Regulation of endothelial cell activity and vascular inflammation by shear stress

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Abstract Atherosclerosis, a chronic inflammatory disease of arteries, develops predominantly at branches, bends, and bifurcations in the arterial tree that are exposed to low or disturbed blood flow. The endothelium is in direct contact with flowing blood and hence is exposed to shear stress, a mechanical force that varies with time, magnitude, and direction, according to vascular pulsatility and anatomy. Bends and bifurcations of arteries that are susceptible to lesion formation are exposed to low/oscillatory shear stress, a mechanical environment that influences vascular physiology by enhancing inflammatory activation and promoting endothelial cell (EC) apoptosis. In contrast, relatively straight, unbranched regions of the arterial tree that are exposed to high shear stress are protected from inflammation, EC death and lesion development. Thus low shear stress may predispose arteries for lesion formation whereas high shear stress may prevent atherosclerosis by enhancing endothelial protection. In this paper, I will summarize some of the molecular mechanisms behind the spatial localization of vascular inflammation and atherosclerosis, emphasizing studies by my research group of two key proinflammatory signaling pathways, the mitogen-activated protein kinase (MAPK) pathway and the nuclear factor-kappa-B (NF-κB) pathway.

Keywords: Atherosclerosis, endothelial cells, shear stress, MAP kinase, NF-κB

1. Introduction

Studies of cultured endothelial cells and analysis of arteries in vivo have revealed that unidirectional high shear stress suppresses proinflammatory activation and leukocyte recruitment, whereas oscillatory low shear stress promotes vascular inflammation (Sheikh et al, 2003; Partridge et al, 2007; Hajra et al, 2000; Passerini et al, 2004; Jongstra-Bilen et al, 2006; Zakkar et al, 2008). Understanding the mechanisms by which shear stress influences proinflammatory signaling is an area of active research. Studies by our group and others have revealed that two key proinflammatory signaling pathways: the mitogen-activated protein kinase (MAPK) pathway and nuclear factor kappa- B (NF-κB) pathway are regulated by the mechanical environment of the cell (Partridge et al, 2007; Hajra et al, 2000; Zakkar et al, 2008; Zakkar et al, 2009; Cuhlmann et al, 2011; Fledderus et al, 2007; Liu et al, 2001; Magid and Davies, 2005; Garin et al, 2007; Hahn et al, 2009; Parmar et al, 2006; Wang et al, 2006; Fledderus et al, 2008).

The MAPK Pathway

The MAPKs are a group of highly conserved serine/threonine protein kinases that influence multiple cellular processes including apoptosis, proliferation and inflammation. The c-Jun N-terminal kinase (JNK) and the p38 MAPK pathways are preferentially activated by inflammatory inflammatory mediators and stress. They promote vascular inflammation in part by activating transcription factors belonging to the activating protein-1 (AP-1) superfamily (including c-Jun and activating transcription factor-2 [ATF2]), which drive transcription of proinflammatory genes such as VCAM-1.

We recently demonstrated that high shear

stress suppresses inflammatory MAPK signaling by inducing MAPK phosphatase-1 (MKP-1), a negative regulator of the MAPK pathway that inactivates p38 and JNK by removing phosphate groups from key residues (Zakkar et al, 2008). Our studies using cultured EC revealed that unidirectional high shear stress markedly induced MKP-1. We concluded that MKP-1 reduces proinflammatory signaling since silencing MKP-1 function in cultured endothelial cells increased p38 activation and enhanced VCAM-1 expression in response to shear stress. These findings were validated in a murine model as genetic deletion of MKP-1 increased activity of JNK and p38 and increased expression of VCAM-1 at protected regions of the mouse aorta. Thus we propose that high shear stress protects arteries from inflammation by inducing persistent endothelial expression of MKP-1, which suppresses the activities of p38 and JNK (Zakkar et al, 2008). In subsequent studies we have found that high shear stress also enhances the activity of nuclear factor erythroid 2-related factor (Nrf2), a transcription factor that induces multiple antioxidant genes and suppresses EC activation (Zakkar et al, 2009). Interestingly, Nrf2 and MKP-1 may co-operate to reduce vascular inflammation at high shear regions – specifically we provide evidence that Nrf2 dampens inflammation by promoting an antioxidant environment that in turn promotes the catalytic activity of MKP-1.

The NF-κB pathway

The transcription factor NF-κB controls multiple processes including immunity, inflammation, cell survival, differentiation and proliferation, and regulation of cellular responses to stress, hypoxia, stretch, and ischemia. NF-κB signaling in endothelial cells typically promotes the recruitment of inflammatory cells to the vessel wall by inducing adhesion proteins, cytokines and other inflammatory molecules. Studies from our group and from others have found that NF-κB expression and activation is exquisitively sensitive to mechanical forces. We recently demonstrated that low oscillatory shear stress can enhance NF-κB expression in EC via a JNK-ATF2 signalling pathway, thus priming regions of arteries for inflammation (Cuhlmann et al, 2011). NF-κB has the potential to exert dual functions since it can induce both antiapoptotic (protective) and proinflammatory genes. Our studies of cultured EC demonstrated that high laminar shear stress enhanced NF-κB-mediated induction of several antiapoptotic genes, whilst simultaneously dampening proinflammatory activation (Partridge et al, 2007). We therefore conclude that the *function* of NF-κB in EC can be influenced by shear stress.

In summary, high shear stress suppresses vascular inflammation and protects against lesion development by modulating both the MAPK pathway and the NF-κB pathway. The underlying mechanism for the anti-inflammatory effects of shear stress involves induction of protective genes (e.g. MKP-1, Nrf2) however further work is now required to further define the molecular mechanisms that control the spatial distribution of vascular inflammation and atherogenesis. Better understanding of these mechanisms will inform novel therapies to promote a protective phenotype in atherosusceptible regions in order to slow or even halt the progression of cardiovascular disease.

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