Translational Medicine
ISSN: 2161-1025

Triggiani et al. Transl Med (Sunnyvale) 2016, 6:1
DOI: 10.4172/2161-1025.1000164

Mini Review Open Access

Radio-Resistance and Cancer Stem Cells: The Glioblastoma Model

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Abstract

Glioblastoma Multiforme (GBM) is the most common and aggressive primary malignancy of the central nervous system (CNS). The best currently available treatment is radical surgical excision followed by association of radiotherapy and chemotherapy with Temozolomide (TMZ). Even with this aggressive treatment, almost all the patients have replace of disease, and this recurrence remains predominately local. The Cancer Stem Cells (CSC) hypothesis assumes the existence of a hierarchic tumour model with just few CSCs responsible both of the tumour growth and of the tumour resistance to radio and chemotherapy. We therefore reviewed some aspects of the complex GBM biology; these factors could potentially, in the future, impact on the design of new translational studies.

Keywords: Glioblastoma; Radiotherapy; Adioresistance; Cancer stem cells

Introduction

Glioblastoma Multiforme (GBM, Astrocytoma Grade IV according to the World Health Organization (WHO) criteria) is the most common and aggressive primary malignancy of the central nervous system (CNS) [1]. Although GBM can occur at any age, adults are more often affected (age range 45–75years); there is a slight male predominance and incidence is 3-4 new cases per year per 100,000 [2]. It usually involves cerebral hemispheres and less frequently in the rest of the CNS. The clinical onset depends on the localization of the tumour and usually includes symptoms of increased intracranial pressure (headache, nausea, vomiting) and symptoms of neuronal irritation (epileptic seizures) [3]. The best currently available treatment is radical surgical excision followed by association of radiotherapy and chemotherapy with Temozolomide (TMZ) [4]. Unfortunately, the prognosis of this tumour remains extremely poor and second-line therapies are still inefficient. The median survival of GMB patients is in the range of 9-12 months, with a 2-year survival rate of only 8%-12% [5].

High grade Gliomas represent the main challenge for the neuro-oncology community for several different reasons: firstly because tumour cells show a particularly aggressive behaviour toward the normal brain tissue, making them an extremely complex target for any kind of therapy. Furthermore, because GBM grows inside an isolated human compartment, separated from the bloodstream by the interposition of the systemic blood-brain barrier, that represent an obstacle to the diffusion of drugs into the brain for the pharmacological point of view.

Despite many successes in different areas of Oncology, prognosis of GBM did not change over the past 30 years, except for a small benefit achieved with the Stupp's protocol [6]. The reason of these failures could be attributed to the lack of knowledge of many different biological aspects of the tumour. Therefore, it is crucial to concentrate the efforts towards a more precise biologic classification of this disease, because these aspects could have an important impact on the ongoing search for new therapies. The emerging idea is that we should try to understand why not all patients with GBM have the same outcome if treated with the same therapeutic protocol. Undoubtedly, even if nowadays we are not able to distinguish them very well, we are treating a number of different pathological and genetic conditions with the same therapies. The Cancer Genome Atlas Network (TCGA) recently identified some genomic abnormalities of GBM. Researchers classified GBM into distinct molecular subgroups by the characterization of a specific gene expression profile and a different set of mutant genes [7]. The different nature of each subtype (classical, proneuronal, neuronal and mesenchymal) could explain both the differences in clinical presentation and the response to standard treatment.

We therefore reviewed some aspects of the complex GBM biology; these factors could potentially, in the future, impact on the design of new translational studies.

Different radiotherapeutic strategies and the request for new biologic targets

After primary resection, the combination of RT plus TMZ is actually the most effective adjuvant therapy for GMB and should be considered in all patients [7]. In particular, the current standard of care for RT in GBM consists in external-beam radiation (EBRT) to a dose of 60 Gy in 2 Gy fractions, given in 6 weeks. The dose is delivered to the surgical cavity with a 2-cm margin at surrounding brain tissue for the clinical target volume (CTV) [8].

Radiotherapy remains a milestone of the treatment of GBM. The studies of the 70s, which firstly demonstrated the efficacy of radiotherapy, employed post-surgery whole brain irradiation by means two parallel opposite fields to a dose of 45-60 Gy in 25-30 fraction [9]. The clinical results, although better than surgery alone, were unsatisfactory both in terms of survival and toxicity (even according to the "old" Emami paper, radio-induced cerebral necrosis TD5/5 for whole brain irradiation is 40 Gy while the TD50/5 is 60 Gy) [10]. Toxicity, in addition to necrosis (not always symptomatic), could also became manifest as asymptomatic leukoencephalopathy, vestibular and auditory damage, visual disorders, pituitary function deficiency and possibly cognitive deficits. The majority of patients treated with whole brain radiotherapy showed a relapse mostly in the same site or close to

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Received November 25, 2015; **Accepted** December 10, 2015; **Published** January 21, 2016

Citation: Triggiani L, Pasinetti N, Pedretti S, Maddalo M, Borghetti P, et al. (2016) Radio-Resistance and Cancer Stem Cells: The Glioblastoma Model. Transl Med 6: 164. doi:10.4172/2161-1025.1000164

it [11]. This empirical observation caused the reduction of the target volume in order to irradiate only the site of disease with margin (site irradiation SI). Although it has never been performed a randomized comparison between whole brain radiotherapy and SI, the introduction of treatment volume reduction did not cause the increase of the relapse rate, and became the standard treatment [12].

Besides reducing target volume, radiation oncologists have tried to improve outcome through different strategies: introduction of different fractionation schedules, innovative techniques with dose escalation, brachytherapy, adrotherapy, functional imaging methods for the detection of different target volumes and, last but not least, the association with different chemotherapeutic agents and targeted therapy. With the exception of concomitant use of TMZ with radiotherapy, none of these strategies has been successful [13]. The literature about the treatment of GBM has been characterized by promising Phase II trials never confirmed in the subsequent Phase III trials. The results coming from the laboratory tests show a high radioresistence of this tumor. The survival curve of GBM cell lines treated with radiotherapy is not linear and is characterized by a large shoulder and low α / β ratio [14]. Theoretically, a larger dose per fraction should therefore be more effective in obtaining a better local control of disease. Moreover, the use of hypo-fractionated RT reduces the overall treatment time. On the other hand, hypofractionated radiotherapy (dose per fraction higher than 2 Gy), has been proved not only to be inefficient [15] but also to increase the risk of brain late toxicity [10]. Some clinical trials used also hyper-fractionated schedules. GBM cells, in fact, are relatively rapid proliferating cells and an increased number of daily fractions would raise the chance of radiating them during a more sensitive cell cycle phase [16]. Furthermore, GBM is a very hypoxic tumor [17]: as small dose per fraction induce cell kill independently by oxygen, these could be advantageously used in hypoxic areas. Unfortunately, in most clinical trials even the "low dose per fraction" strategies did not obtain a statistical significant better overall survival [18]. The modern approach to the treatment of GBM is the association between different new drugs and radiotherapy. The epidermal growth factor receptor (EGFR) is considered one of the most attractive therapeutic targets for GBM. The gene encoding EGFR is amplified in approximately 40% of GBMs, especially in the classical subtype [19]. The EGFR kinase inhibitors gefitinib and erlotinib demonstrated minimal activity for patients with recurrent malignant gliomas [20,21]. A Radiation Therapy Oncology Group (RTOG) phase I/II trial, enrolled 147 patients with newly diagnosed GBM, investigated the combination of gefitinib and radiotherapy and obtained average survival comparable to patients received radiotherapy alone [22].

The majority of the drugs experimented in clinical trials interfere with the vascular epidermal growth factor (VEGF) pathway by directly blocking the receptor or by the use of monoclonal antibody against VGEF (Bevacizumab). GBM blood vessels are structurally and functionally abnormal, creating an adverse microenvironment characterized by low oxygen tension and high interstitial fluid pressure. This microenvironment promotes radioresistance and impairs delivery of chemotherapeutic agents [23]. In animal models, VEGF inhibitors lead to vascular "normalization" by passively removing immature and leaky tumour vessels and by actively remodelling the remaining ones and inducing the resemble of normal vasculature [24]. This promising experimental effect was not confirmed in clinical trials: the addiction of Bevacizumab to radiotherapy and TMZ in prospective clinical trials did not improved survival [25]. Cilengitide, a novel small molecule that selectively blocks activation of the αvβ3 and αvβ5 integrins, has been studied in GBM [26]. Integrins are a family of trans-membrane glycoprotein receptors for cell adhesion molecules that mediate cell-matrix and cell-cell interactions [27]. In GBM, the αvβ3 and αvβ5 integrins are widely overexpressed both in malignant cells and in tumour vasculature and, in addition to VEGF, they are key mediators of angiogenesis and tumour growth [28]. Unfortunately, the results of a phase III trial showed that the addition of Cilengitide to standard adjuvant treatment does not improve progression-free survival and overall survival if compared to RT and TMZ [29]. The results of all these clinical trials underline the high radio-chemioresistence of GBM cells and suggest the hypothesis that particular cell should be the responsible of local recurrence.

Cancer stem cells theory

In consideration of the unsatisfactory clinical results of the previous research lines, of the evident intrinsic radio-resistance and of the already proved interaction between vessels and cell matrix in the post-temozolomide era researchers focused their attention on stem cells theory.

In primary brain tumours, especially in GBM, a cells hierarchy has been proved [30]. In particular, part of the tumour is composed by cells having the same characteristics of neural stem cells called "cancer stem cells (CSCs)".

CSCs share some of their features with those of normal neural progenitors, including the expression of neural stem cells markers, the ability to self-renewal (the ensemble of the process by which stem cells divide themselves in other stem cells – asymmetric division), multi-potency (the ability to differentiate themselves into cells with specific function: in CNS neurons, astrocytes and oligodendrocytes) and long term proliferation. Moreover, CSCs are tumorigenic; they are the unique cancer cells able to initiate and reproduce the original malignancy if xenotransplanted in animal models and for this reason, they are also called tumor-initiating cells (TICs) [31].

The CSC hypothesis assumes the existence of a hierarchic tumour model with just few CSCs responsible both of the tumour growth and of the tumour resistance to radio and chemotherapy [32]. Therefore, this model would explain how a very little number of GBM CSCs escaped to surgery and radio-chemotherapy, could lead to a local relapse of disease.

Cancer stem cells in radiobiology

If the inactivation of the whole population of CSCs is obtained with radiotherapy, permanent local tumour control is achieved. The recognition of these cells could lead to the creation of therapies targeted to them and could solve the problem of the high rate of local relapses. Unfortunately, the identification and isolation of brain CSCs remains difficult and the expression of a single marker cannot be considered as a specific principle for defining CSCs. The neurosphere assay [33] is a valid tool for identification and isolation CSCs. In fact, neurospheres have all the characteristics of CSCs: self-renewal ability, the potential to differentiate into a variety of mature elements including neuronal, astrocytes, and oligodendroglial cells if dissociated in single cell suspension and the ability to generate tumours when transplanted in *in-vivo* models.

The mechanisms underlying CSCs radio-resistance remains unknown. Some laboratory evidences indicate that CSCs are more radio-resistant than non-CSCs. The prevailing hypothesis is that CSCs are more resistant to radiation-induced apoptosis *via* the activation of DNA damage repair mechanisms. Compared to non-stem tumour cells, CSCs recover more rapidly and are able to repair more efficiently DNA damage. This is likely due to the activation of several DNA damage

checkpoint proteins [32].

Nevertheless, this finding is still under investigations and the results are contradictory. A large number of in vitro investigations failed to demonstrate differences of radio-sensitivity of putative CSC marker-positive and marker-negative cells. Some researchers recently have distinguished CSCs using different stem cell markers and have showed that these cells are not more resistant, but can rather be defined a more sensitive group of glioma cells. Only CD44 protein expression, in this study, correlated positively with radioresistance. Therefore, it seems that high levels of stem cells markers do not correlate with resistance to radiotherapy [34].

In several studies, the tumour microenvironment has been shown to play an important role in shaping the features of radioresistance in GMB [35,36]. CSCs are positioned in distinct microenvironments within the tumour, similar to stem cell niches described for neural stem cell. Specifically, CSCs should be are located within vascular and perinecrotic niches and so they are in a region characterized by a peculiar radioresistence [35, 37]. Moreover, CSCs are should be able to protect "their niche" producing several soluble factor [38]. It is commonly accepted that tumour associated parenchymal cells (microglia, vascular cells and peripheral immune cells) directly interact with GBM cells and play a crucial role in the natural history of this disease.

Conclusive remarks

Treatment of GBM remains one of the most challenging fields in clinical oncology. This is substantially due to a lack of knowledge on the biological drivers of the natural history of this malignancy. In fact, even with surgical resection and aggressive treatment (with chemo- and radiotherapy), the prognosis remains very poor. However, due to relentless efforts in basic research, the increasing understanding of the complex biology of GBM and of its pathogenesis produced a variety of novel therapeutic approaches, under study. Investigators around the world are searching for molecular and biologic features of GBM relevant to therapy improvements. To date it is not yet clear how to best incorporate these molecular data into the treatment decision tree for the individual patient.

Finally, the identification of CSCs and of their possible therapeutic implications in GBM generated a paradigm shift in neuro-oncology research. When the molecular and genetic abnormalities of CSCs will be fully elucidated new targeted weapons against CSC-specific targets would probably offer a substantial improvement in the chances of cure for these patients.

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