Key transcriptional regulators of the vasoprotective effects of shear stress

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Summary
Atherosclerotic plaque rupture and subsequent thrombosis is the main cause of sudden coronary death. Remarkably, atherosclerosis only develops in certain predisposed areas of the vasculature. Endothelial cells in these predisposed areas experience low or oscillatory shear stress, which activates the proinflammatory and procoagulant transcription factors activator protein 1 (AP-1) and nuclear factor κB (NFκB), thus inducing a proinflammatory, procoagulant surface. In contrast, healthy endothelial cells that are exposed to prolonged high laminar shear stress, express anti-inflammatory and anticoagulant genes. The key shear stress-induced transcription factors that govern the expression of these genes are Krüppel-like factor 2 (KLF2) and nuclear factor erythroid 2-like 2 (Nrf2). Together KLF2 and Nrf2 govern — 70% of the shear stress-elicited gene sets. Nrf2 potently induces anti-inflammatory/antioxidant enzymes, while KLF2 induces anti-inflammatory and anticoagulant proteins, most specifically endothelial nitric oxide synthase (eNOS) and thrombomodulin (TM). KLF2 also inhibits proinflammatory and antifibrinolytic genes through inhibition of the proinflammatory transcription factors AP-1 and NFκB. The widespread beneficial effects of the key transcription factors KLF2 and Nrf2 on endothelial phenotype, holds the promise that their targeted modulation might lead to a new class of cardiovascular drugs.

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Shear stress

Inverse correlation with atherosclerosis development

Atherosclerosis is a prevalent disease in the Western world and is the underlying cause for acute myocardial infarction (MI) and stroke. These acute events are mainly triggered by rupture of the atherosclerotic plaque and subsequent occlusive thrombosis in 84% of the cases of MI (1). In the remaining cases, either endothelial erosion or its local, strong procoagulant properties are thought to be responsible. Despite the systemic nature of the associated risk factors like smoking, diabetes, hyperlipidemia and hypertension, it is known that atherosclerosis only develops at predisposed sites in the arterial tree (2). This is likely due to the local disturbances in blood flow, as these sites are always near bends and bifurcations in the vasculature. Laminar blood flow occurs in straight parts of arteries and near the outer curvatures of bends and exerts a tangential viscous drag on the vascular endothelium, called shear stress. This laminar flow that is sensed directly by the endothelium is unidirectional and pulsatile, due to the cardiac cycle, and reaches shear stress levels of 15 to 70 dynes/cm² (3). Near bends and bifurcations, the flow is still pulsatile, but highly turbulent and bidirectional, generating oscillatory shear stress of only 0 to 10 dynes/cm². High shear stress induces an atheroprotective and anticoagulant endothelial phenotype, while low or oscillatory shear stress is associated with endothelial dysfunction and atherosclerosis development. This review focuses on the transcriptional activities of endothelial cells in response to shear stress.

Effects on endothelial cells

Different transcriptional responses

In an experimental context, endothelial cells are mostly cultured in the absence of flow, whereas healthy endothelial cells in vivo are exposed to shear stress throughout their entire lifespan. Therefore, it is important to distinguish between short-term shear stress exposure (>24 h) and prolonged shear stress exposure (>24 h), as the first endothelial responses to acute shear stress changes will be less reminiscent of endothelial cell shear stress exposure in vivo. Nonetheless, short-term shear stress exposure gives vital clues about the mechanisms by which endothelial cells sense shear stress and convey these signals. For example, using shear stress exposure of less than one hour, Tzima and colleagues identified a cell-cell junctional complex-dependent sensory complex that detects acute shear stress changes (4). Transcriptional changes specific for an acute shear response are mainly elicited by the activator protein 1 (AP-1) and nuclear factor κB (NFκB) transcriptional complexes (5).

These transcription factors are generally known to induce proinflammatory and procoagulant gene expression and this does not correlate with the anti-inflammatory and anti-coagulant effects of shear stress in vivo. On the contrary, activation of NFκB and AP-1 (consisting of a dimer of ATF2 and c-Jun) by Jun NH2-terminal kinase (JNK) is known to occur specifically in endothelial cells exposed to oscillatory shear stress (6–8). Furthermore, the downstream target genes of these transcription factors, like MCP-1, E-Selectin, ICAM-1 and tissue factor (TF) are known to be expressed by inflamed endothelial cells (9). This phenomenon also leads to the historical misinterpretation of the so-called shear stress responsive element (SSRE) in the promoters of these genes, which turned out to be the NFκB binding site (9). With hindsight, this is not surprising since these experiments were all done with short shear stress stimulation. Long-term shear stress exposure (>24 h), on the other hand, does confer anti-inflammatory and anticoagulant properties to cultured endothelial cells (10). Well-known examples of genes that are induced by long-term shear stress are endothelial...
nitric oxide synthase (eNOS) and thrombomodulin (TM) (11, 12). These proteins are potent anti-inflammatory and anticoagulant molecules and are thought to account for a large part for the anti-inflammatory and anticoagulant properties of healthy endothelial cells.

In the past several studies were performed to identify the differences on the transcriptomic level of endothelial cells exposed to high shear stress compared to control conditions. One of the first studies on this subject showed that in pig aortas, endothelial cells in (atheroprotected) regions of high shear stress have lower expressions of both pro- and anti-inflammatory genes (13). Using a similar full-genome approach, Dekker et al. (14) identified the transcriptomic level of endothelial cells expressing of pro-inflammatory and procoagulant genes. A short-term shear stimulation induces expression of pro-inflammatory and procoagulant genes.

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More recently it was shown that KLF2 improves the nuclear localization of Nrf2 and the combined actions of these two factors constitute about 70% of the shear-stress-induced endothelial gene expression (Fig. 1a) (18).

Shear stress induces KLF2 by raising protein levels through transcriptional activation at the KLF2 promoter, as well as through KLF2 mRNA stabilization (14, 22). Implicated in the transcriptional activation at the KLF2 promoter is myocyte enhancer binding factor 2 (MEF2), which binds to an evolutionary conserved MEF2-binding site (23, 24). MEF2 is activated by the upstream mitogen activated protein kinase (MAPK) signaling cascade consisting of MAPK kinase 5 (MEK5) and extracellular-signal-regulated kinase 5 (ERK5, BMK1) (18). Cofactors that have been implicated in the regulation of MEF2 transcriptional activity at the KLF2 promoter are histone methyl transferases (HMTs) and histone de-acyetylases (HDACs), as well as nucleolin (25). Both activation of the HMT P300/cAMP-response element-binding protein-binding protein-associated factor (PCAF) and nucleolin requires PI3K signaling to enhance MEF2 transcriptional activity on the KLF2 promoter (26). Recruitment of HDACs 4 and 5, however, have been implicated in the inhibition of MEF2-dependent KLF2 transcription by NFκB after stimulation with the inflammatory cytokines IL1 and TNFα (27).

Pharmacological inducers of KLF2 are the well-known 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors, also known as statins (22, 28, 29). The mech-
The mechanism behind this induction is that statins inhibit the addition of a crucial geranyl geranyl pyrophosphate moiety to Rho, thereby relieving the inhibitory effect of Rho on the MEK5/ERK5/MEF2 pathway (29). On the other hand, statins do not stabilize KLF2 mRNA levels like shear stress stimulation does and therefore the statin-mediated induction of KLF2 is not as potent as the shear stress-mediated induction of KLF2 in evoking stable eNOS and TM expression, especially in the presence of TNFα (22).

**Knock-out models**

Nrf2−/− mice are seemingly normal, but show a reduced capacity to cope with oxidative insults (30), while Keap1−/− mice die postnatally due to constitutively active Nrf2 (31). Studies concerning endothelial-specific Nrf2−/− in the context of atherosclerosis are still lacking. KLF2−/− mice have been generated more than a decade ago, but these mice die around embryonic day E13.5 due to blood vessel malformation (32). More recent results show that lack of endothelial KLF2 results in improper recruitment of smooth muscle cells to form a stable blood vessel, giving rise to a lack of vessel tone and ultimately leading to cardiac overload and embryonic lethality (33, 34). Interestingly, the upstream MAPKs MEK5 and ERK5 that induce KLF2 expression have also been found to be essential for proper endothelial function (35, 36).

In addition, MEF2C is a critical mediator in vascular development and MEF2A loss of function mutations have been found to be associated with cardiovascular disease (37, 38). The viable hemizygous KLF2 knockout mouse (KLF2+/−) does not have an apparent endothelial phenotype, which is probably due to compensatory upregulation of KLF4 (39). Still, when crossed to the apolipoprotein E-deficient background, KLF2+/− do have aggravated atherosclerosis development, possibly through increased foam-cell formation.

**Target gene expression and function**

Transcription factors regulate gene expression by directly binding to promoters of target genes. Nrf2 binds to the antioxidant response element (ARE) present in the promoters of many antioxidant enzymes like heme oxygenase 1 (HO1) and NAD(P)H dehydrogenase quinone 1 (NQO1). These direct target genes can also affect the expression of other genes, which therefore constitute indirect targets. In the case of Nrf2 activation, the direct targets enhance the antioxidant capacity of the cell, resulting in less oxidative stress and subsequently in less inflammation.

Direct targets for KLF2 have also been identified, even though a consensus KLF2 binding sequence in promoters of target genes has not yet been identified. The general GC rich KLF binding site 5’-CACC-3’ seems not very specific and can in principle be bound by all 20-odd KLF family members, as well as by other transcription factors such as SP-1. Such GC rich KLF binding sites are present in most of the identified direct targets of KLF2, like eNOS and TM, which are known to be involved in endothelial homeostasis (40, 41). These two proteins both have essential functions in keeping the endothelium anticoagulant and the shear stress-mediated and also the statin-mediated induction of these proteins was shown to be dependent on KLF2 expression (17, 29, 40).
The KLF2-regulated transcriptome probably contains a large amount of indirect targets as well, because KLF2 was reported to regulate the expression of over a thousand genes (24, 42). Most of the anti-inflammatory effects of KLF2 (apart from direct eNOS induction) are probably indirect. For instance, KLF2 was shown to recruit the essential cofactor cyclic AMP response element-binding protein (C3BP/p300) away from NFκB, thereby inhibiting the transcriptional activity of NFκB, leading to attenuation of inflammatory gene expression (41).

Another mechanism by which KLF2 confers its anti-inflammatory actions is through inhibition of nuclear localization of phosphorylated AT1F2, which is essential for inflammatory gene expression in endothelial cells (6). AT1F2 can form a transcriptional complex together with c-Jun, called AP-1 and c-Jun phosphorylation is also inhibited by KLF2 (43). AP-1 is well-known to induce pro-inflammatory and procoagulant gene expression, and can be activated by p38 and JNK MAPK signalling, suggesting that KLF2 interferes with these pro-inflammatory MAPK pathways. Furthermore, KLF2 inhibits pro-inflammatory signalling through the thrombin receptor, and by inhibiting TGF-β signalling, thus potentially preventing the expression of antifibrinolytic PAI-1. The latter occurs through a simultaneous induction of the inhibitory Smad7 and inhibition of the above-mentioned AP-1, which is an essential co-factor for TGF-β signalling, leading to a decrease of Smad4 transcriptional activity and downstream gene expression (43). Other indirect anti-inflammatory effects of KLF2 are elicited through Nrf2 and its antioxidant enzyme target genes, as KLF2 improves the nuclear localization and transcriptional activity of Nrf2 (18).

The changes in expression of many of the KLF2 modulated genes can be classified as anti-coagulant and pro-fibrinolytic (Tab. 1). Especially, the coordinated upregulation of TM and downregulation of the thrombin receptor (F2R, PAR-1) results in a KLF2-mediated hundred-fold lower affinity for thrombin (42, 44). As a consequence, von Willebrand factor (VWF) release from Weibel-Palade bodies is also markedly reduced by KLF2, even though a slight controversy still exists whether KLF2 induces or represses VWF expression on the mRNA level (Tab. 1) (40, 42). Other procoagulant proteins like tissue factor (TF), TF pathway inhibitor 2 (TFPI2) and IL8 are also markedly down-regulated by KLF2, as well as antifibrinolytic proteins like plasminogen activator inhibitor 1 (PAI1). Many of these anticoagulant genes also contain AP-1 sites but not an archetypal KLF-site (CACCC), likely indicating indirect modulation by KLF2 (6), while the direct KLF2 targets TM and eNOS do have GC-rich KLF consensus binding sites (40, 41).

**Future directions, conclusion**

Endothelium is in a constant balance between a pro- or anti-inflammatory and a pro- or anticoagulant state. Prolonged laminar shear stress tips the balance to the anti-inflammatory and antiaggregative side through activation of KLF2 and Nrf2, while oscillatory shear stress and short shear stress exposure tips the balance to the pro-inflammatory and proaggregative state via NFκB and AP-1 (Fig. 1b). Several key issues are still unresolved.

- It remains to be established whether ectopic expression or activation of the anti-thrombogenic and vasoprotective transcription factors KLF2 and Nrf2 in endothelial cells exposed to atheroprone shear stress would indeed tip the balance towards an anti-inflammatory state. As such, this might protect against atherosclerotic development or even arterial thrombosis.

**Conflict of interest**

The authors declare that they have no conflicts of interest.

References


