REVIEW ARTICLE

Ivana Jovčevska: Long-term survival in GBM

Genetic secrets of long-term glioblastoma survivors

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ABSTRACT

Glioblastomas are the most aggressive and lethal primary astrocytic tumors of the central nervous system. They account for 60–70% of all gliomas and the majority are diagnosed in male Caucasian patients at advanced age. Genetic analyses of glioblastoma show a great intra- and inter-tumor heterogeneity, which opens up a debate about its cellular origin. Different types of brain cells, including astrocytes, neural stem cells, oligodendrocyte precursor cells and glioblastoma stem cells are proposed to have a role in tumor initiation and spreading; however, data is still inconclusive. Due to short life expectancy, long-term glioblastoma survivors are defined as patients who live longer than 2 years post-diagnosis. Extreme survivors, living 10 years or more after diagnosis, comprise less than 1% of all patients. Molecular testing indicates genetic differences between short- and long-term survivors with glioblastoma. The most informative are IDH1/2 gene mutations and MGMT promoter methylation, which are associated with a better response to standard clinical care. Moreover, a decreased expression of the CHI3L1, FBLN4, EMP3, IGFBP2, IGFBP3, LGALS3, MAOB, PDPN, SERPING1 and TIMP1 genes has been associated with prolonged survival. In addition, emerging evidence suggests the role of different microRNAs in predicting patient survival. Other factors that may affect the survival of glioblastoma patients include clinical/demographic characteristics such as seizures at presentation, age at diagnosis, and the extent of surgical resection. Because of the small number of long-term survivors with glioblastoma, comparative studies on genetic differences between short and long-term survivors are challenging. To improve patient management and clinical outcomes, a thorough “omics” approach is necessary for identifying differences between short- and long-term survivors with glioblastoma.

KEY WORDS: Glioblastoma; genetics; IDH; MGMT; chromosome 1p/19q; long term survival; extreme survivors
INTRODUCTION

Diverse nature of glioblastoma

The term “glioma” refers to a type of brain tumour which originates in the parenchyma of the central nervous system, more precisely from the supportive glial cells – ependymal cells, astrocytes and oligodendrocytes. According to the cell type they originate from or share histological features with, gliomas are divided into ependymomas, astrocytomas and oligodendrogliomas. Of these, astrocytomas are the most common type of glial tumours. The World Health Organization divides gliomas into four grades starting from pilocytic astrocytoma (grade I), diffuse astrocytoma (grade II), anaplastic astrocytoma (grade III) and glioblastoma (grade IV) (1, 2). Grades III and IV are considered high grade gliomas and represent the majority of brain tumours (3). Glioblastomas are astrocytic tumours with necrosis and microvascular proliferation. Patients suffering from the most malignant type, glioblastoma, usually succumb to the disease in 12 to 18 months after diagnosis (4). Although glioblastoma incidence is very low among all cancer types, 1 per 10 000 cases, with 16% of all the primary brain tumour cases it is the most common brain malignancy which is almost always lethal (5, 6). According to the malignant progression there are two types of glioblastomas – primary, originating de novo, and secondary, evolving from lower-grade gliomas. Based on the presence or absence of isocitrate dehydrogenase (IDH) 1 and 2 gene mutations and chromosome 1p/19q codeletion, in adults, primary glioblastomas are also defined as IDH-wild type, while secondary can either be IDH-mutant and 1p/19q intact, or IDH-mutant and 1p/19q codeleted (7, 8). The most frequent IDH1 mutations are at codon 132 – in 90% of the cases the mutation is R132H. Other known mutations are R132C, R132G and R132S (9). IDH2 mutations are less frequent and occur at codon 172, with R172K being the most common. Primary IDH wild type glioblastomas present with mutations in TERT, PTEN and TP53 as well as amplification of EGFR, PDGFRA, CDK4, CDK6, MDM2 and MDM4,
while secondary glioblastomas show mutations in *IDH*, *TP53*, and *ATRX*, as well as *CDKN2A* deletion (10-12). *IDH* mutations are believed to be the among the first changes that occur in gliomagenesis, and are most commonly accompanied by *TP53* and *ATRX* mutations (13). *ATRX* enables incorporation of histone variant H3.3 into heterochromatin, which results in changes in telomere length and genomic instability (6). A study by Reuss *et al.* reports existence of three distinct glioblastoma sets: chromosome 7p gain and 10q loss with absence of *IDH* mutations and presence of nuclear *ATRX* expression; nuclear *ATRX* loss and/or *IDH* mutations; and nuclear *ATRX* loss with *H3F3A* mutations without *IDH* mutations – an adult glioblastoma subset with similarities to paediatric glioblastoma (14). In addition, expression profiling of glioblastoma specimens defines four different molecular subtypes. A large scale genomic study by Verhaak *et al.* analysed more than 200 glioblastoma samples from three gene expression platforms (Affymetrix HuEx, Affymetrix U133A and Agilent 244K arrays) and identified four robust clusters: classical, mesenchymal, neural and proneural – subtype names were chosen based on the expression of signature genes (15). The distinct genetic events of each subtype were identified by analysing data available from The Cancer Genome Atlas Research Network and are presented in Figure 1 (9, 15-20).

With a bioinformatics approach, Wang *et al.* identified 1520 differentially expressed genes in glioblastoma compared to non-tumour glial cells of epilepsy patients (21). In their work, the pathways most commonly associated to upregulated genes were Wnt, MAPK and ErbB signalling, while p53 signalling, ECM-receptor interaction and antigen processing and presentation were associated with downregulated genes. Of these, Wnt signalling plays a key role in neurogenesis and embryonic brain development, and is able to modulate self-renewal and differentiation of adult tissue stem cells (22). Also, Wnt signalling pathway is commonly dysregulated in tumorigenesis (23). In addition, abnormal activation of the Wnt signalling leads to glioblastoma growth and invasion (24). The TCF/LEF family of Wnt signalling
transcription factors has also been correlated to glioblastoma malignancy (23).

Immunohistochemical staining of glioblastomas resulted in strong expression of TCF-1 and LEF-1 in 51.6% and 71% of analysed samples, respectively. The study by Pečina-Šlaus et al. reports increased expression of transcriptional factors TCF-1 and LEF-1 to be characteristic for malignant gliomas. Other signalling pathways, including RTK, EGFR, PDGFRA, FGFR-1, IGFR-1, NF-κB, MET and SHH are also altered in glioblastoma (24, 25). The reason for presence of such genetic diversity in glioblastomas is not revealed. It is thought to either be a result of different cellular origin, or same cellular origin but different response to signals from the microenvironment.

Glioblastomas are very heterogeneous in nature. They present with cellular and molecular diversity not only among tumours, but also within the same tumour (26, 27). Coexistence of cells with different properties has been proven by numerous genetic studies (28-30) which suggests that glioblastomas may arise from different cell types. Various genetic changes including mutations, chromosomal aberrations and copy number variations in both oncogenes and tumour suppressor genes have been found (7). These observed changes can be either clonal (or early events) – present in all cells even before the malignant transformation, or sub-clonal (or late events) – present in a subset of cells and occurring after the malignant transformation (31). Such genetic diversity implies glioblastoma is not a single condition, but most likely a set of diseases.

**Glioblastoma cellular origin**

Glioblastomas can arise anywhere in the central nervous system; proneural and neural subtypes arise in or near the subventricular zone, mesenchymal and classical subtypes are distal to the subventricular zone, other gliomas arise in the superficial subcortical white matter, while proneural glioblastomas with *IDH* mutations are more likely to occur in the frontal lobes (5, 8). Such correlation between tumour subtype and intracranial location can be
as a result of different cell origin. Glioblastoma cellular origin is controversial and still a matter of debate, where two theories dominate the field: dedifferentiation theory and stem cell theory (Figure 2).

The first proposed mechanism of glioblastoma origin is the “dedifferentiation theory or stochastic model” which states that all cells are equipotent, but under different genetic or epigenetic stimuli some of them can contribute to tumour growth (32, 33). In the past, it was thought that the adult brain does not regenerate and astrocytes were considered the only dividing cells in the adult brain. In addition to this, the GFAP astrocytic marker was frequently found in glioma tissues, which led to the conclusion that astrocytes are the cells of origin of gliomas as reported by Jiang and Uhrbom (1). This theory was questioned after the discovery of self-renewing and multipotent neural stem cells in the late 1990s, which have been successfully isolated from subventricular zone, hippocampus and dentate gyrus of the adult mammalian brain (34-36). Neural stem cells express surface markers nestin, SOX2, CD133 and GFAP (8). Gliomas arise near the subventricular zone which is consistent with the location of neural stem cells in the mammalian brain and this is why neural stem cells were proposed as the cells of origin of gliomas. Furthermore, alterations of EGFR and AKT/PKB signalling pathways, which are important for neural stem cell proliferation and differentiation, are commonly altered in gliomas. At last, oligodendrocyte precursor cells were proposed as the cells of origin of gliomas for several reasons: they are the major dividing cells in adult brain, they can give rise to oligodendrocytes and they are present in the subventricular zone, white and grey brain matter (1). This hypothesis was supported by the frequent alterations in the PDGFRα signalling pathway in gliomas – a pathway which is important for normal oligodendrocyte development. Moreover, oligodendrocyte precursor cell markers NG2, OLIG2 and PDGFR are found in gliomas which proposes a direct link between the tumours and oligodendrocyte precursor cells. The regenerative potential of
astrocytes, neural stem cells and oligodendrocyte precursor cells makes them candidates for cells of origin of gliomas (37). Still, despite all evidence pointing in the directions of these cells, glioblastoma origin remains unsolved.

The second mechanism is the so called “hierarchical model or stem cell theory” which argues that tumours contain a subset of cells, named cancer stem cells, which are able to proliferate, give rise to and reseed a tumour. Cancer stem cells are described as cells able to self-renew and generate more differentiated tumour cells which is believed to be accomplished by asymmetric division – one daughter cells retains stem cell properties while the other differentiates into different types of tumour cells (38). Cancer stem cells have been proven as more resistant to genotoxic treatments which seems to be the cause for tumour recurrence (34, 38). Their resistance to radiation is a result of the higher DNA repair rate as presented by Bao et al. (39). The authors compared early DNA damage checkpoint responses in CD133+ and CD133− cells and found that ionizing radiation initiates activating phosphorylation of ATM, Rad17, Chk1 and Chk2 checkpoint proteins. Moreover, the activating phosphorylation was significantly higher in CD133+ compared to CD133− cells which indicated that CD133+ cells present with greater checkpoint activation to DNA damage. The authors also show that CD133+ cells repair DNA damage more efficiently than CD133− cells. This ability to repair DNA damage more rapidly suggests CD133+ cells are able to survive radiation and reseed a tumour (39). The great self-renewal ability of cancer stem cells favours the accumulation of mutations which ultimately lead to tumour formation and progression (22). However, their existence is difficult to be proven due to lack of specific biomarkers. Usually, for identification of cancer stem cells markers associated with immature cells and normal stem cells are used. Cancer stem cells expressing CD133+ were isolated from glioblastomas, so this molecule was considered as a potential biomarker (36, 40, 41). However, it was later shown that CD133− cells can also give rise to a glioma (42-44). Moreover, cell surface marker
CD15 has also been proposed as a glioma stem cell marker (44, 45). Still, the “gold standard” for identification of cancer stem cells is their ability to give rise to a phenotypically identical tumour as the primary malignancy in immunocompromised mice.

In the case of glioblastomas, it is highly likely that multiple cell lineages are simultaneously present in the tumour. This also suggests that various cell types are responsible for glioma initiation and development. It is thus important to continue investigating glioma cellular origin and how specific cell types can contribute to glioma formation, progression and recurrence (1).

**Clinical management and molecular biomarkers**

The late glioblastoma discovery is a result of its unspecific symptoms (headache, confusion, memory loss and personality changes) which can be accompanied by problems in motor function and speech (46). Diagnosis is performed with computer tomography and magnetic resonance imaging or magnetic resonance spectroscopy, and is confirmed with molecular techniques including immunohistochemistry, Sanger sequencing, fluorescence in situ hybridization and microsatellite analysis (47). Standard of care consists of maximal surgical resection followed by radiation and temozolomide chemotherapy (48, 49). However, even with such an aggressive treatment in 75% – 90% of the glioblastoma cases, the tumour recurs within 7 to 10 months after surgery. Only 9% of glioblastoma patients are still alive 2 years post diagnosis and these are considered long-term survivors (50). Major issues in glioblastoma management are its intracranial location, fast growth, infiltrative nature that leads to incomplete surgical resection, and development of therapy resistance (37). Also, chemotherapy offers limited options due to poor drug penetration through the blood brain barrier (51). Identifying the cell of origin of glioblastoma is of great importance for patient care. If treatment method could be tailored to target a specific subset of cells in every patient, the effectiveness of clinical care can be greatly improved (Figure 3) (5). Besides, targeting
specific cell types can lead to design of novel drugs with minimal toxicity to other non-malignant cells (8).
So far, the only confirmed molecular biomarkers are O⁶-methylguanine-DNA methyltransferase (MGMT) promoter methylation, IDH1/2 mutations and loss of heterozygosity in chromosome 1p/19q (3, 14, 51, 52). The alkylating agent temozolomide causes DNA damages by adding alkyl groups to guanine O⁶ position. This change is effectively repaired by the DNA repair protein MGMT which restores guanine from O⁶-methylguanine and reverses the effect of chemotherapy. Nevertheless, when MGMT promoter is methylated, protein expression is lower which leads to better sensitivity to temozolomide chemotherapy (53). Patient survival with MGMT promoter methylation is significantly higher (21.7 months) than patient survival in cases with non-methylated promoter (15.3 months) (51). IDH mutations are correlated with excessive genome methylation resulting in glioma-specific CpG island methylator phenotype (G-CIMP). They are also commonly associated with MGMT promoter methylation as they occur in 79% of G-CIMP and 46% of non-G-CIMP (3, 51). Moreover, IDH-wild type glioblastomas are often found in brain areas which are difficult to be accessed surgically. IDH mutations alone are barely related to long term survival, but when paired with MGMT promoter methylation they are considered a significant prognostic factor. At last, chromosome 1p/19q codeletion is considered beneficial for elderly patients when they receive PCV chemotherapy. The combination of whole arm 1p/19q codeletion in combination with IDH mutations is favourable. In patients who receive temozolomide chemotherapy the presence of 1p/19q codeletion was linked to longer chemotherapy response duration (54). Contrary, chemoresistant tumours were found to contain both copies of chromosome 1p. Patients with both chromosomes 1p and 19q have 5.7 times higher risk of recurrence when compared to patients with allele losses on these chromosomes (52).
Long term survivors

Gliomas can occur at any age, but the majority arise in older patients. Primary glioblastomas are more common among Caucasian men in advanced age, while lower grade gliomas and secondary glioblastomas are more common in younger adults (aged 45 years and younger) (55). Gender differences are attributed to hormonal changes and genetic features (8). Due to frequent disease recurrence, only 3 to 5% of patients live longer than 3 years after diagnosis (56). Prognosis depends on patients’ age at diagnosis with younger patients having better outcome. This can be partially explained with better overall health, but can also be a result of different molecular and genetic alterations (5). Although glioblastomas from short and long term survivors are histologically the same, their biological and molecular characteristics are remarkably different (57).

In general, gliomas are very aggressive tumours. Astrocytomas are associated with worse prognosis, and oligodendrogliomas show promise for better outcome (5). Long-term survivors comprise approximately 10% of all glioblastoma patients (58). Still, a small number of patients shows strong therapy response and extremely long term survival of 10 years or more. Tykocki and Eltayeb performed literature analysis on clinical studies containing information for extreme survivors – glioblastoma patients surviving 10 years or longer (59). Their systematic review reports 0.71% of all patients present with survival longer than 10 years. Moreover, the authors found a relationship between age at diagnosis and overall survival – for every 4.7 years younger age at diagnosis the overall survival is one year longer after 10 years of survival. In general, 10-year overall survival varies among age groups: 0 – 14, 15 – 39 and 40+ years present with 14.9%, 13.6% and 1.6% long term survivors, respectively. Another investigated factor is the most common clinical symptom of glioblastoma – epileptic seizures. Seizures can either occur as part of the initial diagnosis or as a result of recurrence of the disease. Although they reduce the quality of life, seizures as a
symptom are found to be positively correlated to longer survival (60-62). However, in cases of a longer delay between epileptic seizures as a symptom and the surgical resection, this correlation does not seem to be significant (62, 63).

When it comes to the genetics, reports show glioblastomas with IDH1/2 mutations and MGMT promotor methylation are more responsive to surgical resection and temozolomide chemotherapy, and have better prognosis (57, 64-66). Hartmann et al. compared samples from 33 patients who lived longer than 60 months and patients whose overall survival was not longer than 36 months (67). Their study reports more frequent MGMT promotor methylation (66.7% versus 33.6%) and less frequent IDH1/2 mutations (63.6% versus 96.4% for IDH1 and 96.9% versus 99.2% for IDH2) in the long term survival group. In their study, 1p/19q codeletions were not common and EGFR amplifications were absent. Li et al. analysed 3 microarray datasets containing 8 controls, 58 long term and 135 short term survivors, and found FBLN4, IGFBP2 and CHI3L1 genes to negatively associated with glioblastoma survival (68). Expression levels of the three genes were found to be significantly increased in short term survivors versus long term survivors and normal controls. The authors conclude that increased mRNA levels of FBLN4, IGFBP2 and CHI3L1 mean decreased survival probability. Similar to this, Gerber et al. found that decreased levels of CHI3L1, EMP3, IGFBP2, IGFBP3, LGALS3, MAOB, PDPN, SERPING1 and TIMP1 genes are associated with longer survival (66). The study also shows high MGMT promotor methylation in long term survivors. Although glioblastoma samples could not be classified into a single subtype, many of the obtained genes (CHI3L1, EMP3, PDPN and TIMP1) are linked to the mesenchymal subtype. A study by Michaelsen et al. reports MGMT, IFNG, CXLC9, LGALS4 and CD34 genes as prognostic variables (65). Their further analysis using data available from AVAglio study confirmed high expression only of CD34 in the long term survival group (69). Then, CD34 was tested in concordance with clinical and pathological
features (age, corticosteroid use and MGMT promoter methylation) and showed its association with prolonger overall survival, which was also the case for MGMT mRNA level. An interesting observation is the involvement of ATRX in glioblastoma longevity. Using a gene-targeted next generation sequencing panel Cantero et al. found better prognostic value for patients whose tumours present with ATRX or DAXX mutations in the absence of IDH or H3F3A mutations (70). Similar findings were published by Chaurasia et al. who report statistically better overall survival and progression free survival for patients with ATRX and IDH1 mutant protein expression (ATRX- and IDH+, respectively) (6). Their Kaplan-Meier analysis was supported by the immunohistochemical examination of formalin-fixed paraffin-embedded tissue samples which showed that patients with ATRX-/IDH+ pair have longest overall (42.71 months) and progression free (42.2 months) survival. On the contrary, the combination of ATRX+/IDH- was correlated with the lowest overall (20.7 months) and progression free (16.8 months) survival. Increasing evidence shows microRNAs (miRNAs) have a prognostic value in long term survival (22, 71-73). Hermansen et al. report differences in the miRNA profiles of short- and long-term glioblastoma survival. They have identified four miRNAs (miR-107, hsa-miR-548, miR-3125 and miR-331-3p) whose low levels were associated with shorter survival. However, their results are valid for patients suffering from glioblastomas with unmethylated MGMT promotors (74). Yuan et al. found increased levels of let-7g-5p, miR-139-5p, miR-17-5p and miR-9-3p in samples from long term survivors independent from the MGMT methylation status (57). Patients with high expression levels of these miRNAs lived 88 days longer than patients with low expression levels (439 versus 351 days, respectively).

A case report presents single patient with 20 years survival after glioblastoma (75). The patient, a 45-year-old Caucasian man, was diagnosed with glioblastoma at the age of 25. After initial treatment (surgery and radiation) there were two tumour recurrences, 2 and 20
years after initial diagnosis. Molecular testing was performed only on samples from the last recurrence and revealed MGMT promotor methylation, PTEN and TP53 positive, EGFR and protein kinase AKT negative. Although this isolated case is not enough for drawing general conclusions, the molecular profile of the patient can give information about possible genetic benefits for glioblastoma patients.

In general, younger age is a predictor of better overall survival. Patients diagnosed with glioblastoma below the age of 40 years have a greater chance for long survival, especially above 10 years after diagnosis (76, 77). Another factor affecting the prognosis is the extent of the surgical resection – 12 months progression free survival is increased by 50% with complete tumour resection. However, there are also reports of patients with incomplete resection or biopsy only that survived for more than a decade (59). Besides age and good performance status at diagnosis, adjuvant chemotherapy is also considered beneficial (58). Epileptic seizures are also favourable, probably due to the possibility of an earlier stage diagnosis, when the tumour is smaller and the chance for a total resection is higher.

Regarding the genetic factors, patients with MGMT promoter methylation and IDH1/2 mutations are more common among long term survivors.
CONCLUSION

Glioblastoma is a big medical problem of modern society because of its high mortality rate. Even with aggressive clinical care, long-term glioblastoma survivors comprise less than 15% of all cases. Further extensive research and multidisciplinary “omics” approach will be needed for understanding the natural causes of glioblastoma occurrence. Clinical features like age at diagnosis, presence of seizures as an initial symptom, and extent of surgical resection are known factors that contribute to patient’ life expectancy. However, for better patient management, genetic, epigenetic, transcriptomic and proteomic information should be added to the appearance of early clinical symptoms. So far, IDH mutations and MGMT promoter methylation are the most important molecular factors for determining glioblastoma longevity. Revealing the factors that contribute to patients’ longevity is important for precise diagnosis and correct clinical management of the disease. Due to the small number of patients with long life expectancy, comparative studies about genetic differences between short- and long-term survivors are challenging which makes this phenomenon poorly understood. However, identification of such differences is crucial for establishing the mechanism of glioblastoma pathology.

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DECLARATION OF INTERESTS

The author declares no conflict of interests.
REFERENCES


**FIGURES**

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<td>• Loss of CDKN2A and NF1</td>
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<td>• IDH1 point mutations</td>
<td>• Expression of GABRA1, SLC12A5,</td>
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<td>• Expression of SERPINE, TRADD, RELB and CTGF</td>
<td>• Chromosome 10 loss</td>
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<td>• Presence of mesenchymal markers CHI3L1, MET, CD44 and MERTK</td>
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**FIGURE 1.** Glioblastoma subtypes. The most common genetic changes in the four glioblastoma subtypes (classical, neural, proneural and mesenchymal) as described by Verhaak *et al.* (2010) are given in the scheme.
FIGURE 2. Glioblastoma origin. Schematic representation of the two theories about glioblastoma cellular origin: dedifferentiation theory – all cells have tumorigenic potential, but under different stimuli some of them will contribute to tumour growth; versus stem cell theory – only a fraction of cells, named cancer stem cells, is able to self-renew, initiate and regrow a tumour. Cell figures are for graphical representation only and do not show actual cell shapes.
FIGURE 3. Conventional *versus* targeted therapy. A schematic representation of appearance of symptoms and glioblastoma diagnosis with immunohistochemistry as a molecular technique followed by two possible treatment methods: conventional therapy – aiming at the majority of cells which in most cases leads to tumour recurrence; and targeted therapy – targeting a specific cell type or cell property which will ultimately lead to tumour relapse.