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Elucidation of functionally relevant mediators of the MEK5/Erk5 signaling pathway in human endothelial cells

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The MEK5/ERK5 signaling pathway mediates beneficial effects of laminar flow, which prevents vascular dysfunction and diseases. Activation of this cascade results in a vasoprotective phenotype in primary human umbilical vein endothelial cells (HUVECs) and is associated with decreased migration, changes in cell morphology and enhanced adhesiveness. Our previous observations implicate that activation of the MEK5/Erk5 pathway mimics conditions imposed by shear stress by inducing the Kruppel-like transcription factors 2 and 4 (KLF2; KLF4), which are well-known for their mediation of protective flow responses. However, the functionally relevant secondary effectors of this pathway have widely been unknown. To identify functionally relevant secondary targets of Erk5, we previously performed qRT-PCR-based arrays with human primary ECs expressing constitutively active MEK5D. In contrast to our initial microarray studies, here we focused on secondary ERK5 targets rather than primary response genes. Intriguingly, these arrays revealed a potent repression of the established pro-migratory Rac/Cdc42 effector PAK1. This project established PAK1 as important migratory target regulated by the MEK5/ERK5/KLF2 pathway in endothelial cells. It is shown that three independent conditions result in ERK5 activation: (1) expression of constitutively active MEK5D, (2) exposure to statins (e. g. simvastatin) and (3) endothelial exposure to laminar flow. All three conditions lead to downregulation of PAK1 transcription and protein expression. siRNA studies revealed that the knockdown of KLF2 but not of KLF4 prevented ERK5-mediated PAK1 mRNA inhibition implicating KLF2 as a novel PAK1 repressor. Furthermore, pharmacological inhibition of the MEK5/ERK5 pathway confirmed the role of PAK1 as downstream target of ERK5 in MEK5D-transduced endothelial cells. The data support our earlier observations showing that constitutive ERK5 activation inhibits endothelial migration. Remarkably, this study further demonstrated that both PAK1 re-expression and knock-down of KLF2 rescued the migration capacity of MEK5D-expressing endothelial cells, suggesting their functional relevance downstream of ERK5. Thus, the anti-migratory behavior and changes in cell morphology of active ERK5 are due to the loss of PAK1, indicating the crucial role of PAK1 in governing cell migration and morphology. Collectively, this thesis demonstrates for the first time that PAK1 is a key migration-relevant Erk5 effector regulated by KLF2 but not KLF4. We propose that the observed effects of MEK5/ERK5/KLF2 signalling on migration and loss of PAK1 expression likely exerts a beneficial function and may act as a natural mechanism controlling migration under pathological conditions, such as the progression of atherosclerosis. Our data thus may stimulate future investigations to target PAK1 signalling and to further identify novel drug targets for selective therapeutic interventions to control pathological cell migration occurring during vascular diseases.