IN BRIEF

CHEMOTHERAPEUTICS

A bacterial protein enhances the release and efficacy of liposomal cancer drugs.

Cheong, I. *et al. Science* **314**, 1308–1311 (2006)

Bert Vogelstein and colleagues show that *Clostridium novyi-NT*, an attenuated anaerobic bacterium, selectively infects hypoxic tumour microenvironments. Once established in the tumour, the bacteria can increase the release of liposome-encapsulated chemotherapy drugs such as doxorubicin or irinotecan. Using a combination of the attenuated bacteria and liposomal doxorubicin or liposomal irinotecan resulted in complete tumour regression and increased the survival of mice with large, established human colorectal cancer xenografts or colon CT26 mouse tumours. Furthermore, *Clostridium novyi-NT* increased sixfold the concentration of drug that the tumour cells were exposed to. However, treatment with monotherapies or non-liposomal drugs in combination with *Clostridium novyi-NT* did not increase survival or prolong tumour regression. The secretion of liposomase, the *Clostridium novyi-NT*-specific lipase, was shown to be the probable cause of the liposome-disrupting ability of the bacteria. Unlike other bacterial lipases, liposomase can alter lipid bilayer structures and disrupt liposomes *in vitro*. Therefore, the development of *Clostridium novyi-NT* or liposomase as adjuvant therapies combined with liposomal chemotherapy drugs could substantially improve the specific targeting of tumour cells.

DIFFERENTIATION

GATA-3 maintains the differentiation of the luminal cell fate in the mammary gland.

Kouros-Mehr, H. *et al. Cell* **127**, 1041–1055 (2006)

During puberty, the mammary gland differentiates from multipotent progenitor cells into myoepithelial cells or luminal epithelial cells, the cell type from which breast cancer develops. Zena Werb and colleagues now show that the transcription factor GATA3 is essential to this process. Using an RNA-based genome-wide microarray screen they show that Gata3 is the most highly expressed transcription factor in mouse differentiating and mature mammary epithelium. Furthermore, a conditional Gata3 knockout mouse was used to show that the transcription factor is essential for the pubertal development and maintenance of the mammary gland. Gata3 loss in adult mice resulted in the proliferation of undifferentiated cells and the detachment of existing epithelial cells from the basement membrane. Reduced expression of GATA3 in breast cancers is associated with a poor prognosis. This work suggests that GATA3 loss might have a causal role in breast cancer progression and metastasis.

PREVENTION

Prevention of Brca1-mediated mammary tumorigenesis in mice by a progesterone antagonist.

Poole, A. J. *et al. Science* **314**, 1467–1470 (2006)

How does BRCA1 suppress tumorigenesis in hormone-sensitive tissues? Eva Lee and colleagues show that Brca1/Trp53-deficient mice have a defect in the progesterone receptor (PR)-degradation pathway that results in PR overexpression in mammary epithelial cells. Mammary tumorigenesis was prevented in the Brca1/Trp53-deficient mice by treatment with mifepristone, a progesterone antagonist. Therefore, antiprogesterone treatment might to prevent breast cancer in individuals with BRCA1 mutations.

PROGNOSIS

Prognostic proteasome

Proteasome levels in the blood are increased in patients with malignancies that are known to overexpress components of the ubiquitin–proteasome system. A longitudinal study has now shown that measuring the levels of circulating proteasomes in patients with multiple myeloma (MM) has prognostic value.

Orhan Sezer and colleagues used an enzyme-linked immunosorbent assay (ELISA) based on the 20S catalytic core complex of the proteasome to measure proteasome levels in sera from 141 previously untreated patients with MM. Serum samples were also drawn from 50 healthy individuals and from 20 patients with monoclonal gammopathies of undetermined significance. Compared with both these control groups, patients with

MM had significantly increased levels of circulating proteasomes. In addition, patients with active MM had significantly higher levels than those with smouldering disease.

The authors examined the effect of treatment on circulating proteasome levels in patients with active MM who received first-line chemotherapy. Levels declined significantly in patients who achieved a complete or partial remission after treatment, whereas non-responders showed no change. Importantly, treated patients whose circulating proteasome level returned to normal had significantly prolonged survival relative to responders with a high post-treatment level.

The authors also evaluated the potential of the serum proteasome level to predict patient survival. In univariate analyses of samples from

TUMORIGENESIS

Licensed to cause chaos

Mini chromosome maintenance (MCM) proteins 2–7 are part of the machinery that licenses DNA replication. Naoko Shima and colleagues now show that a single amino-acid change in MCM4 induces breast adenocarcinomas in mice.

Using a chemical mutagenesis screen in mice to identify genes that affect chromosomal stability, the authors had previously found a mutation — *Chaos3* — that results in increased levels of micronuclei in red blood cells, an indicator of chromosome instability; the young mice were otherwise normal. *Chaos3* was mapped to a region that includes *Mcm4*, and sequencing showed a T–A transversion in *Mcm4* that results in a single amino-acid change in a residue that is highly conserved and potentially important for MCM protein interaction.

To investigate further, the authors made a mouse line that carries a mutant *Mcm4* allele (*Mcm4⁻*), in which the highly conserved MCM domain is disrupted. *Mcm4⁻* heterozygotes are normal, with a normal level of micronuclei in red blood cells, but the homozygotes are inviable. A series of matings between *Mcm4⁻* and *Chaos3* mice indicated that *Mcm4⁻* is probably a null allele owing to the more extensive mutation of the protein, whereas *Mcm4^{Chaos3}* is a hypomorphic (reduced activity) allele that might have a dominant function in terms of chromosomal instability.

Further experiments in *Mcm4⁻*; *Mcm4^{Chaos3}* and *Mcm4^{Chaos3}*; *Mcm4^{Chaos3}* mouse embryonic fibroblasts indicated that in standard culture conditions there was no evidence of chromosomal instability. However, if the cells were treated with aphidicolin to induce DNA replication stress, increased levels of chromosomal

patients with active MM, circulating proteasome level was a significant prognostic factor, as were the established indicators β 2-microglobulin, C-reactive protein, chromosomal deletion 13q14 and high-dose chemotherapy. Circulating proteasome level proved to be the most powerful independent prognostic factor on the basis of a multivariate analysis.

This study has convincingly shown that the circulating proteasome level is correlated with advanced disease in patients with MM, is sensitive to disease activity and is useful in predicting survival. Further studies are warranted to establish the value of this marker in monitoring patients treated with the new class of drugs that directly inhibit the ubiquitin–proteasome system.

Edward Wawrzyniczak

ORIGINAL RESEARCH PAPER Jakob, C. *et al.* Circulating proteasome levels are an independent prognostic factor for survival in multiple myeloma. *Blood* 9 November 2006 (doi: 10.1182/blood-2006-04-016360)

breaks were seen. Western blot analyses indicate that the *Mcm4*^{Chaos3} mutation destabilizes the MCM4 protein, and levels of MCM7 were also reduced.

Analysis of the *Mcm4*^{Chaos3} mutation in an inbred mouse strain indicates that this allele is oncogenic in females. Most homozygous *Mcm4*^{Chaos3} virgin mice developed breast adenocarcinomas by 12 months of age.

It has been proposed that a reduced number of licensed DNA replication origins might compromise the fidelity of DNA replication (and therefore chromosomal stability), especially in conditions of replication stress. These findings add weight to this argument and show that the MCM proteins could function as tumour suppressors. Further investigations are required to address why *Mcm4*^{Chaos3} mice are prone to mammary tumours.

Nicola McCarthy

ORIGINAL RESEARCH PAPERS Shima, N. *et al.* A viable allele of *Mcm4* causes chromosomal instability and mammary adenocarcinomas in mice. *Nature Genet.* AOP 3 December 2006 (doi: 10.1038/ng1936)

TUMOUR MICROENVIRONMENT

Remodelling resistance

Patients with glioblastoma invariably fail to achieve long-term survival, which is thought to result from acquired resistance to chemotherapeutics or radiation, and is associated with the expression of transglutaminase 2 (TG2). Keith Rich, David Piwnica-Worms and colleagues show that small-molecule inhibitors of TG2 can overcome resistance to chemotherapy.

TG2 has many functions, including stabilizing fibronectin in the extracellular matrix (ECM). This is thought to enable the interaction of fibronectin with cellular integrins, setting up an anti-apoptotic cell–ECM signalling network that promotes glioblastoma cell survival. Indeed, the inhibition of TG2 in combination with the chemotherapeutic alkylating agent *N,N'*-bis(2-chloroethyl)-*N*-nitrosourea (BCNU) in a mouse glioblastoma xenograft model has previously been shown to increase apoptosis and reduce tumour weight. However, how the inhibition of TG2 activity sensitizes glioblastoma cells to chemotherapy remains unclear.

Using frozen anaplastic astrocytoma and glioblastoma samples, the authors show that TG2 activity and expression is significantly increased compared with normal brain tissue. The increased expression of TG2 in the ECM correlated with an abnormally dense linear assembly of fibronectin. Moreover, using fresh glioblastoma tissue they showed that TG2 and fibronectin are also overexpressed and abnormally organized in regions of the brain that have infiltrating tumour cells. So, is the abnormal expression of fibronectin functionally significant and caused by the increased expression of TG2? The authors show that the TG2 small-molecule inhibitor KCC009 prevents the assembly of fibronectin into dense strands along the cell surface of U87MG glioblastoma cells. Furthermore, mice with orthotopic glioblastoma (derived from injected DBT-bioluminescent glioblastoma cells) that were treated with KCC009 had reduced abnormal fibronectin expression that correlated with reduced TG2 activity. The relationship between fibronectin assembly and TG2 activity was also confirmed by RNA interference of TG2 in U87MG cells.

Finally, the authors investigated whether KCC009 could sensitize DBT orthotopic tumours to BCNU. Using bioluminescence imaging, they showed that only KCC009 in combination with BCNU resulted in a significant reduction in tumour size. The tumours were analysed for levels of apoptosis, and mice treated with KCC009 and BCNU combination therapy had increased levels of apoptosis compared with mice treated with each drug separately. Similarly, monotherapy failed to increase the survival of the mice, unlike treatment with KCC009 and BCNU.

Remodelling the ECM is thought to provide a permissive environment for tumour growth. The authors suggest that KCC009 prevents the TG2-dependent remodelling of fibronectin, thought to function in pro-survival signalling in glioblastomas. Therefore, targeting factors that promote permissive remodelling of the ECM, such as TG2, could be a new approach to sensitizing glioblastoma cells to chemotherapy.

Gemma Alderton

ORIGINAL RESEARCH PAPER Yuan, L. *et al.* Transglutaminase 2 inhibitor, KCC009, disrupts fibronectin assembly in the extracellular matrix and sensitizes orthotopic glioblastomas to chemotherapy. *Oncogene* 13 November 2006 (doi:10.1038/sj.onc.1210048)