RESEARCH HIGHLIGHTS

Mapping receptor tyrosine kinase signalling networks

Receptor tyrosine kinases (RTKs), such as EGFR, PDGFR and c-Met, promote cell survival and proliferation through signalling pathways, including PI3K–Akt, Ras–MAPK, and mTOR–p70S6K. These pathways are commonly dysregulated in human cancers, and the receptors and their downstream effectors remain attractive targets for anti-cancer therapies. Comb and colleagues now use a large-scale proteomics approach to uncover additional members of oncogenic RTK signalling pathways, and evaluate their importance to cancer cell survival (*Sci. Signal.* **3**, ra64; 2010).

Akt, RSK and p70S6K phosphorylate a common basophilic motif (RXRXXS/T); however, its basic nature has complicated efforts to identify substrates by traditional mass spectrometry methods. The authors developed monoclonal antibodies that recognized this phosphorylated motif, and used them, along with anti-phosphotyrosine antibodies, in two different large-scale approaches in cancer cell lines expressing oncogenic EGFR, PDGFRα or c-Met. These techniques permitted identification of over 500 seemingly novel substrates, and revealed a comprehensive and highly interconnected signalling network.

An siRNA-based screen for substrates that modulate cancer cell viability showed that the chaperone protein SGTA — shown here to be a target of Akt2 — was required for the viability of PDGFRα-expressing cells. Additional analyses found that SGTA was critical for PDGFRα stability and that siRNA against SGTA cooperated with the PDGFRα inhibitor Gleevec to kill cancer cell lines. Thus, this study could yield additional targets for the development of anti-cancer therapeutics. EJC

A close look at CLICs

Clathrin-independent endocytosis (CIE) is relatively understudied, compared with clathrin-mediated and caveolar endocytosis. Clathrin-independent carriers (CLICs) are known to bud from the plasma membrane in a dynamin-independent process, and several regulatory and cargo proteins have now been identified. However, basic parameters of CLICs and their contribution to many cellular processes are unknown. A quantitative analysis of CLICs by Parton and colleagues now shows that they mediate the major constitutive endocytic pathway in fibroblasts, and reveals the importance of CLICs in cell migration (*J. Cell Biol.* **190**, 675–691; 2010).

Dynamin inhibitors which blocked all CLIC-independent endocytic traffic enabled the authors to quantify the contribution of the CLIC-dependent CIE pathway to total endocytosis in fibroblasts, showing that CLICs deliver a significant proportion of all endocytic cargo. Electron tomography analyses revealed the complex and heterogenous structure of CLICs, and subcellular fractionation experiments permitted the identification of additional CLIC cargoes. Intriguingly, several cargo proteins have known roles in cell adhesion and migration, suggesting that CLICs might regulate these processes. Indeed, CLICs localized to the leading edge of migrating fibroblasts, and transient inhibition of the CLIC pathway decreased migration in a scratch wound assay. These data show that CLICs are important regulators of plasma membrane integrity and cell migration, and it will be important to determine how CLIC localization becomes polarized in migrating cells. EJC

A DUB in DSB repair

Double strand breaks (DSB) in DNA need to be efficiently recognized and repaired to ensure genomic integrity. Although much is known about the cascade of ubiquitylation events triggered by DSBs, inhibitors of this cascade are less studied. The groups of Durocher and Nakada have now identified a de-ubiquitylation (DUB) enzyme, OTUB1, that suppresses polyubiqitylation mediated by the ligase RNF168 (*Nature* **466,** 941–946; 2010)

DSBs initiate the RNF8-dependent ubiquitylation of histones, which promotes the recruitment of RNF168 and formation of non-degradative K63-linked ubiquitin chains. This in turn leads to recruitment of repair factors such as BRCA1 and 53BP1. The authors identified OTUB1 in an siRNA screen for DUBs whose depletion caused persistence of conjugated ubiquitin foci after ionizing radiation. They found that, conversely, *OTUB1* overexpression reduced chomatin ubiquitylation and 53BP1 focus formation. OTUB1 is known to deubiquitylate K48-linked chains, but overexpression of a catalytically inactive mutant suppressed 53BP1 focus formation as efficiently as wild-type, indicating a non-catalytic role for this DUB. Instead, OTUB1 was found to bind and inhibit the E2 ubiquitin-conjugating enzyme, UBC13, which functions together with RNF168. Importantly, in cells that were rendered defective for DNA repair through inhibitition of ATM, OTUB1 depletion rescued 53BP1 focus formation and repair through homologous recombination. The authors speculate that the physiological role of OTUB1 is to set a threshold for RNF168 activity. CKR

Rotating polarity

In the developing *Drosophila* wing epithelium, planar cell polarity (PCP) directs the orientation of hair outgrowth along the proximal–distal axis. Two new studies shed light on how tissue morphogenesis influences PCP. Eaton and colleagues show that during pupa formation, PCP complex components initially point towards the wing margin and, as development goes on, mechanical forces resulting from a combination of oriented cell divisions, cell elongation and neighbour exchanges re-orients planar polarity in the proximal–distal axis (*Cell* **142,** 773–786; 2010). These events are dependent on the contraction of the hinge region of the wing, as its severing prevents the global re-organization. Using a combination of live-cell imaging and computational analysis, the authors demonstrate that oriented cell divisions and cell elongation are essential for changes in PCP axis orientation, whereas local neighbour exchanges and oriented cell boundary assembly reinforce planar polarity. The authors propose that epithelium cell elongation could drive the realignment of microtubules. Using live-cell imaging of microtubules growing ends, Uemura and colleagues observe such realignment during pupal wing development and show that it depends on the activity of the atypical cadherins, Dachsous and Fat (*Dev. Cell,* 19, 389–401; 2010). Interestingly, Eaton and colleagues also find that loss of Dachsous perturbs-the dynamics observed in the epithelium at pupal stage. How morphogen signalling influences the dynamics of these events will be determined by future research. NLB

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