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METASTASIS

Oxidizing abnormalities

Tumour vessel 'abnormalization' describes the phenomenon by which severe hypoxia and excessive release of angiogenic cytokines leads to the formation of aberrant endothelial linings of tumour blood vessels. Such malformed vessels impair the perfusion and oxygenation of the surrounding tumour tissue, promoting invasion and metastasis. Peter Carmeliet and colleagues identify a regulator of endothelial cell morphology, inhibition of which could be the key to promoting vessel normalization and hence inhibiting metastasis.

EGLN1 (also known as PHD2) is a member of the Phd family of oxygen-sensitive prolyl hydroxylases that regulate the protein levels of the hypoxia-inducible factor-α (HIFα) subunits of the HIF transcription factor complex. HIF expression in tumour cells promotes motility, survival and angiogenesis in low O_2 tensions, being degraded in the presence of O_2 through the actions of Phds. To investigate the role of PHD2 in endothelial cell morphology and vessel normalization, Mazzone, Dettori, Leite de Oliveira and colleagues generated *Phd2*$^{+/-}$ mice (homozygous null mice were unviable). Using either ectopic transplantation of B16 melanoma or Lewis lung carcinoma (LLC) cells or orthotopic transplantation of Panc02 pancreatic carcinoma cells in *Phd2*$^{+/-}$ mice, the authors found that, although the tumours grew at comparable rates to those transplanted into wild-type mice, those in the *Phd2*$^{+/-}$ mice had an encapsulated morphology and metastasis was significantly decreased, hence improving survival. Consistently, using the Panc02 cells they found that tumour cell intravasation and presence in the circulation were 50% and 70% lower, respectively, in *Phd2*$^{+/-}$ mice. Moreover, gene expression analysis of the Panc02 tumours indicated that the expression of HIF target genes was reduced and that hypoxic and necrotic areas within B16 tumours were smaller. They also found that aerobic metabolism — rather than glycolysis — was the preferred energy source in these tumours, indicating that tumours in *Phd2*$^{+/-}$ mice are better oxygenated.

So, how does PHD2 haploinsufficiency improve tumour oxygenation and prevent metastasis? The authors found that tumour blood and lymphatic vessel density, size and area were comparable in all tumour models. However, using fluorescent microspheres in the B16 tumour model, the authors showed that perfusion was increased in *Phd2*$^{+/-}$ mice, suggesting that a tighter endothelial barrier lining the blood vessels might be the cause. Indeed, they found that vessels in tumours within *Phd2*$^{+/-}$ mice were more mature and stable (with less endothelial proliferation and apoptosis) and had higher pericyte coverage. Electron microscopy and whole-mount staining of tumour sections with an endothelial cell marker revealed that endothelial cells formed a continuous lining (termed the phalanx phenotype), whereas tumour-associated vessels within wild-type mice showed signs of abnormalization. Therefore, these data suggest that PHD2 haploinsufficiency regulates endothelial cell morphology, not vessel number, which consequently modifies the aggressiveness of tumours.

This novel phalanx phenotype of endothelial linings in tumour-associated blood vessels prevents tumour cell intravasation and hence dissemination and metastasis. Arguably, PHD2 might provide an excellent target for anti-metastatic drug discovery, provided appropriate targeting strategies to the tumour are used.

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ORIGINAL RESEARCH PAPER Mazzone, M. *et al.* Heterozygous deficiency of *PHD2* restores tumor oxygenation and inhibits metastasis via endothelial normalization. *Cell* 12 Feb 2009 (doi: 10.1016/j.cell.2009.01.020)

FURTHER READING Kaelin, W. G. Jr. The von Hippel–Lindau tumour syuppressor protein: O_2 sensing and cancer. *Nature Rev. Cancer* **8**, 865–873 (2008)