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Accelerating glioblastoma drug discovery: Convergence of patient-derived models, genome editing and phenotypic screening

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ABSTRACT

Patients diagnosed with glioblastoma (GBM) continue to face a bleak prognosis. It is critical that new effective therapeutic strategies are developed. GBM stem cells have molecular hallmarks of neural stem and progenitor cells and it is possible to propagate both non-transformed normal neural stem cells and GBM stem cells, in defined, feeder-free, adherent culture. These primary stem cell lines provide an experimental model that is ideally suited to cell-based drug discovery or genetic screens in order to identify tumour-specific vulnerabilities. For many solid tumours, including GBM, the genetic disruptions that drive tumour initiation and growth have now been catalogued. CRISPR/Cas-based genome editing technologies have recently emerged, transforming our ability to functionally annotate the human genome. Genome editing opens prospects for engineering precise genetic changes in normal and GBM-derived neural stem cells, which will provide more defined and reliable genetic models, with critical matched pairs of isogenic cell lines. Generation of more complex alleles such as knock in tags or fluorescent reporters is also now possible. These new cellular models can be deployed in cell-based phenotypic drug discovery (PDD). Here we discuss the convergence of these advanced technologies (iPS cells, neural stem cell culture, genome editing and high content phenotypic screening) and how they herald a new era in human cellular genetics that should have a major impact in accelerating glioblastoma drug discovery.

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1. Introduction

The prognosis for children and adults suffering from high grade glioma is dismal. An improved understanding of disease biology is urgently needed. Gliomas are a heterogeneous group of tumours, but the higher grade tumours – more commonly known as glioblastoma (GBM) – are invariably driven by cells that display features of neural stem and progenitor cells (Lathia et al., 2015). Many putative genetic and epigenetic drivers of glioma have now been uncovered through systematic genome-wide molecular annotation, opening up a wealth of new directions for fundamental discovery and improved molecular classifications (Brennan et al., 2013; Sturm et al., 2012). This fundamental knowledge will ultimately lead to new treatments and enhanced patient outcomes; however, in the shorter term there remains an urgent unmet need to repurpose existing drugs for use in GBM as well as identify key molecular targets and develop new lead compounds.

During the past five years there have been remarkable advances across several technologies that will enhance glioma discovery research, including: 1) improved cellular models and stem cell culture conditions (iPS cell, neural stem cell and glioma stem cells), 2) CRISPR/Cas genome editing, and 3) cell phenotypic screening platforms. The emergence of these technologies, paralleled by improved understanding of cancer genetic and epigenetic disruptions, should drive development of novel patient-derived cellular models that can be channelled into cell-based chemical and genetic screens in vitro and xenotransplantation models in vivo. Here we discuss each of these areas, particularly how they intersect and might be deployed in the coming years to improve the prognosis for people living with GBM – one of the most lethal human cancers. We focus on chemical screens using patient-derived cellular models, and the opportunities for gene editing to underpin novel cell-based phenotypic assays. Use of CRISPR/Cas for genetic screens has been discussed elsewhere (Agrotis and Ketteler, 2015).

2. Sources of neural stem and progenitor cells

Much effort has been expended over the past few decades by developmental neurobiologists seeking to define the diversity of neural stem and progenitor cell types responsible for construction of the mammalian central nervous system (CNS) (Gage and Temple, 2013). Knowledge of mammalian brain development has largely come from studies of mouse developmental biology and several distinct categories of neural progenitor cells have been identified. The most primitive and earliest-born neural progenitors are termed neuroepithelial cells, and these likely retain the potential to differentiate into a variety of neuronal or glial subtypes. Neuroepithelial cells transit at the onset of neurogenesis into what are now termed apical progenitors (formerly radial glia) that generate neurons, and at later foetal stages glial cells (Taverna et al., 2014). These apical progenitors generate the wave of newborn neuronal populations, but do so via stepwise transitions along a series of distinct intermediate progenitors (Rowitch and Kriegstein, 2010). These major temporal transitions in neural progenitor states/subtypes are superimposed by well-understood patterning events that establish distinct positional identity: e.g. forebrain versus spinal cord, or cortex versus striatum. In the adult mouse brain, two regions have been uncovered in which new neurons are generated throughout adulthood: the hippocampus, and the walls of the forebrain ventricles. A subpopulation of apical progenitors are the founders of adult neural stem cells and these emerge postnatally (Merkle et al., 2004). The reader is pointed to other reviews which cover these topics in more detail (Bond et al., 2015; Kriegstein and Alvarez-Buylla, 2009).

Despite this progress, we still lack a comprehensive understanding of the full diversity of distinct immature populations and their differentiation potential and plasticity. New classes of progenitor are still being uncovered in the mouse (Pilz et al., 2013). Also, inevitably our understanding of the diversity of human neural stem and progenitors has lagged behind that of the mouse, and important species differences are now being uncovered in the repertoire of progenitors and their molecular regulation (Florio et al., 2015; Lui et al., 2011, 2014).

Considerable attention has focussed on the developing cortex, due to its importance in human biology and evolution, and a population of progenitors termed outer radial glia have been described that are thought to drive the massive expansion of the human (but not mouse) cortical surface area (Hansen et al., 2010). Application of single cell transcriptome analysis and epigenetic profiling are now providing a more complete picture of the full range of distinct cell types (Johnson et al., 2015). Open access databases such as Allen Brain Atlas that integrate neuroanatomical and gene expression datasets also provide a wealth of information to understand the genetic and cellular basis of CNS development in mouse and human (Miller et al., 2014). More recently a related effort has been established for GBM (Sunkin et al., 2013). Altogether these ongoing efforts should eventually lead to comprehensive understanding of the gene expression signatures that define the full inventory of distinct neural progenitors.

Pluripotent stem cells (PSCs) – embryonic stem cells (ESCs) and induced PSCs (iPSCs) – are phenotypically similar to the early pre-gastrulation stage human embryo, and therefore provide a valuable tool to explore early human development. Importantly, they also have practical value as a means to produce human neural cell types in the laboratory (Dolmetsch and Geschwind, 2011; Pourquié et al., 2015), providing a potentially unlimited source of neurons and glia that can be utilized in chemical and genetic screening.

Our knowledge of neural development has been useful to guide approaches to generate, expand and differentiate neural stem cells in vitro (Aboody et al., 2011). Neuroepithelial cells emerge early during ES and iPS cell differentiation – mirroring the primitive ectoderm to neural ectoderm developmental transition; these then transit into radial glia/apical progenitors that lose epithelial features such as expression of the cell-cell tight junction marker ZO-1 and acquire a 'rosette'-like appearance in culture (Elkabetz et al., 2008). These in turn go on to differentiate into neurons, and then a later wave of glial differentiation (astrocytes and oligodendrocytes).

It has proven difficult to capture the more primitive neuroepithelial cells and expand them long term. However, mouse or human radial glia-like apical progenitors, whether derived from PSC differentiation, or freshly isolated foetal/adult CNS tissue, can be expanded long-term in culture using the growth factors EGF and FGF-2. These neural stem cells – herein termed 'NS cells' – can be propagated either in suspension culture as 'neurospheres', or using adherent monolayer. The advantages and disadvantages of these in vitro models have been discussed previously elsewhere (Pastrana et al., 2011). NS cells are somewhat restricted in their differentiation capacity and are glial biased, with features more akin to proliferative adult SVZ neural stem cells. It remains unclear to what extent distinct positional and temporal identities are permanently erased by the culture environment, or if some epigenetic memory of their original identity persists. The rest of this article focuses on these NS cell cultures. This cell state most closely corresponds to the glioma stem cells in their patterns of marker expression, glial differentiation bias and requirement for EGFR signaling. Comparisons of NS cells with their malignant GNS cell counterparts can identify tumour-associated pathways.

3. Neural stem cells and brain cancer

Around 10 years ago there was increased interest in the relationship between neural development, neural stem cells and cancer biology. It became clear that many neural stem cell markers were frequently expressed in and required for growth of gliomas, such as OLIG2 (Ligon et al., 2004, 2007). This raised a related issue of whether CNS derived tumours might arise from stem cells gone awry, and whether these putative cancer stem cells are critical to sustaining tumour growth (Stiles et al., 2008). Functional data supporting a hierarchy of tumour cell malignancy came via improved methods for fractionating tumour populations based on neural stem cell markers and interrogating their