Meeting report

Cell signaling and cancer
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A report on the Cancer Research UK London Research Institute Special Conference 'Signal Transduction', London, UK, 14-16 May 2007.

Disruption of intracellular signaling is central to many different diseases, including cancer. A conference on signaling mechanisms held recently in London under the auspices of the charity Cancer Research UK looked at cell-signaling pathways in relation to cancer and how they may point to new therapeutic approaches.

Signaling pathways and signaling mechanisms

The canonical Wnt/β-catenin pathway is implicated in both normal development and disease. For example, APC, a component of the canonical Wnt signaling pathway, was first isolated as a tumor suppressor gene in human colon cancer and activating mutations in β-catenin are found in human colon cancer and melanomas. Two distinct receptors are required for its activation: Frizzled, a seven-span receptor, and the LDL-receptor related proteins 5 or 6 (LRP5 or LRP6). Xi He (Children’s Hospital and Harvard Medical School, Boston, USA) discussed the formation and activation of the Frizzled-LRP5/6 receptor complex. Upon binding to Wnt ligands, LRP6 is phosphorylated on serines (S) in multiple proline (P)-rich PPPSPxS motifs, which is sufficient to activate the pathway. He went on to describe how the two serines in PPPSPxS are phosphorylated by two distinct kinases, casein kinase 1 and glycogen synthase kinase 3 (Gsk3β). A role for Gsk3β in the activation of the pathway was something of a surprise, as its textbook role is to inhibit the pathway by promoting β-catenin phosphorylation and degradation. His group has resolved the problem by showing that membrane-associated Gsk3β activates Wnt signaling, whereas Gsk3β in the cytosol has the opposite effect.

The Ras-activated MAP kinase (MAPK) module is part of many signaling pathways, and RAS is one of the genes commonly found mutated in human cancers. The scaffold protein KSRI (kinase supressor of Ras) is involved in the positive regulation of the MAPK pathway. Upon Ras activation, KSRI translocates from the cytosol to become associated with the plasma membrane, where it interacts with the three kinases of the MAPK pathway, Raf, MEK and ERK, to facilitate their Ras-induced activation. Deborah Morrison (National Cancer Institute, Frederick, USA) described a search for proteins that associate with KSRI and might affect Ras signaling, and the identification of one such protein, casein kinase 2 (CK2), using mass spectrometry. She reported that CK2 interacts with the basic surface of the C1 domain of KSRI, and that disruption of this interaction does not interfere with growth-factor-stimulated KSRI membrane association or its binding to MEK and ERK, but does reduce levels of phosphorylation for two members of the mammalian Raf kinases, C-Raf and B-Raf. In vitro assays showed that the negative-charge regulatory regions (N-regions) of C-Raf and B-Raf are in fact substrates for CK2, suggesting that CK2 acts as a Raf N-region kinase participating in the KSRI complex and contributing to ERK activation.

Modeling and imaging of signaling pathways

Although hundreds of unique proteins are associated with the regulation of cell shape, there is no systems-level understanding of the organization and composition of the signaling pathways that affect them. Norbert Perrimon (Harvard Medical School, Boston, USA) reported a high-content RNA interference (RNAi) screen for signaling mediated by small GTPases, which results in changes in cell shape. Hundreds of pictures of treated cells are taken automatically and classified in a compendium of ‘quantitative morphological signatures’. Using this information, genes were assigned to distinct local signaling networks involved in mechanisms...
that regulate cell adhesion, cell tension and cell protrusion, and several signaling networks were identified as possible regulators of particular morphological changes and behaviors. The new RNAi libraries include two to three double-stranded RNAs per gene in two different concentrations; in addition, subsets of RNAi libraries are now available, such as the ‘best annotated Drosophila melanogaster genes’ or ‘D. melanogaster genes that are phylogenetically conserved with mammalian genes’.

Olli Kallioniemi (Turku Centre for Biotechnology, Turku, Finland) reported RNAi investigations of prostate cancer development. Using libraries of 20,000 small interfering RNAs, RNAi of living cancer cells identified genes essential for the growth of androgen-independent prostate cancer cells. He described a novel RNAi screening platform using miniaturized arrays of 10,000 spots of 150–250 cells each, which can be assessed for parameters such as cell numbers, cell death, or cell cycle behavior. The technique has proved efficient and reproducible, and is compatible with standard analyses and commonly used scanners, microscopes and imagers. Using this platform, breast cancer cell lines have been screened by RNAi and the results combined with data from tumors to reveal genes that are coexpressed in aggressive breast cancers in vivo.

Live-cell imaging is a valuable tool to investigate the dynamics of cellular processes. Discussing the development of biosensors for imaging signaling and cell motility, Klaus Hahn (University of North Carolina, Chapel Hill, USA) described a method for monitoring the endogenous activity of the small GTPase RhoA. The biosensor comprises the Rho-binding domain of the small protein rhotekin covalently linked to cyan fluorescent protein (CFP) and then covalently joined to the amino terminus of full-length RhoA, itself linked to a yellow fluorescent protein (YFP). In cells expressing the construct, activation of RhoA leads to binding of the rhotekin domain, which brings the two fluorescent proteins together to produce a fluorescent resonance energy transfer (FRET) signal. The construct does not interfere with the turnover of the active and inactive state of RhoA or its membrane localization. Hahn’s group has used this approach to monitor the kinetics of the small GTPase Cdc42 and to assess the dynamics of RhoA activity in fibroblasts during membrane protrusion as well as the interaction between RhoA and the microtubule-associated GEF-H1 during cytokinesis.

**Signaling pathways as therapeutic targets**

The phosphatidylinositol-3-OH-kinase (PI3K) pathway is involved in a wide variety of cellular functions, including cell growth, differentiation and survival, glucose metabolism and organization of the cytoskeleton. Dysregulation of this kinase can lead to cancer, and much effort is being invested in identifying components of this pathway as possible targets for therapy. Because the known PI3K inhibitors, such as wortmannin, inactivate all eight mammalian isoforms of PI3K and are toxic, an alternative approach to examining its role was proposed by Bart Vanhaesebroeck (Ludwig Institute for Cancer Research, London, UK). His group has generated kinase-inactive knock-in mice by introducing mutations into the ATP-binding domain of the various class Iα PI3K genes, thus producing isoform-specific inhibition of signaling without the use of drugs. Knock-in mice with a non-functional PI3K catalytic subunit p110α die at embryonic stage E10.5 as a result of vascular defects. Knock-in animals with a non-functional p110δ catalytic subunit, on the other hand, are viable but show reduced immune responses, a confirmation of the role of p110δ PI3K as a regulator of B-cell receptor signaling.

The intestine is a favorite system for studying epithelial stem cells and their relation to cancer. Hans Clevers (Netherlands Institute for Developmental Biology, Utrecht, The Netherlands) reported the discovery of a marker for intestinal stem cells, GPR49 is a G-protein-coupled receptor that is a target of the Wnt signaling pathway and is expressed in cancer cells. In normal gut epithelium it is expressed in the columnar epithelial cells in the base of the crypts. Clevers described the generation of mice in which GPR49-expressing cells can be marked and their descendants traced for several days. In these mice, the GPR49-expressing cells could give rise to all the intestine and stomach epithelium, indicating that GPR49 is a marker for intestinal stem cells. Overacting mutations of GPR49 are implicated in colon cancer, making GPR49 a potential target for colon cancer therapy. Gaining insight on GPR49 function will help in future treatments.

Richard Marais (Institute of Cancer Research, London, UK) discussed the role of the protein kinase BRAF in melanomas. BRAF is mutated in 70% of human melanomas, with a valine to glutamic acid substitution at position 600 (V600E) being the most common mutation; this elevates the kinase activity of BRAF 500-fold. He described the generation of tamoxifen-inducible V600E-BRAF mice and reported that six months after the application of tamoxifen to the skin, these animals develop melanomas, indicating that V600E-BRAF is an initiating oncogene. The transcription factor MITF is critical for normal melanocyte development and function, controlling lineage commitment, proliferation, differentiation and survival, as well as melanin synthesis. Marais showed that in melanoma cells, progression is ensured by the regulation of MITF by BRAF, which involves both transcriptional and post-translational mechanisms. Novel therapeutic approaches to treating melanoma might be developed by targeting BRAF signaling.

The p53 tumor suppressor protein is known as the gatekeeper of genome integrity, directing cells that have suffered DNA damage to undergo apoptosis. p53 is negatively controlled by the protein MDM2, which modulates its transcriptional...
activity and stability. MDM2 is overexpressed in many cancers, and thus inhibiting its interaction with p53 might offer a novel therapeutic approach. To this end, Lyubomir Vassilev (Hoffman-La Roche, Nutley, USA) described the development of nutlins, small-molecule antagonists of the p53-MDM2 interaction. Nutlins specifically interact with MDM2 and free p53 from negative control. Nutlin-3, for example, induces a senescent phenotype in apoptosis-resistant cancer cell lines. Vassilev reported that nutlin-3 activates p53 and restores its cell-cycle arrest function in cell lines derived from solid tumors of various types. Induction of p53-directed apoptosis can also be affected by the protein MDMX, which binds p53. Nutlin-3 does not inhibit p53-DMMX complex formation, but MDM2 is upregulated. MDM2 in turn mediates ubiquitin-dependent degradation of MDMX, leading to destruction of its complex with p53 and activation of apoptosis. Most important, Vassilev reported that nutlin-3 has antitumor activity \textit{in vivo}. Oral administration of nutlin-3 in mice with osteosarcomas or prostate tumors results in reduction of tumor size.

Overall, the meeting highlighted the main avenues of basic research on signal transduction, its relationship to tumorigenesis and the development of new therapeutic approaches and strategies for cancer treatment via targeting members of signaling pathways. The 2008 LRI conference will focus on chromosome biology.

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