
Article:
Serbanovic-Canic, J. orcid.org/0000-0002-8835-1491, Souilhol, C. and Evans, P.C. orcid.org/0000-0001-7975-681X (2019) Shear stress makes its mark on the endothelial genome. Cardiovascular Research, 115 (10). pp. 1449-1451. ISSN 0008-6363

https://doi.org/10.1093/cvr/cvz088

SHEAR STRESS MAKES ITS MARK ON THE ENDOTHELIAL GENOME

Jovana Serbanovic-Canic*, Celine Souilhol*, Paul C. Evans.
(* equal contributions)

Blood flow induces a frictional force (wall shear stress; WSS) on the lumen of vessels that has profound effects on vascular biology. It is a key determinant of the anatomical location of atherosclerotic plaques; regions of arteries exposed to high unidirectional WSS (e.g. thoracic aorta) are protected from atherosclerosis, whereas regions exposed to low magnitude forces that oscillate in direction (e.g. aortic arch) are susceptible to disease[1]. Clinical studies and animal models revealed that WSS also regulates the progression of atherosclerosis and plaque rupture[2, 3] leading to myocardial infarction or stroke. Endothelial cells (EC) play a vital role in this system because they can sense both the magnitude and direction of WSS and convert this 'mechanical code' into appropriate biological responses, including altered transcription of multiple genes. EC populations exposed to high atheroprotective WSS are aligned in the direction of flow and adopt a homogenous quiescent phenotype with minimal turnover. This differs from EC exposed to low oscillatory WSS which induces considerable phenotypic heterogeneity including cells undergoing apoptosis, proliferation, senescence, inflammation and endothelial-to-mesenchymal transition (EndMT) which is a form of cell plasticity[1, 4-6].

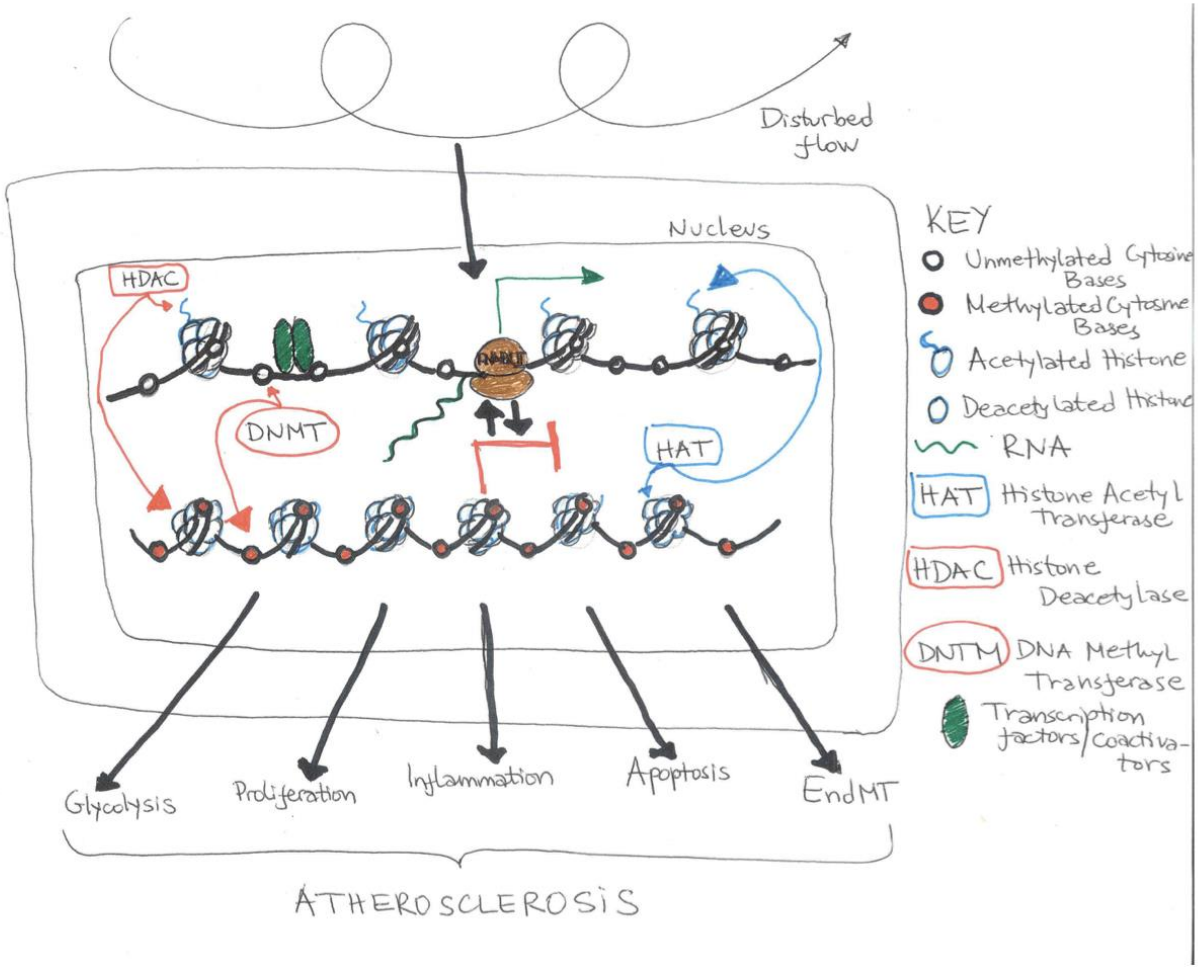
In this issue of *Cardiovascular Research*, Bondareva *et al* elucidate the mechanism linking oscillatory WSS to EC phenotype by analysing its effects on histone H3 acetylation on Lysine 27 (H3K27ac) which is an epigenetic modification associated with active transcription. This was carried out using human umbilical vein EC (HUVEC) which were exposed to oscillatory WSS for 6 hours or cultured under static conditions as a control. Cells were then cross-linked prior to chromatin immunoprecipitation using anti-H3K27ac antibodies and sequencing of the precipitated DNA. A major strength of this hypothesis-free approach is that it detects alterations in H3K27ac at a genome-wide level and without preconceptions. The experiment identified >30,000 H3K27ac peaks (putative regulatory elements) in the endothelial genome and >2,500 of them were exclusively found in EC exposed to oscillatory WSS. Importantly, the authors validated this dataset experimentally by cloning several oscillatory WSS-specific regulatory elements into luciferase reporter constructs and demonstrating that they respond to oscillatory flow. Bondareva *et al* then performed a carefully designed series of bioinformatics studies to identify the functions of genes located near to oscillatory WSS-regulatory elements. They observed enrichment of several gene ontology terms that have been associated with EC dysfunction and atherosclerosis including actin cytoskeleton reorganisation, response to stress, regulation of cell cycle, apoptotic processes and angiogenesis. Similarly, oscillatory WSS-regulatory elements were also overrepresented in several signalling pathways that are known to be flow-sensitive including MAP kinase, PI3K/AKT, WNT, NOTCH and HIPPO (YAP/TAZ)[7-10]. Finally, Bondareva *et al* investigated the transcription regulators associated with oscillatory WSS-regulatory elements by analysing binding motifs and found enrichment of several factors including HIF1α, EGR1,2 and YAP/TAZ (which binds DNA indirectly via RUNX1 and TEAD2,4). These data are consistent with recent studies showing that these molecules are activated by low oscillatory WSS leading to the activation of transcriptional programmes regulating glycolysis, proliferation, inflammation, apoptosis and EndMT[10-12] (Fig. 1). In summary, H3K27ac modification is a key response to oscillatory WSS leading to the activation of multiple genes that participate in signalling and other downstream processes.

It is interesting to compare the observations of Bondareva *et al* with previous studies of flow regulation of DNA methylation, a vital epigenetic mark associated with transcriptional repression (Fig. 1). Dunn *et al* studied the effects of disturbed blood on arterial EC physiology using a partial carotid ligation model in mice[13]. They found that low WSS induces the expression of DNA methyltransferase 1 (an enzyme that catalyzes DNA methylation) resulting in hypermethylation at the promoters of

several flow regulated genes including Homeobox (Hox)A5 and KLF3. On the other hand, Jiang *et al*[14] performed methylated DNA immunoprecipitation sequencing (MeDIP-seq) at high versus low WSS regions of the porcine aorta and identified >5,500 regions of the genome with differential methylation. Functional annotation revealed that differentially methylated genes were enriched for Hox family members, and for regulators of cytoskeletal remodelling, oxidative stress and the ER stress pathway, all of which have been associated with atherosusceptibility[15]. Notably genes regulating cytoskeleton reorganisation and the response to stress were enriched by both DNA methylome and H3K27ac ChIP-seq indicating that they are regulated by both DNA and histone modifications.

Although the study from Bondareva *et al* has illuminated the field of endothelial mechanics, it also has limitations that should be addressed in the following future studies: (1) the data set of Bondareva *et al* should be mined further to identify novel flow-sensitive pathways in addition to those already known, (2) the H3K27ac ChIP-seq data were obtained using cultured HUVEC. While these cells are a useful and well-established model of endothelial biology, they also exhibit differences in behaviour compared to arterial EC exposed to physiological conditions *in vivo*, (3) EC were exposed to oscillatory WSS for 6 h which is an insufficient time point to generate steady state changes in the genomic landscape. To address points 2 and 3, H3K27ac ChIP-seq data should now be generated using arterial EC exposed to oscillatory WSS for extended periods *in vivo* e.g. analysis of porcine aorta[14] or the murine partial ligation model[13], (4) it would also be interesting to investigate how WSS affects histone acetylation including effects on the expression and activity of enzymes that govern this process i.e. histone acetylases and deacetylases, (5) future studies should consider the highly heterogenous phenotypes of EC at atherosusceptible sites. Most omics studies use a pool of cells and therefore information on the signalling pathways and transcriptome in single cells is lost. To overcome this, genomic modifications, such as H3K27ac or DNA methylation, should be assessed at a single cell level thus allowing specific patterns of genomic modifications to be coupled to specific EC phenotypes. Collectively, these studies have the potential to identify new medicines that can slow the progression of atherosclerosis by targeting mechanosensitive epigenetic mechanisms.

Figure 1 Oscillatory shear stress remodels chromatin and activates transcription factors to promote atherogenesis



REFERENCES

1. Kwak BR, Back M, Bochaton-Piallat ML, Caligiuri G, Daemens M, Davies PF, Hoefer IE, Holvoet P, Jo H, Krams R, Lehoux S, Monaco C, Steffens S, Virmani R, Weber C, Wentzel JJ, Evans PC. Biomechanical factors in atherosclerosis: mechanisms and clinical implications. *European Heart Journal* 2014;**35**:3013-+.
2. Stone PH, Saito S, Takahashi S, Makita Y, Nakamura S, Kawasaki T, Takahashi A, Katsuki T, Nakamura S, Namiki A, Hirohata A, Matsumura T, Yamazaki S, Yokoi H, Tanaka S, Otsuji S, Yoshimachi F, Honye J, Harwood D, Reitman M, Coskun AU, Papafaklis MI, Feldman CL, Investigators P. Prediction of Progression of Coronary Artery Disease and Clinical Outcomes Using Vascular Profiling of Endothelial Shear Stress and Arterial Plaque Characteristics The PREDICTION Study. *Circulation* 2012;**126**:172-+.
3. Pedrigi RM, Poulsen CB, Mehta VV, Holm NR, Pareek N, Post AL, Kilic ID, Banya WAS, Dall'Ara G, Mattesini A, Bjorklund MM, Andersen NP, Grondal AK, Petretto E, Foin N, Davies JE, Di Mario C, Bentzon JF, Botker HE, Falk E, Krams R, de Silva R. Inducing Persistent Flow Disturbances Accelerates Atherogenesis and Promotes Thin Cap Fibroatheroma Development in D374Y-PCSK9 Hypercholesterolemic Minipigs. *Circulation* 2015;**132**:1003-1012.
4. Souilhol C, Harmsen MC, Evans PC, Krenning G. Endothelial-mesenchymal transition in atherosclerosis. *Cardiovasc Res* 2018;**114**:565-577.
5. Tricot O, Mallat Z, Heymes C, Belmin J, Leseche G, Tedgui A. Relation between endothelial cell apoptosis and blood flow direction in human atherosclerotic plaques. *Circulation* 2000;**101**:2450-2453.
6. Warboys CM, de Luca A, Amini N, Luong L, Duckles H, Hsiao S, White A, Biswas S, Khamis R, Chong CK, Cheung W-M, Sherwin SJ, Bennett MR, Gil J, Mason JC, Haskard DO, Evans PC. Disturbed Flow Promotes Endothelial Senescence via a p53-Dependent Pathway. *Arteriosclerosis Thrombosis and Vascular Biology* 2014;**34**:985-995.
7. Zakkar M, Chaudhury H, Sandvik G, Enesa K, Luong LA, Cuhlmann S, Mason JC, Krams R, Clark AR, Haskard DO, Evans PC. Increased endothelial mitogen-activated protein kinase phosphatase-1 expression suppresses proinflammatory activation at sites that are resistant to atherosclerosis. *Circulation Research* 2008;**103**:726-732.
8. Maimari N, Pedrigi RM, Russo A, Broda K, Krams R. Integration of flow studies for robust selection of mechanoresponsive genes. *Thromb Haemost* 2016;**115**:474-483.
9. Mack JJ, Mosqueiro TS, Archer BJ, Jones WM, Sunshine H, Faas GC, Briot A, Aragon RL, Su T, Romay MC, McDonald AI, Kuo CH, Lizama CO, Lane TF, Zovein AC, Fang Y, Tarling EJ, de Aguiar Vallim TQ, Navab M, Fogelman AM, Bouchard LS, Iruela-Arispe ML. NOTCH1 is a mechanosensor in adult arteries. *Nature communications* 2017;**8**:1620.
10. Wang L, Luo J-Y, Li B, Tian XY, Chen L-J, Huang Y, Liu J, Deng D, Lau CW, Wan S, Ai D, Mak K-LK, Tong KK, Kwan KM, Wang N, Chiu J-J, Zhu Y, Huang Y. Integrin-YAP/TAZ-JNK cascade mediates atheroprotective effect of unidirectional shear flow. *Nature* 2016;**540**:579-582.
11. Baron V, Adamson ED, Calogero A, Ragona G, Mercola D. The transcription factor Egr1 is a direct regulator of multiple tumor suppressors including TGFbeta1, PTEN, p53, and fibronectin. *Cancer gene therapy* 2006;**13**:115-124.
12. Feng S, Bowden N, Fragiadaki M, Souilhol C, Hsiao S, Mahmoud M, Allen S, Pirri D, Ayllon BT, Akhtar S, Thompson AAR, Jo H, Weber C, Ridger V, Schober A, Evans PC. Mechanical Activation of Hypoxia-Inducible Factor 1alpha Drives Endothelial Dysfunction at Atheroprone Sites. *Arteriosclerosis, thrombosis, and vascular biology* 2017.
13. Dunn J, Qiu H, Kim S, Jjingo D, Hoffman R, Kim CW, Jang I, Son DJ, Kim D, Pan C, Fan Y, Jordan IK, Jo H. Flow-dependent epigenetic DNA methylation regulates endothelial gene expression and atherosclerosis. *Journal of Clinical Investigation* 2014;**124**:3187-3199.

14. Jiang YZ, Manduchi E, Stoeckert CJ, Davies PF. Arterial endothelial methylome: differential DNA methylation in athero-susceptible disturbed flow regions in vivo. *BMC Genomics* 2015;**16**.
15. Civelek M, Manduchi E, Riley RJ, Stoeckert CJ, Jr., Davies PF. Chronic Endoplasmic Reticulum Stress Activates Unfolded Protein Response in Arterial Endothelium in Regions of Susceptibility to Atherosclerosis. *Circulation Research* 2009;**105**:453-U127.