METASTASIS

Coming unstuck

Just as avoiding apoptosis is a crucial component of transformation, the ability to avoid anoikis — apoptosis resulting from lack of adhesion — seems to be required for metastasis. Researchers have only begun to understand this process at the molecular level, but a functional screen designed by Daniel Peeper and colleagues has identified the neurotrophic receptor TRKB as an important factor in both anoikis inhibition and metastasis.

To identify suppressors of anoikis, Peeper's group screened for the ability of rat intestinal epithelial cells transduced with a retroviral cDNA expression library to survive transfer from an adhesive culture to a non-adhesive one. This move resulted in the death of most cells, but surviving clones were considered to be resistant to anoikis. One of these clones was found to carry a cDNA encoding TRKB — a tyrosine kinase receptor that has well-known roles in preventing apoptosis in the developing mammalian nervous system.

The authors showed that overexpression of TRKB in the epithelial cells allowed them to proliferate as large spheroid aggregates in suspension. TRKB is the receptor for brain-derived neurotrophic factor (BDNF), and co-expression of TRKB and BDNF promoted further proliferation. The effects of TRKB seemed to be mediated through inhibition of apoptosis, as TRKB suppressed upregulation of the pro-apoptotic executioner caspase 3 when cells were removed from a substrate. Peeper and colleagues also showed that TRKB expression activated AKT and phosphatidylinositol 3-kinase — signalling molecules that have previously been shown to suppress anoikis.

Resistance to anoikis is an important aspect of metastasis, as tumour cells must acquire the ability to survive while floating in the lymph and blood circulation. TRKB and BDNF are overexpressed in certain human tumour types, including neuroblastoma, and are associated with aggressive behaviour and poor prognosis. *TRKB* has not yet, however, been clearly established as an oncogene. The authors found that when TRKB was overexpressed in non-malignant epithelial cells and then transplanted into nude mice, these cells rapidly formed tumours that metastasized throughout the body. The effect was even more marked with cells that overexpressed both TRKB and BDNF.

The authors propose that TRKB activation is an important event in transformation and the initiation of metastasis. Further experiments are required to determine the exact mechanism by which it allows cells to survive in the absence of substrate. TRK-family inactivating drugs are already under development, and similar screens could be used to identify other metastasis-associated genes.

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References and links

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TUMORIGENESIS

Dependence on independence

The dependence receptors, which include DCC (deleted in colorectal carcinoma), induce apoptosis when not bound by their ligands, such as netrin 1. DCC has been considered a tumour suppressor, but mice lacking one copy of *Dcc* are not predisposed to cancer, so its role is unclear. Patrick Mehlen and colleagues show that overexpressing netrin 1 contributes to colorectal tumorigenesis probably by inhibiting DCC-induced apoptosis, indicating that the tumour-suppressive capacity of DCC depends on the extracellular concentration of netrin 1.

As decreased DCC expression is particularly associated with colorectal carcinoma, Mehlen and colleagues looked at the expression patterns of netrin 1 and DCC in intestinal epithelium. Netrin 1 was found mainly at the base of the crypts of the intestinal villi and decreased in a gradient towards the distal end, whereas DCC was expressed throughout the intestinal epithelium. These observations fit with the proposal that the physiological role of DCC is to regulate cell survival. That is, apoptosis is not triggered by DCC bound by netrin 1 in the proximal part of the villi, which is a site of intense proliferation, but is triggered by DCC when not bound by netrin 1 in the distal part of the villi, which is a site of cell death. To test this, the authors used transgenic mice that overexpressed netrin 1 throughout the intestinal epithelium, and showed that apoptosis was inhibited by 50% compared with normal mice.

So, does netrin 1 regulate DCC-induced tumour suppression in intestinal epithelium? Seventeen percent of the mice overexpressing netrin 1 developed adenomas (early colorectal tumorigenic lesions), compared with none of the control mice, and 43% had focal or diffuse hyperplasia in the colon, compared with only 13% of control animals. The suppression of apoptosis by netrin 1 therefore increases tumorigenesis.

DCC loss is usually a late-stage event in colorectal cancer progression; so, to test whether netrin 1 influences tumour progression, Mehlen and colleagues crossed the mice overexpressing netrin 1 with mice expressing mutant adenomatous polyposis coli (APC). *APC* mutations are an early event in human colorectal tumorigenesis. The *APC*-mutant mice developed mainly low-grade adenomas, but the *APC*-mutant mice overexpressing netrin 1 had a much higher incidence of high-grade adenomas (40% versus 17%). Furthermore, half of these high-grade adenomas were further classified as adenocarcinomas because of the presence of foci of mucosal invasion.

The authors propose that induction of cell death by DCC due to absence of netrin 1 in a region of the gut exposed to repeated mechanical and chemical insults might limit the risk of transformation. Loss of DCC expression in tumours would then reduce cellular dependence on netrin 1 for survival. Further research will investigate the role of other netrin 1 dependence receptors, the UNC5H proteins, which are also expressed in the gut and are putative tumour suppressors.

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Patrick Mehlen's lab: http://www.cgmc.univ-lyon1.fr/EN/eq_mehlenEN.php