



Tumour Modelling and Experimental Medicine

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Oncogenes initiate and sustain carcinogenesis, thus a detailed understanding of oncogene function is needed to design effective strategies to prevent, diagnose, and treat cancer.

We model malignant progression in transgenic mice, focusing on pancreatic cancer and melanoma as prototypical diseases with poor prognoses despite the identification of the predominant oncogene in both diseases. Our approach involves the characterisation of molecular and cellular events following oncogene expression, the identification of cell autonomous and non-cell autonomous pathways in tumour evolution, and the evaluation of therapeutic and diagnostic strategies in these model systems. These preclinical results help determine future clinical investigations.

Cellular consequences of oncogene expression

The expression of oncogenes may be productive, negative or neutral to cellular physiology – for example they can cause proliferative arrest in human and murine pre-neoplasms. This 'oncogene-induced senescence', is suggested to be an important barrier to the development of malignancy. We have characterised in detail the immediate and prolonged effects of conditional endogenous Kras and Braf oncogenes in primary cells, since they are associated with most cases of ductal pancreatic cancer and cutaneous melanoma, respectively. Contrary to recent reports, we find that oncogenic Kras promotes partial transformation of

primary fibroblasts and epithelial cells in culture, and either stimulates proliferation or has no effects in relevant tissues (pancreas, colon, lung) *in vivo*. There is also no evidence of a senescent phenotype in these tissues. Indeed, a direct analysis of markers previously identified in pre-neoplasms as representing the senescent state revealed that most are not specific to arrested or quiescent cells (Figure 1). We are currently evaluating whether oncogenic Kras has deleterious or neutral effects in tissues that are not normally transformed. We expect to extend our studies to oncogenic alleles of Braf as they become available.

Cell autonomous and non-cell autonomous events in carcinogenesis

Both cell autonomous and non-cell autonomous events shape the evolution of carcinogenesis through internal signalling events and homotypic and heterotypic cellular interactions. Whereas such events are difficult to characterise using human specimens, they can be readily pursued with our murine cancer models through a variety of biochemical, cellular and genetic approaches.

We have undertaken both unbiased and targeted approaches to identify important mediators of Kras transformation. A global proteomic and transcriptional evaluation of primary cells following oncogenic Kras expression has shown that alterations in cellular metabolism play an important role in cellular immortalisation in primary cells. The regulation of this altered metabolic response appears to be due to signalling by the MAP kinase cascade, and thus represents a possible therapeutic target in early neoplasms. Our

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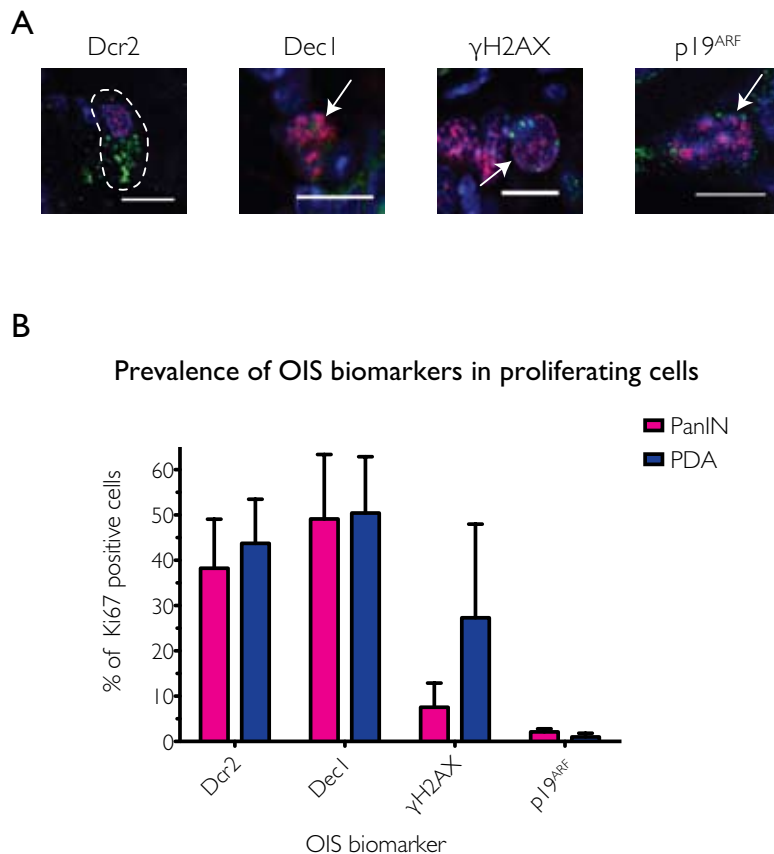


Figure 1. Proliferating PanIN and PDA cells are enriched for OIS biomarker expression. Scale bars: 10 μ m. (a) Coimmunofluorescence images of oncogene-induced senescence biomarkers (Dec1, Dcr2, γ H2AX and p19: green) co-localising with proliferating PanIN epithelial cells, as labelled by Ki67 (red). Nuclei counterstained with DAPI (blue). White arrows denote double positive cells. (b) Quantification of OIS biomarker expression in proliferating PanIN and PDA cells. Half of all proliferating epithelial PanIN and PDA cells express Dcr2 and Dec1 whereas nearly a quarter express γ H2AX. A smaller fraction of proliferating PanIN and PDA cells harbour p19 expression.

targeted approaches include the conditional antagonism of several pathways that may cooperate with oncogenic Kras to promote tumourigenesis. To determine whether specific branches of the Ras signalling pathway are required to sustain tumourigenesis, we are generating several 'inverter alleles' to enable the dissection of the major pathways downstream of mutant Kras following initial tumour formation. As such alleles may be confounded by the caveat of also being hypomorphic for oncogenic Kras function, the conditional gene ablation of the MAPK, PI3K and Ral pathways is being pursued in parallel. Cell biological approaches allow us to explore the non-cell autonomous interactions in developing tumours and thereby identify features that may both suppress and promote tumourigenesis. Our initial efforts have focused on the identification of the cell types that comprise the immune, stromal and vascular constituents of the tumour microenvironment. We recently reported the presence of immature myeloid cells in pancreatic preneoplasms and invasive tumours. Such cells might stimulate tumourigenesis as they have been implicated as potential suppressors of the

acquired immune response. We are also investigating the role of inflammation in the genesis of pancreatic cancer. To do this, we are analysing the importance of the immune system and stromal cells in the generation of the desmoplastic stroma common in preinvasive and advanced pancreatic cancer; and are investigating cell autonomous paracrine and autocrine factors that could stimulate the microenvironment. We are also pursuing whether non-mutant epithelial cells participate in tumourigenesis or are merely neutral bystanders.

Finally, we are investigating novel genes and pathways that facilitate pancreatic cancer initiation and metastasis. This entails high throughput sequencing of spontaneous and transposon-stimulated tumours, and the validation of potential candidates in cell culture and *in vivo*.

Cancer medicine advances in the preclinical and clinical settings

The poor correlation between efficacy in tumour xenografts and clinical outcome has prompted us to evaluate whether the autochthonous models of pancreatic cancer are superior in this regard. In contrast to the uniform response of ectopic tumour xenograft or allograft models, we have shown that our models

of ductal pancreatic cancer closely recapitulate the modest clinical effects of the nucleoside analogue Gemcitabine. Only 10% of ductal pancreatic cancer model mice demonstrated a primary tumour response by radiological monitoring and pharmacodynamic criteria. Gemcitabine-resistant autochthonous tumours displayed a more extensive stromal composition with a lower mean vascular density in comparison to Gemcitabine-sensitive autochthonous tumours and ectopic tumours. Furthermore, pharmacological, radiological and tissue perfusion studies demonstrated that the delivery of Gemcitabine and other small molecules is substantially reduced in autochthonous tumours as compared to ectopic tumours (Figure 1). We are currently pursuing the underlying mechanism for heterogeneity in the tumour microenvironment, and extending these studies to clinical specimens. Studies with a variety of targeted agents are under investigation.

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