Mechanical and Chemical Regulation of Endothelial Cell Polarity

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McCue et al\(^1\) showed by a combination of in vitro and in vivo studies that shear stress regulates planar cell polarity (PCP) of endothelial cells (ECs) and that glycogen synthase kinase 3β (GSK3β) plays a significant role in this process.

**In Vitro Studies on Effects of Shear Stress on PCP and GSK-3β**

The application of shear stress at 15 dyn/cm\(^2\) to cultured porcine aortic ECs caused tubulin acetylation and stabilization of microtubules (MTs), downstream polarization of microtubule-organizing centers (MTOCs), as well as MT orientation and EC elongation in the shear direction. The importance of tubulin dynamics in these shear-induced changes was demonstrated by their elimination by the MT-disrupting agent nocodazole and also the MT-stabilizing agent taxol.

A novel finding is the role of GSK-3β in shear-induced MT orientation. GSK-3β is a regulatory serine/threonine kinase\(^2\) that has high activity under basal state, and its activity is inhibited after serine-9 phosphorylation (p-GSK-3β); this would lead to downstream signaling\(^3\) and MT stabilization,\(^4\) eg, through the binding of adenomatous polyposis cell protein (APC).\(^4,5\) GSK3β can be inhibited by lithium and SB415286, which interfere with the binding of ATP to GSK-3β rather than acting directly on GSK-3β phosphorylation. The evidence that GSK-3β plays a role in mediating the shear-induced MT orientation and cell shape change is as follows. First, shear stress caused an increase in p-GSK-3β (at 1 and 4 hours, though waned at 24 hours). Second, inhibition of GSK-3β by LiCl and SB415286 blocked the shear-induced EC elongation. SB415286 was also shown to reverse the shear-induced downstream localization of MTOCs to become upstream. Third, overexpression of a constitutively active GSK-3β (GSK3\(^{\text{A}}\)), which cannot be phosphorylated, attenuated tubulin acetylation, blocked cell elongation, and caused MTOC distribution to be more random.

Although these results provide evidence that the shear-induced PCP and cell elongation are mediated by GSK3β, several points remain to be elucidated.\(^1\) Thus, GSK3β phosphorylation waned from 4 to 24 hours, whereas tubulin acetylation was higher at 24 than at 4 hours after shearing. The authors postulated that at 24 hours, factors other than GSK3β phosphorylation may participate in the maintenance of shear-induced MT stability. It would be valuable to determine what these factor(s) are. The shear-induced downstream MTOC polarity was observed at 24 but not 6 hours; there is a need to obtain data with more detailed time points to decipher the temporal relationships among the various parameters being studied, especially between GSK-3β activity and tubulin acetylation, as well as MTOC orientation and tubulin stabilization. The authors proposed that the shear-induced responses may involve tight regulation rather than simple suppression of GSK-3β activity, and also the modulation of its intracellular gradients rather than the global absolute level. Investigations on the effects of shear stress with different magnitudes and time variances may help to address these questions. It would also be valuable to develop technologies to dynamically track the spatial and temporal characteristics of GSK3β phosphorylation in relation to tubulin acetylation and PCP regulation, eg, by fluorescence resonance energy transfer.\(^7\)

The reversal of MTOC localization to the upstream side of ECs after inhibition of GSK-3β activity with SB415286 led McCue et al\(^1\) to suggest that the control of EC polarity under shear involves two mechanisms, one dependent on pGSK3β/GSK3β and the other being independent. This explanation implies that the GSK-3β-independent mechanism would cause the positioning of the MTOCs to the upstream side and that this is normally overridden by the GSK-3β-dependent mechanism in response to shear stress. After GSK3β inhibition, shear stress would only activate the GSK3β-independent mechanism and thus cause the upstream localization of the MTOCs. Such dual mechanisms of regulation are plausible but need to be tested by experiments. It would be interesting to determine whether the GSK-3β-independent mechanism responsible for MTOC reversal is related to the maintenance of shear-induced tubulin acetylation at 24 hours when p-GSK-3β has subsided. In this connection, there is the possibility that a short period of GSK-3β phosphorylation may lead to a longer lasting tubulin acetylation. It may be worthwhile to test the effect of varying durations of GSK-3β phosphorylation (by stimuli other than shear stress) on the time course of tubulin acetylation.

Among the various possibilities by which GSK-3β modulates tubulin dynamics, the authors emphasized its capacity to dissociate APC from the + ends of MT.\(^4,5\) As they pointed...
out, it is still unknown how the inhibition of GSK-3β and the resulting APC-MT association affect MT redistribution. The authors did postulate that the GSK-3β inhibition may promote MT binding to proteins/structures at the cell anterior to prevent them from being swept backward by the retrograde flow of actin. In this regard, concurrent investigations of actin dynamics would be useful in view of its role in determining cell morphology and its interactions with MT.8 Other molecules of interest are integrins, focal adhesion proteins, and junctional proteins, as well as the small GTPases cdc424 and Rac,9 which may participate in the shear-induced EC polarization.

In Vivo Studies on the Effects of Shear Stress on PCP and GSK-3β

In rats and pigs the MTOCs of ECs are localized upstream to the nucleus in thoracic aorta and downstream in vena cava, ie, on the heart side for both. In the rabbit, MTOCs are also on the upstream side in carotid artery and downstream side in vena cava but unpolarized in thoracic aorta. In view of the downstream localization of MTOCs of ECs subjected to 15 dyn/cm² shear stress in vitro, it is surprising that the MTOCs are located upstream in the arterial vessels exposed to shear stress on the order of 15 dyn/cm². The MTOCs in the rabbit abdominal aorta switched from being downstream when 5 weeks old to upstream when mature. Thus, EC polarity is vessel- and age-specific.

Investigating the directionality of EC mitosis, McCue et al1 found in immature (3 to 5 weeks old) rabbit carotid artery that the daughter cells aligned along the axis of vessels with normal flow, but non-aligned when the distal end of the artery had been ligated to stop the flow. This flow-aligned mitosis was also observed in vitro. Surgical production of 50% stenosis of mid-abdominal aorta induced a post-stenotic vortex with flow reversal near the vessel wall, and this eliminated the MTOC polarity in both immature (normally downstream) and mature (normally upstream) abdominal aortas. These experiments offer clear evidence for the shear regulation of PCP. Because flow reversal can alter MTOC polarity, it may be interesting to examine MTOC polarity, as well as GSK-3β status, in regions of complex flow patterns in the arterial tree, eg, aortic arch and bifurcations.10

McCue et al1 found that the GSK-3β inhibitor LiCO₃ suppressed PCP of ECs in the abdominal aorta of mature rabbits (upstream polarity), as well as the abdominal aorta and vena cava of immature rabbits (downstream polarity). Furthermore, the suppression of acetylated tubulin staining induced by 1-week carotid ligation in the rabbit was partially restored by LiCO₃.

Although the in vivo studies described above have demonstrated that shear stress can regulate PCP and that this shear-regulation can be mediated by GSK-3β, the opposite directions of MTOC orientations in arteries versus veins cannot be readily explained, nor can their variations with age. Using the concept of dual mechanisms (GSK-3β-dependent and –independent) derived from in vitro studies, it is tempting to postulate that the difference between the directions of PCP between arteries and veins results from the differential preponderance of these two mechanisms attributable to different characteristics of shearing (eg, pulsatility and magnitude) and/or intrinsic differences in the properties of these vessels. To distinguish these two possibilities, it may be interesting to study the effects of imposing arterial hemodynamic characteristics on veins by A-V anastomosis. The variations in PCP with aging might also be interpreted in terms of a change in the relative preponderance of the two mechanisms resulting from age-induced variations in the forces experienced by EC attributable to differences in flow pattern and/or viscoelastic properties of vessel wall.

Summary and Significance

The regulation of EC polarity and directional migration by mechanical forces is an important determinant of vascular functions such as remodeling and wound repair.11 McCue et al1 have demonstrated that shear stress can modulate MTOC polarity and MT stability in vitro and in vivo and that GSK3β plays a significant role in this process. This is an important novel finding in the mechanotransduction mechanism controlling EC polarity under flow. A summary of their in vitro findings is given in the Figure.

The results of this study raise a number of questions that require additional experimentation to further elucidate the mechanism of shear-regulation of PCP and the role of GSK3β. These include the difference in EC polarity between arteries and veins in vivo, the difference in polarity between ECs in arteries in vivo and ECs subjected to arterial-level shear stress in vitro, the change in EC polarity with aging, the nature of the GSK-3β–independent mechanism in mediating the shear-induced EC polarity and its relation with the GSK-3β–dependent mechanism, the effects of the characteristics of shear stress (pulsatility, magnitude, and time course), and the spatial and temporal characteristics of GSK-3β phosphorylation. Other questions to be addressed are the significance of the upstream versus downstream polarity of the MTOCs and the relations between MT stability and other cytoskeletal elements and various regulatory pathways.

In summary, McCue et al1 have introduced a novel mechanism for the shear-induced EC orientation, and this will open new directions for studying molecular mechanisms of
EC functions that will contribute to the understanding of vascular homeostasis in health and disease.

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References


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