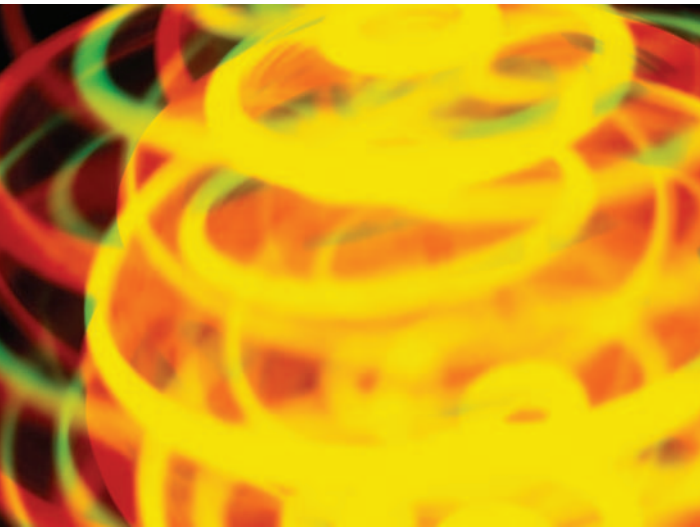


MULTIPLE MYELOMA

Establishing order from chaos



“ the use of this ‘omics’ approach has identified a large number of amplifications and deletions in which the cancer-relevant gene is not yet known. ”

Multiple myeloma (MM) remains largely incurable, with only 10% of patients surviving 10 years after diagnosis. Part of the problem is that MM is a highly heterogeneous disease, characterized by many changes in chromosome structure and number. Ron DePinho and colleagues sought to distinguish the cancer-associated changes in MM more fully by using an integrated oncogenomic approach.

MM, which arises because of a clonal proliferation of plasma (antibody-secreting) cells, can be split into two categories: non-hyperdiploid and hyperdiploid, the latter of which tends to have a better prognosis. DePinho and colleagues initially used high-resolution array comparative genomic hybridization (aCGH) to identify

copy-number aberrations in plasma cells from 67 newly diagnosed patients with MM. The authors developed an algorithm based on unsupervised methodologies and were able to group the aCGH results into a maximum of 4 distinct molecular subclasses (k1–k4). This provided molecular evidence that MM is a heterogeneous disease and that the MM hyperdiploid class can be further subdivided into two molecular subclasses (k1–k2).

The authors then asked whether there was any difference in the survival of patients grouped as k1 or k2 — k1 patients showed longer event-free survival and, to a lesser extent, overall survival. So, what are the chromosomal changes that determine this outcome? Comparison of the k1 and k2 genomic patterns identified several prominent changes indicating that a gain of chromosome 11 (ch11) is associated with a favourable outcome, but a gain of ch1q and/or loss of ch13 is associated with a poor clinical outcome.

Further evidence of the biological significance of the k1 and k2 subclasses was provided by gene-set enrichment analysis of the relevant transcriptomes. Although *TP53*, *KRAS*, *FRAP* and components of the proteasome pathway were altered in both k1 and k2 subgroups, deregulation of pathways such as sonic hedgehog, and deregulation of *RAC1* were only seen in the k2 samples. These findings need further validation.

However, not all of the important changes involved gross chromosomal alterations, so the authors also used the aCGH data to look for discrete minimal common regions (MCRs) that contained recurrent, highly focal copy-number alterations. Eighty-seven of the most disease relevant MCRs comprised 47 DNA amplifications and 40 DNA deletions. Fourteen of these MCRs were associated with poor survival.

As copy-number alterations influence gene-expression levels, the authors used integrated RNA-expression analyses to look for oncogenic expression patterns for every gene altered in an MCR. Of the 2,151 genes analysed, 30% were significantly overexpressed, narrowing down the search for oncogenic candidates in MM. These included genes with a known function in MM such as *MYC*, *ABL1* and *MCL1*, and other genes with no previous link to MM, including anaphase-promoting complex subunit 2 and F-Box protein 3, and many genes involved in ribosome biogenesis and protein synthesis.

Interestingly, the use of this ‘omics’ approach has identified a large number of amplifications and deletions in which the cancer-relevant gene is not yet known. Therefore the authors suggest that there might be many cancer genes that are yet to be discovered that could provide new therapeutic targets as well as useful prognostic markers for MM.

Nicola McCarthy

ORIGINAL RESEARCH PAPER Carrasco, D. R. et al. High-resolution genomic profiles defines distinct clinico-pathogenetic subgroups of multiple myeloma patients. *Cancer Cell* 9, 313–325 (2005).

RESEARCH HIGHLIGHTS ADVISORS

AVI ASHKENAZI Genentech, Inc., South San Francisco, CA, USA
JOSE BASELGA Vall d'Hebron University Hospital, Barcelona, Spain
ANTON BERNIS Netherlands Cancer Institute, Amsterdam, The Netherlands

MARIA BLASCO Spanish National Cancer Centre (CNIO), Madrid, Spain
RON DEPINHO Harvard Medical School, Boston, MA, USA
GLENN DRANOFF Dana–Farber Cancer Institute, Boston, MA, USA

RAKESH JAIN Massachusetts General Hospital, Boston, MA, USA
CHRISTOPH LENGAUER Novartis Institute for Biomedical Research Inc., Cambridge, MA, USA
LANCE LIOTTA National Cancer Institute, Bethesda, MD, USA

JOHN D. POTTER Fred Hutchinson Cancer Research Center, Seattle, WA, USA
DAVID SIDRANSKY Johns Hopkins University School of Medicine, Baltimore, MD, USA

BERT VOGELSTEIN The Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD, USA
ROBERT WEINBERG Whitehead Institute for Biomedical Research, Cambridge, MA, USA
ZENA WERB University of California at San Francisco, CA, USA

RNA INTERFERENCE

New targets on the cards

Molecular targets in cancer need to be specific — healthy cells must be left undamaged. Louis Staudt and colleagues have devised an RNA interference (RNAi) screen to discover such specific targets and have tested it on diffuse large-B-cell lymphoma (DLBCL).

Until now most RNAi screens of cancer have uncovered tumour suppressors because they attempt to save cells from a cytotoxic or cytostatic influence. This new screen

uncovers oncogenes because it looks for short RNAs that kill cancer cells.

The authors created a library of inducible RNAi constructs in retroviral vectors, targeting about 2,500 genes. Each construct has a different barcode sequence that allows it to be tracked. The library was introduced into cancer cell lines, and in half the resultant transgenic cells the short RNA was induced. Microarrays containing the barcode sequences were used to measure the abundance of each construct in the two populations. If the abundance of any construct decreases in the induced population relative to the

control then that construct must be affecting the survival of the cells.

To look for specific oncogenes, the authors carried out their screen on four different B-cell-like DLBCL lines — two of the activated type and two of the germinal-centre type. They reasoned that the knockdown of a gene that decreased survival of both lines of one type, but neither line of the other, would be specific and could be targeted without affecting most of the healthy cells.

The most promising target that was uncovered by this approach was CARD11 (which is involved in nuclear factor κ B (NF κ B) activation) the knockdown of which only affected activated B-cell-like DLBCL cells. The authors carried out further experiments to validate this target, including monitoring the knockdown with green fluorescent protein (GFP) and using microarrays to assay



GENOMIC INSTABILITY

Beyond Boveri

The gain or loss of whole chromosomes (aneuploidy) is the most frequently identified genomic abnormality in cancer, but the mechanism through which this arises is poorly understood. Now, Thea Tlsty and colleagues show that the generation of aneuploid daughter cells can result from the generation of too many centrosomes through a pathway that involves the tumour suppressor p16^{INK4a}.

The classic hypothesis by Theodor Boveri, published in 1914, proposed that aneuploidy results from an increase in the number of centrosomes — organelles that organize the poles of the mitotic spindle. Tlsty and colleagues investigated this further through the use of a variant population of primary human mammary epithelial cells (vHMECs) that accumulate additional centrosomes with continued population doublings and become aneuploid. They showed that the additional centrosomes are not due to polyploidy, and that inhibiting DNA synthesis with hydroxyurea (HU) led to the acquisition of too many centrosomes in these cells, which indicates that

“ p16^{INK4a} has a key function in centrosome biology ”

centrosome duplication and DNA replication are uncoupled.

The loss of p16^{INK4a} expression is a distinguishing characteristic of vHMECs, so could this have a causal role in the acquisition of additional centrosomes? RNA interference with the mRNA that encodes p16^{INK4a} in normal HMECs (which express p16^{INK4a} and do not accumulate additional centrosomes) resulted in an increased proportion of HMECs with additional centrosomes after exposure to HU. Conversely, transfection of vHMECs with a plasmid that expresses wild-type p16^{INK4a} inhibited the generation of additional centrosomes following exposure to HU. So, the acquisition of additional centrosomes because of the loss of p16^{INK4a} activity most probably explains the production of aneuploid daughter cells.

Closer examination of the additional centrosomes by immunocytochemistry revealed that, although still functional, a statistically significant fraction contained only one centriole. This indicates that these additional centrosomes are generated by centriole-pair splitting and that p16^{INK4a} must therefore prevent this during S phase.

Cyclin-dependent kinase 2 (CDK2) is known to regulate DNA synthesis and centrosome duplication, so does the

p16^{INK4a} pathway involve CDK2? Tlsty and colleagues showed that inhibiting CDK2 activity prevented the acquisition of additional chromosomes in vHMECs treated with HU. Furthermore, their data indicate that p16^{INK4a} regulates CDK2 activity by interacting directly with the cyclin-dependent kinase inhibitor p21.

So, p16^{INK4a} has a key function in centrosome biology, acting through CDK2 to prevent centriole-pair splitting and to couple the DNA-replication and centrosome-duplication cycles. Loss of p16^{INK4a} could give cells a proliferative advantage under stress conditions: the transient inhibition



the reduction in the expression of NF κ B-pathway genes that is caused by *CARD11* knockdown.

This revealed that *CARD11* has an important role in B-cell lymphoma and is a potential molecular target, and also demonstrated a new method for target discovery.

The authors propose that such screens will be used to classify cancers according to the proteins that are responsible for proliferation and death in each type. It might also reveal pathways previously not known to be involved in these processes. This will aid the selection of targets for drug discovery and also assist in the search for the mutations that underlie each cancer type.

Patrick Goymer

ORIGINAL RESEARCH PAPER Ngo, V. N. et al. A loss-of-function RNA interference screen for molecular targets in cancer. *Nature* 29 March 2006 (doi:10.1038/nature04687)

of DNA synthesis would lead to the acquisition of additional centrosomes and, consequently, the generation of aneuploid daughter cells. This disruption in gene dosage might provide the necessary pro-proliferation and anti-apoptotic mechanisms required for tumorigenesis.

Rebecca Robey,
Assistant Editor, Oncogene

ORIGINAL RESEARCH PAPER McDermott, K. M. et al. p16INK4a prevents centrosome dysfunction and genomic instability in primary cells. *PLoS Biol.* 4, 350–365 (2006)

WEB SITE

Thea Tlsty's home page: http://cc.ucsf.edu/people/tlsty_thea.html



EXPRESSION PROFILING

Developing patterns

Gliomas are currently diagnosed using histopathological criteria; the main prognostic factors are tumour grade and age of the patient. There is now some evidence that gliomas might arise from neural-stem-like cells, so Heidi Phillips and colleagues set out to define the pattern of disease progression in gliomas in relation to stages of neurogenesis, and to establish molecular-signature prognostic models.

First, the authors profiled 76 samples from newly diagnosed cases of anaplastic astrocytoma and glioblastoma using microarrays. Three clusters of tumour samples were identified with differential expression of 108 survival-related genes — 35 of which were robust markers of the three tumour subtypes. One subtype was found to express genes associated with the normal brain and the process of neurogenesis (known as the proneural subtype), another expressed proliferation-associated genes (the proliferative subtype) and a third subtype expressed genes associated with angiogenesis and mesenchymal origin (the mesenchymal subtype). Patients with the proneural subtype had better prognosis (median survival 174.5 weeks) than those with either the proliferation (60.5 weeks) or mesenchymal subtypes (65 weeks). Profiling of an independent set of 31 glioblastoma cases confirmed the prognostic value of this classification. The authors then compared the signatures of 26 pairs of matched specimens — primary and recurrent astrocytoma from the same patient — and found that on recurrence tumours tend to shift towards the mesenchymal phenotype.

So, what are the main features of each subtype, and how do they relate to neurogenesis? The poor prognosis subtypes showed features of tumour cell proliferation or angiogenesis that were almost absent from the better-prognosis proneural subtype. In addition, although poor-prognosis subtypes expressed markers of undifferentiated neural stem cells and/or transit amplifying cells (progenitor cells), the proneural subtype expressed markers of neuroblasts or neurons. At the genomic level, most of the proliferative and mesenchymal tumours had losses on chromosome 10, which includes the *PTEN* (phosphatase and tensin homologue) locus, and gains on chromosome 7, which contains the *EGFR* (epidermal-growth-factor receptor) locus, whereas proneural tumours did not have these alterations. Notch pathway elements, including *DLL3*, were



overexpressed in proneural tumours. The authors then investigated whether the phenotypes associated with changes in the Notch pathway and the AKT pathway (which is activated by changes in *PTEN* and *EGFR*) were directly associated with patient survival, and found that levels of *PTEN* and *DLL3* mRNA formed a highly significant predictive model of survival in high-grade astrocytoma. These findings are intriguing, as both the Notch and AKT pathways have been implicated as key regulators in neurogenesis.

The authors propose a model for human gliomas in which all molecularly defined subtypes arise from cell types of similar origin, but that differential activation of signalling pathways leads some tumours to maintain more undifferentiated neural-stem-cell-like or transit-amplifying-cell-like phenotypes, whereas others adopt a phenotype closer to that of neuroblasts or immature neurons. Further confirmation of the correlation between stem-cell biology and glioma aggressiveness should produce a useful molecular-signature prognostic model.

Ezzie Hutchinson

ORIGINAL RESEARCH PAPER Phillips, H. et al. Molecular subclasses of high-grade gliomas predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell* 9, 157–173 (2006)

In the news

EARLY OPTIMISM

Trials of lapatinib ditosylate, a new drug for herceptin-resistant breast cancer, have proved so beneficial that GlaxoSmithKline (GSK) has ended the study early and is proceeding to obtain regulatory approval.

Only one in five breast tumours respond to herceptin, so there is an urgent need for alternative therapies. In this phase III study, 321 women with advanced herceptin-resistant breast cancer were given lapatinib ditosylate in combination with capecitabine, an existing chemotherapeutic agent. GSK say that they have observed a 50% delay in cancer growth in the treated women and have therefore, in consultation with the Independent Data Monitoring Committee, halted patient recruitment. "We are extremely encouraged by these data, which suggest that Tykerb [lapatinib ditosylate] may offer significant benefit as an oral medication in combination with chemotherapy" said Paolo Paoletti of GSK (<http://www.timesonline.co.uk>, 4 April 2006).

This means that the drug might become available much sooner than expected. "On the basis of this and other data we now plan to file [for regulatory approval] in the US and Europe during the second half of 2006" said Paoletti (<http://www.washingtonpost.com>, 10 April 2006).

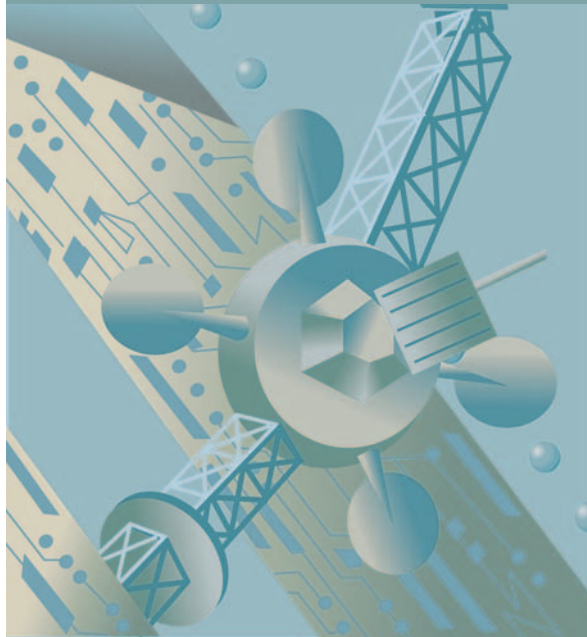
Lapatinib ditosylate is a small-molecule dual-kinase inhibitor that targets both EGFR and ERBB2. For this reason, it could also be used in lung cancer, and GSK are actively pursuing this possibility. Another advantage is that, unlike traditional chemotherapy, it is orally active.

Sarah Rawlings, of the UK's Breakthrough Breast Cancer, expressed cautious optimism from those outside the pharmaceutical industry: "This could be another option in the increasing armoury of breast [cancer] drugs available. We look forward to hearing in more detail the possible benefits and side-effects" (<http://www.eveningnews.co.uk>, 4 April 2006).

Patrick Goymer

LUNG CANCER

Transducing a mutant signal



The aberrant activation of the epidermal-growth-factor receptor (EGFR) is a contributing factor to the development of lung cancer. James Alvarez and colleagues now show that the EGFR-mediated activation of one member of a family of transcription factors seems to be crucial for this process.

The activation of EGFR induces a cascade of signal-transduction pathways that includes the signal transducer and activator of transcription (STAT) family. There is good evidence that STAT3 (and STAT5) is activated in response to EGFR activity, so the authors investigated whether STAT3 activity is necessary for the oncogenic effects of mutant EGFR.

STAT3 is activated as a result of phosphorylation on tyrosine 705

STEM CELLS

Distinguishing features

The similarities between normal adult stem cells and cancer stem cells pose a problem for therapies that are designed to target the latter. Now, two papers published in *Nature* have shown that *Pten*-deletion has opposite effects on normal haematopoietic stem cells (HSCs) and leukaemic stem cells (LSCs), making it possible to target this difference with rapamycin to eliminate the LSCs without also damaging HSCs.

The *PTEN* tumour-suppressor gene is often deleted or otherwise inactivated in cancers. Therefore, Sean J. Morrison and colleagues and Linheng Li and colleagues studied the effects of deleting it in stem cells. They used an inducible deletion system in a mouse model and found that deleting *Pten* in adult mice caused myeloproliferative disease followed by frank leukaemia.

HSCs from healthy mice can be transferred to irradiated mice, where they reconstitute multi-lineage bone

“ these results open up the possibility of therapies that target leukaemia stem cells without affecting healthy stem cells. ”

marrow. Both sets of authors tested the ability of *PTEN*-deficient HSCs to do this and found that they initially caused multi-lineage reconstitution but this ability decreased rapidly over time, in marked contrast to control HSCs. This is consistent with the authors' findings that *Pten* deletion increases the proliferation of HSCs above a level that is sustainable in the long term. By contrast, when neoplasms or whole bone marrow from *PTEN*-deficient mice were transferred, the recipients frequently developed leukaemia and died. This implies that *PTEN* is necessary for the maintenance of HSCs but not of LSCs, even though the latter might have developed from the former.

Rapamycin, which targets the *PTEN* downstream effector mTOR, has been used in the treatment of patients with acute myeloid leukaemia. Morrison and colleagues therefore tested the effect of rapamycin on their *PTEN*-deficient

and serine 727. The authors showed that this phosphorylation is dependent on EGFR kinase activity in NIH3T3 fibroblasts expressing EGFR mutants that are found in non-small-cell lung cancer (NSCLC). However, when verifying these findings in two human NSCLC cell lines with *EGFR* mutations, only phosphorylation of serine 727 proved to be dependent on the kinase activity of mutant EGFR. Nevertheless, the use of short interfering RNAs to inhibit *STAT3*, or dominant-negative *STAT3* mutants, showed that *STAT3* activity enhanced the survival of these NSCLC cell lines. Moreover, inhibition of *STAT3* increased the sensitivity of these cells to the EGFR inhibitor gefitinib.

If *STAT3* does mediate the oncogenic effects of mutant EGFR, then one would expect *STAT3* target genes to be expressed in lung tumours that have mutant EGFR. To analyse this, the authors sequenced *EGFR* in 127 lung

adenocarcinomas for which microarray gene-expression data were already available. By comparing the tumours with the EGFR kinase mutations with those without, the authors were able to show, using a previously identified activated *STAT3* gene-signature set from breast cancer samples, that lung adenocarcinomas with mutant EGFR showed enrichment for the activated *STAT3* gene-signature profile.

The authors conclude that studying mutant EGFR-mediated signal transduction, including activation of *STAT3*, should help to further our understanding of the response of lung tumours to EGFR inhibitors. Their findings also indicate that the use of *STAT3*-based inhibitors might have a beneficial effect.

Nicola McCarthy

ORIGINAL RESEARCH PAPER Alvarez, J. V. *et al.* Signal transducer and activator of transcription 3 is required for the oncogenic effects of non-small-cell lung cancer-associated mutations of the epidermal growth factor receptor. *Cancer Res.* 15 March 2006 (doi: 10.1158/0008-5472.CAN-05-3757).

mice. Treatment with rapamycin prevented the mice from developing leukaemia and also prolonged the life of mice that had established disease. Importantly, bone-marrow cells from these treated mice did not cause leukaemia in transplanted recipients, showing that rapamycin somehow kills the LSCs. These authors also showed that rapamycin restores the function of PTEN-deficient HSCs.

Although the precise mechanism behind these effects remains unclear, these results open up the possibility of therapies that target LSCs without affecting healthy stem cells.

The HSC-like properties of LSCs had previously been thought to make this unfeasible.

Patrick Goymer

ORIGINAL RESEARCH PAPERS Yilmaz, O. H. *et al.* Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. *Nature* 5 April 2006 (doi:10.1038/nature04703) | Zhang, J. *et al.* PTEN maintains haematopoietic cells and acts in lineage choice and leukaemia prevention. *Nature* 23 April 2006 (doi:10.1038/nature04747)



IN BRIEF

▶ EPIGENETICS

A truncating mutation of HDAC2 in human cancers confers resistance to histone deacetylase inhibition

Ropero, S. *et al.* *Nature Genet.* 16 April 2006 (doi: 10.1038/ng1773)

Ropero *et al.* have identified a truncating mutation in histone deacetylase 2 (*HDAC2*) in tumours with microsatellite instability. This mutation causes a loss of *HDAC2* enzymatic activity and renders cells more resistant to the anti-cancer effects of histone-deacetylase inhibitors. So, determining the *HDAC2* mutational status of patients might indicate suitability for treatment with HDAC inhibitors.

▶ TUMOUR SUPPRESSORS

The tumour suppressors Merlin and expanded function cooperatively to modulate receptor endocytosis and signalling

Maitra, S. *et al.* *Curr. Biol.* 16, 702–709 (2006)

How does receptor regulation control cell proliferation? Maitra *et al.* investigate this in developing *Drosophila* imaginal epithelium. They show that the tumour suppressors Merlin (*Mer*) and expanded (*ex*) function together to maintain steady-state levels of signalling and adhesion receptors, and that the loss of these proteins can cause the hyperactivation of associated signalling pathways. Moreover, *Mer/ex* double mutants display defective receptor clearance from the cell surface. So, the loss of these proteins might cause tumorigenesis through the deregulation of cell-surface receptors involved in cell proliferation.

▶ THERAPY

Circulating endothelial cell kinetics and viability predict survival in breast cancer patients receiving metronomic chemotherapy

Mancuso, P. *et al.* *Blood* 16 March 2006 (doi: 10.1182/blood-2005-11-4570)

New approaches are required to monitor the efficacy of anti-angiogenic therapeutic strategies. Mancuso *et al.* show that circulating endothelial cell (CEC) kinetics and viability might predict clinical outcome in patients receiving anti-angiogenic chemotherapy. CEC numbers were significantly increased in patients for whom a clinical benefit was observed compared with those who had no overall clinical benefit. This was because of an increased fraction of apoptotic CECs, most probably arising from the tumour vasculature, in patients with a clinical benefit.

▶ TELOMERES

DNA methyltransferases control telomere length and telomere recombination in mammalian cells

Gonzalo, S. *et al.* *Nature Cell Biol.* 26 March 2006 (doi: 10.1038/ncb1386)

Elongated telomeres and errors in DNA methylation are both common features of cancer cells. Gonzalo *et al.* describe a previously unknown role for mammalian DNA methyltransferases (DNMTs) in telomere-length control. They show that DNMT-deficient mouse embryonic stem cells have elongated telomeres and that their subteleromeric chromatin is demethylated compared with wild-type controls. The loss of DNMTs also resulted in increased telomeric recombination, which might affect telomere length.

Trial watch

REDUCED SKIN RASH WITH EGFR INHIBITOR

Panitumumab is the first fully humanized monoclonal antibody against the epidermal growth-factor-receptor (EGFR) to go into phase III trials for the treatment of colorectal cancer — the first results of this trial were presented at the annual meeting of the American Association for Cancer Research (AACR) on April 3 by Marc Peeters.

A total of 463 patients with metastatic colorectal cancer who had failed standard chemotherapy, including oxaliplatin and irinotecan, and who had $\geq 1\%$ tumour cells positive for membrane expression of EGFR, were randomized to receive panitumumab plus best supportive care (BSC) or BSC alone.

A significant improvement in progression-free survival on panitumumab was seen — patients receiving panitumumab had a 46% lower relative progression rate compared with those receiving BSC alone. A partial response was seen in 8% of patients on panitumumab and 28% had stable disease, compared with 0% with partial response or 10% with stable disease on BSC. In the study, patients who progressed on BSC could crossover to receive panitumumab on a separate protocol — 174 of the 232 patients on BSC crossed over and panitumumab also showed clinical benefit in this group. Interim analysis of overall survival did not show any significant improvement on panitumumab, but this is probably because of confounding by the crossover data. As with other EGFR inhibitors, the most frequent side effect was skin rash, but, unlike with cetuximab, only $< 1\%$ of patients discontinued treatment because of this side effect.

James Abruzzese discussed these data and noted that, although the effect of panitumumab occurred very early in the trial, by 20 weeks the effect seemed to be decreasing rapidly, possibly because resistance to the antibody was emerging. Panitumumab is also being studied in trials with either the FOLFOX or FOLFIRI combination-chemotherapy regimen plus the anti-vascular endothelial-growth-factor antibody bevacizumab, versus the same therapy without the addition of panitumumab.

WEBSITE : <http://www.aacr.org>

ACTIVITY IN IMATINIB-RESISTANT LEUKAEMIA

Dasatinib differs from imatinib in that it binds both the active and inactive forms of the ABL kinase domain of BCR-ABL. It also binds kinases such as SRC, KIT and PDGFR. Because of this less stringent binding activity, it retains activity against many imatinib-resistant kinase-domain mutants. At the annual meeting of the AACR on April 3, Charles Sawyers presented the preliminary results of four ongoing international phase II single-agent studies in chronic myeloid leukaemia (CML) and Ph+ acute lymphoblastic leukaemia (ALL).

The 6-month response rates showed complete haematological response rates of 90% in patients with chronic phase CML, 59% in accelerated CML, 32% in myeloid blast-crisis CML and 35% in Ph+ ALL. The adverse events were similar in all patient groups. Of 481 patients enrolled in these phase II studies, 211 patients had mutations in BCR-ABL. However, 19 patients with mutations at T315I, which is deep in the ATP-binding pocket of BCR-ABL and causes resistance to imatinib, did not respond to dasatinib. There are limited data on patients who relapsed on dasatinib — some patients had the T315I mutation, but others had different mutations not seen after the development of imatinib resistance.

Jose Baselga discussed these data and speculated that the ideal therapy for CML might be a combination of imatinib, dasatinib and a T315I inhibitor. The Food and Drug Administration will review dasatinib for accelerated approval for the treatment of imatinib-resistant leukaemias on June 2.

WEBSITE : <http://www.aacr.org>

MICRORNAS

Tiny new oncogenes

A new screen for the function of human microRNAs (miRNAs) has implicated two of them as oncogenes. Reuven Agami and colleagues have shown that miR-372 and miR-373 co-operate with oncogenic Ras to overcome the need for p53 loss in the transformation of cells, and that this occurs in some testicular germ-cell tumours.

The authors created a library of vectors that expresses most cloned human miRNAs, and also constructed an array with which to detect the expression of the members of this library. They then virally transduced cultured fibroblasts with both their library and oncogenic Ras. Cells with wild-type p53 respond to oncogenic Ras by senescing, and the loss of p53 is usually needed to overcome this antiproliferative response. However, the expression of two miRNAs — miR-372 and miR-373 (which are homologues of each other) — allowed the cells to continue proliferating.

These results were independently validated, and further study showed that cells transduced with these miRNAs were less likely to have a senescent phenotype and had a proliferative advantage even without oncogenic Ras.

To test the mechanism by which this occurs, the authors looked at the levels and activity of p53 in these transformed cells, but found them unchanged. Instead, they found that the activity of cyclin-dependent kinase 2 (CDK2) was not reduced. Normally, the activation of p53 in response to oncogenic Ras leads to the inhibition of CDK2, but this can be relieved by miR-372 and miR-373. So, what are the direct targets of the miRNAs that mediate this effect? The genes that were found to be downregulated by the miRNAs in a microarray study were analysed for miRNA-target sequences. The most promising candidate was large tumour-suppressing homologue 2 (*LATS2*). An immunoblot assay verified that *LATS2* is a target of the miRNAs, and luciferase assays with the 3' untranslated region of the gene showed that *LATS2* is directly downregulated by the two miRNAs. This might be the first step towards a full mechanistic explanation of the phenomenon.

Do miR-372 and miR-373 have a role in the development of tumours in patients? The authors looked for miR-372 and miR-373 expression in primary cells and cell lines from testicular germ-cell tumours, as these are predominantly p53-positive. Most samples expressed the miRNAs, and all of those that did contained wild-type p53. Interestingly, two of the four tumours that did not express the miRNAs had inactivating mutations in the *TP53* gene, an extremely rare event in this type of tumour. By contrast, expression of the miRNAs was rare in somatic-cell tumours.

These results demonstrate a new mechanism for bypassing senescence in p53-wild-type tumours. It remains to be seen how widespread oncogenic miRNAs are, but the study has also provided a system for uncovering the roles of other miRNAs in tumorigenesis.

Patrick Goymer

ORIGINAL RESEARCH PAPER Voorhoeve, P.M. et al. A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumours. *Cell* **124**, 1169–1181 (2006)

FURTHER READING Esquela-Kerscher, A. & Slack, F.J. Oncomirs — microRNAs with a role in cancer. *Nature Rev. Cancer* **6**, 259–269 (2006)

ONCOGENES

How much?

MYC is known to be oncogenic when overexpressed in mouse models, but just how much MYC does it take to make a tumour?

To answer this question, Suzanne Cory and colleagues produced a series of transgenic mice that expressed different levels of MYC under the control of regulatory elements of the mouse *Vav* gene. This gene directs the expression of MYC in diverse haematopoietic cell types, whether early progenitors or mature progeny.

The authors found that the rate of tumour development correlated with the expression levels of MYC: MYC17 animals, which expressed the highest level of MYC, had a median survival time of 8 weeks, compared with 41 weeks for MYC10 and 58 weeks for MYC12 mice, which expressed the lowest levels of MYC.

The nature of the malignancies also depended on the level of expression. MYC17 mice developed disseminated T-cell lymphomas, but all the MYC12 and most of the MYC10 mice developed histiocytic sarcomas composed of

cells from the monocyte–macrophage lineage. Interestingly, these sarcomas resemble rare human histiocytic sarcomas, which can progress to malignant histiocytosis. Whether MYC is involved in this disease is unclear, but such an analysis, state the authors, might be worthwhile.

A small subset of the MYC10 mice developed early onset T-cell lymphomas, much like the MYC17 mice, indicating that MYC might need to exceed a threshold to transform T cells. To investigate this, the authors bred the MYC10 animals to homozygosity. All of these mice developed T-cell lymphomas, showing that a mere doubling of MYC expression levels can change the tumour type.

The authors also found that crossing the heterozygous MYC10 mice with transgenic *BCL2* mice significantly increased the incidence of T-cell lymphomas, indicating that T-cell transformation might well require anti-apoptotic mutations in addition to high levels of MYC expression.

The authors conclude that cells of the monocyte–macrophage lineage are highly susceptible to transformation by a relatively modest increase in MYC expression levels, but there is a higher threshold required for the MYC-mediated transformation of T cells. Further

investigation of these models might identify the MYC-target genes that are required to transform these different cell types.

Nicola McCarthy

ORIGINAL RESEARCH PAPER Smith, D. P. et al. MYC levels govern haematopoietic tumour type and latency in transgenic mice. *Blood* 14 March 2006 (doi: 10.1182/blood.2006.01.0172)



EXPRESSION PROFILING

Small but influential



MicroRNAs (miRNAs) are small non-coding RNA gene products with key roles in regulating the translation and degradation of mRNAs. The biological function of most miRNAs is not fully understood, but miRNAs have been implicated in human tumorigenesis. Curtis Harris and colleagues have now found that lung cancers have unique miRNA-expression profiles that differentiate them from normal lung tissue and correlate with patient survival.

The miRNA-expression profiles of 104 pairs of primary lung cancers and corresponding normal lung tissue were analysed, and 43 miRNAs were found to be differentially expressed between the two groups. A multivariate permutation test showed that the probability of identifying these miRNAs by chance was nil. Several of these miRNAs were associated with fragile sites

— preferential sites of translocation, deletion and amplification — that are often altered in lung cancer. Six miRNAs were found in both non-small-cell lung cancer (NSCLC) adenocarcinomas and squamous-cell carcinomas, and another six were expressed differentially in these two histological types of NSCLC. Solution hybridization detection for mature miRNAs and real-time RT-PCR for precursor miRNAs validated the microarray analysis findings: *has-mir-21* and *has-mir-205* were upregulated and *has-mir-126** was downregulated in lung cancer tissue compared with normal lung tissue.

So, do the miRNA-expression profiles correlate with prognosis of lung cancer patients? Patients with high expression of five specific miRNAs and low expression of one of three other miRNAs, including

has-let-7a-2, had significantly worse prognosis. In addition, three miRNAs — *has-mir-155*, *has-mir-17.3p* and *has-mir-20* — were associated with the survival of 41 patients with stage I adenocarcinoma. Kaplan–Meier survival analysis showed that high *has-mir-155*, low *has-let-7a-2* and disease stage were particularly significant prognostic factors. These expression-signature data were also confirmed using real-time RT-PCR on an independent set of patients with adenocarcinoma.

Some of the miRNAs that were identified in this study have been implicated in other cancers and linked to the regulation of oncogenes — miR-155 is implicated as a collaborator of MYC, and the let-7 family negatively regulates Ras. Additional studies are required to confirm the targets of these miRNAs and elucidate their function in lung carcinogenesis.

Ezzie Hutchinson

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