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TUMOUR SUPPRESSORS

At the hypusine of the crime


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As multiple genes are typically affected by the genomic amplifications and deletions that occur in various cancers, functional studies are required to distinguish the driver from the passenger gene lesions. A new *in vivo* RNA interference (RNAi) screen has identified tumour suppressor genes for lymphoma, including an unexpected link to post-translation modification by the amino acid hypusine.

Scott Lowe and colleagues compiled a list of 323 genes that are recurrently deleted in human B cell non-Hodgkin's lymphoma. To identify the bona fide tumour suppressor genes, they generated a library of short hairpin RNAs (shRNAs) targeting the mouse orthologues of these genes. The shRNAs were transduced into mouse Eμ-*Myc* haematopoietic stem and progenitor cells *in vitro* in pools of 100 shRNAs. Tail-vein injection of these cells into sublethally irradiated mice was carried out to test which gene knockdowns co-operate with the Eμ-driven MYC overexpression in B cells to generate B cell lymphomas. High-throughput sequencing was used to assess the representation of shRNA-encoding genomic integrants in the resultant lymphomas, and thus to find positively selected gene knockdowns. Overall, Scuoppo *et al.* identified nine new tumour suppressor genes, which were validated as accelerators of disease progression when tested individually in the same model.

Of the nine genes, two pairs with functional connections were particularly interesting. Mediator complex subunit 4 (*Med4*) and cyclin C (*Ccnc*) are components of a transcription-associated complex. Additionally, *S*-adenosylmethionine decarboxylase 1 (*Amd1*) and eukaryotic translation initiation factor 5A (*Eif5a*) are in a common polyamine pathway that leads to the post-translational modification of eIF5A by hypusine. Currently, eIF5A and the related eIF5A2 are the only known targets of this modification. Interestingly, the simultaneous knockdown of AMD1 and eIF5A resulted in a greater acceleration of tumorigenesis than either single knockdown, implying that multiple lesions in a single pathway can cooperate during tumorigenesis.

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From a secondary shRNA screen focused on polyamine pathway members, the authors found that additional enzymes involved in hypusination — spermidine synthase (*Srm*) and deoxyhypusine synthase (*Dhps*) — were also lymphoma suppressors, and lymphomas generated in the presence of knockdown of hypusination enzymes showed less eIF5A hypusination relative to control lymphomas. Although the molecular explanation for why hypusinated eIF5A suppresses lymphomagenesis is unclear, knockdown experiments suggested that it might promote apoptosis through the upregulation of the proapoptotic protein BAX.

Finally, human lymphoma data revealed a significant association between the hemizygous co-deletion of genes involved in hypusination, such as *AMD1* and *eIF5A*. These genes are essential, so the combined hemizygous deletions may provide developing tumours with maximal tumorigenic effects without compromising essential functions.

The view that multiple genes affected by chromosomal lesions — including physically linked genes in a single deletion — might cooperate to promote tumorigenesis is progressively gaining momentum, and is supported by a recent *Proceedings of the National Academy of Sciences* paper from the same laboratory studying deletions in liver cancer. It is noteworthy that the lymphoma suppressor genes were identified on the basis of the phenotypes of single-gene-knockdowns; the extent to which discovery power might be boosted by expanding RNAi screens to interrogate combinatorial knockdowns remains to be determined.

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ORIGINAL RESEARCH PAPER Scuoppo, C. *et al.* A tumour suppressor network relying on the polyamine–hypusine axis. *Nature* 10 Jun 2012 (doi:10.1038/nature11126)
FURTHER READING Xue, W. *et al.* A cluster of cooperating tumor-suppressor gene candidates in chromosomal deletions. *Proc. Natl Acad. Sci. USA* **109**, 8212–8217 (2012)