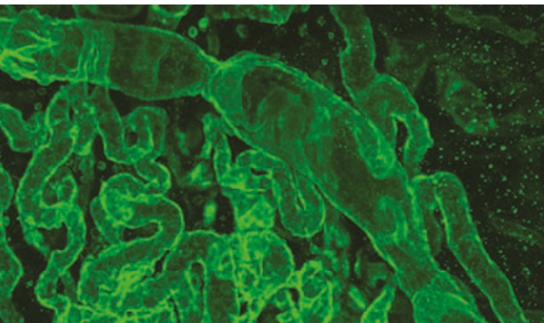
ANGIOGENESIS

All tied up

Blood vessels that feed developing tumours do not show the highly organized vascular pattern that is seen in normal tissues. Sunyoung Lee and colleagues show that this might, in part, be explained by the differing actions of matrix-tethered and untethered vascular endothelial growth factor (VEGF).



Once secreted from the cell, VEGF becomes associated with the extracellular matrix (ECM) through the heparin-binding or ECM-binding domain in its carboxyl terminus, and is thought to act in a paracrine fashion. VEGF is released from the ECM during matrix breakdown, which is mediated by enzymes such as the heparinases and plasmin. Matrix metalloproteinases (MMPs) have also been implicated in ECM breakdown and the liberation of VEGF, but by an unclear mechanism. Therefore, Lee and colleagues asked if MMPs function like plasmin and release VEGF by degrading matrix proteins or if, in fact, they cleave VEGF itself.

The authors found that the matrix-bound form of VEGF is efficiently cleaved by MMP3, 7, 9 and 19, into a stable, soluble cleavage product, $VEGF_{113}$. Antibody studies on ascites isolated from patients with ovarian cancer verified that $VEGF_{113}$ is present *in vivo*. Moreover, using matrix-assisted laser desorption time-of-flight mass spectrometry, they demonstrated that $VEGF_{113}$ contains the VEGF receptor (VEGFR)-binding domain, which is released from the ECM-binding domain by MMPs.

$VEGF_{113}$ is able to interact with VEGFR and to induce its phosphorylation and activation like wild-type VEGF. So, to analyse the biological function of $VEGF_{113}$, the researchers tested wild-type VEGF, $VEGF_{113}$ and a non-MMP cleavable form — VEGF(Δ108–118) — in angiogenesis assays *in vitro*. All these forms of VEGF could induce angiogenesis, but, notably, they induced different patterns of blood-vessel growth. The wild-type form of VEGF induced tortuous blood vessels similar to those seen normally in tumours in which VEGF acts without other angiogenic factors. However, $VEGF_{113}$ produced very wide and leaky blood vessels (see accompanying picture), and VEGF(Δ108–118) produced thin, highly interconnected blood vessels.

In vivo, xenotransplants of human cancer cell lines expressing these different forms of VEGF resulted in different rates of tumour growth. The tumours expressing $VEGF_{113}$ did not grow well and remained pale, whereas those expressing VEGF(Δ108–118) grew faster than those expressing wild-type VEGF.

MOUSE MODELS

Knocking in is the new knocking out

In the beginning there was the transgenic mouse; then came the gene knockout mouse; then the regulatable transgenic mouse; and now we have the knock-in, knockout mouse. Gerard Evan and colleagues have made a $p53ER^{TAM}$ mouse in which the endogenous *Trp53* gene has been replaced by one that encodes a p53 fusion protein, the function of which is solely dependent on the presence of an artificial ligand, 4-hydroxytamoxifen (4-OHT).

Why go to the extensive trouble of constructing such a mouse? Is the wealth of data from existing models not enough for addressing the complex issues of tumorigenesis? Sadly not, because as our mouse models have evolved, so has the complexity of the questions that we wish to address.

Take p53 for example. The p53 pathway is disrupted frequently in human tumours, a reflection, perhaps, of the fact that p53 integrates myriad signals generated by cells in response to stress and pathological stimuli. Initially, researchers complacently thought that p53, as a transcription factor, would act through the regulation of specific, readily identifiable gene networks. However, it is now abundantly evident that p53 is regulated, and elicits many of its effects, subtly at the protein level. So if you could make a mouse in which p53 remains under the control of its endogenous gene promotor, where the protein is made at normal physiological levels in all cells, but that protein is non-functional until an exogenous source of ligand is provided, would such a mouse really provide answers to our new questions? The $p53ER^{TAM}$ knock-in mouse (*Trp53*$^{K1/K1}$) is viable and expresses a regulatable p53 protein, and has already begun to shed new light on p53 function. The *Trp53*$^{K1/K1}$ mice develop normally in the absence of 4-OHT but go on to develop lymphoid tumours with similar kinetics to standard p53-knockout animals, demonstrating that $p53ER^{TAM}$ is essentially non-functional in the absence of 4-OHT. In the presence of 4-OHT, $p53ER^{TAM}$ is functional, but, crucially, is not activated. On exposure of either *Trp53*$^{K1/K1}$ mouse embryo fibroblasts (MEFs) or adult mice to DNA damage in the presence of 4-OHT, p53 functions as expected — p53 target genes are expressed and apoptosis is induced. Moreover, on withdrawal of 4-OHT, p53 function is negated, indicating that p53 functionality in these animals requires the constant presence of 4-OHT.

With these ground rules established, the authors have started to examine the temporal regulation of p53 in response to deregulated oncogene expression and DNA damage. RAS, when expressed in cultured MEFs, induces replicative senescence due to p53 activation. In *Trp53*$^{K1/K1}$ MEFs that express activated HRAS, RAS deregulation persistently generates a p53 activation signal and p53 function is required to maintain replicative senescence in most cells. However, in response to DNA damage, such as γ-radiation, the signal to activate p53 is transient. Restoration of p53 function triggers apoptosis in irradiated *Trp53*$^{K1/K1}$ mice for only 48–72 hours after radiation exposure. This indicates that such damage is efficiently repaired and questions the requirement of p53 in the resolution of radiation-induced damage.

These initial data are already beginning to probe our previous ideas about p53 biology and functionality during tumorigenesis. By raising more questions in the future, we might at last be in a position where we can effectively manipulate this fascinating and frustrating tumour-suppressor gene.

Nicola McCarthy

References and links

ORIGINAL RESEARCH PAPER Christophorou, M. A. *et al.* Temporal dissection of p53 function *in vitro* and *in vivo*. *Nature Genet*. 29 May 2005 (doi:10.1038/ng1572)

© 2005 Nature Publishing Group