Welcome to IOTA's news bulletin: WINDOW on Glioblastoma

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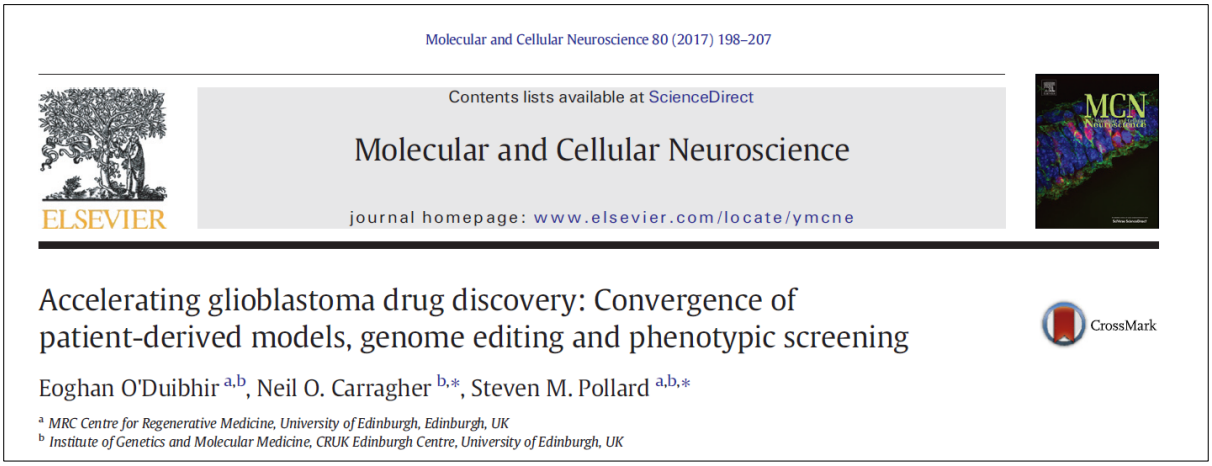
Glioblastoma stem cells and drug discovery



Executive summary

High grade glioblastoma (GBM) is treated using surgery, radiation and chemotherapy, but despite this, overall patient survival is very limited. In efforts to understand why this is the case, scientists have shown that a population of cells in GBM are resistant to treatment and eventually emerge to form further tumours. These glioblastoma 'stem cells' are therefore attractive targets for drug therapy. This article describes how these stem cells may be grown in the laboratory and how they can be used to gain information on specific vulnerabilities they possess which can be exploited in the clinic. The cells can also be used to discover novel chemicals as starting points in the development of future treatments.

Background

Gliomas, including the aggressive and lethal type IV glioblastomas (GBM) form complex brain tumours with features that limit the efficacy of current medical interventions. A typical treatment regimen involves surgery, radiation and chemotherapy with the alkylating agent temozolomide, but overall survival is only 15 to 19 months.[1] This dismal prognosis is due in part to the heterogeneity of tumour cell types, including a population of cancer stem cells that are resistant to therapy and drive the relapse of disease after apparently successful initial intervention. An understanding of the biology of these GBM stem cells is therefore an essential prerequisite for developing novel treatments for overcoming the resistance problem. Given the positioning of stem cells at the head of a hierarchy of cellular development, it seems appropriate that this first in a series of *Window on Glioblastoma* articles should be devoted to these cells in gliomas. We focus on a recent review from Steven Pollard's lab at the University of Edinburgh who describe the use of GBM stem cells to identify novel molecular targets and drug candidates through technologies such as cellular reprogramming, genome editing and phenotypic screening.[2]

Neural stem cells

Neuroepithelial cells are the developmental precursors of both neurons and glial cells via apical progenitor cells and are therefore the earliest cell lineage for normal brain development. Detailed transcriptome and epigenetic analysis of human neural cell development involving these different cell types is underway providing a baseline for comparison with the transformed cell types arising in neural cell tumours, including gliomas.[3] Cancer stem cells (CSCs) arise from epithelial-mesenchymal transition (EMT) during development (for a review see [4]). For cells to be considered as CSCs, they must exhibit:

- sustained self-renewal
- persistent proliferation
- tumour initiation upon secondary transplantation

They may also express normal stem cell markers and generate progeny of multiple lineages (see [5] for more background on glioma CSCs).

"Arguably the most critical question for GBM is whether the putative cancer stem cells are hijacking and exploiting the self-renewal pathways that underpin normal neural stem cell self-renewal, as this information could eventually be exploited to halt tumour growth and relapse after therapy. Indeed, many of the essential transcriptional and epigenetic regulators of neural stem cells are highly expressed in gliomas and have clear functional importance in sustaining tumour growth."

This quote from O'Duibhir et al [2] is central to exploiting GBM stem cell biology for therapeutic ends.

GBM stem cell cultures

The serum-free growth media supplemented with EGF and FGF-2 used for expanding untransformed neural stem cells can also be used with their GBM counterparts. The latter can be grown as adherent monolayers (which simplifies drug screening) while retaining the key properties of stem cells that were listed in the previous section.[2] Other systems have been described, including those producing suspension cell cultures called neurospheres.[6] The nature of the cells and the format of the cell culture assays used is of critical importance for choosing the best system for mimicking the tumour environment *in vivo*. This, and related topics, will be covered in future *WINDOW* articles. In the meantime, we focus on 'patient-derived cellular models and matched controls: a unique opportunity for gliomas'.[2]

While large collections of cell lines have been established and used for functional and molecular studies, the ideal situation would be to have isogenic cell lines matched to each GBM stem cell culture. This is not practical using normal tissue, except in rare cases where surgically-removed brain tissue is available, although using pluripotential stem cell (iPS) technology to reprogramme a patient's dermal fibroblasts is an attractive alternative.[7]

Genome editing

If panels of GBM stem cells and matched neural stem cells are available, genome editing using CRISPR-Cas technologies can be used to selectively add or remove genes for a number of applications.[8] These include:

- Identification of new molecular targets through genome-wide CRISPR screens
- Engineering of candidate driver mutations into normal NS cells
- Reversion of genetic drivers to wild-type in GSCs
- Creation of useful live cell reporters (epitope tags or fluorescent proteins) or biosensors for cell based phenotypic screening

Specific examples of genes that may be added or deleted are summarised in the following table [2]:

Procedure	Examples
Driver oncogene deletion in GSCs	EGFR, PDGFR, CDK4
Revert to wild type	TP53, H3F3A, TERT (promoter), IDH1/2
Luciferase reporter	Live cell imaging
eGFP, mCherry or BFP2 reporters	Readout of SOX2, FOXG1, OLIG2 promoters

Cell-based phenotypic screening

The availability of GBM stem cells and matched normal controls in reasonable numbers and an appropriate microtitre plate assay provide a good basis for phenotypic screening of drug candidates. The pros and cons of screening purified molecular targets *vs* whole cells or tissues has recently been discussed in depth.[9] There remain technical issues, but advances in microscopy and high content screening are now having an impact on preclinical drug discovery. Microscopes and imaging can be automated and the readout data analysed using advanced statistical methods. Box 2 in O'Duibhir [2] shows an impressive list of commercially available imaging systems.

Platform	Vendor
Epifluorescent High content platforms	
ImageXpress MicroXLS	Molecular Devices
CellInsight	Thermo Fisher
ArrayScan	Thermo Fisher
IN Cell Analyzer 2200	GE Healthcare
ScanR	Olympus
WiScan	IDEA Bio-Medical Ltd
Cytation 5	BioTek
Cellavista	SynenTec Bio Services
Laser Scanning imaging cytometer	
Acumen Cellista	TTP Labtech
Confocal High content platforms	
Operetta	Perkin Elmer
Opera Phenix	Perkin Elmer
ImageXpress Ultra	Molecular devices
IN Cell Analyzer 6000	GE Healthcare
Yokogawa CQ1	Wako Automation
Yokogawa CV700	Wako Automation
"Live-cell" high-content imaging platforms tailored for long-term kinetic studies	
IncuCyte-ZoomTM	Essen Bioscience
Cell-IQ	CM Technologies
BioStation-CT	Nikon Instruments

Improvements in automation and throughput of cell-based screens allows the investigator to increase the number of parameters being simultaneously investigated rather than looking at single data points. Examples are kinetic imaging and multi-parametric phenotypic profiling. In the former, key cellular events (such as mitosis) can be interrogated in detail over time, thus revealing potentially interesting targets and chemical modulators.[11] Multi-parametric phenotypic profiling allows comparisons to be made between different cell types based on phenotypic fingerprints created from high content screening assays. This is well illustrated by the study of Caie et al[12] in which cytoskeletal, nuclear and cellular morphology data are acquired simultaneously from 3 different cancer cell lines and a control, and are used to classify panels of standard anticancer drugs. This, perhaps unsurprisingly, revealed that compounds disrupting the cytoskeleton and DNA-damaging agents were associated together in specific clusters. However, the protein synthesis inhibitor emetine was shown to disrupt cytoskeletal architecture only in cells with mutations in the p53 oncogene, thereby providing new avenues for research into tumour invasiveness.

Matching chemical structure to phenotypic response

The random nature of many compound screening projects leads inevitably to false positives based on 'promiscuous' binding to cellular proteins. This problem is particularly acute when using whole cells in phenotypic screens, so careful selection of compound libraries is essential. O'Duibhir et al list some sources of chemical libraries that presumably have been curated in such a way as to minimize false positives.

Published Kinase Inhibitor set available from GSK
An open access tool of 367 annotated small molecule kinase inhibitors. [Drewry et al., 2014 and Elkins et al., 2016]

Bioactive Compound Library from Selleck
Commercially available customizable library of 1902 bioactive compounds. [Mei et al., 2014 and Moisan et al., 2015]
http://www.selleckchem.com/screening/chemical-library.html

StemSelect
Library of 303 pharmacologically active, structurally diverse small molecules targeting a variety of pathways involved in proliferation, migration and differentiation. Available from Merck Millipore.

InhibitorSelect I, II & III
243 well-characterized protein kinase inhibitors spread over three 96 well plates. Also available from Merck Millipore.

Phenotypic toolbox from BioAscent
Composed of FDA approved drugs, reference compounds and diverse lead-like molecules in a small combined library.
http://www.bioascent.com/phenotypic/

Epigenetic chemical probes from the Structural Genomics Consortium (SGC)
More than 30 open access high quality tool compounds targeting a variety of epigenetic regulators. [Brown et al., 2015]
http://www.thesgc.org/chemical-probes

Of course, chemical space is so vast that there is a danger of missing completely novel chemotypes if the element of randomness is excluded. Large-scale cancer cell line screening projects are generating large datasets of inhibition data for many compounds and cell types, thus providing a starting point for more detailed phenotypic screening.[13] It is timely that the compound classes relating to different mechanisms of action can be applied to the well-defined GBM stem cell populations described by O'Duibhir et al. Their use contrasts favourably with that of transformed cell lines that may bear little relation to those in the originating tumour (and in any case, may not be stem cells).

Concluding remarks

We have highlighted a review from Steve Pollard's lab in Edinburgh which clearly lays out the basic technology for producing GBM stem cells and isogenic controls as well as applying gene editing and high-content screening approaches for drug discovery. This is expected to yield many useful starting points for drug development, through the discovery of both novel targets and novel compounds. This is clearly an important and highly practical application of GBM stem cell biology. However, there is clearly much still to be done with these cells.[5,14].

Finally, it is worth commenting on a possible disconnect between gene expression in GBM cells cultured *in vitro* and those present in the tumour microenvironment. Although not specifically mentioning GBM stem cells, Miller et al [15] have shown that gene expression in primary explanted GBM cells is significantly different to that occurring in xenograft models where human cells are transplanted into mouse brains. The molecular insights revealed by their study will be the topic of another *Window on Glioblastoma* article, but in the present context, it highlights the need for well-defined *in vivo* models to validate the results of GBM stem cell screens, anticipating the fate of promising lead compounds once they enter the clinic.

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