



Lincoln's Inn Fields

Signal Transduction

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The Signal Transduction Laboratory is interested in the mechanisms by which regulatory signals affecting the growth and survival of cells are transmitted from receptors at the cell surface to target enzyme systems within the cell. We study both the normal function of these signalling pathways and also the defects in their regulation found in cancerous cells.

Investigation of mechanisms of transformation by Ras oncogenes

Much of the work in the laboratory has focused on the *RAS* family of oncogenes and the signalling pathways that they control. *RAS* genes are activated by point mutation in some 20% of human tumours and are known to play a key role in the establishment of the transformed phenotype. While the early signalling pathways activated by Ras are now well characterised and the transcriptional programmes they induce have been documented in detail using microarray technology (e.g. Schulze *et al.*, *Genes & Development* 2001; 15: 981 and Schulze *et al.*, *Mol. Biol. Cell* 2004; 15: 3450), it remains a major challenge to understand later events in oncogene-induced signalling and, in particular, which regulated genes are important in the establishment of the transformed phenotype. In order to investigate novel aspects of these pathways in cancer cells, especially those with activated *RAS* oncogenes, we have employed a functional genomics approach using post-transcriptional gene silencing by genome-scale libraries of RNA interference agents.

Two approaches to screening have been applied. In one, genes corresponding to a large fraction of the genome are systematically silenced one by one, allowing identification of genes required for a particular aspect of the transformed phenotype. An example of a successful high throughput screen from the lab published recently (Swanton *et al.*, 2007 *Cancer Cell*; 11: 498) studied the effect of knock down of expression of different genes on the sensitivity of cancer cells with activated *RAS* oncogenes to common chemotherapeutic agents. This has led to the identification of proteins that might be potential therapeutic targets for overcoming resistance of tumours to existing drugs. In particular, targeting a ceramide transport protein, COL4A3BP/CERT, results in multidrug sensitisation in several tumour lines, with less effect on untransformed cells, suggesting that it could be a promising chemosensitisation target. The suitability of CERT as a drug target is currently being investigated in collaboration with Cancer Research Technology.

In the second approach – a selective screen using retroviral RNA interference vectors – many genes are silenced at the same time in a mixed pool of cells, with cells only surviving that acquire the desired phenotype as a result of knock down of a specific gene. These cells, along with the RNAi sequence they carry, are then identified as they emerge at the end of the screen. An example of this approach was published previously (Nicke *et al.*, *Molecular Cell* 2005; 20: 673) in which we identified MINK, a MAP4 kinase acting in the p38 stress activated protein kinase pathway, as a protein required for *RAS* oncogene induced senescence in ovarian epithelial cells. A novel adaptation of this methodology allows the identification of shRNA sequences lost from a population of cells under selective pressure by the use of high throughput sequencing of barcodes in the shRNA library to give a digital readout of library sequence representation. This

methodology is being used currently in the lab to address the mechanisms whereby tumour cells acquire resistance to targeted therapeutic agents such as small molecule inhibitors of EGF receptor and IGF-I receptor tyrosine kinases, a phenomenon that represents a major limitation of novel therapies in the clinic.

A whole genome-scale screen has now been completed searching for synthetic lethal interactions between gene silencing and activation of the Ras oncogene, comparing a colon cancer cell line containing an activated KRAS allele with a normal derivative in which this has been deleted by homologous recombination. This has uncovered proteins whose therapeutic targeting might be expected to provide differential toxicity towards tumour versus normal cells. The relationship between synthetic lethality with Ras oncogene activation and oncogene addiction to activated Ras is also being studied with hits from this screen.

The role of phosphatidylinositol 3-kinase in Ras-driven oncogenesis

Ras proteins signal through direct interaction with a number of effector enzymes, including type I phosphatidylinositol (PI) 3-kinases. Although the ability of Ras to control PI 3-kinase has been well established in manipulated cell culture models, evidence for a role of the interaction of endogenous Ras with PI 3-kinase in normal and malignant cell growth *in vivo* has been lacking. We have generated mice with mutations in the *Pi3kca* gene encoding the PI 3-kinase catalytic p110 α isoform that block its ability to interact with Ras (Gupta *et al.*, 2007 *Cell*; 129: 957). Cells from these mice show proliferative defects and selective disruption of signaling from certain growth factors to PI 3-kinase. The mice also display defective development of the lymphatic vasculature due to reduced signalling from VEGF-C to PI 3-kinase. Most importantly, the mice are highly resistant to endogenous *KRAS* oncogene induced lung tumourigenesis and *HRAS* oncogene induced skin carcinogenesis. The interaction of Ras with p110 α is thus required

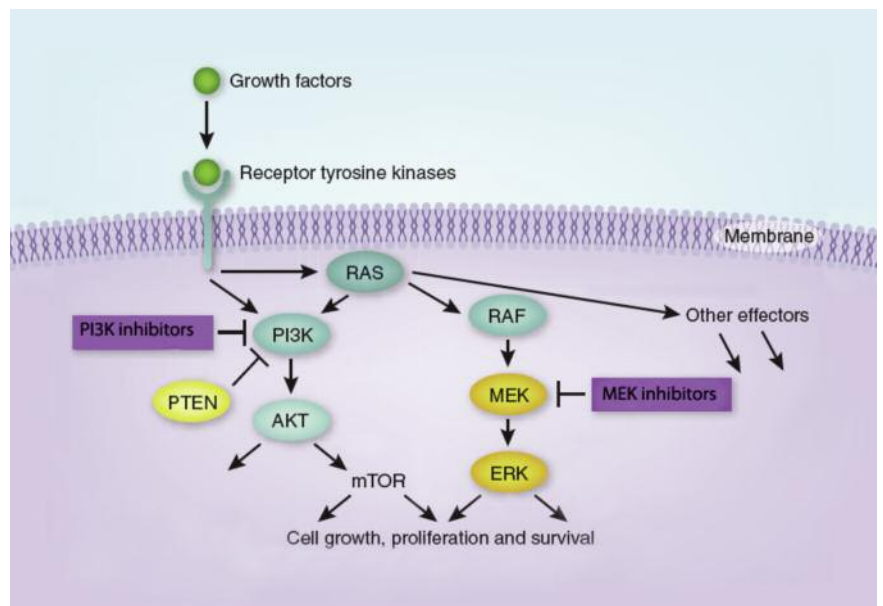


Figure 1. Ras signaling pathways in cancer therapy. Growth factors activate RAS through receptor tyrosine kinases, leading to stimulation of the RAF-MEK-ERK kinase cascade and also the PI3K pathway and other pathways involved in the control of cellular growth, proliferation and survival. Activation of the signaling network commonly occurs in human tumors through mutation of the components shown in blue or the deletion of those in yellow. Using a combination of PI3K and MEK inhibitory drugs may block the growth of RAS-driven tumours. Reproduced from (Downward, 2008, *Nature Medicine*; 14: 1315) with permission.

in vivo for certain normal growth factor signaling and for Ras-driven tumour formation. The demonstration of the importance of the Ras/PI 3-kinase interaction in tumourigenesis raises the prospect that agents that disrupt this interaction might have particular value in cancer therapy.

This work is being further pursued by the generation of mice with inducible expression of the inactivating mutation in the Ras binding domain of p110 α so that the requirement of this interaction for tumour maintenance, rather than simply tumour initiation and development, can be assessed. In addition, the effect of this mutation in PI 3-kinase on tumourigenesis driven by other oncogenes acting upstream of Ras, such as EGF receptor, is also being studied.

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