Commentary

Do we now have a relevant animal model for breast cancer?

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Recent advances in manipulating targeted genes in a tissue-specific manner have opened the way to the development of relevant mouse models for the molecular dissection of the events leading to breast cancer. However, when judging the appropriateness of any given mouse model, it is important to remember that breast cancer comprises a heterogeneous group of diseases characterized by different sets of genetic mutations, histopathological types and metastatic potentials, often within the same primary tumour mass. It is unlikely that any single mouse model will be able to mimic all these aspects of human breast cancer but this does not invalidate their use in studying specific aspects of the disease. Mouse models are particularly valuable for defining the molecular pathways participating in mammary epithelial cell transformation and disease progression, for identifying modifier genes that affect penetrance of the manipulated gene and for testing various therapeutic and preventative approaches. The paper by Xu *et al* [1] in a recent edition of *Nature Genetics* describes a new model that offers promise in several respects.

To put the new model in perspective, we need briefly to consider the historical background on mouse models of human cancers. From the 1950s, much effort has been put into describing and classifying spontaneous, viral- and carcinogen-induced mammary tumours in rats and mice, and these models have proven value in toxicology and drug testing. Mice infected with the mouse mammary tumour virus (MMTV) have played a large part in our understanding of insertional mutagenesis and activation of oncogenes leading to mammary tumourigenesis [2]. However, only a few of the human homologues of these genes are mutated in human breast cancers although the signalling pathways through which these genes act have been implicated. In carcinogen-induced rat mammary tumours there is a high incidence of *ras* mutations, which are very rare in human breast cancers. Thus, although these models are valuable tools for the dissection of the complex signalling pathways through which these genes act, they do not necessarily represent the exact genetic events that precipitate human breast cancers.

Transgenic technology has recently facilitated the development of an entirely new set of genetically engineered mouse models that can be used to define the transforming potential of genes implicated in human breast cancer [3,4]. The seminal work of Stewart *et al* [5] has shown that a *c-myc* transgene expressed in the mammary gland under the regulation of the MMTV long-terminal repeat (LTR) induces mammary tumours. Webster *et al* [6] have extended this work, and demonstrate how sophisticated targeted mutagenesis can be applied to dissecting signalling pathways.

The paper by Xu *et al* [1] now demonstrates the power of conditional mutagenesis to specifically delete a gene relevant to human hereditary breast cancer (*BRCA1*) from the mouse mammary gland. *BRCA1* mutations are known to account for a significant proportion of familial breast cancers. BRCA1 contains a region that interacts with RAD51, a homologue of bacterial RecA, which is involved in DNA repair, and is believed to be important in maintaining genetic stability. Homozygous loss of *BRCA1* in human tumours is thought to allow the accumulation of mutations in other genes, eventually resulting in tumourigenesis. However, progress in studying the effects of *BRCA1* deletion or mutation on breast development and breast cancer has been delayed because of the lack of an animal model. Mice bearing homozygous null mutations of *Brca1* die before embryonic day 9, whereas mice heterozygous for *Brca1* deletions do not develop

Apc = adenomatous polyposis coli; LTR = long-terminal repeat; MMTV = mouse mammary tumour virus; Wap = whey acidic protein.

mammary tumours. The paper by Xu *et al [1]* demonstrates one way forward that uses a conditional knockout approach to mutate the intact allele in the mammary glands of mice bearing heterozygous deletions of *Brca1*. This new method uses the Cre-*loxP* system to induce mutations in a tissue- and temporal-specific manner [7], and mimics human disease by producing mice in which one of the two *Brca1* genes has been disabled while the other carries a mutation that enables it to be disabled in mammary tissue later in the life of the mouse. Specifically, it induces mammary tissue specific deletion of *Brca1* exon 11 (which encodes the region that interacts with RAD51) under the control of either an *MMTV-Cre* or a whey acidic protein *(Wap)-Cre* transgene. The *MMTV* and *Wap* promoters are maximally activated during pregnancy and lactation; the Cre-*loxP* system excises specific DNA sequences under the control of these tissue-specific promoters. The model mice thus lose the Brca1 repair function on pregnancy and lactation.

The resulting *Brca1* conditional knockout appears to model the molecular mechanism of *BRCA1* involvement in human breast cancer. The mice in which Brca1 function has been ablated in this way develop mammary-specific developmental abnormalities and, after a long latency period, mammary tumours. The molecular pathology of these tumours resembles that of the carcinomas arising in human carriers of *BRCA1* mutations. A common feature of the tumours that develop in the mice is aneuploidy and genetic instability as indicated by chromosomal translocations. The tumours show rearrangements or translocations of chromosome 11, and it is stated that rearrangements of other chromosomes are found. Human *BRCA1*-associated tumours also show frequent chromosomal aberrations [8–10]. It is interesting that two out of three tumours arising in the conditional *Brca1* knock-out mice have abnormalities in the *Trp53* gene. The shortened latency of tumour development produced by introducing a loss of function *Trp53* allele provides further support for the important role of *TP53* mutations in tumourigenesis in the *BRCA1* mutant background as described in human tumours [11]. Thus, the Xu mouse is a true breakthrough as it is the first model in which the mechanisms of genetic instability and resultant tumourigenesis in the *Brca1*-deficient mammary gland can be studied. These mice will undoubtedly be of value in elucidating the early genetic lesions that promote breast tumourigenesis. Furthermore, they should be useful for investigating the effects of an array of suspected agents in breast cancer because of their increased sensitivity to DNA-damaging insults.

The comparative histopathology is an important element in validating a mouse model and has implications for deducing the stem cell of origin, and predicting future behaviour of the tumour. The differences in the histopathology of the tumours arising in mice compared to those in women have been a major limitation of many mouse models, especially as they suggest a different target cell population for the initiating event. Comparisons have been complicated by the lack of an internationally accepted terminology for both the normal glandular structure and the tumours that arise in the mammary glands of rats and mice. In transgenic models, interpretation of pathology is further complicated by the currently used promoters, which may direct recombination to cells at different stages of differentiation or to cell lineages different from those commonly mutated in human cancers. The *Wap* promoter is expressed primarily during pregnancy and lactation throughout the mammary tree and one advantage is that the lumenal cell population only is the target. The MMTV-LTR, also used to generate *Brca1* conditional knockouts, has the disadvantage of being active in many tissues. Despite these caveats, however, it is clear that the tumours arising in the *Brca1*-deficient animals have many of the morphological features seen in human breast cancers. It will now be important to evaluate the model in terms of invasion of these tumours, lymph node involvement and the pattern of dissemination, as mouse models of metastasis to organs other than the lung, such as the brain and bones, are very much needed. This could be difficult to ascertain in the Xu model, given the long latency to tumourigenesis, but will be well worth the effort. It will also be important to assess the patterns of expression of molecular markers such as the receptors for oestrogen and progesterone. The faithfulness of the Xu model in this respect will determine its value for biochemical analyses and treatment studies. Now that it has been shown that *Brca1*-deficient mice do develop mammary tumours, future models may be prepared using new, more selective promoters as they are identified.

Counsellors working with women who carry *BRCA1* mutations can only give them a statistical probability of the likelihood of developing cancer by a given age; this is an unsatisfactory basis for making high-stake decisions regarding preventative strategies. Differences in penetrance among different ethnic populations are becoming apparent. These can be attributed either to specific *BRCA1* mutations or to allelic variants in modifier loci. Inbred mice are undoubtedly the most powerful experimental system for identifying modifier genes and an example of their usefulness is the identification of *Pla2g2a*, encoding a secretory phospholipase, as a major modifier of intestinal neoplasm formation in adenomatous polyposis coli (*Apc*)-deficient mice [12]. However, the conditionally mutant *Brca1* allele was generated using outbred mice, which limits immediate use for mapping modifiers. The *MMTV-Cre* and *Wap-Cre* transgenic lineages are also on segregating backgrounds, compounding the complications for mapping already presented by the cross needed to produce the recombined allele. This particular model is therefore not yet ideal for detecting additional predisposing genes.

Finally, transgenic mice have been used successfully in the development of gene-based therapies such as the farnesyl transferase inhibitor approach to treating *ras*-mediated tumours. The work in Mark Greene's laboratory, showing that *ErbB2*-expressing mammary tumours could be inhibited *in vivo* by treatment with monoclonal antibodies to the receptor [13], was an important step in the development of Herceptin® (Genentech Inc, South San Francisco, California, USA). Because the *Brca1*-deficient mice appear to model the molecular mechanisms of human *BRCA1*-associated tumours, they should be valuable to the development of gene-based preventative or therapeutic strategies. In particular, they may be useful for testing therapies that induce apoptosis through *Trp53*-independent pathways.

Clearly, the ability to delete *Brca1* in a tissue-specific manner is a breakthrough in the development of animal models of the molecular mechanisms of *BRCA1*-associated breast cancer in humans. This alone will make it a very useful model for understanding how cells that have lost *Brca1* become transformed, and for testing treatments aimed at blocking that process. There will be a lot of excitement in the scientific community as the remaining elements of this model are evaluated and we would encourage Xu *et al* to make the mice available to the community so that different aspects of its ability to model human disease are efficiently tested.

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