THERAPEUTIC TARGETS

TORpedoing

Since the nineteenth century, torpedoes have been used to seek and destroy ships. Rapamycin — an inhibitor of mammalian target of rapamycin (mTOR) — acts similarly, destroying tumour cells by inducing apoptosis. But how does this antibiotic have such a specific effect on tumours? Peter Houghton's group report in *Molecular Cell* that rapamycin-induced apoptosis is caused by prolonged activation of the c-Jun amino-terminal kinase (JNK) signalling pathway, but only in cells that lack functional p53.

Cells lacking p53 undergo apoptosis in response to rapamycin and are protected if p53 function is restored. So, as the JNK cascade is implicated in p53-dependent apoptosis, the authors wondered whether this pathway was activated by rapamycin. Using Rh30 cells — human rhabdomyosarcoma cells that express an inactive form of p53 — the authors showed that JNK activation and hyperphosphorylation of c-JUN occurred and was sustained for 5 days, the timeframe for maximal apoptosis. This activation was mTOR-dependent, as Rh30 cells expressing a rapamycin-resistant mutant of mTOR did not respond to the antibiotic. So, how is mTOR linked to the JNK signalling cascade?

As the cellular effects of mTOR are mediated by two distinct pathways — S6K1 or 4E-BP1 — the authors wondered which was affected by rapamycin. They used an Rh30 rapamycin-resistant clone, expressing low levels of 4E-BP1, or a revertant of this clone, expressing levels similar to the wild-type cells, and found that only the revertant clone showed hyperphosphorylation of c-JUN in response to rapamycin. So, 4E-BP1 is essential for this process. But does c-JUN hyperphosphorylation, by itself, cause rapamycin-induced apoptosis?

Expression of dominant-negative c-Jun mutants or deletion of c-Jun in *Trp53*-null mouse embryonic fibroblasts (MEFs) completely repressed rapamycin-induced apoptosis. So, loss of c-Jun rescues *Trp53*-null cells from rapamycin-induced apoptosis. To establish if p53 protects cells by blocking JNK activity, the authors restored functional p53 or expressed WAF1 — a downstream effector of p53 — in Rh30 cells and showed that c-JUN was only transiently hyperphosphorylated, compared with sustained activation in normal Rh30 cells. Moreover, c-Jun hyperphosphorylation differed markedly with genotype, as it was sustained in *Trp53*-null MEFs and was transient in wild-type MEFs. So, both P53 and WAF1 suppress sustained phosphorylation of c-Jun. Transactivation of other p53-responsive genes was inadequate for p53-mediated protection against rapamycin-induced apoptosis, as Waf1-expressing MEFs overexpressing p53 showed no suppression of c-Jun phosphorylation.

Apoptosis signal-regulating kinase (ASK) functions upstream of JNK, and c-JUN hyperphosphorylation was increased in Rh30 cells expressing wild-type, but not a dominant-negative ASK, indicating that ASK mediates rapamycin-induced apoptosis. Using immunoprecipitation experiments, the authors showed that ASK interacts with WAF1 to negatively regulate ASK–JNK signalling. So, when p53 is present, or WAF1 is constitutively expressed, the activation of JNK is blocked by formation of the ASK–WAF1 complex. But, in cells lacking functional p53 or expression of WAF1, the JNK cascade is triggered and results in apoptosis of cancer cells.

These findings indicate that rapamycin analogues or other inhibitors of mTOR could be useful for developing selective therapy to seek and destroy *TP53*-null tumours.

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References and links

ORIGINAL RESEARCH PAPER Huang, S. *et al.* Sustained activation of the JNK cascade and rapamycin-induced apoptosis are suppressed by p53/p21^{Cip1}. *Mol. Cell* **11**, 1491–1501 (2003)

Peter Houghton's lab: www.stjude.org/molecular-pharmacology

IN BRIEF

ANGIOGENESIS

Role of Raf in vascular protection from distinct apoptotic stimuli.

Alavi, A. *et al. Science* **301**, 94–96 (2003)

Alavi *et al.* investigated angiogenic pathways in endothelial cells (ECs) activated by vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) showing that VEGF prevents receptor-mediated apoptosis, whereas bFGF protects cells from stress-mediated death. RAF1— associated with EC survival — is activated by p21-activated protein kinase (PAK) or SRC kinase. Mutation analysis of RAF1 showed that PAK is crucial for bFGF-mediated protection from apoptosis, whereas SRC is essential for the protection mediated by VEGF. These findings indicate that RAF1 is important for EC survival during angiogenesis.

IMMUNOTHERAPY

Immunotherapy of established tumors using bone marrow transplantation with antigen gene-modified hematopoietic stem cells.

Cui, Y. *et al. Nature Med.* **9**, 952–958 (2003)

If tumour antigens can be efficiently transferred to antigen-presenting dendritic cells (DCs) *in vivo*, cancer vaccines will be more effective for immunizing against tumours. Cui *et al.* used haematopoietic stem cells (HSCs) engineered to express tumour-antigen-encoding genes and transplanted them into irradiated mice, generating efficient transgene expression in the mouse DCs. Administration of DC-activating agents and mature T cells with the transplanted HSCs, led to marked expansion of antigen-specific T cells and successful treatment of established tumours.

MOUSE MODELS

Acute mutation of retinoblastoma gene function is sufficient for cell cycle re-entry.

Sage, O. J. *et al. Nature* **424**, 223–228 (2003)

Germline alteration of genes in mouse models is often used to study human cancer. However, Sage *et al.* show that a conditional knockout of the retinoblastoma (*Rb*) gene in mice does not have the same effect as germline loss of Rb function. The authors show that acute loss of Rb is sufficient for cell-cycle entry. Use of conditional knockouts might enable more accurate study of the effects of spontaneous mutations in human cancers.

TUMOUR SUPPRESSORS

OPCML at 11q25 is epigenetically inactivated and has a tumor-suppressor function in epithelial ovarian cancer.

Sellar, G. C. *et al. Nature Genet.* **34**, 337–343 (2003)

Loss of heterozygosity at 11q25 has been shown to be associated with sporadic epithelial ovarian cancer. Sellar *et al.* have now identified a candidate tumour-suppressor gene at this locus — *OPCML* — that is a member of the immunoglobulin-domain-containing GPI-anchored cell-adhesion molecules.