

Recent Advances in Glial Tumours



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Epidemiological data suggests that glioma incidence in adults is on the increase.¹ Why gliomas occur at all in the brain is still an interesting question - after all the brain is relatively protected from the environment by the blood brain barrier and is thought to have a very low proliferative potential compared with other organs. Recent advances in our understanding of gliomas revolve around the cancer stem cell theory and the cytogenetic and epigenetic control of these tumours. This article briefly outlines the general pathology of gliomas and describes these recent advances in the glioma field.

General pathology of gliomas

Primary CNS tumours are most often composed of cells that resemble glial cells, and are hence called gliomas. For example, tumours of various grades with cells resembling astrocytes, termed astrocytomas, are the most common glioma (See Figure 1 for examples of different grades of astrocytoma). The least aggressive of these tumours (grade I) are pilocytic astrocytomas with elongated processes and few mitotic figures, classically occurring in children. Grade II astrocytomas often

present in young adults with an average survival of five years after diagnosis and histologically comprise cells with bland nuclei and fibrillary processes. By contrast anaplastic astrocytomas (grade III) show obvious cellular atypia and mitotic activity and have a worse clinical prognosis. The most aggressive astrocytoma grade IV glioblastoma, is seen mainly in elderly adults and has a survival often measured in months. The other major categories of glioma include oligodendrogliomas and ependymomas, which display histological similarities to their putative glial origin.²

Cancer stem cell theory

The cancer stem cell was originally described in the field of haematological malignancies in acute myeloid leukaemia³ and has now been described in many solid tumours, including gliomas.^{4,5,6} By definition, cancer stem cells are a population of cells within a tumour that can divide infinitely and have the capacity to differentiate and therefore show similarities to 'normal' stem cells outwith the cancer field. These cancer stem cells are of great interest because they may represent the population of cells within a tumour that are resist-

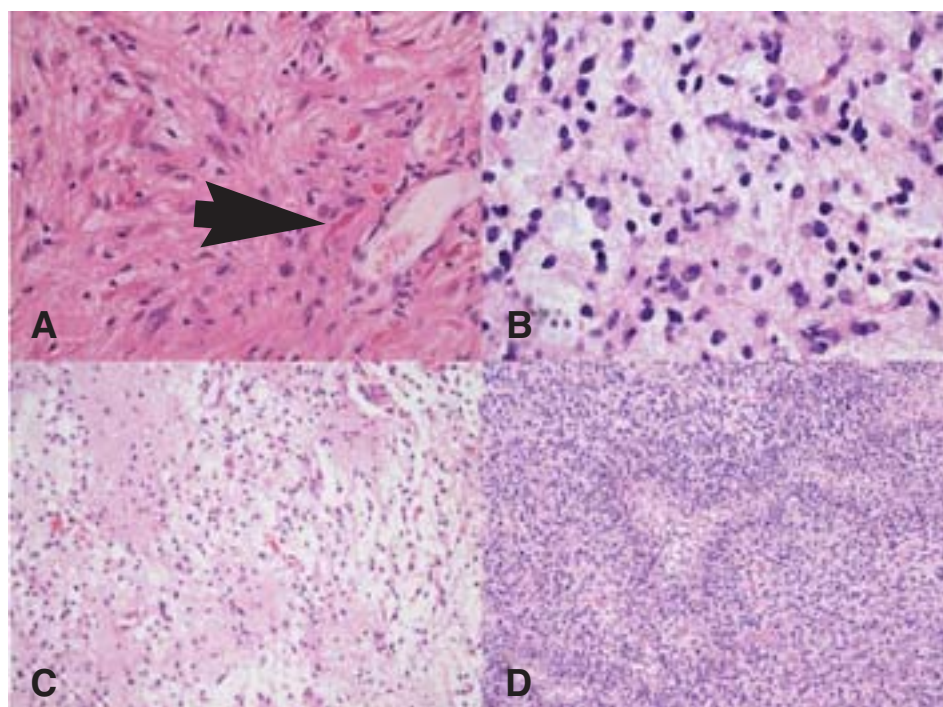


Figure 1: Astrocytic tumours stained with H&E.

A. (x20) Grade I Pilocytic astrocytoma showing elongated piloid cells and corkscrew eosinophilic Rosenthal fibres (arrow). B. (x 40) Grade II Diffuse astrocytoma showing bland nuclei with fibrillary processes. C. (x 40) Grade III Anaplastic astrocytoma with increasing pleomorphism and mitotic activity. D. (x10) Grade IV Glioblastoma with pseudopalisading of tumour cells around areas of necrosis.

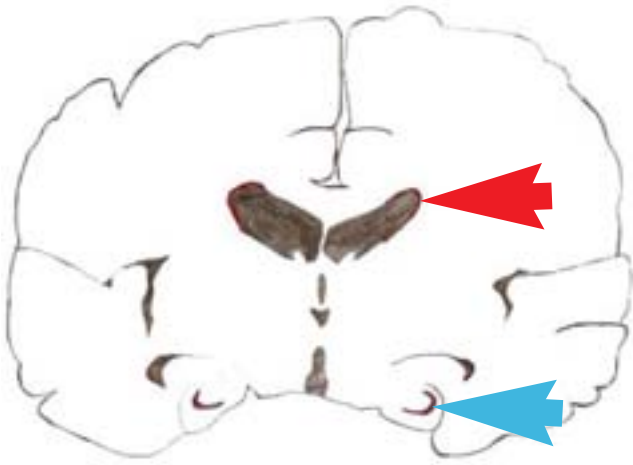


Figure 2: Putative adult neural stem cell niches: Subventricular Zone (red arrow) in the walls of the lateral ventricle and Dentate Fascia Subgranular Zone (blue arrow) of the Hippocampus. While cancer stem cells show in vitro similarities to neural stem cells, the cell of origin or tumour-initiating cell in adult gliomas remains unclear.

ant to therapy and responsible for tumour relapse. Whether cancer stem cells actually develop from pre-existing human neural stem cells or represent cells which have reacquired a stem-like state as a by-product of tumourigenesis or in vitro culture conditions remains controversial.^{4,5,6}

Normal human neural stem cells are thought to reside within the brain mostly in the subventricular zone lining the lateral ventricles and dentate gyrus of the hippocampus (see Figure 2).^{7,8} These stem cells persist throughout adulthood into old age and may divide symmetrically for self-renewal and asymmetrically to produce neurons, astrocytes and oligodendrocytes. Direct isolation of human neural stem cells from the human foetal brain using the cell surface marker CD133 by flow cytometry has been reported to produce human foetal brain cells that differentiate, persist and incorporate into the brain.⁷ The precise function of CD133, also known as prominin, remains unclear, however it was originally shown to be a haemopoietic stem cell marker.⁹ More recently the Dirks group and others have used CD133 to isolate a population of brain tumour stem cells within glioblastoma.¹⁰⁻¹⁴ Original papers suggested that CD133-positive stem-like cells were the only population of cells within the glioblastoma that were capable of producing tumours when transplanted into the brains of immunodeficient mice.¹³ More recent studies suggest that this may not always be the case.¹⁴

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The question whether there is a universal glioma cancer stem cell, or whether different subtypes of glioma contain different stem cells remains unanswered. Work by Gilbertson's group suggests that the stem cell may vary according to the nature of the original tumour. He has shown that subtypes of a different type of glial tumour (ependymoma) may derive from radial glia at different locations at different stages of development in the nervous system. It is possible that radial glia may represent the stem cell of ependymomas. These cells express CD133, RC2 and nestin, which are present on radial glia and human neural stem cells.¹⁵

It seems that glioma stem cells are dependent on their microenviron-

ment in order to maintain stem cell properties. There is evidence to suggest that endothelial cells interact and secrete factors in vitro that maintain cells in a stem-like state.¹⁶ Increasing the number of endothelial cells expands the population of self-renewing cells and their tumourigenic properties. It is therefore possible that stem-like-cells survive within a vascular niche.

Recent exciting work has shown that in vitro it is possible to make certain types of glioma initiating stem cells differentiate into neuronal type cells using manipulation of the Bone Morphogenetic Protein (BMP) pathway, which is involved in differentiation of human embryonic stem cells to human neural stem cells.¹⁷ This study demonstrates a major differentiation block in a subset of glioblastoma caused by the Polycomb repressor complex (PRC)-mediated epigenetic silencing of the BMP1B promoter, analogous to early embryonic neural stem cells. Although this is a radical departure from traditional therapy using surgery, chemo- and radiotherapy, the ability to control glioma cells by forcing them to undergo a differentiation programme represents a major conceptual advance in the field.

Cytogenetics and epigenetics of glioma

The most recent major advance in our understanding of glioblastoma has been achieved by the Cancer Genome Atlas (TCGA) pilot project. This aims to assess the value of large-scale multidimensional analysis of molecular characteristics in human cancer and to provide data rapidly to the research community. They recently reported the interim integrative analysis of DNA copy number, gene expression and DNA methylation aberrations in 206 glioblastomas.¹⁸ They studied alterations in the RTK/RAS/PIK pathway, and p53 and RB signalling pathways, involved in the control of cell cycle and apoptosis in the majority of glioblastomas. RTK/Ras/PIK signalling was altered in 88%, p53 signalling altered in 87% and RB signalling altered in 78% of cases. This study appears to blow apart the conventional dogma that primary and secondary gliomas derive by different restricted genetic pathways. It implicates different players in glioblastoma pathogenesis including ERBB2, NF1 and PIK3R1, which are different components of growth factor and cell cycle control pathways (See Table 1). This study also examined the methylation status of MGMT, a repair protein that specifically removes promutagenic alkyl groups from DNA. This has been implicated in drug resistance since it reduces the cytotoxicity of alkylating agents such as temozolomide - an alkylating agent used to treat glioblastoma. Patients with newly diagnosed glioblastomas with MGMT methylation respond well to temozolomide. The alkylated DNA results in cycles of futile mismatch repair, leading to cell death. The study

showed that treated glioblastomas were under a strong selective pressure to lose mismatch repair function predicting that such patients would eventually develop treatment resistance. The authors suggest that combining alkylating agents with an agent designed to target mismatch-repair-deficient cells as a first-line therapy may minimise the emergence of drug resistance.¹⁸

There has been recent progress in gliomas traditionally thought to lack karyotypic abnormalities. Such genetic abnormalities may be key events in the development of these tumours. In particular a tandem repeat has been identified in most pilocytic astrocytomas which produces a novel

oncogenic BRAF fusion gene, involved in growth factor stimulated tyrosine kinase pathways which transforms fibroblasts in vitro.¹⁹ A different type of glioma (subependymoma) also carries cytogenetic abnormalities in a proportion of cases.²⁰ As the sensitivity of these techniques increases and we head inexorably

to whole genome sequencing of tumours, the number of abnormalities identified will further increase.

Conclusions

The cancer stem cell theory predicts that not all tumour cells are equal. We may have the

ability to control glioma cells by forcing them to differentiate. This represents a major conceptual advance in the field, but it is still controversial. Major new insights into the genetics and epigenetics of gliomas will hopefully direct a new era of patient-specific combined molecular therapies. ♦

Table 1: Glossary showing a selection of putative candidates and pathways involved in gliomagenesis

Candidate Molecule	Function of Pathway
CD133 (prominin 1/PROM1)	Transmembrane molecule, function still unclear, expressed on haemopoietic neural stem cells. First used to isolate brain tumour stem cells from glioblastoma. Found in a range of tumour types.
RC2	Marker of radial glia (the progenitor of glial and neuronal lineages). Expressed on the putative ependymoma stem cell.
Nestin	Intermediate filament expressed in developing CNS, PNS and myogenesis. Upregulated in some pathological states, a putative marker of ependymoma stem cells and expressed in glioblastoma and other tumour types.
BMP (Bone Morphogenetic Protein)	Member of the Transforming Growth Factor Beta family of secreted ligands. Originally described in bone formation, but has pleotropic effects on the developing nervous system. BMPs have differing effects on glioma stem cells including promoting differentiation in certain tumour lines.
RTK/Ras/PIK	Receptor tyrosine kinases (RTKs) are cell surface receptors for many polypeptide growth factors, cytokines and hormones eg Epidermal Growth Factor (EGF) and Fibroblast Growth Factor (FGF). Receptor tyrosine kinases are key regulators of normal cellular processes and have a critical role in the development and progression of many types of cancer via Ras, PIK and other modulators.
p53	('guardian of the genome') The transcription factor encoded by the TP53 gene. p53 regulates the cell cycle and thus functions as a tumour suppressor that is involved in preventing many cancers including gliomas.
RB	Retinoblastoma protein is involved in cell cycle control and is a well known tumour suppressor gene involved in the formation of certain gliomas.
ErbB2	Member of Epidermal Growth Factor Receptor Tyrosine Kinase family (see above Receptor Tyrosine Kinases).
NF1	(neurofibromin), an intracellular cell signal molecule. A negative regulator of the Ras oncogene and implicated in tumourigenesis outwith the neurofibromatosis type 1 syndrome, including in glioblastomas.
PIK	Phosphoinositide kinases- family of related enzymes that phosphorylate phosphoinositide lipids generating lipid second messengers involved in cell growth, differentiation, survival, proliferation and migration.
MGMT	Repair protein that specifically removes promutagenic alkyl groups in DNA. Implicated in drug resistance since it reduces the cytotoxicity of alkylating agents.
Ras, BRAF	Part of a signal transduction pathway that regulates cell growth, elevated in approximately 30% of human cancers. RAS is mutated in approximately 15% of human cancers.
RAF	There are three RAF proteins, A-RAF, B-RAF and C-RAF. Many different types of B-RAF mutations have been found in human cancers. Activating mutations stimulate proliferation of cells and protect them from apoptosis. B-RAF has recently been implicated in the formation of pilocytic astrocytomas.

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