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Since it has become clear that adhesion receptors are trafficked through the endosomal pathway and that this can influence their function, we have managed to obtain detailed descriptions of the molecular machinery responsible for internalising and recycling integrins. We have focused much of our recent work on the role played by the Rab11-effector, Rab-coupling protein (RCP) in coordinating the trafficking of integrins and growth factor receptors, and how this drives invasion and metastasis. Recently, we have shown that mutated forms of the tumour suppressor protein, p53 drive invasion by activating RCP-mediated trafficking. In addition to this, we have found that the chloride intracellular channel protein-3 (CLIC3) controls a novel vesicular transport route that returns lysosomally targeted integrins to the plasma membrane, and that CLIC3 acts as an independent prognostic indicator in pancreatic cancer. We are currently evaluating RCP and CLIC3 as potential targets for anti-metastatic therapy.

### Mutant p53 drives cancer invasion via activation of Rab-coupling protein and diacylglycerol kinase-mediated trafficking of $\alpha 5 \beta 1$ integrin

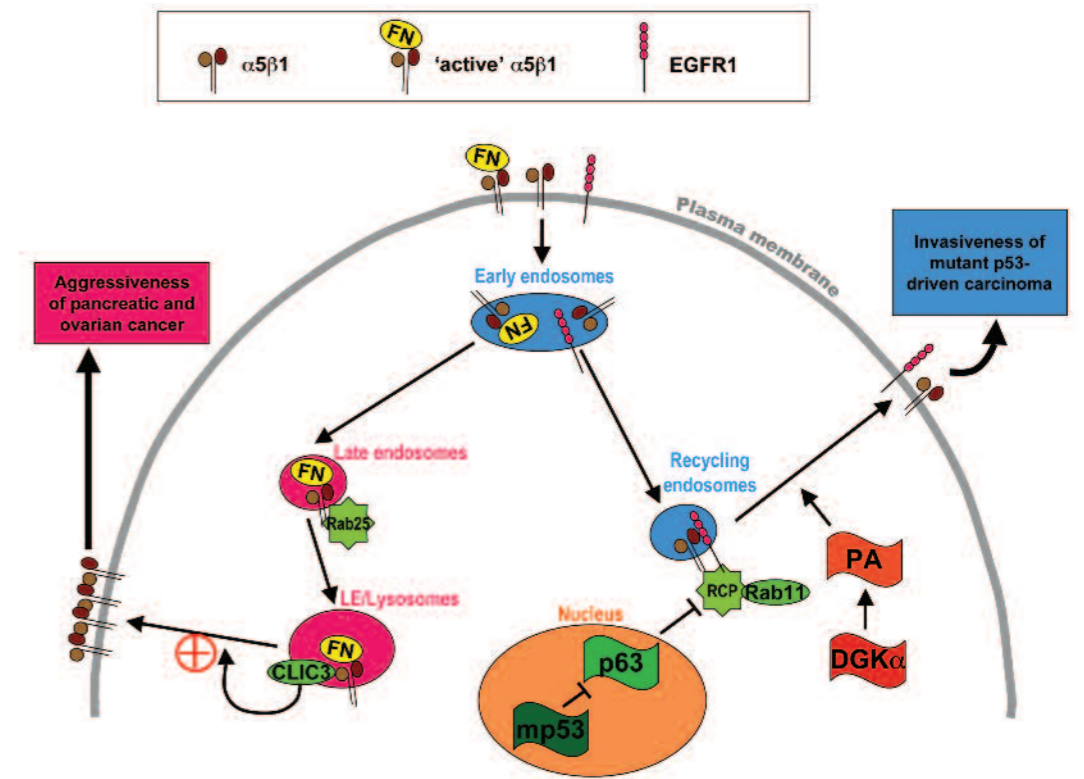
The Rab GTPase effector protein, Rab-coupling protein (RCP) associates with  $\alpha 5 \beta 1$  integrin and plays an important role in guiding integrin transport from recycling endosomes to the plasma membrane and thus contributes to movement of tumour cells in fibronectin-rich, three-dimensional microenvironments. Moreover, the importance of RCP in cancer progression is further illustrated by the finding that it is frequently overexpressed in human breast cancers.

In about half of all human cancers the tumour suppressor p53 protein is either lost or mutated, frequently resulting in the expression of a transcriptionally inactive mutant p53 protein. Loss of p53 function is well known to influence cell cycle checkpoint controls and apoptosis. But it is now clear that p53 regulates other key

stages of metastatic progression, such as cell migration and invasion. Moreover, recent data suggests that expression of mutant p53 is not the equivalent of p53 loss, and that mutant p53s can acquire new functions to drive cell migration, invasion and metastasis. We have now established that mutant p53s (acting via inhibition of the p53-related protein, p63) promote the association of RCP with internalised  $\alpha 5 \beta 1$  and the EGF receptor. The resulting increase in the rate at which these internalised receptors are transported back to the cell surface influences signalling through pro-invasive kinases such as PKB/Akt to promote tumour cell migration and invasion.

RCP contains a C2 domain that is known to bind to the acidic lipid metabolite phosphatidic acid (PA). PA can be produced either by the action of phospholipase D on membrane phospholipids, or an enzyme called diacylglycerol kinase alpha (DGK $\alpha$ ), which phosphorylates diacylglycerol to PA. Further investigation of the

**Figure 1**  
Integrin trafficking pathways that drive cancer invasion. 'Left-hand' pathway: Fibronectin-bound, 'active' conformation  $\alpha 5 \beta 1$  integrin is internalised into early endosomes and then transported from this compartment to late endosomes under control of Rab25. Upon arrival at the late endosomes/lysosomal compartment,  $\alpha 5 \beta 1$  forms a close association with CLIC3, which enables the integrin to return from lysosomes to the plasma membrane. CLIC3 is required for Rab25-driven invasion and dictates the aggressiveness of pancreatic adenocarcinoma. 'Right-hand' pathway:  $\alpha 5 \beta 1$  integrin that is not ligand-bound is trafficked to recycling endosomes where it forms a complex with Rab-coupling protein (RCP) and the EGF receptor (EGFR1). The assembly of this complex is strongly promoted by oncogenic mutants of p53 via the inhibition of p63. The EGFR1/ $\alpha 5 \beta 1$ /RCP complex then recycles to the plasma membrane under control of the phosphatidic acid (PA)-producing enzyme diacylglycerol kinase alpha (DGK $\alpha$ ). RCP/DGK $\alpha$ -dependent recycling is required for mutant p53 to drive cancer invasion.



mechanism by which RCP carries integrins and growth factor receptors to the plasma membrane during tumour invasion has revealed an essential role for DGK $\alpha$  in this process. Indeed, we have found that DGK $\alpha$  is required to mediate transport of RCP vesicles from the perinuclear region to the tips of invading pseudopods as cells move through three-dimensional microenvironments, highlighting this enzyme as a potential target for inhibition of mutant p53-driven tumour cell invasion.

We are currently using transgenic mice that have targeted disruptions in the genes for RCP and/or DGK $\alpha$  to evaluate the role of the mutant p53/RCP/DGK $\alpha$  axis in controlling invasion and metastasis in mouse models of cancer, and are intending to combine these transgenic approaches with in vivo imaging to gain a clearer picture of the contribution of integrin recycling to the dissemination and spread of cancer.

### The chloride intracellular channel protein-3 (CLIC3) drives cancer invasion by recycling lysosomally targeted integrins

Rab25 is a small GTPase of the Rab11 family that is known to control endosomal trafficking and the return of internalised receptors to the plasma membrane. Recent reports that Rab25 can act as either a promoter or suppressor of tumourigenesis highlight the need for greater understanding of how it controls integrin

trafficking. We have shown that Rab25 permits the sorting of ligand-occupied, active conformation  $\alpha 5 \beta 1$  integrin to late endosomes/lysosomes. Novel photoactivation and biochemical approaches show that integrins thus sorted are not degraded but rapidly recycled from late endosomes/lysosomes to the plasma membrane. This requires the chloride intracellular channel protein-3 (CLIC3); a protein resident in late endosomes/lysosomes, of previously unknown function, that is upregulated in Rab25-expressing cells. CLIC3 exists in close proximity with active  $\alpha 5 \beta 1$  in late endosomes/lysosomes and is required for cancer cell invasion into fibronectin-containing, three-dimensional matrices. Clinical data indicate that CLIC3 is expressed in ovarian cancers, pancreatic ductal adenocarcinoma (PDAC) and pancreatic intraepithelial neoplasia (PanIN) but is absent from the normal pancreas and tumour-associated stroma. Moreover, there is a strong correlation between high expression of CLIC3 (at both the mRNA and protein levels) and poor prognosis in operable cases of PDAC. These findings identify CLIC3 both as a regulator and component of a novel vesicular transport route that returns lysosomally targeted integrins to the plasma membrane, and as an independent prognostic indicator in pancreatic cancer.

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