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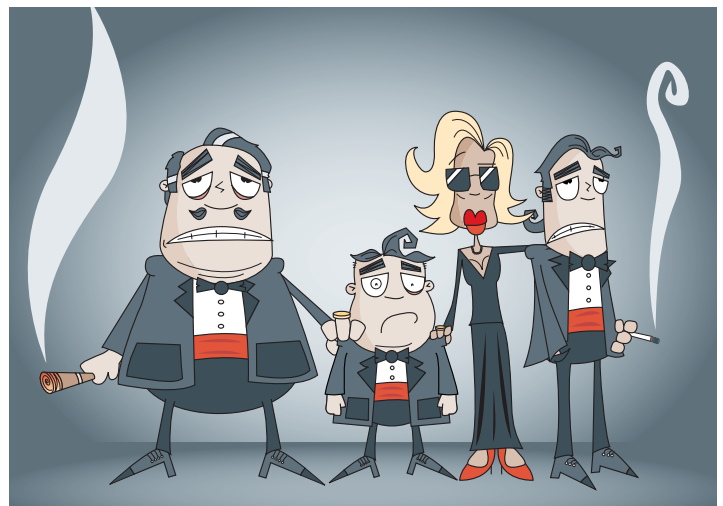
## GENETICS

# Family connections

Cancer pathogenesis often involves a subtle interplay between both genetic and environmental factors. A recent study by Kari Stefansson and colleagues highlights the value of combining cancer incidence statistics with extensive genealogical data to tease out information about the relative contribution of these factors in different cancers and about the familial distribution of cancer.

Using data from the Icelandic Cancer Registry and deCODE Genetics' genealogical database of Icelanders — two large and comprehensive data sets — the authors showed that, in 22 of the 27 cancers studied, close relatives of cancer patients were more likely than the general population to develop the same cancer. Moreover, for 8 of these 22 cancers there was still a significantly increased risk of an individual developing the disease even if only distant relatives (third- to fifth-degree relatives) had cancer.

So which cancers showed this 'familial clustering'? The highest risks (over a threefold increased risk) for close relatives were for lymphoid leukaemia, Hodgkin's disease, and cancers of the thyroid, meninges, lip, testis and larynx. Except for thyroid cancer, these cancers were among the least prevalent sites in the study. This is probably because rarer tumours are more likely to be caused by a trait determined by the action of a single gene with high penetrance



(a Mendelian trait), whereas common cancers are genetically complex. Also, the very fact that they are widespread in the general population means that common cancers have a high baseline frequency, which limits the power of risk estimates for these cancers in subsets of data.

The authors also investigated how the presence of a particular cancer within a family influenced the chances of a relative developing cancer at a different site. They found significantly increased familial clustering between different cancer sites in both close and distant relatives. Stomach and prostate cancer were most commonly associated with other cancers, followed by colon, ovarian and cervical cancers. Although some of the connections between cancer sites could be explained by known high-risk genes in heritable syndromes, others could not. For example, familial clusters were shown between lung, oesophageal, cervical and stomach cancers. The authors propose that this 'excess familiarity' between cancer sites can be explained by gene–environment

interactions. That is, the same environmental factor(s) might interact with the same genetic factor(s) to generate different cancers (for example, smoking in lung and cervical cancer), or different environmental factor(s) might interact with the same genetic factor(s) to induce different cancers (for example, smoking in lung cancer and human papilloma virus in cervical cancer).

It is important to keep in mind that even for the highest-risk cancers, the absolute increased risk for relatives remains small. Nevertheless, genetic counsellors will certainly be interested in this study and the implication that, in some cases, cancer should be considered in a broader familial context. The results are also valuable in that they indicate associations between previously unrelated cancers, so highlighting avenues for further investigation.

*Oliver Childs*

## References and links

**ORIGINAL RESEARCH PAPER** Amundadóttir, L. T. *et al.* Cancer as a complex phenotype: pattern of cancer distribution within and beyond the nuclear family. *PLoS Med.* **1**, e65 (2004)

## IN THE NEWS

**Ready for the end?**

A pilot study in America is investigating if the illegal drug ecstasy will help terminally ill cancer patients to cope better with demanding end of life issues.

Anecdotal stories indicate that people who have taken ecstasy when dying from cancer felt able to talk to family and friends about death-related subjects that they were unable to tackle previously, noted John Halpern, the psychiatrist at Harvard Medical School leading the trial ([www.telegraph.co.uk](http://www.telegraph.co.uk), 13 January 2005). The 4 month Food and Drug Administration approved trial will recruit 12 terminally ill cancer patients to assess if taking ecstasy, which is known to give users a euphoric feeling — increased empathy, energy and sexual arousal — helps alleviate a patient's fear of death.

However, concerns have been raised. "There's more research coming in all the time pointing out that there really is adverse effects of using these illicit drugs", stated Jeanette Tait of the Australian Medical Association Queensland public health committee ([www.theaustralian.news.com.au](http://www.theaustralian.news.com.au), 30 December 2004). Yet others have expressed guarded support for the trial, "...when taken in the context of carefully structured and approved research protocols and facilitated by individuals with expertise, adverse effects can be contained to a minimum", said psychiatrist Charles Grob at Harbour-UCLA Medical Center, Los Angeles (<http://seattletimes.nwsource.com/>, 28 December 2004).

John Halpern summed up the aim of this trial by saying "This is not about trying to create some sensationalistic storm. This is about trying to help these patients in a meaningful way" ([www.cbsnews.com](http://www.cbsnews.com), 27 December 2004).

Nicola McCarthy

## CANCER GENETICS

## Tumour-suppressor super models

Point mutations in the tumour-suppressor gene *TP53* cause Li–Fraumeni syndrome, which predisposes patients to a broad spectrum of malignancies, particularly sarcomas and carcinomas. However, the range of tumours seen in these patients cannot be explained simply by the loss of wild-type p53 function. Now, two research groups have generated mouse models that closely resemble Li–Fraumeni syndrome and have used these models to investigate why the *TP53* mutations seen in a wide range of human cancers are so oncogenic.

Mice that lack p53 develop lymphomas and sarcomas but not carcinomas, and these tumours tend not

to metastasize. Furthermore, p53 is an unusual tumour suppressor because it is commonly altered through missense mutation rather than deletion. So, Kenneth Olive and co-workers produced mice with missense point mutations in two of the most commonly mutated p53 codons in human cancer: *Trp53*<sup>R172H</sup> affects the overall structure of the p53 DNA-binding domain, whereas *Trp53*<sup>R270H</sup> affects a residue that makes direct contact with DNA. Although *Trp53*<sup>R270H/-</sup> and *Trp53*<sup>R172H/-</sup> mice developed distinct tumour spectra, both developed different tumour phenotypes compared with *Trp53*-



knockout mice, indicating that missense *Trp53* mutants have pro-tumorigenic or oncogenic functions that cannot be explained simply by the loss of wild-type p53. In particular, strains carrying these two mutant alleles developed metastatic carcinomas and are therefore more accurate models of Li–Fraumeni syndrome.

The possibility that mice carrying *Trp53* missense mutations are useful models of Li–Fraumeni syndrome was further supported by work carried out by Gene Lang and

## ONCOLYTIC VIRUSES

## Export license

Several viruses have been engineered with the capacity to replicate in and exclusively kill cancer cells, although the precise molecular mechanism behind the selectivity of these oncolytic viruses has not always been clear. While studying the oncolytic adenovirus ONYX-015, O'Shea and colleagues have found that tumour cells have alterations in RNA export pathways, revealing a previously unidentified and therapeutically interesting target that governs the selectivity of this virus.

Cells seem to have evolved a defence mechanism to deal with virus infection — stabilization and activation of the tumour suppressor p53 can provoke the premature apoptosis of the infected cell, limiting both viral replication and spread. Therefore, many viruses have evolved

mechanisms to subvert the host p53 response. Given that the p53 pathway is mutated in a wide range of cancers, ONYX-015 was designed to specifically replicate in tumour cells that lacked a functional p53 pathway — the E1B-55K viral gene product that targets the tumour suppressor p53 for degradation is deleted in this virus. Surprisingly though, ONYX-015 has efficacy in tumour cells irrespective of their p53 status, prompting O'Shea and colleagues to investigate further.

Initially the authors looked at ONYX-015 infection in normal primary human epithelial lines with a wild-type p53 pathway. As expected, ONYX-015 was unable to successfully replicate and p53 was stabilized in the nucleus. Surprisingly though, this stabilized form of p53 was not active and did not induce apoptosis, indicating that viral products other than E1B-55K can restrict p53 activation. So, the authors next addressed the p53-independent functions of E1B-55K, which include the shutdown of host protein synthesis allowing late

viral protein production. By using a set of adenoviruses that were deficient in specific functions of E1B-55K, the authors found that ONYX-015 was unable to induce late viral protein production due to a defect in late viral RNA export from the nucleus. But why should this be different in cancer cells? All the tumour cells that support the full lytic viral replication of ONYX-015 compensated for the defect in late viral RNA export, showing that normal and tumour cells differ markedly in this respect.

These data provide not only a further opportunity to understand how p53 can be inactivated, but also indicate a need to investigate whether the export of late viral RNA shares some characteristics with the export of RNAs important in growth control and tumorigenesis.

Nicola McCarthy

 **References and links**

**ORIGINAL RESEARCH PAPER** O'Shea *et al.* Late viral RNA export, rather than p53 inactivation, determines ONYX-015 tumor selectivity. *Cancer Cell* **6**, 611–623 (2004)

**FURTHER INFORMATION**  
ONYX pharmaceuticals web site:  
[http://www.onyx-pharm.com/products/onyx\\_015.html](http://www.onyx-pharm.com/products/onyx_015.html)

colleagues, who also generated mice that possess the *Trp53*<sup>R172H</sup> structural mutation. The results from the two laboratories show that the same *Trp53* mutation causes different tumour spectra in different mouse strains; whereas Olive and co-workers found that *Trp53*<sup>R172H/+</sup> mice developed more carcinomas than *Trp53*<sup>+/-</sup> mice, the *Trp53*<sup>R172H/+</sup> mice generated by Lang *et al.* developed metastatic tumours.

Lang and colleagues also found that *Trp53*<sup>R172H/R172H</sup> and *Trp53*<sup>R172H/+</sup> mouse embryonic fibroblasts grow faster, have more DNA synthesis and have greater transformation potential than *Trp53*<sup>+/+</sup>, *Trp53*<sup>+/-</sup> or *Trp53*<sup>-/-</sup> cells, again supporting the idea that mutant p53 proteins function differently to wild-type p53. So, how do missense mutant p53 proteins exert their oncogenic effects?

p53 interacts with its family members p63 and p73, which themselves activate several p53 target

genes in response to DNA damage. Both groups found evidence that p53<sup>R172H</sup> interacts with and inhibits endogenous p63 and p73 in cell lines that are derived from mouse tumours expressing this protein. Lang and colleagues also found that the disruption of p63 and p73 causes increased transformation of *Trp53*<sup>-/-</sup> cells and augments DNA synthesis to levels seen in *Trp53*<sup>R172H/R172H</sup> cells. The researchers conclude that the ability of mutant p53 to bind and inhibit p63 and p73 could explain why mutant p53 is more detrimental than the lack of p53, and why *TP53* missense mutations — rather than deletions of *TP53* — are so commonly found in human tumours.

Jenny Bangham

### References and links

**ORIGINAL RESEARCH PAPERS** Lang, G. A. *et al.* Gain of function of a p53 hot spot mutation in a mouse model of Li–Fraumeni syndrome. *Cell* **119**, 861–872 (2004) | Olive, K. P. *et al.* Mutant p53 gain of function in two mouse models of Li–Fraumeni syndrome. *Cell* **119**, 847–860 (2004)

## IN BRIEF

### EARLY DETECTION

Sensitive, non-invasive detection of lymph node metastases.

Harisinghani, M. G. & Weissleder, R. *PLoS Med.* **1**, 202–209 (2005)

Using a nanoparticle-enhanced lymphotropic magnetic resonance imaging (LMRI) technique, the authors compared the magnetic tissue parameters of normal lymph nodes with those of patients with metastases from a range of primary tumour types. They identified unique magnetic tissue parameters that could accurately distinguish metastasis-containing nodes from negative nodes, with a sensitivity of 98% and a specificity of 92%.

### PROGNOSIS

Transcriptional activation of integrin  $\beta 6$  during the epithelial–mesenchymal transition defines a novel prognostic indicator of aggressive colon carcinoma.

Bates, R. C. *et al.* *J. Clin. Invest.* 20 Jan 2005 (doi:10.1172/JCI200523183)

Using a spheroid model of colon carcinoma to identify factors that mediate tumour progression, Bates *et al.* found that upregulation of the integrin- $\alpha\beta 6$  mediates epithelial–mesenchymal transition and tumour invasiveness. An analysis of almost 500 human colorectal carcinoma samples revealed that high expression levels of this receptor were associated with reduced patient survival, making it a useful marker for early-stage disease.

### CANCER STEM CELLS

Sustained hedgehog signalling is required for basal cell carcinoma proliferation and survival: conditioned skin tumorigenesis recapitulates the hair growth cycle.

Hutchin, M. E. *et al.* *Genes Dev.* 29 Dec 2004 (doi:10.1101/gad.1258705)

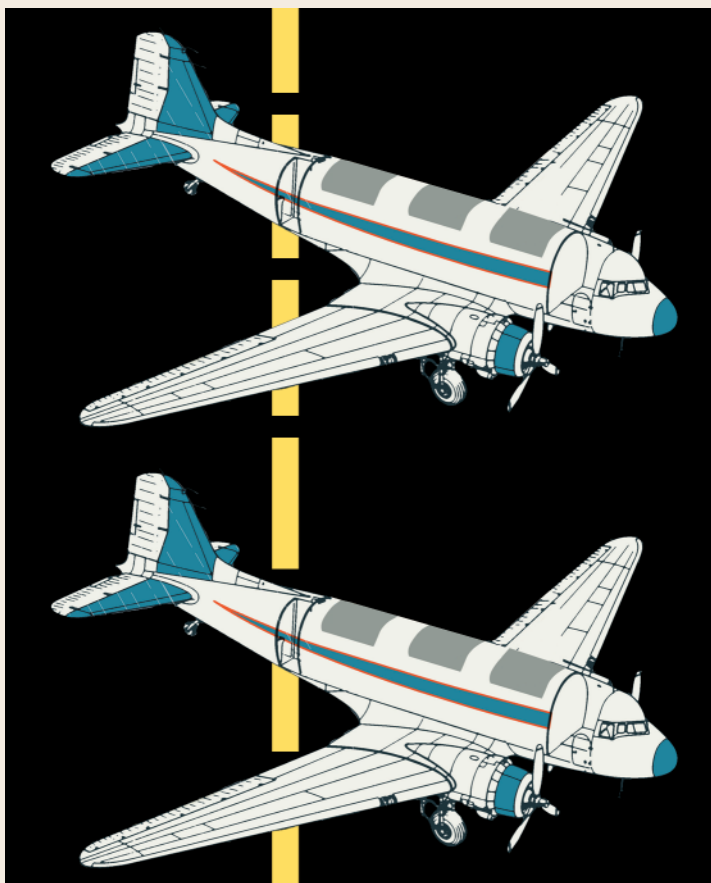
Sustained Hedgehog (HH) signalling leads to the development and maintenance of basal-cell carcinoma (BCC) in the skin. These authors found that a small subset of tumour cells failed to die when HH signalling was inhibited. These cells remained as a non-proliferative population with the capacity to give rise to many epidermal cell lineages and to reform BCCs after reactivation of the HH pathway, indicating that they might be cancer stem cells.

### EARLY DIAGNOSIS

Molecular and genetic analysis of disseminated neoplastic cells in lymphangioleiomyomatosis.

Crooks, D. M. *et al.* *Proc. Natl Acad. Sci. USA* **101**, 17462–17467 (2005)

Lymphangioleiomyomatosis (LAM) causes lung degeneration, renal angiomyolipomas and lymphatic abnormalities. LAM lesions involve the proliferation of smooth-muscle-like LAM cells, which vary in their appearance and are difficult to detect. These authors have developed a cheap and non-invasive way of detecting LAM cells. They show that loss of heterozygosity of the gene tuberous sclerosis complex 2 allows disseminated, potentially metastatic LAM cells to be identified in the body fluids of LAM patients.



## IMMUNOTHERAPY

# Memory

The objective of any T-cell-based cancer treatment is to induce not only tumour eradication, but also to maintain this state long term. This requires the establishment of immunological memory.

Daniel Powell and colleagues have studied the therapy-induced immune response in patients with malignant melanoma that has proved refractory to standard treatments. These patients were treated with immunodepleting chemotherapy (cyclophosphamide and fludarabine) for 7 days before the adoptive transfer of their own *ex vivo* expanded tumour-infiltrating lymphocytes. Responding patients who subsequently had detectable tumour-reactive T cells in both the tumour-infiltrating and peripheral-blood cell populations were studied further.

Absolute numbers of CD8<sup>+</sup> effector T cells peaked 1 week after adoptive cell transfer and high numbers of antigen-specific T cells

persisted in the peripheral blood of all patients nearly 2 months after transfer. Analysis of these cells *in vitro* demonstrated that they had some of the markers associated with effector memory T cells such as CD45RO expression and lack of expression of the chemokine receptor CCR7 along with L-selectin (CD62L). Immediate upregulation of the interleukin 7 receptor- $\alpha$  was also evident, as was the increased expression of the co-stimulatory molecule CD28.

CD27 is another co-stimulatory molecule implicated in T-cell differentiation and memory generation. Initially, in most patients, CD27 was not expressed, consistent with the activation of the patient's T cells *ex vivo*, which leads to the downregulation of this molecule. However, over time, CD27-CD28<sup>+</sup> T-cell subsets were lost and replaced by a stable



population of CD27<sup>+</sup>CD28<sup>+</sup>CD8<sup>+</sup> T cells in patients who showed a T-cell-mediated antitumour response.

Powell and co-authors conclude that transferred T cells that express CD27 *in vivo* early after stimulation *ex vivo* are more likely to survive and to give rise to the effector memory T-cell subset. Although only six patients were assessed, these results indicate that further studies that address why this particular subset of cells persists in these patients are warranted.

Nicola McCarthy

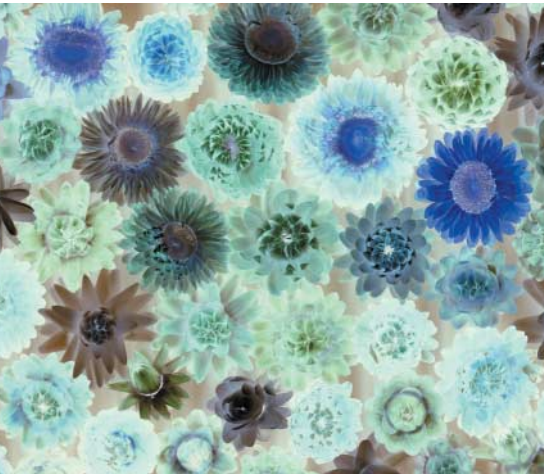
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**ORIGINAL RESEARCH PAPER** Powell, D. J. Jr, Dudley, M. E., Robbins, P. F. & Rosenberg, S. A. Transition of late-stage effector T cells to CD27<sup>+</sup> CD28<sup>+</sup> tumor-reactive effector memory T cells in humans after adoptive cell transfer therapy. *Blood* **105**, 241–250 (2005)

**FURTHER READING** Dudley, M. E. & Rosenberg, S. A. Adoptive-cell-transfer therapy for the treatment of patients with cancer. *Nature Rev. Cancer* **3**, 666–675 (2003)

## DNA REPAIR

# Lethal variations



To trigger cell death, some chemotherapeutic agents cause severe DNA damage; however, if the trigger does not function properly, DNA damage might itself cause rapidly replicating cells to become cancerous. James Allan and colleagues have now identified genetic variation that is associated with the carcinogenic effects of chemotherapy.

The xeroderma pigmentosum group D (*XPD*) gene encodes a DNA helicase involved in nucleotide-excision repair (NER). One common *XPD* variant — the codon 751 lysine-to-glutamine variant — is predicted to affect protein function and alter cellular responses to specific types of DNA damage. Because NER is involved in the pathogenesis of some myeloid leukaemias, Allan and colleagues postulated that the efficiency of *XPD* NER might affect the efficacy of chemotherapy used to treat leukaemia and also the risk of developing leukaemia following chemotherapy for pre-existing cancer.

The researchers tested 341 elderly patients who were being given chemotherapy for acute myeloid leukaemia (AML). Following chemotherapy, survival was significantly lower among patients who had the glutamine variant, leading the researchers to suggest that the *XPD* codon 751 polymorphism could be used as a prognostic marker in elderly AML patients.

The researchers went on to find that the glutamine-encoding allele is significantly over-represented in individuals who develop AML after treatment for a pre-existing cancer with alkylating chemotherapeutic agents, which cause DNA damage that induces the NER machinery. By contrast, they found no association between the glutamine-encoding allele and AML patients who developed the disease after radiotherapy. This makes perfect sense;

radiotherapy-induced DNA single and double-strand breaks do not initiate NER.

But why are people with the *XPD* codon 751 glutamine variant susceptible to developing AML? The researchers suggest that *XPD* is either directly or indirectly involved in triggering alkylating-agent-induced apoptosis, not only in cancerous leukaemia cells, but also in normal bone-marrow cells. One hypothesis is that the codon 751 polymorphism affects the ability of *XPD* to interact with p53 and induce p53-dependent apoptosis. Alternatively, the codon 751 variant might indirectly modulate myeloid-cell death by affecting the efficiency of the NER of chemotherapy-induced pre-toxic DNA lesions.

As such, the inability to trigger cell death following chemotherapy will not only protect leukaemic cells from apoptosis, resulting in the poor prognosis of leukaemia patients, but might also prevent damaged bone-marrow cells from dying, resulting in an increased risk of AML. The authors point out that future work on the pathways that trigger cell death will be required to better understand therapy-related leukaemogenesis.

Jenny Bangham

## References and links

**ORIGINAL RESEARCH PAPER** Allan, J. M. *et al.* Genetic variation in *XPD* predicts treatment outcome and risk of acute myeloid leukaemia following chemotherapy. *Blood* **104**, 3872–3877 (2004)

## CARCINOGENESIS

## Catch-22

BCL2 overexpression leads to inhibition of apoptosis, so promoting carcinogenesis. Now, Ho Jin You and colleagues show that BCL2 also stimulates mutagenesis through its independent function in cell-cycle arrest, by suppressing DNA repair.

The authors first investigated the effect of treatment with the alkylating agent MNNG on a cell line with inducible BCL2. When BCL2 expression was induced the cells were more resistant to MNNG-induced death than control cells, and spontaneous mutagenesis and mutagenesis caused by MNNG were also higher. Decrease of mismatch-repair (MMR) activity correlated with the degree of induction of BCL2. Cells expressing a BCL2 mutant that is unable to induce cell-cycle arrest were more resistant to MNNG mutation than either control cells or cells expressing an anti-apoptotic deficient BCL2 mutant. Moreover, only cells expressing the anti-apoptotic deficient BCL2 mutant had reduced MMR activity. This indicates that BCL2-induced cell-cycle arrest is important for the BCL2-mediated suppression of MMR, but that the anti-apoptotic function is not involved.

So how does BCL2 suppress MMR? The authors discovered that the mRNA of the MMR protein MSH2 was significantly decreased in cells after BCL2 induction. As MSH2 is an E2F-responsive gene, the

authors investigated whether inactivation of this transcription factor could mediate the BCL2-induced suppression of MSH2. BCL2-expressing cells had more E2F1 bound to phosphorylated RB — which keeps E2F1 inactive — than when BCL2 expression was not induced, and binding of E2F1 to the MSH2 promoter was reduced. Moreover, when small interfering RNAs that target E2F1 were added to the cells, MSH2 expression and MMR activity were reduced in BCL2-expressing cells.

You and colleagues confirmed that the same mechanisms of MMR suppression occurred in two human B-cell lymphoma cell lines that overexpress BCL2, but not in another B-cell lymphoma cell line that does not express BCL2. These data indicate that far from cell-cycle arrest protecting cells from oncogenesis, it actually induces mutagenesis. These findings might help to explain why cancer incidence increases exponentially with the ageing process, in which senescent cells (with irreversible growth arrest) accumulate.

Ezzie Hutchinson

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**ORIGINAL RESEARCH PAPER** You, C. *et al.* Bcl-2 expression suppresses mismatch repair activity through inhibition of E2F transcriptional activity. *Nature Cell Biol.* 26 Dec 2004 (doi:10.1038/ncb1215)

**WEB SITE**

Ho Jin You's lab:

[http://www.chosun.ac.kr/~rcpm/korea/si\\_pe\(3\).htm](http://www.chosun.ac.kr/~rcpm/korea/si_pe(3).htm)

## TRIAL WATCH

## Metastasis profiles

A gene-expression signature of primary tumours from patients with head and neck squamous-cell carcinoma (HNSCC) is better at detecting the presence of lymph-node metastases than the current diagnostic procedure. This study is the first to independently validate a primary tumour expression signature that can reliably detect the presence of metastases in local lymph nodes, and might offer patients better therapeutic options.

To identify a predictive gene-expression signature for lymph-node metastases, Frank Holstege and colleagues analysed 82 primary HNSCC tumours, 45 from patients whose tumours were known to be metastatic and 37 from patients known to be lymph-node negative (N0). Genes expressed differentially in at least 30 samples were selected (1,986 genes) and used to build a predictor. A supervised classification approach was applied to establish a classifier without bias towards the training set, and 102 genes were found to be optimal in predicting the presence or absence of lymph-node metastases.

The accuracy of this set of genes was validated on independent tumours. Expression profiles were generated for an additional 22 samples, and the metastatic status of 19 of these was accurately predicted, with no false negatives. This predictor performed better than the current clinical procedure of lymph-node biopsy in these patients (86% versus 68%). The authors also observed that performance increased when more recent samples were analysed, indicating that long-term storage of tumour tissue has adverse effects on gene-expression profile analysis.

Detecting local lymph-node metastases is important in patients with HNSCC, as their presence or absence determines the treatment regimen. Most patients have the primary tumour removed and patients with lymph-node metastases undergo the additional surgical removal of lymph nodes in the neck and other associated muscles, veins and nerves in the region — termed 'radical neck dissection'. Only 10–20% of patients are considered to be N0, although this status is difficult to determine — one-third of patients diagnosed as N0 are found to not be so. As a result, most clinics perform a selective neck dissection on these patients, which involves removal of a restricted set of lymph nodes. Although less rigorous than radical neck dissection, this procedure can cause many complications and results in overtreatment for patients who are truly N0 and undertreatment for patients who are later found to have lymph-node metastases. The findings of Holstege and colleagues can therefore save many patients from unnecessary surgery and significantly improve the treatment for patients who are currently incorrectly diagnosed as N0.

The experiments also identified possible new metastasis-associated genes. Over half of the predictor genes have not been previously associated with tumorigenesis or metastasis, providing starting points for new investigations. These included genes encoding extracellular-matrix and cell-adhesion components, such as members of the plakin family. Surprisingly, more genes in the predictor set were downregulated than upregulated, indicating that a loss of many cellular activities is also an important aspect of metastasis.

**ORIGINAL RESEARCH PAPER** Roepman, P. *et al.* An expression profile for diagnosis of lymph node metastases from primary head and neck squamous cell carcinomas. *Nature Genet.* 37, 182–186 (2005)



## METASTASIS

## Deciphering signatures



What is the basis of the tissue tropism shown by metastatic cells? Joan Massagué and colleagues now report a gene-expression signature that distinguishes breast cancer cells that metastasize to bone from those that metastasize elsewhere.

Massagué and colleagues have previously shown that the human breast cancer cell line MDA-MB-231 has a poor-prognosis metastasis signature and have subsequently identified and validated another set of genes in this cell line that specifically mediates bone metastasis in the mouse. In this study, single-cell-derived progenies (SCPs) derived from MDA-MB-231 were introduced into the arterial circulation of immunodeficient mice. The development of bone metastases was followed by transducing the SCPs with a triple-modality reporter gene and tracking the metastatic cells with bioluminescence imaging and fluorescence microscopy. Bone was the main site of tumour growth, but the SCPs varied in the aggressiveness of their growth in bone and this correlated

with whether or not they expressed the bone-metastasis gene set. A few SCPs grew in the adrenal glands and, if tail-vein injection was used, some SCPs grew in the lung. SCPs that grew well at one site did not necessarily grow well at other sites, consistent with the hypothesis that growth at metastatic sites is increased by genes that confer favourable tumour–stroma interactions at that site.

So are there differences in gene expression that can account for the variability in metastatic activity of the SCPs? The authors found 286 genes that differed more than twofold in their expression between SCPs. Classification of the gene-expression profiles showed that SCPs that had different primary metastatic tropisms — bone or lung — or were weakly metastatic formed distinct clusters. These three clusters were significantly closer to each other than to the profile of a normal human breast epithelial cell line. These data support the idea that distinct gene-expression patterns are responsible for variation in metastatic tropism.

## ONCOGENES

## Addicted to MYC?

Studies in a range of model systems have indicated that tumours can become 'addicted' to the oncogenes that initiated them, so it might be possible to treat cancer by transiently targeting a single dominant pathway. For example, inactivation of *MYC* in experimental tumour systems has been shown to reverse the malignant properties of a range of tumour types. Lewis Chodosh and colleagues report, however, that this is not the case for mammary adenocarcinoma cells, making targeted therapy more of a challenge than expected.

Several transgenic mouse models have been used to show that even advanced-stage tumours can remain dependent on pathways activated by individual oncogenes such as *MYC* or *RAS*. So targeting a single one of these pathways would lead to regression of tumours, in spite of many other genetic and

epigenetic alterations. These findings are at odds with clinical findings, however, as patients with metastatic cancers of epithelial tissues are rarely cured by a single agent or even by combination therapy. Chodosh and colleagues developed a transgenic mouse model of breast cancer that more closely resembles a human epithelial tumour type — as *MYC* amplification occurs in 5–15% of human breast cancers, they used an inducible promoter to overexpress this oncogene specifically in the mammary glands.

These mice develop mammary adenocarcinomas, but in contrast to other mouse models of *MYC* activation, less than half of these tumours regressed following *MYC* transgene downregulation — most of these tumours rapidly acquire the ability to grow in the absence of *MYC* overexpression. Furthermore, over half of the fully regressed tumours recurred spontaneously, without *MYC* overexpression, at the site of the original tumour. And the tumours that did not spontaneously recur did rapidly grow back when *MYC* was briefly re-expressed, indicating the presence of residual cells that are only one step away from re-acquiring their full malignant potential. So

transient inactivation of this oncogene would not be sufficient to stop tumour progression of mammary epithelial cells.

What allows these tumours to survive in the absence of *MYC*? Chodosh's group showed that many *MYC*-independent mammary tumours harboured *KRAS* mutations, but multiple other mechanisms are likely to underlie *MYC* independence. The authors propose that in some types of cancer, such as haematological cancers or sarcomas, *MYC* inactivation leads to cell differentiation, which can prevent tumour recurrence. The fact that mammary tumour cells and other epithelial cell types do not undergo terminal differentiation following *MYC* downregulation might explain their resistance to *MYC* inactivation. So oncogene dependence seems to be context dependent, and common human epithelial cancers are likely to require chronic and combination treatments with targeted agents.

Kristine Novak

 **References and links**

**ORIGINAL RESEARCH PAPER** Boxer, R. B., Jang, J. W., Sintasath, L. & Chodosh, L. A. Lack of sustained regression of c-MYC-induced mammary adenocarcinomas following brief or prolonged *MYC* inactivation. *Cancer Cell* **6**, 577–586 (2004)

To investigate the relevance of these findings to the behaviour of disease in humans, 63 primary breast cancers were examined for expression of 50 genes from the bone-metastasis gene set that are also present in the poor-prognosis signature. Hierarchical clustering did not distinguish between tumours that had metastasized to bone and those that had not. However, when the analysis was restricted to those tumours that were known to have metastasized, the 50-bone-metastasis gene set did distinguish a bone-metastasis cluster from a lung-metastasis cluster.

Confirmatory studies could lead to an accurate predictor of bone-metastasis tropism in primary breast cancers, which would be valuable in the effective management of breast cancer patients.

Ezzie Hutchinson

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**ORIGINAL RESEARCH PAPER** Minn, A. J. *et al.* Distinct organ-specific metastatic potential of individual breast cancer cells and primary tumors. *J. Clin. Invest.* **115**, 44–55 (2005)

#### WEB SITE

Joan Massagué's lab:  
<http://www.hhmi.org/research/investigators/massague.html>



#### THERAPEUTICS

## On the TRAIL of death

Histone-deacetylase inhibitors (HDACIs) have successfully entered clinical trials, but the basis of their antitumour activity is not clear. Two papers published in the January issue of *Nature Medicine* indicate that HDACIs increase the expression of the death-receptor ligand TRAIL in cancer cells, leading to tumour-cell death.

Histone deacetylases (HDACs) regulate transcription by altering chromatin structure and can also modify individual protein function. Their activity is frequently altered in human tumours. The best-characterized example of this is evident in myeloid leukaemia cells, where the oncogenic, chromosomal translocation fusion protein products PML–RAR or AML1–ETO function to silence genes and transform cells by interacting with HDACs.

Insinga and colleagues treated mice with PML–RAR-induced acute promyelocytic leukaemia (APL) with the HDACI valproic acid and compared their response with standard therapy for APL, all-*trans* retinoic acid. Both drugs prolonged the survival of these mice, but through different mechanisms — all-*trans* retinoic acid primarily induced the terminal differentiation of the leukaemic blast cells, whereas valproic acid induced massive blast-cell apoptosis. The authors found that the pro-apoptotic activity of valproic acid is not due to the inhibition of the PML–RAR-induced degradation of the tumour-suppressor gene product p53, but that treatment with HDACIs induces the selective upregulation of the death receptors DR5 and FAS and their cognate ligands TRAIL and FASL. Blocking access of these ligands to their receptors through blocking antibodies prevented valproic-acid-triggered apoptosis *in vitro* and RNA-interference studies confirmed this result *in vivo*.

These authors show further that the effect of HDACIs is reproduced in other mouse leukaemic models and in a subset of freshly isolated human leukaemic blasts.

Nebbio and colleagues examined the action of three HDACIs — including the benzamide derivative MS275 — on a human leukaemic cell line and a large number of blasts from patients with acute myeloid leukaemia (AML). They found that TRAIL expression and resultant apoptosis were induced and, in addition, that MS275 induced cell-cycle arrest, upregulation of the cell-cycle inhibitor p21 (WAF1) and differentiation of the leukaemic cells. To examine the contribution of p21 and TRAIL to HDACI antitumour activity, these authors used RNA interference to knockdown the expression of these proteins. Their results show that p21 specifically induces HDACI-mediated growth arrest and that TRAIL induces the acute apoptotic response through the death-receptor pathway. Moreover, irrespective of their genetic defects, most *ex vivo* cultured AML blasts from patients responded to HDACI exposure. Nebbio and co-workers also showed that MS275 induces the expression of the TRAIL gene *TNFSF10* by inhibiting promoter-resident HDAC1 and HDAC2 and recruiting and acetylating the transcription factors SP1 and SP3, allowing formation of a transcriptionally active complex.

Significantly, both groups show that no apoptosis was evident in the normal myeloid progenitors tested, despite their intrinsically higher levels of TRAIL expression. Both conclude that the HDACI-mediated selective TRAIL expression and apoptosis seen in myeloid leukaemic cells warrants further investigation and has implications for the treatment of other human tumours.

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#### References and links

**ORIGINAL RESEARCH PAPERS** Insinga, A. *et al.* Inhibitors of histone deacetylases induce tumor-selective apoptosis through activation of the death receptor pathway. *Nature Med.* **11**, 71–76 (2005) | Nebbio, A. *et al.* Tumor-selective action of HDAC inhibitors involves TRAIL induction in acute myeloid leukemia cells. *Nature Med.* **11**, 77–84 (2005)

