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CANCER

Keeping it real to kill glioblastoma

The results of *in vitro* and *in vivo* screens to identify genes that are essential for the survival of a type of brain cancer show almost no overlap, underlining the need for caution when interpreting *in vitro* studies.

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Glioblastoma is the most deadly form of brain cancer in adults, and is responsible for more than 12,000 brain-tumour diagnoses in the United States each year[1]. Extensive characterization of its molecular basis, largely made possible through massive genomic studies[2,3], has provided a comprehensive understanding of the cancer-causing genes and aberrant pathways that underlie glioblastoma development, relapse and resistance. But putative therapies that target these genes and pathways have shown little efficacy in clinical trials[4], motivating investigation of alternative targets. In a paper online in *Nature*, Miller *et al.*[5] report results from an elegant, systematic screen, which aimed to identify therapeutic targets for glioblastoma that regulate the epigenome — a collection of heritable chemical modifications that control gene activity without altering the underlying DNA sequence.

Epigenetic modifications include those on DNA (for instance, the presence of methyl groups on cytosine residues), and those on histone proteins (including methylation, acetylation and phosphorylation), around which DNA is packaged. Collectively, these modifications dictate whether genes are expressed or silent, typically in a cell-type-specific manner. Enzymes called epigenetic modifiers are responsible for establishing, maintaining, reading and removing these modifications in a highly orchestrated, multifaceted manner.

Cancer biologists have taken a keen interest in the role of epigenetic modifiers in tumour development, largely because mutations in the genes that encode these enzymes are prevalent in a wide range of cancers[6]. Various classes of epigenome-targeting drug have created considerable enthusiasm of late, and clinical trials of all phases are under way for such drugs[7].

Miller *et al.* sought to investigate the cellular requirements for epigenetic modifiers in glioblastoma (Fig. 1). The authors used cancerous cells derived from people with glioblastoma, which they either grew in culture or transplanted into the brains of mice. They used these models to perform parallel *in vitro* and *in vivo* screens to look for those genes on which cancer cells depend by introducing RNA molecules that inhibit the expression of particular genes (one RNA in each glioblastoma cell). The authors grew the cells for two to three weeks, and looked for genes whose inhibition caused cell depletion, compared with controls in which the inhibitory RNAs were inactive. Finally, they compared inventories of genes depleted under the two experimental conditions.

Surprisingly, the researchers found that the candidate gene classes identified differed vastly between experiments, with only three genes picked out under both conditions. Cells grown in culture, where the nutrients required for tumour-cell proliferation are abundant, depended on genes encoding epigenetic modifiers that regulate cell metabolism and macromolecule production. By contrast, cells propagated in mice depended on a class of gene that encodes epigenetic modifiers involved in the regulation of transcriptional pause release — a mechanism by which gene transcription is restarted after a pause. Epigenetic modification of transcriptional pause release allows the timing, rate and magnitude of transcriptional responses to be highly coordinated under certain physiological conditions[8]. The targets of this class of modifier include stress-response genes, and the authors suggest that activation of these genes enables tumour cells *in vivo* to adapt to the harsh conditions of their microenvironment.

Among the top hits emerging from the *in vivo* dependency screen was the gene Jumonji C-domain-containing protein 6 (*JMJD6*), which encodes a bifunctional enzyme that can modify molecules by removing specific methyl groups or adding hydroxyl

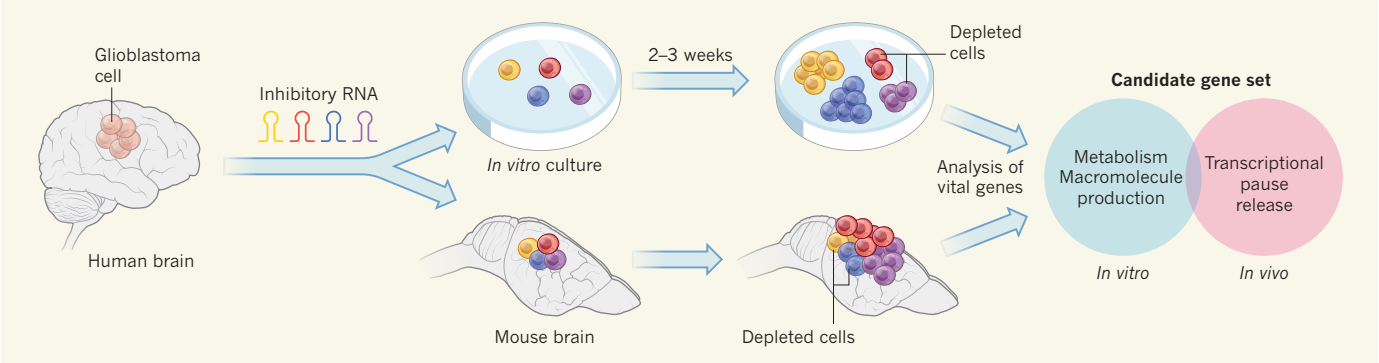

Figure 1 | Parallel screens for essential genes. Miller *et al.*[5] took cells from patients with the cancer glioblastoma, and introduced different inhibitory RNA molecules into different cells (colour coded). Each RNA molecule inhibited expression of a gene involved in epigenetic modification — regulation of chemical modifications on DNA and associated proteins that alter gene activity without changing the underlying DNA sequence. The authors grew the cells in culture or in the brains of mice for two to three weeks, then analysed cell depletion, to see which genes must be properly expressed for normal glioblastoma-cell growth. The *in vivo* and *in vitro* results showed almost no overlap. Genes vital for growth from the *in vitro* screen related mostly to cell metabolism and macromolecule production, whereas those from the *in vivo* screen related to a regulatory process in gene transcription called pause release. (Adapted from Fig. 1 of the paper[5].)

groups. Miller *et al.* demonstrated that the JMJD6 protein binds to active regulatory DNA sequences that control the expression of stress-response genes in their glioblastoma models. Epigenetic modification by JMJD6 involves[9] coordination of transcriptional pause release, promoting gene transcription; presumably, activation of stress-response genes by JMJD6 promotes tumour-cell survival. Targeted inhibition of *JMJD6* or related factors reproducibly prolonged the survival of mice with glioblastomas, but had no detectable effect on glioblastoma cells in culture.

The authors' results have implications that extend far beyond the specifics of the current report. The overwhelming majority of similar dependency screens reported in the literature were conducted *in vitro*. Most use cancer-cell lines that were established decades ago and propagated in standard tissue-culture conditions, which involve nutrient-containing culture medium mixed with growth-factor-containing serum. In the current study, the *in vitro* screen used patient-derived cells grown under serum-free conditions — conditions that are arguably more faithful, physiologically, to the real-life conditions in which tumours grow — and still showed little overlap with the *in vivo* results.

This lack of congruence raises serious concerns about the validity of results obtained using standard *in vitro* approaches, even if such results have subsequently been reproduced in other cell lines. Similar context-dependent readouts have been observed in other *in vivo* dependency studies[10,11], underlining the need for careful consideration of the experimental system used when conducting such screens.

Miller and co-workers' findings add to a growing list of potential epigenetic therapies for cancer. Drugs that inhibit the activity of proteins of the BET family of epigenetic modifiers have yielded promising results across a range of cancers and are now being evaluated in early clinical trials[12,13]. Among those causing the biggest stir are drugs that target BRD4, which (in addition to other roles) functions in concert with JMJD6 to regulate transcriptional pause release. BRD4 inhibitors have shown preliminary efficacy in glioblastoma cells and mouse models[14,15], consistent with the effects of JMJD6 inhibition reported in the current study.

Although Miller and colleagues' work suggests that inhibiting transcriptional pause release might be an attractive option for treating glioblastoma, translation of these early results to the clinic is a long way off. There are currently no drugs approved by the US Food and Drug Administration that specifically modulate transcriptional pause release. However, so far no targeted approaches to treating this universally fatal form of brain cancer have provided any appreciable survival advantage to patients. Strategies such as those implicated in the current study are most certainly worth a closer look. ■

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