Pleiotropic treatment of pro-atherogenic endothelium: are SIRT1 and EZH2 promising candidates?

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ABSTRACT

Cardiovascular diseases are the leading cause of mortality worldwide and account for almost 17.7 million deaths worldwide annually. Atherosclerosis is one of the main underlying pathologies of coronary artery and cerebrovascular diseases. Current atherosclerosis care is well developed at the emergency room or in the operation theatre by performing percutaneous intervention and vascular grafting per occasion when local atherosclerosis plaque cause infarction or severe ischemia. Moreover, preventative and follow-up therapies of atherosclerosis are predominantly limited by the reduction of cholesterol levels and inhibiting platelet aggregation.

It is well established that endothelial dysfunction is the major initiating event in atherogenesis and continues throughout atherosclerosis progression, yet no endothelial cell-specific therapies are available for the treatment of atherosclerosis. In this perspective, we review the contribution of the endothelium to atherogenesis and postulate that the dysregulation of epigenetic enzymes aggravate endothelial dysfunction in a pleiotropic fashion. We propose that targeted delivery of a SIRT1 activator or an EZH2 inhibitor to the pro-atherogenic endothelium might reduce the atherosclerosis development and prevent from the life-threatening complications.
I. THE ATHEROSCLEROTIC ENDOTHELIUM

The endothelium forms the innermost layer of all blood vessels and is a major regulator of vascular homeostasis. The endothelium plays a critical role in the regulation of vascular permeability, leukocyte trafficking, vascular tone, inflammation, thrombogenesis, fibrinolysis and angiogenesis. The healthy quiescent endothelium mediates vascular homeostasis by the inhibition of unwanted inflammation, blood clotting and vasoconstriction and the maintenance of the vascular barrier, whereas endothelial dysfunction refers to a proinflammatory, prothrombotic and vasoconstrictive state of the endothelium wherein vascular permeability if often increased. Endothelial dysfunction is the initial stage of atherosclerosis (1). Dysfunctional endothelial cells facilitate lipid accumulation in the vessel wall, leukocyte extravasation, the secretion of pro-inflammatory cytokines, vasoconstriction, thrombogenesis and the accumulation of fibrous elements in the vessel wall, which form the basic elements of atherogenesis (2, 3).

Even before the first anatomical evidence of atherosclerotic plaque formation, endothelial dysfunction is appeared in hypercholesteremic children (Familial hypercholesterolemia) and young adult smokers (4). Moreover, the contribution of the endothelium to the pathogenesis of atherosclerosis has been established in a clinical long-term follow-up study, which compared non-obstructive coronary artery disease patients in which endothelial function was severely impaired to patients with only mild or moderate endothelial dysfunction. This study revealed that the group of patients with severe endothelial dysfunction has a higher incidence of cardiac events compared to the patients with mild and moderate endothelial dysfunction, implying the importance of endothelial dysfunction in the progression of atherosclerosis to cardiovascular events (5).

**Figure 1. Endothelial cells are a pivotal mediator of atherogenic pathways.** Located on luminal side of the blood vessels, endothelial cells regulate smooth muscle cell contraction and platelet activation. Forming a barrier between the blood and the underlying tissue, the endothelium plays a crucial role in the selective recruitment of leukocytes and the lipid accumulation in the vessel wall.
The classical risk factors for the development of atherosclerosis, such as physical inactivity, obesity, diabetes, hypertension, smoking, dyslipidemia and aging (6) act at the systemic level, yet atherosclerotic lesions preferentially develop in areas where endothelial cells are exposed to low oscillatory flow, suggesting that focal risk factors for the development of atherosclerosis exist (7-9). The vascular areas at risk, the so-called atheroprone regions, are commonly found at the outer wall of vascular bifurcations and the inner wall of vascular curvatures. Interestingly, the induction of blood flow disturbances in animals by for instance arteriovenous fistula (10), aortic ligation/constriction model (11) and partial carotid ligation (12) induces intimal hyperplasia and the development of a neointima even in absence of systemic atherosclerosis risk factors. A distinct gene expression profile is observed in endothelial cells exposed to high laminar shear stress vs low oscillatory shear stress in the human and porcine aorta (13, 14).

Endothelial cells in atheroprone areas produce less nitric oxide (NO) compared to endothelial cells at atheroprotected sites (15, 16). Furthermore, atheroprone, or low oscillatory shear stress induces a proinflammatory phenotype in endothelial cells (17). Inflammation is crucial in atherosclerosis development, progression and plaque stability (extensively reviewed (18, 19)). The inflammatory reaction at the atheroprone site increases the endothelial permeability to circulating lipids and initiates leukocyte recruitment, wherein the expression of leukocyte adhesion molecules by endothelial cells crucially regulates inflammatory cell influx into the forming atherosclerotic plaque (20). Attracted by monocyte chemotactic protein-1, monocytes transmigrate through the vessel wall, differentiate into macrophages and start to take up oxidized LDL (ox-LDL) and other cholesterol esters using their scavenger receptors, thereby differentiating in foam cells (21, 22). The foam cells which release more chemokines, cytokines and reactive oxygen species aggravating disease progression (23). Besides this fatty streak formation and inflammation, a hallmark of the initial stages of atherosclerosis is intimal hyperplasia or neointimal formation. Medial smooth muscle cells, adventitial fibroblasts and circulating fibrocytes are all implicated as origin of neointimal cells, however, an increasing body of evidence suggest that upon TGFβ and inflammatory activation, endothelial cells might acquire a mesenchymal-like or fibroproliferative phenotype and migrate into the neointima (24, 25). Endothelial lineage-tracing studies indicate that luminal endothelial cells undergo a process called Endothelial-Mesenchymal Transition (EndMT) and form myofibroblast-like cells that accumulate in the neointima and fibrous cap of atherosclerotic lesions (26, 27). EndMT is a cellular transdifferentiation process wherein endothelial cells lose the expression of endothelial cell-specific markers while the expression of mesenchymal cell markers is induced. Moreover, at the functional level, endothelial cells lose the ability to produce NO, loosen their endothelial cell-cell junctions, transit from a quiescent to a (hyper)proliferative state, acquire migratory and contractile properties and start to produce extracellular matrix components, culminating in enhanced leukocyte diapedesis, intimal lipid accumulation, intimal accumulation of fibroproliferative cells and the accumulation of fibrotic elements(28, 29). Also ageing is the major non-modifiable risk factor for the development of atherosclerosis. Cellular senescence is the phenomenon by which cells cease to divide in response to telomere shortening ageing or biochemical damages (e.g. ROS accumulation and DNA damage)(30).
Senescent endothelial cells adopt pro-inflammatory, pro-thrombotic phenotype and lose their cell-cell junction and regenerative capacity (31). Senescent endothelial cells are found in the atherosclerotic lesion (32), which indicates that endothelial senescence might contribute to the development and aggravation of atherosclerotic lesions.

From the above, we can conclude that endothelial dysfunction (i.e. endothelial oxidative stress, mesenchymal transition and senescence) plays a pivotal role in atherogenesis and therefore postulate that the endothelium might serve as an efficacious therapeutic target cell for anti-atherogenic therapies. In this perspective, we discuss the potential to ameliorate atherogenesis via the restoration of endothelial homeostasis using epigenetic drugs.

### II. CURRENT ATHEROSCLEROSIS TREATMENTS

Current medical treatments to prevent atherosclerosis development, progression and plaque rupture encompass lipid lowering and the prevention of blood clotting (Figure 2) and emerging anti-inflammatory therapies are currently under clinical investigations to increase the efficacy of anti-atherosclerosis treatment. Below, we discuss the currently available therapeutic agents and their rationale as an anti-atherogenic agent.

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<tr>
<th>Pathway Treatment</th>
<th>Complication Treatment</th>
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<td>Non-uniform/turbulent flow</td>
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<td>Accumulation of lipids</td>
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<td>Inflammation/leukocyte recruitment</td>
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<td>Accumulation of fibrous elements</td>
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<td>Surgical - vascular grafting</td>
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Figure 2. Current anti-atherosclerosis therapies. Established atherogenic pathways are depicted in blue bars and if treatments are currently available to counteract these pathways, it is depicted in the according brown bars. Current anti-atherogenic therapies successfully preclude dyslipidemia, platelet aggregation and systemic inflammation, however the fundamental problem “endothelial dysfunction” is not sufficiently addressed therapeutically.

ATHEROSCLEROSIS PATHOGENESIS THERAPIES

II.1 HYPERCHOLESTEROLEMIA:

The principal current pharmaceutical intervention for the treatment of atherosclerosis aims to reduce the lipid risk. As evidenced by epidemiological cohort studies as well as the clinical trials and meta-analysis, increasing levels of low density lipoproteins-C (LDL-C) associate strongly to the development of atherosclerosis and other cardiovascular diseases (extensively condensed in (33)). Extensive basic and clinical research has supported the dyslipidemia hypothesis and several groups of lipid lowering medications are currently available in the clinical practice.

Statins: Although a reduction in dietary cholesterol intake is able to reduce the serum cholesterol level, over two-thirds of serum cholesterol is synthesized in the liver. Statins, also known as HMG-CoA reductase inhibitors, act by reducing the liver’s production of cholesterol via the inhibition of the conversion of HMG CoA to mevalonic acid (34). Besides reducing serum LDL-C levels, statins offer anti-inflammatory (35) effects and increase endothelial NO production primarily through the activation of the endothelial nitric oxide synthase (eNOS) (36), which might alleviate endothelial oxidative stress. These pleiotropic effects might explain why statins outperform other lipid lowering drugs and statins are considered as the first-choice medicament to reduce lipid risk in the secondary prevention of multiple CVD. The reduction of LDL cholesterol by 1.0 mmol/L with statins reduces the risk of a major vascular events (myocardial infarction or coronary death, stroke, coronary revascularization) by 25%, regardless of the baseline LDL cholesterol level (37). According to the European Society for Cardiology (ESC) clinical guideline for dyslipidemias (ESC), statin treatment is recommended when patients have a LDL-C level greater than 3.0 mmol/L or have a (very) high 10-year risk to develop a fatal cardiovascular event (38).

PCSK9 inhibitors (Evolocumab, Bococizumab and Alirocumab): Although statins are the most-effective therapy available now for lowering LDL-C level, in part of the treatment population, the desired LDL-C level can’t be reached with the maximal tolerated dose of statin therapy. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is an enzyme which regulates the degradation of LDL-receptors (LDLR) in the liver. Monoclonal antibodies against PCSK9 reduce the degradation of LDL receptors and increase the clearance of the LDL-C (39). On the other hand, atorvastatin treatment reciprocally increased PCSK9 protein levels in serum by 34% compared to the placebo controlled group (40). This data suggested that PCSK9 inhibition in combination with statin treatment can further decrease LDL-C levels. Phase III clinical trial results proved that combination of statin and PCSK9 inhibitors can further decrease the LDL-C level and combination therapies are recommended if necessary (38, 41).
**Cholesterol absorption inhibitors (Ezetimibe):** Ezetimibe reduces the absorption of cholesterol from the intestine via a mechanism involving the Niemann-Pick C1-like 1 (NPC1L1) protein on the gastrointestinal epithelial cells (42). Ezetimibe itself can decrease LDL-C around 15-22% and in combination with statin treatment and additional 15-20% LDL-C lowering observed (38).

LDL-C reduction through the above-mentioned medications is one of the most efficient secondary prevention to decrease both relative and absolute risk. In certain extend, several clinical trial results indicated these beneficial effect is achieved not only through the lipid lowering, but also reducing the inflammation, implying that anti-inflammatory medications are important part of atherosclerosis treatment (43).

II.2 ANTI-INFLAMMATORY AGENTS

Inflammation plays critical role in the development and progression of atherosclerosis (18, 19) and the discovery of drugable targets that reduce inflammation in atherosclerosis has been a topic of intense research in cardiovascular medicine for several decades. Inflammation regulatory pathways such as interleukin-1 (IL-1), tumor necrosis factor α (TNF α), interleukin-6 (IL-6) are extensively targeted by selective inhibitors and monoclonal antibodies and some medicaments are in Phase III clinical trials. Also vascular targeted antioxidants, selective phospholipase A2 (PLA2) inhibitors, adhesion molecule inhibitors, serpines/sirtuins, FLAP inhibitors, 5-LO inhibitors, CCL2-CCR2 inhibitors and other molecules underwent extensive experimental and clinical research (extensively reviewed (44, 45)) Recently, promising results were reported by the CANTOS trial using monoclonal antibody named canakinumab.

**Canakinumab:** Canakinumab is a monoclonal antibody against Interleukin 1β (IL1β) and an approved drug for treating cryopyrin-associated periodic syndrome (CAPS) (46). IL1β is released from macrophages and one of the main mediators of innate immunity. In Canakinumab-treated patients, markers of inflammation such as Interleukin 6 (IL-6) and high sensitivity CRP (hs-CRP) were decreased without changes in their lipid profile (47) resulting in an reduced cardiovascular event risk score. A randomized double blinded clinical trial result indicated that canakinumab significantly lowers the occurrence of cardiovascular events and cardiovascular deaths compared to the placebo control group. However, an increasing incidence of fatal infections, sepsis and mild thrombocytopenia was associated with Canakinumab treatment (48).

II.3 ANTI-THROMBOTIC AGENTS

Arterial thrombosis is commonly initiated by the rupture of an atherosclerotic plaque which triggers platelet aggregation and thrombus formation (49). This process is called atherothrombosis and is the main cause of mortality in atherosclerosis. Hence, inhibiting platelet aggregation (anti-aggregants) and inhibiting blood coagulation (anti-coagulants) are pivotal parts of anti-atherosclerosis treatment, especially in the late stages. The damaged endothelium recruit platelets and enables primary and secondary hemostasis. In contrast, the quiescent healthy endothelium prevents these thrombogenic processes via prostaglandin I2 activation (50) and NO induction (51).
**Thromboxane A2 inhibitor (Aspirin):** Low dose aspirin (acetylsalicylic acid) inhibits platelet cyclooxygenase which is vital enzyme for thromboxane A2 generation. Thromboxane A2 triggers platelet aggregation and adhesion. The long-term usage of antiplatelet therapies shown to reduce vascular events around 25% among patients who have already experienced occlusive vascular diseases (52). In primary prevention trials, aspirin usage reduced the frequency of cardiovascular events over 12% in patients that have a myocardial infarction in their history (53).

**P2Y12 inhibitors (Clopidegril, Ticagrelor, Prasugrel, Cangrelor):** Inhibiting the P2Y12 receptor blocks the binding of extracellular adenosine diphosphate (ADP) to its receptor, which prevents thrombocyte aggregation. The P2Y12 inhibitor (clopidegril) combined with aspirin reduced serious vascular events by 20% in myocardial infarction patients with ST-segment elevation. (54). Clopidegril is prodrug which is metabolized and converted into its active form by Cytochrome P 450 enzyme (CYP). Patients who have different isoforms of the CYP enzyme respond different to clopidegril treatment (49). Prasugrel, another P2Y12 inhibitor, acts faster and was shown to reduce recurrent vascular events and stent complications compared to clopidegril after angioplasty and PCI (55).

**GPIIIb/IIIa inhibitors (Tirofiban, Eptifibatide, Abciximb):** GPIIb/IIIa inhibitors(GPIs) are potent and rapid acting antiplatelet drugs. The GPIs target the αIIbβ3 integrin on the platelet membrane, thereby inhibiting platelet aggregation (56). Meta-analysis indicted that 30-day death or myocardial infarction was moderately decreased after using these medications compared to the placebo group, the efect was highly pronounced in patients undergoing PCI (57).

**PAR-1 inhibitors (Voraxapar):** The novel class of antiplatelets drugs are developed to inhibit protease activated receptors (PAR-1) which mediates thrombin-induced platelet activation. Interestingly, the PAR-1 receptors are not only present at the platelets but also at endothelial cells, smooth muscle cells and fibroblasts (58). A phase III clinical trial indicates that Voraxapar addition to the standard treatment can decrease the risk of cardiovascular death and ischemic events, but moderate and severe bleedings occur more often (59).

From the above, it becomes evident that among the core atherogenic pathways, only lipid accumulation, inflammation and platelet aggregation are addressed by currently available therapeutic agents and therapeutic agent in development. As emphasized above, the endothelium plays a pivotal role in all atherogenic pathways, yet no endothelial-targeted therapy is available. In the next section, we elaborate on how endothelial-specific epigenetic molecules might offer a potential therapeutic beneit for patients suffering from atherosclerosis.

### III. ENDOTHELIAL SPECIFIC PRO-ATHEROGENIC TREATMENT

Endothelial cells play a crucial role in the development and progression of atherosclerosis. Stimulated by uniform laminar flow, the endothelial cells acquire healthy quiescent phenotype that precludes atherosclerosis pathways (24). In contrast, disturbed flow alters the endothelial phenotype, which enables atherosclerosis pathways. This phenotypic shift might be the consequence of differential gene expression regulated by epigenetic modifications. An important feature of the epigenetics is the reversibility.
Following the fact that epigenetic molecules are well established as mediators of health and disease, epigenetic enzymes or their activator/inhibitors can be exploited as therapeutic target (60, 61).

III.1. EPGENETIC MOLECULES ARE PROMISING CANDIDATES TO TREAT THE PRO-ATHEROGENIC ENDOTHELIUM

Epigenetics refers to heritable yet stable changes in genome function resulting from changes in the chromatin without alterations in the underlying DNA sequence. In other words “changing the cells’ phenotype without changing genotype” (62). Epigenetic modifications can explain how one fertilized egg gives rise to more than 200 different cell types that compose the human body (63). Moreover, epigenetics explain novel mechanisms for complex and chronic diseases such as diabetes (64), cancer (65, 66) and cardiovascular diseases (67). Epigenetic traits consist of several interconnected parameters, i.e. histone modifications and DNA methylation (68).

On average, a human cell has a 2-metre long DNA molecule. Cell size and timely transcriptional activity requires organized folding of the DNA. In the nucleus, the DNA strand is 1.7 times coiled around an octamer of core histone proteins, forming the nucleosomes. (H2A, H2B, H3 and H4, 2 copies of each) (69). Core histone proteins contain a globular domain and an amino terminal tail which can undergo post-transcriptional modifications such as acetylation, methylation (lysines/arginines), phosphorylation, sumoylation, ubiquitylation, ADP ribosylation, deamination, proline isomerization and other modifications (70). Many of these modifications are known to play functional roles in gene expression. The functional role of lysine acetylation and methylation of histone core proteins on transcriptional level are well studied. For instance, histone acetylation is associated with transcriptional activation by amongst others neutralizing the basic charges of lysine residues (71), whereas the consequence of histone methylation depend on the specific lysine or arginine residue that is methylated. Methylation of H3K27 and H3K9 correlate with the transcriptional repression, but methylation of H3K4 and H3K36 correlates transcriptional activation (72). Thus, histone modifications affect gene expression via altering chromatin structure and accessibility.

_DNA methylation_ refers to the addition of a methyl group (-CH3) on 5th carbon atom of cytosine of the DNA. When DNA methylation occurs at gene promoter areas rich in cytosine and guanidine residues (so-called CpG islands) linked to the transcriptional repression (73). However, 5 methylcytosine (5mC) also found in gene body (transcribable region) and related to the supportive function in transcription (74, 75). The contribution of epigenetic mechanisms to atherosclerosis development is under extensive research. Here, we focus on the contribution of epigenetic modifications in the early stages of atherosclerosis and question if reversing those changes may have anti-atherosclerosis capacity.

One of the main epigenetic features found in early atherogenesis is DNA hypomethylation. CpG islands in newly forming atherosclerosis lesions are mostly hypomethylated compared to non-atherogenic vessel areas. However, several hypermethylated genes also be identified (76). By using high performance liquid chromatography analysis, the 5mC content was 3.2%±0.2 in healthy arteries and declined to 2.9%±0.1 in advanced atherosclerosis lesions (77).
The finding was supported by the study results implicating that 84% of differentially methylated promoter sites were hypomethylated (de-methylated) in femoral artery atherectomy samples compared to the non-sclerosed mammary artery samples (78). These studies indicate that DNA hypomethylation is dominantly occurring during atherosclerosis.

Human carotid artery atherosclerosis samples showed increased acetylation (active mark) of H3K9 and H3K27 in endothelial cells in early stage of atherosclerosis and this increment was consistently kept high in advanced atherosclerotic plaques. Quantitative PCR result revealed that the expression of histone acetyltransferase GCN5L is elevated in advanced atherosclerotic plaques compared to control. Also, H3K4 methylation (active mark) was increased in endothelial cells in early stage maintained high during the advanced atherosclerosis (79). This finding matches with the previous finding from Wierda et al (80), who showed increased H3K4 methylation and expression of the H3K4 writer MLL2/4 in endothelial cells in atherosclerotic lesions compared to non-atherogenic sites. The methylation level of H3K27 (repressive mark) is higher in early stages of atherosclerosis and normalizes during the advanced atherosclerotic plaques in endothelial cells (79). It is interesting phenomenon that concurrent increment of H3k27 methylation, H3k27 acetylation and H3k4 methylation was observed in early stage of atherosclerosis, which may imply the presence of “bivalent domains” (81) that might contribute to endothelial dysfunction and atherosclerosis. However, it is still elusive whether these opposing modifications are occurring at the same gene locus or reflect epigenetic regulation across different loci.

The above-mentioned data indicate that during atherosclerosis, the epigenome of the endothelial cells changes, and it raises the question if we can we ameliorate atherosclerosis progression via reversal of these modifications? Here we demonstrate the beneficial effects of epigenetic therapies by exemplifying two histone modifying enzymes as molecules for targeting the pro-atherogenic endothelium and promote endothelial homeostasis.

**EZH2- ENHANCER OF ZESTE HOMOLOGUE 2**

**Rationale:** Methylation of H3K27 is increased during the early stage of atherosclerosis in endothelial cells. Thereby, decreasing Ezh2 might be beneficial to ameliorate pro-atherogenic endothelium via reducing the methylation of H3K27.

Enhancer of zeste homologue 2 (Ezh2) is the catalytic subunit of Polycomb Repressive Complex 2 (PRC 2). In mammals, Polycomb Repressive Complex 2 core subunits are EED, SUZ12 and EZH2/EZH1. EZH2 and its close homologue EZH1 have SET domain which encompasses its histone methyltransferase activity. EZH2 trimethylates lysine 27 on N terminal tail of histone 3 protein. H3K27me3 act as docking site for chromobox-domain (CBX) of Polycomb Repressive Complex 1. H3K27me3 is a repressive chromatin mark that leads to the formation of condensed chromatin and transcriptional silencing of the target gene. (82) The rationale of targeting EZH2 in the endothelium to ameliorate atherogenesis is summarized in Table 1. Shear stress regulates the protein expression of histone methyltransferase EZH2. Under atheroprotective -laminar flow, EZH2 protein expression is low, thereby inducing a quiescent phenotype in endothelial cells (83). Elevated serum homocysteine is one of the independent risk factors of the atherosclerosis and has adverse effects on endothelial cells.
Interestingly, homocysteine enhanced fat accumulation and increased EZH2 and H3K27me3 levels are found in atherosclerosis-prone APOE-/- mice (84). The metabolic conversion of homocysteine Hcy-thiolactone induces the expression of EZH2 in a dose-dependent manner in endothelial cells (85). Moreover, LDL-C can reduce the expression of KLF2 – a well-established antiatherogenic transcription factor - which can be precluded by inhibiting EZH2 (86). These findings indicate that the elevated expression levels of EZH2 during atherogenesis is detrimental for endothelial homeostasis and might aggravate atherogenesis. One of the main representatives of the statin therapies, i.e. simvastatin decreased the transcriptional and translational levels of EZH2 in colorectal cancer cells. This finding suggest that some beneficial effects of statin outside the lipid-lowering effects might be achieved through the reduction of EZH2 in endothelial cells (87).

Besides the endothelium, elevated expression of EZH2 in macrophages enhances foam cell formation via ABCA1 gene promoter DNA methylation(88) and EZH2 affects DNA methylation in polycomb target gene areas via modulating DNMTs (89). Based on the above, EZH2 inhibition might be beneficial to endothelial homeostasis and may ameliorate atherosclerosis progression.

SIRT1- NAD+ DEPENDENT DEACETYLASE GROUP III

Rationale: Acetylation of H3K9 and H3K27 is increased during the early and advanced stages of atherosclerosis in endothelial cells. Thereby increasing histone deacetylase SIRT1 might be beneficial via reversing the acetylation of H3K9 and H3K27.

Sirtuin 1, the mammalian ortholog of yeast Sir2, is a nicotinamide adenine dinucleotide (NAD) dependent deacetylase. SIRT1 removes acetyl group from histone tails and non-histone proteins. Higher expression levels of SIRT1 positively correlate with lifespan in yeast, flies and mice.(90) SIRT1 activation protects cardiomyocytes from endoplasmic reticulum (ER) stress-induced apoptosis by attenuating PERK/eIF2α pathway activation. The rationale of using SIRT1 to ameliorate atherogenesis is exemplified in Table 1.

SIRT1 is also a shear stress responsive protein. Atheroprotective - uniform laminar flow induces SIRT1 protein expression, while static or oscillatory shear stress inhibits SIRT1 expression(91). As mentioned above, inflammation plays a key role in endothelial dysfunction and atherogenesis. NF-κB is the core transcription factor of inflammation and inflammation mediated responses. SIRT1 can de-acetylate and deactivate NF-κB, thereby inhibiting inflammation(92). Also endothelial senescence contributes to the atherosclerosis development and SIRT1 induction was shown to prevent from H₂O₂-induced endothelial senescence(93). Moreover, SIRT1 elevates NO production in endothelial cells. Albeit that the SIRT1-dependent favorable effects on the endothelium are pleiotropic, in synergy these effects enable endothelial homeostasis and might offer therapeutic benefit in atherosclerosis.

Several clinical trials have demonstrated and the SIRT1 activator SRT2014 can decrease serum LDL levels (94) via decreasing the PCSK9 secretion of from hepatic cells (95). Interestingly, SIRT1 modulates DNA methylation and the target genes overlap with the Polycomb group proteins (96), implying an interconnection between these two epigenetic enzymes.
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<th>SIRT1- NAD dependent histone deacetylase (eraser)</th>
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<tr>
<td><strong>Non-uniform/turbulent flow</strong></td>
<td>Laminar flow decreases Ezh2 and upregulation of Ezh2 post-transcriptionally inhibit MAPK7 activity</td>
<td>Laminar flow increases SIRT1 and its activity Static/oscillatory shear stress inhibits SIRT1 level</td>
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<td><strong>Lipid accumulation</strong></td>
<td>Homocystein induced atherosclerosis via upregulating EZH2 and H3K27me3 in APOE-/- mice</td>
<td>SIRT1 activator SRT3025 reduces serum LDL-C via reducing hepatic PCSK9 secretion</td>
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<td><strong>Inflammation / leukocyte recruitment</strong></td>
<td>Overexpression of EZH2 induces lipid accumulation in macrophages by methylating ABCA1 gene promoter thereby accelerate atherosclerosis progression in apoE-/- mice</td>
<td>Hyperglycemia induced endothelial dysfunction is prevented via SIRT1 dependent P66shC downregulation SIRT1 deacetylase NFκB thereby prevent chronic inflammation</td>
</tr>
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<td><strong>Blood clotting</strong></td>
<td>EZH2 knockdown prevents LDL induced downregulation of thrombomodulin (TM) thereby prevents from the unnecessary platelet aggregation</td>
<td>H2O2 induced endothelial senescence rescued by the SIRT1 SIRT1 downregulates PAI thereby prevents replicative senescence</td>
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<td><strong>Endothelial senescence</strong></td>
<td>Ezh2 regulates sm22a/TAGLN expression</td>
<td>SIRT1 modulates EMT in cancer</td>
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<td><strong>Accumulation of fibrous element (EndMT)</strong></td>
<td>EZH2 knockdown prevents LDL induced downregulation of the NO decline through the KLF2 promoter methylation</td>
<td>SIRT1 induce NO production via increasing eNOS production Vitamin D rescues endothelial cells from oxidative stress mek/erk- SIRT1 cascade SIRT1 promotes mitochondrial biogenesis via activation of PGC1α</td>
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<tr>
<td><strong>DNA methylation</strong></td>
<td>Ezh2 directly controls DNA methylation</td>
<td>SIRT1 affects DNA methylation of polycomb group target genes</td>
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Table 1. EZH2 and SIRT1 role in endothelial homeostasis and contribution to the development of atherosclerosis
III.2. AVAILABILITY OF THE EPIGENETIC ENZYME MEDICAMENTS

The current therapeutic strategies and possibilities of using epigenetic molecules for the treatment of cancer and other diseases have been reviewed previously (103, 104) and several studies are already using EZH2 and SIRT1 as epigenetic targets in clinical trials. (see table 2)

The possibility of using histone methyltransferases and demethylases (105) especially EZH2 inhibitors (106) in cancer therapy have been reviewed previously. EZH2 inhibitors are being tested in Phase I/II clinical trials in cancer field, but they are not yet used in trials for atherosclerosis. (Table 2). Compared to the EZH2, SIRT1 activators are well known in field of the cardiovascular medicine. Several SIRT1 activators are recognized and being tested and cardiovascular outcomes were measured (Table 2). Resveratrol, a well-known activator of SIRT1 was used in cancer, neurological disorder, cardiovascular diseases, diabetes and other diseases clinical trials (extensively reviewed in (107)).

<table>
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<tr>
<th>Drug name</th>
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<th>Indication</th>
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<tr>
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<td>EZH2 inhibitor</td>
<td>II</td>
<td>INI1-negative tumors</td>
<td>NCT02601950</td>
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<td>GSK2816126</td>
<td>EZH2 inhibitor</td>
<td>I</td>
<td>Diffuse B cell Lymphoma, other Non- Hodgkin Lymphoma, solid tumors and multiple myeloma</td>
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<td>SRT2104</td>
<td>SIRT1 activator</td>
<td>I</td>
<td>60-80 years old males</td>
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<td>SIRT1 activator</td>
<td>II</td>
<td>Otherwise healthy smokers</td>
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</table>

Table 2. EZH2 inhibitors and SIRT1 activators in clinical trials. Showing early phase clinical trials using above mentioned epigenetic enzymes – implying these therapeutic molecules are already available and tolerated to use in human.

From above mentioned results we can see that EZH2 inhibition (open chromatin) and SIRT1 induction (closed chromatin) can be beneficial in providing endothelial homeostasis thereby slowing down atherosclerosis progression. Moreover, small molecules that inhibit EZH2 or activate SIRT1 are available in clinical trials which warrants the adaptation of cardiovascular endpoints in these trials. Moreover, given the current safety record of these experimental medicines, the clinical testing in the context of atherosclerosis could be performed in the near future.
Figure 3. SIRT1 activator and EZH2 inhibitor based treatment of atherosclerotic endothelium. We explained how epigenetic molecules can be promising candidates to treatment pro-atherogenic endothelium using two pre-exemplary molecules namely SIRT1 and EZH2. Together with the current medications, our proposed pro-atherogenic endothelium treatment may slow down the atherosclerosis progression and prevent from the life-threatening complications.

Although the chromatin modeling consequence of these two enzymes is controversial, the effect is target gene dependent. Epigenetic modifications can be reversed and “Epigenetic editing” is an emerging research field in medicine (extensively reviewed (108, 109)). Epigenetic repression and epigenetic activation are successfully accomplished by using Zinc Finger Proteins (ZFP), Transcription- Activator-Like Effectors (TALEs) arrays or Clustered Regulatory Interspaced Short Palindromic Repeats (CRISPR). Moreover, the newly edited modifications are shown sustainability through the cell division (110).
III.3. targeted approach to endothelial cells in atheroprone areas

Since atherosclerosis lesions develop exclusively at vascular branches and curvatures, the ideal treatment would be to target the affected endothelial cells at these atheroprone areas only. Moreover, lineage committed cell have a distinct epigenome landscape including DNA methylation and histone modifications, (111, 112) which might make the systemic application of epigenetic drugs harmful to non-target cells in the human body. Promising liposome-based drug delivery approaches are available that might be suitable to deliver therapeutic agents to the pre-atherogenic activated endothelium exclusively (113). For instance, SAINT-O-Somes directed to microvascular endothelial cells expressing VCAM-1 (114) successfully inhibited inflammatory genes in microvascular endothelial cells without toxic effects liver and kidney (115). Also, E-selectin targeted immunoliposomes successfully abrogated ANCA-induced glomerulonephritis via targeted delivery of siRNA against NF-kB in glomerular endothelial cells (116). Some studies used specific peptides for the targeted treatment approach. For example, the ICAM-targeted CLIRRTSIC peptide was successful in targeting endothelial cells in disturbed flow exposed areas in vivo (117). Outcome of endothelial cells-specific SIRT1 overexpression was tested in ApoE-/- mice; this study revealed an enhancement of the endothelium-dependent vasodilation and less atherosclerosis lesion development (118).

Conclusion

Endothelial dysfunction is a critical component of the development of the atherosclerosis. Current atherosclerosis treatment encompasses lipid lowering, inhibiting platelet aggregation and anti-inflammatory drugs, however there is no treatment available that targets pathway “endothelial dysfunction”. In this review, we proposed the targeting of two epigenetic pathways (i.e. SIRT1 and EZH2) to ameliorate atherogenesis and exemplify a number of established medicaments that would allow for rapid clinical valorization. Moreover, we set forth a future strategy that utilizes a cell-targeted strategy using drug carriers that might further enhance endothelial homeostasis and ameliorate atherosclerosis development.
CHAPTER 2

REFERENCES


PLEIOTROPIC TREATMENT OF PRO-ATHEROGENIC ENDOTHELIUM


