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CANCER

The evolution of genetically engineered mouse models of cancer

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Mouse models of cancer have taught us much about how cancer develops. They have been instrumental in, and some would argue essential for, verifying theories of cancer biology that were initially developed in cultured cells. However, as our understanding of the complexity of tumour biology has increased, the limitations of using mice to model human cancer have become evident. But mice still offer the promise of testing a new hypothesis under replicated *in vivo* conditions, and few would question that findings from *in vitro* studies need to be verified *in vivo*. So how can we improve genetically engineered mice (GEM) so that they are more relevant to the conundrums that we are now trying to resolve? GEM need to evolve further to accurately reflect all the components of a human tumour if they are to have a greater role in the bench-to-bedside continuum. Humanizing GEM, alongside the insightful use of current genetic technology, should ensure that this progression is successfully achieved.

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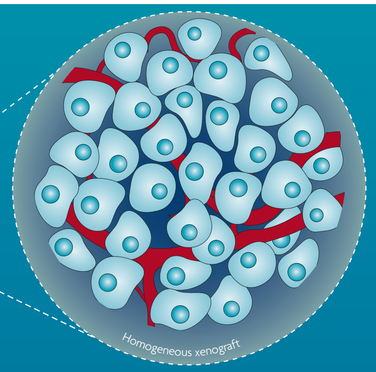
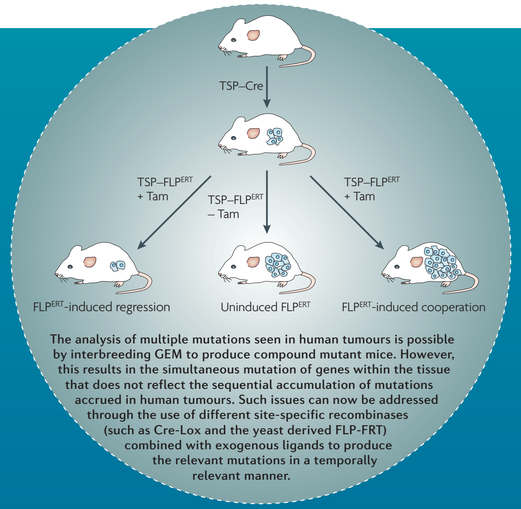
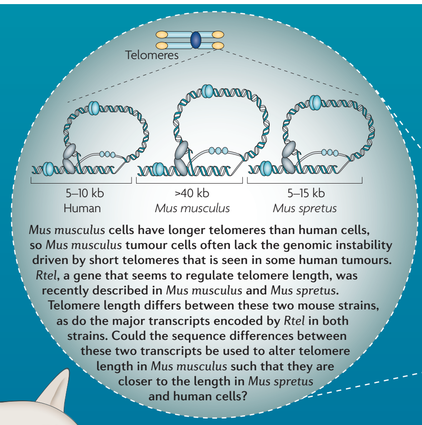
Animal culture

Although initially useful, xenograft models of human cancer do little to replicate the real disease and are essentially an *in vivo* Petri dish. Xenografts show loss of the normal tumour architecture and often consist of a dominant clone that was not evident in the primary tumour. Moreover, the vascular and lymphatic systems are not well established in xenografts and there is an aberrant immune response. It is therefore not surprising that xenografts have an altered response to chemotherapeutic drugs. The time for reliance on such models to determine the response to a new therapy has passed.





The analysis of multiple mutations seen in human tumours is possible by interbreeding GEM to produce compound mutant mice. However, this results in the simultaneous mutation of genes within the tissue that does not reflect the sequential accumulation of mutations accrued in human tumours. Such issues can now be addressed through the use of different site-specific recombinases (such as Cre-Lox and the yeast derived FLP-FRT) combined with exogenous ligands to produce the relevant mutations in a temporally relevant manner.



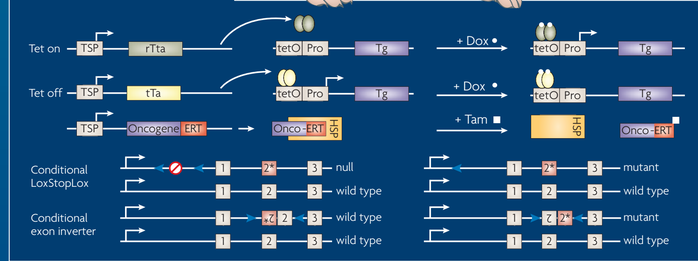
Mus musculus cells have longer telomeres than human cells, so *Mus musculus* tumour cells often lack the genomic instability driven by short telomeres that is seen in some human tumours. *Rtel*, a gene that seems to regulate telomere length, was recently described in *Mus musculus* and *Mus spretus*. Telomere length differs between these two mouse strains, as do the major transcripts encoded by *Rtel* in both strains. Could the sequence differences between these two transcripts be used to alter telomere length in *Mus musculus* such that they are closer to the length in *Mus spretus* and human cells?

Genetically engineered mice (GEM)

GEM are now many and varied. Initial GEM relied on the overexpression of a transgene — either an oncogene or dominant negative tumour-suppressor gene — within a specific tissue through the use of ectopic promoter and enhancer elements, such as the immunoglobulin heavy chain enhancer in *Eμ–Bcl2* or *Eμ–Myc* transgenics. The capacity to regulate the function of a transgene through the use of exogenous ligands, such as doxycycline to regulate transcription (the Tet system), or tamoxifen to regulate protein function, have enabled the temporal regulation of oncogene expression and the demonstration of 'oncogene addiction' in a tissue. For example, the regulation of *Kras* and *Hras* by doxycycline demonstrated a role for these oncoproteins in the induction and maintenance of lung cancer and melanoma, respectively. However, such models are still under the regulation of an ectopic promoter. Knockout and knockin technologies heralded the era of endogenous GEM, in which mutant genes are under the control of the endogenous promoter and enhancer sequences.

Such technologies also enabled the loss of tumour-suppressor genes in cancer, such as those observed in human familial syndromes, to be replicated in mice. However, most studies to date have not reproduced the human condition (for example, loss of *Nf2* does not induce neurofibromatosis type 2 schwannomas in mice). Conditional models have shown more promise. These enable the deletion or expression of a gene within a specific tissue, under the control of their endogenous promoter through the use of recombinases such as Cre-Lox and FLP-FRT. This can also be combined with ligand-dependent activation of Cre through the use of the tamoxifen-dependent Cre-ERT to achieve greater temporal control.

Genetics of GEM

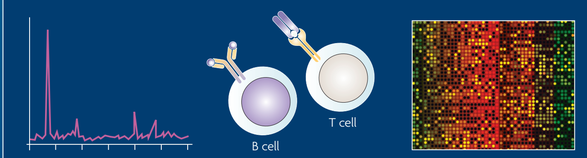


More advanced GEM

Human tumours are thought to arise from a cell sustaining one initial mutation, from which more mutated cells arise. However, the oncogenic events in many GEM occur in all cells of the tissue, and the tumour cells do not evolve in the context of normal surrounding cells. The use of Cre-expressing viruses at low titres has enabled the activation and silencing of genes in just a few cells, resulting in hyperplastic foci surrounded by normal cells. Cre-Lox technology is also being used to introduce and model the effects of genetic changes within the tumour microenvironment.

Humanized mice

A long-standing criticism of genetically engineered mouse models of cancer is that important physiological processes in mice differ substantially from those in humans. So, why not make GEM more human? Currently, mice are being engineered that harbour human genomic loci including non-coding regulatory elements, genes involved in the immune response and genes that regulate drug metabolism and protein glycosylation. All of these modifications should enable mRNA profiles, cellular and serum biomarkers, changes in tumour metabolism and relevant alterations in the tumour microenvironment to more closely model changes that occur during tumorigenesis in humans, and therefore should translate much more effectively into the clinic.



Cancer Research UK Cambridge Research Institute

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The Cancer Research UK Cambridge Research Institute (CRI) was officially opened by Her Majesty the Queen in February 2007. The Institute is located on the Cambridge Biomedical Campus in the heart of the Cambridge research community. This allows researchers in the CRI to interact extensively with clinicians in Addenbrooke's hospital and with scientists working both in the University and the surrounding biotechnology cluster. A virtual Cambridge Cancer Centre provides a framework to bring together scientists from across Cambridge that are interested in cancer research.

The Institute is devoted to translational cancer research, driving the development of new approaches to early detection, prevention and treatment. Our research is built around three themes:

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- Biology of epithelial tissues and early stages of cancer development
- Interactions between emerging cancer cells and normal cells
- Mechanisms of faulty gene control

New technology-driven research
- Molecular imaging
- Genomics
- Bioinformatics and bio-molecular computing

Clinical research
- Solid tumours (including breast, prostate and pancreas)
- Improved clinical trials
- Population-based studies in screening and prevention

Researchers at the Institute are supported by excellent core services including genomics, bioinformatics, imaging, microscopy and histopathology.

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Abbreviations

Dox, doxycycline; ERT, oestrogen receptor that responds only to tamoxifen; FLP, flipase recombination enzyme; FLT, flipase recognition target; GEM, genetically engineered mice; HSP, heat shock protein; rTta and tTa, tetracycline-regulatable transcription factors; Tam, tamoxifen; Tet, tetracycline; Tg, transgene; TSP, tissue-specific promoter.

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