Drugs for targeting transcription factors in GBM: OLIG2 as a case study

Executive summary
In trying to find new targets for drugs to treat cancers like glioblastoma, scientists often look at the basic components of tumour cells to see how they differ from their normal counterparts. One difference is in the action of proteins called transcription factors (TFs) that bind to DNA and increase or reduce the expression of specific genes that code for proteins. TFs are often ‘switched on’ in cancers and this article describes one of them, OLIG2, which is associated with glioblastoma cells. Using both computer analysis and laboratory science, the authors of the highlighted paper show how they have identified chemical compounds that block the action of OLIG2 in glioblastoma cells and from which a candidate molecule has been selected for further development as a medicine. In addition, the methods they use are of interest to investigators wanting to inhibit other classes of transcription factors using small chemical molecules.

Background
The human genome encodes more than 1,500 transcription factors (TFs), each binding to specific regions of DNA, as regulators of gene expression. Not surprisingly therefore, dysregulation of TF action is a feature of many cancers and TF proteins are potential drug targets for these diseases. Several transcription factors (particularly those relating to neuronal development) have been implicated in glioblastoma (e.g. in Window on Glioblastoma articles). Here, we highlight the OLIG2 transcription factor, a basic HLH (helix-loop-helix) protein associated with glioblastoma cells. Using both computer analysis and laboratory science, the authors of the highlighted paper show how they have identified chemical compounds that block the action of OLIG2 in glioblastoma cells and from which a candidate molecule has been selected for further development as a medicine. In addition, the methods they use are of interest to investigators wanting to inhibit other classes of transcription factors using small chemical molecules.

OLIG2 in glioblastoma
OLIG2 (oligodendroglial lineage marker) is expressed in progenitor cells that give rise to neurons and to myelinating oligodendrocytes during neuronal development and is present in 100% of diffuse gliomas regardless of grade. According to Mehta et al, OLIG2 is implicated in the DNA damage response to drugs and radiation that is orchestrated by the P53 oncogene. Glioblastomas are resistant to radiation and genotoxic drugs, and yet the P53 gene is structurally intact in the majority of adult high grade primary gliomas. OLIG2 suppresses the acetylation of P53, thereby affecting the latter’s interaction with downstream promoters and resulting protective functions. In studies on mouse glioma formation in vivo, OLIG2 is implicated in glioma cell proliferation. Ablation of this gene causes a downregulation of PDGF receptor alpha and upregulation of the EGFR receptor (EGFR) and sensitisation of the tumours to EGFR inhibitors. Taken together, these and other findings suggest that pharmacological inhibition of OLIG2 function in GBM may provide clinical benefit.

Inhibition of OLIG2 dimerisation
TF function can be inhibited by interfering with either protein-protein or protein-DNA interactions. Tsigelny et al employed a virtual screening approach to find inhibitors of OLIG2 dimerisation. Protein-protein interactions of this type present a major challenge to small molecule drug discovery, so the successful identification of OLIG2 dimerisation inhibitors (<600 MW, with micromolar potency) is a significant achievement. A common approach to finding inhibitory compounds of this type is to use in silico methods to identify a single ‘hotspot’ on the target protein to act as a guide for building chemical scaffolds. Unfortunately, this method has largely failed because the binding surface may contain multiple ‘daughter’ or subpharmacophores within a much larger area. The authors had already developed this multiple pharmacophore concept to identify (among others) protein interface pharmacophores as drug candidates.
In the absence of a crystal structure for OLIG2, daughter pharmacophores in OLIG2 were identified through homology modelling of the related TFs NeuroD1 and the E47 isoform of E2A whose structures were available. Virtual screening was used to identify compounds that interact with features in the parental and daughter pharmacophores of OLIG2, shown left.

The Open NCI Chemical Structure Database of 260,071 compounds (as of April 2012) was used as a starting point. Compounds with molecular weight > 600 were excluded and the search yielded 1840 compounds (1.3% hit rate) predicted to fit 4 of 5 features belonging to the parental pharmacophore. 147 compounds were theoretically predicted to fit 4 out of 5 features of the parental and all features of daughter pharmacophores. They were then clustered into groups according to conserved structural features, shown in the figure below (Figure 4A in 5).

<table>
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<th>Cluster C (code5)</th>
<th>Cluster D (code8)</th>
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<td>SKOG113</td>
<td>SKOG121</td>
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The IC50 values for GBM growth inhibition, to be discussed later, are indicated in red in each panel. Compounds which showed the most potent activity in subsequent GBM cell-based screens were mainly those that were predicted to fit all hypothesized pharmacophores.

Selection and biological activity of lead compounds

Ninety-five compounds were synthesized and tested for antiproliferative activity on murine Ink4a/arf EGFR-vIII glioma stem cells, since these express OLIG2 at elevated levels. Twenty-two compounds exhibited micromolar potency (see Figure) with SKOG102 being the most active. SKOG102 also inhibited tumour sphere formation in cultures of primary human cells derived from GBM stem cells. Experiments were then performed to correlate this in vitro activity to inhibition of OLIG2 itself. The biological activity of SKOG102 was compared with the actual expression of OLIG2 in different cell types using RT-PCR and western blotting. Interestingly, the primary GBM cells used in this study (GBM 4 and 8) expressed significantly higher levels of OLIG2 than the U87 GBM cell line commonly used for drug screening. Genes that are modulated by OLIG2 such as p21 (down regulated) and OMG (upregulated) were respectively induced and inhibited by SKOG102 in a dose dependent manner. Temozolomide had no effect on these OLIG2 gene effects at 100 micromolar concentration implying that drugs based on inhibition of OLIG2 would act independently of this standard chemotherapeutic agent. Thermal shift and electrophoretic mobility shift assays were used to show that SKOG102 binds directly to the OLIG2 protein and prevents its binding to DNA.

Activity of SKOG102 on GBM stem cells

Patient-derived stem cell cultures were treated with either the pharmacological OLIG2 inhibitor SKOG102 or a short hairpin RNA (shRNA) designed to downregulate OLIG2 expression. Both approaches inhibited the expression of stem cell markers nestin and CD133, as well as the oligodendrocyte lineage markers 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNPase), myelin-basic protein (MBP), oligodendrocyte myelin glycoprotein (OMG), and proteolipid protein 1 (plp1). Tsigelny et al suggest that this compound (or approach in general) could be used to change the GBM stem cell compartment and control tumour growth. They also undertook xenograft studies using human GBM stem cells engrafted into the flank, or later in the brain. In both cases, tumours derived from the implanted GBM4 cells were significantly reduced in size compared with controls. Most importantly, SKG102 could cross the blood brain barrier.

Concluding remarks

Transcription factors such as OLIG2 are potential targets in GBM, but unlike enzymes and other ‘druggable’ targets, are difficult target for small molecule discovery. The authors of the paper highlighted in this article have applied in silico protein analysis and virtual screening technology in a creative way that overcomes some of the problems encountered in this field. By identifying compounds that interact with a series of daughter sites on a large pharmacophore, they have found compounds that appear to act directly on OLIG2. The lead compound affects GBM stem cell growth in xenograft models, encouraging further evaluation in ADMET and safety studies. The observation that OLIG2 inhibition in GBM cells may confer sensitivity to inhibition by EGFR-blocking drugs is particularly interesting. Whatever the fate of these compounds as drugs, this study demonstrates that it is possible to find leads for transcription factors, a difficult class of drug target with considerable potential in modulating the growth and development of brain tumours and other cancers showing developmental plasticity.
References

10) http://cactus.nci.nih.gov/download/nci/