TRANSGENIC TECHNOLOGY

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The Transgenic Technology Laboratory uses molecular genetic approaches to help analyse the function of genes in cancer development and progression. By using both genome editing and gene targeting, we are able to make precise genetic alterations into endogenous genes in stem cells. These methods allow us to introduce changes into cells which accurately reproduce the mutations observed in human cancers.

Making better models of clinically relevant cancers

Embryonic stem (ES) cells have some valuable attributes, which make them very useful tools to allow us to study the role of gene mutations in cancer. Firstly, ES cells have very high rates of homologous recombination, a process which enables us to introduce precise genetic changes into endogenous genes. So, by taking advantage of homologous recombination, we can copy mutations seen in human cancers directly into genes in ES cells. This makes it possible for us to study how these mutations affect the structure of the associated proteins and how these differences affect function in cells and tissues.

A second attribute that ES cells possess is that they can differentiate to form cells from a wide variety of different tissues. So, once we have stem cells carrying the genetic changes we want to study, we can then differentiate these into cells from a relevant tissue. So, for example, if we want to study the consequences of a mutation originally detected in liver cancer, we can analyse liver cells derived from the altered stem cells.

During the year, we have collaborated with other groups at the Institute to generate a variety of different kinds of alleles. These include conditional knockouts, point mutations and inducible genetic markers. In addition to making small changes to endogenous stem cell genes, where human and mouse gene differ significantly, we can now replace the entire endogenous mouse gene with its human equivalent. Consequently, using this approach, we can test the outcome of mutations directly in the appropriate context of the human gene, and this ensures that the changes we make directly imitate the mutations discovered in human cancers.

In vivo CRISPR for the study of models of Hepatocellular Carcinoma (HCC)

Over the last decade, liver cancer incidence rates have increased by almost half (45%) in the UK and are expected to reach 15 cases per 100,000 people by 2035 (Cancer Research UK). Various genetically engineered mouse models of HCC have been developed which allow the study of the multiple genetic changes occurring at each stage of progression towards tumour formation as well as provide the possibility to examine treatment strategies. However, complicated genetic models can be laborious and time-consuming to create and require extensive breeding strategies. For this reason, we attempted to test if we could generate mutations of known tumour suppressor genes directly within the liver using genome editing technology.

A Myc and β-catenin mouse model of HCC was used as a basis for the study, in which Cre recombinase is used to activate Myc expression as well as to remove exon 3 of β-catenin. These genetic changes are used to mimic the early stages of HCC. Additionally, these mice were bred to include Cre inducible expression of Cas9. Cas9 is a CRISPR-associated (Cas) endonuclease, or enzyme, that functions as "programmable molecular scissors" to cut DNA at a site which is determined by a guide RNA. An Adeno-associated virus (AAV) expressing a low-dose Cre was provided to activate all three mutations in the liver. At the same time CRISPR guides designed against critical coding regions of two tumour suppressor genes (A and B) were also provided virally. These guides should direct the Cas9 enzyme to cause mutations in the two tumour suppressor genes. Following the introduction of mutations, the suppressor genes should co-operate with Myc and β-catenin to accelerate the rate of tumour progression in the model. After administration of the viruses expressing Cre recombinase and the guide RNAs, mice were then monitored for liver tumour growth and subsequent tumours were analysed for gene mutations and examined histologically.

Mice, in which guides for both tumour suppressor genes were provided, were found to reach end point significantly sooner, than those in which no CRISPR guides were provided. Median survival was around 100 days for the mice who received the A and B guide RNAs compared to 300 for the control mice without the guides (Figure 1a). Mice, in which a guide to only one suppressor gene (tumour suppressor A) was provided also had median survival which was much lower than the controls (137 days). Tumour tissue was sequenced, and various mutations identified within the tumour DNA in both tumour suppressor genes. These mutations were discovered at the exact sites where Cas9 was directed by the guide RNAs. No mutations were found at the same regions in the control mice. This suggests Cas9 works to introduce mutations directly into tissues. Histological staining was also used to identify knock-down of tumour suppressor gene A in tumour tissue of these mice (Figure 1b). Tumour growth was exhibited in all mice which were administered with CRISPR guides and mutations were also identified in all of these mice suggesting high rates of Cas9 mutation of these genes. Overall, *in vivo* CRISPR has been demonstrated to be a potentially valuable tool for cancer mouse genetics.

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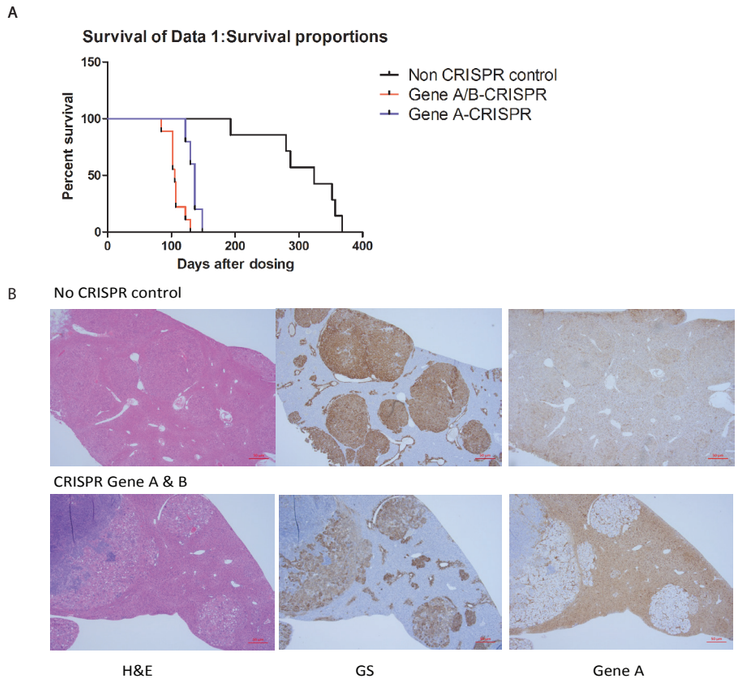

Figure 1
A Survival curve of Myc/Bcat HCC mice administered with AAV CRISPR for tumour suppressor genes A and B (Red), Gene A only (Blue) and a non-CRISPR control (Black). P Value, 0.002 ***. **B** Histological analysis of tumour samples from mice where no guide provided (top panel) H&E, GS and then stained against tumour suppressor gene A. Bottom panel shows a mouse in which guides were provided against both tumour suppressor gene A and tumour suppressor gene B, knock-down of tumour suppressor gene A can be seen within the tumours.