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Review

The role of epigenetics in the endothelial cell shear stress response and atherosclerosis☆

Jessilyn Dunn[a], Rachel Simmons[a], Salim Thabet[b], Hanjoong Jo[a,b,*]

[a] Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, USA
[b] Division of Cardiology, Georgia Institute of Technology and Emory University, USA

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ABSTRACT

Currently in the field of vascular biology, the role of epigenetics in endothelial cell biology and vascular disease has attracted more in-depth study. Using both in vitro and in vivo models of blood flow, investigators have recently begun to reveal the underlying epigenetic regulation of endothelial gene expression. Recently, our group, along with two other independent groups, have demonstrated that blood flow controls endothelial gene expression by DNA methyltransferases (DNMT1 and 3A). Disturbed flow (d-flow), characterized by low and oscillating shear stress (OS), is pro-atherogenic and induces expression of DNMT1 both in vivo and in vitro. D-flow regulates genome-wide DNA methylation patterns in a DNMT-dependent manner. The DNMT inhibitor 5-Aza-2′deoxycytidine (5Aza) or DNMT1 siRNA reduces OS-induced endothelial inflammation. Moreover, 5Aza inhibits the development of atherosclerosis in ApoE$^{-/-}$ mice. Through a systems biological analysis of genome-wide DNA methylation patterns and gene expression data, we found 11 mechanosensitive genes which were suppressed by d-flow in vivo, experienced hypermethylation in their promoter region in response to d-flow, and were rescued by 5Aza treatment. Interestingly, among these mechanosensitive genes, the two transcription factors *HoxA5* and *Klf3* contain cAMP-response-elements (CRE), which may indicate that methylation of CRE sites could serve as a mechanosensitive master switch in gene expression. These findings provide new insight into the mechanism by which flow controls epigenetic DNA methylation patterns, which in turn alters endothelial gene expression, regulates vascular biology, and induces atherosclerosis. These novel findings have broad implications for understanding the biochemical mechanisms of atherogenesis and provide a basis for identifying potential therapeutic targets for atherosclerosis.

This article is part of a Directed Issue entitled: Epigenetics dynamics in development and disease.

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* Corresponding author at: John and Jan Portman Professor, Coulter Department of Biomedical Engineering, Georgia Tech and Emory University, 1760 Haygood Drive, Health Sciences Research Bldg E170, Atlanta, GA 30322, USA.
E-mail addresses: hjo@bme.gatech.edu, hjo@emory.edu (H. Jo).

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1. Blood flow regulates endothelial cell biology and atherosclerosis

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Atherosclerosis, an inflammatory disease of the arterial wall, is the major cause of heart attack, stroke, and peripheral arterial disease. Atherosclerosis typically occurs in curves or branches in the vasculature due to the effect of disturbed blood flow (d-flow) on endothelial gene expression, which naturally arises as a consequence of the geometry and is known to induce endothelial cell (EC) dysfunction.

Blood flow generates shear stress on vascular endothelial cells. Unidirectional, laminar shear stress (LS, or s-flow) is crucial for normal vascular function, whereas d-flow, characterized as low and oscillatory shear stress (OS), causes vascular dysfunction and disease (Akimoto et al., 2000; Garcia-Cardena et al., 2001a; Chatzizisis et al., 2007a; Chien and Shyy, 1998; He et al., 2008). When compared to s-flow, ECs have drastically altered gene expression patterns both in vivo and in vitro (White et al., 2011; Gimbrone et al., 2000; Chappell et al., 1998; Dhawan et al., 2010; Abumiya et al., 2002; Topper et al., 1996). Atherosclerosis preferentially develops in areas of d-flow, where the dysfunctional EC phenotype initiates and perpetuates plaque accumulation (Garcia-Cardena et al., 2001b). Epigenetics control aberrant gene expression in many diseases including cancer and cardiovascular disease, but the mechanism of flow-induced epigenetic gene regulation via DNA methylation has not been well studied until very recently.

2. Mechanosensitive endothelial gene expression

High throughput, genome-wide gene expression studies have shown that shear stress regulates endothelial gene expression in vitro and in vivo (Garcia-Cardena et al., 2001a; Chien and Shyy, 1998; Chien, 2007; Davies, 1995; Nagel et al., 1994; Skogsberg et al., 2008; Chen et al., 2001). These studies have identified numerous shear-responsive regulatory pathways as well as novel mechanosensitive genes and functional gene clusters (Tarbell et al., 2014; Kwak et al., 2014).

Shear stress is able to be translated from the cell surface (luminal, junctional, and basal) through a variety of mechanosensors, including ion channels, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and xanthine oxidase (XO), receptor tyrosine kinases, G proteins, cell/cell and cell/matrix (integrins) adhesion complexes, the glycocalyx, caveolae, and cytoskeletal elements (Chatzizisis et al., 2007b). Once the shear stress stimulus is applied to the cell, various intracellular pathways are triggered in a process known as mechanotransduction. Interestingly, many of these pathways converge on common signaling pathways, such as the mitogen-activated protein kinase (MAPKs) pathway and the phosphatidylinositol-3-OH kinase PI3K/Akt pathway (Li et al., 2005). The MAPK pathway in particular can be activated through integrins, NADPH oxidase, or ion channels, among others. Integrins activated by mechanical stimuli phosphorylate and activate a complex of kinases (FAK, c-Src, Shc, paxillin, and p130CAS), adaptor proteins (Grb2, Crk), and guanine nucleotide exchange factors (Sos, C3G), which ultimately lead to the activation of Ras. When Ras becomes activated, this leads to the activation of MAPKs. The extracellular signal-regulated kinases (ERK1/2), members of the MAP kinase family, then activate transcription factors (c-myc, c-jun, c-fos, p62TCF) and/or eNOS (Traub and Berk, 1998). Furthermore, ion channels activate the MAPK pathway through protein kinase C (PKC) (Traub and Berk, 1998) whereas NADPH oxidase activates the MAPK pathway through ROS (Hwang et al., 2003). An additional example of such is the activation of platelet endothelial cell adhesion molecule-1 (PECAM-1). PECAM-1 directly transmits mechanical force at the junctional surface and VEGFR2 (in complex with PECAM-1 and VE-cadherin) activates PI3K. After PI3K is activated, Akt is also activated, and this leads to cytoskeletal arrangement so that ECs exposed to anti-atherogenic LS align in the direction of flow and ECs exposed to pro-atherogenic OS have activation of Rac1, which in turn leads to increased ROS and NF-κB becomes activated. Thus, in OS, these same mechanosensors lead to the expression of pro-atherosclerotic genes such as ICAM1 by activating NF-κB. (Tzima et al., 2002; Tzima et al., 2005). Furthermore, the PI3K/Akt pathway can converge with the same integrins that the MAPKs interact with and can lead to activation of eNOS (Shyy and Chien, 2002).

These shear-responsive pathways often activate the MAPK and PI3K/Akt pathways differentially in response to LS vs. OS (Chien, 2007), Gimbrone 2000). In laminar shear, atheroprotective genes such as eNOS (Topper et al., 1996), the Kruppel-like factor family (Klf2 and Klf4) (Wang et al., 2006), Nrf2 (Takabe et al., 2011a), and superoxide dismutases (Mn-SOD, EC-SOD) (Topper et al., 1996), become upregulated. eNOS becomes phosphorylated and activated by Akt via a PI3K-dependent pathway (Dimmeler et al., 1999) and leads to an anti-atherogenic phenotype in ECs by producing NO, which prevents EC expression of MCP-1 and plays a role in maintaining vessel tone (Zeiher et al., 1995). Klf2 also plays a role as a potent anti-atherogenic protein. It has been reported to prevent thrombin-mediated induction of multiple cytokines (such as MCP-1), as well as inhibiting inflammation and apoptosis, maintaining cell shape, and maintaining vessel tone (Nayak et al., 2011). Klf2 becomes upregulated by LS through myocyte enhancer factor 2 (MEF2) transcription, which in turn is upregulated either directly through HDAC4/5 inhibition (Wang et al., 2010) or indirectly through LS effect on the MAPK pathway (namely MEK5 and ERK5) (Parmar et al., 2006). Finally, Nrf2, another anti-atherogenic transcription factor, becomes upregulated in LS through the negative regulation of Keap1. Nrf2 exerts its anti-atherogenic effects by upregulating cytoprotective genes that contain antioxidant response elements (AREs) (Takabe et al., 2011a). As opposed to LS, in oscillatory shear stress, ECs express pro-inflammatory cytokines such as MCP-1 (Shyy et al., 1994) and inflammatory cell adhesion molecules such as VCAM1 and ICAM1 (Nagel et al., 1994). Furthermore, ECs express other pro-inflammatory proteins such as NADPH oxidase (Takabe et al., 2011a; Takabe et al., 2011b), which mediates its effects through ROS, and bone morphogenetic protein (BMP4) (Rhee et al., 2010). MCP-1 contains a phorbol ester (TPA)-responsive element (TRE) in its promoter region, which was also found to be shear-sensitive. Shyy et al. discovered that MAPKs can control AP-1 mediated transcriptional control of this element (Shyy et al., 1995). Similarly, VCAM1 and ICAM1 also contain these shear-responsive elements in their promoters (Nagel et al., 1994). Finally, NADPH oxidase is in part regulated through the MAPK pathway as well via c-Jun NH2 kinase (JNK) (Takabe et al., 2011a; Takabe et al., 2011b). Ultimately, these findings have demonstrated that laminar, unidirectional s-flow or LS upregulates "atheroprotective" genes and downregulates "pro-atherogenic" genes while disturbed, reversing, or stagnant d-flow or OS results in the opposite phenomenon of enhancing pro-atherogenic genes and suppressing atheroprotective genes.

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