TUMOUR SUPPRESSORS

The PUMA effect

The p53-regulated protein PUMA is a pro-apoptotic member of the BCL2 family and is required for p53-activated apoptosis in some contexts. However, whether it also has a role in preventing tumorigenesis is unclear. Scott Lowe and colleagues now reveal that PUMA is required for tumour suppression by p53 in response to specific oncogenes.

PUMA is crucial for p53-activated cell death in mouse embryonic fibroblasts (MEFs), indicating that it might also be required for tumour suppression by p53 in these cells. To determine this, the authors tested whether loss of *Puma* expression mimics the increase in tumour formation in the absence of p53 that is seen in MEFs expressing the oncogenes *Ras* and *E1a*. Lowe and colleagues used an RNA interference (RNAi) method in which *Puma*-specific short hairpin RNAs (shRNAs) are expressed from retroviral vectors, allowing sustained inhibition of gene expression. MEFs expressing *Puma* shRNAs and either *Ras* alone or both *Ras* and *E1a* were injected into mice. When cells expressed both oncogenes, inhibition of PUMA expression resulted in high levels of tumour formation — similar to those seen for cells lacking p53 — whereas cells transduced with a control retrovirus failed to form tumours. However, for cells expressing *Ras* alone, blocking *Puma* expression did not stimulate tumour formation. So, although PUMA is required for tumour suppression by p53 in response to a combination of *Ras* and *E1a*, it is not essential for the response to *Ras* alone.

Is PUMA required for tumour suppression in response to other oncogenes? *Eμ-Myc* haematopoietic stem cells (HSCs) form B-cell lymphomas when injected into mice as they express *Myc* under the control of an immunoglobulin heavy-chain promoter. This lymphomagenesis is accelerated when p53 expression is inhibited by RNAi. The authors tested whether the same effect is seen when PUMA expression is blocked. All mice injected with *Eμ-Myc* HSCs expressing *Puma* shRNAs developed lymphomas, compared with 40% of mice injected with cells carrying a control retrovirus. Similar to the effects of p53 inhibition, the cells lacking PUMA expression also formed lymphomas at an increased rate. Lowe and colleagues confirmed that PUMA inhibition cooperates with *Myc* expression to promote tumorigenesis, as only one-sixth of mice injected with wild-type HSCs expressing *Puma* shRNAs developed lymphomas.

So, PUMA is required to suppress tumorigenesis in some contexts, such as expression of MYC or a combination of E1A and RAS, but not in others, such as expression of RAS alone. Further studies should increase our understanding of how different p53 targets contribute to tumour suppression in response to specific oncogenic stimuli, with implications for how this key pathway could be manipulated in the treatment of different types of cancer.

Louisa Flintoft

References and links

ORIGINAL RESEARCH PAPER Hemann, M. T. *et al.* Suppression of tumorigenesis by the p53 target PUMA. *Proc. Natl Acad. Sci. USA* **101**, 9333–9338 (2004)

WEB SITE

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http://www.cshl.org/public/SCIENCE/lowe.html

GENE THERAPY

Special delivery

Hypoxic areas of tumours, and particularly of metastases, are notoriously resistant to chemo- or radiotherapies. Jiwu Wei *et al.* have developed a scheme to specifically deliver a cytotoxic gene product to hypoxic lung metastases, through embryonic endothelial progenitor cells (EPCs) that usually contribute to tumour vasculogenesis.

EPCs arise in the bone marrow and are recruited to sites of active neovasculogenesis by vascular endothelial growth factor (VEGF) and other factors. As they have been shown to contribute to the growing tumour vasculature, Wei *et al.* investigated if they could be used to deliver therapeutics to hypoxic tumours. They specifically chose to use EPCs isolated from mouse embryos, rather than adult EPCs, because they can be easily grown in culture and genetically manipulated. Also, as embryonic EPCs do not express major histocompatibility complex class I molecules, they are not rejected by the host's immune system.

Wei *et al.* showed that when these cells were injected into the tail veins of mice, they localized primarily to lung metastases that developed from transplanted osteosarcomas or Lewis lung carcinomas, but were also found in liver and kidney metastases. The EPCs homed mostly to poorly vascularized metastases, which were found to be hypoxic and expressed high levels of VEGF. So could these EPCs be used to deliver a cytotoxic 'suicide' gene to these metastases? The authors stably transfected EPCs with the yeast cytosine deaminase gene, fused to uracil phosphoribosyl transferase. This fusion enzyme converts the prodrug 5-fluorocytosine (5-FC) into the cytotoxic compound 5-fluorouracil (5-FU), which can diffuse into the interstitial space and mediate a cytotoxic effect on surrounding tumour cells.

Wei *et al.* found that mice with established multiple lung metastases lived significantly longer after treatment with suicide-gene-harbouring EPCs and 5-FC, compared with controls. Up to 90% of all lung metastases were targeted by the cells, and the treatment was not found to cause any toxic effects or embryonic tumours. The mice, however, eventually succumbed to metastases formed in other organs, as well as the non-hypoxic, well-vascularized metastases that were not efficiently infiltrated by the EPCs. Another complication of the system was that it did not kill tumour cells immediately — it required time for the EPCs to incorporate into the tumour vasculature, to express the suicide gene and to kill bystander cells. Furthermore, control EPCs that did not carry the suicide gene actually promoted tumour vascularization and growth. Therefore, one crucial aspect for future clinical use will be the inclusion of safeguards to ensure that the cytotoxic system becomes activated in all EPCs.

Kristine Novak

References and links

ORIGINAL RESEARCH PAPER Wei, J. *et al.* Embryonic endothelial progenitor cells armed with a suicide gene target hypoxic lung metastases after intravenous delivery. *Cancer Cell* **5**, 477–488 (2004)

FURTHER READING Rafii, S. *et al.* Vascular and haematopoietic stem cells: novel targets for anti-angiogenesis therapy? *Nature Rev. Cancer* **2**, 826–835 (2002)