Biology

Cancer

EMSY: the missing link in sporadic breast cancer?

A recent study [1] has made headway towards elucidating the role of BRCA in non-familial breast cancers. Using a yeast two-hybrid screen, a team led by Tony Kouzarides has identified a protein, EMSY, which has the ability to interact with the N-terminal transactivation domain of BRCA2. EMSY appears to be an entirely novel protein; however, it does contain an evolutionarily conserved domain, ENT, which is also present in some *Arabidopis* proteins. In addition, EMSY has the ability to bind proteins possessing a 'Royal Family' domain, suggesting that it is involved in chromatin remodelling.

The study showed that EMSY can both repress BRCA2 mediated transactivation and potentially modulate the ability of BRCA2 to repair DNA damage. These results indicate that EMSY might antagonize BRCA2 function. The *EMSY* gene maps to a region, 11q13.4-5, which is frequently amplified in sporadic breast cancers. Accordingly, *EMSY* was found to be amplified in 13% of breast cancers analyzed, with a co-incident increase in the mRNA expression level.

These suggest a mechanism whereby BRCA could be affected in sporadic breast cancer in the absence of gene mutation. The fact that EMSY and BRCA2 physically interact and regulate similar biological functions, taken in conjunction with the observation that BRCA mutation and EMSY amplification are both associated with the same pathology, indicate that these proteins are components of a common pathway. The goal now will be to determine how this pathway is subverted during tumourigenesis and whether EMSY is indeed the missing link in sporadic breast cancer.

1 Hughes-Davies, L. *et al.* (2003) EMSY links the BRCA2 pathway to sporadic breast and ovarian cancer. *Cell* 115, 523–535

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Targets and Mechanisms

Regulation of the IκB kinase complex by multiple ubiquitination events

In a recent study, Tang *et al.* describe activation of the IκB kinase (IKK) complex by ubiquitination of the regulatory subunit IKKγ in response to TNF-α [2]. This finding demonstrates that IKK activity can be regulated by covalent modification other than phosphorylation and provides a rationale for the ubiquitination-dependence of IKK activity observed during the identification of this kinase.

Now, Carter *et al.* report that the catalytic subunit IKKβ, too, undergoes signal-induced ubiquitination [3]. They found that, unlike ubiquitination of IκB and IKKγ, which each receive a chain of ubiquitin monomers, IKKβ ubiquitination appears to involve the attachment of only a single molecule of ubiquitin. This monoubiquitination depends on prior phosphorylation of IKKβ on serine residues in the activation loop, suggesting that phosphorylation and ubiquitination are closely coordinated events in the activation of this kinase.

Thus, IKK seems to be regulated by polyubiquitination of IKKγ as well as monoubiquitination of IKKβ. Although activation of TAK1, a serine–threonine protein kinase upstream of IKK in IL-1/Toll-like and TNF receptor signaling, had previously been shown to be ubiquitination-dependent, IKK is the first NF-κB-activating kinase found to be modulated by direct ubiquitination as TAK1 is not itself ubiquitinated. Instead, according to a recent publication [4], two adaptor proteins (TAB2 and TAB3), which are involved in TAK1 activation and link TAK1 to TRAF6, appear to be ubiquitinated by this ubiquitin ligase in response to interleukin-1.

2 Tang, E.D. *et al.* (2003) A role for NF-kappaB essential modifier/IkappaB kinase-gamma (NEMO/IKKgamma) ubiquitination in the activation of the IkappaB kinase complex by tumor necrosis factor-alpha. *J. Biol. Chem.* 278, 37297–37305
3 Carter, R.S. *et al.* (2003) Signal-induced ubiquitination of I kappaB kinase-beta. *J. Biol. Chem.* 278, 48903–48906
4 Ishitani, T. *et al.* (2003) Role of the TAB2-related protein TAB3 in IL-1 and TNF signalling. *EMBO J.* 22, 6277–6288

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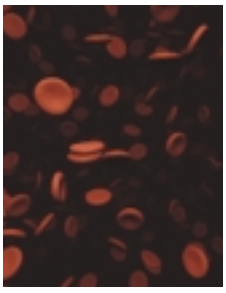
G_{13} protein: an essential mediator of platelet activation



Platelet activation has a major role in hemostasis and also underlies arterial thrombosis leading to myocardial infarction or stroke. Several agonists, including adenosine diphosphate, thrombin and thromboxane A_2, cause platelet activation that involves a shape-change reaction, during which they become spherical and extrude a pseudopodia-like structure, facilitating the secretion of granulate contents as well as inside-out activation of the fibrinogen receptor, integrin $\alpha_{IIb}\beta_3$, which results in platelet aggregation. Such activators activate receptors that are coupled to heterotrimeric G proteins.

However, it has been reported that $G\alpha_{12}/G\alpha_{13}$ are activated by various platelet stimuli. Although $G\alpha_{12}$ and $G\alpha_{13}$ are similar in amino acid sequence and biochemical properties and are involved in Rho activation, studies indicate that they have functional differences. The most striking difference was demonstrated in $G\alpha_{13}$ gene knockout mice, which showed embryonic lethality due to the defect of vascular system formation.

To avoid embryonic lethality of mice lacking $G\alpha_{13}$ and to study whether G_{12} and G_{13} proteins have a specific role in platelet activation, Moers *et al.* [5] used *Cre/loxP*-mediated recombination to conditionally inactivate *Gna13*, the gene encoding $G\alpha_{13}$ alone or in a $G\alpha_{12}$-deficient background. They found that lack of $G\alpha_{13}$, but not $G\alpha_{12}$, is required for the induction of shape-change and aggregation of platelets in response to activators. However, the defective aggregation response is not caused by impaired inside-out activation of the fibrinogen receptor, integrin $\alpha_{IIb}\beta_3$.

This paper provides evidence that $G\alpha_{13}$ has an important role for platelet activation in hemostasis and thrombosis. Furthermore, as $G\alpha_{13}$ deficiency protected animals against thrombosis, the inhibition of $G\alpha_{13}$-mediated signaling pathway in platelets

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could be a promising strategy to prevent or treat platelet activation in thrombosis.

5 Moers, A. *et al.* (2003) G_{13} is an essential mediator of platelet activation in hemostasis and thrombosis. *Nat. Med.* 9, 1418–1422

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Cellular garbage removal: regulation of aggresome formation by HDAC6

Aggresomes are a deposition of misfolded and aggregated proteins that can not be degraded close to the microtubule-organizing centre (MTOC) in the cytosol. Aggresomes are formed upon dynein-mediated transport of their constituents along microtubules.

In a recent study [6], Kawaguchi *et al.* show that a histone deacetylase, HDAC6, is a component of aggresomes. HDAC6 is an unconventional histone deacetylase; it is microtubule-associated and regulates microtubule acetylation and chemotactic cell motility, beyond its function in regulation of gene expression and chromatin dynamics.

Using RNAi technology, a correlation between HDAC6 expression and dynein binding could be established, suggesting that HDAC6 recruits cargo, that is, aggregates of misfolded proteins, to dynein, so that these can be transported towards and deposited close to the MTOC. The crucial role of HDAC6 in this process is underscored by the finding that aggresome formation is diminished in HDAC6-knockout cells. Moreover, these cells were found to be hypersensitive to stress induced by misfolded proteins.

A peculiar point of this work is that a form of HDAC6 that is catalytically inactive, but otherwise intact, does not support aggresome formation. This finding could be explored further, as the role of the enzymatic activity of HDAC6 in these processes remained somewhat unclear. It will also be interesting to determine whether gene expression mediated by HDAC6 is affected in the context of aggresome formation, or if the role of this histone deacetylase remains limited to its function in bridging of polyubiquitinated protein aggregates with dynein and their transport along microtubules to the cytoplasmic garbage bin.

6 Kawaguchi, Y. *et al.* (2003) The deacetylase HDAC6 regulates aggresome formation and cell viability in response to misfolded protein stress. *Cell* 115, 727–738

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Bioinformatics

Chloroquine resistance reversers: hydrophobic interaction with PfCRT

Current evidence strongly supports the argument that chloroquine resistance in *Plasmodium falciparum* is caused by mutations in its resistance transporter (PfCRT). A recent computational study by David Warhurst suggests that PfCRT acts as a channel, extruding chloroquine from the parasite food vacuole, its likely site of action.

In this study [7], Warhurst has computed the mean Eisenberg hydropathy values, side-chain volumes and charges of the mutated PfCRT residues in a series of chloroquine-resistant (CQR) and -sensitive (CQS) strains of *P. falciparum*. A strong linear correlation was found between the IC_{50} of desethyl amodiaquine (DAQ) in CQR parasites and the mean hydrophobicity of groups close to the crucial mutated residue K76T. The activity of this drug increases as the hydrophobicity of mutated groups in PfCRT increases. A similar correlation is found between the IC_{50} of DAQ and the mean molecular side chain volumes of the mutated groups near the crucial site. In this case, the drug becomes more active as the side chains become larger.

The findings suggest that DAQ can enter the PfCRT channel, but then binds to it through hydrophobic interaction. This binding prevents DAQ exiting the food vacuole and increases its biological activity.

These finding suggest that PfCRT mutants that have more hydrophilic groups will exhibit more cross-resistance with CQ. Warhurst has predicted the IC_{50} values for DAQ for a series of field isolates based on available PfCRT sequences. These predictions are in good agreement with observed values. If these conclusions are correct, extensive use of amodiaquine might select for such cross-resistant strains.

7 Warhurst, D.C. *et al.* (2003) Polymorphisms in the *Plasmodium falciparum* chloroquine-resistance transporter protein links verapamil enhancement of chloroquine sensitivity with the clinical efficacy of amodiaquine. *Malaria J.* 2, 31–43

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Neuroscience

Endocannabinoid-induced inhibition under physiological conditions

Endocannabinoids are released from postsynaptic neurons to inhibit synaptic activity. A robust endocannabinoid-mediated retrograde inhibition is now demonstrated in response to synaptic stimulation in the cerebellum that did not spread to other synapses.

Brown *et al.* [8] took transverse sections of rat cerebellar vermis for whole-cell current clamp recording of Purkinje cells after stimulation of parallel fibers without blocking inhibitory currents. Presynaptic fiber stimulation evoked endocannabinoid inhibition, which was completely blocked by the CB1 receptor antagonist AM251.

The measurement of response in the postsynaptic cell is an indirect measure of endocannabinoid effects as they inhibit transmission at the presynaptic terminal, so the authors measured the calcium influx in the parallel fibers. The results show reproducible inhibition of presynaptic calcium entry that is blocked by AM251 and the CB1 receptor agonist, WIN 55,212-2, produced a maximum inhibition. When presynaptic calcium was measured with simultaneous postsynaptic potential recording, the AMPA receptor antagonist, NBQX and the mGluR1 antagonist, CPCCOEt, reduced the endocannabinoid inhibition of transmission.

Stimulation of the parallel fibers evoked a spatially and temporally restricted endocannabinoid response in the Purkinje cell dendrites with no spread to unstimulated areas. The calcium buffer, BAPTA, and the G-protein inhibitor, GDP-βS, were included in the patch pipette and blocked the inhibition showing that there was no spread of endocannabinoids from adjacent cells. These results have shown direct testing of the presynaptic effect of endocannabinoids.

8 Brown, S.P. *et al.* (2003) Brief presynaptic bursts evoke synapse-specific retrograde inhibition mediated by endogenous cannabinoids. *Nat. Neurosci.* 6, 1048–1057

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Microbiology

New places to hide: Salmonella and dendritic cells

Infections with *Salmonella typhimurium* generally occur via oral uptake of contaminated food or water. It is not fully understood how *S. typhimurium* can spread systemically after oral infection, but phagocytic cells have been considered as possible vehicles. *Salmonella* induces two type III secretion systems (TTSS) to invade and survive inside eukaryotic cells. Most of the corresponding genes are located on *Salmonella* pathogenicity islands 1 (SPI-1) and 2 (SPI-2). SPI-2 deficient mutant strains are dramatically attenuated in systemic virulence and reveal reduced proliferation in infected eukaryotic cells.

S. typhimurium is efficiently taken up by dendritic cells (DC) and bacterial antigens are presented by infected DC. A recent study describes the intracellular activities of this bacterial pathogen within DC [9]. Jantsch *et al.* demonstrate that virulence genes of *S. typhimurium* are induced after uptake by DC. Furthermore, a functional TTSS is assembled by intracellular bacteria residing in the parasitophorous vacuole within DC, influencing intracellular trafficking of the bacteria. Both wild-type and an SPI-2-deficient strain persisted to an equal extent in DC over 24 hours but did not proliferate.

These data suggest that the pathogen-containing vacuole in DC is a unique compartment distinct from that in other phagocytic cells. It does not allow proliferation of intracellular *Salmonella* but is permissive for the biosynthesis and secretion of virulence proteins. This specialized vacuole might be a consequence of the function of DC to sample, transport and present antigens. The authors speculate that this compartment is used by intracellular pathogens for distribution in the host organism. Intracellular persistence in DC might be a requirement for the systemic spread of a pathogen.

9 Jantsch, J. *et al.* (2003) Intracellular activities of *Salmonella enterica* in murine dendritic cells. *Cell. Microbiol.* 5, 933–945

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Miscellaneous

Acute regulation of liver metabolism

How the fasted liver responds to refeeding – where the tissue redirects carbohydrate flux from net glucose production to net up-take for storage and utilization – has been the subject of much research for the past 40 years.

Recently, Chu *et al.* [10] addressed this question when they demonstrated that hepatic glucokinase (GK) moves from the nucleus to the cytosol within 10 minutes of an intraduodenal infusion of glucose into rats. Their studies further revealed that insulin could stimulate GK translocation in the same timeframe and that the GK regulatory protein (GKRP) did not translocate to the cytosol.

Their data suggest that disruption of the GK–GKRP complex and translocation of GK to the cytosol are essential components of the acute response of the liver to a meal. These data are complemented by the report of Jin *et al.* that, during the same acute response, the liver also increases the content of fructose-2,6-bisphosphate (F-2,6-P2), a potent activator of 6-phosphofructo-1-kinase (PFK-1), thus up-regulating glycolysis within minutes of an oral gavage in rats [11].

These data suggest that, during the acute response, the liver specifically upregulates the disposal of glucose via glycolysis by concurrent activation of glucose phosphorylation (GK) and the committing step to glycolysis (PFK-1). This is accomplished without inhibition of FBP-1, even when F-2,6-P2 is high, consistent with earlier observations that hepatic glycogen is synthesized primarily by the indirect pathway during the acute response.

These observations are important because it is precisely this response that appears to be compromised in type 2 diabetes mellitus, in which the downregulation of post-prandial hepatic glucose output fails.

10 Chu, C.A. *et al.* (2003) Rapid translocation of hepatic glucokinase in response to intraduodenal glucose infusion and changes in plasma glucose and insulin in conscious rats. *Am. J. Physiol. Gastrointest. Liver Physiol.* (E-pub ahead of print; http://ajpgi.physiology.org)
11 Jin, E.S. *et al.* (2003) Increased hepatic fructose 2,6-bisphosphate after an oral glucose load does not affect gluconeogenesis. *J. Biol. Chem.* 278, 28427–28433

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Business

Collaborations

Aventis and Avalon form oncology collaboration

Aventis (http://www.aventis.com) and Avalon Pharmaceuticals (http://www.avalonrx.com) have entered into a collaboration for the identification, discovery and validation of druggable screening targets. Avalon will provide Aventis with a subset of its library of >200 identified amplicons that have been discovered through cytogenetic analysis.

Thierry Hercend, Head of Aventis Oncology Research, said: 'Through this alliance… Aventis hopes to generate innovative drugs targeting amplified oncogenes, a promising class of caner drug targets.'

Ken Carter, President and Chief Executive Officer of Avalon, said: 'We are excited about this agreement, which allows us to advance our cancer target

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