TUMOUR SUPPRESSORS

Conflict resolution

The activity of the peroxisome-proliferator-activated receptor (PPAR) family members has been implicated in various tumour types, although the evidence for one of these proteins — PPARβ/δ — has been contradictory. Raymond DuBois and colleagues have therefore used a mouse model of colorectal cancer, along with a Pparβ/δ selective agonist, to more clearly demonstrate the role of Pparβ/δ in tumour growth.

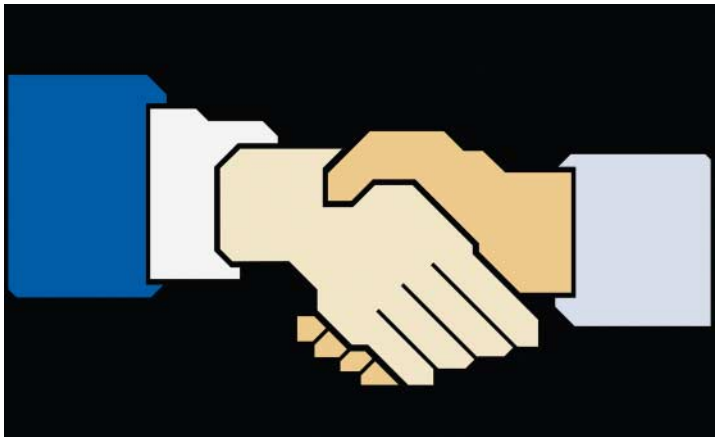
PPARs are a family of nuclear hormone receptors that function as ligand-activated transcription factors. PPARβ/δ is involved in development, wound healing, fatty-acid metabolism and repression of the inflammatory response. More importantly, the expression and activity of PPARβ/δ are increased after loss of the adenomatous polyposis coli (APC) tumour suppressor, which implicates it in the pathogenesis of colorectal cancer. To support this, a study showed that loss of both *PPARβ/δ* alleles from a colorectal cancer cell line slowed tumour growth. A separate study, however, reported that disruption of *Pparβ/δ* did not affect polyp formation in Apc^{min} mice — a model of intestinal polyposis that progresses to colorectal cancer, in which *Apc* is mutated. DuBois and colleagues therefore tested another approach to this problem — they treated Apc^{min} mice with the selective Pparβ/δ agonist GW501516.

Pparβ/δ is expressed primarily in the intestinal epithelial cells of both the normal intestinal epithelia and adenomas of Apc^{min} mice. Treating these mice with GW501516 led to a twofold increase in polyp number in the small intestine, but no change in the number of colon polyps. The mice that were treated with the Pparβ/δ agonist also showed a five-fold increase in polyps larger than 2 mm, so Pparβ/δ activation seems to affect the rate of polyp growth more than polyp formation. The polyps in these mice also had a slightly higher degree of dysplasia, indicating a more advanced stage of progression.

How does Pparβ/δ activation promote tumour growth? Although GW501516 had no effect on proliferation of colorectal cancer cells *in vitro*, it suppressed apoptosis in a dose-dependent manner. DuBois concluded that Pparβ/δ stimulates the growth and development of intestinal adenomas by activating anti-apoptotic pathways in intestinal epithelial cells.

This finding has important implications for the clinic, as activating ligands of PPARβ/δ (including GW501516) are in the later stages of development as drugs to treat dyslipidaemia syndromes, obesity and atherosclerosis. PPARβ/δ agonists should be administered with caution, as they might increase the risk of cancer in individuals with familial adenomatous polyposis — a disease in which *APC* mutations lead to a high incidence of colorectal cancer.

Kristine Novak



References and links

ORIGINAL RESEARCH PAPER Gupta, R. A. *et al*. Activation of nuclear hormone receptor peroxisome proliferator-activated receptor-δ accelerates intestinal adenoma growth. *Nature Med*. 1 Feb 2004 (doi:10.1038/nm993)

FURTHER READING Michalik, L., Desvergne, B. & Wahli, W. Peroxisome-proliferator-activated receptors and cancers: complex stories. *Nature Rev. Cancer* **4**, 61–70 (2004)

WEB SITE
Raymond DuBois' lab:
https://medschool.mc.vanderbilt.edu/facultydata/php_files/show_faculty.php?id3=760

WEB WATCH

COSMIC collection

- http://www.sanger.ac.uk/perl/CGP/cosmic

Somatic mutations in more than 260 genes have been identified from studies of human cancers, and a huge amount of data has been generated from this work. Rather than researchers spending hours carrying out literature searches or visiting various specialist databases, a single, comprehensive source of information on cancer-related somatic mutations would clearly be a good thing. In response to this need, the Cancer Genome Project, based at The Wellcome Trust Sanger Institute, launched a new web site on 4 February 2004. COSMIC (Catalogue of Somatic Mutations in Cancer) will bring these data together in one accessible, freely available resource.

COSMIC allows researchers to select their gene of interest and displays a map of where mutations occur in the amino-acid sequence. It also gives structural and functional information on protein domains and provides a list of samples containing each mutation, as well as a comprehensive list of publications for each altered site. Information can alternatively be accessed starting with tissue type, so that data are displayed for the genes that are mutated in each tissue. Data of interest can be exported in several useful formats, including text, HTML and Microsoft Excel spreadsheets.

The COSMIC web site will eventually contain data on all genes that are associated with human cancer. So far, details for four of these — *BRAF*, *HRAS*, *KRAS2* and *NRAS* — have been catalogued. This already provides data on 57,444 tumours and 10,647 mutations, giving an idea of the huge amount of information this project will eventually bring together.

Louisa Flintoft

THERAPEUTICS

Overcoming inhibition

Ever since resistance to apoptosis emerged as an influential pathway in cancer, targeting the mechanisms that allow tumours to avoid the same fate as normal cells has been proposed as a potent anticancer strategy. In *Cancer Cell*, John Reed and colleagues validate this principle by describing how small-molecule inhibitors that remove an important 'brake' in apoptosis can strip tumours of their immortality.

The ultimate effectors of programmed cell death are the caspase family of proteases. Normally, caspases are kept in check by members of the inhibitor of apoptosis (IAP) family, which bind to and inactivate caspases until they are needed. Caspases are overexpressed in tumours, but so are IAPs, and, therefore, failure to activate caspases could create resistance to apoptosis.

So, Reed and colleagues screened a library of around one million compounds for binding to one of the best characterized of the IAPs: XIAP. XIAP inhibits apoptosis at a distal step in the apoptosis pathway — at the convergence of cell-death pathways that are activated by mitochondria-dependent and mitochondria-independent stimuli.

Eight polyphenylurea-based compounds were identified that bind to the BIR2 domain of XIAP

TUMORIGENESIS

Nuisance neighbours

Disruptive neighbours can cause turmoil in any community and, in cancer, abnormal changes in one cell type can lead to tumorigenesis in other nearby cells. Reporting in *Science*, Harold Moses and colleagues now describe a new mechanism for this, showing that loss of transforming growth factor-β (TGF-β) signalling in stromal fibroblasts leads to oncogenic changes in adjacent epithelial cells.

It is well known that disrupting the normal interactions between epithelial cells and fibroblasts in the underlying stroma can lead to tumorigenesis, but the signalling pathways that are involved in this are poorly understood. To investigate a potential role of Tgf-β signalling in stroma–epithelium interactions, Moses and co-workers made transgenic mice in which the gene encoding the Tgf-β type II receptor (Tgf-βRII) — a crucial component of Tgf-β signalling — is specifically inactivated in fibroblasts (*Tgfbr2*^{fspKO} mice). Increased proliferation of both fibroblasts and epithelial cells was seen in prostate tissue from these mice, with prostate epithelial cells also showing neoplastic characteristics. In the forestomach, even more marked effects on epithelial cells were seen, with invasive squamous-cell carcinomas developing in 100% of *Tgfbr2*^{fspKO} mice.

But how does loss of Tgf-β signalling in fibroblasts trigger tumorigenesis in adjacent epithelia? Hepatocyte growth factor (Hgf) is one target of Tgf-β signalling, so Moses and colleagues analysed Hgf signalling in *Tgfbr2*^{fspKO} animals. Cultured fibroblasts from *Tgfbr2*^{fspKO} mice were found to secrete three times more of the active form of Hgf than cells from control mice. Furthermore, increased levels of the phosphorylated, active form of the Hgf receptor — c-Met — were seen in prostate and forestomach epithelial cells from *Tgfbr2*^{fspKO} mice.

The authors also showed increased expression of the tumour promoter c-Myc and decreased levels of the cyclin-dependent kinase inhibitors Waf1 and Kip1 in prostate and forestomach tissue from *Tgfbr2*^{fspKO} mice. This indicates that when Tgf-β signalling is disrupted in fibroblasts, increased Hgf signalling in neighbouring cells might lead to changes in the

— which is responsible for the inactivation of caspase-3 and caspase-7 — and reversed caspase inhibition. (XIAP also suppresses an upstream initiator caspase-9 through the BIR3 region, but the authors decided to target a more downstream mechanism.)

The most active of these compounds induced apoptosis in a range of tumour cell lines and primary leukaemia cells *in vitro*, but showed little toxicity in normal cells. These compounds also sensitized tumour cells to the anticancer treatments etoposide, doxorubicin and paclitaxel. Inactive structural analogues had no effect on these tumour cells.

The induction of cell death by the XIAP antagonists was blocked by the universal caspase inhibitor zVAD-fmk and was reduced by overexpressing XIAP. Cell death was unaffected, however, by overexpression of the upstream apoptosis suppressors BCL-X_L and CRMA, which shows that targeting such a distal point in the apoptosis pathway bypasses many upstream defects in apoptosis-regulatory mechanisms in tumours. Delivered at modest doses, the XIAP antagonists also suppressed growth of established tumours in mouse xenograft models, with little toxicity to normal cells.

The results indicate that tumours have an intrinsic drive to activate caspases, and that inhibition of IAPs allows apoptosis to occur in tumours with little or a lesser effect in normal cells. The compatibility of XIAP antagonists with established anticancer drugs, and their ability to suppress tumour growth *in vivo*, provides a rationale to investigate pharmacokinetic and toxicological profiles for these compounds as single agents or as combined therapy.

Simon Frantz, Associate Editor (News),
Nature Reviews Drug Discovery

References and links

ORIGINAL RESEARCH PAPER Schimmer, A. D. *et al.* Small-molecule antagonists of apoptosis suppressor XIAP exhibit broad antitumor activity. *Cancer Cell* **5**, 25–35 (2004)
FURTHER READING Reed, J. C. Apoptosis-based therapies. *Nature Rev. Drug Discov.* **1**, 111–121 (2002) | Salvesen, G. S. & Duckett, C. S. IAP proteins: blocking the road to death's door. *Nature Rev. Mol. Cell Biol.* **3**, 401–410 (2002)

expression of proteins such as c-Myc, Waf1 and Kip1 that lead to uncontrolled proliferation. In support of this, the overexpression of c-Myc was shown to co-localize with that of active c-Met in epithelial cells.

So, it seems that in addition to its well-known influence on tumorigenic processes in cells in which it acts directly, Tgf-β also functions as an indirect suppressor of epithelial tumorigenesis through its effects on neighbouring fibroblasts. The question of why the effects of loss of Tgf-β signalling were seen in the prostate and forestomach, but not in other organs, remains to be investigated.

Louisa Flintoft

References and links

ORIGINAL RESEARCH PAPER Bhowmick, N. A. *et al.* TGF-β signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. *Science* **303**, 848–851 (2004)
FURTHER READING Siegel, P. M. & Massagué, J. Cytostatic and apoptotic actions of TGF-β in homeostasis and cancer. *Nature Rev. Cancer* **3**, 807–820 (2003)

IN BRIEF

EARLY DETECTION

Visualization of tumors and metastases in live animals with bacteria and vaccinia virus encoding light-emitting proteins.

Yu, Y. A. *et al. Nature Biotechnol.* 8 Feb 2004 (doi:10.1038/nbt941)

Yu *et al.* show that microorganisms can preferentially survive and replicate in tumours. Bacteria and vaccinia virus engineered to express green fluorescent protein were visualized by real-time imaging in tumour-bearing rodents. Two days after injection, light emission was only observed in tumours and metastases, and after 45 days was still present in the primary tumour. So, microorganisms might be useful for cancer detection and treatment.

METASTASIS

Treatment of terminal peritoneal carcinomatosis by a transducible p53-activating peptide.

Snyder, E. L., Meade, B. R., Saenz, C. C. & Dowdy, S. F. *PLoS Biol.* **2**, 1–8 (2004)

Metastatic disease is difficult to treat. An alternative to gene therapy is systemic delivery of tumour suppressors. This approach is also limited, however, as the large proteins cannot cross the plasma membrane. Snyder *et al.* have delivered a p53-activating peptide to mice with terminal metastatic disease using peptides containing a protein transduction domain. p53 was activated in cancer cells, but not normal cells, and resulted in increased lifespan and disease-free animals.

THERAPEUTICS

Application of gene expression-based high-throughput screening (GE-HTS) to leukemia differentiation.

Stegmaier, K. *et al. Nature Genet.* 8 Feb 2004 (doi:10.1038/ng1305)

Stegmaier *et al.* developed GE-HTS as a cell-based approach to screen chemical libraries for compounds that regulate biological processes. They used GE-HTS to identify compounds that cause differentiation of acute myeloid leukeamia (AML) cells. Of the 1,739 compounds screened, 8 induced the GE-HTS AML differentiation signature and could induce at least one hallmark of differentiation assayed by conventional methods. This approach will be useful for dissecting the mechanisms that regulate AML differentiation.

IMMUNOTHERAPY

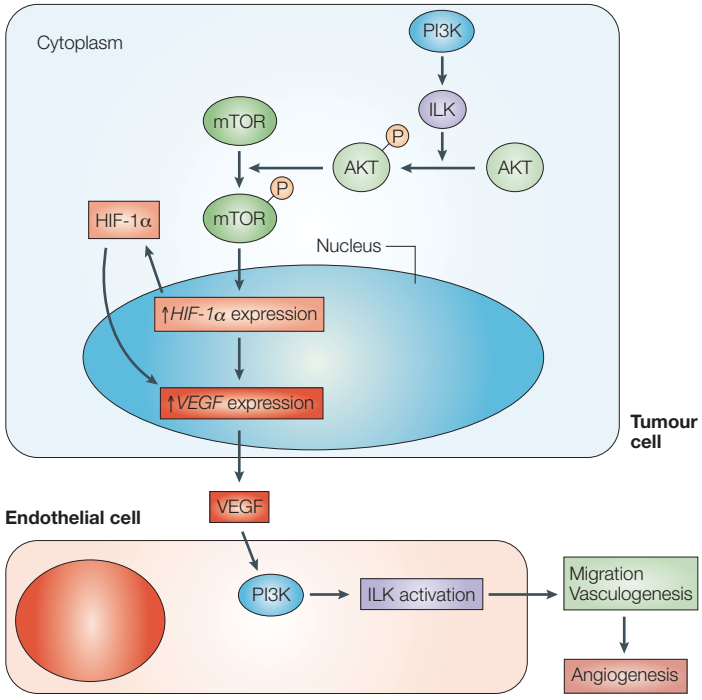
High vaccination efficiency of low-affinity epitopes in antitumor immunotherapy.

Gross, D.-A. *et al. J. Clin. Invest.* **113**, 425–433 (2004)

Cancer vaccines should help the immune system to recognize tumour cells. But, most tumour-associated antigens — which are used to make the vaccines — are also expressed on normal cells and cause autoimmunity to develop. Gross *et al.* report that low-affinity epitopes of TERT, a protein preferentially expressed in cancer cells, induce tumour immunity without causing autoimmunity. So, selection of low-affinity epitopes might overcome the problems associated with existing cancer vaccines.

ANGIOGENESIS

Going for the double



Angiogenesis inhibitors have great potential as anticancer therapies, but have so far given disappointing results in clinical trials. Reporting in *Cancer Cell*, Shoukat Dedhar and colleagues reveal that integrin-linked kinase (ILK) has two key roles in tumour angiogenesis, and is therefore a promising new target for anti-angiogenic therapies.

Vascular endothelial growth factor (VEGF) is crucial for tumour angiogenesis, as it is produced by tumour cells and promotes the proliferation and migration of endothelial cells. VEGF expression can be stimulated by the phosphatidylinositol-3-kinase (PI3K) pathway. PI3K stimulates the phosphorylation and activity of AKT, which, in turn, increases the synthesis of transcription factors such as hypoxia-induced factor-1α (HIF-1α), to upregulate VEGF expression.

As ILK is directly upstream of AKT in the PI3K pathway, Dedhar and colleagues investigated whether it is also required for tumour angiogenesis. Overexpression of Ilk in rat intestinal epithelial cells led to increased Vegf expression compared with control cells, and to increases in the levels of phosphorylated Akt and Hif-1α-mediated transcription. Conversely, a small-interfering RNA (siRNA) directed against *Ilk* suppressed Vegf expression. The authors also showed ILK is required for high levels of VEGF expression in the PC3 human prostate cancer cell line, and went on to show that this involves the activation of mTOR, a target of AKT. So, ILK has a key role in the ability of tumour cells to stimulate VEGF expression through its effects on AKT activation (see figure).

To test the potential of ILK inhibition as an antiangiogenic therapy, the authors exposed PC3 cells and DU145 cells — another prostate cancer cell line — to an ILK inhibitor. This suppressed both VEGF and HIF-1α expression in a dose-dependent manner.

As ILK is known to promote cell motility in response to growth factors, Dedhar and colleagues tested whether ILK might also function in endothelial cells in VEGF-mediated migration and vasculogenesis. Human umbilical-vein endothelial

METASTASIS

Migratory cues

Like birds flying south for the winter, cancer cells can migrate from the site of the primary tumour and disseminate around the body. But what signals turn a non-invasive cancer cell into a metastatic cell? Using a unique *in vitro* system that models the early stages of breast cancer, Joan Brugge and colleagues show that ERBB2 and transforming growth factor-β (TGF-β) work together to cause invasion and migration of breast cancer cells.

Under certain culture conditions, MCF10A cells that are engineered to express activated ERBB2 (10A.B2 cells) produce structures that share properties with ductal carcinoma *in situ* (DCIS), an early-stage, non-invasive breast cancer. So, 10A.B2 cells were infected with retroviral vectors expressing breast-cancer-associated cDNAs to identify factors that could promote metastasis. Only TGF-β1 and TGF-β3 increased migration of the activated 10A.B2 cells, indicating that ERBB2 and TGF-β might cooperate to induce migration. Soluble TGF-β had a similar effect, although to a lesser extent, indicating that autocrine TGF-β stimulation is particularly important. Furthermore, this induction of migration seems to be dependent on ERBB2, as TGF-β1 and TGF-β3 had no effect on MCF10A cells expressing the activated epidermal growth factor receptor ERBB1.

Various assays confirmed that TGF-β1 and TGF-β3 expression induced migration and invasion of activated 10A.B2 cells. As the extracellular signal-regulated kinase ERK is implicated in migration and is activated by ERBB2 and TGF-β, the authors investigated ERK activity in the TGF-β-expressing 10A.B2 cells. Phosphorylation of both ERK and the ERK kinase MEK was increased in these cells compared with controls, indicating that TGF-β and ERBB2 increase ERK activation. This was investigated further by expressing TGF-β and activated MEK in the absence of activated ERBB2; migration and invasion were substantially increased, compared with controls expressing activated MEK alone. So, MEK activation is insufficient to induce migration and invasion, but it can substitute for ERBB2 in the presence of TGF-β. MEK inhibitors reduced migration of TGF-β-expressing 10A.B2 cells by 85% and invasion by 65%, confirming that ERK activation is required for the synergism between TGF-β and ERBB2.

How might ERBB2 and TGF-β induce this migration? Cultured medium from activated TGF-β-expressing 10A.B2 cells induced migration of MCF10A cells, but mixing cultured medium from cells expressing either TGF-β or activated ERBB2 alone did not enhance migration, indicating that TGF-β and ERBB2 must cooperate to produce soluble migratory factors. ERBB1-specific antibodies reduced the stimulating activity of the cultured medium by 50–60%, indicating that other pathways contribute to migratory activity.

As about 80% of DCIS lesions express ERBB2, this work indicates that TGF-β expression could represent one step in the process that turns a non-invasive tumour into metastatic breast cancer.

Emma Croager

References and links

ORIGINAL RESEARCH PAPER Seton-Rogers, S. E. *et al.* Cooperation of the ErbB2 receptor and transforming growth factor β in induction of migration and invasion in mammary epithelial cells. *Proc. Natl Acad. Sci. USA* **101**, 1257–1262 (2004)

WEB SITE

Joan Brugge's lab:
http://cellbio.med.harvard.edu/faculty/brugge/

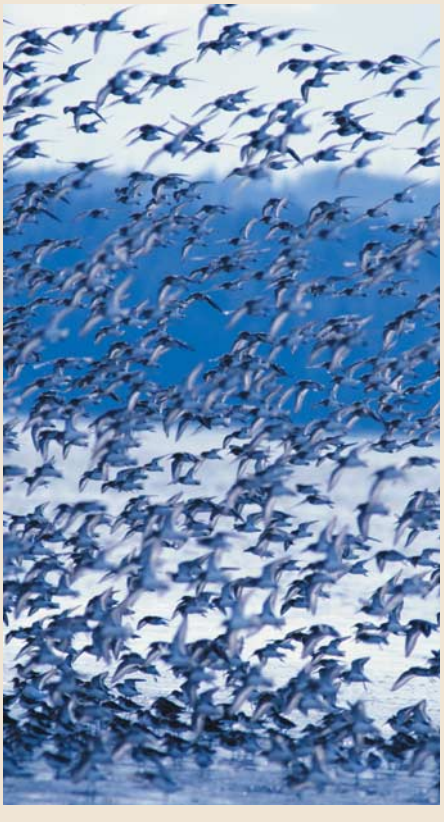
cells (HUVECs) that were exposed to VEGF showed increased ILK activity. This was dependent on PI3K, as this effect was blocked by the addition of a PI3K inhibitor. Furthermore, inhibition of ILK function suppressed the migration and proliferation of HUVECs in response to VEGF. The authors also showed that ILK inhibitors block VEGF-stimulated angiogenesis in two standard assays.

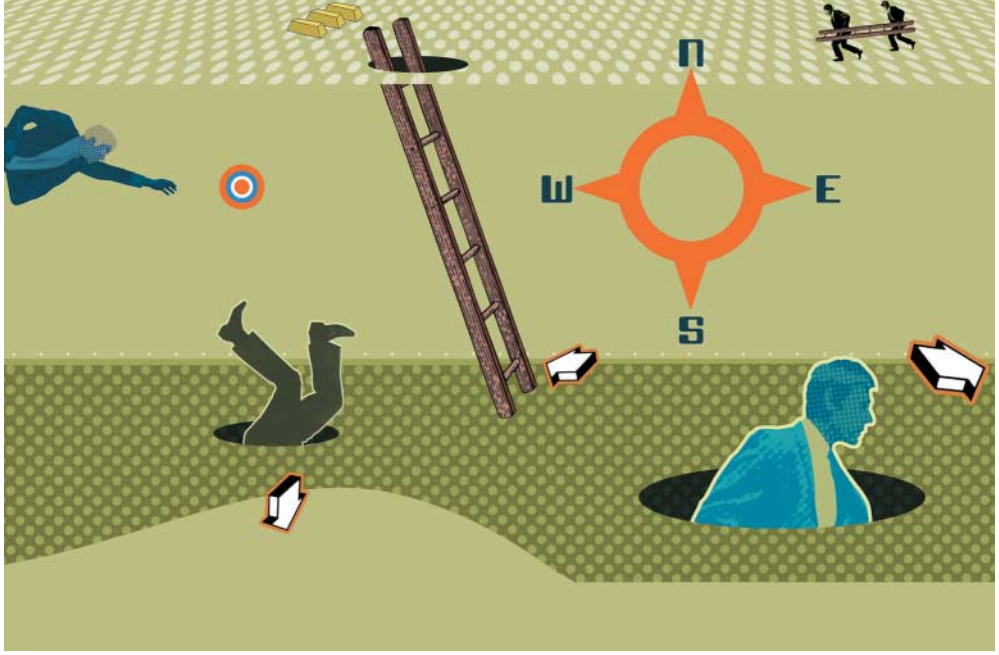
A mouse xenograft model was used to test the effects of ILK inhibitors *in vivo*. Nude mice were injected with PC3 cells, and animals bearing well-established tumours were treated with an ILK inhibitor. Treated mice showed a reduced density of tumour-associated blood vessels and a decrease in tumour mass, as compared with untreated mice, and did not show any obvious side-effects. These results indicate that ILK, with its dual role in tumour angiogenesis, might prove to be a useful target for anticancer therapies.

Louisa Flintoft

References and links

ORIGINAL RESEARCH PAPER Tan, C. *et al.* Regulation of tumor angiogenesis by integrin-linked kinase (ILK). *Cancer Cell* **5**, 79–90 (2004)





TUMOUR SUPPRESSORS

A topsy-turvy world

Tumour suppressors normally inhibit cell proliferation, but one seems to act in the opposite manner. Chun-Ming Chen and Richard Behringer report in the February issue of *Genes & Development* that it is the loss of OVCA1 that causes proliferation defects — only when its loss is combined with that of p53 is its tumour-suppressive activity unleashed.

The genes that cause ovarian cancer are largely unknown, so the identification of a region of chromosome 17 that is frequently lost was an important find. The *OVCA1* gene has since been shown to reside at that location, but it has yet to be shown to act as a tumour suppressor. The authors had previously identified the mouse orthologue, and continued their studies to understand the function of Ovca1.

They generated knockout mice, but these either died during development or shortly after birth. The embryos showed developmental defects and were generally smaller. Transplanting the ovaries into kidney capsules of wild-type mice allowed their development to be followed over time, and although smaller, development seemed normal and there was no evidence of carcinomas.

The growth defect could be caused by a decrease in proliferation or an increase in apoptosis, so *Ovca1*-null mouse embryonic fibroblasts (MEFs) were established to answer this question. The MEFs grew poorly, but there was no sub-G1 population, which would be indicative of apoptosis. Cell-cycle analysis revealed that fewer cells were in S phase, and this corresponded with a decrease in phosphorylated Rb, which would explain the inability of the cells to enter S phase.

But this is an unusual property for loss of a tumour suppressor, so might elimination of a checkpoint allow the cells to recover from this proliferation defect? The authors found that *Ovca1*$^{-/-}$*Trp53*$^{+/-}$ MEFs also had a reduced S-phase population, but *Ovca1*$^{-/-}$*Trp53*$^{-/-}$ MEFs grew normally. Loss of p53 could not rescue the developmental defects though — the mice still died soon after birth.

The next important question was whether Ovca1 actually did act as a tumour suppressor *in vivo*. Almost 60% of *Ovca1*$^{+/-}$ mice developed a range of tumours by two years, with an average latency of 92 weeks. This was shortened to 52 weeks in *Ovca1*$^{+/-}$*Trp53*$^{+/-}$ mice, and the incidence was increased to 72%. Importantly, the loss of one *Ovca1* allele increases the tumour incidence when compared with *Trp53*$^{+/-}$ mice, and although the tumour incidence in *Ovca1*$^{+/-}$*Trp53*$^{-/-}$ and *Trp53*$^{-/-}$ mice is the same, more mice have numerous tumours when they have lost one *Ovca1* allele. The tumour spectrum is also somewhat different in the *Ovca1* heterozygous mice.

So, despite its role as a positive regulator of cell-cycle progression, it seems that OVCA1 is, indeed, a tumour suppressor. Further work is needed to understand exactly how it functions in normal cells, and how its loss accelerates tumour progression when p53 is also lost.

Emma Greenwood

References and links

ORIGINAL RESEARCH PAPER Chen, C.-M. & Behringer, R. R. Ovca1 regulates cell proliferation, embryonic development, and tumorigenesis. *Genes Dev.* **18**, 320–332 (2004)

FURTHER READING Jensen, M. R. & Helin, K. OVCA1: emerging as a *bona fide* tumor suppressor. *Genes Dev.* **18**, 245–248 (2004)

WEB SITE

Richard Behringer's lab:
http://www3.mdanderson.org/~genedev/behringer.html

IN THE NEWS

Deodorant debate rages on Public concern has once again been raised over the link between underarm deodorants and cancer, after a recent report that frequent underarm shaving combined with deodorant use could increase the risk of breast cancer.

The study, which was carried out by Kris McGrath from Northwestern University, USA, surveyed the underarm-hygiene habits of 437 women with breast cancer. Women who shaved their underarms more than twice a week and applied deodorant more than once a week were almost 15 years younger when they were diagnosed with breast cancer than those who used neither regimen. Consistent with previous studies, McGrath found no link with a younger age of breast cancer diagnosis when either shaving or deodorant was used alone.

This finding was published shortly after a report from Philippa Darbre, Reading University, UK, showed that traces of parabens — preservatives used in cosmetics, food and pharmaceutical products — in breast tumours. Although there is no proof that parabens cause cancer and most deodorants no longer contain these compounds, Darbre said "Their detection in human breast tumours is of concern since parabens have been shown to mimic the action of the female hormone oestrogen, [which] can drive the growth of human breast tumours" (*Reuters*, 12 January 2004).

Darbre is excited by McGrath's work and claims "It is a landmark publication because it provides the first epidemiological evidence for a link between the use of antiperspirants/deodorants and breast cancer development" (*NewScientist.com*, 24 January 2004). It is clear that more studies will be needed to resolve this controversial issue.

Emma Croager

MATHEMATICAL MODELS

Accelerating cancer understanding

So, cancer incidence increases with age…doesn't it? Although this longstanding assumption is mostly true, incidence in many tissues departs from the expected linear log–log plot. In a recent article from *Current Biology*, Steven Frank uses mathematical models to explore how clonal expansion and the number of cell lineages in a tissue might explain the departures from the standard plot that occur in breast and prostate cancer.

The typical log–log curves show the rate of cancer at different ages, and Frank began by plotting the slope of the rate curve at each point. This provides the age-specific acceleration of cancer — acceleration can decline even though the frequency of cases increases, because acceleration measures how fast the frequency of cases rises with age. The acceleration plots show that breast cancer acceleration is highest early in life, acceleration then steadily declines with age. By contrast, the acceleration of prostate cancer rises rapidly to a peak at ~40 years, and then drops just as rapidly through later life (see figure). Frank first developed a general set of equations to model that cancer arises when a certain number of rate-limiting mutations occur within a cell lineage — this standard multistage model was thought to give a constant acceleration throughout life — he then attempted to explain the observed deviations from expected constant acceleration.

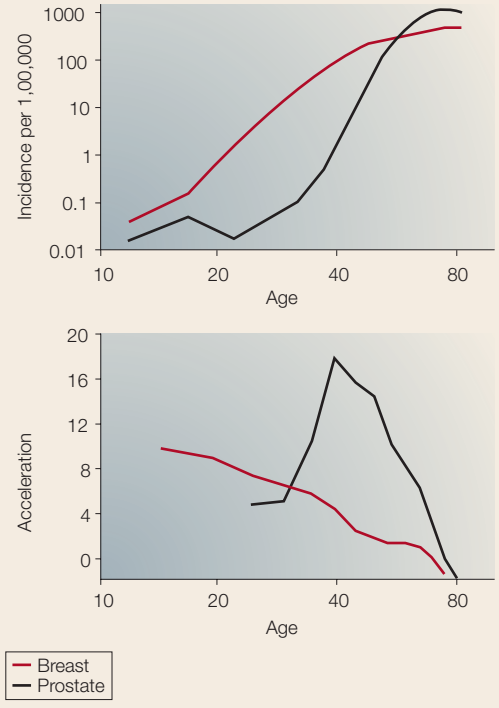


Figure modified, with permission, from Frank, S. © (2004) Cell Press.

He hypothesized that the decline in acceleration at later ages could be caused by the fact that individual cell lineages accumulate mutations, so that they need fewer steps to become tumorigenic. The number of lineages that are present in a tissue would affect this. If there were more lineages, only a few would become transformed, so most would have ~0 mutations; however, if there were fewer lineages, more of these would have to undergo some of the steps towards cancer in order for the total incidence to be the same. The number of mutations in each lineage would therefore be higher, and there would be a corresponding decrease in acceleration. This mimics the situation in breast cancer, which could be explained by the tissue having fewer lineages either because there are fewer stem cells than in other tissues or because precancerous lineages frequently expand at the expense of neighbouring lineages.

The clonal expansion of a cell population could also influence the acceleration of cancer. For example, if the rate of expansion is slow, the rate at which a lineage acquires the next rate-limiting mutation would accelerate slowly over time, causing a peak of acceleration in midlife. The more rapid clonal expansion, the earlier the peak in acceleration. Also, as the size of the clone increases, the peak of acceleration increases, but only to a certain extent. When the number of cells reaches a certain size, the probability that a mutation will occur after a short time is so high that further clonal expansion can not further increase the rate. Finally, the number of rounds of clonal expansion could also affect the acceleration of cancer. When three rounds of clonal expansion occur — because different mutations cause waves of proliferation — the peak acceleration is greatly increased, and this could explain the high acceleration of cancer in midlife that is observed in prostate cancer.

So, Steven Frank goes beyond mere descriptions of known processes here. He investigates the observation that the age-specific acceleration of cancer varies according to the tissue type of origin and provides models to explain these. Although these are not the only possible solutions, he suggests specific experiments that could be used to test these hypotheses and suggests alternatives that could also be considered. This type of instructive use of mathematical models should aid the understanding of other cancer processes.

Emma Greenwood

References and links

ORIGINAL RESEARCH PAPER Frank, S. Age-specific acceleration of cancer. *Curr. Biol.* **14**, 242–246 (2004)
WEB SITE Steven A. Frank's home page: http://stevefrank.org/
FURTHER READING Michor, F., Iwasa, Y. & Nowak, M. A. Dynamics of cancer progression. *Nature Rev. Cancer* **4**, 197–205 (2004).

PHARMACOGENETICS

Small change, large effect

Single-nucleotide polymorphisms might be useful in predicting the efficacy of anticancer therapy, but evidence of exactly how these small changes in DNA can have such important consequences is lacking. Kyoung-Jin Sohn and colleagues have focused on the C667T polymorphism in methylenetetrahydrofolate reductase (*MTHFR*), which is present in about 35% of the North-American population. They have discovered that the altered enzyme affects the concentration and distribution of folates in cancer cells, which, in turn, alters the chemosensitivity to antifolate drugs.

MTHFR catalyses the conversion of 5,10-methylene THF to 5-methyl THF. The 667T variant of *MTHFR* is less active, so 5,10-methylene THF accumulates. The authors tested whether the polymorphism altered the effectiveness of the antifolate drugs 5-fluorouracil (5-FU) and methotrexate (MTX) — which are used widely to treat breast and colon cancer — and correlated with their known mechanisms of action.

The authors took a colon cancer and a breast cancer cell line with endogenous 667C *MTHFR* and transfected them with the 667T variant. Enzyme activity was 35% lower in the 667T *MTHFR* cells and the intracellular distribution of folates had also changed: levels of 5-methyl THF were about 12% lower and 5,10-methylene THF levels were about 10% higher. These cells grew faster than 667C variant cells, probably because 5,10-methylene THF is required for DNA synthesis.

So, how sensitive are the 667T variant *MTHFR* cells to antifolate drugs? The authors postulated that the effect of 5-FU, which acts by forming an inhibitory complex with thymidylate synthase and 5,10-methylene THF, would be increased by the presence of the polymorphism in cancer cells, and this was the case both *in vitro* and in xenograft models. By contrast, the authors thought that the effect of MTX, which acts by decreasing levels of 5,10-methylene THF, might be compromised by the polymorphism, and this also proved true for the breast cancer cell line. There was no difference in chemosensitivity to MTX in the 667T variant colon cancer cells, but as colon cancer does not usually respond to MTX, this was not that surprising. Interestingly, if MTHFR expression and activity was more completely inhibited by transfecting with antisense to *MTHFR*, the colon cancer cells were more resistant to MTX.

These data provide evidence of a functional consequence of the C677T *MTHFR* polymorphism in response to chemotherapy and support the view that this polymorphism might be a useful pharmacogenetic marker for providing tailored chemotherapy to patients with cancer.

Ezzie Hutchinson

References and links

ORIGINAL RESEARCH PAPER Sohn, K.-J. *et al.* Effect of the methylenetetrahydrofolate reductase C667T polymorphism on chemosensitivity of colon and breast cancer cells to 5-fluorouracil and methotrexate. *J. Natl Cancer Inst.* **96**, 134–144 (2004)

FURTHER READING Ulrich, C. M., Robien, K. & McLeod, H. L. Cancer pharmacogenetics: polymorphisms, pathways and beyond. *Nature Rev. Cancer* **3**, 912–920 (2003)

WEB SITE

Young-In Kim's lab: http://www.utoronto.ca/nutrisci/faculty/kim.html

TRIAL WATCH

Debugged

Chronic *Helicobacter pylori* infection is associated with gastric cancer, but could its eradication in chronic carriers prevent gastric cancer? Wong *et al.* performed the first prospective, randomized, placebo-controlled, population-based study to determine whether *H. pylori* eradication in a high-risk population reduced the incidence of gastric cancer.

The study was performed in a high-risk region of southern China and involved 1,630 carriers of *H. pylori* infection — 988 of whom did not have any precancerous lesions, as determined by endoscopic evaluation. *H. pylori* was eradicated in the participants through combination treatment with omeprazole and antibiotics. After a follow-up of 7.5 years, the authors report that the incidence of gastric cancer was similar among participants who received *H. pylori* eradication treatment and those who received placebo (7 and 11 cases, respectively). In those carriers who did not have precancerous lesions, however, eradication of *H. pylori* seemed to prevent the development of gastric cancer — none of the treated patients developed gastric cancer, whereas six in the placebo group did.

Although *H. pylori* eradication seems to be effective in preventing gastric cancer in high-risk populations, further studies are required to determine the effects in low-risk populations, and also to determine the long-term effects of antibiotic treatment in patients with precancerous lesions.

ORIGINAL RESEARCH PAPER Chun-Yu Wong, B. *et al. Helicobacter pylori* eradication to prevent gastric cancer in a high-risk region of China — a randomized controlled trial. *JAMA* **291**, 187–194 (2004)

Insufficient evidence

It is a common belief that participating in clinical trials leads to a better outcome for patients with cancer, regardless of whether they are assigned to the control or experimental group of the study. But, researchers at the Dana–Farber Cancer Institute have found little evidence to support this 'trial effect' and advocate that it should no longer be emphasized as an incentive for patient recruitment.

Peppercorn *et al.* systematically looked for studies that presented primary data comparing the outcome of patients with cancer, within and outside clinical trials. The identified studies were critically evaluated for evidence of a trial effect using a conceptual framework designed by the authors. Only 14 of the 26 identified studies showed evidence of improved outcome for trial participants, but most were unreliable, as they did not effectively control for study bias such as co-morbidity, socio-economic status and performance status. Eight studies compared trial participants with non-trial participants who met the criteria for trial selection, but only three showed improved outcome for trial participants. In addition, the studies showing improved outcome tended to involve children with cancer, patients treated before 1986 and patients with haematological malignancies.

This work indicates that there is insufficient evidence to confirm that the trial effect exists. Even though there might be no immediate benefit from participating in a clinical trial, it is important for trial participants to realize the benefit for future patients.

ORIGINAL RESEARCH PAPER Peppercorn, J. M., Weeks, J. C., Cook, E. F. & Joffe, S. Comparison of outcomes in cancer patients treated within and outside clinical trials: conceptual framework and structured review. *Lancet* **363**, 263–270 (2004)