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Current strategies in the development of new therapies for malignant disease are based on exploiting our increasing understanding of the molecular and cellular basis of cancer development and progression. Work in our group is aimed at developing novel laboratory models that will allow us to understand the biological function of key tumour suppressor genes and oncogenes *in vivo* in both normal tissues and tumours.

We also aim to identify and characterise the signalling pathways that are deregulated at the early stages of pancreatic cancer as well as during the development and progression of the invasive and metastatic phenotype. Using these models, we will determine how potential anti-cancer agents might best be evaluated in subsequent clinical trials.

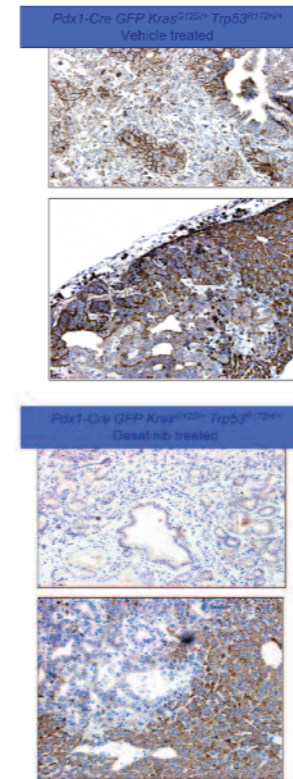
Infiltrating ductal carcinoma of the pancreas is the fifth commonest cancer and the fourth commonest cause of cancer deaths in the UK. Aggressive invasion and early metastases are characteristic of the disease, such that 90 percent of patients have surgically unresectable disease at the time of diagnosis. Furthermore, most systemic therapies are largely ineffective in advanced, inoperable disease and the estimated five-year overall survival is less than 5 percent. Gemcitabine has modest clinical benefit and a marginal survival advantage in patients with advanced pancreatic cancer and has become standard of care for patients with advanced disease. However, the median survival of patients with advanced pancreatic cancer remains poor, being less than six months. In addition, the majority of the selected patients who undergo potentially curative resection for small, localised lesions inevitably develop recurrent or metastatic disease, presumably due to the presence of distant micro-metastases at initial diagnosis. Adjuvant (post-operative) chemotherapy can improve outcome, although overall survival remains disappointing. Consequently, the development of more effective strategies to treat pre-invasive pancreatic cancer, micro-metastatic disease and advanced disease is of paramount importance.

Our work aims to develop strategies for early detection of pre-invasive disease, to evaluate putative anti-invasive therapies with the aim of improving relapse-free and overall survival following resection of invasive pancreatic cancer, and to develop a 'personalised medicine' approach to treatment of pancreatic ductal adenocarcinoma models from a range of genetic backgrounds that ultimately might influence the management of advanced disease in the human population.

### Evaluation of putative anti-invasive therapies in pancreatic cancer models

One aim of our work is to define the mode of action of novel anti-cancer drugs that are currently in clinical evaluation and testing the hypothesis that these agents may have anti-migratory and hence anti-invasive and/or anti-metastatic properties. For this work, we are using clinically relevant, pharmacologically active anti-cancer agents as experimental tools. These have been chosen because they perturb pathways implicated in tumour cell invasion. We are using a model of invasive and metastatic pancreatic cancer that closely mimics the human disease. We have adapted this model to generate primary and metastatic tumours that express green fluorescent protein, which can therefore be directly visualised both at the whole body level and also at the level of single cells at the invading tumour margins. We are using this model to help us understand how tumour cell migration and invasion are controlled, how this is linked to the development of metastasis and how best to evaluate anti-invasive agents in the clinic.

**Figure 1**  
Dasatinib inhibits Src kinase activity in the *Pdx1-Cre, ZIEGFP, LSL-Kras<sup>G12D</sup>, LSL-Trp53<sup>R172H</sup>* model as determined by decreased phospho-SrcY416 expression by immunohistochemistry.



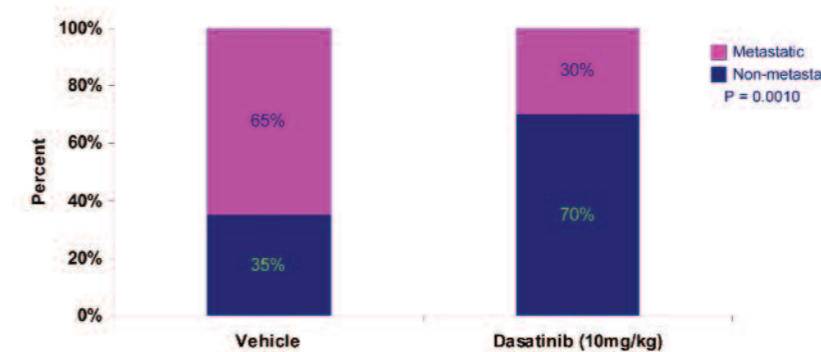
Initially we have used dasatinib, an inhibitor of several kinases including Src family kinases and BCR-ABL. We have demonstrated that levels of Src expression and activity are significant indicators of vascular invasion, lymph node status and prognosis following resection in human pancreatic ductal adenocarcinomas (PDACs), suggesting that Src kinase may be a relevant target for therapeutic intervention following resection of PDAC.

Using the the *Pdx1-Cre, ZIEGFP, LSL-Kras<sup>G12D</sup>, LSL-Trp53<sup>R172H</sup>* model, we demonstrated that Src was expressed in normal ducts within the pancreas and this expression was maintained in pancreatic intra-epithelial neoplasia (PanIN) lesions and PDACs. The activity of Src was very low in normal ducts and early PanIN lesions, with a marked increase in late, high grade PanINs, which was maintained in the invasive PDAC. Thus, we demonstrated that Src activity was upregulated during progression to invasive PDAC in this model.

Using cell lines generated from PDACs harvested from this model, we showed that treatment with dasatinib *in vitro* resulted in a dose-dependent inhibition of Src kinase activity. Dasatinib had no effect on cell proliferation at concentrations that inhibit Src kinase activity, however at these Src kinase-inhibitory doses, dasatinib inhibited migration and invasion of PDAC cells. *In vivo*, treatment with dasatinib significantly reduced the number of *Pdx1-Cre, ZIEGFP, LSL-Kras<sup>G12D</sup>, LSL-Trp53<sup>R172H</sup>* with metastases compared with those treated with vehicle control. However, dasatinib did not improve survival when compared with vehicle control due to the morbidity of the primary tumour burden. We speculate that administration of dasatinib after potentially curative resection of localised disease could reduce the risk of metastases and improve overall survival.

We are now investigating how best to exploit agents that target a number of pathways implicated in pancreatic cancer development and progression, and propose to extend these studies to models of malignant melanoma in collaboration with Owen Sansom and Karen Blyth.

**Figure 2**  
Dasatinib significantly reduces the development of metastases in the *Pdx1-Cre, ZIEGFP, LSL-Kras<sup>G12D</sup>, LSL-Trp53<sup>R172H</sup>* model compared with vehicle control.



### The biological function of key tumour suppressor genes and oncogenes in PDAC

We have also begun to develop novel models with a range of genetic backgrounds in collaboration with Owen Sansom's group. These models will help us understand the biological function of key tumour suppressor genes and oncogenes *in vivo* in both normal tissues and tumours.

For example, in collaboration with the Sansom group, we have shown that although *Kras<sup>G12D</sup>* is one of the major oncogenic drivers of PDAC, most *Kras<sup>G12D</sup>* expressing pancreatic cells are selectively lost from the tissue and those that remain form premalignant lesions. We showed that loss or mutation of *Trp53* allows retention of the *Kras<sup>G12D</sup>* expressing cells and drives rapid progression of these premalignant lesions to PDAC. This progression is consistent with failed growth arrest and/or senescence of premalignant lesions, since a mutant of p53, *p53<sup>R172H</sup>*, which can still induce p21 and cell cycle arrest, is resistant to PDAC formation. We also showed that despite similar kinetics of primary tumour formation, mutant *p53<sup>R172H</sup>*, as compared with genetic loss of p53, specifically promotes metastasis.

We have also investigated the consequences of LKB1 deficiency in the pancreas through targeted heterozygous deletion of LKB1 in combination with endogenous targeted expression of the oncogenic *Kras<sup>G12D</sup>*, again in collaboration with the Sansom group. We demonstrated that LKB1 haplo-insufficiency cooperates with *Kras<sup>G12D</sup>* to cause PDAC. Mechanistically, we showed that LKB1 deficient, *Kras<sup>G12D</sup>*-induced tumours exhibited reduced levels of the tumour suppressors p53 and p21, and we proposed that this reduction in p53 and p21 allows *Kras<sup>G12D</sup>*-bearing cells to overcome a senescent barrier to tumour formation. Moreover, haplo-insufficiency for p21 also synergised with *Kras<sup>G12D</sup>* to drive PDAC.

Using a human tissue microarray of resected PDAC specimens from patients, we found that LKB1 expression was decreased in around 20 percent of human PDACs and significantly correlated with low p21 expression and poor prognosis. All tumours that had low levels of LKB1 also had low levels of p21 and these tumours did not express mutant p53. Our data reveal a novel LKB1/p21 axis that suppresses pancreatic ductal adenocarcinoma following *Kras* mutation *in vivo*. Downregulation of LKB1 may therefore serve as an alternative to p53 mutation to drive pancreatic cancer *in vivo*.

Publications listed on page 76