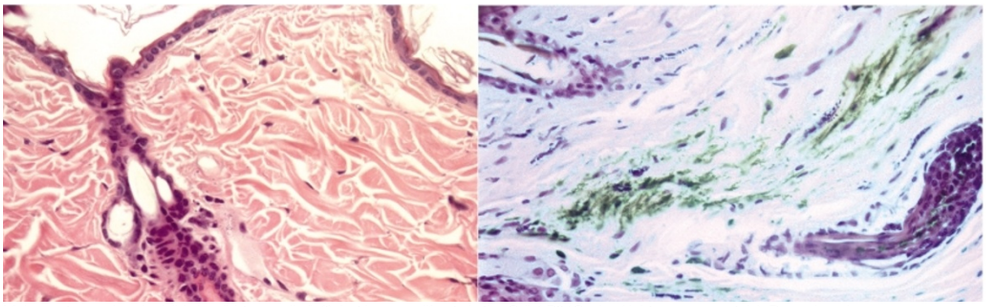
MOUSE MODELS

# Of mice and moles



Melanocytes are barely visible in wild-type cells (left), but hyperproliferate in the *Cdk4 R24C* mice (right; see green stain). Reproduced with permission © (2001) *Proc. Natl Acad. Sci. USA.*

Invasive melanoma is notoriously difficult to treat, but the generation of mouse models of the disease would facilitate the development of vaccines and expedite screening for new drugs. Sotillo *et al.*, reporting in the 6 November issue of *Proceedings of the National Academy of Sciences*, describe such a mouse model.

Most human tumours have deregulated CDK4 activity, usually due to cyclin D1 overexpression or inactivation of the INK4 cyclin-dependent kinase inhibitors, but a point mutation in CDK4 has also been identified in patients with both spontaneous and familial melanoma. Mutation of this residue (R24C) prevents CDK4 from binding to the INK4 proteins.

To investigate the role of R24C in melanoma susceptibility, the authors generated knock-in mice that express *Cdk4 R24C*. They develop multiple tumours, but do not show melanocyte hyperproliferation (see picture). However, on treatment with the skin carcinogens 7,12-dimethylbenz[*a*]anthracene (DMBA) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA), they develop more, and larger, skin papillomas than wild-type mice, which frequently progress to invasive carcinomas. Nevi (moles), which are precursors of melanoma, are also more common in the carcinogen-treated *Cdk4 R24C* mice, and 70% of these mice developed melanomas.

Interestingly, although the draining and regional lymph nodes were enlarged and pigmented, distant metastases were not found. This could be because the mice die before distant metastasis occurs, or because they lack distant metastatic potential.

So, is loss of the Ink4 inhibitors at the *Cdkn2a* and *Cdkn2b* loci also necessary for the generation of melanoma? Sotillo *et al.* suggest not, as Southern blotting of the melanomas from *Cdk4 R24C* mice did not reveal deletion or rearrangement of the loci, or promoter methylation in the the genes encoding Ink4a, Ink4b and Arf. In addition to this, immunostaining of Ink4a showed that it was present in all tumours analysed.

*Cdk4 R24C* mice are insensitive to all members of the Ink4 family, so which confer susceptibility to melanoma? The authors investigated tumour incidence following carcinogen treatment in mice deficient in Ink4b and Ink4c, as well as in *Ink4aΔ2,3* mice — which produce neither Ink4a nor Arf. *Ink4aΔ2,3* mice have an increased susceptibility to lymphomas, so all die by 10–12 weeks — before the incidence of melanoma could be evaluated — and Ink4b-deficient mice do not exhibit a higher rate of melanomas than wild-type mice. Ink4c-deficient mice, however, have an increased incidence of melanomas, but not of papillomas. The incidence of melanoma is still lower than in carcinogen-treated *Cdk4 R24C* mice, indicating that their susceptibility to melanoma is probably also due to loss of interaction with Ink4a, which is supported by results from human tumours and other melanoma mouse models (see further reading).

Now, with several melanoma mouse models available, it is time to use them to generate new treatment opportunities for melanoma.

*Emma Greenwood*

## References and links


**ORIGINAL RESEARCH PAPER** Sotillo, R. *et al*. Invasive melanoma in Cdk4-targeted mice. *Proc. Natl Acad. Sci. USA* **98**, 13312–13317 (2001)

**FURTHER READING** Krimpenfort, P. *et al*. Loss of $p16^{Ink4a}$ confers susceptibility to metastatic melanoma in mice. *Nature* **413**, 83–86 (2001) | Sharpless, N. E. *et al.* Loss of $p16^{Ink4a}$ with retention of $p19^{ARF}$ predisposes mice to tumorigenesis. *Nature* **413**, 86–91 (2001) | Sotillo, R. *et al*. Wide spectrum of tumors in knock-in mice carrying a Cdk4 protein insensitive to INK4 inhibitors. *EMBO J.* (in the press)

**WEB SITE**

**Encyclopedia of Life Sciences:** www.els.net
melanoma


BREAST CANCER

# Team effort

**Why has it been so difficult to model hereditary breast cancer in mice? Homozygous loss of either of the known breast-cancer susceptibility genes, *Brca1* and *Brca2*, is not compatible with life, whereas mice bearing truncation mutations die or develop lymphomas. Jos Jonkers and colleagues now describe how they have overcome these problems by developing a conditional knockout of *Brca2*, using the *cre–lox* system.**

**The authors targeted exon 11 of *Brca2*, which encodes its Rad51-binding BRC repeats. Binding to Rad51 is necessary for Brca2 to carry out its poorly understood function in DNA repair. To generate a tissue-specific conditional mutant, the authors put the *cre* gene under the control of the keratin 14 (K14) promoter, which is active in stratified epithelia such as those in mammary gland and skin. By crossing *K14cre* mice with mice carrying the $Brca2^{\Delta 11}$ allele, mice were bred in which both *Brca2* alleles were mutated in tissues where the K14 promoter is active. Surpsisingly, these mice did not develop tumours any more frequently than wild-type mice.**

**So what else is needed to tip their apparently normal mammary tissue towards tumour formation? The tumours of women with *BRCA2* mutations often show loss of *TP53*; could this situation be reproduced by crossing *Brca2* mutant mice with conditional *Trp53* knockout mice? The conditional *Trp53* knockouts generated by the authors were tumour prone but didn't develop any mammary tumours. In stark contrast, mice that were homozygous for both mutations in epithelia developed tumours of the mammary gland and skin. Mice with just one functional copy of either *Brca2* or *Trp53* in epithelium also developed tumours, but with a longer latency. In all the mice lacking one *Trp53* allele, the second allele was lost in the tumours, but in those lacking two *Trp53* alleles, only about half lost the second *Brca2* allele.**

**Loss of both copies of *Trp53*, then, is necessary for tumour formation in mice lacking *Brca2*. Another intriguing finding is that there is nothing unique about mammary tissue, as the double knockouts developed tumours in skin. Further experiments will uncover how these two important tumour suppressors normally collaborate to protect against cancer.**

***Cath Brooksbank***

## References and links


**ORIGINAL RESEARCH PAPER** Jonkers, J. *et al.* Synergistic tumour suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. *Nature Genet.* **29**, 418–425 (2001)

**FURTHER READING** Scully, R. & Livingston, D. In search of the tumour suppressor functions of BRCA1 and BRCA2. *Nature* **408**, 429–432 (2000)