Expression and Function of Ephrin Receptor B2 in Human Atherosclerosis: a Ligand Independent Guidance Cue

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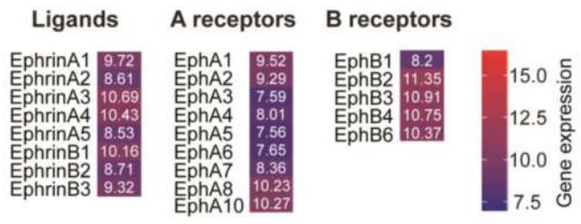
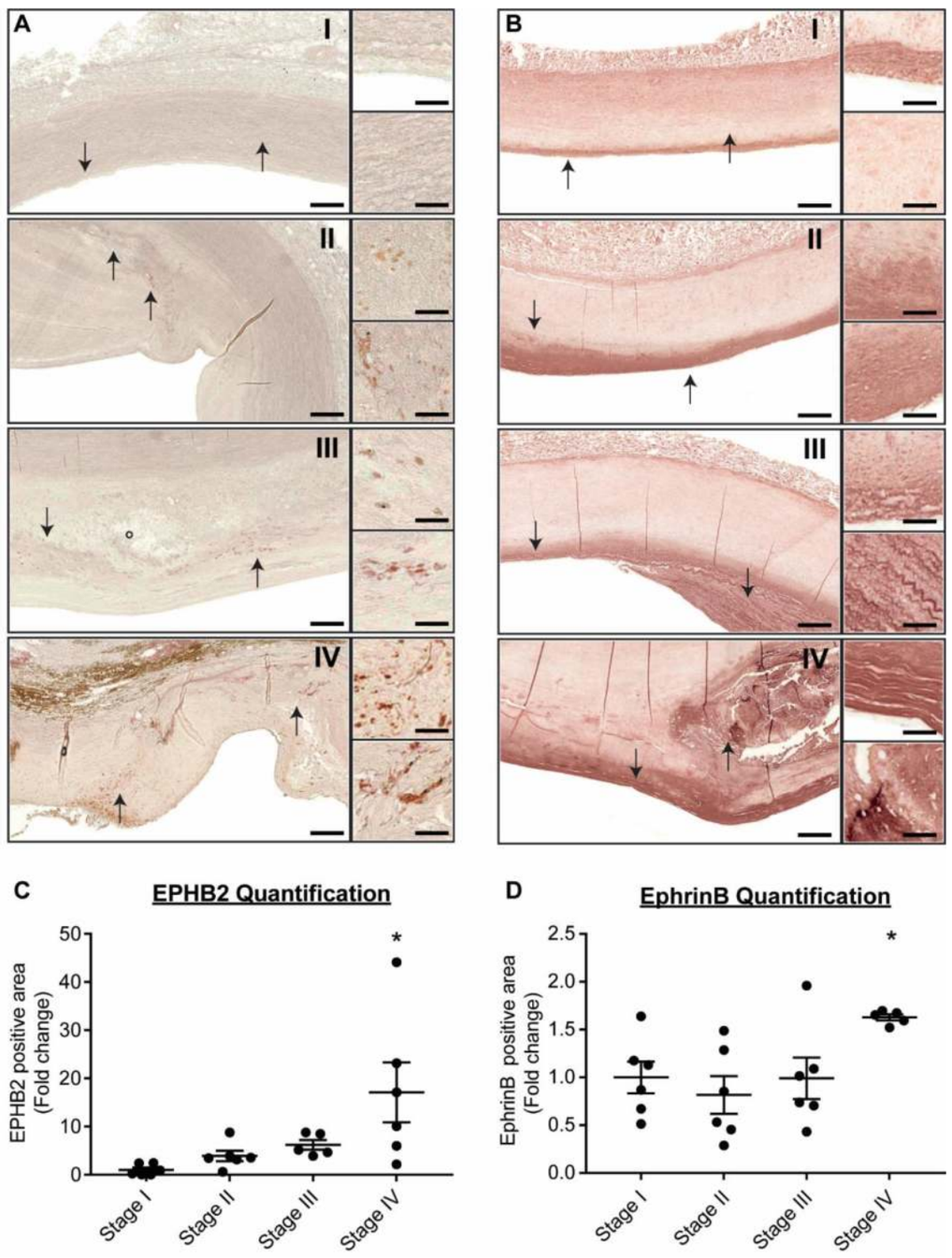
Neuroimmune guidance cues in atherosclerosis

Ephrin receptors (EPHs) and their ligands are related to a plethora of physiological and pathological processes including atherosclerosis. Preliminary data of our lab showed Ephrin Receptor B2 (EPHB2) to be the most abundantly expressed EPH receptor on monocytes, one of the culprit cell types in atherosclerosis. Combined with the observation that the EPHB2 receptor gene is located on a myocardial infarction locus, we hypothesized that monocytic EPHB2 plays a role in atherosclerosis, and set out to investigate the expression of EPHB2 in human atherosclerosis and its function in human monocytes.



Increased expression of EPHB2 and EphrinB in progressive human atherosclerotic lesions

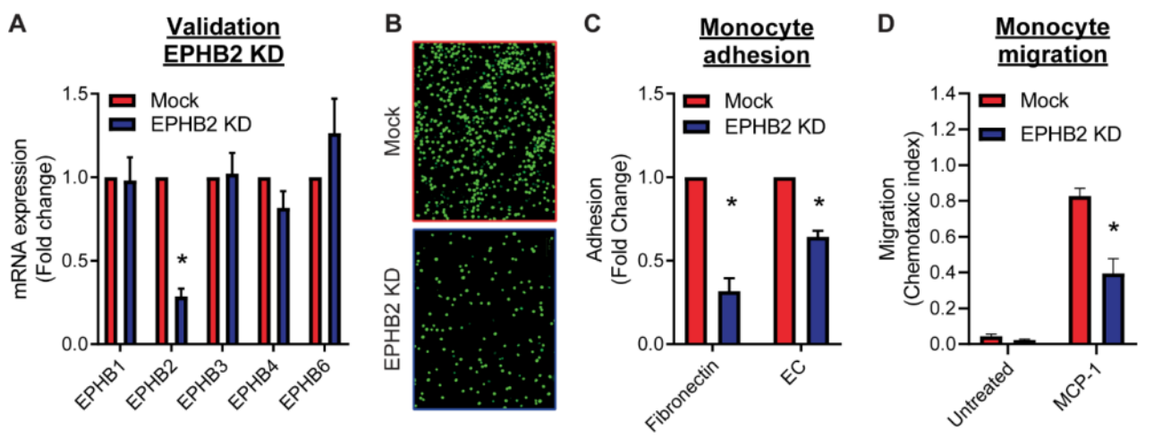


Immunohistochemical staining of human aortic tissue sections with varying stages of atherosclerosis for the receptor EPHB2 and its ligands EphrinB, showed an increase in expression of EPHB2 in the more progressive stages of disease (A/C). Expression of the EphrinB ligands was high in all stages of plaque progression but significantly increased in the most severe stage of disease (B/D). Data is expressed as mean ± SEM *p<0.05.

Conclusion

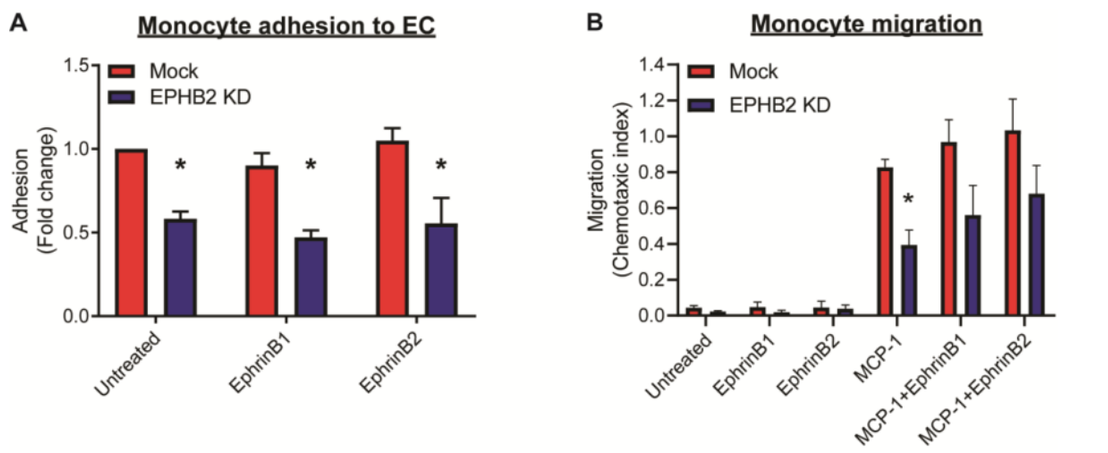
In our current study we have shown that expression of EPHB2 and its ligand EphrinB in atherosclerotic lesions is driven by atherosclerosis progression. In addition we have shown that EPHB2, independent of its ligands, facilitates monocyte adhesion and migration via regulation of the cytoskeleton of the cells and FAK phosphorylation, which *in vivo* might promote monocyte accumulation and thereby atherogenesis.

Decreased monocyte adhesion and migration upon knockdown of EPHB2



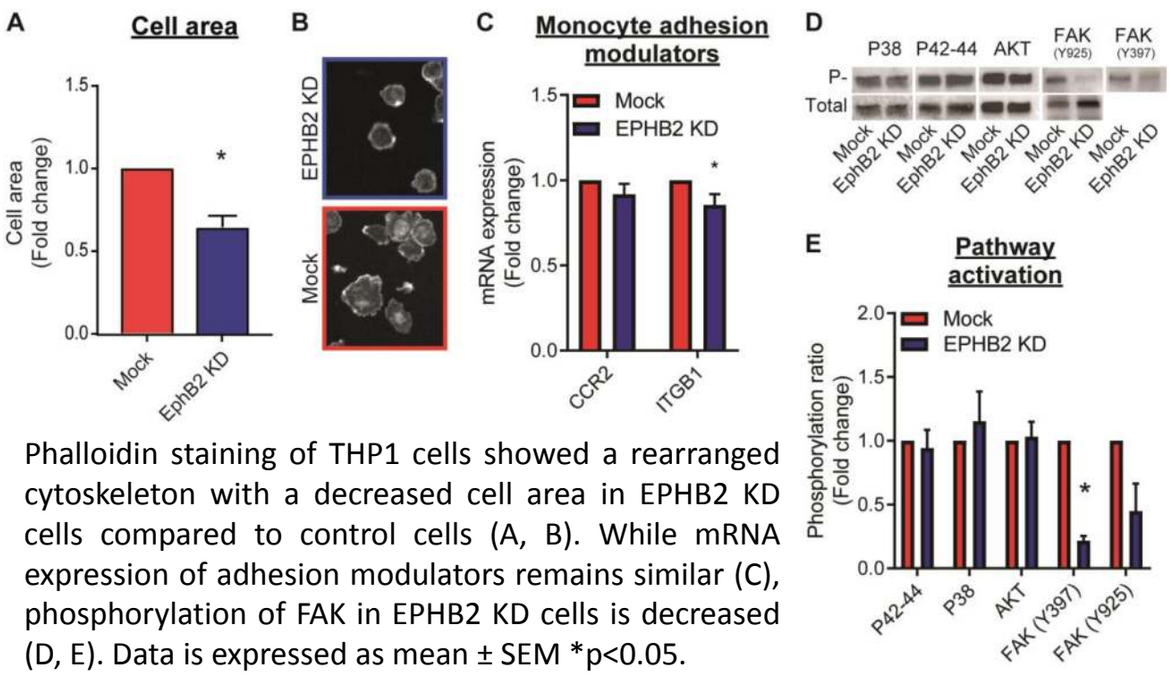
Specific lentiviral KD of EPHB2 in THP1 monocytes (A) resulted in a decreased capacity of monocytes to adhere to either fibronectin or a monolayer of HUVECs (B, C). In addition transendothelial migration of monocytes towards MCP-1 is diminished in monocytes with a knockdown of EPHB2 (D). Data is expressed as mean ± SEM *p<0.05.

Effect of EPHB2 KD on monocyte adhesion and migration is ligand independent



Stimulation of control or KD THP1 monocytes with recombinant EphrinB1 or EphrinB2 ligand did not alter the adherence of monocytes to endothelial cells (A), neither did migration towards MCP-1 change when either EphrinB1 or EphrinB2 was present (B). Data is expressed as mean ± SEM *p<0.05.

EPHB2 KD alters cellular morphology and phosphorylation patterns



Phalloidin staining of THP1 cells showed a rearranged cytoskeleton with a decreased cell area in EPHB2 KD cells compared to control cells (A, B). While mRNA expression of adhesion modulators remains similar (C), phosphorylation of FAK in EPHB2 KD cells is decreased (D, E). Data is expressed as mean ± SEM *p<0.05.

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