TUMOUR SUPRESSORS

Benign association

Hamartomas are benign lesions that develop in a range of dominantly inherited tumour syndromes, including tuberous sclerosis complex (TSC) and Peutz–Jeghers syndrome (PJS). Two groups now have evidence for a molecular link between TSC and PJS.

Although developing in different tissues, the hamartomas seen in patients with either TSC or PJS have a similar histology. One of the known phosphorylation targets of the PJS tumour-suppressor kinase LKB1 is the energy-sensing AMP kinase (AMPK), which is activated by high AMP (low ATP) levels. TSC2 — a tumour suppressor involved in TSC and a regulator of target of rapamycin (TOR) kinase — is a direct target of AMPK, prompting Corradetti *et al.* and Shaw *et al.* to look closely at the molecular pathways disrupted through the loss of *Lkb1*.

Both groups initially verified that LKB1 can negatively regulate phosphorylation of the TOR kinase substrates S6 kinase and 4EBP1. Phosphorylation of both of these proteins was increased in cells with non-functional LKB1. Both groups then examined the regulation of this pathway by manipulating the activity of AMPK. Shaw and co-workers used an AMP mimetic to stimulate AMPK and showed that TSC2 was phosphorylated only in the presence of functional LKB1. Corradetti and colleagues used an AMPK inhibitor to show that LKB1 was unable to alter the phosphorylation status of S6 kinase in the absence of functional AMPK.

What is the physiological relevance of these findings? TOR is a component of the energy-sensing pathway and $Tsc2^{-/-}$ cells undergo apoptosis when deprived of glucose, a response that is blocked by the TOR inhibitor rapamycin. Both groups demonstrate the same sensitivity and response to rapamycin in $Lkb1^{-/-}$ cells in the absence of glucose. Corradetti and colleagues showed that, like $Tsc2^{-/-}$ cells, $Lkb1^{-/-}$ cells secrete increased levels of vascular endothelial growth factor, which is attenuated in the presence of rapamycin. Shaw and colleagues have also found that $Lkb1^{+/-}$ mice develop intestinal hamartomas that mimic those arising in patients with PJS and these also have increased levels of TOR activity.

Therefore, the similarities between PJS and TSC are because of LKB1 and TSC2 belonging to the same kinase signalling pathway. Their affect on TOR also indicates that rapamycin and its analogues could be used to treat hamartomas in these patients. However, despite these similarities, intriguing questions remain. Why, for example, do patients with PJS develop hamartomas in the intestine, whereas patients with TSC have widespread tissue involvement? One explanation might be that loss of TSC2 function affects more than energy signalling pathways; $Tsc2^{-/-}$ cells also fail to respond to growth-factor-mediated pathways that remain intact in $Lkb1^{-/-}$ cells.

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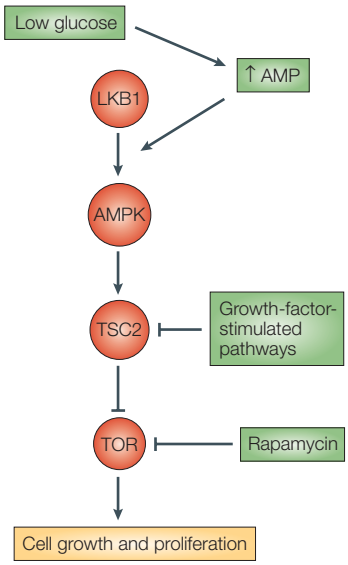

References and links

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ANGIOGENESIS

Recruitment drive

Vascular endothelial growth factor (VEGF) is important in tumour angiogenesis, but whether it is essential that cancer cells themselves express VEGF is unclear. A recent paper in *The EMBO Journal* shows that tumours can recruit stromal cells to carry out this function.

Jianying Dong, Napoleone Ferrara and colleagues generated *Vegf*-null mouse embryonic fibroblasts (MEFs), which were immortalized and then transformed with oncogenic *Ras*. These cells formed tumours on injection into mice, which were about half the size of those formed by a parental VEGF-expressing cell line that was similarly immortalized and transformed. The *Vegf*-null tumours successfully induced angiogenesis, with only a slight decrease in vessel density compared with tumours formed by parental cells.

Although these tumours do not express VEGF themselves, VEGF mRNA and protein were present in the tumours, but at markedly lower levels than in those formed by the parental cell line. Could stromal cells recruited to these tumours be the source of VEGF expression? This was confirmed by *in situ* hybridization using a probe specific for the exon of the *Vegf* gene that was deleted in the *Vegf*-null MEFs, showing that the transcript co-localized with tumour-associated stromal cells.

The authors used an anti-VEGF antibody to test whether the small amounts of VEGF produced by recruited stromal cells were responsible for angiogenesis and tumour growth in the *Vegf*-null tumours. Treatment with the antibody resulted in a decrease in tumour mass of up to 62%, indicating that the stroma-derived VEGF does have an important role.

How do tumours recruit VEGF-expressing stromal cells? Fibroblasts are an important component of the tumour-associated stroma and are known to express VEGF. The authors showed that conditioned medium obtained from *Vegf*-null tumour cells stimulated migration and proliferation of a fibroblast cell line *in vitro*. Fractionation of the medium revealed a peak of activity corresponding to PDGFA — a member of the platelet-derived growth factor family. Consistent with a role of this protein in fibroblast recruitment, *Pdgfa* expression was seen throughout *Vegf*-null tumours, whereas expression of the mRNA encoding its receptor — PDGFRα — was localized to stromal cells. In addition, a soluble form of PDGFRα inhibited tumour growth by 50%, confirming a crucial role for signalling through this receptor.

These results have important implications for anticancer treatments that inhibit angiogenesis. The fact that tumour cells can recruit VEGF-expressing fibroblasts, as well as producing VEGF themselves, indicates that the signalling pathways involved in both of these mechanisms might need to be blocked to completely inhibit angiogenesis and tumour growth.

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References and links

ORIGINAL RESEARCH PAPER Dong, J. *et al.* VEGF-null cells require PDGFRα signaling-mediated stromal fibroblast recruitment for tumorigenesis. *EMBO J.* 1 July 2004 (doi:10.1038/sj.emboj.7600289)