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MOUSE MODELS

Novel *in vivo* RNAi screen in mice

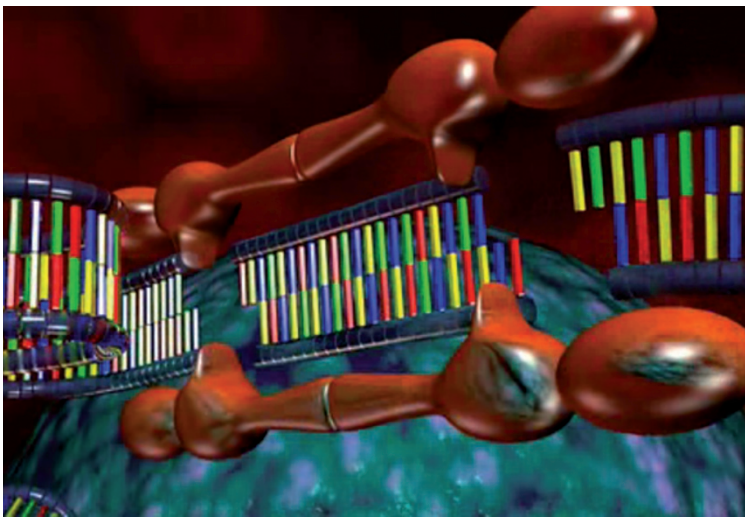
> This method eliminates many of the caveats that are typically associated with culture screens

Using an innovative RNA interference (RNAi) screening method in mice, a recent study has uncovered genes that are required for normal skin growth and skin cancer development.

Beronja *et al.* previously developed an ultrasound-guided *in utero* lentiviral transduction method to target embryonic skin epithelium *in vivo* with pools of lentiviral short hairpin RNAs (shRNAs). In this new study, the authors extend the above method to conduct parallel genome-wide screens of 78,000 shRNAs for genes required for normal skin growth in wild-type embryos, and genes required for hyperplastic skin growth in embryos that express oncogenic *Hras* in skin cells. They successfully identified ~1,800 genes required for normal growth and 160 genes required for hyperplastic growth.

The top two candidates that emerged from their screen were β-catenin and mixed-lineage leukaemia translocated to 6 gene (*Mllt6*). Deregulation of β-catenin is known to be involved in several cancer types, including skin cancer, whereas *Mllt6* has only previously been implicated in the development of leukaemias.

Interestingly, knockdown of β-catenin was found not only to reduce oncogene-mediated proliferation in *Hras* mice but also to increase skin cell proliferation in wild-type mice. This increased proliferation was found to be dependent on the adhesion properties of β-catenin and independent of WNT signalling. Ablation of *Mllt6* mirrored the outcome of β-catenin loss with respect to decreasing proliferation in *Hras* mice, but had no effect on proliferation in wild-type mice.



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The authors extended these findings to human squamous cell carcinoma (SCC) samples, and found that both proteins were expressed in SCCs. Moreover, tumour development was delayed when either β-catenin or MLLT6 expression was reduced both in adult mice with *Hras*-driven skin cancer and in xenograft mouse models. In addition, an inducible method of shRNA depletion for either β-catenin or *Mllt6* resulted in partial regression of established skin tumours. Together, these results suggest that β-catenin and MLLT6 are important for both tumour initiation and tumour maintenance.

Intriguingly, the investigators showed that knockdown of either β-catenin or *Mllt6* in oncogenic *Hras*-expressing embryos affected similar sets of mRNA transcripts. In addition, depletion of β-catenin on its own reduced *Mllt6* transcript levels. Furthermore, using immunohistochemistry, β-catenin and MLLT6 were shown to exhibit similar expression patterns in human tumour samples. An interesting avenue for future research will be to determine whether these results collectively reflect a functional interaction between β-catenin and *Mllt6*.

Additional targets identified in the screen included chromatin modifiers that have been shown to have key roles in other malignancies and are therefore attractive prospects for further investigation.

Overall, this paper shows that the large-scale *in vivo* knockdown of genes is possible in mice, when cells are in their native environment. This method eliminates many of the caveats that are typically associated with culture screens, and could provide new insights into genes involved in the biology of skin growth and skin carcinogenesis.

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ORIGINAL RESEARCH PAPER Beronja, S. *et al.* RNAi screens in mice identify physiological regulators of oncogenic growth. *Nature* http://dx.doi.org/10.1038/nature12464 (2013)