ANGIOGENESIS

A happy medium?

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Epsins are membrane proteins that are thought to function as adaptors between ubiquitinated proteins that are targeted for endocytosis and the clathrin endocytotic machinery. A new study shows that epsins can function as regulators of angiogenesis through the endocytosis of vascular endothelial growth factor receptor 2 (VEGFR2). Although angiogenesis is normally crucial for tumour progression, the angiogenesis that occurs in the absence of epsins is surprisingly

tumour suppressive through the generation of a highly dysfunctional tumour vasculature.

Combined germline deletion of epsin 1 and epsin 2 in mice is embryonic lethal, with embryos displaying vascular defects. To further investigate these vascular functions, Hong Chen, Wang Min and colleagues generated mice with floxed epsin 1 and null epsin 2 alleles; double deficiency for these genes specifically in endothelial cells was achieved by driving the expression of the Cre recombinase from the vascular-endothelial cadherin (*VEcad*; also known as *Cdh5*) promoter. These mice were more resistant to the growth of subcutaneous allografts of melanoma and lung cancer cells, and orthotopic allografts of glioblastoma, as well as to spontaneous tumours such as carcinogen-induced colorectal cancer and large T antigen-driven prostate cancer, compared with wild-type mice. Whereas normal organ vasculature was relatively unaffected by epsin deficiency, the tumour vessels were enlarged, disorganized and leaky, probably resulting in impaired oxygen and nutrient supply to the tumours.

So, what is the molecular explanation for these effects? In the tumour vasculature, VEGFR2 was found to be upregulated in tumours from epsin-deficient mice. Given the endocytic roles of epsins, the authors investigated whether epsins might normally promote the internalization and degradation of VEGFR2 and thus the downregulation of VEGF signalling. Indeed, in wild-type endothelial cells stimulated with

VEGF *in vitro*, epsin 1 was found to bind to ubiquitinated VEGFR2, and immunofluorescence and biochemical analyses revealed that this resulted in VEGFR2 endocytosis. However, in endothelial cells that lacked epsin 1 and epsin 2, VEGFR2 endocytosis was defective; this led to enhanced proliferative and migratory responses to VEGF stimulation *in vitro*, and to increased angiogenesis in response to ectopic VEGF expression *in vivo*.

Overall, this work suggests a model in which a 'medium' level of VEGF signalling is required to support tumour growth, and that a lack of epsin 1 and epsin 2 leaves vessels hypersensitive to VEGF stimulation and the resulting dysfunctional and tumour-suppressive angiogenesis. In support of this, injecting a VEGFR2 inhibitor into tumour-bearing mice was tumour promoting in mice that were deficient for epsin 1 and epsin 2 but was tumour suppressive in wild-type mice.

It will be interesting to see whether targeting epsins, with the aim of disrupting the tumour vasculature, will be a promising therapeutic strategy. Key challenges include assessing the safety of systemic (rather than endothelial-specific) epsin targeting, whether it will be effective for controlling established tumours and determining when the pro-angiogenic effects will be tumour-promoting rather than tumour-suppressive.

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ORIGINAL RESEARCH PAPER Pasula, S. et al. Endothelial epsin deficiency decreases tumor growth by enhancing VEGF signaling. *J. Clin. Invest.* 26 Nov 2012 (doi:10.1172/JCI64537)