EPHB4 EXPRESSION ALONG ADULT MICROVASCULAR NETWORKS: EPHB4 IS MORE THAN A VENOUS-SPECIFIC MARKER

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Introduction: The Eph receptors and their membrane-bound ephrin ligands play essential roles during embryonic vascular development. EphB4 and ephrinB2 are also considered to be markers of arterial/venous identity in the embryo, as EphB4 is expressed predominately by venous endothelial cells and ephrinB2 is expressed by arterial endothelial cells. Despite the evidence for EphB4 and ephrinB2 regulation of vascular network formation during embryonic development, their expression patterns and functional roles during adult microvascular remodeling remain unclear. Thus, the objective of this study was to characterize the cellular distribution of EphB4 expression along the hierarchy of unstimulated and remodeling adult rat mesenteric microvascular networks.

Methods: Mesenteric tissues (unstimulated or stimulated using either a 20-minute exteriorization model or mast cell degranulation model) were harvested from 300-g adult male Fisher rats, and stained for various combinations of EphB4 and the vascular cell markers smooth muscle alpha-actin, NG2, and isolectin/CD-31. Positive EphB4 staining was confirmed by comparison to IgG and secondary-only controls. The distribution of EphB4 expression was compared to the cell-specific markers along arteriole, venule, and capillary segments for each mesenteric microvascular network (n=32). Arterial/venous expression of EphB4 was also evaluated in rat subcutaneous tissue (n=3) and spinotrapezius muscle (n=2). In unstimulated networks containing a main arterial/venous pair, EphB4 staining intensity on blind-ended capillary sprouts was compared to that on capillaries connecting arterioles to venules. Representative connecting capillaries (n=34) and a corresponding number of randomly selected capillary sprouts were analyzed using ImageJ software, to obtain a numerical value indicative of the fluorescence intensity of EphB4 staining (termed “fluorescence intensity index”).

Results: EphB4 expression along adult rat microvessels is confined to endothelial cells, with expression undetectable on NG2- and/or smooth muscle alpha-actin-expressing perivascular cells (vascular smooth muscle cells and pericytes). EphB4 expression was detected on 100% of the main feeding arterioles and draining venules examined. Additionally, 100% of the blind-ended capillary sprouts examined in both unstimulated and actively remodeling vascular networks stained positively for EphB4. The fluorescence intensity of EphB4 immunostaining on blind-ended capillary sprouts was significantly greater than the EphB4 staining along capillaries that connected to both an upstream arteriole and a downstream venule (mean fluorescence intensity index = 25 for capillary sprouts, mean fluorescence intensity index = 6 for capillaries connecting arterioles to venules; $p < 0.001$).

Conclusions: This study provides a detailed analysis of the spatial and cellular expression patterns of EphB4 throughout an entire adult microvascular tree. Our findings indicate that EphB4 is not a ubiquitous marker of arterial/venous polarity in the adult microcirculation, but instead is expressed by endothelial cells of both arterioles and venules. Moreover, prominent expression, as detected by fluorescence intensity, of EphB4 along capillary sprouts as compared to connecting capillaries suggests that EphB4 is upregulated along capillaries with an angiogenic phenotype and may play a role in the capillary sprouting process.

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