TUMORIGENESIS

A transforming combination

Human cells are notoriously difficult to transform. Unlike rodent cells, which can be transformed with just a couple of oncogenes, human cells require several genetic lesions, including viral oncoproteins such as the SV40 large T and small T antigens, which disrupt both the p53 and RB tumour-suppressor pathways. Now, Gordon Peters and colleagues report in *Cancer Cell* that a new combination of just four genetic alterations can transform human diploid fibroblasts, and these leave the p53 pathway intact.

Culturing human cells results in a stress-induced, INK4A-dependent cell-cycle arrest, so the authors investigated the requirement for genetic lesions that induce transformation in Leiden cells, in which INK4A is specifically deleted. They had previously shown that immortalizing these cells with the telomerase subunit TERT and introducing the oncogene *HRAS* (LTR cells) allowed them to grow as anchorage-independent colonies. They now extended these observations by expressing, in LT cells, either MYC alone (LTM) or MYC and RAS (LTRM). These could proliferate in 0.2% agarose, so were also able to grow without anchorage. Western-blot analysis confirmed that members of the p53 pathway — ARF, p53 and WAF1 (also known as p21) — were expressed, so the cells could overcome the p53 checkpoint.

So, LTR, LTM and LTRM cells could all form anchorage-independent colonies, but were they fully transformed? Only the LTRM cells had a transformed phenotype — they were smaller and more rounded — and resulted in tumours on subcutaneous injection into nude mice. However, as only 5/16 innoculations gave rise to tumours, and the latency was relatively long — 59–98 days — another alteration might have occurred.

The phenotype of the tumour cells was further analysed by re-plating them into tissue culture. They had reduced adhesion and produced autocrine growth factors — they could proliferate in low serum. The tumours also had high levels of p53 and its targets, MDM2 and WAF1.

TUMORIGENESIS

A bad influence

In groups of friends that have become set in their ways, it often takes the influence of an outsider to shake things up. In a similar way, signals from the environment are required in colon cancer to stimulate cells to become invasive. In a new study, Ancy Leroy and colleagues now provide evidence that gut bacteria are one such outside influence that contributes to triggering invasiveness in colon tumour cells.

Leroy and co-workers found that incubating human colon cancer cells with *Listeria monocytogenes*, a gut pathogen, increased their ability to invade a collagen gel. This increased invasiveness was due to the production of a soluble factor, as the same effect was produced by incubating cells with filtered supernatant obtained from bacterial cultures. The ability of bacteria to produce a pro-invasive factor is not restricted to *L. monocytogenes*, as incubation of colon cancer cells with conditioned media from cultures of *Escherichia coli*, *Salmonella typhimurium* and bacteria that were isolated from colon tumour biopsies also had a stimulatory effect on invasiveness.

To identify the pro-invasive factor that is producd by *L. monocytogenes*, Leroy and colleagues isolated fractions of the growth-culture supernatant that retained the ability to stimulate invasiveness. This led to the identification of a 13-mer peptide with pro-invasive properties that corresponded to a region of the bovine β-casein protein — a principal component of milk products. The authors found that a bacterial metalloprotease, Mp1, is required for the production of this pro-invasive factor from larger β-casein-derived peptides. In addition to this bacterial enzyme, the production of the pro-invasive peptide was also shown to be dependent on a serine protease that is associated with the type I collagen gel used for the invasion assay.

To provide evidence that all the factors that are required for this process are present *in vivo*, the authors incubated tumour biopsies from patients with colon cancer with a 33-mer β-casein peptide containing the smaller pro-invasive factor. In the absence of a collagen gel or experimentally added bacteria, the colon cancer tissue was able to generate the 13-mer peptide. Addition of an antibiotic to the sample, however, abolished the production of the pro-invasive factor. This indicates that both the host serine protease and the bacteria that are required for the cleavage of β-casein peptides are present in the guts of patients with colon cancer.

Leroy and colleagues also studied the mechanism by which the pro-invasive peptide stimulates increased motility in colon tumour cells, and found that expression of a dominant-negative form of CDC42 or a constitutively active RHOA protein abolished invasion in response to the β-casein 13-mer. This indicates that the pro-invasive peptide that is produced by the combined efforts of the bacterial and host enzymes stimulates increased motility by modulating the activities of RHO-family GTPases.

The link between gut microflora and the development of colon cancer has been established for some time, but the precise roles of microorganisms in this disease are poorly understood. This study indicates a mechanism by which gut bacteria trigger invasiveness of tumour cells and provides a new insight into the way that bacteria and dietary factors influence the progression of human colon cancer.

Louisa Flintoft

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As the tumour cells had maintained a functional p53 pathway, might they also have avoided the aneuploidy that frequently characterizes tumour cells? A combination of multiplex fluorescence *in situ* hybridization and comparative genomic hybridization revealed that although the cells were normally diploid, two changes — on chromosomes 18 and 20 — were frequently observed.

Although these results leave us with some unanswered questions, they do provide a more physiological starting point for examining the mechanisms and consequences of transformation, and highlight some differences between the processes that occur in mouse and human cells.

Emma Greenwood

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WEB SITE

Gordon Peter's lab: http://science.cancerresearchuk.org/research/loc/london/lifch/petersg/

GLIOBLASTOMA

Cooperation is the key

The *RAS* and *AKT* oncogenes are frequently upregulated in glioblastomas, and experiments in glial progenitor cells indicate that they cooperate to induce glioblastoma formation. But what process do these two signalling pathways regulate? In the October issue of *Molecular Cell*, Eric Holland and colleagues show that, more than affecting transcription, these oncogenes cooperate to increase the translation of specific messenger RNAs that encode proteins important for cancer development.

Holland and colleagues used the RCAS/tv-a system to infect mouse glial progenitor cells with either constitutively active Kras, constitutively active Akt, or both. Kras activated Erk, which in turn activated the translation initiation factor eIF4E, whereas Akt activated TOR, which inhibits the eIF4E inhibitor 4E-BP and activates S6 ribosomal protein (S6RP) — also important in translation initiation. Interestingly, Kras increased the ability of Akt to inhibit 4E-BP and to activate S6RP, but the mechanism is unknown at present. The same pathways are activated in human glioblastoma cell lines, which confirms the relevance of this model.

So, these results confirm a causal link between the combined activity of Ras and Akt, and translation. They might act by differentially altering the translational efficiency of specific mRNAs, and the authors investigated this hypothesis by comparing total mRNA levels with that of polysomal mRNA when Ras or Akt were inhibited. Blocking either the Ras pathway with the Mek inhibitor U0126, or the Akt pathway with the PI3K inhibitor LY294002 and the TOR inhibitor rapamycin for two hours, had very little effect on total mRNA levels, as measured on a 12,488 gene array. Using a threefold change in levels as a cut-off, inhibiting the Ras pathway altered the expression of 12 genes and inhibiting the Akt pathway altered the expression of four genes. However, these genes were involved in cancer-relevant pathways, so should contribute to tumour development. By contrast, hundreds of mRNAs were lost from the polysome fraction when either Ras or Akt were inhibited for the same amount of time.

To obtain an unbiased profile of how Akt and Ras affect the generation of polysomal mRNA, the authors generated a normalized polysomal mRNA by comparing total and polysomal mRNA from seven different cell types — active Ras and Akt; active Ras; active Akt; active Ras and Akt with Ras pharmacologically inhibited; active Ras and Akt with Akt pharmacologically inhibited; and two controls. A refined analysis of these, using strict selection criteria, revealed that 219 mRNAs were associated more with polysomes when both Akt and Ras were activated. Less strict selection criteria resulted in 324 mRNAs and, together, these form a 'union set' of 426 mRNAs. Many of these encode components of signalling pathways and other biological functions that are known to be important in tumorigenesis.

A final question was whether the association with polysomes actually reflected a change in protein synthesis. A comparison of the hybridization values from the array analysis of total and polysomal mRNA with metabolic radiolabelling to determine the synthesis of candidate genes confirmed that it did in most cases.

So, it seems that Ras and Akt cooperate to alter the rate of synthesis of specific mRNAs, and their oncogenic effects could, largely, be through translation, rather than transcription.

Emma Greenwood

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Eric Holland's lab: http://www.mskcc.org/prg/prg/bios/640.cfm