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Continuous Plasma S1p-dependent Signaling by Apically Polarized S1Pr1 Supports Endothelial Barrier Function

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Sphingosine-1-phosphate, generated by sphingosine kinases (Sphk1 and 2), acts at its receptor S1Pr1 to maintain vascular homeostasis. The gradient of S1P concentration (low micromolar in plasma vs. low nanomolar in tissue) suggests two non-mutually exclusive models via which S1P maintains the endothelial barrier: 1) plasma S1P communicates continuously with the endothelium to maintain vascular integrity and regulate vascular leak, or 2) compartmentalization of S1P and S1Pr1 enables sensing of plasma extravasation and a dynamic response to leak. To distinguish between these models, we examined endothelial responses to apical vs. basolateral application of S1P in vitro and S1Pr1 subcellular localization in vivo. Addition of S1P to the upper (apical) chamber of transwells containing EA.hy926 endothelial monolayers triggered ERK activation. Surprisingly, addition of these agonists to the lower (basolateral) chamber was without effect. Similar results were obtained with the S1Pr1 agonist SEW2871, but the PAR1 agonist SFLLRN triggered ERK activation from either chamber. By immunofluorescence staining and surface biotinylation of EA.hy926 cells, S1Pr1 protein was present predominantly on the apical surface. Similarly, staining of mouse trachea microvessels revealed S1Pr1 on CD31-positive membranes apical, but not basal, to the endothelial cell nucleus. S1Pr1 staining co-localized with the apical membrane marker podocalyxin but not the basal marker β1-integrin. The level of S1Pr1 protein in endothelial cells in mice lacking plasma S1P (Sphk1 flox/-; Sphk2 -/-; Mx-1 cre) was twice that in littermate controls. Deletion of S1Pr1 from the endothelium (S1Pr1 flox/-; Cdh5 Cre) resulted in a 6-fold increase in vascular permeability compared to littermate controls. Preliminary data suggests that S1Pr1 inactivation alters the phosphorylation state of VE-cadherin. These results suggest that S1Pr1 is polarized to the luminal membrane of endothelial cells where it is continuously activated but only partially downregulated by plasma S1P and that ongoing activation of S1Pr1 by S1P in plasma is required to maintain endothelial barrier function.

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