Nature Reviews Drug Discovery | Published online 30 Mar 2017; doi:10.1038/nrd.2017.55

ANTICANCER DRUGS

All roads lead to EZH2 inhibition

Enhancer of zeste homologue 2 (EZH2) — the catalytic subunit of Polycomb repressive complex 2 (PRC2) — mediates transcriptional silencing through trimethylation of histone H3 lysine 27 (H3K27me3). Mutation or overexpression of EZH2 has been linked to numerous types of cancer, and EZH2 inhibitors are being evaluated in clinical trials as potential anticancer drugs. Now, two studies in mouse models of diffuse intrinsic pontine glioma (DIPG) and bladder cancer — two types of cancer with limited treatment options — have identified different vulnerabilities that can be exploited by targeting EZH2.

Mohammad *et al.* investigated the role of a lysine-to-methionine (K27M) mutation in histone H3 that is observed in up to 80% of DIPGs. H3-K27M has been shown to inhibit PRC2 activity by binding to EZH2, but H3-K27M-mutant DIPGs still exhibit some PRC2 activity.

To assess whether this residual activity is required for tumour growth, the authors used a mouse model in which they showed that EZH2 is required for the maintenance of DIPG. They then inhibited EZH2 in primary human DIPG cell lines that expressed H3-K27M by using two different inhibitors, GSK343 and EPZ-6438. H3-K27M cell lines showed reduced proliferation in response to EZH2 inhibition compared with wild-type H3K27 cell lines. This effect was associated with decreased H3K27me3 enrichment on the cyclin-dependent kinase inhibitor 2A (*CDKN2A*) promoter and a corresponding increase in the expression of the tumour-suppressor protein INK4A (also known as p16), which suggests that INK4A could be involved in the response to EZH2 inhibition. Indeed, cells in which the INK4A pathway is non-functional were insensitive to EZH2 inhibition, which indicates that INK4A status can predict the response to EZH2 inhibitors.

> “These studies add to the rapidly growing evidence that indicates the therapeutic potential of targeting EZH2”

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In a similar approach, Ler *et al.* investigated the functional consequences and therapeutic targetability of inactivating mutations in the histone demethylase KDM6A, which are frequently found in urothelial bladder carcinoma. KDM6A counteracts EZH2 activity by removing methyl groups from H3K27me3. KDM6A mutations resulted in sustained repression of PRC2-regulated genes, whereas there was no change in levels of EZH2 when comparing wild-type and mutant KDM6A urothelial bladder carcinoma samples.

This observation led the authors to hypothesize that urothelial bladder carcinoma with KDM6A loss may depend on continued PRC2–EZH2 activity for cell proliferation. Unlike wild-type KDM6A cells, KDM6A-null cells were sensitive to EZH2 inhibition with GSK343. Furthermore, reintroducing functional KDM6A, but not KDM6A with a mutation in the catalytic domain, could rescue GSK343-inhibited cell proliferation in KDM6A-null cells. Accordingly, loss of KDM6A rendered the previously unresponsive wild-type KDM6A cells sensitive to EZH2 inhibition. KDM6A-null cells also responded to other EZH2 inhibitors, such as GSK126 and EPZ-6438.

Finally, the authors tested the effect of pharmacological inhibition of EZH2 in several *in vivo* models of urothelial bladder carcinoma. Pretreatment of KDM6A-null cells with GSK343 before subcutaneous implantation into nude mice delayed tumour growth and improved overall survival of these mice. Growth of KDM6A-null engrafted tumours and KDM6A-null patient-derived tumours was substantially inhibited by treatment with GSK503 compared with wild-type KDM6A engrafted tumours. GSK503 treatment seemed well tolerated, with the mice showing normal behaviour and only minor transient decreases in body weight.

These studies add to the rapidly growing evidence that indicates the therapeutic potential of targeting EZH2 and expand the horizons of EZH2 inhibitors already in development.

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ORIGINAL ARTICLES Mohammad, F. *et al.* EZH2 is a potential therapeutic target for H3K27M-mutant pediatric gliomas. *Nat. Med.* http://dx.doi.org/10.1038/nm.4293 (2017) | Ler, D. L. *et al.* Loss of tumor suppressor KDM6A amplifies PRC2-regulated transcriptional repression in bladder cancer and can be targeted through inhibition of EZH2. *Sci. Transl Med.* **9**, eaai8312 (2017)