CANCER GENETICS

CRISPR screens go *in vivo*

CRISPR–Cas9 is a powerful system for gene disruption and gene editing that has recently been applied for genome-wide functional screens *in vitro*. A new study shows that CRISPR–Cas9 screens are feasible *in vivo* and can be used to identify tumour suppressor genes.

Chen, Sanjana *et al.* used a mouse lung cancer cell line with defined cancer driver mutations (*Kras*$^{G12D/+}$,*Trp53*$^{-/-}$ and *Dicer1*$^{+/-}$) that is known to form primary tumours in immunocompromised mice but is poorly metastatic. Using lentiviruses, they sequentially transduced an *in vitro* population of these cells with a construct expressing the Cas9 nuclease, and then with a genome-wide library of 67,405 constructs expressing guide RNAs that direct Cas9 to defined genomic locations for inactivation of protein-coding or microRNA-encoding genes.

> “useful candidates for further functional testing”

The investigators subcutaneously injected these cells into immunocompromised mice and found that the cells formed primary tumours faster and also metastasized more frequently to various organs than the same cell line without the guide RNA library. This implies that inactivation of some of the target genes promotes tumorigenesis and metastasis.

To identify which inactivated genes were responsible for the increased tumorigenesis and metastasis, the authors sequenced the different guide-RNA-expressing constructs in the initial injected cell population, primary tumour samples and metastases. They identified various constructs that became reproducibly enriched as the cancers advanced, indicating that inactivation of the corresponding target genes was promoting tumorigenesis and/or metastasis of those subclones. Some of the gene hits for primary tumour growth (such as *Pten* and *Cdkn2b*) are well-characterized tumour suppressors, thus validating the approach, whereas others (such as *Mgmt* and *Med16*) are useful candidates for further functional testing. Furthermore, hits for which knockout accelerates metastasis also comprised both well-known tumour suppressors (*Nf2*, *Pten* and *Cdkn2a*) and less well-characterized genes (*Trim72*, *Fga*, *mir-152* and *mir-345*).

In follow-up work, Chen, Sanjana *et al.* validated the scoring genes by showing that different guide RNAs targeting these genes enhanced metastasis formation when tested individually in the same *in vivo* assay that was used in the screen. Moreover, the investigators also formed a focused pool of 524 guide RNAs targeting 53 of the top gene hits from the screen in order to track the competitive dynamics of tumour subclones harbouring lesions in these candidate tumour suppressor genes.

This technology could be applied to various other mouse models of cancer, either using this genetic loss-of-function setup or using Cas9 variants for screens based on transcriptional modulation.

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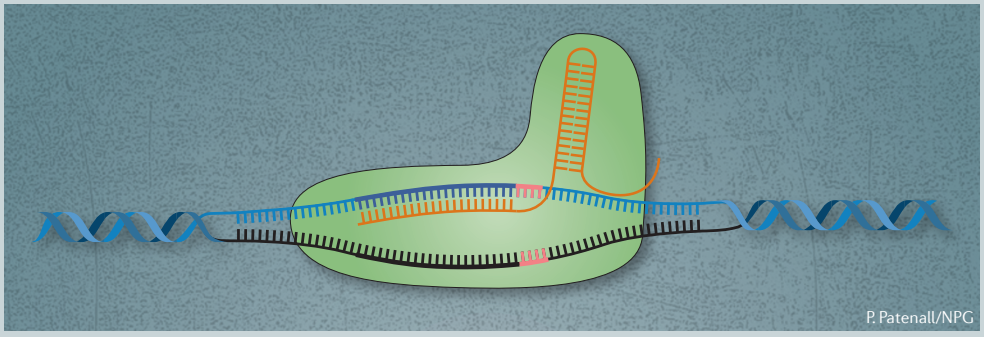

P. Patenall/NPG

ORIGINAL RESEARCH PAPER Chen, S., Sanjana, N. E. *et al.* Genome-wide CRISPR screen in a mouse model of tumor growth and metastasis. *Cell* http://dx.doi.org/10.1016/j.cell.2015.02.038 (2015)