Biology of Glioblastoma Multiforme

Subjects: Oncology

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Glioblastoma multiforme (GBM) represents a highly aggressive malignancy of the brain which leads to early patient lethality. Malignant GBM cells develop from glia and gradually acquire specific mutations and epigenetic changes associated with several disctinct phenotypic features such as differently expressed and localized cytoskeletal components (in particular microtubules) and deregulated cell cycle via defunct checkpoints. While the use of traditional microtubular targeting agents (eg. taxanes) in treatment of GBM is limited due to several reasons, newly repurpused compounds such as benzimidazole carbamates may offer a new perspective by inducing mitotic catastrophe in GBM. Mitotic catastrophe is nowadays viewed as a way of elimination of genomically unstable cells via diverse cellular endpoint phenotypes and its exploration in potential treatment of GBM is the subject of this entry.

glioblastoma multiforme | mitotic catastrophe | cell death | microtubule-targeting agents | benzimidazole carbamates

1. Introduction

Malignant tumors of the central nervous system (CNS) comprise both cases arising mostly in the brain and to a minor extent in other parts of the CNS as well as metastatic malignancies originating from other tissues and/or anatomical parts in the body. In the former group of conditions, the most frequently occurring are malignant gliomas (accounting for up to 80% of adult brain tumors), which are traditionally categorized according to their cellular origin, histopathological features and clinical manifestation. Using these criteria, World Health Organization classifies gliomas into four groups-grades, with each of them reflecting the level of malignant phenotypes associated with glioma cells. Typically, grade I gliomas are largely viewed as benign with relatively good patient prognosis if it is possible to remove the tumor mass surgically, while higher-grade gliomas show increasingly pathological features and behavior, resulting in their diffuse spread throughout the brain, resistance to therapy and incurred damage to the brain tissues, leading to ultimate and rather fast patient lethality [1]. The most malignant and aggressive type of glioma, i.e., grade IV glioma, is termed glioblastoma multiforme (GBM), which represents approximately 60% of all brain tumors in adults. Despite the fact that GBM global incidence is considered statistically low (5–10 cases per 100,000 people), its biological and genetic heterogeneity combined with exceptional aggressiveness and very ineffective available therapies result in poor prognosis for patients whose survival rate even upon the best clinical management rarely exceeds 15 months following the initial diagnosis [2].

Given the lack of efficient cytostatics or modern molecular target-specific compounds in the treatment of GBM owing to their limited access via hematoencephalic barrier and/or due to the intrinsic or acquired resistance of malignant astrocytes, drugs inducing mitotic catastrophe might offer a new, efficient alternative to the existing clinical management of this at present incurable malignancy.

2.Data, Model, Applications and Influences

2.1. Molecular Classification of GBM

Technological advancements in molecular diagnostics and, in particular, use of gene expression profiling, have been instrumental in our understanding of GBM diversity, leading to the identification of its four major subtypes (i.e., proneural, neural, classical, and mesenchymal) [3]. One characteristic molecular difference between primary and secondary GBMs is the mutational state of isocitrate dehydrogenase (IDH) genes, with IDH wild-type being present most frequently in the primary GBM, whereas IDH mutant type associates more commonly with the secondary GBM [1]. Both GBM types next harbor several typical genetic alterations in key genes regulating growth factors, cell cycle regulators, DNA repair, survival and cell migration, with corresponding associated upregulated or downregulated signaling pathways [4][5]. In addition, a number of less explored genetic changes such as copy number alterations in other genes on the corresponding chromosomes have been identified in individual GBM types alongside differences in DNA methylation [6], histone acetylation and expression of non-coding RNAs [7]. Such evidence increasingly contributes to the more specific typing of individuals diagnosed GBMs and enables detailed appreciation of often less robust molecular signatures hitherto not acknowledged, not only in newly diagnosed cases but also in recurrent tumors. Accordingly, it may be expected that new types of GBMs will be identified in the future based on unique molecular changes as proposed recently [4][5].

2.2. Cytoskeleton of Astrocytes and Malignant GBM Cells

Astrocytes originate from radial glial cells and through a series of steps they mature and migrate to the designated position in the brain [8]. There they begin to assume their final spongy stellate morphology, which involves, among other things, extensive changes in their cytoskeleton. These include dense packing of microtubules (MTs) and their accumulation in the main cellular processes and remodeling of contractile actin fibers in favor of Arp2/3-dependent branched actin arrays [9] with associated shifts in corresponding regulatory signaling pathways; i.e. inhibition of Rock-RhoA axis and activation of Rac1 [10]. Similar to MTs, intermediate filaments (IFs) localize mostly into astrocytic processes of mature cells but unlike MTs and microfilaments (MFs) they show differential expression at different stages of development. Thus non-mature astrocytes are positive for vimentin and synemin while mature astrocytes express glial fibrillary acidic protein (GFAP) and vimentin [11].

In GBM cells the reported changes entail all cytoskeletal elements and their regulation. Still, at present the dynamics of these changes is not thoroughly mapped since they firstly occur in a cell-autonomous manner but at later stages they are no doubt significantly influenced by tumor microenvironment, in particular hypoxia [12]. Thus

currently cytoskeletal alterations in malignant glioma cells are reported individually and do not faithfully recapitulate the entire progress of GBM development nor distinguish between heterogeneous cell clones present in this tumor.

2.3. Cell Cycle

The ability of eukaryotic cells to reproduce by the process of cell division relates to a series of events which are known under the collective term cell cycle. The main purpose of cell cycle is to ensure accurate DNA replication (S phase) and final generation of two identical daughter cells (M phase). Together, there are three major control points or checkpoints recognized in eukaryotic cells; the first occurring near the end of G_1 phase, the second at the G_2/M phase transition, and the third (also called spindle assembly checkpoint - SAC) placed at the metaphase-to-anaphase transition.

Malignant GBM cells have been reported to harbor multiple genetic abnormalities leading to deregulation of cell cycle via defunct checkpoints. Specifically, in about 50 % of GBM cases, p16 was reported to be deleted or, alternatively, silenced by methylation [13]. Similarly, the expression of Rb protein may frequently be absent in GBM cells too [14].

Mutations and other changes in protein p53 and its dependent pathways as part of both G_1 and G_2/M checkpoint signaling have also been detected in GBM although their frequency and importance for development and maintenance of malignant phenotype differ in relation to the GBM type [15][16].

Various chromosomal aberrations seen in advanced malignancies including GBM suggest their likely origin from aberrant mitoses [17]. This in turn points at possible presence of defects in the SAC signaling and activity.

2.4. Checkpoint Inhibition

GBM cells show aberrant cycling and increased proliferation, which is associated with deregulated checkpoints as outlined above. Accordingly, these behaviors started to be exploited as a therapeutic target once the basic principles of chemo and radiotherapy were established [18]. Thus until today, the specific treatments in this field aim to interfere with (1) cellular components and events linked with cell cycle and cell division such as DNA integrity and replication, mitotic spindle activity and contractile ring formation and function or target (2) individual molecules regulating cell cycle progress and cell division as reviewed in [19]. The mechanism of action of many traditional (i.e. MTAs) as well as newer targeted agents (i.e. CDK, aurora kinase or polo-like kinase inhibitors) involves G_2/M inhibition [19]. This approach presents several advantages. Firstly, despite ongoing discovery of new classes of antineoplastics, many traditional compounds (i.e. MTAs) continue to be standards in curative and palliative oncological care [20]. Secondly, these agents may synergize with the current standards in GBM therapy, i.e. temozolomide or radiation, and enhance their DNA damaging effects or sensitize malignant cells to them [21][22]. Thirdly, since many of these compounds interfere with mitosis, they may enhance instability of mitosis emerging cells to ultimately bring their demise via the process of mitotic catastrophe.

2.5. Mitotic Catastrophe

Mitotic catastrophe (MC) represents a sequence of events which acts to prevent genomic instability of cells via inducing mitosis-linked delayed cell death or permanent cell cycle arrest with subsequent senescence. As such under physiological circumstances MC functions as one of oncosuppressive mechanisms, which has recently gained the considerable interest among biomedical scientists due to its potential to eliminate potential or nascent tumor cells. At least three scenarios of MC have been described [23] in which (1) the cell might activate cell death machinery in the presence of elevated cyclin B1 levels, i.e. while it is still in mitosis or (2) the cell is firstly allowed to complete mitosis and in the subsequent interphase may undergo cell death, in a delayed manner. This particular instance is referred to as mitotic slippage or mitotic checkpoint adaptation. And, finally, (3) the cell is firstly allowed to complete mitosis and in the subsequent interphase develops the senescent phenotype [24].

Diverse factors may trigger MC including DNA damage, checkpoint inhibition, general stress (i.e. hyperthermia) as well as mitosis-addressing agents (i.e. MTAs or small molecule inhibitors) [25]. MTAs induce MC by their interference with mitotic spindle, which leads to perturbations in spindle assembly checkpoint (SAC), incorrect segregation of chromosomes and activation of the corresponding signaling. This signaling may include the activation of protein p53 and its dependent circuits, Bcl-2 family proteins and various execution substrates (i.e. caspases) whose individual wiring determines the cellular endpoints [26].

MC and its role in suppression of GBM cells, in particular in the context of MTAs has not been intensively researched so far. In human GBM cells U87-MG (U87), D54, H80, H247, H392, H397, H502, H566, and the mouse GL261 glioma cell line, mebendazole demonstrated cytotoxicity, with low IC_{50} values. Mebendazole reduced microtubule polymerization in exposed GBM cells and significantly extended mean survival in syngeneic and xenograft orthotopic mouse glioma models [27]. Based on these results, a clinical trial with the aim of finding the highest dose of mebendazole that can be safely given to people with high grade glioma in combination with the current standard of care (temozolomide) without causing severe side effects was started in April 2013 with the nowadays set primary completion in September 2016 and estimated study completion in September 2025. In this intervention single group study, mebendazole will be given to patients three times every day orally with meals on a 28 day cycle. Apart its primary objective; i.e. to determine the maximum tolerated dose of mebendazole in combination with temozolomide (TMZ) given after surgery and the standard radiation and TMZ treatment in patients with newly diagnosed malignant gliomas, the overall patients survival (10 years frame) will be measured (https://clinicaltrials.gov/ct2/show/NCT01729260). Another member of benzimidazole family, flubendazole, has been found effective against two human glioma cell lines SF-268 and T-98G in which it induced G_2/M cell cycle arrest, upregulated p53 expression and reduced cyclin B1 and p-cdc2 expression. This activity led to cell apoptosis via downregulation of Bcl-2 expression. Flubendazole also successfully suppressed the growth of glioma xenograft models in mouse [28].

3. Conclution

Here we provided ample evidence on the complexity of GBM origin, development, and behavior, which do reflect the complicated terrain where we aspire to interfere. Conversely, a number of unique features of GBM cells, namely the extent and specificity of cytoskeletal (microtubular) reprogramming, offer an attractive target of possible

intervention. MC is nowadays viewed as a way of elimination of genomically unstable cells via diverse cellular endpoint phenotypes and as such represents an attractive platform for the development of novel antineoplastic agents. In addition, MC in target cells may be induced with considerably lower concentrations of employed agents, which is very beneficial due to the reduction of side-effects-related toxicity. Finally, MC may be successfully employed as an additional effect of combined therapies, which would maximize the clinical efficiency upon minimized toxicities or off-target effects. Their future potential and application in treatment protocols will most likely be in chemotherapy or radiotherapy sensitization.

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