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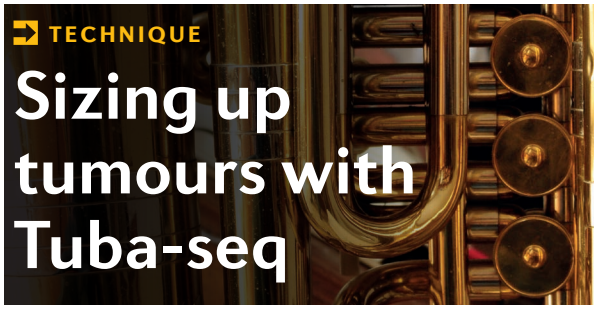

TECHNIQUE

Sizing up tumours with Tuba-seq

Sergio Amiti/Moment/Getty

Precise determination of tumour growth is a prerequisite for estimating the functional importance of tumour suppressor alterations in cancer. A study in *Nature Methods* now describes the development of Tuba-seq, an approach that integrates tumour barcoding with high-throughput sequencing, to precisely quantify the size of individual tumours *in vivo*.

The new method relies on the use of lentiviral Cre-vector libraries that contain DNA barcodes to stably tag individual lesions with a unique identifier. In a genetically engineered mouse model of human lung cancer, transduction of lung epithelial cells leads to Cre recombinase-mediated expression of oncogenic Kras. Expression of this oncogene induces the simultaneous development of multiple tumours, each now tagged with a unique 15-nucleotide (nt) barcode. In a first set of experiments, the Kras-driven tumours were analysed side-by-side with lung tumours that carried additional deficiencies in the tumour suppressor genes *Lkb1* or *p53*. High-throughput sequencing was performed on bulk tumour DNA, and read counts from barcodes were converted to estimated cell numbers based on mixed-in 'benchmark' cells. Tuba-seq accurately determined the number of neoplastic cells in all tumours in parallel, finding them to be more than 1,000-fold different in cell number. The size distribution of tumours further provided insight into the different mechanisms of action of p53 and LKB1 (also known as STK11) in suppressing tumour growth.

> "read counts of barcodes were converted to estimated cell numbers"

Having demonstrated the utility of Tuba-seq, Rogers, McFarland, Winters *et al.* set out to adapt the method for multiplexing, this time using CRISPR–Cas9-mediated inactivation of 11 known or suspected tumour suppressors in the Kras-driven lung adenocarcinoma model. Lentiviral vectors carried an 8-nt tag specific for an encoded guide RNA, as well as the 15-nt barcode for cancer cell quantification. In this experimental setup, in which different tumour suppressor genotypes were present within the same mouse, size and genotype of tumours were simultaneously determined by sequencing the guide RNA-specific barcode tag. Lkb1, Rb1, Cdkn2a and Apc were confirmed as tumour suppressors in Kras-driven lung cancer growth, and novel potential lung tumour suppressors (splicing factor Rbm10 and the methyltransferase Setd2) were found.

With its exceptional level of precision and reproducibility, Tuba-seq will be a powerful tool to determine the impact of loss-of-function mutations on tumour growth *in vivo*. Moreover, this approach can likely be adapted to other types of cancers and scaled up to even more extensively multiplexed libraries.

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Nature Communications

ORIGINAL ARTICLE Rogers, Z. N., McFarland, C. D., Winters, I. P. *et al.* A quantitative and multiplexed approach to uncover the fitness landscape of tumor suppression *in vivo*. *Nat. Met.* http://dx.doi.org/10.1038/nmeth.4297 (2017)