A Turbulent path to plaque formation

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Plaque deposits, which obstruct blood flow, are usually found in curved regions of the arteries where blood flow is turbulent. A study identifies a pathway that is specifically activated by turbulent blood flow and triggers the formation of plaques.

Atherosclerosis is a disease that is characterised by the gradual build-up of plaque, which is a collection of cells, fatty deposits and cholesterol, in major arteries. When plaques become large enough, they can obstruct blood flow to major organs and cause heart attacks or strokes.[1] Plaques are not distributed uniformly in arteries, but rather have "preferred" locations in which they develop. In this issue, Huang *et al* provide a mechanism that could explain why some areas of the vasculature develop plaques, while others are protected.

The patchy distribution of atherosclerosis was first reported by a number of groups in the 1960s, who collectively described an association between plaque formation and arterial blood mechanics. Caro *et al* put forth the hypothesis that the appearance of early plaques is coincident with regions in the arteries that experience low and disturbed blood flow patterns.[2] We now know that haemodynamic forces play a fundamental role in atherosclerosis development (reviewed in [3]), with shear stress, the frictional force due to blood flow, being the most important. Laminar shear stress with a significant forward direction is found in straight regions of the vasculature and is considered to be beneficial/atheroprotective. On the contrary, curved regions of the vasculature, such as bifurcations and branch points, exhibit disturbed blood flow and are more susceptible to the development of plaques (atheroprone). Endothelial cells, the cells that line blood vessels, are endowed with the unique capability of not only sensing but also distinguishing between these two types of blood flow. The process of endothelial

mechanotransduction refers to the ability of the endothelium to detect the mechanical force of blood flow and “translate” it into a number of meaningful biochemical signalling pathways that will ultimately define their function and behaviour and decide whether a plaque will be promoted or inhibited.

To determine how mechanotransduction leads to distinct phenotypes in different regions of the arteries, it is necessary to understand the molecular mechanisms that are activated by atheroprotective *vs* atheroprone blood flow. Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) have received a lot of attention as master regulators in the Hippo signalling cascade that controls organ size as well as in tumour suppression (reviewed in [4]). In a seminal paper published in Nature, Dupont *et al* revealed a fundamental role for YAP/TAZ in mechanotransduction, reporting that YAP/TAZ act as sensors or checkpoints for mechanical stimuli.[5] Although previous studies implied an involvement of YAP/TAZ in atherogenesis due to the upregulation of two of its transcriptional targets in atherosclerotic arteries,[6,7] direct evidence linking YAP/TAZ to endothelial mechanotransduction and atherosclerosis was lacking until now.

In this study, Huang *et al.* demonstrate that YAP/TAZ activity is differentially regulated by flow, such that atheroprone flow increases, whereas atheroprotective flow decreases YAP/TAZ activity. YAP/TAZ is inhibited in cultured endothelial cells subjected to uniform shear stress and in regions of the vasculature where blood flow is laminar, such as the thoracic aorta. On the contrary, disturbed flow enhances YAP activity both in cultured endothelial cells and in the inner aortic arch *in vivo* (Fig1). These studies were further corroborated in the rat abdominal cross-clamping model, which generates regions of uniform and disturbed shear stress in the same vessel *in vivo*.[9] The constricted region, with high uniform shear stress, exhibited low YAP activity, whereas the reverse was seen in the downstream region, where flow is non-laminar. RNA sequencing analysis and cellular assays revealed that YAP/TAZ promotes activation of several downstream inflammatory pathways, including

the atherogenic JNK pathway, suggesting that YAP/TAZ activation sets the stage for the initiation of atherosclerosis.

How does atheroprotective laminar shear stress inhibit YAP/TAZ activity? Huang *et al* describe a pathway that involves several molecules known to participate in mechanotransduction: they show that laminar shear stress inhibits YAP/TAZ activity by modulating integrin-Gα13-RhoA signalling. In this model, laminar shear stress promotes integrin activation and the association between integrin β3 and Gα13, RhoA inhibition and downstream YAP inactivation. How this fits with the previously reported proatherogenic role of integrin β3[Refs 10-12] remains to be determined and is a topic worthy of further investigation.

Is any of this relevant *in vivo*? Huang and colleagues extended their studies using a mouse model of atherosclerosis, as well as patient samples. Using ApoE knockout mice (in which a crucial gene required for cholesterol metabolism is deleted, making them more susceptible to the development of plaques when fed on high fat diet),[13] they demonstrated that YAP activity and TAZ levels were enhanced in the aortae of animals with atherosclerotic plaques. Similar observations were made in human atherosclerotic aortae. To further validate these findings, Huang *et al.* generated ApoE knockout transgenic mice in which YAP was over-expressed specifically in the endothelium. After four weeks of feeding them a high fat diet, these transgenic mice showed significantly increased atherosclerosis than control animals. Furthermore, the authors knocked down endothelial expression of YAP in ApoE knockout mice using CRISPR/Cas9 technology, and subjected them to disturbed shear stress (by partial ligation of the carotid artery).[14] They showed that endothelium-specific YAP knockdown resulted in significantly less atherosclerosis compared to control animals. Similar results were obtained with TAZ knockdown using an shRNA approach. Thus, both gain- and loss-of-function experiments establish the importance of YAP/TAZ in atherosclerosis.

Statins, cholesterol-lowering drugs, are the most commonly prescribed medicines in Western countries and the first line therapy for patients with cardiovascular disease. Interestingly, statins have been shown to regulate the YAP/TAZ pathway[15,16] but whether they enhance atheroprotection via this pathway was unknown. Huang and colleagues have shown that simvastatin failed to suppress the expression of pro-inflammatory genes in constitutively active-YAP/TAZ transfected cells, thus suggesting that the anti-inflammatory and anti-atherogenic effects of statins are most likely mediated by inhibition of YAP/TAZ activity.

The present study provides novel evidence that endothelial activation of the oncogenes YAP/TAZ by atheroprone flow present in curved regions of the vasculature triggers atherosclerosis. Uniform blood flow on the contrary, suppresses YAP/TAZ activity and is atheroprotective. The finding that knockdown of YAP/TAZ activity in the endothelium decelerated the onset of atherosclerosis, together with the fact that statins most likely confer their therapeutic effects via YAP/TAZ inhibition, suggest that targeting this pathway may be of potential therapeutic benefit for the treatment of atherosclerosis.

It is well established that atherosclerosis is a complex multifactorial disease, where inflammation most probably occurs through multiple pathways. Is suppression of YAP/TAZ sufficient to ameliorate atherosclerosis? Is it possible to target YAP/TAZ exclusively in the endothelial compartment in clinics? How will this affect signalling via the critical Hippo pathway? Which molecules within the Integrin-YAP/TAZ-RhoA cascade will prove to be the best therapeutic targets? All these are questions that need to be answered before a potential therapy based on YAP/TAP inhibition can be devised.

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