Discovery of new epigenetic targets by screening engineered GBM cells in vivo

Executive summary

Drug molecules for treating glioblastoma are usually discovered by testing collections of chemical compounds to identify those that can inhibit cancer cell growth in vitro (i.e. in a test tube or equivalent), assuming that the cells behave in the same way as in the tumour itself (i.e. in vivo). The current study shows that this assumption is not necessarily true. By complex genetic engineering, the authors identify a new series of genes (and their corresponding proteins) as potential targets for GBM drug discovery.

Background

A fundamental question in glioblastoma (GBM) research is why patients treated with drugs that are highly effective in preclinical models show poor clinical responses. In the last Window on Glioblastoma article, we highlighted GBM stem cells as the tumour cell population responsible for re-emergence of tumours after initial drug treatments and discussed how these cells can be isolated and used in drug discovery programmes. One reason for treatment failure could be the difference in behaviour of GBM cells (of whatever type) in vitro compared to those within authentic GBM tumours in patients in vivo, where GBM tumours occur in a microenvironment of stromal cells and stress conditions that are difficult to fully replicate in vitro. This Window article reviews a recent paper in Nature by Miller et al (together with an accompanying News & Views article) which directly addresses the in vitro/vivo issue and, in the process reveals some potential epigenetic targets for GBM drug development.1,2

Screening for essential genes in GBM

The strategy employed in this paper by Miller et al 1 employs parallel screening of GBM cells cultured in vitro and compares data from the same tumour explants cultured in mouse brain xenografts as an in vivo model. Their idea was to identify specific genetic "dependencies" for each condition by retroviral transduction of a panel of 1,586 inducible shRNAs (short-hairpin RNAs) targeting 406 known chromatin and transcriptional regulators. The technology employed for this tour-de force included reporter gene expression and cell sorting, described in detail in the paper. Treated cells were either cultured with EGF and B-FGF under serum-free conditions in vitro, or else engrafted intracranially into mice (immunodeficient NOD/SCID/Il2r-γ) for growth in vivo. Xenograft models used in GBM research will be discussed in more detail in a future article. The rigorous nature of the in vivo study design is noteworthy, with consideration of sex differences (31 females and 30 males were examined), estimation of statistical power, and blinded controls for key stages. Measurement of cell depletion in cultures or xenografts, through sequencing the 'barcodes' associated with individual shRNA vectors, provided a list of genes that were important in each condition.

Differences in GBM cells under in vitro and in vivo growth conditions

The results of the above experiments showed that there was almost no overlap between the gene panels regulating in vitro cultures, where nutrients are plentiful, and in vivo xenografts where cells are highly stressed within the tumour mass. In the former case, genes encoding metabolic regulators scored highly, in contrast to the in vivo situation, where genes controlling RNA transcription (specifically the phenomenon of RNA pause release) were more important. The general findings are summarised in the table below where the epigenetic modifier genes are grouped according to their importance in vitro or in vivo (from Figure 1d in 2):

<table>
<thead>
<tr>
<th>Cultured cells</th>
<th>Intracranial tumour cells</th>
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<tr>
<td>Protein complex subunit organization</td>
<td>Regulation of DNA-templated transcription, elongation</td>
</tr>
<tr>
<td>Protein complex biogenesis</td>
<td>DNA-templated transcription, elongation</td>
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<tr>
<td>Positive regulation of macromolecule</td>
<td>Transcription elongation from RNA Pol II promoter</td>
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<td>metabolic process</td>
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Top gene dependency hits in intracranial tumours

55 genes were upregulated more than 2.5-fold in both proneural GBM528 and mesenchymal GBM3565 cells grown intracranially, as well as in tumours from patients with primary glioblastoma. Twelve genes encoding transcription elongation factors were prioritised and used to interrogate independent gene expression datasets from primary tumours and the Ivy Glioblastoma Atlas Project (Ivy GAP) dataset, which provides intratumour microenvironment-
specific expression. The most significant validated hits (in descending order) were: JMJD6, BRD4 and DOT1L, with JMJD6 being selected for further evaluation.

Transcriptional pause release and epigenetic modifiers
Promoter-proximal pausing of RNA polymerase II (Pol II) is a critical regulatory event subsequent to Pol II initiation on a large set of genes and is achieved mainly through the action of the P-TEFb complex, which phosphorylates at least three targets, including the NELF subunit of NELF, the Spt5 subunit of DSIF, and serine 2 of RNA Pol II carboxyl-terminal domain (CTD) (reviewed in 1).

JMJD6 (Jumonji C-domain-containing protein 6, also known as PTDSR or PSR) was originally identified as a phosphatidylserine receptor but has subsequently been shown to possess arginine demethylase and lysyl-5-hydroxylase enzymatic activity. It also physically associates with the BET family protein BRD4 to regulate pause release of pol II by binding to distal enhancers. A BRD4 inhibitor has already been shown to inhibit GBM cells in preclinical models, thus providing support for investigating JMJD6 inhibition as a therapeutic target in glioblastoma. In their paper, Miller et al have provided ample evidence for this protein being of pharmaceutical interest, so the question now is whether it is amenable to inhibition with small molecule drugs, and what aspect of its function to inhibit.

JMJD6 as a drug target
The JMJD6 protein has a chequered and confusing history, even being implicated in the replication of foot and mouth disease virus in cells. The protein appears to be essential for normal embryonic development through its involvement in several key biological processes. In addition to the transcriptional pausing of relevance here, it regulates alternative splicing of RNA in ways that are both dependent and independent on its enzymatic activity. Unsurprisingly, drug development for this target is ‘under the radar’ if being pursued at all. However, other epigenetic targets are revealed using the strategy described in this paper, DOT1L (mammalian homologue of disruptor of telomeric silencing-1) being a prime example. DOT1L is the only enzyme responsible for mono-, di-, and trimethylation of the ε-amino group on H3K79, an activating mark with respect to gene transcription. Inhibitory compounds for this target are under active clinical development for cancer; for example, Pinometostat (formerly EPZ-5676) is being developed by Epizyme in collaboration with Celgene and has completed Phase I clinical trials for leukaemia. While these studies are quite removed from glioblastoma treatment, there may be possibilities for repurposing these compounds in other cancers such as GBM.

Concluding remarks
The paper by Miller et al is a significant piece of work in that it highlights some key differences between GBM cells grown in culture and those in vivo. The title of the accompanying News and View article says it all: ‘Keeping it real to kill glioblastoma’. In fact, this is not the first time that such an approach has been used to uncover genes that are relevant to cancer in vivo (see for example 11) so there are precedents. The study focussed on identifying a set of genes involved in epigenetic regulation which provided enough work for 25 authors. It would be interesting to see what other gene dependencies might emerge if other shRNAs are employed. The genes they did uncover related to transcriptional pausing and were consistent with the idea of tumour cells being subjected to stress in the brain and modifying their transcriptional programmes as a result.

Perhaps the last words should go to Northcott: “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”.
References
3) Ivy GAP. The Cancer Imaging Archive. https://wiki.cancerimagingarchive.net/display/Public/Ivy+GAP
10) http://www.epizyme.com/programs/pinometostat/