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TUMOUR VIRUSES

A genetic switch

Kaposi's sarcoma is a malignant tumour that commonly arises in immunocompromised individuals as the result of infection with Kaposi's-sarcoma-associated herpesvirus (KSHV), but little is known about what cells it arises from or how it progresses. Two articles in the July issue of *Nature Genetics* use gene-expression profiling to show that KSHV activates expression of lymphatic-specific genes, indicating new therapeutic avenues.

Kaposi's sarcoma mainly affects cells of the skin, and most tumour cells express endothelial-cell markers. Chris Boshoff and colleagues used gene-expression analysis to compare the profiles of Kaposi's-sarcoma biopsy samples to normal skin cells. They found that Kaposi's-sarcoma cells expressed a large percentage of lymphatic endothelial cell (LEC)-associated genes, such as vascular endothelial growth factor receptor 3 (*VEGFR3*), angiopoietin 2 (*ANG2*), podoplanin and *CD206*. They also expressed some genes that were specific to blood-vessel endothelial cells (BECs). Similarly, Detmar's group used gene-expression analysis to show that KSHV infection of human dermal microvascular endothelial cells resulted in upregulation of 70% of lymphatic-lineage-specific genes.

So, which cells become infected with KSHV? Boshoff's group assayed viral infection and replication in several vascular cell types and found that LECs, followed by BECs, were the most susceptible to KSHV infection. They concluded that when the virus infects vascular endothelial cells, they become transcriptionally reprogrammed to express primarily lymphatic-vessel-specific genes, although some BEC-specific genes are also induced. The resulting change in cellular phenotype might help the virus to replicate more efficiently, exploiting LEC differentiation pathways to promote its own life cycle. When this differentiation goes awry, Kaposi's sarcoma develops. This is reminiscent of human papillomavirus (HPV)-induced cervical cancer, where HPV exploits squamous-cell differentiation to complete its life cycle.

What are the cellular targets of the virus that can cause this switch in gene expression? The homeobox gene *PROX1* is a master gene that controls lymphatic-vessel development and differentiation, and is specifically expressed by LECs. Detmar's group showed that KSHV infection resulted in an eight-fold upregulation of *PROX1*. Boshoff also observed that *PROX1* was moderately upregulated in Kaposi's-sarcoma cells, relative to normal skin.

So, KSHV seems to reprogramme endothelial-cell transcriptomes towards a primarily lymphatic phenotype. This is likely to be partially mediated by viral upregulation of *PROX1*, although both authors predict that other cellular or viral genes are also likely to be involved. As lymphangiogenic molecules such as ANG2 and VEGFD are upregulated in the plasma of patients with Kaposi's sarcoma, they might be good candidates for treatment with newly developed anti-lymphangiogenic therapies.

Kristine Novak

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ONCOGENES

Two of a kind

Cytoskeletal proteins regulate cell adhesion and motility, and some have even been shown to mediate cell survival. In the June issue of *Cancer Cell*, Rakesh Kumar and colleagues report the surprising finding that two cytoskeletal proteins, p21-activated kinase 1 (PAK1) and the dynein light chain 1 (DLC1), interact to promote the survival and tumorigenic potential of breast cancer cells.

Kumar and colleagues began their studies by looking for new substrates of PAKs. PAKs phosphorylate RHO GTPases to control cytoskeletal organization, and also phosphorylate and inactivate the pro-apoptotic protein BAD to promote survival. In a yeast two-hybrid screen of a mammary-gland cDNA library, they found that PAK1 interacts directly with, and also phosphorylates, DLC1 — a component of the dynein motor complex. DLC1 not only regulates the microtubule-dependent motor function of dynein, but has also been shown to bind and inhibit activity of the pro-apoptotic protein BimL.

So what happens when these two pro-survival signalling proteins get together? Kumar and colleagues expressed normal and mutant forms of the proteins in a breast cancer cell line, and showed that the interaction between PAK1 and DLC1 is required for cell-cycle progression and survival. Cells that overexpressed either PAK1 or DLC1 were able to undergo anchorage-independent growth, indicating a malignant phenotype, and also led to oestrogen-independent tumour growth when transplanted into nude mice, unlike control cells. A DLC mutant that lacked the PAK1 phosphorylation site did not show tumorigenic potential in mice. Therefore, PAK1 phosphorylation of DLC1 seems to be required for cell survival and tumour formation. Furthermore, DLC1 levels were increased in 90% of the human breast tumour samples that the authors analysed.

How does PAK1 activation of DLC1 inhibit apoptosis and promote tumorigenesis? The authors propose a model whereby DLC1 sequesters BimL to the microtubules. Following pro-apoptotic signals, DLC1–BimL dimers are released and are free to inhibit BCL2, leading to cell death. When cells are exposed to growth factors or other survival signals, PAK1 becomes activated, leading to phosphorylation of DLC1 and BimL. This prevents the ability of the DLC1–BimL dimer to interact with and inhibit BCL2, leading to cell survival. So, increased levels of either PAK1 or DLC1 could promote cell survival and tumorigenesis. Further experiments are required to support this model and to investigate the role of these proteins in other tumour types.

Kristine Novak

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WEB SITE

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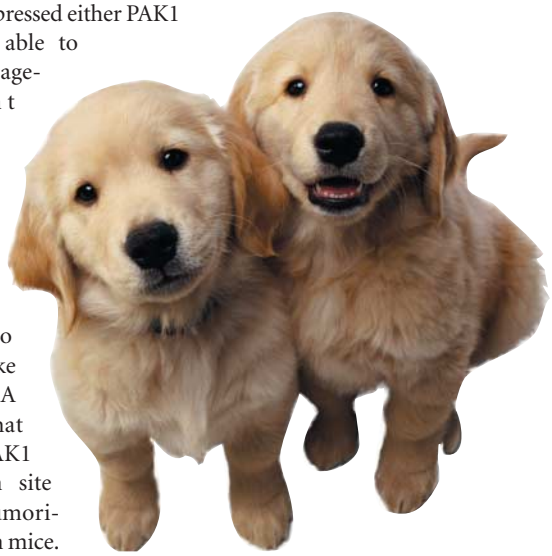


IN BRIEF

BREAST CANCER

NF-κB activation in human breast cancer specimens and its role in cell proliferation and apoptosis.

Biswas, D.K. *et al*. *Proc. Natl Acad. Sci. USA* **101**, 10137–10142 (2004)

Oestrogen-receptor-negative breast tumours represent a significant therapeutic hurdle because of a lack of effective molecular targets. Biswas *et al.* show that tumours that are oestrogen-receptor-negative but ERBB2-positive express increased levels of activated NF-κB. Suppression of NF-κB induced apoptosis in proliferating breast carcinoma cells, indicating that it might be a therapeutic target in these tumours.

TUMORIGENESIS

SMYD3 encodes a histone methyltransferase involved in the proliferation of cancer cells.

Hamamoto, R. *et al. Nature Cell Biol.* 4 July 2004 (doi:10.1038/ncb1151)

Methylation of DNA and its associated histones is important for the activation and repression of transcription. Hamamoto *et al.* show that *SMYD3* is overexpressed in most colorectal and liver tumours. A specific domain in SMYD3 was found to methylate histone H3 and — as part of a complex of proteins that includes RNA polymerase II — SMYD3 regulates the transcription of several important cell-cycle control genes. The deregulated expression of SMYD3 in these tumours might be a key factor in their development.

THERAPEUTICS

Histone deacetylase inhibitors specifically kill nonproliferating tumour cells.

Burgess, A. *et al. Oncogene* 5 July 2004 (doi:10.1038/sj.onc.1207893)

Burgess and colleagues show that histone deacetylase inhibitors (HDIs) kill non-proliferating tumour cells, but not normal cells. The mechanism of cytotoxicity in non-proliferating cells involves the activation of the cyclin-dependent kinase inhibitor WAF1 — the same pathway that is activated by HDIs in proliferating cells — but non-proliferating cells take longer to activate the apoptosis machinery. Therefore, HDIs might be useful in the treatment of slowly proliferating tumours.

GENE EXPRESSION

Inhibition of fatty acid synthase (FAS) suppresses *HER2/neu* (*erbB-2*) oncogene overexpression in cancer cells.

Menendez, J. A. *et al. Proc. Natl Acad. Sci. USA* 2 July 2004 (doi:10.1073/pnas.0403390101)

Levels of fatty-acid synthase (FAS) — an essential enzyme in the conversion of dietary carbohydrates to fatty acids — increase during breast cancer development, and hyperactivity of FAS is associated with aggressive disease. Javier Menendez *et al.* show that increased FAS activity in breast and ovarian cancer cell lines has an active role in malignant transformation by regulating key oncogenes, such as *ERBB2*.

IN THE NEWS

Smoke signals

Smokers die on average 10 years earlier than non-smokers; so concludes a landmark study published in the *British Medical Journal* (26 June 2004). This and other findings of the now-famous prospective study of the long-term smoking habits of over 34,000 British male doctors are the culmination of 50-years research into the effects of cigarette smoking in this cohort.

"Since the study began in 1951, tobacco has killed around 100 million people globally", commented Alex Markham of Cancer Research UK (*http://news.bbc.co.uk*, 25 June 2004). But quantification of the risk of smoking has been limited. The results of this study provide some sobering facts and figures for smokers: "It is clear that consistent cigarette smoking doubles mortality throughout adult life" (*Reuters*, 22 June 2004), remarked Richard Doll, the Oxford University professor who initiated the study and first discovered the link between smoking and lung cancer.

However, the news is not all bad: "... we also know that stopping smoking will significantly limit the harm" (*San Francisco Chronicle*, 23 June 2004), said Richard Peto, Doll's colleague of 30 years on the study. In fact, the study found that stopping smoking at age 50 added 6 years to life expectancy. Furthermore, stopping before the age of 30 avoids almost all hazard associated with smoking.

The study conclusions are stark for those who continue to smoke, but also signal to those who are keen on quitting that it is not too late to do so. As Peto remarked, "Smoking kills people and stopping works" (*Reuters*, 22 June 2004).

Oliver Childs

TUMORIGENESIS

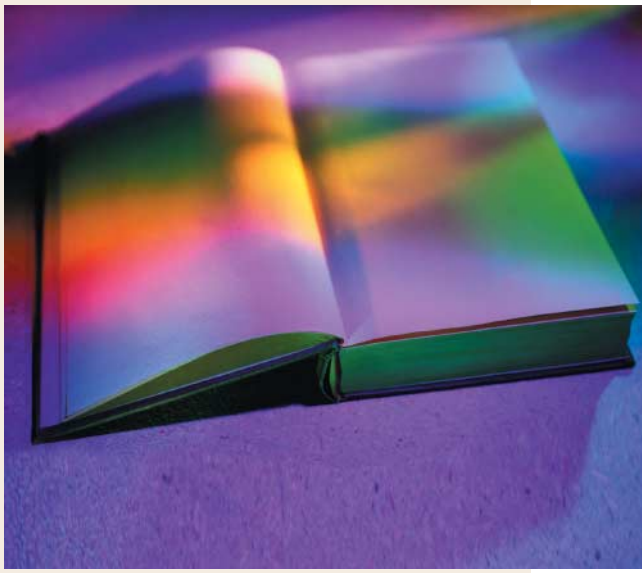
An original beginning

The retinoblastoma tumour suppressor (*RB*) is a key regulator of the cell cycle; its loss prevents cell-cycle arrest and induces apoptosis in many tissues. These findings prompted speculation that the tumour 'cell of origin' for retinoblastoma would be one that has acquired an anti-apoptotic mutation. However, two separate groups have reached the similar and surprising conclusion that, in mice, the cell of origin for this tumour is naturally resistant to the effects of *Rb* loss.

The laboratories of Rod Bremner and Tyler Jacks have closely examined how retinal development is subverted through *Rb* loss, leading to tumour formation. Modelling retinoblastoma in mice is difficult because loss of both *Rb* alleles in all tissues is lethal and $Rb^{+/-}$ mice develop pituitary rather than retinal tumours. Previous evidence from an earlier mouse model implied that the RB-like protein p107 can compensate for *Rb* loss in the retina. So, these two groups have used mice specifically deficient for RB function in the retina and crossed them with mice lacking expression of either of the *Rb*-like genes *p107* or *p130*.

Retinal cells pass through three basic developmental stages — expansion of progenitors, differentiation into seven post-mitotic precursor retinal cell types and terminal differentiation. Bremner and co-workers mapped retinal cell fates in mice with *Rb* function conditionally absent in the progenitor cells of the peripheral retina ($\alpha Cre/Rb^{lox/lox}$) and also crossed these mice with $p107^{-/-}$ mice. The $Rb^{-/-}p107^{-/-}$ mice developed retinoblastoma. Staining for the precursor cell types in the retina of embryonic and neonatal mice showed that all seven cell types were evident, indicating that *Rb* loss does not cause a defect in differentiation. Extensive apoptosis as well as large numbers of S-phase cells were evident in the mutant mice, particularly at developmental time points where terminal differentiation of retinal precursors occurs.

Jacks and co-workers also studied retinas with a conditional *Rb* deletion. In one approach, *Rb* function was disrupted within almost all cells of the retina, nervous system and other tissues (*Rb* mosaics) and these mice were crossed with $p107^{-/-}$ or $p130^{-/-}$ mice. The *Rb* mosaic $p130^{-/-}$ mice developed retinoblastoma with histology very similar to human tumours, indicating that, like p107, p130 might compensate for *Rb* loss in mice. In another approach, Jacks and colleagues used the same $\alpha Cre/Rb^{lox/lox}$ mice as the Bremner lab to accurately determine retinal cell fate in the absence of *Rb* only. They observed similar results to the Bremner lab, concluding that apoptotic and S-phase cells corresponded primarily with terminally differentiating cells. Given that loss of *Rb* predisposes terminally differentiating retinal cells to apoptosis, which cells survive to go on to form tumours?



Both groups found that four terminally differentiated cell types — ganglion, rod, cone and bipolar cells — were lost, whereas some horizontal, Muller glia and amacrine cells survived. The survival of amacrine cells might explain why *Rb* loss leads to amacrine-rich retinoblastomas in $Rb^{-/-}p107^{-/-}$ or $Rb^{-/-}p130^{-/-}$ mice.

But why do amacrine cells survive when other cell types do not? Both research groups conclude that amacrine cells are more resistant to *Rb* loss and can survive the many rounds of replication that result before they terminally differentiate. Therefore, the retinoblastoma cell of origin arises from a pool of intrinsically apoptosis-resistant differentiating precursors with extended, but finite, division capacity. These cells presumably undergo further mutations to evade terminal differentiation. Discovering this particular property of the cell of origin should help identify both further mutations that are involved in the development of human retinoblastoma and novel tumour therapy targets.

Nicola McCarthy

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Rod Bremner's lab: http://vsrp.uhnres.utoronto.ca/Bremner.html

Tyler Jacks' lab: http://web.mit.edu/biology/www/facultyareas/facresearch/jacks.shtml

TUMOUR SUPRESSORS

Benign association

Hamartomas are benign lesions that develop in a range of dominantly inherited tumour syndromes, including tuberous sclerosis complex (TSC) and Peutz–Jeghers syndrome (PJS). Two groups now have evidence for a molecular link between TSC and PJS.

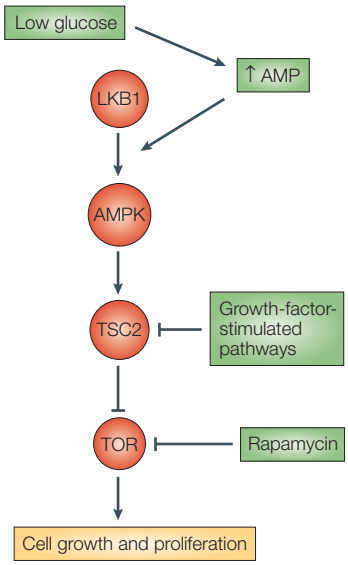
Although developing in different tissues, the hamartomas seen in patients with either TSC or PJS have a similar histology. One of the known phosphorylation targets of the PJS tumour-suppressor kinase LKB1 is the energy-sensing AMP kinase (AMPK), which is activated by high AMP (low ATP) levels. TSC2 — a tumour suppressor involved in TSC and a regulator of target of rapamycin (TOR) kinase — is a direct target of AMPK, prompting Corradetti *et al.* and Shaw *et al.* to look closely at the molecular pathways disrupted through the loss of *Lkb1*.

Both groups initially verified that LKB1 can negatively regulate phosphorylation of the TOR kinase substrates S6 kinase and 4EBP1. Phosphorylation of both of these proteins was increased in cells with non-functional LKB1. Both groups then examined the regulation of this pathway by manipulating the activity of AMPK. Shaw and co-workers used an AMP mimetic to stimulate AMPK and showed that TSC2 was phosphorylated only in the presence of functional LKB1. Corradetti and colleagues used an AMPK inhibitor to show that LKB1 was unable to alter the phosphorylation status of S6 kinase in the absence of functional AMPK.

What is the physiological relevance of these findings? TOR is a component of the energy-sensing pathway and $Tsc2^{-/-}$ cells undergo apoptosis when deprived of glucose, a response that is blocked by the TOR inhibitor rapamycin. Both groups demonstrate the same sensitivity and response to rapamycin in $Lkb1^{-/-}$ cells in the absence of glucose. Corradetti and colleagues showed that, like $Tsc2^{-/-}$ cells, $Lkb1^{-/-}$ cells secrete increased levels of vascular endothelial growth factor, which is attenuated in the presence of rapamycin. Shaw and colleagues have also found that $Lkb1^{+/-}$ mice develop intestinal hamartomas that mimic those arising in patients with PJS and these also have increased levels of TOR activity.

Therefore, the similarities between PJS and TSC are because of LKB1 and TSC2 belonging to the same kinase signalling pathway. Their affect on TOR also indicates that rapamycin and its analogues could be used to treat hamartomas in these patients. However, despite these similarities, intriguing questions remain. Why, for example, do patients with PJS develop hamartomas in the intestine, whereas patients with TSC have widespread tissue involvement? One explanation might be that loss of TSC2 function affects more than energy signalling pathways; $Tsc2^{-/-}$ cells also fail to respond to growth-factor-mediated pathways that remain intact in $Lkb1^{-/-}$ cells.

Nicola McCarthy



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ANGIOGENESIS

Recruitment drive

Vascular endothelial growth factor (VEGF) is important in tumour angiogenesis, but whether it is essential that cancer cells themselves express VEGF is unclear. A recent paper in *The EMBO Journal* shows that tumours can recruit stromal cells to carry out this function.

Jianying Dong, Napoleone Ferrara and colleagues generated *Vegf*-null mouse embryonic fibroblasts (MEFs), which were immortalized and then transformed with oncogenic *Ras*. These cells formed tumours on injection into mice, which were about half the size of those formed by a parental VEGF-expressing cell line that was similarly immortalized and transformed. The *Vegf*-null tumours successfully induced angiogenesis, with only a slight decrease in vessel density compared with tumours formed by parental cells.

Although these tumours do not express VEGF themselves, VEGF mRNA and protein were present in the tumours, but at markedly lower levels than in those formed by the parental cell line. Could stromal cells recruited to these tumours be the source of VEGF expression? This was confirmed by *in situ* hybridization using a probe specific for the exon of the *Vegf* gene that was deleted in the *Vegf*-null MEFs, showing that the transcript co-localized with tumour-associated stromal cells.

The authors used an anti-VEGF antibody to test whether the small amounts of VEGF produced by recruited stromal cells were responsible for angiogenesis and tumour growth in the *Vegf*-null tumours. Treatment with the antibody resulted in a decrease in tumour mass of up to 62%, indicating that the stroma-derived VEGF does have an important role.

How do tumours recruit VEGF-expressing stromal cells? Fibroblasts are an important component of the tumour-associated stroma and are known to express VEGF. The authors showed that conditioned medium obtained from *Vegf*-null tumour cells stimulated migration and proliferation of a fibroblast cell line *in vitro*. Fractionation of the medium revealed a peak of activity corresponding to PDGFA — a member of the platelet-derived growth factor family. Consistent with a role of this protein in fibroblast recruitment, *Pdgfa* expression was seen throughout *Vegf*-null tumours, whereas expression of the mRNA encoding its receptor — PDGFRα — was localized to stromal cells. In addition, a soluble form of PDGFRα inhibited tumour growth by 50%, confirming a crucial role for signalling through this receptor.

These results have important implications for anticancer treatments that inhibit angiogenesis. The fact that tumour cells can recruit VEGF-expressing fibroblasts, as well as producing VEGF themselves, indicates that the signalling pathways involved in both of these mechanisms might need to be blocked to completely inhibit angiogenesis and tumour growth.

Louisa Flintoft

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CELLULAR IMMORTALITY

Early old age

Werner syndrome is an autosomal recessive disease caused by inactivation of the gene encoding the DNA helicase WRN and is characterized by premature ageing, genomic instability and increased non-epithelial cancer incidence. It is thought that the erosion of telomeres — structures that cap chromosomes and are essential for chromosomal stability — has a role in the pathogenesis of this syndrome. Ron DePinho, Sandy Chang and colleagues have developed a mouse model that is null for both *Wrn* and the RNA component of telomerase (*Terc*) — an enzyme essential for telomere maintenance — that shows many of the classic features of Werner syndrome.

WRN is involved in DNA recombination, replication and repair, and hyper-recombination and numerous chromosomal aberrations have been observed in individuals with Werner syndrome. DePinho and Chang hypothesized that a combination of impaired DNA repair and telomere dysfunction might drive Werner syndrome pathogenesis.

The authors carried out successive intercrosses between $Wrn^{-/-}$ mice and $Terc^{-/-}$ mice to produce cohorts with progressively shorter telomeres and increasing telomere dysfunction. In first- and second-generation $Terc^{-/-}$ mice, *Wrn* status had no impact on clinical appearance, but the fourth- to sixth-generation $Terc^{-/-}Wrn^{-/-}$ mice had lower body weights and shorter survival times than $Terc^{-/-}Wrn^{+/+}$ mice. Although healthy in early life, by 12–16 weeks of age many of the $Terc^{-/-}Wrn^{-/-}$ mice had features of premature ageing, including Werner-syndrome-related diseases. Increased apoptosis in gastrointestinal crypt cells and increased numbers of fused chromosomes in bone-marrow cells were seen in successive generations of $Terc^{-/-}Wrn^{-/-}$ mice. This reinforced a link between genomic instability due to WRN loss and telomere dysfunction.

So, how did these genotypes affect the cancer phenotype of these mice? The prematurely aged late-generation $Terc^{-/-}Wrn^{-/-}$ mice were

IMMUNOLOGY

Receptors and effectors

Effective therapies for non-Hodgkin's B-cell lymphoma aim to deplete the B-cell population in patients. However, the precise mechanism by which the humanized immunoglobulin G1 (IgG1) antibody therapy rituximab kills B cells was previously unknown. Jungi Uchida *et al.* now reveal the mechanism involved.

Rituximab, which targets a B-cell-specific antigen called CD20, could affect many aspects of the immune response, including antibody, effector-cell-(macrophage and natural killer cell) and complement-dependent cytoxicity; the disruption of CD20 signalling pathways; and the induction of apoptosis. Previous studies have looked at the mechanisms *in vitro* or in circulating human B cells only. So, the authors developed a mouse model for anti-CD20 immunotherapy using 12 mouse anti-mouse CD20 monoclonal antibodies (mAbs) to study each of the possible mechanisms. All these antibodies bound to B cells in the CD20 wild-type mice and depleted both the circulating and splenic B-cell compartments. The effectiveness of mAb-induced B-cell depletion correlated closely with mAb isotype — a single injection of an IgG2a mAb (MB20-11) depleted more than 95% of blood B cells and more than 93% of splenic B cells. None of the antibodies had any effect in $Cd20^{-/-}$ mice.

Immune effector cells express three different Fc receptor classes for IgG. FcγRI is the highest-affinity receptor and binding of IgG to it triggers phagocytosis by macrophages and cytotoxicity by natural killer cells. Although treatment of mice deficient in either FcγRI or FcγRIII with MB20-11 did deplete B cells, treatment of mice deficient in both FcγRI and FcγRIII did not deplete B cells. This shows that binding to one of these receptors is important for efficacy of anti-CD20 mAbs. Next, the authors looked at complement-deficient mice to assess the role of complement in B-cell depletion by anti-CD20 mAbs. *In vitro*, the antibodies caused B-cell lysis and apoptosis only in the presence of complement. However, *in vivo*, there was no difference in the ability of any mAb to induce B-cell killing in the wild-type or complement-knockout mice.

So, Fc receptors are crucial for the efficiency of anti-CD20 mAbs; but what are the effectors of this response? When mice lacking T cells or natural killer cells were treated with MB20-11, more than 96% of B cells were depleted. However, similar treatment of macrophage-deficient mice did not cause significant depletion of circulating or splenic B cells.

The authors conclude that a likely mechanism of B-cell depletion by anti-CD20 mAbs is FcγR-mediated phagocytosis of mAb-coated B cells by macrophages. This knowledge should help to understand the response and resistance to rituximab therapy and the development of effective methods to enhance the benefits of therapies for non-Hodgkin's lymphoma.

Ezzie Hutchinson

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not particularly prone to cancer (presumably because they died before cancers developed), but the first- to third-generation *Terc*$^{-/-}$*Wrn*$^{-/-}$ mice did have an increased incidence of osteosarcomas and soft-tissue sarcomas, usually developing at about 63 weeks. *Terc*$^{-/-}$*Wrn*$^{+/+}$ mice developed tumours later (around 85 weeks), and these were mainly lymphomas.

The study of this compound-mutant model of Werner syndrome supports the view that WRN is involved in telomere dynamics and that inactivation of this protein forms the basis of ageing phenotypes that target slowly proliferating mesenchymal tissues.

Ezzie Hutchinson

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WEB SITE

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METASTASIS

Stick or twist?

For many years the similarities between the migratory properties of metastatic cancer cells and embryonic stem cells have been noted. In a recent *Cell* paper, Weinberg and colleagues now show that *TWIST* — a master gene that controls epithelial–mesenchymal transition (EMT) in embryogenesis — is required for metastasis in epithelial-derived breast tumours.

Weinberg and colleagues have exploited a breast tumour model that uses four spontaneously arising mouse breast tumour cell lines with distinctive metastatic properties. The four lines range from having no metastatic potential to being highly metastatic. By comparing microarray expression profiles of the primary breast tumours arising from each of these lines *in vivo*, the authors have identified genes that show altered expression patterns as metastatic potential increases. As well as identifying genes that are known to be involved in metastasis, such as the gene encoding matrix metalloproteinase 9, they also isolated *Twist* as the second most robustly upregulated gene when comparing metastatic with non-metastatic tumours.

To investigate the contribution of *Twist* to metastasis in this mouse model, the authors used short interfering RNAs to suppress expression of *Twist* in the most metastatic cell line. Suppression of *Twist* did not affect primary breast tumour formation, but did significantly suppress metastasis by inhibiting both the capacity of the cells to enter blood vessels and their ability to establish micrometastases within the lung.

How might *TWIST* influence the capacity of a cell for metastasis? TWIST is an evolutionarily conserved transcription factor that regulates tissue reorganization during embryogenesis and, importantly, enables cells to migrate. Therefore, Weinberg and colleagues asked if *TWIST* might confer migratory properties to human mammary epithelial cells. Cells expressing *TWIST* developed a spindle-like, fibroblastic morphology, and had reduced cell–cell contacts, which correlated with reduced expression of adherens-junction proteins such as E-cadherin and β-catenin. This altered morphology is one of the hallmarks of cells that undergo EMT and is closely associated with migratory properties.

Is *TWIST* involved in human breast cancer? The authors compared the levels of *TWIST* expression in metastatic and non-metastatic human breast cancer cell lines and showed that only metastatic lines expressed *TWIST*. Moreover, microarray analysis of *TWIST* expression in specific breast cancer subtypes — ductal, mixed ductal/lobular and lobular carcinoma — revealed that 70% of the latter type expressed high levels of *TWIST*. Interestingly, lobular carcinoma cells show many of the hallmarks of EMT cells, prompting the authors to examine E-cadherin mRNA expression in these tumours — the level was substantially reduced. These findings correlate with the aggressive, invasive nature of lobular breast carcinomas.

TWIST is also overexpressed in diffuse-type gastric cancers, which also show infiltrative growth and reduced levels of E-cadherin. Therefore, the authors speculate that E-cadherin expression might be directly suppressed by TWIST and that this is one mechanism through which TWIST promotes invasive tumour growth.

Nicola McCarthy

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WEB SITE

Robert A. Weinberg's Lab: http://web.wi.mit.edu/weinberg/pub/

TRIAL WATCH

Immunotherapy boost

A cancer vaccine designed to stimulate an immune response against the tumour antigens carcinoembryonic antigen (CEA) and mucin 1 (MUC1) has entered a Phase III clinical trial for the treatment of patients with metastatic pancreatic cancer.

The vaccine, called PANVAC-VF, was developed by Therion Biologics Corporation. It includes recombinant vaccinia and fowlpox viruses that co-express CEA and MUC1, which are expressed by over 90% of pancreatic tumour cells. The vaccine also incorporates the costimulatory molecules B7.1, ICAM1 and LFA to enhance and sustain the antitumour immune response.

In Phase I studies of 22 patients with advanced pancreatic cancer, 20 of whom had metastatic disease, vaccination with PANVAC-VF resulted in a median overall survival of 7.9 months and at least 5.3 months. This is in comparison to an anticipated median overall survival of approximately 3 months, based on historical controls. The most common adverse event was a reaction at the injection site, but no serious side effects were observed.

A multicentre, randomized, controlled Phase III trial is underway, enrolling 250 patients with advanced pancreatic cancer who have not responded to treatment with gemcitabine. The primary end point will be overall survival, compared with palliative chemotherapy or best supportive care. The study will also determine safety, quality of life, change in serum tumour antigen levels, response rate and disease stabilization.

Immunotherapies targeted at CEA and MUC1 have also been tested in patients with other tumours that are known to overexpress the CEA and MUC1 antigens, including breast, lung and colorectal cancers. Therion and the National Cancer Institute are now planning up to 18 additional studies with PANVAC-VF in patients with these cancers.

ORIGINAL RESEARCH PAPER Schuetz, T. *et al.* Two phase I studies of prime-boost vaccinations with vaccinia-fowlpox vaccines expressing CEA, MUC-1, and TRICOM costimulatory molecules (B7.1/ICAM-1/LFA-3) in patients with advanced pancreatic cancer. *Am. Soc. Clin. Oncol.* abstract 2564 (2004)

Cardiotoxicity reduced

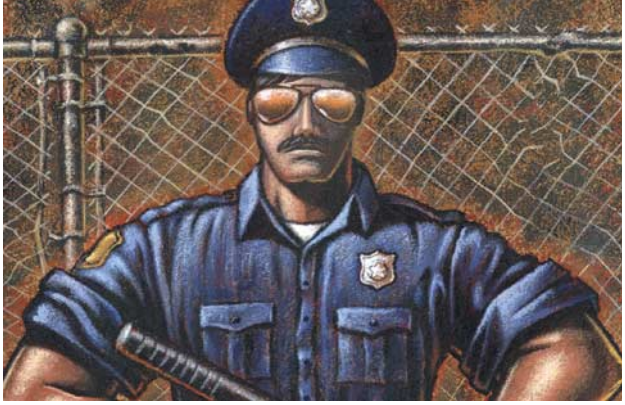
A novel anthracycline, pixantrone, has been given fast-track status by the Food and Drug Administration for relapsed, aggressive non-Hodgkin's lymphoma. Anthracyclines, such as doxorubicin, are part of standard treatment for non-Hodgkin's lymphoma together with cyclophosphamide, vincristine and prednisone (CHOP). However, anthracyclines are associated with cumulative heart damage that limits their use in patients previously treated with anthracyclines and prohibits their use in many others.

Pixantrone (made by Cell Therapeutics Inc.) is easier to administer than doxorubicin and has not shown the same risk of cardiac damage. The drug design was based on the anthracycline mitoxantrone — the OH groups thought to cause free-radical production and cardiotoxicity were removed and replaced with nitrogen. The molecule is still planar, so the substitution does not effect intercalation into DNA, where the drug causes its damage.

Cell Therapeutics tested single-agent pixantrone in 30 patients with aggressive non-Hodgkin's lymphoma who had already received 300 mg/m^2 doxorubicin, which would normally make them ineligible for further anthracycline treatment. Five patients had a complete response and four had a partial response; responses lasted an average of 11 months. Pixantrone is now being studied in combination, replacing doxorubicin in CHOP, and preliminary results of 22 patients have shown 13 with complete responses and four with partial responses. Pixantrone was well tolerated, with neutropaenia as the dose-limiting toxicity.

Other trials, including a Phase II trial of pixantrone with rituximab in relapsed indolent non-Hodgkin's lymphoma, are ongoing.

WEB SITE http://www.cticseattle.com/prod_frame-iprod-pix.htm



TUMOUR SUPPRESSORS

A guardian and a suppressor

There are some fundamental processes in biology that we would expect to be conserved across all species. And yet some of them use a surprising variety of molecular mechanisms. A recent report by McPherson *et al.* shows that the MUS81 endonuclease, which is involved in processing branched DNA structures in yeast, such as those found in stalled replication forks, is required in mammals for genomic stability and tumour suppression.

To clarify the role of *Mus81 in vivo*, the authors knocked out the gene in mice. Based on the role of Mus81 in yeast, they expected the mice to show meiotic recombination defects, such as infertility. To their surprise, the animals were fertile with no defects in gametogenesis. Normal gene targeting in the *Mus81*$^{-/-}$ embryonic-stem-cell lineage, and B- and T-cell lineages (the ontogeny of which requires DNA rearrangements), confirmed that *Mus81* is not required for a cell to cope with double-stranded DNA breaks.

But *Mus81* knockout mice do have a phenotype — mutant embryonic stem cells are hypersensitive to the alkylating agent mitomycin C, indicating that the gene might be involved in repairing mitomycin-C-induced DNA interstrand crosslinks. *Mus81* also seems to act as a haploinsufficient genome caretaker — loss of even one copy of *Mus81* leads to aneuploidy and other chromosomal defects.

Although, at first glance, mutant mice seem to be normal, the authors found that only 27% of homozygotes and 50% of heterozygotes were healthy and survived through their first year. Many had tumours, mainly non-Hodgkin's lymphomas, that at the cellular level were associated with aneuploidy. Because *Mus81* homozygotes and heterozygotes were equally susceptible to cancer, both copies of *Mus81* must be required for its tumour-suppressor function — just as two copies are required for genome integrity.

Although MUS81 is not alone in being a haploinsufficient tumour suppressor, it is at odds with the common view that tumorigenesis requires the loss of both copies of a tumour suppressor. Mechanistically speaking, the genomic instability caused by *Mus81* haploinsufficiency might facilitate tumorigenesis, for example, in preneoplastic lymphocytes. However, it remains to be seen whether the model that the authors propose is correct — that is, that a 50% reduction in the amount of MUS81 protein in heterozygotes is not sufficient to resolve intermediate DNA structures that form during DNA repair.

Magdalena Skipper
Editor, Nature Reviews Genetics

References and links

ORIGINAL RESEARCH PAPER McPherson, J. P. *et al.* Involvement of mammalian Mus81 in genome integrity and tumour suppression. *Science* **304**, 1822–1826 (2004)