



## Invasion and Metastasis

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Invasion and metastasis, the leading causes of cancer deaths, often occur before diagnosis thereby creating serious problems for treatment and management of the disease. They result from complex manifestations of normal cellular activities, which are controlled by transcription factors that become deregulated during tumorigenic progression. The aim of our work is to define the changes in gene expression that result in invasion and metastasis and to validate some of these as targets for the development of novel cancer diagnostics and therapeutics.

#### Transcription factors drive invasion and metastasis

Although many transcription factors are important for the acquisition of invasive and metastatic potential increased AP-1 activity is often crucial (Ozanne *et al.*, *Oncogene* 2007; 26: 1). In tumour cells, AP-1 helps to integrate signals from activated oncogenes, growth factors and extracellular matrix by coordinating changes in gene expression that enhance tumour progression. The viral oncogenes *v-fos* and *v-jun*, which encode components of AP-1, transform a variety of cells and induce locally invasive tumours, while inactivation of AP-1 activity in tumours suppresses invasion. Our research aims to identify AP-1 regulated genes that contribute to the invasive potential of tumours.

Gene expression profiling of telomerase-immortalised human fibroblasts (TIFs), rendered invasive by expression of *v-fos* or

*Ha-ras*, demonstrated that a Rac-specific guanine nucleotide exchange factor (GEF), P-Rex1 was upregulated by both oncogenes (Scott *et al.*, *Mol. Cell. Biol.* 2004; 24: 1540). In collaboration with Heidi Welch (Babraham Institute, Cambridge), we continue to investigate the role of P-Rex1. Expression of P-Rex1 in TIFs (TIF-P-Rex1), through Rac1 activation, causes a rearrangement of the actin cytoskeleton that reduces actin stress fibres and enhances lamellipodia with active membrane ruffling. The activity of P-Rex1 in TIF cells is dependent upon serum. Under serum-starved conditions, TIF-P-Rex1 cells revert to a normal appearance with increased F-actin and loss of membrane ruffling lamellipodia. The addition of serum or PDGF, but not HGF or EGF, stimulates the rearrangement of the actin cytoskeleton. In two-dimensional cultures, the TIF-P-Rex1 cells migrate rapidly but with little persistence. However, if a chemotactic gradient of serum or PDGF is generated, the cells respond by migrating almost exclusively towards the attractant. In three-dimensional cultures, TIF-P-Rex1 cells also use a gradient of serum or PDGF to stimulate their migration. HGF and EGF fail to stimulate three-dimensional migration. The response of TIF-P-Rex1 cells to PDGF correlates with dramatic and prolonged PDGF stimulation of PI3K activation. In contrast, EGF and HGF give a rapid but weak, short-lived activation. Thus, it is the cells response to PDGF signalling that is responsible for P-Rex1 mediated cytoskeleton rearrangements and three-dimensional migration.

Mutational analysis of P-Rex1 revealed that actin cytoskeleton rearrangement depends on the presence of a functional GEF domain but that invasion requires an intact protein. The closely related homologue of P-Rex1, P-Rex2 also renders TIFs invasive but another Rac specific GEF, TIAM1 did not, nor did active Rac1, even though both mediated similar actin cytoskeleton rearrangements. These results demonstrate that the ability of P-Rex1 to enhance invasion is dependent upon the pathways that control its activation in response to a chemotactic gradient. P-Rex1 is

consistently upregulated in human melanoma-derived cells compared to normal melanocytes (collaboration with Channing Der, University of North Carolina). The role of P-Rex1 in melanoma cell invasion is to mediate between mesenchymal and amoeboid forms of three-dimensional migration. Suppression of P-Rex1 only inhibits invasion of melanoma cells that use the mesenchymal mode of migration (Fig. 1). However, increasing the expression of P-Rex1 in melanoma cells that use amoeboid migration switches them to a mesenchymal mode and enhances invasion. In collaboration with Owen Sansom (Beatson Institute) and Heidi Welch, we are using mouse models to test the role of P-Rex1 in melanocyte migration during development and melanomagenesis. In C57black mice, deletion of *P-Rex1* results in a white belly spot that is characteristic of a defect in melanoblast migration. In a melanoma model driven by *tyrosinase-N-Ras<sup>Q61K</sup>*, in an *INK4a<sup>-/-</sup>* background, mice develop metastatic melanomas within six months. We are using this model with the *P-Rex1<sup>-/-</sup>* mice. In preliminary experiments, *P-Rex1<sup>-/-</sup>* mice displayed a defect in their response to expression of *N-Ras<sup>Q61K</sup>*, illustrated by a failure of the stimulated melanocytes to migrate into the white belly, feet and tail. Results from these studies suggest that there is no difference in the time of onset or the number of melanomas that develop in *P-Rex1<sup>+/+</sup>* or *P-Rex1<sup>-/-</sup>* mice. However, it is too soon to determine if there is a difference between the mice in terms of metastases.

We have demonstrated that AP-1 regulates a multigenic invasion programme where both up- and down-regulated genes cooperate to facilitate invasion, in part by altering the activity of proteins encoded by genes not differentially expressed (Ozanne *et al.*, *Oncogene* 2007; 26: 1, Spence *et al.*, *Mol. Cell. Biol.* 2006; 26: 1480). We are focusing on genes that contribute to pseudopod elongation, the means by which mesenchymal cells migrate through three-dimensional extracellular matrices. Fibronectin is downregulated in a variety of tumour-derived cell lines and exposure to fibronectin suppresses invasion. However, in other tumour-derived cell lines, fibronectin is upregulated and enhances invasion. We have demonstrated that in *Fos*-transformed cells, the downregulation of fibronectin results in integrin  $\alpha 5 \beta 1$  suppression of Rho-Rho kinase activity and enhancement of pseudopod elongation and invasion. Jim Norman's group (Beatson Institute) has demonstrated that for tumour-derived cell lines where fibronectin enhances invasion, the activity of  $\alpha 5 \beta 1$  is modified by its binding to Rab25 so that it also suppresses Rho-Rho kinase signalling. In collaboration with Jim's group, we have demonstrated that the expression of Rab25 in *Fos*-transformed cells results in the cells responding to fibronectin as an invasion enhancer (Caswell *et al.*, *Dev. Cell* 2007; 13: 496).

Kelch-related protein 1 (Krp1) is necessary for pseudopod elongation and invasion (Spence *et al.*, *Mol. Cell. Biol.* 2006; 26: 1480), and binds to Lasp-1. RNAi or dominant negative

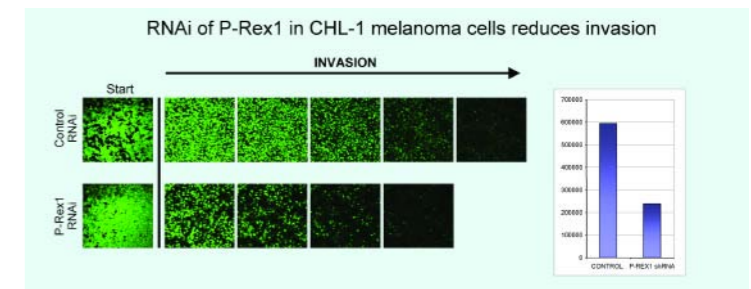


Fig. 1: P-Rex1 expression is required for the invasion of the melanoma derived cell line CHL-1. Reduction in the expression of P-Rex1 by RNAi technology decreases the three-dimensional migration of CHL-1 cells through the extracellular matrix, Matrigel. The panel of pictures of CHL-1 cells (green) in the presence of control RNAi or P-Rex1 specific RNAi. The bar chart is the quantification of invasion of CHL-1 cells stably expressing P-Rex1 specific shRNA or control shRNA.

mutants of Lasp-1 inhibit pseudopod elongation and invasion. The interaction of Krp1 and Lasp-1 is inhibited by the exposure of the cells to fibronectin and the integrin-mediated activation of Rho-Rho kinase signalling. To better understand the interaction of Krp1 and Lasp-1, we used peptide arrays to identify two sites on Krp1 involved in binding to Lasp-1; one in the back domain and the other in the fifth kelch repeat near the carboxy terminus of the protein. Through an alanine scan, we identified the amino acids that were important in the interaction of Krp1 with Lasp-1. In collaboration with Neil Issac (University of Glasgow), we determined the protein structure of part of the back domain and the five kelch repeats of Krp1. This revealed a novel six-bladed beta propeller composed of one non kelch blade derived from the back domain and the five kelch repeats. The two separate binding sites identified by the peptide array analysis comprise a single interface on the protein structure, where the first kelch-like blade contacts the fifth kelch repeat (Fig. 2).

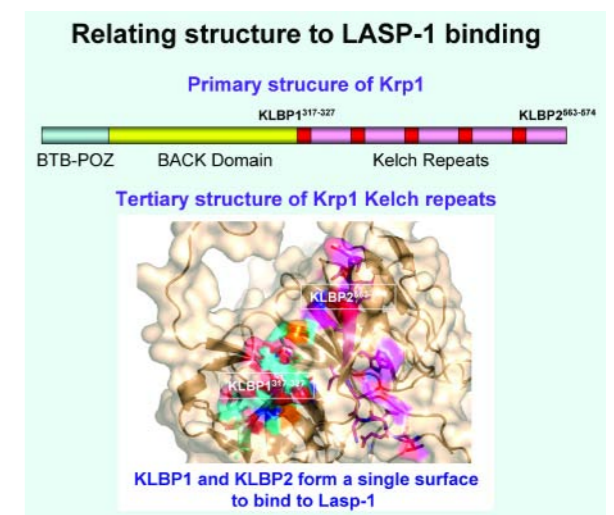


Fig. 2: Two linear peptides of Krp1 bind to Lasp-1. These binding sites are separated by 237 amino acids in the primary structure of Krp1. However, the tertiary structure of the Kelch domain of Krp1 demonstrates that both KLBP1 and KLBP2 form a single surface for interacting with Lasp-1.

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