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A signal to collapse

The aptly named carboxy-terminal fragment of perlecan, endorepellin, has anti-angiogenic activity and, reporting in *The Journal of Cell Biology*, Gregory Bix *et al.* have come a few steps closer to finding out how it does this. An interaction with the collagen receptor $\alpha_2\beta_1$, followed by unique intracellular signalling, causes actin stress fibres and focal adhesions to break down and stops endothelial-cell migration in its tracks, thereby preventing capillary formation.

The authors cultured human umbilical vein endothelial cells (HUVECs) in a three-dimensional environment with cells that produced either full-length endorepellin or fragments of it. Soluble endorepellin and its carboxy-terminal laminin-G-like (LG3) domain stopped the HUVECs from making endothelial tubes. Endothelial cells that were artificially induced to secrete endorepellin couldn't form tubes either.

Why might this be? One reason was that endorepellin and LG3 caused the disassembly of actin stress fibres and focal adhesions of endothelial cells that were grown on collagen I. The authors considered that, as endorepellin was already known to prevent endothelial cells from attaching to collagen I, integrins might be involved. Endorepellin and LG3 caused $\alpha_2\beta_1$ clustering, concomitant with the collapse of actin bundles in HUVECs. This was prevented by pre-treating the cells with an anti- $\alpha_2\beta_1$ antibody. And, because endothelial

cells could attach (albeit weakly) to endorepellin-coated dishes and this could be blocked by anti- $\alpha_2\beta_1$ antibodies, the authors surmised that $\alpha_2\beta_1$ is a functional endorepellin receptor. Indeed, binding studies showed that endorepellin and LG3 interacted with the α_2 I integrin domain, and therefore interfered with the binding of collagen I to $\alpha_2\beta_1$ integrin.

Collagen encourages endothelial tubes to form by interacting with $\alpha_2\beta_1$ and reducing the concentration of intracellular cyclic AMP [cAMP]_i and the activity of protein kinase A (PKA). Endorepellin and LG3 — as Bix *et al.* predicted — reversed these effects in HUVECs. When PKA activity was inhibited, though, the endorepellin- and LG3-induced actin collapse was blocked, which indicated that PKA was required downstream of endorepellin. Similarly, reducing the concentration of Ca²⁺ blocked the collapse, consistent with the presence of Ca²⁺-binding sites within the LG

domains of endorepellin and the fact that cations are known to be required for integrin-mediated signalling.

Endostatin, a carboxy-terminal fragment of collagen XVIII, also has anti-angiogenic activity, and it disrupts actin stress fibres and focal adhesions by downregulating the Rho-family GTPase RhoA. By contrast, endorepellin failed to affect Rho signalling. Instead, it induced a sustained phosphorylation of focal adhesion kinase (FAK) in HUVECs, as well as a transient phosphorylation followed by a downregulation of the mitogen-activated protein kinase p38 and a downstream effector, heat-shock protein 27. These data are particularly interesting in light of FAK and p38 already being implicated in the development of blood vessels.

Katrin Bussell

References and links

ORIGINAL RESEARCH PAPER Bix, G. *et al.*

Endorepellin causes endothelial cell disassembly of actin cytoskeleton and focal adhesions through $\alpha_2\beta_1$ integrin. *J. Cell Biol.* **166**, 97–109 (2004)