Tumour biology

An epigenetic fulcrum tipped in cancer

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Two studies show that some cancers are driven by genetic changes in the NSD3 protein that alter its enzymatic activity. Biochemical and structural characterization hints at a route to pharmacological reversal. **See p.498 & p.504**

Gene expression is dynamic, but tightly regulated. Cells use a variety of mechanisms to control when and where individual genes are turned on and off. When those mechanisms falter, the resulting aberrant gene expression often leads to cancer. One way in which genes are controlled is through the addition of various chemical marks to DNA and DNA-associated proteins. Yuan *et al.*[1] (page 504) and Li *et al.*[2] (page 498) now show that one of the enzymes that deposit such marks, NSD3, is mutated and hyperactive in some human cancers — a finding that suggests new avenues for treating people with the diseases.

In cells, DNA is wound around clusters of proteins called histones, forming structures known as nucleosomes. This allows the genetic material to be neatly compacted within the nucleus. The DNA and histones can undergo extensive modifications, in which various chemical groups are covalently added to, or removed from, the DNA bases and the tails of the histones. Those changes are referred to as epigenetic modifications: they do not alter the underlying genetic sequence, but instead constitute a signal that informs cellular machinery about which genes should be turned on and off.

The enzymes that add or remove epigenetic modifications are commonly overexpressed or mutated in cancer, pointing to their roles in initiating and maintaining the disease. However, the biochemical mechanisms underlying such dysregulation are only beginning to be understood. Yuan *et al.* and Li *et al.* tackle this issue, defining a cancer-promoting function for NSD3 — an enzyme that transfers methyl groups to specific amino acid residues of histones.

Yuan *et al.* use mouse models and human tumour specimens to define the *NSD3* gene as a cancer-causing oncogene in lung squamous cell carcinoma (oncogenes are genes that, when mutated, lead to uncontrolled cell multiplication). The authors find that, in human tumours, NSD3 becomes dysregulated, either by an increase in the number of copies of it in the genome, or by missense mutations (those that alter its amino acid sequence) that increase its catalytic activity. NSD3 dimethylates (adds two methyl groups to) the 36th amino acid residue in histone H3 (a lysine residue dubbed H3K36)[3], and the authors discovered that tumours with hyperactive NSD3 have elevated levels of H3K36 dimethylation.

Using genomic-profiling techniques, Yuan *et al.* found that oncogenic NSD3 catalyses the dimethylation of H3K36 in the protein-coding region of cancer-promoting genes, which in turn leads to higher rates of expression of those genes (Fig. 1a). An important set of experiments suggests that an increase in the copy number of *NSD3* (which is much more common than missense mutations) causes lung cancer cells to become 'addicted' to the catalytic function of NSD3, in that its loss impedes cell growth through a decrease in H3K36 dimethylation and decreased expression of cancer-driving genes (Fig. 1b). Collectively, the experiments point to NSD3-mediated catalysis in these tumours as a possible drug target.

In the accompanying study, Li *et al.* use cryo-electron microscopy to solve the structures of normal and oncogenic mutant forms of NSD3 bound to a nucleosome. One of their major achievements is in explaining the long-observed preference of NSD proteins for methylating H3K36 in the context of a nucleosome, rather than in free histones[3]. The authors find that, in the absence of a substrate, the substrate-binding site of NSD3 is blocked by one of the protein's own loops. Several interactions with the nucleosome surface and with partially unwound DNA cause this loop to become displaced, freeing up space for the histone H3 tail to enter NSD3's catalytic site.

The structural analysis also reveals how cancer-causing mutations in NSD3 lead to the formation of new hydrogen bonds with histones, explaining the enhanced catalytic activities of these mutants. These atomic-resolution structures of NSD3, as well as enzymology studies reported in the new work, will provide a foundation for structure-guided screens to identify small-molecule NSD3 inhibitors. Notably, a study[4] published last year described small molecules that covalently inhibit the methyltransferase function of NSD1

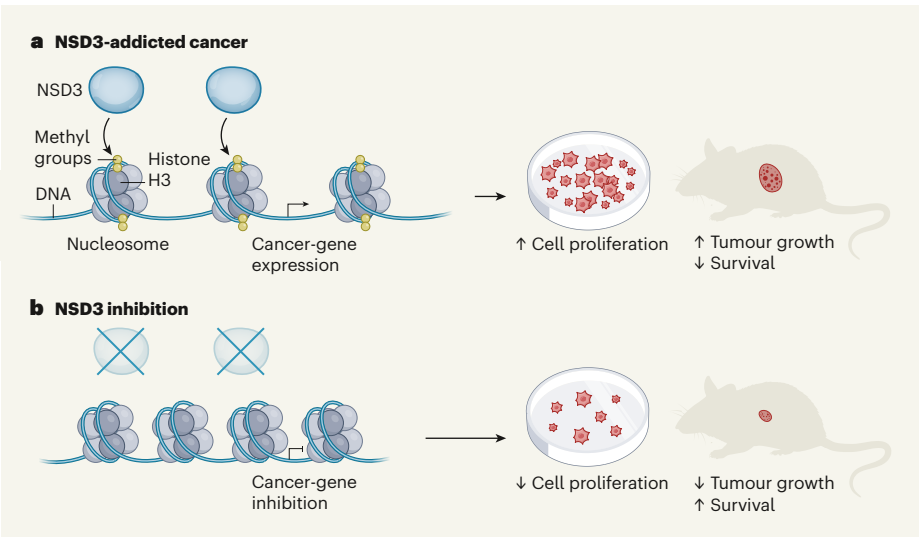

Figure 1 | Deregulation of histone methylation in human cancer. The enzyme NSD3 is a histone methyltransferase — it dimethylates (adds two methyl groups to) histone H3 proteins, around which DNA is packaged. Yuan *et al.*[1] have found that NSD3 is dysregulated in human squamous cell lung carcinoma, and Li *et al.*[2] have unravelled the structural mechanism involved. **a**, In 'NSD3-addicted cancers', the enzymatic activity of NSD3 is increased through an increase in the number of copies of the *NSD3* gene, or through mutations that cause hyperactive enzyme activity (not shown). This leads to an increase in H3 dimethylation and the expression of cancer-associated genes. In cell culture, this results in increased cell proliferation, and in mice, it results in increased tumour growth and a decrease in the animals' survival. **b**, Inhibiting NSD3 might provide a targeted treatment for those cancers. Depletion of NSD3 leads to a decrease in H3 dimethylation, decreased expression of cancer genes, reduced cell growth in culture and fewer tumours in mice.

– a proof-of-concept that selective targeting of NSD proteins is feasible.

The current studies highlight remarkable parallels between NSD3 and the known role of NSD2 in promoting lymphoid cancers, which also occurs through mutations and gene rearrangements that enhance H3K36 dimethylation[5,6]. These commonalities are reinforced by Li and colleagues, who show that the catalytic domains of NSD2 and NSD3 have similar atomic structures. Moreover, in cells, missense mutations in the two enzymes cause similar increases in the methylation of H3K36. This finding begs the question of whether NSD2 and NSD3 mutations lead to identical effects, or whether there are differences in the mechanisms that target these two enzymes to specific genes. An understanding of the functional overlap between different H3K36 methyltransferases will be essential to predict and prevent resistance to targeted inhibitors. A related question is whether NSD2- and NSD3-activated tumours have common cellular characteristics and responses to drugs, despite occurring in different cell types.

There are hundreds of covalent histone modifications that have regulatory functions. However, a substantial body of evidence points to the methylation of H3K36 in particular as a focal point for epigenetic dysregulation in cancer – an idea reinforced by the two new papers. At least four H3K36 methyltransferases are now known to be bona fide genetic drivers of human cancer, functioning as oncogenes (NSD2 and NSD3), tumour suppressors (SETD2, for instance[7]), or both (NSD1; refs 8, 9), depending on the cellular context. Also, certain mutations in histone-encoding genes promote cancer by inhibiting the catalytic function of H3K36 methyltransferases[9,10]. By considering the current studies in this broader context, it becomes apparent that too much or too little H3K36 methylation can profoundly affect the development of cancer.

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A dominant feature of all of these mutated methyltransferases and histones is the tight association with specific tumour-cell lineages: each mutation that deregulates H3K36 methylation is largely confined to a particular cancer type. That suggests a physiological role for the methylation of H3K36 in controlling the identity of normal cells – a convenient molecular switch that is exploited by tumour cells to achieve uncontrolled cell growth. The status of H3K36 methylation as an epigenetic fulcrum, the common target of many enzymes, poses a substantial impediment to advancing therapeutics for cancer. Nonetheless, Yuan *et al.* and Li *et al.* highlight how targeting both NSD2 and NSD3 could allow the normalization of H3K36 methylation in lymphoid and lung tumours, respectively, while avoiding the tumour-promoting consequences that would be expected from inhibiting all H3K36 methyltransferases at once.

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1. Yuan, G. *et al. Nature* **590**, 504–508 (2021).
2. Li, W. *et al. Nature* **590**, 498–503 (2021).
3. Li, Y. *et al. J. Biol. Chem.* **284**, 34283–34295 (2009).
4. Huang, H. *et al. Nature Chem. Biol.* **16**, 1403–1410 (2020).
5. Kuo, A. J. *et al. Mol. Cell* **44**, 609–620 (2011).
6. Popovic, R. *et al. PLoS Genet.* **10**, e1004566 (2014).
7. Fahey, C. C. & Davis, I. J. *Cold Spring. Harb. Perspect. Med.* **7**, a026468 (2017).
8. Wang, G. G., Cai, L., Pasillas, M. P. & Kamps, M. P. *Nature Cell Biol.* **9**, 804–812 (2007).
9. Papillon-Cavanagh, S. *et al. Nature Genet.* **49**, 180–185 (2017).
10. Lu, C. *et al. Science* **352**, 844–849 (2016).

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