The Transgenic Technology Laboratory uses molecular genetic techniques to analyse gene function during the onset and progression of cancers. By using gene targeting or genome editing, we can make accurate genetic changes at precise locations in specialised stem cells. It allows us to make accurate models of clinically relevant cancers by introducing changes identical to those discovered in human cancer cells. Moreover, recent advances in these technologies give the possibility to introduce multiple genetic changes, simultaneously. Thereby, we can study how combinations of mutant alleles interact and thus, enable the development and progression of cancer.

Making better models of clinically relevant cancers

Stem cells have a number of useful properties, which make them suitable for studying the role of gene mutations in cancer. Firstly, homologous recombination, a process where endogenous genes can be replaced with copies carrying precise genetic changes, works relatively efficiently in these cells. This allows us to introduce genetic changes into genes in stem cells, identical to those uncovered in human tumours. We can, therefore, study how these genetic alterations affect the function of the altered proteins in intact cells and tissues. Secondly, stem cells can develop or differentiate to produce a wide variety of different cell types found in various tissues. Having introduced genetic changes into stem cells, we can then differentiate the cells to analyse the role of these alterations in relevant cells from the tissue where the original mutation was identified.

This year, we have collaborated with a number of groups at the Institute to generate a variety of different allele types in stem cells, including, for example, conditional knockouts and point mutations. Furthermore, advances in techniques have enabled us to replace the original genes in stem cells with their human genomic equivalents. This means that we can now introduce mutations directly within the appropriate genetic setting, ensuring that these changes more precisely reflect the mutations associated with human disease.

Figure 1
Repairing alleles by genome editing

[Diagram of the repair of the condition allele of Runx2 Small guide RNA (sgRNA) were designed on either side of the Neo selectable cassette. The guide directed Cas9 to excise the selectable cassette but leaving the loxp sites intact. (i) Graph of weights of mice homozygous or heterozygous for the repaired Runx2 allele and their wild-type littermates. Following removal of the cassette the mice no longer displayed the hypomorphic phenotype and were the same size as their wild-type littermates. (ii) Diagram of the insertion of an additional loxp site into the Ccr1 locus. This was achieved by designing a small guide RNA directing Cas9 to cut the DNA downstream of exon 2. A repair template was also introduced which allowed incorporation of an additional loxp into the cut site by the DNA repair pathway. (iii) Recombination of the modified Ccr1 locus in response to Cre recombinase. (iv) PCR of genomic DNA for Cre recombinase or PCR identifying mice carrying the modified Ccr1 allele. (v) Recombination was seen in mice carrying both the modified Ccr1 allele and Cre recombinase, demonstrating that the additional loxp had been inserted into the Ccr1 gene.]

Reusing old broken alleles

The continued development of genome editing techniques presents an opportunity for us to approach projects in a different way. Gene targeting requires a DNA intermediate to make alterations in endogenous genes. However, with genome editing approaches, alterations can be made directly to endogenous genes without the requirement for a DNA intermediate. This enables us to modify genes in ways that are not feasible using standard gene targeting techniques.

One circumstance where genome editing provides a significant advantage over gene targeting is the introduction of small modifications to already existing targeted alleles. In the examples outlined below, the use of genome editing allowed us to modify targeted loci already in use, enabling them to function better, or in one case to work in the way that they were originally intended.

The first allele where we used gene editing to repair, was a conditional allele of Runx2. This allele had been correctly targeted, introducing loxp sites on either side of exon 3, but also had introduced a selectable neomycin (Neo) marker cassette as part of the gene targeting procedure. This cassette had been impossible to remove. Furthermore, as this cassette had included a strong transcriptional promoter and had been inserted directly in the middle of the Runx2 gene between exons 3 and 4, it had interfered with the expression of the endogenous Runx2 gene. This had resulted in a hypomorphic allele that had displayed some of the mutant phenotypes even before Cre recombinase expression disrupted the gene by recombination at the loxp sites.

Altering this, would have meant to start again and create a new allele from scratch. However, using gene targeting, we had the opportunity to remove the selectable marker cassette from the already existing Runx2 allele. This was achieved by designing two small guide RNAs which directed Cas9 nuclease activity to either side of the selectable marker cassette, taking care to leave the two loxp sites intact. The Cas9 nuclease cut precisely on either side of the selectable marker cassette, effectively excising the cassette and leaving the resultant gap to be filled by the DNA repair pathway.

This way, we removed the selectable marker from the Runx2 allele, leaving the loxp sites intact, but the allele could also function as a conditional allele. Furthermore, we showed that animals carrying the newly engineered allele did not display any of the hypomorphic phenotypes of the original allele, but instead were the same size as their wild-type counterparts (Figure 1B).

In the second example, we modified an allele of the gene Ccr1. This gene was missing an essential loxp site at the 3' end of exon 2, that would have allowed the gene to work as a conditional allele. Again, to repair this allele by gene targeting would have required generating a new allele from scratch. However, we repaired the existing allele using genome editing by inserting a small 34 base pair loxp site downstream of exon 2. This was achieved by designing a small guide RNA, directing Cas9 nuclease to cleave DNA just downstream of exon 2 of the Ccr1 gene. In order to facilitate the incorporation of the additional loxp site, we introduced a small repair template which included the loxp site of exon 2, allowing the allele to be used as a conditional allele. Furthermore, we were able to show that the expression of Cre recombinase allowed Cre recombination at the locus, which should alter the expression of the Ccr1 gene.

Taken together, these examples showed how genome editing broadens the range of tools we have available to generate functional alleles for cancer modelling. We are now able to approach projects in new ways, and accomplish them in a more effective and ethical manner, ultimately saving valuable time and resources.