TUMOUR SUPPRESSORS

Adaptor protein connects to cancer

Adaptor proteins mostly function as flexible molecular scaffolds that mediate protein–protein and protein–lipid interactions in signalling pathways. Writing in *Nature Immunology*, Alexandra Flemming and colleagues propose that an adaptor protein that is expressed during B-cell development — the SLP65 protein — is a tumour suppressor that limits the proliferation of precursor B cells and promotes their differentiation into mature cells.

B cells develop in a stepwise manner, from progenitor B cells to precursor (pre-) B cells to mature cells. A key checkpoint in pre-B-cell development is the surface expression of a pre-B-cell receptor (pre-BCR), which is needed to signal the selection and proliferation of these cells. The cells then differentiate, lose the pre-BCR and express the BCR of mature cells. It was known that mice lacking the Slp65 adaptor have more pre-B cells and fewer mature B cells than normal. This hinted at a role for SLP65 in pre-B cells, and Flemming *et al*. set out to investigate this.

The authors first isolated pre-B cells from the bone marrow of wild-type and *Slp65*$^{-/-}$ mice, growing them for short periods *in vitro* in the presence of interleukin-7 (IL-7). They found that the mutant cells showed a greater proliferative capacity than wild-type cells, and that a larger proportion expressed the pre-BCR — indeed, the authors also show that Slp65 usually downregulates the surface expression of this receptor. As one function of the pre-BCR is to signal proliferation, could the mutant cells multiply without it? The authors crossed *Slp65*$^{-/-}$ mice with mice lacking part of the pre-BCR, and found that isolated bone-marrow-derived B cells proliferated slowly. So, the increased proliferation of *Slp65*$^{-/-}$ B cells requires the high surface expression of pre-BCRs.

Flemming *et al*. then cultured *Slp65*$^{-/-}$ pre-B cells for longer periods, and showed that a signalling pathway involving the mitogen-activated protein kinase ERK, which is required for proliferation, was active. In addition, withdrawing IL-7 led to differentiation, as with wild-type cells, but adding back Slp65 markedly enhanced differentiation.

CELL-CYCLE PROGRESSION

Endless cycling

Stem cells and cancer cells share certain properties, such as plasticity and self-renewal, which indicates that they might have common cellular machineries. Tsai and McKay now report in *Genes & Development* a nucleolar mechanism that regulates cell-cycle progression in stem cells and cancer cells.

To investigate the mechanism that underlies the proliferative state of stem cells, Tsai and McKay took advantage of the precise differentiation kinetics of dissociated central nervous system (CNS) stem cells in tissue culture. They constructed a subtractive library from which they identified a novel nucleolar protein — nucleostemin — which was highly enriched in cortical stem cells but absent in serum-differentiated cells. Nucleostemin was also present in embryonic stem cells and several human cancer cell lines.

Tsai and McKay showed that, during CNS development, nucleostemin is expressed before nestin expression peaks — nestin is an intermediate filament protein that is characteristic of neuroepithelial precursors — and is downregulated when the expression of the proliferative marker PCNA and the nucleolar protein B23 is still high. This means that cells continue to proliferate after nucleostemin expression is lost, and that nucleostemin downregulation occurs before the differentiation of neurons and glia. So, nucleostemin expression does not reflect the immediate proliferative state, but is characteristic of an early multipotential state.

To understand the functional role of nucleostemin, Tsai and McKay carried out small inhibitory RNA (siRNA) knockdown experiments in which nucleostemin expression was reduced. Compared with the control cultures, the percentage of non-dividing cells was increased in transfected cortical stem cells and the U2OS cancer cell line, indicating that nucleostemin is required for maintaining the proliferative capacity. Intriguingly, overexpression of nucleostemin also caused cells to exit the cell cycle — which is similar to the loss-of-function phenotype.

Tsai and McKay then set out to further dissect the molecular mechanism of nucleostemin function. Deletion studies showed that the amino-terminal basic region of nucleostemin is important for its nucleolar localization and that its two GTP-binding motifs regulate the nucleolar integrity.

Overexpression of mutants lacking the GTP-binding motifs blocked DNA replication, indicating that dysregulation of GTP binding hinders cell-cycle progression in late S phase. Overexpression of these mutants also caused an increase in cell death, compared with wild-type nucleostemin, and were partially rescued by deletion of the amino-terminal basic domain. In addition, when the GTP-binding-domain deletion mutants were expressed in p53-null cells, no significant increase in cell death was found.

So how is p53 correlated to nucleostemin? Tsai and McKay showed that nucleostemin can bind p53 in glutathione-*S*-transferase (GST) pulldown and co-immunoprecipitation assays, and that the interacting region maps to the amino-terminal basic domain, which explains the rescue phenotype.

Tsai and McKay hypothesize that nucleostemin forms a complex with other nucleolar proteins when it is in a non-GTP-bound state and becomes dissociated on binding to GTP. The interaction of nucleostemin with p53, which presumably takes place in the nucleoplasm, represents a GTP-regulated and stem-cell/cancer-cell-specific control mechanism of cell-cycle progression.

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References and links

ORIGINAL RESEARCH PAPERS Tsai, R. Y. L. & McKay, R. D. G. A nucleolar mechanism controlling cell proliferation in stem cells and cancer cells. *Genes Dev*. **16**, 2991–3003 (2002)

A small but significant percentage of the *Slp65*$^{-/-}$ mice developed solid tumours, mostly close to the scapula, and splenomegaly; all these tumours consisted solely of pre-B cells expressing pre-BCRs. The authors propose that the increased proliferation of mutant pre-B cells seen in culture causes this increase in tumours. But they also suggest that, as the proportion of tumours is small, increased expression of pre-BCRs is not sufficient for tumorigenesis; secondary mutations are required, and are given greater opportunity to occur. Whether proliferating pre-B cells are prone to such mutations is a question for the future.

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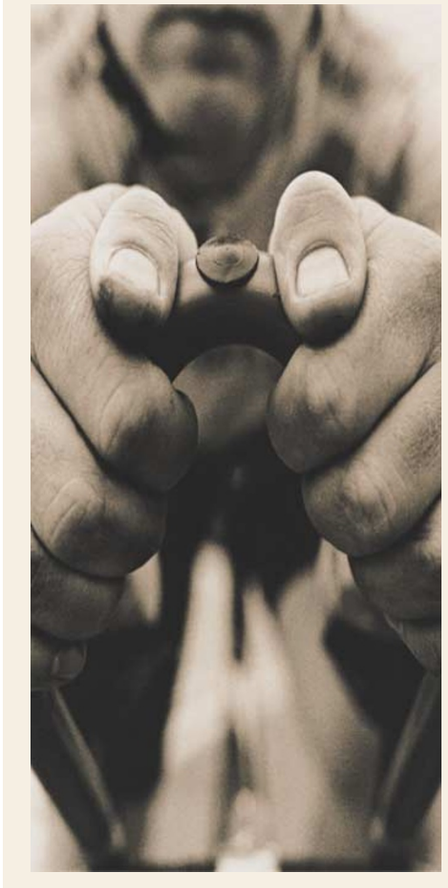
References and links

ORIGINAL RESEARCH PAPER Flemming, A., Brummer, T., Reth, M. & Jumaa, H. The adaptor protein SLP-65 acts as a tumor suppressor that limits pre-B cell expansion. *Nature Immunol.* 18 Nov 2002 (doi:10.1038/ni862).



TRANSFORMATION

Telomerase — the third element?

Unlike mouse cells, primary human cells are refractory to oncogenic transformation — transformation requires a specific combination of three genetic elements (the *HRAS-V12* oncogene, the SV40 early region and the catalytic subunit of telomerase (*TERT*)) as opposed to a combination of two oncogenes in mouse cells. But what characteristic might explain this difference? The relative ease of immortalization of mouse cells — because they have longer telomeres and express telomerase — is one possibility, and a requirement for telomere maintenance and immortality in human cells is supported by the fact that *TERT* is the third element. However, in the November issue of *Cancer Cell*, Yvette Seger *et al.* investigate this premise, and show that TERT does not have to be one of the three elements.

Expression of *HRAS-V12* alone causes irreversible growth arrest, and adenovirus *E1A* is one of the few oncogenes that can rescue this phenotype; in fact, expression of *HRAS-V12* and *E1A* is sufficient for transformation of mouse cells, so the authors investigated whether this oncogenic combination could also transform primary human fibroblasts — BJ cells. They first investigated whether cells expressing *HRAS-V12* and *E1A* showed anchorage-independent growth — a characteristic of transformation — and found that they did. Co-expression of *HRAS-V12* with *E1A* deletion mutants confirmed that E1A must maintain the ability to interact with p300, p400 and the retinoblastoma (RB) family. Interestingly, despite showing characteristics of transformation, these cells are not able to form tumours when injected into immunocompromised mice. So, what other element might be required for this function?

The SV40 early region is known to abrogate both the RB and p53 pathways, so the authors investigated whether expression of the oncogene *MDM2*, which inhibits p53, could confer tumorigenic potential on the *HRAS-V12*- and *E1A*-expressing BJ cells. Triple-infected cells (BJ/ERM cells) were injected into immunocompromised mice and were able to generate tumours with a similar latency to human cancer cell lines.

So, can transformation really occur in the absence of telomerase activity or an alternative telomere-maintenance strategy? Telomerase activity could not be detected using the TRAP assay in the BJ/ERM cells, and they also do not seem to be immortal — they undergo 'crisis' and adopt a senescent phenotype after prolonged culture. Similarly, the tumours that are formed from these cells do not generally express *TERT*, as shown by reverse-transcriptase polymerase chain reaction, and do not have telomerase activity, as shown by the TRAP assay. On explantation into culture, BJ/ERM tumour cells undergo crisis, which is indicative of a lack of telomere maintenance, and telomeric fluorescence *in situ* hybridization revealed that the telomeres continued to be eroded during tumour growth, confirming that telomeres were not maintained by the alternative (ALT) recombination-based mechanism.

Karyotypic analysis of chromosomes from explanted BJ/ERM tumour cells reveals many chromosomal abnormalities, which are characteristic of the end-to-end chromosome fusions that occur as telomeres shorten. This type of chromosomal instability could accelerate the tumorigenic process.

So, unlike previous transformation protocols, this one does not require telomerase activity or immortalization, demonstrating that immortality is not an obligate characteristic of a cancer cell.

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References and links

ORIGINAL RESEARCH PAPER Seger, Y. R. *et al.* Transformation of normal human cells in the absence of telomerase activation. *Cancer Cell* **2**, 401–413 (2002)

WEB SITE

Greg Hannon's lab: http://www.cshl.org/gradschool/hannon_html