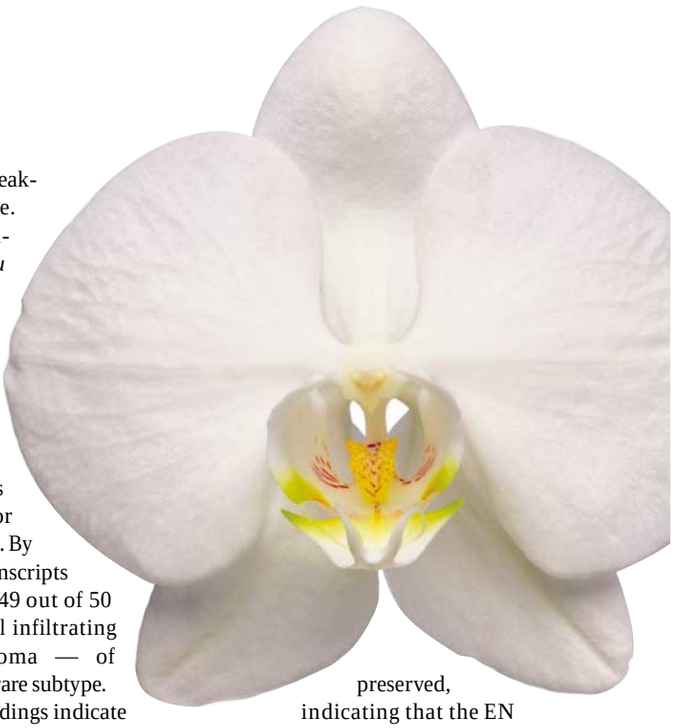
HIGHLIGHTS

TRANSLOCATIONS

Rare simplicity



Single genetic lesions underlying transformation are seldom found in epithelial cancers because of the genetic complexity of the disease. However, Tognon *et al.* now report a translocation event that forms a dominantly acting oncogene and causes a rare form of breast cancer — secretory breast carcinoma (SBC).

Secretory carcinoma accounts for less than 1% of all breast cancers and occurs in patients as young as 3 years old — patients are usually cured, but a mastectomy or chemotherapy is often required. It is generally accepted that specific fusion genes are associated with specific tumour types, but when the authors saw that a translocation — which they had previously identified in paediatric mesenchymal tumours — was the only karyotypic abnormality in an SBC in one 6-year-old patient, they decided to investigate other cases.

The translocation — between the *ETV6* transcription factor on chromosome 12 and the protein tyrosine kinase domain of the neurotrophin-3 receptor *NTRK3* on chromosome 15 — results in constitutive activation of wild-type NTRK3, which activates the RAS–mitogen-activated protein kinase and the phosphatidylinositol 3-kinase–AKT pathways for mitogenesis and cancer survival.

The authors detected *ETV6–NTRK3* fusion transcripts in tumour specimens from 11 out of 12 further patients with SBC, all of whom had the identical breakpoint sequence. Dual-colour fluorescence *in situ* hybridization (FISH) analysis showed that all samples of SBC that were available for analysis were positive for the fusion gene. By contrast, no transcripts were found in 49 out of 50 cases of typical infiltrating ductal carcinoma — of which SBC is a rare subtype.

So, these findings indicate that the *ETV6–NTRK3* gene fusion is a non-random rearrangement in SBC, but does the translocation product (EN) cause transformation? Tognon and colleagues transfected two immortalized non-transformed mouse epithelial cell lines — the Scg6 cell line has mesenchymal features, and Eph4 has a stable epithelial phenotype — with an *ETV6–NTRK3* retroviral construct. Both cell lines expressed the construct and showed a transformed phenotype, whereas cells transfected with vector alone did not. In addition, when these cells were injected into nude mice, the EN-expressing cells formed tumours, whereas the cells transfected with vector alone did not. Histopathology showed that the original phenotype was preserved, indicating that the EN product — which had only been associated with mesenchymal malignancies before — does not block epithelial differentiation potential.

The authors have established that *ETV6–NTRK3* is a dominantly acting oncogene in SBC. Furthermore, this research challenges the dogma that fusion genes are only associated with one of the three germ layers, as the EN product has been previously found in mesenchymal tumours, and now in an epithelial malignancy.

Ezzie Hutchinson

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IN THE NEWS

From mouse to man

With the publication of the draft sequence of the C57BL/6J mouse genome in the 5 December issue of *Nature*, a powerful new resource has become available to the cancer-research community. "The entire biomedical research community can for the first time fully use this resource to tackle human diseases", said Dr Jane Rogers of the Sanger Institute, UK (bbc.co.uk).

The international sequencing consortium estimates that the 2.5 billion nucleotide genome contains 30,000 genes, 99% of which have a human homologue — this is the first time that the genomes of two mammals have been available for comparison (covered in the New York Times, 5 Dec 2002). Some 96% of the mouse genes lie in regions that are 'syntenic' with human chromosomes. In an accompanying News and Views article (*Nature* **420**, 515–518 (2002)), Mark Boguski says "the conservation of synteny between mouse and human chromosomes will allow effective cross-reference of the location of any genetically mapped traits in the mouse with genes in the orthologous regions of the human genome. This will greatly accelerate the isolate of disease genes".

The genome sequence will also make mice a better model for mutation-based screening assays. In a News and Views article in the January issue of *Nature Genetics*, Tim O'Brien and Rick Woychik discuss how the genome sequence will also help with the design and generation of targeted mutations produced by homologous recombination.

The sequencing effort has also led to the discovery of about 1,200 new genes that have human homologues, many of which are likely to have undiscovered cancer-related functions.

Kris Novak

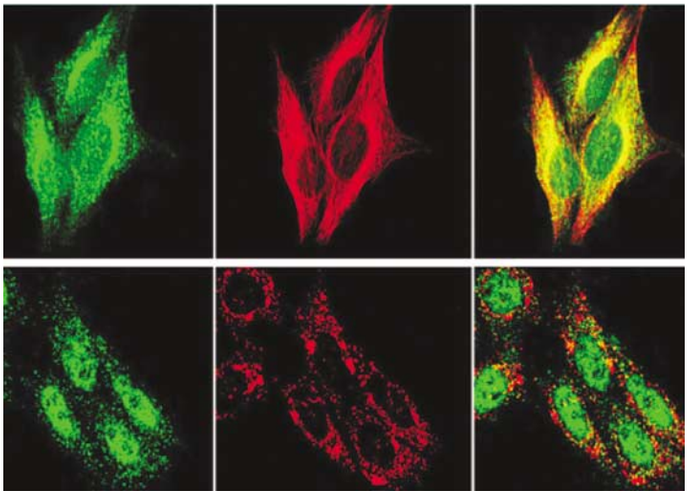
TUMOUR SUPPRESSORS

The stabilizing influence of VHL

Inactivation of the von Hippel–Lindau (*VHL*) tumour-suppressor gene is linked to the development of several different tumour types in humans, including tumours of the kidney, retina, central nervous system and the adrenal gland. Despite identification of the gene almost 10 years ago, we are only now beginning to understand how VHL functions in the cell and how its mutation leads to tumour development. Reporting in the January issue of *Nature Cell Biology*, Krek and colleagues have now uncovered a novel function for VHL — microtubule stabilization — and show that disruption of this function is linked to the development of a specific subtype of VHL disease.

So far, the best-characterized function of VHL has been as a component of an E3 ubiquitin ligase complex, which mediates degradation of the hypoxia-inducible factor (HIF) under normoxic conditions. Although there are some links between this and particular subtypes of VHL disease, the extent to which this function of VHL contributes to tumour progression is unclear. More recently, VHL has also been implicated in extracellular-matrix formation and cell-cycle progression.

The importance of the new work from Krek and colleagues is that they identify a novel cytoskeletal function, which is specific to an isoform of VHL that has not previously been linked to VHL tumour development.



VHL localization depends on an intact microtubule network. VHL_{30} (green) co-localizes with the microtubule network (β-tubulin; red) in HeLa cells (top panel). Co-localization is shown in yellow. This localization of VHL_{30} is disrupted when microtubules are depolymerized with colcemid (bottom panel).

METASTASIS

Stay or go?

At what point do tumour cells acquire their metastatic potential? Are certain primary tumours prone to metastasis, or is the ability to take up residence in a foreign tissue a characterstic of only a few cells that have managed to break free from their primary tumour host? A gene-profiling study, published by Todd Golub and colleagues in the January issue of *Nature Genetics*, begins to answer these questions.

The authors analysed the gene-expression profiles of 12 metastatic adenocarcinoma nodules from tissues such as lung, breast, prostate, colorectum, uterus and ovary, and compared them with expression profiles of 64 primary adenocarcinomas representing the same spectrum of tumours. They identified 128 genes that distinguished primary from metastatic adenocarcinomas. Metastasis was associated with the upregulation of a number of genes that regulate protein translation (*SNRPF, EIF4EL3, HNRPAB* and *DHPS*). Other upregulated genes seemed to come from the non-epithelial component of the tumour, such as those that encode type I collagens, indicating the importance of the stroma in regulating metastasis. Analysis of additional tumours revealed a similar metastatic gene signature.

This metastasis-associated gene-expression pattern was also present in some primary tumours, so did this mean that these tumours were destined to metastasize? The authors found that patients with primary tumours that expressed the metastatic gene profile had significantly shorter survival times than cancer patients whose tumours did not. This means that some primary tumours already have the propensity for metastasis as early as the time of diagnosis.

The authors also looked for the metastasis-associated gene signature in other tumour types, and found that their pattern could be used to predict metastatic potential of small stage I primary breast adenocarcinomas, prostate adenocarcinomas and medulloblastomas. This indicates that there are generic gene-expression programmes associated with the metastatic process in different tumours. Notably, the gene-expression profile was not able to predict metastasis in patients with diffuse large-B-cell lymphoma. This might be because haematopoietic malignancies have special mechanisms for spreading

VHL exists as two isoforms: the longer VHL_{30} isoform and the shorter VHL_{19} isoform that results from internal initiation at methionine 54. By raising antibodies that are specific for each isoform, Krek and colleagues revealed that, whereas the shorter isoform localizes predominantly to the nucleus, the longer VHL_{30} isoform co-localizes with the cytoplasmic microtubule network and depends on an intact microtubule network for this.

To address the functional significance of this localization, the authors tested the effect of VHL binding on microtubule dynamics and found that it mediates microtubule stabilization, protecting microtubules against nocodazole treatment. One important distinction is that this function of VHL seems to be independent of its ability to form an active E3 ligase complex. So what is the relevance, if any, of this role to the tumour-suppressor function of VHL? To test this, the authors looked at the effects of different *VHL* mutations associated with each disease subtype on microtubule stabilization. Intriguingly, only mutations associated with type 2A VHL disease (and one associated with type 2C disease), which is characterized by a high risk of developing adrenal-gland tumours and cerebellar haemangioblastomas, were abrogated in microtubule stabilization.

How this function of VHL might contribute to tumour development remains to be seen, but from this work a new model for VHL function is beginning to emerge. The authors propose that each of the two VHL isoforms has a distinct role. Whereas the shorter isoform resides in the nucleus and is required as part of an E3 ligase complex to regulate HIF under normoxic conditions, the longer isoform has a novel E3-independent function in the cytoplasm, mediating microtubule stability. Exactly how loss of microtubule stabilization by VHL contributes to tumour progression remains to be seen.

Alison Schuldt

Associate Editor, Nature Cell Biology

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FURTHER READING Kaelin, W. G. Jr. Molecular basis of the VHL hereditary cancer syndrome. *Nature Rev. Cancer* **2**, 673–682 (2002)

throughout the blood vessels and lymphatic system.

Golub and colleagues admit that although their outcome predictor was statistically significant, it was still imperfect, and suggest that additional factors are involved in determining tumour behaviour. But the discovery of an expression signature that can be used to classify a subset of primary solid tumours as premetastatic will be useful not only in determining prognosis, but also in designing therapies to stop the spread of tumours.

Kristine Novak

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WEB SITE

Todd Golub's web site: http://www-genome.wi.mit.edu/cancer/

IN BRIEF

ONCOGENES

High frequency of *BRAF* mutations in nevi.

Pollock, P. M. *et al. Nature Genet.* 25 Nov 2002 (doi:10.1038/ng1054)

BRAF encodes an oncogenic kinase that is involved in the RAS–RAF–MAPK signalling pathway. Earlier this year, *BRAF* was found to be mutated in malignant melanoma, but how early in the transformation process does this occur? Pollock *et al.* now show that mutations in *BRAF* occur very early in melanoma pathogenesis — at the nevi stage. Some 82% of nevi had an activating mutation in *BRAF*, resulting in the amino-acid substitution V599E, indicating that this is a crucial step in the initiation of melanoma.

CHECKPOINTS

53BP1 functions in an ATM-dependent checkpoint pathway that is constitutively activated in human cancer.

DiTullio, R. A. *et al. Nature Cell Biol.* **4**, 998–1002 (2002)

53BP1 localizes to double-strand breaks following irradiation, indicating that it might be a checkpoint protein. RNAi of 53BP1 showed that it is required for the ATM-dependent phosphorylation of certain substrates after DNA damage, and for the G2–M checkpoint. Interestingly, in several cancer cell lines that have mutant *TP53*, 53BP1 foci form even in the absence of irradiation, which has led the authors to suggest that an activated checkpoint pathway might provide a selective pressure for mutation of *TP53*.

TUMORIGENESIS

Highly penetrant, rapid tumorigenesis through conditional inversion of the tumor suppressor gene *Snf5*.

Roberts, C. W. M. *et al. Cancer Cell* **2**, 415–425 (2002)

The SWI/SNF chromatin remodelling complex might act as a tumour suppressor, but definitive evidence has been lacking. Now, a reversible inactivating conditional allele of *Snf5* — a core subunit of SWI/SNF — has been generated to investigate this. Inactivation of *Snf5* results in the formation of tumours — T-cell lymphomas and rare rhaboid tumours — in 100% of mice with an average latency of 11 weeks, confirming that it does act as a tumour suppressor.

THERAPEUTICS

Using cyclooxygenase-2 inhibitors as molecular platforms to develop a new class of apoptosis-inducing agents.

Zhu, J. *et al. J. Natl Cancer Inst.* **94**, 1745–1757 (2002)

COX2 inhibitors act as chemopreventive drugs by sensitizing cancer cells to apoptosis, but why do agents that inhibit COX2 to a similar extent show different potencies against cancer cells? A systematic chemical approach to modify the structures of celecoxib and rofecoxib was used to generate compounds that could be tested for their ability to induce apoptosis of prostate cancer cells. The structural requirements for COX2 inhibition are different from those for apoptotic induction — which occurs by downregulating AKT and ERK2 — so existing COX2 inhibitors could be modified to maximize their ability to kill cancer cells.

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.

Arianne Heinrichs
Senior Editor, Nature Reviews
Molecular Cell Biology

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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.

Amanda Tromans
Senior Editor, News & Views, Nature

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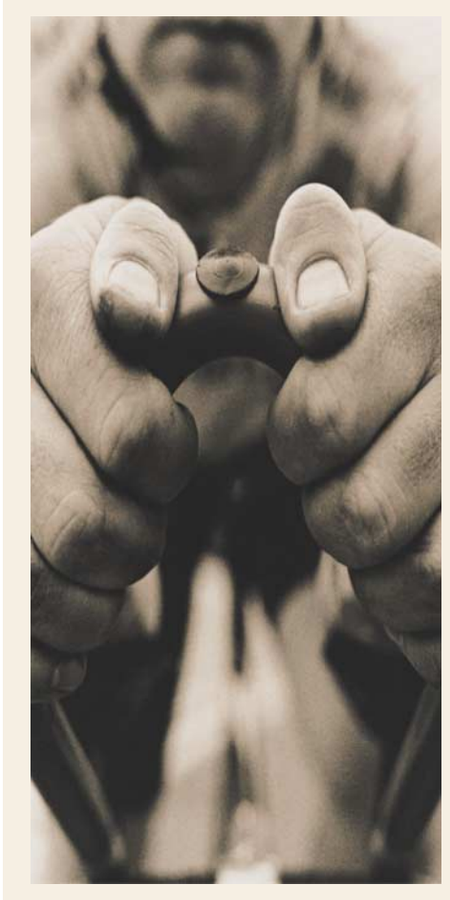

TRANSFORMATION

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.

Emma Greenwood

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WEB SITE

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

ANGIOGENESIS

Highs and lows

The rationale behind the frequent administration of chemotherapeutic drugs at low doses — high-time or metronomic dosing — is to prevent time for repair of damage to the tumour vasculature, thereby deriving increased therapeutic benefit. Logically, haematopoietic cells and gut epithelial tissues should also sustain more damage because of the lack of recovery time between cycles of chemotherapy, but, interestingly, such side effects, at least in the short term, seem to be much less severe. Bocci and colleagues provide an explanation for this as they show, in *Cancer Research*, that cycling endothelial cells are inherently more sensitive than other cells to continuous low-dose therapy, and suggest that this might therefore be an optimal way of delivering certain types of anti-angiogenic therapies, especially those using conventional chemotherapeutic drugs.

Bocci *et al*. exposed human tumour cells, fibroblasts and endothelial cells to daily low concentrations of chemotherapeutic drugs for up to 6 days — analogous to the protracted metronomic protocols used in patients. They observed specific inhibition of endothelial-cell proliferation with taxanes and cyclophosphamide after 6 days, but not after 24 hours, using concentrations of 10–100 pM and 1–100 nM, respectively. By contrast, normal fibroblasts and breast cancer cell lines were not affected. Not all drugs showed this differential effect — doxorubicin had similar antiproliferative activity against both endothelial cells and breast cancer cells. In addition, the authors observed that treated endothelial cells had a higher level of apoptosis than the cancer cell lines or fibroblasts.

So, prolonged exposure times, once an effective dose of drug has been reached, are crucial for cell kill in high-time chemotherapy regimens and might be selective for endothelial cells. This type of schedule might create an anti-angiogenic therapeutic window, and could be used to treat tumours that are resistant to the very drugs that are used for low-dose chemotherapy or to decrease host toxicity without reducing efficacy. It remains to be seen whether other types of normal cells are also highly sensitive to metronomic dosing.

Ezzie Hutchinson

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WEB SITE

Robert S. Kerbel's lab: http://medbio.utoronto.ca/faculty/kerbel.htm

SENESCENCE

Stopping the cycle

There are several different ways to stop cancer-cell proliferation. The most obvious approach is to induce apoptosis, and many chemotherapeutic agents are known to do just that. Less is known, however, about how to induce senescence — a state of terminal cell-cycle arrest — in cancer cells.

In normal cells, replicative senescence results from loss of telomeric repeats after several rounds of cell division. This generates a DNA-damage signal that activates p53. Some anti-cancer drugs induce cell senescence by inducing this DNA-damage response in both normal and malignant cells, but these drugs can lead to unwanted side effects.

Dimitri Lodygin *et al*. therefore looked for signalling pathways that were specifically down-regulated during replicative senescence. They reasoned that pharmacological inhibition of these pathways should induce senescence in tumour cells. Using microarray analysis, they found that several components of the cGMP signalling pathway were downregulated during replicative senescence of primary human diploid fibroblasts. So, could a compound such as 6-anilino-5,8-quinolinequinone (LY83583, or LY), which is known to inhibit cGMP production, induce senescence?

Lodygin *et al*. showed that treatment of fibroblasts with LY completely and irreversibly inhibited proliferation of the cells by blocking S-phase entry. Microarray analysis of LY-treated cells revealed a significant overlap between the genetic profiles of cells during replicative senescence and after LY treatment. One gene that was induced under both conditions was *CDKN1A*, which encodes an inhibitor of cyclin-dependent kinases — called WAF1 (also known as p21). LY also induced WAF1 expression and prevented proliferation in colorectal cancer, breast cancer and melanoma cell lines, indicating its therapeutic potential.

Experiments in cGMP-null cell lines revealed that LY's effects depended on induction of WAF1. LY did not, however, activate the DNA-damage/p53 pathway, as LY treatment of p53-deficient cells still resulted in WAF1 upregulation and senescence. It is not clear whether LY upregulation of WAF1 occurs through its effects on the cGMP signalling pathway, or through some other mechanism.

As LY induces cell-cycle arrest in a p53-independent manner, it might be useful in treating tumours that have p53 mutations. Inactivation of WAF1, on the other hand, has not been reported in cancer cells. In cells with disruptions in the RB pathway, LY induced apoptosis, rather than cell-cycle arrest, so LY could also be used to treat tumours that have RB defects. The ability of LY to induce senescence without inducing DNA damage indicates that it might also have fewer side effects than current chemotherapeutic agents.

Kristine Novak

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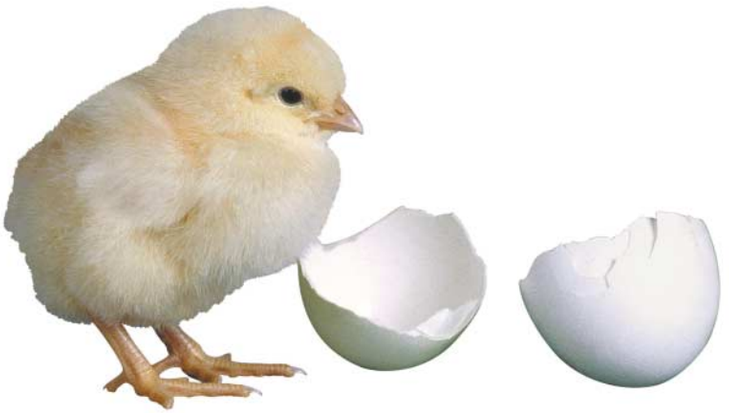
WEB SITE

Heiko Hermeking's lab: http://www.biochem.mpg.de/hermeking/

GENOMIC INSTABILITY

What came first?



Colorectal cancer is genetically well defined, and is known to progress through a defined series of stages, but what is the initial event that sets cells along this tumorigenic pathway? Mutation of the *APC* tumour-suppressor gene is certainly an early event, and chromosomal instability (CIN) is also thought to be a driving force of tumorigenesis, but whether CIN occurs early in tumorigenesis — perhaps even causing loss of heterozygosity of the second *APC* allele — is, at present, unknown. Martin Nowak *et al*., reporting in *Proceedings of the National Academy of Sciences*, have now devised a stochastic mathematical model to investigate this question.

They started by considering the six states, with respect to *APC* inactivation and CIN, that any cell in the colonic crypt could be in. Cells could have none, one or two functional *APC* alleles, in the presence or absence of CIN. They then determined how, and with what rate, a cell could progress from one state to the next by considering parameters such as mutation rate, loss of heterozygosity rate in normal and CIN cells, reproductive rate of $APC^{-/-}$ and CIN cells, and the number of dominant CIN genes.

They calculated the probability that the system reaches the state X_2 (*APC* inactivation occurs in the absence of CIN) or Y_2 (*APC* inactivation occurs in the presence of CIN) first, and therefore whether CIN could cause inactivation of the second *APC* allele. For the second scenario to occur, the number of CIN genes must exceed a certain threshold value. This number depends on several factors, including the selective cost of CIN and the effective number of stem cells per crypt. For a wide range of realistic parameter values, the crucial number of CIN genes is as low as 1–10.

So, under certain conditions, and if the number of dominantly acting CIN genes in the human genome exceeds a certain number, it should be possible for CIN to inactivate the first tumour-suppressor gene in colorectal cancer. The more time-consuming process — confirming this hypothesis — remains to be undertaken.

Emma Greenwood

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TRIAL WATCH

Infectious enthusiasm

The sexually transmitted human papillomavirus (HPV) is associated with 50% of cervical cancer cases and is considered to be a potent human carcinogen. In the *New England Journal of Medicine*, Laura Koutsky and colleagues show that a vaccine that prevents persistent HPV16 infection can also reduce the incidence of cervical cancer.

In this double-blind study, 768 women of ages 16–23 received three doses of an HPV16 vaccine that consists of virus-like particles — viral coats without the DNA component. A total of 765 other women received a placebo control and the women were followed for a median of 17.4 months after completing the vaccination regimen.

Some 41 cases of HPV16 infection occurred in the placebo group, and these included nine cases of virus-related cervical intraepithelial neoplasia. Amazingly, none of the women that received the vaccine developed persistent HPV16 infections or virus-associated cervical neoplasia. The vaccine is therefore 100% effective in protecting women from HPV16 and preinvasive cancer. It was also well-tolerated and the immunized women generated high levels of antibodies against the virus.

HPV vaccines are urgently needed, as cervical cancer ranks second as a cause of cancer-related deaths in women. More than 450,000 cases are diagnosed each year worldwide, and therapeutic approaches are limited. Although the incidence of this cancer has been reduced by screening, 50% of cervical cancers that occur in the United States develop in women who have been screened. Furthermore, many women in developing countries do not have access to screening programmes. A safe and effective HPV vaccine could therefore overcome these obstacles to cervical cancer prevention. There is no evidence, however, that this vaccine will reverse cervical cancer once it has developed.

Nearly 20 different types of HPV have been associated with cervical cancer, and Koutsky *et al*. show that vaccination against one will not protect against another. Similar vaccination approaches might be developed to prevent the spread of these other viruses. The task is not as overwhelming as it seems, as only five HPVs — types 16, 18, 31, 33 and 45 — are responsible for most cervical cancer cases. In an accompanying editorial, Christopher P. Crum (Brigham and Women's Hospital, Boston) predicts that widespread vaccination against these five strains could reduce the number of cervical cancer deaths by 95%.

ORIGINAL RESEARCH PAPER Koutsky, L. A. *et al*. A controlled trial of a human papillomavirus type 16 vaccine. *N. Engl. J. Med*. **21**, 1645–1650 (2002)