Clinical Neurology and Neurosurgery 170 (2018) 120–126

Contents lists available at ScienceDirect

Clinical Neurology and Neurosurgery

journal homepage: www.elsevier.com/locate/clineuro

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

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ARTICLE INFO

Keywords:
Glioblastoma
MGMT
IDH1
IDH2
TERT
BRAF
High grade gliomas
Molecular biology

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 Tumors, 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 patients' 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.

1. Introduction

Glioblastoma (GBM) is the most common glioma in adults, with incidence increasing by 3% per year [1]. According to the World Health Organization Classification of Central Nervous System Tumours, GBM is considered a grade IV tumor due to its malignant behavior [2].

Historically, radiotherapy (RT) alone following surgery resulted in 3- and 5-year survival rates of 4.4 and 1.9%, respectively [3]. 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) [4]. Moreover, adjuvant temozolomide (TMZ) therapy, significantly increased the long-term survivors [3].

The past years have seen remarkable advances in GBM research, especially regarding tumor biology, but this has failed to allow significant improvements of 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 [1,3,4].

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 [2]. This novel classification paradigm of diffuse gliomas allows to identify patients with significantly different outcomes, paving the way to more tailored

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https://doi.org/10.1016/j.clineuro.2018.05.015
Received 20 March 2018; Received in revised form 4 May 2018; Accepted 13 May 2018

treatments. However, this represents just a biginning 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 patients' 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.

2. 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".

3. Results

3.1. 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 [4] 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 150–200 mg/m^2. The addition of TMZ resulted in a 3-year OS of 16% and 5-year OS of 9.8% [3]. 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 [5]. 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 [6].

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 [7]. Repetitive futile rounds of mismatch repair are thought to result in a state of chronic strand breaks, which triggers an apoptotic response [8].

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 [9,10], 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 [10].

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.

3.2. 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 [11,12]. 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 [12–14]. 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 [13,15].

EGFR amplification has no prognostic impact on OS when considered alone [11,13]. 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 [16].

Up to now, different molecules have been developed to target EGFR signaling pathway. 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 TKIs 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 [17].

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) [18,19].

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 [20].

3.3. IDH1/IDH2 mutation and 1p19q codeletion

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 [8,21,22], but also in a gain of enzyme function of the NADPH-dependent reduction of ketoglutarate to 2-hydroxyglutarate, which accumulates in IDH1 mutated cells [23]. IDH1 mutation rate is highly variable among glioma