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TRANSGENIC TECHNOLOGY

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The Transgenic Technology Laboratory uses molecular genetic methods to investigate the consequences of genetic alterations in contributing to the onset and progression of cancer. We can introduce precise genetic changes into specialised stem cells using techniques such as gene targeting or genome editing. These methods allow us to build accurate models of human cancers by the introducing into stem cells identical genetic alterations to those discovered in cancers. Refinements in the technology allows us to introduce multiple genetic changes into cells at the same time. This allows us to analyse how combinations of mutations can interact to enable the development of cancer.

Improving our models of human cancer

We use embryonic stem cells to enable us to decipher the role that different mutations can play in causing growth of cancers. The cells have high rates of homologous recombination, which allow us to introduce specific genetic alterations into these cells with relatively high efficiency. This allows us to effectively copy the exact genetic changes uncovered at the root of human cancers and incorporate these into our models. This can give us an understanding of how these genetic changes can change the function of proteins in intact cells and tissues. Stem cells can also be differentiated into a wide variety of different specialised cells found in different tissues. This allows us to establish how these genetic changes affect the behaviour of cells from the particular tissue in which the mutation was originally uncovered. For example, we can analyse how mutations uncovered in liver cancers affect the biology of liver cells.

A TAZ KO mouse model of Barth syndrome

One such genetic alteration, which specifically affects heart cells, is a truncating mutation in the gene Tafazzin (*Taz*). This mutation also causes an inherited mitochondrial disorder called Barth Syndrome (BTHS). BTHS is a rare mitochondrial disease resulting in a range of variable clinical features including cardiomyopathy, neutropenia, muscle myopathy and metabolic defects. Barth syndrome is caused by mutations in the *Taz* gene. Here at the Beatson Institute, we generated the first conditional model of this gene in order to allow its further analysis and study. The *Taz* KO cells exhibit abnormal mitochondrial function as a result of a defect in the mitochondrial membrane. As a result of these defects, *Taz* KO heart cells are not able to work as effectively as wild-type (WT) cells, identical to the clinical consequences of BTHS seen in affected individuals. As well as this, animals carrying a *Taz* KO mutation show a reduction in growth and an inability to gain weight, even when being fed a high-fat diet.

Mitochondria in Cancer

Mitochondria are responsible for regulating the production of cellular energy, providing amino acid building blocks for new cells as well as controlling redox homeostasis and apoptosis. Functioning mitochondria are therefore essential for tumourigenesis, and the depletion of mitochondria from tumour cells has been shown to compromise this process. For this reason, we were interested in what effect the *Taz* KO allele would have on tumour progression.

Taz KO and Hepatocellular Carcinoma (HCC)

HCC is the most common form of liver cancer, most often found in people with long-term liver diseases and common in people who drink large amounts of alcohol or who have an accumulation of fat in the liver. As animals carrying a *Taz* KO mutation are shown to be resistant to the effects of fatty diet, we believed this was a good cancer model to use for our investigation. We collaborated with another group at the Institute in order to breed the *Taz* KO allele onto the inducible Myc/BCat model of HCC. In this study the proto-oncogene MYC is overexpressed and B-Catenin is mutated directly in hepatocytes of these animals using a liver-specific adenovirus. Over the course of five months and following sampling, it was established that animals carrying the *Taz* KO allele had a significantly lower tumour burden within the liver relative to mice wild-type at the *Taz* locus (Figure 1). The tumours within the Taz KO mice were found to be similar immunohistologically to those found within the WT mice but were smaller, suggesting either these were appearing later or growing slower. Ongoing analysis is underway to investigate this further.

Additionally, this year we have continued to collaborate with a number of other groups at the Institute to generate a wide variety of different new models. These include point mutations, conditional knockouts and protein tags. In addition, we have established new methods that allow the replacement of entire endogenous stem cell genes with their human equivalent. This ensures that the genetic alterations that we make in stem cells are carried out in the precise genomic context, and as a consequence, these changes more accurately model the outcome of the mutations associated with human disease.

Publications listed on page 111

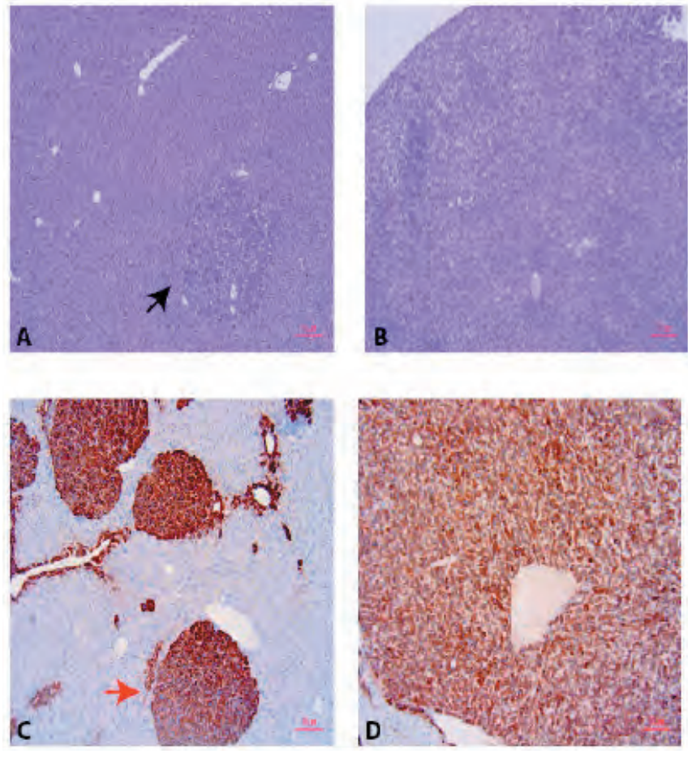

Figure 1
Liver tissue histology
H&E staining of liver tissue taken from induced mice either *Taz* KO (A) or *Taz* WT (B). WT tissue shown to be almost entirely tumour tissue compared with KO where smaller tumours are present (black arrow). Glutamine Synthase (GS) staining of liver tissue taken from either *Taz* KO (C) or *Taz* WT (D) showing that both *Taz* KO and *Taz* WT tumours exhibit β-catenin activation; however, much smaller growths of tumour tissue are present within the *Taz* KO sample (red arrow).