Focal adhesion kinase phosphorylation in flow-activation of endothelial NF-κB. Focus on “Focal adhesion kinase modulates activation of NF-κB by flow in endothelial cells”

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Focal adhesion kinase (FAK) is believed to be a key event in the activation of NF-κB binding protein seen in FAKfl/fl were abolished or reduced in els), and nuclear accumulation of sterol regulatory element-phosphorylation, ICAM-1 expression (at mRNA and protein lev-

Thus, the shear-induced increases in NF-κB Ser536 phosphorylation, ICAM-1 expression (at mRNA and protein levels), and nuclear accumulation of sterol regulatory element-binding protein seen in FAKfl/fl were abolished or reduced in FAK−/−. These effects could be partially rescued by the transfection of wild-type FAK. The use of small interfering RNA (siRNA) also inhibited the shear-induction of Ser536 phosphorylation in bovine aortic ECs. In contrast to their differential responses in the shear-induction of Ser536 phosphorylation, the two groups of mice showed comparable results in the shear-induction of nuclear translocation of p65 and phosphorylation of endothelial nitric oxide synthase, ERK, and JNK. The FAK dependence of shear-induced NF-κB Ser536 phosphorylation and the FAK independence of shear-induced NF-κB p65 translocation are summarized in Fig. 1 (top).

While shear flow caused Ser536 phosphorylation but not nuclear translocation of NF-κB, chemical stimuli such as TNF-α and H2O2 had comparable effects between FAK−/− and FAKfl/fl for both Ser536 phosphorylation and nuclear translocation of NF-κB (10). Thus, these two types of responses of the NF-κB system to a chemical stimulus such as TNF-α are both independent of FAK, as shown in Fig. 1 (bottom). It would be interesting to establish the differences in signaling pathways involved in such differential responses to mechanical shear vs. chemical stimuli.

Wang et al. (11) have reported the involvement of different signaling pathways by ECs in response to mechanical and chemical stimuli (shear and VEGF, respectively) following the activation of VEGF receptor 2 (Flk-1). VEGF induced a rapid association of Flk-1 with Nckβ but shear stress did not. Both SU1498 (a specific inhibitor of Flk-1) and Nckβmut (a negative mutant of Nckβ) blocked the VEGF-induced ERK and JNK activities. Only SU1498, but not Nckβmut, inhibited the shear-induced ERK activity. Furthermore, neither SU1498 nor Nckβmut had significant effects on the shear-induced JNK activity, which can be blocked by inhibitors of Src family kinase and Ras-associated protein kinase. In this case, mechanical (shear) stress and chemical (VEGF) stimuli diverge at the receptor Flk-1 in terms of the recruitment of the adapter protein Nckβ, and they have differential effects on the downstream signaling molecules, e.g., ERK and JNK.

These findings of differential effects of mechanical vs. chemical stimuli raise the possibility of activation in different cellular compartments. Thus, it is possible that the phosphorylation of NF-κB occurs in regions close to the focal adhesions (FAs) without having to involve translocation into the nucleus? Using quantitative total internal reflection fluorescence microscopy and green fluorescent protein-FAK, Ferko et al. (6) have shown that differences in elastic properties between the nucleus and the cytoplasm, as well as between the juxtaposition of constrained regions (e.g., FAs) and unattached regions, may provide mechanisms of stress amplification in sheared ECs. Del Alamo et al. (5) have demonstrated anisotropy of intracellular rheology of ECs subjected to laminar shear. Such microdomains of stress distribution may play a role in the subcellular localization of mechanotransduction events that would not occur following chemical stimulation.

The interplay between mechanical and chemical stimuli has also been demonstrated by the shear stress-inhibition of the increases in NF-κB binding activity in EC nuclei and the consequent proinflammatory gene expression induced by two forms of chemical stimulation: treatment of ECs with TNF-α (2) and response of ECs to IL-1β and IL-6 released from cocultured synthetic-type smooth muscle cells (3). Given the findings of Petzold et al. (10), it would be interesting to assess the roles of FAK and NF-κB Ser536 phosphorylation in the interplay of mechanical and chemical stimuli in these situations (2, 3).

FAK is known to play a significant role in cell adhesion, migration, proliferation, and survival, and FAK-mediated NF-κB activation has been suggested to play favorable roles in cardiac protection during ischemia-reperfusion (7) and fibroblast survival in cytokine-induced apoptosis (8). The findings by Petzold et al. (10) raise the possibility that the differential roles of FAK in shear- vs. cytokine-induced NF-κB activation may contribute to the regulation of a variety of EC functions in health and disease.

It has been suggested that NF-κB activation may play an important role in feedback control of FAK phosphorylation in
response to interferon-γ-inducible GTPase (9). While the shear-induced integrin activation leads to FAK phosphorylation, FAK is not involved in integrin activation (10), suggesting that there is no feedback loop in ECs involving these molecules, at least under short-term shearing. Petzold et al. (10) stated that the effect of short-term shearing is more akin to disturbed flow, which is known to be opposite to that of long-term shearing with a sustained direction (1).

Wang et al. (12) have shown that shear-induced NF-κB translocation is blocked by SU1498 and by inhibition of phosphatidylinositol 3-kinase (a molecule downstream to Cbl). Disruption of the actin cytoskeleton and inhibition of FAK or Src also suppressed the shear-induced NF-κB translocation, suggesting the involvement of the actin cytoskeleton and key tyrosine kinases in the shear-induced NF-κB translocation. In view of the involvement of integrin in the signaling process in Petzold et al. (10), it would be interesting to know whether actin is also required for the shear activation of FAK and NF-κB Ser536 phosphorylation. While integrin is involved in the shear activation of NF-κB phosphorylation, it is not clear whether it is involved in TNF-α activation.

The use of FAK−/− in comparison to FAK−/− mice by Petzold et al. (10) is an excellent approach. In the FAK−/− mouse aortic endothelial cells, however, there was a higher baseline value for NF-κB phosphorylation as compared with FAK−/−, and this difference is also seen in the silencing experiments. These findings raise the question whether FAK normally has a suppressing effect on Ser536 phosphorylation.

In the study by Petzold et al. (10), FAK is shown to mediate the NF-κB phosphorylation and ICAM-1 expression induced by shear stress. The finding by Crosara-Alberto et al. (4) that FAK also mediates the inflammatory responses induced by mechanical stretch further indicates the critical role of FAK in mechanotransduction under these different types of mechanical stimuli.

In summary, the article by Petzold et al. (10) has presented a novel mechanism for the shear-activation of NF-κB and its downstream effects, and as a good article should, it has posed interesting questions that are worthy of further investigation.

REFERENCES