**The post-surgical era of GBM: How molecular biology has impacted on our clinical management. A review**

# The post-surgical era of GBM: how molecular biology has changed our clinical management. A review.

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ABSTRACT

Glioblastoma (GBM) is the most common glioma in adults, with incidence increasing by 3% per year. According to the World Health Organization Classification of Central Nervous System Tumours, GBM is considered a grade IV tumor due to its malignant behavior.
The aim of this review is to summarize the main biological aspects of GBM. In particular, we focused our attention on those alterations which have been proven to have an impact on patient's outcome, mainly in terms of overall survival (OS), or on the tumor response to therapies. We have also analyzed the cellular biology and the interactions between GBM and the surrounding environment.

KEYWORDS

"Glioblastoma", "MGMT", "IDH1", "IDH2", "TERT", "BRAF", "High grade gliomas", "molecular biology"

## INTRODUCTION

Glioblastoma (GBM) is the most common glioma in adults, with incidence increasing by 3% per year [38]. According to the World Health Organization Classification of Central Nervous System Tumours, GBM is considered a grade IV tumor due to its malignant behavior.[1]

Historically, radiotherapy (RT) alone following surgery resulted in 3- and 5-year survival rates of 4.4 and 1.9%, respectively [71]. These results remained fundamentally unchanged until the start of the century when the results of a landmark trial led by the National Cancer Institute of Canada (NCIC) and the European Organization for Research and Treatment of Cancer (EORTC) were published: addition of concurrent and adjuvant oral temozolomide (TMZ) to standard RT achieved a significant improvement in overall survival (OS) [72]. Moreover, adjuvant temozolomide (TMZ) therapy, significantly increased the long-term survivors [71].

The past years have seen remarkable advances in GBM research, especially regarding tumor biology, but this has failed to allow significant improvements in its prognosis. Nevertheless, our improved knowledge allowed us to better understand the observed differences in patients' response to treatments: for example, the 2-year survival in patients with tumors that have

*MGMT* promoter methylation has increased almost 5 times compared to other patients who do not present this genetic hallmark [38, 71, 72].

Our modern knowledge about GBM molecular biology is extensive because, since its early beginning, the field of neuro-oncology has focused on trying to understand the molecular basis of brain tumors and of GBM in particular, considered its frequency and malignancy. We now have abundant information about the molecular biology of glioma cells, including many potential targets for therapeutics. For instance, we now know that the main molecular pathway of signal transduction that drives glioma growth is made up of several components: the growth factor receptors (GFR) on the cell surface functioning as a "docking station" for growth signals; a system of secondary messengers within the cells that is activated by GFRs; a common convergence point for many signal transduction pathways which is represented by DNA to activate expression of cancer-associated genes (oncogenes) and the protein products of those oncogenes that define the malignant phenotype (cell proliferation, angiogenesis, tumor invasiveness). Each component of this molecular pathway is a potential target for therapeutics. These achievements are so important in the understanding of the biological and clinical behavior of gliomas, that the diagnostic entities provided by the latest WHO classification are based upon an integration of histological features and molecular hallmarks.[1] This novel classification paradigm of diffuse gliomas allows to identify patients with significantly different outcomes, paving the way to more tailored treatments, but this represents just a first step: we are still far from satisfying results in terms of outcome for all people who suffer from this aggressive pathology.

The aim of this review is to summarize the main biological aspects of GBM. In particular, we focused our attention on those alterations which have been proven to have an impact on patient's outcome, mainly in terms of overall survival (OS), or on the tumor response to therapies. We have also analyzed the cellular biology and the interactions between GBM and the surrounding environment.

## MATERIALS AND METHODS:

A literature search using PubMed MEDLINE database was performed. The search terms "Glioma", "Glioblastoma", "High grade glioma" were combined with "MGMT", "IDH1", "IDH2", "TERT", "BRAF", "biomarkers", "molecular", "therapy", "monoclonal antibody".

## RESULTS:

### MGMT promoter methylation

For many years, a glioma therapy dogma held that surgery and RT were the only two therapeutic modalities that improved the OS of patients with GBM, with only 10% of patients surviving 2 years. In 2005 a pivotal European/Canadian study by Stupp et al [72] described the addition of TMZ to surgery and RT. The Stupp protocol includes TMZ at 75 mg/m$^2$ on days 1 through 42 with concomitant RT, followed by TMZ on days 1 through 5 of 28 for 6 consecutive months as adjuvant therapy at a dose of 150 to 200 mg/m$^2$. The addition of TMZ resulted in a 3-year OS of 16% and 5-year OS of 9.8% [71]. This resulted in TMZ being

approved by the Food and Drug Administration (FDA) and subsequently other drug regulatory authorities around the world, as well as establishing this combined therapy as the standard treatment for this condition.

TMZ is one of a series of imidazotetrazinone derivatives that is spontaneously activated into the active metabolite 5-(3-methyl)1-triazen-1-yl-imidazole-4-carboxamide (MTIC) at physiological pH in aqueous solution. The mechanism of action of this drug is based on the reaction of water with the electropositive C4 atom of TMZ that opens the heterocyclic ring, releasing MTIC and carbon dioxide. MTIC is biologically unstable and degrades into methyldiazonium ion, a reactive methylating compound. Like the chloroethylnitrosoureas, with which they have a common range of preclinical activity, imidazotetrazinones act as major groove-directed DNA-alkylating agents [18]. They are base-selective and preferentially bind the middle guanine residue of a GGG sequence. The sites of methylation on DNA are the N7 atoms on guanine, O3 on adenine, and O6 on guanine [28].

Although O6-methylguanine represents only a minority of adducts formed by TMZ, it has a critical role in the cytotoxic action of the drug and it is the initial site of attack on DNA of other active agents against malignant gliomas, such as the cross-linking chloroethylnitrosoureas. O6-methylguanine in itself is not lethal to cells; it does not inhibit processes such as DNA replication or transcription. However, the preferred base pairing during DNA replication results in incorporation of thymine instead of cytosine opposite O6-methylguanine. The mismatch repair pathway of the cell recognizes this mismatch and excises the aberrant thymine residue in the daughter strand. However, unless the methyl adduct is removed from the guanine, thymine is likely to be reinserted on the opposite strand. The mismatch repair pathway has a key role in signaling the initiation of apoptosis in response to O6-methylguanine [37] Repetitive futile rounds of mismatch repair are thought to result in a state of chronic strand breaks, which triggers an apoptotic response [57].

6-O-Methylguanine-DNA Methyltransferase (MGMT) gene encodes for a DNA repair enzyme that provides resistance to alkylating CTs such as TMZ. Because MGMT transcription can be silenced by promoter methylation in tumor cells [23, 27], it is widely assumed that MGMT promoter methylation in patient tumors causes decreased MGMT protein expression, thereby abrogating the DNA repair activity necessary for TMZ resistance. In the presence of methylation of MGMT promoter, the 2-year survival of patients treated with RT and TMZ improved to 47%, a 5-fold increase compared with RT alone [27].

Thus, MGMT promoter methylation is a predictive biomarker of response for treatment with alkylating drugs and it can be used to guide the adjuvant treatments in specific settings, like in older patients (>70 year-old) which are at higher risk of developing toxicities due to the concomitant RT/TMZ treatment.

EGFR

EGFR is a transmembrane glycoprotein that plays a critical role in tumor progression, invasion, angiogenesis and CT resistance. Following ligand binding, multiple signal pathways are triggered. The main ones are phosphatidyl inositol-3-kinase (PI3K)/Akt and the mammalian target of rapamycin (mTOR)(PI3K/AKT/mTOR) or Ras/Raf/MAPK. These signal pathways are able to promote the inhibition of autophagy and apoptosis [52, 74]. In this way, the effectiveness of TMZ therapy is reduced.

In glioma, EGFR presents many different alterations: it can be overexpressed, amplified or constitutively activated. EGFR gene amplification has been found in up to 60% of all GBMs. Moreover, in nearly half of these cases the gene is rearranged, which results in an increase in basal activity [13, 36, 74]. The most common EGFR variation in GBM is the EGFRvIII, a deletion of 267 aminoacids in the extracellular domain. EGFRvIII is incapable of binding any known ligand, but it is constitutively active and stimulates glioma proliferation through proteinkinaseA (PKA) dependent activity. In GBM EGFR amplification is frequently accompanied by EGFR overexpression and 97.7% of GBMs with non-amplified EGFR do not show EGFR overexpression [13, 68].

EGFR amplification has no prognostic impact on OS when considered alone [7, 75]. However, in GBM harboring no TERT mutation, patients with EGFR wild type have been shown to have a mean survival twice superior to that of patients with EGFR amplification [42].
The relevance of EGFR for glioma cell migration has long been known [43] and accordingly, the highest expression of EGFR was demonstrated on the infiltrative edge of tumors [44, 45].
Up to now, different molecules have been developed to target EGFR signaling pathway and attempts have been made to address the lack of extracellular receptor [13, 52]. Small molecule tyrosine kinase inhibitors (TKIs) targeting signal transduction as well as monoclonal antibodies against EGFR have been investigated as anti-tumor agents. Despite the availability of several compounds that are approved for a broad spectrum of diseases, none is approved for glioblastoma, which is a result of numerous negative clinical trials. For the leading representatives of this group, erlotinib, gefitinib, afatinib, and lapatinib, trials have not shown efficacy either alone or in combination[76].

The development of monoclonal antibodies recognizing EGFR can serve not only to interfere with ligand binding, thus inactivating signaling, but also to ferry conjugate toxins into the cells, like the currently investigated depatuxizumb mafodotin (ABT-414).
A purely immunological approach to target the EGFRvIII has also been attempted by vaccination approaches, using a unique antigenic epitope arising within the mutant protein sequence. Unfortunately, however, the pivotal phase III trial for newly diagnosed glioblastoma with rindopepimut failed to show overall efficacy, with the results still being evaluated for subgroup efficacy [50].

## IDH1/IDH2 mutation and 1p19q deletion

Recurrent point mutations in codon 132 of the gene encoding human cytosolic NADPH dependent isocitrate dehydrogenase 1 (IDH1) have been described in nearly 40% of gliomas. Such mutations result not only in a dramatic decrease of IDH1 activity [57, 64, 77], but also in a gain of enzyme function of the NADPH-dependent reduction of ketoglutarate to 2-hydroxyglutarate, which accumulates in IDH1 mutated cells [17]. IDH1 mutation rate is highly variable among glioma histologic subtypes, from 5% in adult primary GBMs up to 86% in anaplastic oligodendrogliomas [77], according to the pre-2016 WHO classification criteria. An IDH2 mutation is found in up to 5% of gliomas and lead to the same change of enzyme function, while IDH3 represents the mitochondrial isoform and no known pathogenic mutations of this isoform are known to date [77].

The prognostic impact of IDH mutations on patient's outcome is considerable. IDH mutations constitute one of the most important prognostic marker in lower grade gliomas and help distinguish de novo GBMs (frequently IDH wild-type) from secondary GBMs (more often characterized by IDH mutation) [64]. Moreover, IDH mutations could have a predictive value. IDH1/IDH2, indeed, protect cells against oxidative stress by producing NADPH that, among other functions, reduces glutathione. Cells with mutated IDH1/IDH2 alleles could thus be more sensitive to treatment-induced oxidative cellular damage [43].

IDH1 and IDH2 mutation status of a large series of gliomas has been analyzed in past years using different sequencing techniques. Whether the occurrence of IDH1 or IDH2 mutation correlates with 1p19q status has also been investigated [43]. Since the publication by Cairncross and Jenkins in 2008 [11], it is known that the codeletion of chromosome arms 1p and 19q characterizes a subtype of oligodendroglial tumors with better prognosis and higher chemosensitivity compared to brain tumors which do not present this pattern. The explanation of this peculiarity could be a different origin of those neoplasms. In the WHO 2016 brain tumor classification the presence of IDH mutation and 1p19q codeletion leads to a diagnosis of oligodendroglioma, even if astrocytic features are present. This molecular signature is so relevant that in 2016 WHO classification a IDH-mutated 1p19q codeleted tumor is considered an anaplastic oligodendroglioma even of in presence of microvascular proliferation and necrosis.

The importance of 1p19q codeletion is not only prognostic but also predictive; indeed, the radiological response to chemotherapy (CT) is strongly associated with loss of chromosome 1p. Tumors that responded to CT were likely to have lost chromosome 1p and tumors with chromosome 1p loss were all chemosensitive. Conversely, all chemoresistant tumors that were evaluated for allelic loss of chromosome 1p were discovered to retain both copies of chromosome 1p [12].

In contrast, the association of chromosome 19q loss and CT response did not result statistically significant in the literature. However, patients whose tumors had lost both chromosomes 1p and 19q had a significantly greater chance of responding to chemotherapy than patients whose tumors lacked such combined losses.

The association between losses of chromosomes 1p and 19q and response to chemotherapy was also related to a significantly longer recurrence-free survival after chemotherapy [12]. Patients whose tumors retained both copies of chromosome 1p had a recurrence rate after chemotherapy that was 4.3 times greater than that of patients whose tumors had lost chromosome 1p. Furthermore, patients whose tumors retained chromosome 19q alleles had a rate of recurrence or death after CT that was 5.6 times greater than that of patients with tumors lacking alleles on chromosome 19q. Finally, patients whose tumors retained both chromosomes 1p and 19q had a relative risk of recurrence or death after CT that was 5.7 times greater than that of patients with combined allele losses on chromosomes 1p and 19q [12].

Studies that analyzed large case series demonstrated rather compellingly that IDH1/IDH2 mutation is a constant feature in gliomas with complete 1p19q codeletion [17, 43, 77]. This interesting finding has a direct diagnostic significance for clinicians: a glioma without an

IDH1/IDH2 mutation is extremely unlikely to have a true 1p19q signature and the possibility of partial deletions should be evaluated; moreover, these findings confirm that IDH1/IDH2 mutation is a highly favorable prognostic factor for gliomas of any grade [43]. This indicates that both alterations contribute to the favorable outcome. Some other evidence in the literature suggests a synergistic association between these two alterations. This synergy may play a key role in the oncogenesis of a restricted population of glial cells or of their precursors through unknown mechanisms.

One hypothesis is that IDH1/IDH2 impairment favors DNA double-strand break occurrence and more specifically, peri-centromeric 1p19q codeletion/translocation [43]. At least 2 mechanisms may be involved: the first is the decrease of reduced glutathione that increases oxidative damage and therefore genomic instability. The second is related to the decrease of alfa-ketoglutarate, the product of IDH catalysis, that activates a broad range of dioxygenases including histone demethylase. It is now confirmed by numerous studies that histone methylation is an important modification linked to both transcriptional activation and repression; it also regulates stability of heterochromatin and thereby genome integrity [48, 67]. In this setting, it has been speculated that the modification of pericentric heterochromatin on chromosomes 1 and 19 creates the conditions for 1q19p translocation (28). Another hypothesis is that 1p19q codeletion has transforming properties only in the context of IDH1/ IDH2 inactivation [43]. Exploring these hypotheses by functional studies will be the next step of IDH biology studies.

Unlike other common genetic alterations found in gliomas, such as TP53 mutations or EGFR amplification, both complete 1p19q codeletions and IDH1/IDH2 mutations seem to be restricted to glial tumors (with the exception of a 10% rate of IDH2 mutation in acute myeloid leukemia and IDH1/IDH2 in chondrosarcoma and in cholangiocarcinoma) [51]; in addition, both are related to good outcome, and both are tightly associated with a proneural pattern of expression [21].

## BRAF

BRAF is a human proto-oncogene that encodes a protein called B-Raf. B-Raf has a paramount role in growth signal transduction. This protein is a serine/threonine-protein kinase, part of the Raf kinase family, that plays a crucial role in regulating the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signaling pathway [31]. Mutations in the proto-oncogene BRAF, the most common of which is V600E, result in constitutive activation of the kinase with subsequent uncontrolled cell proliferation and tumorigenesis.

Certain subtypes of brain tumor seem to have a higher frequency of BRAF V600E, as demonstrated in several recent studies [31, 35, 65]. This mutation has also been related to a variety of cancers, including melanoma and papillary thyroid carcinoma [45, 62]. In both adults and

children, up to 20% of extra-cerebellar pilocytic astrocytomas (PA) and 60–80% of pleomorphic xanthoastrocytomas (PXA) harbor BRAF V600E mutation [19, 31, 61, 65]. Moreover, BRAF V600E is mutated in up to 50% of epithelioid glioblastoma cases [40], a glioblastoma variant associated with an aggressive clinical course and a higher rate of leptomeningeal disseamination.
In the last years, more knowledge about the role of BRAF mutation in gliomas has been acquired; however, very few studies have addressed the prognostic value of this mutation in glioma patients [31]. Published results of these few studies show that BRAF V600E was associated with a significantly improved OS, while the effect on tumor progression was not statistically significant [73]. Age seems to have a key role in the relationship between patient outcome and BRAF V600E mutation [32, 53, 78]: it is associated with a better survival in pediatrics (0–16 years) and young adults (17– 35 years), but does not have prognostic value in adult patients (>35 years). This finding suggests that the prognostic implication of BRAF V600E in glioma might depend on patient age. Additionally, BRAF V600E mutation showed no association with response to radiotherapy or chemotherapy [32]

Based on these findings, BRAF V600E targeting molecules are currently under study and could lay the foundations for new and more effective drugs. Specifically, vemurafenib, a BRAF inhibitor approved in 2011 by the United States Food and Drug Administration for therapy of melanoma with a V600E mutation, has already been used with an off-label indication in a young patient with diagnosis of GBM with extraordinary results [60]. Dabrafenib and other molecules are currently under analysis to overcome resistances [8, 10]. More studies are needed to better comprehend the potentialities of these site specific BRAF inhibitors in GBM therapy.

## TERT

The TERT (telomerase reverse transcriptase) gene codes for a highly specialized reverse transcriptase, that catalyzes the extension of chromosome ends by adding hexamer repeats [6, 14]. Telomerase activity and TERT expression are low in normal tissues; such condition is linked to cell senescence. Cancers cells are instead characterized by pronounced telomerase activity, allowing for the maintenance of telomere length, therefore escaping senescence [66, 70]. TERT promoter region is known to be mutated in different types of tumors [26, 33, 34, 47]. High grade gliomas are among the most frequently affected neoplasms [1, 5, 39, 47]. These mutations occur in two key positions, enhancing TERT promoter activity by generating a consensus binding site for transcription factors [33, 34]. A recent study by Labussiere et al. [42] has shown that TERT promoter mutation is an independent factor of poor outcome in GBMs. This is not due to the association of IDH mutation with TERTp wild-type status as previously believed [54]. However, the impact of TERT promoter mutation is even stronger in patients with IDH mutation than in patients with IDH wild-type.

TERT mutations are much more common in primary versus secondary GBMs and are inversely correlated with IDH1/IDH2 mutations.

TERT promoter mutations are usually absent in lower grade IDH mutated astrocytoma, but frequently this kind of neoplasia shows ATRX mutations and alternative lengthening of telomeres (ALT) phenotype, a telomerase-independent telomere maintenance mechanism [5, 39,

47]. IDH wild-type lower grade gliomas are associated with a often dismal prognosis, however a subset without TERT or ATRX mutations may correspond to a subgroup with better prognosis (Ceccarelli M, Barthel FP, Malta TM, et al.; TCGA Research Network. Molecular profiling reveals biologically discrete subsets and pathways of progression in diffuse glioma. Cell. 2016;164(3):550–563.).

In different studies, the 1p/19q co-deletion was associated with mutations in TERT promoter [Kaloshi G, Benouaich-Amiel A, Diakite F, Taillibert S, Lejeune J, Laigle- Donadey F, Renard MA, Iraqi W, Idbaih A, Paris S et al (2007) Temozolomide for low-grade gliomas: predictive impact of 1p/19q loss on response and outcome. Neurology 68:1831–1836. doi:10.1212/01.wnl.0000262034.26310.a2, van den Bent MJ, Brandes AA, Taphoorn MJ, Kros JM, Kouwenhoven MC, Delattre JY, Bernsen HJ, Frenay M, Tijssen CC, Grisold W et al (2013) Adjuvant procarbazine, lomustine, and vincristine chemotherapy in newly diagnosed anaplastic oligodendroglioma: long-term follow-up of EORTC brain tumor group study 26951. J Clin Oncol 31:344–350. doi:10.1200/JCO. 2012.43.2229]. In the study of Lee et al. 96.9% of patients with oligodendrogliomas had a TERT promoter mutation. However, the presence of this molecular alteration is associated with favorable outcomes among patients with IDH-mutant 1p/19q codeleted oligodendrogliomas [Adult infiltrating gliomas with WHO 2016 integrated diagnosis: additional prognostic roles of ATRX and TERT Melike Pekmezci1,2 · Terri Rice3 · Annette M. Molinaro3,4 · Kyle M. Walsh3,4 · Paul A. Decker5 · Helen Hansen3 · Hugues Sicotte5 · Thomas M. Kollmeyer6 · Lucie S. McCoy3 · Gobinda Sarkar6 · Arie Perry1,3 · Caterina Giannini6 · Tarik Tihan3 · Mitchel S. Berger3 · Joseph L. Wiemels4,8 · Paige M. Bracci4 · Jeanette E. Eckel- Passow5 ·Daniel H. Lachance7 · Jennifer Clarke3 · Jennie W. Taylor3 · Tracy Luks8 · John K. Wiencke3,9 · Robert B. Jenkins6 · Margaret R. Wrensch3,9], thus the prognostic role of TERT promoter mutations depends on the overall molecular profile of the tumor.

GBM & Stem Cells

It has been well established that in the adult brain there are both neural stem cells (NSC) and glial progenitor cells in multiple regions. NSC, which are multipotent and self-renewing, have been isolated from the subventricular zone (SVZ) [63], the lining of the lateral ventricles, the dentate gyrus [22], within the hippocampus, and the subcortical white matter [49, 55]. The largest of these germinal regions in humans is the SVZ that contains a population of astrocytes that can function as NSC [63].

NSC and progenitor cells commonly possess shared features with central nervous system cancer, including a robust proliferative potential and a diversity of progeny. Moreover, NSC are regulated by the same cellular pathways that are active in many brain tumors [59]. Consequently, they exhibit attributes that are characteristic of gliomas, including high motility, association with blood vessels and white matter tracts, immature antigenic phenotypes, and activation of “developmental” signaling pathways [20, 56, 69].

Germinal regions such as the SVZ have long been proposed as sources of gliomas [24]. Many gliomas are either periventricular or contiguous with the SVZ, and they frequently express the progenitor-cell marker nestin [3, 16]. These initial observations suggested that gliomas can originate from NSC populations, but the genetic alterations necessary for stem-cell and progenitor-cell transformation are only now being uncovered.

Recently, progenitor cells activated with Akt and KRas, signal transduction proteins associated with human GBMs, were shown to form brain tumors with histologic features of GBM in rodents [29].

The genetic and cellular diversity within each glioma may be due to secondary changes within tumor sub clones, but it is possible that the origin is a multipotent tumor cell.

The cause of mixed cell gliomas is not likely to be the independent transformation of two differentiated cells but, rather, the transformation of a single, bi- or multipotent progenitor cell [15]. For example, a tumor with an oligoastrocytoma morphology may originate from a bipotent adult oligodendrocyte type-2 astrocyte progenitor cell, which normally generates both astrocytic and oligodendrocytic progeny in vitro [58]. The transformation of such a population of progenitor cells, which are already capable of self-renewal through asymmetric cell division, could generate gliomas with astrocytic, oligodendroglial, or mixed phenotypes [46]. It is important to note that the loss of heterozygosity on chromosomes 1p and 19q in both the components of gliomas with mixed oligoastrocytic morphology has been reported [41], suggesting that there is a common cell of origin.

In vivo gene transfer of the polyomavirus middle T antigen, an oncogene that activates multiple signal-transduction pathways involved in gliomagenesis, into differentiated, GFAP-positive astrocytes has been shown to result in the production of both oligodendrogliomas and astrocytomas [30]. Thus, the cell of origin of gliomas not only may be capable of producing multiple cell types, including oligodendrocytes and astrocytes, but also may resemble an astrocyte in terms of phenotype. It remains unknown whether such a multipotent astrocyte is related to the astrocytic neural stem cells lining the SVZ of the adult human lateral ventricles, as recently described [63].

Regarding mixed-cell gliomas, there is also the question of how the cellular environment directs gliomagenesis. Since gliomas can contain non-neoplastic astrocytes and endothelial cells within their stroma, a potentially important factor in the formation of gliomas may be the germinal niche recreated by the coexistence of neoplastic and non-neoplastic cells of the central nervous system. This relationship is highly evocative of the environmental interplay known to exist between adult NSC and the supporting cells of the SVZ and dentate gyrus [4].

Although the presence of multipotent progenitor cells can explain the heterogeneity of brain tumors, the diversity of their progeny may be limited by the blocked-differentiation phenomenon that was first observed in human leukemias [9]. This process entails an accrual of immature, self-renewing progenitor cells in which cell proliferation and maturation are uncoupled. The transformed progenitor cells divide rapidly, but their progeny are incapable of complete differentiation [25]. Thus, the tumor phenotype may be defined by the direction and degree of differentiation of the transformed progenitor population.

Concerning this point Altieri at al. observed a strong correlation between the expression of some of the molecular features explained above and the onset site of GBM. In their series, the authors tested the differences between GBMs removed from the right frontal lobe and the left frontal lobe and they discovered a predictive value of left frontal lobe involvement for a lower value of Ki67 (a proliferation index) by 8.99%.

They also assessed that frontal involvement is predictive of IDH1 mutant tumors, while tem-

poral location is predictive for wild-type lesions (if the temporal lobe is involved, they estimated a probability of 73% of finding IDH1 wild type). Not only, they proceeded differentiating the right side from the left side and found a higher predictive value for the right frontal lobe for IDH1 mutant tumors (odds ratio of 8), while they found no difference between right or left temporal lobe involvement.

Their study showed that parietal involvement is predictive for MGMT methylation and insular location is predictive for unmethylated HGG. No predictive role of patient age was found for Ki67 value or MGMT methylation, but there was a predictive value for IDH1 mutation when tumors involved the temporal lobe.

In another recent study published in Nature, Lan and colleagues [44] attempted to identify mechanisms driving intra tumoral heterogeneity by tracking the clonal evolution of glioblastoma cells and decoding the complex tumor hierarchies. Gliomas are in fact constantly changing, evolving based on built-in developmental and epigenetic programs or adapting as a result of therapy and local microenvironmental constraints.

Tumor cells in different regions of the tumor and in recurrent tumors appear to arise from distinct clones with specific mutations and variable therapeutic response [2]; these clones are however present in the glioma environment early on.

Thus, targeting the dynamic genomic and cellular landscape of gliomas using a single data point in space and time is erroneous and is likely to fail. In order to hit this moving target, we must first be able to anticipate routes of tumor development based on genetic blueprints and predetermined developmental hierarchies.

In the same article Lan at colleagues demonstrate that after birth tumor cells follow a stochastic process and have an equal probability of either duplication or death. This cell population demonstrated a 3-step unidirectional evolutionary process: a clone can transition from a slow cycling stem-like state to a rapidly cycling progenitor state and subsequently to a non-dividing state. The authors also found that most tumor cells undergo asymmetric divisions, although rare instances of symmetric divisions are possible and required in order to maintain clonal dynamics.

Standing these considerations, the authors proceeded to investigate various methods of targeting the proliferative hierarchy. Having identified the spontaneous evolution of tumor clones, they then investigated the impact of chemotherapy on clonal expansion. To this end, xenografts were treated with TMZ, the standard of care in GBM. Based on susceptibility to TMZ, they identified two distinct tumor cell phenotypes with divergent behaviors: the vast majority of clones were sensitive to TMZ (group A), while a small subset of cells was TMZ resistant (group B). Importantly, group B comprised outlier clones that did not follow the negative binominal growth pattern.

The finding of a built-in tumor resistance mechanism has capital importance, because it implies that: 1) treatment-naïve tumors will always recur after chemotherapy, and 2) strategies attempting to prevent resistance to TMZ will be invariably unsuccessful because group B cells resistant to chemotherapy are the putative cause of tumor regrowth and recurrence.

Discovering group B-targeted molecules will be the next step and some research groups are already working on this. If drugs targeting TMZ resistant cells were developed, they could

potentially have a tremendous impact over GBM therapy and overall survival.

## CONCLUSIONS

In conclusion, it is clear that, despite the many advancements made in the wide field of GBM molecular biology and the new therapies that have been developed based on this knowledge, the road towards complete comprehension of this terrible disease is still long.

Our acquisitions have brought to the development of protocols and specific drugs to fight against this neoplasm that is still today the most deadly brain tumor; but this isn't enough.

We need more knowledge, more research and many researchers because, as it appears clearly from this review, it is only with basic research on molecular biology that we could, in a future that we hope is not too far, shed new light on GBM and achieve at last a good survival and, why not, complete healing from this terrible but still undiscovered disease.

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