

## **Functional Role for EphB4 in Vascular Patterning during Angiogenesis of the Chick Chorioallantoic Membrane**

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The Eph receptors and their ephrin ligands are known to be important regulators during embryonic vascular development, and recent evidence suggests they also play a role in post-natal angiogenesis. Previously, our lab investigated EphB4 expression in both quiescent and remodeling adult rat mesenteric microvascular networks. Our results show that EphB4 is significantly upregulated on blind-ended capillary sprouts as compared to capillaries that connect a venule to an arteriole. The prominent expression of EphB4 along capillary sprouts, as detected by immunohistochemistry, indicates that EphB4 may play a role in capillary sprouting. To investigate this possibility, we used the chick chorioallantoic membrane (CAM) assay to investigate the effects of inhibiting EphB4 function on angiogenesis. CAMs were treated with PBS vehicle control, goat IgG control, or an anti-EphB4 neutralizing antibody. Membranes were harvested and evaluated for a spoke-wheel vascular pattern, the characteristic angiogenic response in the CAM assay. The vascular length density of radially and non-radially oriented vessels was also quantified. Only 14% of the CAMs treated with the anti-EphB4 antibody displayed a spoke-wheel vascular pattern, while 89 % of the PBS-treated CAMs and 86% of the IgG-treated CAMs displayed a spoke-wheel pattern. Results from representative CAMs (n=4) indicate that the percentage of total vascular density non-radially oriented is significantly greater in EphB4-treated CAMs ( $87\% \pm 11.5\%$ ) as compared to both PBS and IgG-treated CAMs ( $16.5\% \pm 2.9\%$  and  $22.3\% \pm 9.6\%$  respectively,  $P < 0.001$ ). This difference in the overall pattern and orientation of the vasculature indicates that EphB4 is necessary for a normal angiogenic response in the CAM assay. Our results suggest that EphB4 plays an important role in the patterning of the vasculature during angiogenesis.