxisome proliferator-activated receptor gamma coactivator (PGC) -1α promoter methylation [168]. Physical activity can modify the genome in the peripheral vascular bed [169]. Exercise induces an increase in free radical production and concurrently improves antioxidative capacity, resulting in activation of signalling cascades that modulate the physiological adaptations of the peripheral vascular genome [10, 170]. For example, apoptosis associated spec-like protein (ASC) modulates the expression of pro-inflammatory cytokines such as IL-1β and IL-18. Chronic moderate exercise was shown to attenuate the age-dependent decrease in ASC promoter methylation, and thereby decrease pro-inflammatory cytokine expression [171].

Exercise training has been associated with an increase in the levels of global DNA methylation in peripheral blood cells, furthermore the level of DNA methylation increases with increasing amounts of exercise [172, 173]. A single intense exercise bout results in acute remodelling through promoter methylation of exercise-responsive genes within skeletal muscle. Muscle specific miRNAs called myomirs are also upregulated by a single bout of endurance exercise and reversibly down regulated by 12-week endurance training [174]. A study showed decreased whole genome methylation in skeletal muscle biopsies obtained from healthy sedentary men and women after acute exercise [175]. Exercise induced a dose-dependent expression with a marked hypomethylation on the promoter of several metabolic genes. Exercise training has also been shown to alter DNA methylation in a chronic way, in the skeletal muscles biopsies collected after a 6 months training program [176]. A previous study showed that the DNA methylation profiles of several genes involved in diverse

metabolic pathways were differentially methylated in response to chronic exercise. Furthermore, chronic exercise also led to increased DNA methylation of a large number of genes in adipose tissue [176]. On the contrary, an exercise intervention study in middle aged healthy, sedentary men showed that majority of the genes showed decreased DNA methylation within skeletal muscle [177], supporting the fact that epigenetic modifications are likely tissue and population specific.

(a) Exercise induced epigenetic alterations relevant to CVD

Previous literature suggests a role for exercise in promoting stroke rehabilitation, and indicates that exercise training protects neural cells from inflammation, apoptosis, and oxidative stress and can enhance choline acetyltransferase activity. Recent rodent animal model studies highlight the importance of exercise induced epigenetic modification in the brain [178-180]. Previously, voluntary exercise in adult rats was shown to increase acetylated H3 within the hippocampus [178]. In adolescent male mice, voluntary wheel running for 1 week resulted in increased global H3 acetylation in both the hippocampus and cerebellum. In contrast, the expression pattern of DNMTs and HDAC were decreased in both regions following exercise [179]. Similarly, in another study, a single exercise session decreased both DNMT3b and DNMT1 levels in young adult rats [180]. The exercise protocol was shown to reduce H3-K9 methylation levels in young adult rats, while the single session reversed the changes on H3-K9 methylation levels induced by aging [180]. Furthermore, a forced exercise protocol ameliorated aging-related memory decline, decreased pro-inflammatory markers and increased histone H4 acetylation levels in the hippocampus of 20-months-old rats [181]. A study investigating the effects of antecedent exercise on functional recovery following MCAO in adult male rats showed that antecedent treadmill exercise prior to focal cerebral ischemia had beneficial effects [182]. Antecedent exercise exerted neuroprotective effects against ischemic brain injury by improving motor performance and decreasing the levels of calpain gene expression. Together, these results suggest that an imbalance in the key epigenetic modulators might be linked to the capacity of brain to recover after ischemia induced by occlusion and that patient specific exercise rehabilitation has to be carefully tailored.

(b) Exercise induced epigenetic alterations relevant to PAD

Supervised exercise programs have been consistently demonstrated to improve walking time and walking distance in PAD patients [183, 184]. Supervised exercise leads to alterations in skeletal muscle gene expression. In addition exercise induced elevation of reactive oxygen species (ROS) are believed to be important in promoting angiogenesis [185]. Global lysine 36- H3 acetylation, a site associated with transcriptional elongation, was shown to be increased following 60 mins of cycling [186]. Furthermore, during exercise HDAC4 and 5 were exported from the nucleus, thereby removing their transcriptional repressive function. In addition, activation of two protein kinases that induce phosphorylation-dependent class IIa HDAC nuclear export, namely the AMP-activated protein kinase (AMPK) and the calcium-calmodulin-dependent protein kinase II (CaMKII), was also induced in response to exercise [186]. These protein kinases phosphorylate HDACs, promoting transcription factor binding and subsequent initiation of transcriptional activity of exercise-responsive genes such as myogenin, muscle creatine kinase (MCK) and glucose transporter 4 (GLUT4) [187, 188]. Furthermore, the effects of exercise training was blocked in transgenic mice showing overexpression of HDAC5 further suggesting that epigenetic transcriptomic regulation is important in controlling the effects of exercise [189].

Evidence for rapid and reversible DNA methylation in response to exercise is currently emerging [190]. The human ten eleven translocation (TET)-1 genes encodes an enzyme that catalyses the conversion of 5mC to 5-hydroxy methyl cytocine (5hmC), and plays a crucial role in active demethylation [191]. It has also been suggested that miRNA expression is differentially regulated in subjects that respond favourable and unfavourable to exercise suggesting that taking the epigenetic profile into consideration, will help the patients before subjecting them to exercise rehabilitation programs [192].

10. Future directions

Recently arginase 2 (Arg2), NO production and endothelial function were identified to be regulated by HDAC2 [193]. Thus therapeutic activation of HDAC2 represents a novel method of correcting endothelial dysfunction and atherosclerosis. Lysine acetylation of eNOS is a PTM. HDAC3 antagonizes aspirin-stimulated endothelial NO production by reversing aspirin-induced lysine acetylation of eNOS [194]. This interesting observation suggests that selective pharmacological HDAC inhibition may be a way to improve the efficacy of drugs such as aspirin in the treatment of stroke and PADs.

Exploring epigenetic mechanisms in pathologies is gaining more momentum within scientific research. The fact that the epigenetic modifications can be reversed makes them an ideal target for drug treatment. In the oncology field, epigenetic drugs have already entered the clinical arena and methylation patterns are used as biomarkers to subtype and stage various cancers [195, 196]. HDACis are being investigated in clinical trials for the treatment of solid and hematological malignancies [197, 198]. There are potential limitations in applying these drugs to CVD and PAD however. These medications lack specificity for example. HDACis also influence non-histone proteins making their effects widespread. Furthermore a lot of HDACs form co-repressor complexes in order to silence gene activity, which again amplifies the potential non-specific effect that HDACi technology can have within the cell.

The role of DNMTs, HDACs, HATs and miRNAs in transcriptional regulation is becoming increasingly clear. The complexity of the interactions however means applying findings in a safe way to patients is not straightforward Thus elucidating the cause and effect of these modifications depends on further through preclinical and clinical studies. It should also be noted that translating preclinical findings to clinical setting is also not simple, since the various components of the epigenetic machinery may be functionally different in animal models of CVD and PAD. Realistically, in order for epigenetic drugs to be applied to patients with CVD and PAD there is a need for more specific interventions which can be applied in a safe and effective way to patients with a number of co-morbidities (**Fig 3**). Such developments likely require improved understanding of the epigenetic mechanisms involved in these diseases.

Acknowledgement

a) Acknowledgments: None. b) Sources of Funding: This work is funded in part by grants from the National Health and Medical Research Council (1021416, 1020955, 1003707 and 1000967), the Queensland Government and the Townsville Hospital Private Practice Trust. JG holds a Practitioner Fellowship from the National Health and Medical Research Council, Australia (1019921) and a Senior Clinical Research Fellowship from the Queensland Government. The funding bodies played no role in generation of the data presented in this publication. c) Disclosures: None.

References:

1. Norgren, L., Hiatt, W.R., Dormandy, J.A., Nehler, M.R., Harris, K.A., Fowkes, F.G., and Group, T.I.W. (2007) Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). J Vasc Surg. **45** Suppl S: p. S5-67
2. Warlow, C., Van Gijn, J., Dennis, M., Wardlaw, J., Bamford, J., Hankey, G., Sandercock, P., Rinkel, G., Langhorne, P., Sudlow, C., Rothwell, P. (2008) Practical Management. 3rd ed. publishing, O.B
3. Mackay, J., Mensah, G. (2004) The global burden of cerebrovascular disease The Atlas of Heart Disease and Stroke, World Health Organization; 1 edition, ISBN-10: 9241562765
4. Arnold, T.E., Kerstein, M.D. (2000) Secondary distal extension of infrainguinal bypass: long-term limb and patient survival. Ann Vasc Surg. **14**, 5, 450-456
5. Lawall, H., Bramlage, P., and Amann, B. (2011) Treatment of peripheral arterial disease using stem and progenitor cell therapy. J Vasc Surg. **53**, 2. 445-453
6. Fadini, G.P., Agostini, C., and Avogaro, A. (2010) Autologous stem cell therapy for peripheral arterial disease meta-analysis and systematic review of the literature. Atherosclerosis. **209**, 1. 10-17
7. Norgren, L., Hiatt, W.R., Dormandy, J.A., Nehler, M.R., Harris, K.A., Fowkes, F.G., Group, T.I.W., Bell, K., Caporusso, J., Durand-Zaleski, I., Komori, K., Lammer, J., Liapis, C., Novo, S., Razavi, M., Robbs, J., Schaper, N., Shigematsu, H., Sapoval, M., White, C., White, J., Clement, D., Creager, M., Jaff, M., Mohler, E., 3rd, Rutherford, R.B., Sheehan, P., Sillesen, H., and Rosenfield, K. (2007) Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). Eur J Vasc Endovasc Surg. **33** Suppl 1. 61-75
8. Carmeliet, P. (2000) Mechanisms of angiogenesis and arteriogenesis. Nat Med. **6**, 4. 389-395
9. Baccarelli, A., Ghosh, S. (2012) Environmental exposures, epigenetics and cardiovascular disease. Curr Opin Clin Nutr Metab Care. **15**, 4. 323-329
10. Whayne, T.F. (2014) Epigenetics in the development, modification, and prevention of cardiovascular disease. Mol Biol Rep. PMID: 25205125
11. Allfrey, V.G., Faulkner, R., Mirsky, A.E. (1964) Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. Proc Natl Acad Sci U S A. **51**. 786-794
12. Gurley, L.R., Walters, R.A., Barham, S.S., Deaven, L.L. (1978) Heterochromatin and histone phosphorylation. Exp Cell Res. **111**, 2. 373-383
13. Ambros, V. (2004) The functions of animal microRNAs. Nature. **431**. 350-355
14. Mousa, A.A., Cappello, R.E., Estrada-Gutierrez, G., Shukla, J., Romero, R., Strauss, J.F., 3rd, and Walsh, S.W. (2012) Preeclampsia is associated with alterations in DNA methylation of genes involved in collagen metabolism. Am J Pathol. **181**, 4. 1455-1463
15. Dhar, K., Rakesh, K., Pankajakshan, D., Agrawal DK. (2013) SOCS3 promotor hypermethylation and STAT3-NF-κB interaction downregulate SOCS3 expression in human coronary artery smooth muscle cells. Am J Physiol Heart Circ Physiol. **304**, 6. H776-785
16. Sorianello, E., Soriano, F.X., Fernández-Pascual, S., Sancho, A., Naon, D., Vila-Caballer, M., González-Navarro, H., Portugal, J., Andrés, V., Palacín, M., Zorzano, A. (2012) The promoter activity of human Mfn2 depends on Sp1 in vascular smooth muscle cells. Cardiovasc Res. **94**, 1. 38-47
17. Zelko, I.N., Stepp, M.W., Vorst, A.L., and Folz, R.J. (2011) Histone Acetylation Regulates the Cell-Specific and Interferon-gamma-Inducible Expression of Extracellular Superoxide Dismutase in Human Pulmonary Arteries. Am J Respir Cell Mol Biol. **45**, 5. 953-961

18. Li, L., Xie, J., Zhang, M., and Wang, S. (2009) Homocysteine harasses the imprinting expression of IGF2 and H19 by demethylation of differentially methylated region between IGF2/H19 genes. Acta Biochim Biophys Sin (Shanghai). **41**, 6. 464-471
19. Dunn, J., Qiu, H., Kim, S., Jjingo, D., Hoffman, R., Kim, C.W., Jang, I., Son, D.J., Kim, D., Pan, C., Fan, Y., Jordan, I.K., Jo, H. (2014) Flow-dependent epigenetic DNA methylation regulates endothelial gene expression and atherosclerosis. J Clin Invest. **124**, 7. 3187-3199
20. Quentmeier, H., Eberth, S., Romani, J., Weich, H.A., Zaborski, M., Drexler, H.G. (2012) DNA methylation regulates expression of VEGF-R2 (KDR) and VEGF-R3 (FLT4). BMC Cancer. **12**,19. doi: 10.1186/1471-2407-12-19
21. Zhang, D., Chen, Y., Xie, X., Liu, J., Wang, Q., Kong, W., Zhu, Y. (2012) Homocysteine activates vascular smooth muscle cells by DNA demethylation of platelet-derived growth factor in endothelial cells. J Mol Cell Cardiol. **53**, 4. 487-496
22. Zhang, B.K., Lai, .Y.Q., Niu, P.P., Zhao, M., Jia, S.J. (2013) Epigallocatechin-3-gallate inhibits homocysteine-induced apoptosis of endothelial cells by demethylation of the DDAH2 gene. Planta Med. **79**, 18. 1715-1719
23. Fish, J.E., Matouk, C.C., Rachlis, A., Lin, S., Tai, S.C., D'Abreo, C., and Marsden, P.A. (2005) The expression of endothelial nitric-oxide synthase is controlled by a cell-specific histone code. J Biol Chem. **280**, 26. 24824-24838
24. Rao, X., Zhong, J., Zhang, S., Zhang, Y., Yu, Q., Yang, P., Wang, M.H., Fulton, D.J., Shi, H., Dong, Z., Wang, D., Wang, C.Y. (2011) Loss of methyl-CpG-binding domain protein 2 enhances endothelial angiogenesis and protects mice against hind-limb ischemic injury. Circulation **123**, 25. 2964-2974
25. Robinson, C.M., Neary, R., Levendale, A., Watson, C.J., Baugh, J.A. (2012) Hypoxia-induced DNA hypermethylation in human pulmonary fibroblasts is associated with Thy-1 promoter methylation and the development of a pro-fibrotic phenotype. Respir Res. **13**, 74. doi: 10.1186/1465-9921-13-74
26. Hu, B., Gharaee-Kermani, M.M., Wu, Z., Phan, S.H. (2011) Essential role of MeCP2 in the regulation of myofibroblast differentiation during pulmonary fibrosis. Am J Pathol. **178**, 4. 1500-1508
27. Hu, B, Gharaee-Kermani, M.M., Wu, Z., Phan, S.H. (2010) Epigenetic regulation of myofibroblast differentiation by DNA methylation. Am J Pathol. **177**, 1. 21-28
28. Klein, D., Benchellal, M., Kleff, V., Jakob, H.G., Ergün, S. (2013) Hox genes are involved in vascular wall-resident multipotent stem cell differentiation into smooth muscle cells. Sci Rep. **2178**, 3. doi: 10.1038/srep02178
29. Hu, C.J., Chen, S., Yang, D.I., Lin, T.N., Chen, C.M., Huang, T.H., Hsu, C.Y. (2006) Promoter region methylation and reduced expression of thrombospondin-1 after oxygen-glucose deprivation in murine cerebral endothelial cells. J Cere Blood Flow Metab. **26**,12. 1519-1526
30. Montes de Oca, A., Madueno, J.A., Martinez-Moreno, J.M., Guerrero, F., Munoz-Castaneda, J., Rodriguez-Ortiz, M.E., Mendoza, F.J., Almaden, Y., Lopez, I., Rodriguez, M., and Aguilera-Tejero, E. (2010) High-phosphate-induced calcification is related to SM22alpha promoter methylation in vascular smooth muscle cells. J Bone Miner Res. **25**, 9. 1996-2005
31. Siuda, D., Zechner, U., El Hajj, N., Prawitt, D., Langer, D., Xia, N., Horke, S., Pautz, A., Kleinert, H., Förstermann, U., Li, H. (2012) Transcriptional regulation of Nox4 by histone deacetylases in human endothelial cells. Basic Res Cardiol. **107**, 5. 283. doi: 10.1007/s00395-012-0283-3
32. Kumar, A., Kumar, S., Vikram, A., Hoffman, T.A., Naqvi, A., Lewarchik, C.M., Kim, Y.R., Irani, K. (2013) Histone and DNA methylation-mediated epigenetic downregulation of

endothelial Kruppel-like factor 2 by low-density lipoprotein cholesterol. Arterioscler Thromb Vasc Biol. **33**, 8. 1936-1942
33. Gomez, D., Coyet, A., Ollivier, V., Jeunemaitre, X., Jondeau, G., Michel, J.B., and Vranckx, R. (2011) Epigenetic control of vascular smooth muscle cells in Marfan and non-Marfan thoracic aortic aneurysms. Cardiovasc Res. **89**, 2. 446-456
34. Seo, J., Kim, Y.O, Jo, I. (2009) Differential expression of stromal cell-derived factor 1 in human brain microvascular endothelial cells and pericytes involves histone modifications. Biochem Biophys Res Commun. **382**, 3. 519-524
35. Harris, T.A., Yamakuchi, M., Ferlito, M., Mendell, J.T., Lowenstein, C.J. (2008) MicroRNA-126 regulates endothelial expression of vascular cell adhesion molecule 1. Proc Natl Acad Sci U S A. **105**, 5. 1516-1521
36. Zhu, N., Zhang, D., Chen, S., Liu, X., Lin, L., Huang, X., Guo, Z., Liu, J., Wang, Y., Yuan, W., Qin, Y. (2011) Endothelial enriched microRNAs regulate angiotensin II-induced endothelial inflammation and migration. Atherosclerosis. **215**, 2. 286-293
37. Kuehbacher, A., Urbich, C., Zeiher, A.M., Dimmeler, S. (2007) Role of Dicer and Drosha for endothelial microRNA expression and angiogenesis. Circ Res. **101**, 1. 59-68
38. Fasanaro, P., D'Alessandra, Y., Di Stefano, V., Melchionna, R., Romani, S., Pompilio, G., Capogrossi, M.C., Martelli, F. (2008) MicroRNA-210 modulates endothelial cell response to hypoxia and inhibits the receptor tyrosine kinase ligand Ephrin-A3. J Biol Chem. **283**, 23. 15878-15883
39. Chen, K.C., Wang, Y., Hu, C.Y., Chang, W.C., Liao, Y.C., Dai, C.Y., Juo, S.H. (2011) OxLDL up-regulates microRNA-29b, leading to epigenetic modifications of MMP-2/MMP-9 genes: a novel mechanism for cardiovascular diseases. FASEB J. **25**, 5. 1718-1728
40. Bird, A. (2002) DNA methylation patterns and epigenetic memory. Genes and Development. **16**. 6-21.
41. Tahiliani, M., Koh, K.P., Shen, Y., Pastor, W.A., Bandukwala, H., Brudno, Y., Agarwal, S., Iyer, L.M., Liu, D.R., Aravind, L., Rao, A. (2009) Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science. **324**, 5929. 930-935
42. Ramchandani, S, Bhattacharya, S., Cervoni, N., Szyf, M. (1999) DNA methylation is a reversible biological signal. Proc Natl Acad Sci U S A. **96**, 11. 6107-6112
43. Eom, G.H., Kook, H. (2014) Posttranslational modifications of histone deacetylases: Implications for cardiovascular diseases. Pharmacol Ther. **143**, 2. 168-180
44. Jenuwein, T., Allis, C.D. (2001) Translating the histone code. Science. **293**, 5532. 1074-1080
45. Pons, D., de Vries, F.R., van den Elsen, P.J., Heijmans, B.T., Quax, P.H., and Jukema, J.W. (2009) Epigenetic histone acetylation modifiers in vascular remodelling: new targets for therapy in cardiovascular disease. Eur Heart J. **30**, 3. 266-277
46. Santos-Rosa, H., Schneider, R., Bannister, A.J., Sherriff, J., Bernstein, B.E., Emre, N.C., Schreiber, S.L., Mellor, J., Kouzarides, T. (2002) Active genes are tri-methylated at K4 of histone H3. Nature. **419**, 6905. 407-411
47. Mathiyalagan, P., Chang, L., Du, X.J., El-Osta, A. (2010) Cardiac ventricular chambers are epigenetically distinguishable. Cell Cycle. **9**, 3. 612-617
48. Villeneuve, L., Reddy, M.A., Lanting, L.L., Wang, M., Meng, L., Natarajan, R. (2008) Epigenetic histone H3 lysine 9 methylation in metabolic memory and inflammatory phenotype of vascular smooth muscle cells in diabetes. Proc Natl Acad Sci U S A. **105**, 26. 9047-9052
49. Winter, J., Jung, S., Keller, S., Gregory, R.I., and Diederichs, S. (2009) Many roads to maturity: microRNA biogenesis pathways and their regulation. Nat Cell Biol. **11**, 3. 228-234

50. Lewis, B.P., Burge, C.B., and Bartel, D.P. (2005) Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell. **120**, 1. 15-20
51. Guo, H., Ingolia, N.T., Weissman, J.S., and Bartel, D.P. (2010) Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature. **466**, 7308. 835-840
52. Place, R.F., Li, L.C., Pookot, D., Noonan, E.J., and Dahiya, R. (2008) MicroRNA-373 induces expression of genes with complementary promoter sequences. Proc Natl Acad Sci U S A. **105**, 5. 1608-1613
53. Kim, D.H., Saetrom, P., Snove, O., Jr., and Rossi, J.J. (2008) MicroRNA-directed transcriptional gene silencing in mammalian cells. Proc Natl Acad Sci U S A. **105**, 42. 16230-16235
54. Marinova, Z., Ren, M., Wendland, J.R., Leng, Y., Liang, M.H., Yasuda, S., Leeds, P., Chuang, D.M. (2009) Valproic acid induces functional heat-shock protein 70 via Class I histone deacetylase inhibition in cortical neurons: a potential role of Sp1 acetylation. J Neurochem. **111**, 4. 976-987
55. Marinova, Z., Leng, Y., Leeds, P., Chuang, D.M. (2011) Histone deacetylase inhibition alters histone methylation associated with heat shock protein 70 promoter modifications in astrocytes and neurons. Neuropharmacology. **60**, 7-8. 1109-1115
56. Hunsberger, J.G., Fessler, E., Wang, Z., Elkahloun, A.G., Chuang, D.M. (2012) Post-insult valproic acid-regulated microRNAs: potential targets for cerebral ischemia. Am J Transl Res. **4**, 3. 316-332
57. Amir, R.E., Van den Veyver, I.B., Wan, M., Tran, C.Q., Francke, U., and Zoghbi, H.Y. (1999) Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat Genet. **23**, 2. 185-188
58. Chen, L.J., Wei, S.Y., and Chiu, J.J. (2013) Mechanical regulation of epigenetics in vascular biology and pathobiology. J Cell Mol Med. **17**, 4. 437-448
59. Choi, J.H., Nam, K.H., Kim, J., Baek, M.W., Park, J.E., Park, H.Y., Kwon, H.J., Kwon, O.S., Kim, D.Y., and Oh, G.T. (2005) Trichostatin A exacerbates atherosclerosis in low density lipoprotein receptor-deficient mice. Arterioscler Thromb Vasc Biol. **25**, 11. 2404-2409
60. Okamoto, H., Fujioka, Y., Takahashi, A., Takahashi, T., Taniguchi, T., Ishikawa, Y., and Yokoyama, M. (2006) Trichostatin A, an inhibitor of histone deacetylase, inhibits smooth muscle cell proliferation via induction of p21(WAF1). J Atheroscler Thromb. **13**, 4. 183-191
61. Dje N'Guessan, P., Riediger, F., Vardarova, K., Scharf, S., Eitel, J., Opitz, B., Slevogt, H., Weichert, W., Hocke, A.C., Schmeck, B., Suttorp, N., and Hippenstiel, S. (2009) Statins control oxidized LDL-mediated histone modifications and gene expression in cultured human endothelial cells. Arterioscler Thromb Vasc Biol. **29**, 3. 380-386
62. Jiang, Y.Z., Jimenez, J.M., Ou, K., McCormick, M.E., Zhang, L.D., and Davies, P.F. (2014) Hemodynamic disturbed flow induces differential DNA methylation of endothelial kruppel-like factor 4 promoter in vitro and in vivo. Circ Res. **115**, 1. 32-43
63. Hastings, N.E., Simmers, M.B., McDonald, O.G., Wamhoff, B.R., and Blackman, B.R. (2007) Atherosclerosis-prone hemodynamics differentially regulates endothelial and smooth muscle cell phenotypes and promotes pro-inflammatory priming. Am J Physiol Cell Physiol. **293**, 6. C1824-1833
64. Fan, J., Alsarraf, O., Dahrouj, M., Platt, K.A., Chou, C.J., Rice, D.S., and Crosson, C.E. (2013) Inhibition of HDAC2 protects the retina from ischemic injury. Invest Ophthalmol Vis Sci. **54**, 6. 4072-4080
65. Wellmann, S., Bettkober, M., Zelmer, A., Seeger, K., Faigle, M., Eltzschig, H.K., and Buhrer, C. (2008) Hypoxia upregulates the histone demethylase JMJD1A via HIF-1. Biochem Biophys Res Commun. **372**, 4. 892-897

66. Pollard, P.J., Loenarz, C., Mole, D.R., McDonough, M.A., Gleadle, J.M., Schofield, C.J., and Ratcliffe, P.J. (2008) Regulation of Jumonji-domain-containing histone demethylases by hypoxia-inducible factor (HIF)-1alpha. Biochem J. **416**, 3. 387-394
67. Saco, T.V., Parthasarathy, P.T., Cho, Y., Lockey, R.F., and Kolliputi, N. (2014) Role of epigenetics in pulmonary hypertension. Am J Physiol Cell Physiol. **306**, 12. C1101-C1105
68. Chen, W., Bacanamwo, M., and Harrison, D.G. (2008) Activation of p300 histone acetyltransferase activity is an early endothelial response to laminar shear stress and is essential for stimulation of endothelial nitric-oxide synthase mRNA transcription. J Biol Chem. **283**, 24. 16293-16298
69. Zhang, L., Qin, X., Zhao, Y., Fast, L., Zhuang, S., Liu, P., Cheng, G., and Zhao, T.C. (2012) Inhibition of histone deacetylases preserves myocardial performance and prevents cardiac remodeling through stimulation of endogenous angiomyogenesis. J Pharmacol Exp Ther. **341**, 1. 285-293
70. Semenza, G.L. (2000) Hypoxia, clonal selection, and the role of HIF-1 in tumor progression. Crit Rev Biochem Mol Biol. **35**, 2. 71-103
71. Kim, M.S., Kwon, H.J., Lee, Y.M., Baek, J.H., Jang, J.E., Lee, S.W., Moon, E.J., Kim, H.S., Lee, S.K., Chung, H.Y., Kim, C.W., and Kim, K.W. (2001) Histone deacetylases induce angiogenesis by negative regulation of tumor suppressor genes. Nat Med. **7**, 4. 437-443
72. Chen, S., Sang, N. (2011) Histone deacetylase inhibitors: the epigenetic therapeutics that repress hypoxia-inducible factors. J Biomed Biotechnol. **2011**. 197946. doi: 10.1155/2011/197946
73. Johnson, A.B., Denko, N., and Barton, M.C. (2008) Hypoxia induces a novel signature of chromatin modifications and global repression of transcription. Mutat Res. **640**, 1-2. 174-179
74. Chen, H., Yan, Y., Davidson, T.L., Shinkai, Y., and Costa, M. (2006) Hypoxic stress induces dimethylated histone H3 lysine 9 through histone methyltransferase G9a in mammalian cells. Cancer Res. **66**, 18. 9009-9016
75. Lee, S.H., Kim, J., Kim, W.H., and Lee, Y.M. (2009) Hypoxic silencing of tumor suppressor RUNX3 by histone modification in gastric cancer cells. Oncogene. **28**, 2. 184-194
76. Beyer, S., Kristensen, M.M., Jensen, K.S., Johansen, J.V., and Staller, P. (2008) The histone demethylases JMJD1A and JMJD2B are transcriptional targets of hypoxia-inducible factor HIF. J Biol Chem. **283**, 52. 36542-36552
77. Yang, J., Ledaki, I., Turley, H., Gatter, K.C., Montero, J.C., Li, J.L., and Harris, A.L. (2009) Role of hypoxia-inducible factors in epigenetic regulation via histone demethylases. Ann N Y Acad Sci. **1177**. 185-197
78. Krieg, A.J., Rankin, E.B., Chan, D., Razorenova, O., Fernandez, S., and Giaccia, A.J. (2010) Regulation of the histone demethylase JMJD1A by hypoxia-inducible factor 1 alpha enhances hypoxic gene expression and tumor growth. Mol Cell Biol. **30**, 1. 344-353
79. Glazer, P.M., Hegan, D.C., Lu, Y., Czochor, J., and Scanlon, S.E. (2013) Hypoxia and DNA repair. Yale J Biol Med. **86**, 4. 443-451
80. Lu, Y., Chu, A., Turker, M.S., and Glazer, P.M. (2011) Hypoxia-induced epigenetic regulation and silencing of the BRCA1 promoter. Mol Cell Biol. **31**, 16. 3339-3350
81. Tudisco, L., Della Ragione, F., Tarallo, V., Apicella, I., D'Esposito, M., Matarazzo, M.R., and De Falco, S. (2014) Epigenetic control of hypoxia inducible factor-1alpha-dependent expression of placental growth factor in hypoxic conditions. Epigenetics. **9**, 4. 600-610
82. Chen, D., Fang, F., Yang, Y., Chen, J., Xu, G., Xu, Y., and Gao, Y. (2013) Brahma-related gene 1 (Brg1) epigenetically regulates CAM activation during hypoxic pulmonary hypertension. Cardiovasc Res. **100**, 3. 363-373

83. Fang, F., Chen, D., Yu, L., Dai, X., Yang, Y., Tian, W., Cheng, X., Xu, H., Weng, X., Fang, M., Zhou, J., Gao, Y., Chen, Q., and Xu, Y. (2013) Proinflammatory stimuli engage Brahma related gene 1 and Brahma in endothelial injury. Circ Res. **113**, 8. 986-996
84. Wu, X., Chen, P.S., Dallas, S., Wilson, B., Block, M.L., Wang, C.C., Kinyamu, H., Lu, N., Gao, X., Leng, Y., Chuang, D.M., Zhang, W., Lu, R.B., Hong, J.S. (2008) Histone deacetylase inhibitors up-regulate astrocyte GDNF and BDNF gene transcription and protect dopaminergic neurons. Int J Neuropsychopharmacol. **11**, 8. 123-134
85. Yoshida, M., Kijima, M., Akita M., Beppu, T. (1990) Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. J Biol Chem. **265**, 28. 17174-17179
86. Langley, B., D'Annibale, M.A., Suh, K., Ayoub, I., Tolhurst, A., Bastan, B., Yang, L., Ko, B., Fisher, M., Cho, S., Beal, M.F., Ratan, R.R. (2008) Pulse inhibition of histone deacetylases induces complete resistance to oxidative death in cortical neurons without toxicity and reveals a role for cytoplasmic p21(waf1/cip1) in cell cycle-independent neuroprotection. J Neurosci. **28**, 1. 163-176
87. Wang, B., Zhu, X., Kim, Y., Li, J., Huang, S., Saleem, S., Li, R.C., Xu, Y., Dore, S., Cao, W. (2012) Histone deacetylase inhibition activates transcription factor Nrf2 and protects against cerebral ischemic damage. Free Radic Biol Med. **52**, 5. 928-936
88. Okamoto, H., Fujioka, Y., Takahashi, A., Takahashi, T., Taniguchi, T., Ishikawa, Y., and Yokoyama, M. (2006) Trichostatin A, an inhibitor of histone deacetylase, inhibits smooth muscle cell proliferation via induction of p21(WAF1). J Atheroscler Thromb **13**. 183-191
89. Fleiss, B., Nilsson, M.K., Blomgren, K., Mallard, C. (2012) Neuroprotection by the histone deacetylase inhibitor trichostatin A in a model of lipopolysaccharide-sensitised neonatal hypoxic-ischaemic brain injury. J Neuroinflammation **9**, 70. 2094-2070
90. Hsieh, J., Nakashima, K., Kuwabara, T., Mejia, E., Gage, F.H. (2004) Histone deacetylase inhibition-mediated neuronal differentiation of multipotent adult neural progenitor cells. Proc Natl Acad Sci USA. **101**, 47. 16659-16664
91. Gul, H., Marquez-Curtis, L.A., Jahroudi, N., Lo, J., Turner, A.R., Janowska-Wieczorek, A. (2009) Valproic acid increases CXCR4 expression in hematopoietic stem/progenitor cells by chromatin remodeling. Stem Cell Dev. **18**, 6. 831-838
92. Sinn, D.I., Kim, S.J., Chu, K., Jung, K.H., Lee, S.T., Song, E.C., Kim, J.M., Park, D.K., Kun Lee, S., Kim, M., Roh, J.K. (2007) Valproic acid-mediated neuroprotection in intracerebral hemorrhage via histone deacetylase inhibition and transcriptional activation. Neurobiol Dis. **26**, 2. 262-272
93. Ren, M., Leng, Y., Jeong, M., Leeds, P.R., Chuang, D.M. (2004) Valproic acid reduces brain damage induced by transient focal cerebral ischemia in rats: potential roles of histone deacetylase inhibition and heat shock protein induction. J Neurochem. **89**, 6. 1358-1367
94. Wang, Z., Leng, Y., Tsai, L.K., Leeds, P., Chuang, D.M. (2011) Valproic acid attenuates blood-brain barrier disruption in a rat model of transient focal cerebral ischemia: the roles of HDAC and MMP-9 inhibition. J Cereb Blood Flow Metab. **31**, 1. 52-57
95. Wang, Z., Tsai, L., Munasinghe, J., Leng, Y., Fessler, E.B., Chibane, F., Leeds, P., Chuang, D.M. (2012) Chronic valproate treatment enhances postischemic angiogenesis and promotes functional recovery in a rat model of ischemic stroke. Stroke. **43**, 9. 2430-2436
96. Faraco, G., Pancani, T., Formentini, L., Mascagni, P., Fossati, G., Leoni, F., Moroni, F., Chiarugi, A. (2006) Pharmacological inhibition of histone deacetylases by suberoylanilide hydroxamic acid specifically alters gene expression and reduces ischemic injury in the mouse brain. Mol Pharmacol. **70**, 6. 1876-1884

97. Faraco, G., Pittelli, M., Cavone, L., Fossati, S., Porcu, M., Mascagni, P., Fossati, G., Moroni, F., Chiarugi, A. (2009) Histone deacetylase (HDAC) inhibitors reduce the glial inflammatory response in vitro and in vivo. Neurobiol Dis. **36**, 2. 269-729
98. Kim, H., Leeds P, Chuang DM. (2009) The HDAC inhibitor, sodium butyrate, stimulates neurogenesis in the ischemic brain. J Neurochem. **110**, 4. 1226-1240
99. Rubenstein, R.C., Zeitlin, P.L. (1998) A pilot clinical trial of oral sodium 4-phenylbutyrate (Buphenyl) in deltaF508-homozygous cystic fibrosis patients: partial restoration of nasal epithelial CFTR function. Am J Respir Crit Med. **157**, 2. 484-490
100. Qi, X., Hosoi, T., Okuma, Y., Kaneko, M., Nomura, Y. (2004) Sodium 4-phenylbutyrate protects against cerebral ischemic injury. Mol Pharmacol. **66**, 4. 899-908
101. Jin, G., Bausch, D, Knightly, T, Liu, Z, Li, Y, Liu, B, Lu, J, Chong, W, Velmahos, GC, Alam, HB. (2011) Histone deacetylase inhibitors enhance endothelial cell sprouting angiogenesis in vitro. Surgery. **150**, 3. 429-435
102. Kwon, H.J., Kim, M., Kim, M.J., Nakajima, H., Kim, K.W. (2002) Histone deacetylase inhibitor FK228 inhibits tumor angiogenesis. Int J Cancer. **97**, 3. 290-296
103. Piekarz, R.L., Robey, R., Sandor, V., Bakke, S., Wilson, W.H., Dahmoush, L., Kingma, D.M., Turner, M.L., Altemus, R., Bates, S.E. (2001) Inhibitor of histone deacetylation, depsipeptide (FR901228), in the treatment of peripheral and cutaneous T-cell lymphoma: a case report. Blood. **98**, 9. 2865-2868
104. Kogure, K., Kato, H. (1993) Altered gene expression in cerebral ischemia. Stroke. **24**, 12. 2121-2127
105. Endres, M., Meisel, A., Biniszkiewicz, D., Namura, S., Prass, K., Ruscher, K., Lipski, A., Jaenisch, R., Moskowitz, M. A., Dirnagl, U. (2000) DNA methyltransferase contributes to delayed ischemic brain injury. J Neurosci. **20**, 9. 3175-3181
106. Endres, M., Fan, G., Meisel, A., Dirnagl, U., Jaenisch, R. (2001) Effects of cerebral ischemia in mice lacking DNA methyltransferase 1 in post-mitotic neurons. Neuroreport. **12**, 17. 3763-3766
107. Liu, P., K, Hsu, C.Y., Dizdaroglu, M., Floyd, R. A., Kow, Y.W., Karakaya, A., Rabow, L. E., Cui, J. K. (1996) Damage, repair, and mutagenesis in nuclear genes after mouse forebrain ischemia-reperfusion. J Neurosci. **16**, 21. 6795-6805
108. Giffard, R., Yenari, MA. (2004) Many mechanisms for hsp70 protection from cerebral ischemia. J Neurosurg Anesthesiol. **16**, 1. 53-61
109. Kim, A.K., Kim, M.H., Kim, S., Oh, W., Hong, H.K., Kang, K.S., Kim, B.S., and Kim, D.I. (2011) Stem-cell therapy for peripheral arterial occlusive disease. Eur J Vasc Endovasc Surg. **42**, 5. 667-675
110. Sica, V., Williams-Ignarro, S., de Nigris, F., D'Armiento, F.P., Lerman, L.O., Balestrieri, M.L., Maione, C., Palagiano, A., Rossiello, L., Ignarro, L.J., and Napoli, C. (2006) Autologous bone marrow cell therapy and metabolic intervention in ischemia-induced angiogenesis in the diabetic mouse hindlimb. Cell Cycle. **5**, 24. 2903-2908
111. Hellingman, A.A., Zwaginga, J.J., van Beem, R.T., Te, R.M.S.M.C., Hamming, J.F., Fibbe, W.E., Quax, P.H., and Geutskens, S.B. (2011) T-cell-pre-stimulated monocytes promote neovascularisation in a murine hind limb ischaemia model. Eur J Vasc Endovasc Surg. **41**, 3. 418-428
112. Turunen, M.P., Lehtola, T., Heinonen, S.E., Assefa, G.S., Korpisalo, P., Girnary, R., Glass, C.K., Vaisanen, S., and Yla-Herttuala, S. (2009) Efficient regulation of VEGF expression by promoter-targeted lentiviral shRNAs based on epigenetic mechanism: a novel example of epigenetherapy. Circ Res. **105**, 6. 604-609
113. Palii, C.G., Vulesevic, B., Fraineau, S., Pranckeviciene, E., Griffith, A.J., Chu, A., Faralli, H., Li, Y., McNeill, B., Sun, J., Perkins, T.J., Dilworth, F.J., Perez-Iratxeta, C., Suuronen, E.J., Allan, D.S., and Brand, M. (2014) Trichostatin A enhances vascular repair by

injected human endothelial progenitors through increasing the expression of TAL1-dependent genes. Cell Stem Cell. **14**, 5. 644-657
114. Ueda, H., Matsunaga, H., and Halder, S.K. (2012) Prothymosin alpha plays multifunctional cell robustness roles in genomic, epigenetic, and nongenomic mechanisms. Ann N Y Acad Sci. **1269**. 34-43
115. Spallotta, F., Tardivo, S., Nanni, S., Rosati, J.D., Straino, S., Mai, A., Vecellio, M., Valente, S., Capogrossi, M.C., Farsetti, A., Martone, J., Bozzoni, I., Pontecorvi, A., Gaetano, C., and Colussi, C. (2013) Detrimental effect of class-selective histone deacetylase inhibitors during tissue regeneration following hindlimb ischemia. J Biol Chem. **288**, 32. 22915-22929
116. Rahmatzadeh, M., Liu, H.B., Krishna, S.M., Gaspari, T.A., Welungoda, I., Widdop, R.E., and Dear, A.E. (2014) A Novel Agent with Histone Deacetylase Inhibitory Activity Attenuates Neointimal Hyperplasia. Cardiovasc Drugs Ther. **28**, 5. 395-406
117. Kawano, N., Emoto, M., Mori, K., Yamazaki, Y., Urata, H., Tsuchikura, S., Motoyama, K., Morioka, T., Fukumoto, S., Shoji, T., Koyama, H., Okuno, Y., Nishizawa, Y., Inaba, M. (2012) Association of endothelial and vascular smooth muscle dysfunction with cardiovascular risk factors, vascular complications, and subclinical carotid atherosclerosis in type 2 diabetic patients. J Atheroscler Thromb **19**, 3. 276-284
118. Villeneuve, L.M., Natarajan, R. (2010) The role of epigenetics in the pathology of diabetic complications. Am J Physiol Renal Physiol. **299**, 1. F14-25
119. Krishna, S.M., Dear, A., Craig, J.M., Norman, P.E., and Golledge, J. (2013) The potential role of homocysteine mediated DNA methylation and associated epigenetic changes in abdominal aortic aneurysm formation. Atherosclerosis. **228**, 2. 295-305
120. Becker, A., Kostense, P.J., Bos, G., Heine, R.J., Dekker, J.M., Nijpels, G., Bouter, L.M., and Stehouwer, C.D. (2003) Hyperhomocysteinaemia is associated with coronary events in type 2 diabetes. J Intern Med. **253**, 3. 293-300
121. Okura, T., Miyoshi, K., Irita, J., Enomoto, D., Nagao, T., Kukida, M., Tanino, A., Kudo, K., Pei, Z., and Higaki, J. (2014) Hyperhomocysteinemia is one of the risk factors associated with cerebrovascular stiffness in hypertensive patients, especially elderly males. Sci Rep. **4**. 5663. doi: 10.1038/srep05663
122. Heneghan, H.M., Sultan, S. (2008) Homocysteine, the cholesterol of the 21st century. Impact of hyperhomocysteinemia on patency and amputation-free survival after intervention for critical limb ischemia. J Endovasc Ther. **15**, 4. 399-407
123. Sharma, P., Kumar, J., Garg, G., Kumar, A., Patowary, A., Karthikeyan, G., Ramakrishnan, L., Brahmachari, V., and Sengupta, S. (2008) Detection of altered global DNA methylation in coronary artery disease patients. DNA Cell Biol. **27**, 7. 357-365
124. Talaulikar, V.S., Arulkumaran, S. (2011) Folic acid in obstetric practice: a review. Obstet Gynecol Surv. **66**, 4. 240-7.
125. Jiang, X., Yang, F., Tan, H., Liao, D., Bryan, R.M., Jr., Randhawa, J.K., Rumbaut, R.E., Durante, W., Schafer, A.I., Yang, X., Wang, H. (2005) Hyperhomocystinemia impairs endothelial function and eNOS activity via PKC activation. Arterioscler Thromb Vasc Biol. **25**, 12. 2515-2521
126. De Smet, C., Lurquin, C., Lethe, B., Martelange, V., Boon, T. (1999) DNA methylation is the primary silencing mechanism for a set of germ line- and tumor-specific genes with a CpG-rich promoter. Mol Cell Biol. **19**, 11. 7327-7335
127. Kruman, I.I., Culmsee, C., Chan, S.L., Kruman, Y., Guo, Z., Penix, L., Mattson, M.P. (2000) Homocysteine elicits a DNA damage response in neurons that promotes apoptosis and hypersensitivity to excitotoxicity. J Neurosci. **20**, 18: p. 6920-6.
128. Yan, T.T., Li, Q., Zhang, X.H., Wu, W.K., Sun, J., Li, L., Zhang, Q., Tan, H.M. (2010) Homocysteine impaired endothelial function through compromised vascular

endothelial growth factor/Akt/endothelial nitric oxide synthase signalling. Clin Exp Pharmacol Physiol. **37**, 11. 1071-1077
129. Werstuck, G.H., Lentz, S.R., Dayal, S., Hossain, G.S., Sood, S.K., Shi, Y.Y., Zhou, J., Maeda, N., Krisans, S.K., Malinow, M.R., and Austin, R.C. (2001) Homocysteine-induced endoplasmic reticulum stress causes dysregulation of the cholesterol and triglyceride biosynthetic pathways. J Clin Invest. **107**, 10. 1263-1273
130. Savji, N., Rockman, C., Skolnick, A.H., Guo, Y., Adelman, M.A., Riles, T., Berger, J.S. (2013) Association between advanced age and vascular disease in different arterial territories: a population database of over 3.6 million subjects. J Am Coll Cardiol. **61**,16. 1736-1743
131. Bjornsson, H.T., Sigurdsson, M., Fallin, M.D., Irizarry, R.A., Aspelund, T., Cui, H., Yu, W., Rongione, M.A., Ekström, T.J., Harris, T.B., Launer, L.J., Eiriksdottir, G., Leppert, M.F., Sapienza, C., Gudnason, V., Feinberg, A.P. (2008) Intra-individual change over time in DNA methylation with familial clustering. JAMA. **299**, 24. 2877-2883
132. Magenta, A., Cencioni, C., Fasanaro, P., Zaccagnini, G., Greco, S., Sarra-Ferraris, G., Antonini, A., Martelli, F., Capogrossi, M.C. (2011) miR-200c is upregulated by oxidative stress and induces endothelial cell apoptosis and senescence via ZEB1 inhibition. Cell Death Differ. **18**, 10. 1628-1639
133. Bhaumik, D., Scott, G.K., Schokrpur, S., Patil, C.K., Orjalo, A.V., Rodier, F., Lithgow, G.J., Campisi, J. (2009) MicroRNAs miR-146a/b negatively modulate the senescence-associated inflammatory mediators IL-6 and IL-8. Aging (Albany NY). **1**, 4. 402-411
134. Ito, T., Yagi, S., Yamakuchi, M. (2010) MicroRNA-34a regulation of endothelial senescence. Biochem Biophys Res Commun. **398**, 4. 735-740
135. Menghini, R., Casagrande, V., Cardellini, M., Martelli, E., Terrinoni, A., Amati, F., Vasa-Nicotera, M., Ippoliti, A., Novelli, G., Melino, G., Lauro, R., Federici, M. (2009) MicroRNA 217 modulates endothelial cell senescence via silent information regulator 1. Circulation. **120**, 5. 1524-1532
136. Rupérez, M., Lorenzo, O., Blanco-Colio, L.M., Esteban, V., Egido, J., Ruiz-Ortega, M. (2003) Connective tissue growth factor is a mediator of angiotensin II-induced fibrosis. Circulation. **108**, 12. 1499-1505
137. Yu, Z., Kong, Q., Kone, B.C. (2010) CREB trans-activation of disruptor of telomeric silencing-1 mediates forskolin inhibition of CTGF transcription in mesangial cells. Am J Physiol Renal Physiol. **298**, 3. F617-624
138. Lipson, K., Wong, C., Teng, Y., Spong, S. (2012) CTGF is a central mediator of tissue remodeling and fibrosis and its inhibition can reverse the process of fibrosis. Fibrogenesis Tissue Repair. **S24**. eCollection 2012
139. Breitling, L.P. (2013) Current genetics and epigenetics of smoking/tobacco-related cardiovascular disease. Arterioscler Thromb Vasc Biol. **33**, 7. 1468-1472
140. Zöchbauer-Müller, S., Lam, S., Toyooka, S., Virmani, A.K., Toyooka, K.O., Seidl, S., Minna, J.D., Gazdar, A.F. (2003) Aberrant methylation of multiple genes in the upper aerodigestive tract epithelium of heavy smokers. Int J Cancer. **107**, 4. 612-616
141. Ostrow, K.L., Hoque, M.O., Loyo, M., Brait, M., Greenberg, A., Siegfried, J.M., Grandis, J.R., Gaither Davis, A., Bigbee, W.L., Rom, W., Sidransky, D. (2010) Molecular analysis of plasma DNA for the early detection of lung cancer by quantitative methylation-specific PCR. Clin Cancer Res. **16**, 13. 3463-3472
142. Begum, S., Brait, M., Dasgupta, S., Ostrow, K.L., Zahurak, M., Carvalho, A.L., Califano, J.A., Goodman, S.N., Westra, W.H., Hoque, M.O., Sidransky, D. (2011) An epigenetic marker panel for detection of lung cancer using cell-free serum DNA. Clin Cancer Res. **17**, 13. 4494-4503

143. Breitling, L.P., Yang, R., Korn, B., Burwinkel, B., Brenner, H. (2011) Tobacco-smoking-related differential DNA methylation: 27K discovery and replication. Am J Hum Genet. **88**, 4. 450-457
144. Zochbauer-Muller, S., Lam, S., Toyooka, S., Virmani, A.K., Toyooka, K.O., Seidl, S., Minna, J.D., and Gazdar, A.F. (2003) Aberrant methylation of multiple genes in the upper aerodigestive tract epithelium of heavy smokers. Int J Cancer. **107**, 4. 612-616
145. Yanagawa, N., Tamura, G., Oizumi, H., Endoh, M., Sadahiro, M., and Motoyama, T. (2011) Inverse correlation between EGFR mutation and FHIT, RASSF1A and RUNX3 methylation in lung adenocarcinoma: relation with smoking status. Anticancer Res. **31**, 4. 1211-1214
146. Breton, C.V., Byun, H.M., Wenten, M., Pan, F., Yang, A., and Gilliland, F.D. (2009) Prenatal tobacco smoke exposure affects global and gene-specific DNA methylation. Am J Respir Crit Care Med. **180**, 5. 462-467
147. Suter, M., Ma, J., Harris, A., Patterson, L., Brown, K.A., Shope, C., Showalter, L., Abramovici, A., and Aagaard-Tillery, K.M. (2011) Maternal tobacco use modestly alters correlated epigenome-wide placental DNA methylation and gene expression. Epigenetics. **6**, 11. 1284-1294
148. Heil, M., Eitenmuller, I., Schmitz-Rixen, T., and Schaper, W. (2006) Arteriogenesis versus angiogenesis: similarities and differences. J Cell Mol Med. **10**, 1. 45-55
149. Cai, W., Schaper, W. (2008) Mechanisms of arteriogenesis. Acta Biochim Biophys Sin (Shanghai). **40**, 8: p. 681-692
150. Carmeliet, P. (2005) Angiogenesis in life, disease and medicine. Nature. **438**, 7070. 932-936
151. Katoh, M. (2013) Therapeutics targeting angiogenesis: genetics and epigenetics, extracellular miRNAs and signaling networks (Review). Int J Mol Med. **32**, 4. 763-767
152. Buysschaert, I., Schmidt, T., Roncal, C., Carmeliet, P., and Lambrechts, D. (2008) Genetics, epigenetics and pharmaco-(epi)genomics in angiogenesis. J Cell Mol Med. **12**, 6B. 2533-2551
153. Findeisen, H.M., Gizard, F., Zhao, Y., Qing, H., Heywood, E.B., Jones, K.L., Cohn, D., and Bruemmer, D. (2011) Epigenetic regulation of vascular smooth muscle cell proliferation and neointima formation by histone deacetylase inhibition. Arterioscler Thromb Vasc Biol. **31**,4. 851-860
154. Sambucetti, L.C., Fischer, D.D., Zabludoff, S., Kwon, P.O., Chamberlin, H., Trogani, N., Xu, H., and Cohen, D. (1999) Histone deacetylase inhibition selectively alters the activity and expression of cell cycle proteins leading to specific chromatin acetylation and antiproliferative effects. J Biol Chem. **274**, 49. 34940-34947
155. Mathew, O.P., Ranganna, K., and Yatsu, F.M. (2010) Butyrate, an HDAC inhibitor, stimulates interplay between different posttranslational modifications of histone H3 and differently alters G1-specific cell cycle proteins in vascular smooth muscle cells. Biomed Pharmacother. **64**, 10. 733-740
156. Owens, G.K., Kumar, M.S., and Wamhoff, B.R. (2004) Molecular regulation of vascular smooth muscle cell differentiation in development and disease. Physiol Rev. **84**, 3. 767-801
157. Singh, N., Trivedi, C.M., Lu, M., Mullican, S.E., Lazar, M.A., and Epstein, J.A. (2011) Histone deacetylase 3 regulates smooth muscle differentiation in neural crest cells and development of the cardiac outflow tract. Circ Res. **109**,11: p. 1240-1249
158. Muzic, V., Katusic Bojanac, A., Juric-Lekic, G., Himelreich, M., Tupek, K., Serman, L., Marn, N., Sincic, N., Vlahovic, M., and Bulic-Jakus, F. (2013) Epigenetic drug 5-azacytidine impairs proliferation of rat limb buds in an organotypic model-system in vitro. Croat Med J. **54**, 5. 489-495

159. Shimamura, M., Nakagami, H., Sata, M., Takaoka, M., Azuma, J., Kiomy Osako, M., Koriyama, H., Kurinami, H., Wakayama, K., Miyake, T., and Morishita, R. (2013) Unique remodeling processes after vascular injury in intracranial arteries: analysis using a novel mouse model. J Cereb Blood Flow Metab. **33**, 8. 1153-1159
160. Shiva Shankar, T.V., Willems, L. (2014) Epigenetic modulators mitigate angiogenesis through a complex transcriptomic network. Vascul Pharmacol. **60**, 2. 57-66
161. Martinelli, S., Kanduri, M., Maffei, R., Fiorcari, S., Bulgarelli, J., Marasca, R., and Rosenquist, R. (2013) ANGPT2 promoter methylation is strongly associated with gene expression and prognosis in chronic lymphocytic leukemia. Epigenetics. **8**, 7. 720-729
162. Liu, X., Zheng, N., Shi, Y.N., Yuan, J., and Li, L. (2014) Thyroid hormone induced angiogenesis through the integrin alphavbeta3/protein kinase D/histone deacetylase 5 signaling pathway. J Mol Endocrinol. **52**, 3. 245-254
163. Ungvari, Z., Tucsek, Z., Sosnowska, D., Toth, P., Gautam, T., Podlutsky, A., Csiszar, A., Losonczy, G., Valcarcel-Ares, M.N., Sonntag, W.E., and Csiszar, A. (2013) Aging-induced dysregulation of dicer1-dependent microRNA expression impairs angiogenic capacity of rat cerebromicrovascular endothelial cells. J Gerontol A Biol Sci Med Sci. **68**, 8. 877-891
164. Mann, J., Chu, D.C., Maxwell, A., Oakley, F., Zhu, N.L., Tsukamoto, H., and Mann, D.A. (2010) MeCP2 controls an epigenetic pathway that promotes myofibroblast transdifferentiation and fibrosis. Gastroenterology. **138**, 2. 705-14, 714 e1-4
165. Pang, M., Zhuang, S. (2010) Histone deacetylase: a potential therapeutic target for fibrotic disorders. J Pharmacol Exp Ther. **335**, 2. 266-272
166. Catania, A., Iavarone, C., Carlomagno, S.M., and Chiariello, M. (2006) Selective transcription and cellular proliferation induced by PDGF require histone deacetylase activity. Biochem Biophys Res Commun. **343**, 2. 544-554
167. Ling, C., Ronn, T. (2014) Epigenetic adaptation to regular exercise in humans. Drug Discov Today. **19**, 7. 1015-1018
168. Alibegovic, A.C., Hojbjerre, L., Sonne, M.P., van Hall, G., Stallknecht, B., Dela, F., and Vaag, A. (2009) Impact of 9 days of bed rest on hepatic and peripheral insulin action, insulin secretion, and whole-body lipolysis in healthy young male offspring of patients with type 2 diabetes. Diabetes. **58**, 12. 2749-2756
169. Sanchis-Gomar, F., Garcia-Gimenez, J.L., Perez-Quilis, C., Gomez-Cabrera, M.C., Pallardo, F.V., and Lippi, G. (2012) Physical exercise as an epigenetic modulator: Eustress, the "positive stress" as an effector of gene expression. J Strength Cond Res. **26**, 12. 3469-3472
170. Bloch, W., Suhr, F., and Zimmer, P. (2012) Molecular mechanisms of exercise-induced cardiovascular adaptations. Influence of epigenetics, mechanotransduction and free radicals. Herz. **37**, 5. 508-515
171. Nakajima, K., Takeoka, M., Mori, M., Hashimoto, S., Sakurai, A., Nose, H., Higuchi, K., Itano, N., Shiohara, M., Oh, T., and Taniguchi, S. (2010) Exercise effects on methylation of ASC gene. Int J Sports Med. **31**, 9. 671-675
172. White, A.J., Sandler, D.P., Bolick, S.C., Xu, Z., Taylor, J.A., and DeRoo, L.A. (2013) Recreational and household physical activity at different time points and DNA global methylation. Eur J Cancer. **49**, 9. 2199-2206
173. Zhang, F.F., Cardarelli, R., Carroll, J., Zhang, S., Fulda, K.G., Gonzalez, K., Vishwanatha, J.K., Morabia, A., and Santella, R.M. (2011) Physical activity and global genomic DNA methylation in a cancer-free population. Epigenetics. **6**, 3. 293-299
174. Nielsen, S., Scheele, C., Yfanti, C., Akerstrom, T., Nielsen, A.R., Pedersen, B.K., and Laye, M.J. (2010) Muscle specific microRNAs are regulated by endurance exercise in human skeletal muscle. J Physiol. **588**, Pt 20. 4029-4037

175. Barres, R., Yan, J., Egan, B., Treebak, J.T., Rasmussen, M., Fritz, T., Caidahl, K., Krook, A., O'Gorman, D.J., and Zierath, J.R. (2012) Acute exercise remodels promoter methylation in human skeletal muscle. Cell Metab. **15**, 3. 405-411
176. Ronn, T., Volkov, P., Davegardh, C., Dayeh, T., Hall, E., Olsson, A.H., Nilsson, E., Tornberg, A., Dekker Nitert, M., Eriksson, K.F., Jones, H.A., Groop, L., and Ling, C. (2013) A six months exercise intervention influences the genome-wide DNA methylation pattern in human adipose tissue. PLoS Genet. **9**, 6. e1003572
177. Nitert, M.D., Dayeh, T., Volkov, P., Elgzyri, T., Hall, E., Nilsson, E., Yang, B.T., Lang, S., Parikh, H., Wessman, Y., Weishaupt, H., Attema, J., Abels, M., Wierup, N., Almgren, P., Jansson, P.A., Ronn, T., Hansson, O., Eriksson, K.F., Groop, L., and Ling, C. (2012) Impact of an exercise intervention on DNA methylation in skeletal muscle from first-degree relatives of patients with type 2 diabetes. Diabetes. **61**,12. 3322-3332
178. Gomez-Pinilla, F., Zhuang, Y., Feng, J., Ying, Z., and Fan, G. (2011) Exercise impacts brain-derived neurotrophic factor plasticity by engaging mechanisms of epigenetic regulation. Eur J Neurosci. **33**, 3. 383-390
179. Abel, J.L., Rissman, E.F. (2013) Running-induced epigenetic and gene expression changes in the adolescent brain. Int J Dev Neurosci. **31**, 6. 382-390
180. Elsner, V.R., Lovatel, G.A., Moyses, F., Bertoldi, K., Spindler, C., Cechinel, L.R., Muotri, A.R., and Siqueira, I.R. (2013) Exercise induces age-dependent changes on epigenetic parameters in rat hippocampus: a preliminary study. Exp Gerontol. **48**, 2. 136-139
181. Lovatel, G.A., Elsner, V.R., Bertoldi, K., Vanzella, C., Moyses Fdos, S., Vizuete, A., Spindler, C., Cechinel, L.R., Netto, C.A., Muotri, A.R., and Siqueira, I.R. (2013) Treadmill exercise induces age-related changes in aversive memory, neuroinflammatory and epigenetic processes in the rat hippocampus. Neurobiol Learn Mem. **101**. 94-102
182. Heo, M., Kim, E. (2013) Beneficial effects of antecedent exercise training on limb motor function and calpain expression in a rat model of stroke. J Phys Ther Sci. **25**, 8. 943-946
183. Mascarenhas, J.V., Albayati, M.A., Shearman, C.P., and Jude, E.B. (2014) Peripheral arterial disease. Endocrinol Metab Clin North Am. **43**, 1. 149-166
184. Gardner, A.W., Poehlman, E.T. (1995) Exercise rehabilitation programs for the treatment of claudication pain. A meta-analysis. JAMA. **274**, 12. 975-980
185. Radak, Z., Zhao, Z., Koltai, E., Ohno, H., and Atalay, M. (2013) Oxygen consumption and usage during physical exercise: the balance between oxidative stress and ROS-dependent adaptive signaling. Antioxid Redox Signal. **18**, 10. 1208-1246
186. McGee, S.L., Fairlie, E., Garnham, A.P., and Hargreaves, M. (2009) Exercise-induced histone modifications in human skeletal muscle. J Physiol. **587**, Pt 24. 5951-5958
187. McKinsey, T.A., Zhang, C.L., Lu, J., and Olson, E.N. (2000) Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation. Nature. **408**, 6808. 106-111
188. Smith, J.A., Kohn, T.A., Chetty, A.K., and Ojuka, E.O. (2008) CaMK activation during exercise is required for histone hyperacetylation and MEF2A binding at the MEF2 site on the Glut4 gene. Am J Physiol Endocrinol Metab. **295**, 3. E698-704
189. Potthoff, M.J., Wu, H., Arnold, M.A., Shelton, J.M., Backs, J., McAnally, J., Richardson, J.A., Bassel-Duby, R., and Olson, E.N. (2007) Histone deacetylase degradation and MEF2 activation promote the formation of slow-twitch myofibers. J Clin Invest. **117**, 9. 2459-2467
190. Rasmussen, M., Zierath, J.R., and Barres, R. (2014) Dynamic epigenetic responses to muscle contraction. Drug Discov Today. **19**, 7. 1010-1014
191. Tahiliani, M., Koh, K.P., Shen, Y., Pastor, W.A., Bandukwala, H., Brudno, Y., Agarwal, S., Iyer, L.M., Liu, D.R., Aravind, L., and Rao, A. (2009) Conversion of 5-

methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science. **324**, 5929. 930-935
192. Davidsen, P.K., Gallagher, I.J., Hartman, J.W., Tarnopolsky, M.A., Dela, F., Helge, J.W., Timmons, J.A., and Phillips, S.M. (2011) High responders to resistance exercise training demonstrate differential regulation of skeletal muscle microRNA expression. J Appl Physiol (1985). **110**, 2. 309-317
193. Pandey, D., Sikka, G., Bergman, Y., Kim, J.H., Ryoo, S., Romer, L., and Berkowitz, D. (2014) Transcriptional regulation of endothelial arginase 2 by histone deacetylase 2. Arterioscler Thromb Vasc Biol. **34**, 7. 1556-1566
194. Jung, S.B., Kim, C.S., Naqvi, A., Yamamori, T., Mattagajasingh, I., Hoffman, T.A., Cole, M.P., Kumar, A., Dericco, J.S., Jeon, B.H., and Irani, K. (2010) Histone deacetylase 3 antagonizes aspirin-stimulated endothelial nitric oxide production by reversing aspirin-induced lysine acetylation of endothelial nitric oxide synthase. Circ Res. **107**, 7. 877-887
195. Coppede, F. (2014) Epigenetic biomarkers of colorectal cancer: Focus on DNA methylation. Cancer Lett. **342**, 2. 238-247
196. Litzow, M.R. (2011) Novel therapeutic approaches for acute lymphoblastic leukemia. Hematol Oncol Clin North Am. **25**, 6. 1303-1317
197. Kelly, W.K., Richon, V.M., O'Connor, O., Curley, T., MacGregor-Curtelli, B., Tong, W., Klang, M., Schwartz, L., Richardson, S., Rosa, E., Drobnjak, M., Cordon-Cordo, C., Chiao, J.H., Rifkind, R., Marks, P.A., and Scher, H. (2003) Phase I clinical trial of histone deacetylase inhibitor: suberoylanilide hydroxamic acid administered intravenously. Clin Cancer Res. **9**, 10 Pt 1. 3578-3588
198. Kim, J., Kim, J.Y., Song, K.S., Lee, Y.H., Seo, J.S., Jelinek, J., Goldschmidt-Clermont, P.J., and Issa, J.P. (2007) Epigenetic changes in estrogen receptor beta gene in atherosclerotic cardiovascular tissues and in-vitro vascular senescence. Biochim Biophys Acta. **1772**, 1. 72-80

LEGEND TO FIGURE

Figure 1. Epigenetic control of gene expression.

There are three key epigenetic mechanisms that regulate gene expression (i) methylation of CpG islands, mediated by DNA methyltransferases, (ii) histone protein modifications and (iii) microRNAs. The various epigenetic modifications control gene activation and silencing affecting gene expression.

Abbreviations: CpG, Cytosine phosphate Guanine; meCpG, Methylated CpG islabd; DNMTs, DNA methyltransferases; miRNAs, microRNAs;

Figure 2. Relevance of epigenetic marks to the effect and response to ischemic injury.

A simplified diagram highlighting the different types of epigenetic marks and the complexity of various epigenetic gene regulation mechanisms and the cell types involved in ischemic injury development and resolution.

Abbreviations: ROS, Reactive oxygen species; Hcy, Homocysteine; VSM, Vascular smooth muscle cells; Mir; MicroRNAs

Figure 3. Epigenetic factors affecting occlusive cerebral and peripheral arterial diseases.

A number of risk factors common to both CVD and PAD have been identified to cause epigenetic changes. Epigenetic alterations influence common pathologic responses including inflammation, ischemia, hypoxia and shear stress. Risk factors, such as diabetes, aging and life style habits such as smoking and physical inactivity, modulate the epigenome promoting maladaptive changes.