REVIEW ARTICLE

Glioblastoma cancer stem cells: heterogeneity, microenvironment and related therapeutic strategies

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Glioblastoma Multiforme (GBM) is an incurable malignancy. GBM patients have a short life expectancy despite aggressive therapeutic approaches based on surgical resection followed by adjuvant radiotherapy and concomitant chemotherapy. Glioblastoma growth is characterized by a high motility of tumour cells, their resistance to both chemo/radio-therapy, apoptosis inhibition leading to failure of conventional therapy. Cancer Stem Cells (CSCs), identified in GBM as well as in many other cancer types, express the membrane antigen prominin-1 (namely CD133). These cells and normal Neural Stem Cells (NSC) share surface markers and properties, i.e. are able to self-renew and differentiate into multiple cell types. Stem cell self-renewal depends on microenvironmental cues, including Extracellular Matrix (ECM) composition and cell types. Therefore, the role of microenvironment needs to be evaluated to clarify its importance in tumour initiation and progression through CSCs. The specific microenvironment of CSCs was found to mimic in part the vascular niche of normal stem cells. The targeting of GMB CSCs may represent a powerful treatment approach. Lastly, in GBM patients cancer-initiating cells contribute to the profound immune suppression that in turn correlated with CSCs STAT3 (CD133 +). Further studies of microenvironment are needed to better understand the origin of GMB/GBM CSCs and its immunosuppressive properties. Copyright © 2010 John Wiley & Sons, Ltd.

KEY WORDS — stem cells, glioblastoma, cancer stem cells (CSCs), glioma heterogeneity, CSCs niche, microenvironment

INTRODUCTION

Malignant gliomas are highly lethal brain tumours. Recently the World Health Organization (WHO) offered a novel classification of gliomas in four grades according to their degree of malignancy and morphological features.[1] Low Grade (I and II) gliomas cells are well differentiated; they bear histological similarity to astrocyte and oligodendrocyte. High Grade (III and IV) Glioma (HGG) cells are more anaplastic, resembling immature astrocytes, or oligodendrocytes, or a mixture of both.[1] HGGs include Glioblastoma Multiforme (GBM) and Anaplastic Astrocytoma (AA); both are highly invasive and display high chemoresistance leading to tumour recurrence post surgery.[2] The prognosis for patients with GBM remains dismal, as median survival duration after diagnosis varies from 6 month to 2 years. This is largely due to the inability of current treatment strategies to address the highly invasive nature of this disease.[2]

Most cancers comprise a heterogeneous population of cells with different proliferative potential as well as the ability to reconstitute the tumour after their transplantation in immunodeficient mice.[3] Recently, there is increasing evidence that tumour bulk mass contain a population of cells with stem-like characteristics so called Cancer Stem Cells (CSC), that give rise to a diverse mixture of more differentiated tumour cells.[4] CSCs are multipotent, have the property of self-renewal and are believed to be cancer initiating cells that are responsible for tumour maintenance, recurrence and therapy resistance.[5,6] Tumours recently described as having CSC populations include cancers of the blood,[7] breast,[8] brain,[9] pancreas,[10] neck,[11] prostate[12] and colon.[13,14]

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Following the identification of tumour stem cells in leukaemias and breast cancers,[8,15] CSCs were identificated and isolated in glioblastoma surgical.[10,16] These cells were found to express membrane surface markers typical of normal Neural Stem Cells (NSC), such as CD133 and nestin, and to give origin to spheres similar to neurospheres. CSC were self-renewing and proliferating *in vitro*, and could be induced to differentiate into neuronal, astroglial or oligodendroglial cells.[17–19] CSCs implanted into the brain of immunodeficient animals were shown capable to generate a new tumour, whereas CD133 negative GBM cells failed to do so.[17]

Similarly to the normal stem cells, CSCs were demonstrated to depend on local microenvironment or niches[20–23]; these ones are formed by cells and Extracellular Matrix (ECM). Cells provide a complex milieu that supports and directs the specific functions of the organ. In cancer, subversion of normal microenvironment might provide a starting point for the development of cancer. In glioblastomas, the niche comprises surrounding vasculature, that provides direct cell contacts and secretes factors that maintain stem cells in a quiescent state thereby regulating self-renewal and multipotency.[24–26] CSCs and the microenvironment are integral parts of the tumour; therefore analysis of their role may be as important as microarray or proteomic investigation of transformed glial or neuronal cells within brain tumour.

Glioblastoma cells cultured under the same culture conditions showed a variety of different growth characteristics and molecular profiles.[27] According to gene expression profiling studies at least two subtypes of glioblastomas can be distinguished: one subtype in which prevailed genes associated with neural development, and a second subtype with gene expression pattern associated with the ECM.[27–29] Thus, these stem cell subsets are not entirely defined by the cells expressing NSC markers and contain different cell populations.

The concept of tumour stem cells and their niche may lead to novel understanding of tumour biology, and design of novel treatments targeted towards these cells. It is therefore important to characterize the various populations that contribute to tumour formation.

GLIOBLASTOMA MULTIFORME

Malignant astrocytic gliomas such as glioblastomas are the most common and lethal intracranial tumours. These cancers exhibit a relented malignant progression characterized by widespread invasion throughout the brain, resistance to traditional and newer targeted therapeutic approaches, destruction of normal brain tissue and death.[30] Recently, gliomas were classified and subtyped on the basis of histopathological features and clinical presentation. Grade I tumours are biologically benign and can be cured if they can be surgically resected; grade II tumours are low-grade malignancies that may undergo long lasting clinical courses, never the less early diffuse infiltration of the surrounding brain renders them incurable by surgery; grade III tumours

exhibit increased anaplasy and proliferation over grade II tumours and are more rapidly fatal; grade IV tumours exhibit more aggressive features of malignancy, including vascular proliferation and necrosis. These grade IV tumours are recalcitrant to both radio- and/chemotherapy, thereby they are usually lethal within 12 months. GMB (grade IV) is the most common and biologically aggressive; its hallmark features are uncontrolled cellular proliferation, diffuse infiltration, propensity for necrosis, robust angiogenesis, intense resistance to apoptosis and genomic instability. GBM invades the surrounding parenchyma and destroys functional architecture of the brain, eventually superseding compensatory mechanisms to give rise to the clinical consequences like seizures, nausea, headaches, imbalance and hemiparesis.[23,31] On the basis of clinical presentation, GBMs have been further subdivided into primary or secondary GBM subtypes. Primary GBMs account for a great majority of cases in older patients, while secondary GBMs are quite rare and tend to occur in patients below the age of 45 years. Primary GBMs present in an acute *de novo* manner with no evidence of prior symptoms or antecedent lower grade pathology. In contrast, secondary GBMs derive consistently from the progressive transformation of lower grade astrocytomas, with 70% of grade II gliomas transforming into grade III/IV disease within 5–10 years of diagnosis. Remarkably, despite their distinct clinical histories, primary and secondary GBMs are morphologically and clinically indistinguishable as reflected by an equally poor prognosis when adjusted for patient age. However, although these GBM subtypes achieve a common phenotypic endpoint, recent genomic profiles have revealed strikingly different transcriptional patterns and recurrent DNA copy number aberrations between primary and secondary GBM as well as new disease subclasses within each category.[10,28,32]

Immunohistochemical markers have also been shown to aid in predicting the clinical course for certain classes of tumours. GBMs with intact expression of the PTEN (Phosphatase and Tensin homologue deleted on chromosome 10) and EGFRvIII proteins (detailed in next section) correlated with increased Epidermal Growth Factor Receptor (EGFR) inhibitor response and progression-free survival compared with those tumours expressing EGFRvIII but lacking PTEN.[33] Also, patients with EGFR protein expression, mutant or wild-type, have been identified for the sake of targeting EGFR therapy to the appropriate patient population. Furthermore, combined loss of the short arm of chromosome 1 and the long arm of chromosome 19 is an analytical tool already widely used in the management of oligodendroglial gliomas, but its role in the evaluation of astrocytic gliomas such as GBM is not yet well defined.[1,34]

Malignant cells often disseminate throughout the brain, making them exceedingly difficult to target thus reaching all intracranial neoplastic foci. As a consequence, tumour recurrence is inevitable despite aggressive surgery and adjuvant radiotherapy and/or chemotherapy. Gliomas typically consist of morphologically diverse cells expressing a wide variety of differentiated and undifferentiated

markers. Heterogeneity of glial tumours and their tendency towards fast malignant progression are coupled with the ability of glioma cells to migrate away from a tumour mass into normal brain tissue where they generate multiple new foci and recurrent growth.[35] It is widely held belief that tumour behaviour could be predicted from its cellular composition. However, until now study of the basic morphology and phenotype of brain tumours has only yielded a limited amount of knowledge of clinical behaviour of the tumours. It is therefore important to characterize tumour-initiating subpopulations to identify biological markers able to predict individual prognosis, and to develop specifically directed therapies.

Initially it was considered that glioblastoma arose from astrocytic precursors and was genetically characterized by amplification of EGFR and expression of glial fibrillary acidic protein (GFAP).[36] GFAP is highly specific for cells with astrocytic differentiation and is widely used as a reliable marker in the immunohistochemical diagnosis and differentiation of brain tumours including glioblastoma.[1]

Recent evidence have suggested that tumour organization could be described similarly to the hierarchy of stem cells and various progenitor cells that are locally restricted to the stem cell niche.[37] Demonstrations that the adult human forebrain contains an abundant source of NSCs[38] and that human GBMs contain tumourigenic neural stem-like cells[17,35,39] indicate that neural stem and/or progenitor cells are a plausible origin for human gliomas and have given rise to speculations that more effective therapies will result from targeting stem cell-like component of GBMs.[31,40]

CANCER STEM CELLS

CSCs have been defined in analogy to normal stem cells, as cells that have the capacity to self-renew giving rise to another malignant stem cell as well as to undergo differentiation to give rise to the variety of non-tumourigenic cells found in the tumour. The main property of the CSCs is anyway the ability to reconstitute the original tumour upon the transplantation in immuno-compromised mice.[41] The cell of origin for CSC still remains unclear; they may derive from normal tissue-resident stem cells. Likewise, they may arise from mature cells that acquired ability to self-renew as a result of oncogenic mutations.[4,8] However, normal stem cells are attractive candidates for the cells of origin of tumours, because these cells are long-lived and have primed self-renewal ability, allowing the oncogene to initiate uncontrolled proliferation more easily.[18] The identification of the cell of origin for tumours will permit to understand how the molecular alterations lead to the cancer, and how we can target those alterations for treatment, or prevent them from occurring.

Evidence of existence of CSCs initially arose from studies of Acute Myelogenous Leukaemia (AML), among which a subset of leukaemic cells (Leukaemic Stem Cells, LSCs) was identified to give rise to AML in immunodefficient mice, and which displayed a similar cell surface phenotype to normal Hematopoietic Stem Cells (HSCs).[7,15,42] LSCs isolated by Fluorescence-Activated Cell Sorting (FACS) from human AML were able to initiate leukaemia in transplanted mice.[7] Evaluating CD34+ AMLs it was found that CD34 + CD38- fraction was highly enriched for leukaemia-initiating activity in transplanted recipients, while both CD34 + CD38 + and CD34-fractions did not initiate leukaemia.

Al-Hajj et al.[43] were the first to identify and isolate stem cell population from solid tumour in a breast cancer. Stem-like cells were isolated on the basis of their cell surface phenotype, which was CD44+ CD24-/low and able to initiate the tumour in vivo, whereas the remaining cell population from these tumours were not.

Similarly, CSC population was also identified in brain tumours (GBMs and medulloblastoma)[35,44] surgical specimens and it was shown to contain clonogenic cells that form neurosphere-like aggregates. Subsequently, it was demonstrated that GBM CSCs could be detected by the expression of normal NSCs markers: CD133 and nestin.[9,17,38] Singh et al.[39] reported that few CD133+ cells from human brain tumour could initiate new tumours in the brains of immunodeficient mice, while CD133- cells could not have a tumour-initiating activity. Like the NSCs, these cells form neurospheres cultured in serum-free medium supplemented with EGF and FGF and could be induced to differentiate into all neuronal lineages expressing the markers of mature neurons, astrocytes and oligodendrocytes.[9,16,35,39,45] Thus, CSCs share many properties of normal stem cells. Both tumourigenic and normal stem cells have extensive proliferating potential and give rise to the heterogeneous population of more differentiated cell types. However, if in normal tissues the pathways of self-renewal and differentiation are tightly controlled, in tumours, probably due to continuing mutagenesis, stem cells display aberrant growth and differentiation capacity.[46] Thus, it is difficult to categorize tumour cells according to the hierarchical pyramid of normal development. CSCs refer to a subset of tumour cells that has the ability to self-renew, generate the diverse cells that comprise the tumour and sustain tumourigenesis. Understanding the regulation of normal stem cell self-renewal and differentiation mechanisms is also fundamental for understanding tumour growth and formation.

CD133 AND ITS POSSIBLE PROGNOSTIC VALUE

For a long time the researchers have been looking for reliable markers in studing brain tumours that allow the identification and characterization of brain CSCs. Singh et al.[39] have proposed CD133, a marker of normal NSC, as an antigen that may be used in order to enrich the CSC population in glioblastomas and medulloblastomas. Subsequently, other groups have demonstrated that CD133+ cells were able to recapitulate the original tumour with similar phenotypic properties.[17,47]

CD133 (prominin-1) is a transmembrane glycoprotein discovered on a hepatoma cell surface; it is normally expressed on hematopoietic stem cells,[48] endothelial

precursor cells[49] and NSC.[50] It was demonstrated that CD133 + CD34+ cells are functionally non-adherent precursors that have the capacity to differentiate into mature endothelial cells and could contribute to postnatal lymphangiogenesis and/or angiogenesis.[49] Five alternative promoters, three of which are partially regulated by methylation, drive the transcription of prominin-1.[51]

Although CD133 has been used to isolate human stem cells from various tissues due to its rapid down-regulation upon cell differentiation, its function remains unclear.[52,53] CD133 localization in membrane protrusions suggests an involvement in the dynamic organization of such protrusions and therefore in the mechanisms influencing cell polarity, migration and interaction of stem cells with neighbouring cells and/or ECM; however experimental proofs are lacking. It is also not known whether CD133 has a role in self-renewal and differentiation of stem cells.

CD133+ population of glioblastomas have been shown to have stem cell properties *in vitro*[51] and to initiate and drive the tumour *in vivo*[17,51] strongly suggesting that CD133+ cells may be the brain tumour initiating cells. The frequency of this expression marker could vary from 5 to 30% in glioblastomas.[18] Moreover, it was shown that enhanced CD133 expression was correlated with the poor prognosis and the decreased survival of the patient.[54–56] Zeppernick et al. [56] showed that the proportion of CD133+ cells and their organization in clusters were significant prognostic factors in brain gliomas of different WHO grades. In particular, significant differences were observed between survival estimates of patient with grade III gliomas containing <1% of CD133+ cells and >1%. All the patients with >1% CD133+ cells relapsed rapidly, having short progression-free time and overall survival. In contrast only one third of the patients with <1% of CD133+ cells demonstrated tumour reccurence. Moreover, the frequency of CD133+ cells was shown to increase with tumour grade; indeed glioblastomas may express the marker in more than 25% of cells, whereas tissue sections of grade II gliomas were devoid of immunoreactive cells. Thus, the authors suggest that CD133 expression could serve as a prognostic factor for tumour regrowth, malignant progression and patient survival.[56] Likewise Pallini et al. demonstrated that both generation of GMB CSCs *in vitro* and the presence of CD133 +/Ki67+ cells could have a prognostic value. The patients whose tumours generated CSCs *in vitro* expressing CD133 and Ki67 had an unfavourable outcome and shorter survival in comparison with patients with tumours not generating CSCs.[55] The ability to generate *in vitro* CSCs may distinguish a severe prognostic subtype of glioblastoma responsible for disease progression and recurrence that may be crucial for the development of new and effective therapeutic strategies.

IMMUNOLOGICAL PROPERTIES OF GBM-ASSOCIATED CSCS AND STAT3

Malignant brain tumours have been shown to have the capability to evade immune surveillance and prevent antitumour immune responses, which may lead to continued growth and increased malignancy.[57] It was demonstrated that glioma-associated CSCs contribute to the immunosupression in glioma patients by both cell-to-cell contacts and secreted products resulting in the inhibition T-cell proliferation and activation, induction regulatory T cells, and intiation T-cell apoptosis. These immunosuppressive properties were diminished on altering the differentiation state of CSCs.[58] Moreover, recent studies have found that the activated form of Signal Transducer and Activator of Transcription 3 (STAT3) was a key mediator of immuno-suppression in GBM.[59,60] Sherry et al. investigated the immune-suppressive properties of CD133+ cancer-initiating cells from GBM patients. The STAT3 pathway was constitutively active in these clones and the immunosuppressive properties were markedly diminished when the STAT3 pathway was blocked in the cancer-initiating cells.[59] Another study has shown that STAT3 regulates growth and proliferation of GBM CSCs.[60] The treatment of GBM-CSCs with two chemically distinct small molecule inhibitors of STAT3 DNA-binding inhibited cell proliferation and the formation of new neurospheres from single cells. Genetic knockdown of STAT3 using a short hairpin RNA also inhibited GBM-CSCs proliferation and neurosphere formation, confirming that these effects were specific to STAT3. Thus, there is increasing evidence that GBM CSCs contribute to the immunosuppression. The therapeutic strategies that induce acquisition of more differentiated phenotype by GBM CSCs and block STAT3 pathway could be a potential approach for CSC-directed therapy of GBM.

NOTES ABOUT THERAPY RESISTANCE

GBM is highly resistant to the conventional therapies including chemotherapy and ionizing radiation.[2,5,6] Despite the fact that current treatments involve combined approach and may eradicate most of a tumour mass, resistance and relapse are still primary causes of poor effectiveness. Recent studies revealed that CSCs may contribute to therapy resistance in GBM.[2,5,6,61,62] Bearing the properties of normal stem cells, cancer stem-like cells not only provide insight into tumour oncogenesis but can also explain clinical resistance of these tumours to the conventional therapies. Clinically it is observed that tumours respond to the treatments only to recur with renewed aggression. Although therapeutic agents kill most of the cells in a tumour, CSCs may be left behind and then contribute to tumour recurrence. Thus, several studies have provided evidence that GMB CSCs display significant resistance to the conventional chemotherapic agents.[2,6] Similarly to normal stem cells, CSCs commonly express drug pumps such as Adenosine Triphosphate (ATP)—binding cassette transporters (ABC-transporters), including Multidrug Resistance Transporter 1 (MDRT1) and Breast Cancer Resistance Protein (BCRP). BCRP and MDRT1 have been implicated in specifically expelling chemotherapic agents from cells and may mediate chemotherapy resistance when expressed by CSCs. To evaluate the chemosensitivity of GMB cells, Eramo and

colleagues have treated stem cell clones derived from different GMB patients with commonly used antineoplastic drugs and assessed the rate of cell death. After 48 h of treatment with chemotherapic agents GBM cells displayed a marked resistance to all the compounds used and were able to proliferate, although at levels slightly lower than those used for untreated cells.[2] The CD133+ cells contribute to the tumour's resistance to chemotherapy, that is correlated to the overexpression of drug resistance genes such as BCRP1 and DNA-mismatch repair genes, such as MGMT (Methyl Guanine Methyl Transferase), as well as genes related to the inhibition of the apoptosis.[6,63]

Radiation therapy is considered the most effective non-surgical intervention for glioblastomas. However, these tumours invariably recur after radiation therapy resulting in patient's death. Therefore, determination of the mechanisms of radioresistance in GMB could lead to advances in the treatment of cancer. Bao[5] has investigated GBM CSCs radioresistance both in vitro and in vivo using short-term cultures derived from primary human specimen and xenografted tumours. It was shown that glioma CSC population was enriched after irradiation and that irradiated CSCs had survival advantages relative to the non-CSC population. CSCs were then able to give origin to the tumours that had both CSCs and more differentiated non-CSCs. Moreover, radioresistant tumours displayed a more enriched percentage of CD133+ cells than the parent cell population. Authors supposed that CSC-enriched cell population might avoid radiation induced apoptosis through activation of DNA damage repair mechanisms, including the phosphorilation of checkpoint kinases Chk1 and Chk2, which initiate cell cycle arrest and attempted repair. Radiation caused equal damage to all cancer cells, but CSCs repaired the damages more rapidly then matched non-stem cells.[5] Altogether these data demonstrate that CD133+ cells may play an important role in CSC resistance to chemo- and radiotherapies. By understanding the mechanisms that allow CSCs to resist conventional therapies, it may be possible to find ways to manipulate to become sensitive to these therapies.

GLIOBLASTOMA SUBTYPES

Recent evidence show that GBM cells cultured under similar conditions can display heterogeneous growth characteristics and molecular profiles, suggesting that they may either arise from different cell types or from similar cells that have acquired different genetic alterations.[28,35,64] Ignatova et al. first reported heterogeneous nature of clonally expanded glioblastoma stem cells. Clonal populations demonstrate the presence of different transcripts specific for undifferentiated cells, neurons and astroglia.[35] Further study provided the evidence that CD133+ cells represent only a subset of primary glioblastomas. Beier et al. cultured tumour cells from 22 glioblastomas in stem cell conditions and demonstrated different growth characteristics of differentiation properties. Moreover, the subpopulation of CD133- tumour cells also possessed stem cell-like properties. Both

subtypes were tumourigenic after implantation into nude mice.[64] It was also reported that the distinct regions of the same GMB comprise two diverse subpopulations of CSCs. These subpopulations were greatly different in their growth properties and tumour-initiating ability.[65] Recent findings suggest that glioblastoma stem cell differentiation may not be restricted to tissues of ectodermic origin but can also be induced to differentiate into mesenchymal cell types.[66,67] Chondro-osteogenic potential in glioblastoma CSCs was expressed both in vitro under specific culture conditions and in vivo upon heterotrophic grafting in mice. However, mesenchymal differentiation of GBM CSCs did not occur in vitro under serum-induced stimulation. On the basis of their differentiation potential, glioblastoma CSCs could be distinguished into two major categories: one subset of tumours whose CSCs exhibit both neural and chondrogenic potential, and another subset with differentiation potential limited to the neuronal lineage.[66] Likewise, gene expression profile studies showed that different molecular subtypes of glioblastomas could be distinguished.[27–29] Such molecular classifications can be of prognostic value or provide guiding decisions about disease management. Thus, Phillips et al.[28] reported classification of three main glioblastoma subtypes defined by gene expression signature. These molecular signatures were associated with tumour aggressiveness as well as with disease progression or could be related to the differences in signalling pathways implicated in gliomagenesis. One subtype expressed neurodevelopmental genes termed as proneural (PN) and could be associated with better prognosis. Two other subtypes characterized by their resemblance to either highly proliferative cell lines (Prolif) or tissues of mesenchymal origin (Mes) showed the expression signature for ECM-related genes. Authors suggested that Prolif and Mes tumour types were associated with either a rapid rate of cell division and enhanced survival of tumour afforded by neovascularisation.

Günter et al.[27] distinguished only two glioblastoma subtypes suggesting that the expression of neurodevelopmental genes as opposed to ECM genes might be crucial for the full stem-like phenotype. Although established under identical conditions glioblastomas gave rise to two distinct subtypes in long-term cultures. Four cell lines (cluster-1) shared similar gene expression patterns, associated with neural development and displayed a full stem-like phenotype with spherical growth in vitro, expression of CD133, neuro-glial differentiation capacity and 100% of tumourigenicity in vivo. Other five cell lines (cluster-2) shared a different gene expression pattern and displayed only a restricted stem-like phenotype with expression signature enriched for ECM genes. Interestingly, most of the cluster-2 cell lines showed no detectable CD133+ cells. However, these cell lines contained cells expressing neural stem/progenitor cell markers Sox2 and nestin. Moreover, these cell lines were clonogenic and could give rise to cells expressing high level of neurofilament-M and galactocerebroside under differentiating conditions, but not GFAP positive cells.

A recent study suggested that CD133 is a the marker of quiescent cell population maintained at dormant-like stage

but also able to spontaneously enter into the proliferative cell cycle and generate highly proliferative and angiogenic CD133- cells.[68] Thus these CD133- GMB progenitor cells may be considered as the true effector cells involved in tumour propagation and vascularisation. Probably, these cells are also able to shift towards mesenchymal phenotype. Interestingly, the inverse process may also takes place. After intracerebral engraftment into nude rats CD133- cells were able to reconstitute the original tumour. Moreover, after several passages *in vivo* these cells upregulated the expression of CD133.[69] Thus, CD133 expression was not required for tumour initiation, but it might be involved in tumour progression. It became evident that brain CSCs are not represented by one particular phenotype. Furthermore, the ability to form tumours depends not only on properties inherent to the CSCs, but also on the host microenvironment which plays important role in tumour initiation and progression.[25,70]

VASCULAR NICHE, HYPOTHESIS OR REALITY

The main features of normal stem cells are the ability to self-renew and to differentiate into many cell types; these features are tightly regulated by the microenvironment or 'niche'.[20,21] A stem cell niche is an interactive structural unit, organized to facilitate cell-fate decisions in a proper manner. Key signalling events are patterned to occur in the right place at the right time. In the adult brain NSCs were shown to be concentrated around blood vessels where they have access to signalling molecules, nutrition and possibility to use nascent vasculature for the migration.[71] Similarly, glioblastoma CSCs are situated in 'vascular niches'; these tightly regulate supply of oxygen and nutrition, and at the same time regulate self-renew and differentiaiton.[25,26,72] However, the existence of GMB CSCs and vascular stem cell niches in the normal brain may have a sinister role in tumour progression; the formation of abnormal stem cell niches that maintain CSCs. Vascular niche and CSCs represent integral parts of the cancer facilitating invasion and expansion.[73] Calabrese and collegues identified a population of Nestin +/ Cd133+ cells located in areas of increased microvessels density.[24] These cells were proliferating and distributed into the brain towards endothelial vascular tubes. Authors suggest that tumour vasculature generates specific niche microenvironment that promote formation and maintenance of brain CSCs.[24] Further it was provided the evidence of reciprocal relationship between GBM CSCs and their microenvironment. CSCs were shown not only to receive the signals from surrounding niche but also capable of modulating it.[74] Thus, Bao et al.,[74] have demonstrated that CSCs stimulated angiogenesis by secreting VEGF and it depended on the factors secreted by vascular niche. Calabrese et al.[24] confirmed that CSCs generated VEGF and other factors to induce angiogenesis; moreover they showed that CSCs depended on the factors brought by vasculature. Furthermore, increasing number of endothelial cells or blood vessels led to expansion of CSC population and accelerated growth of the malignancy.[24] Whereas

treatment of GBM CSCs with bevacizumab blocked their ability to induce endothelial cell migration in culture and iniate tumours *in vivo*.[74] Probably, in this way CSCs mimic the normal stem cells which are also dependent on the microenvironment of vascular niches and have potent angiogenic properties.[70]

Indeed niches control stem cell function. It would be obvious that CSCs should be located within these regulatory microenvironments. However, there is evidence that vascular niche in brain tumours is abnormal and contributes directly to the generation of CSCs and tumour growth.[24] CSCs population was expanded and tumour growth accelerated by increasing the number of endothelial cells or blood vessels in xenografts; in contrast, antiangiogenic therapies depleted the CSCs from xenografts and arrested tumour growth.[24,72] Thus, GMB CSCs and vascular niche may give positive feed-back to each other in order to promote tumour maintenance and expansion.

As well as regulating stem cell proliferation and cell-fate decisions niche may also play a protective role, shielding stem cells from environmental insults.[24] For example, it was demonstrated that endothelial cells can protect CSCs from radiation damage.[75,76] Further studies provided evidence that endothelial cells contributed to chemioresistance of CSCs.[77] Hence, vascular microenvironment might protect CSCs from chemio- and radiotherapies, enabling these cells to reform an initial clinical response.[77]

THERAPEUTIC IMPLICATIONS

The traditional therapies including surgery, radiotherapy and chemotherapy usually provide only palliative effect on GMB, probably because they target proliferating non-tumourigenic cells, whereas CSCs are mostly quiescent and thus resistant to conventional therapies. They could therefore provide the reservoir for potential tumour recurrence.[5,70] Recent studies focused on identification and characterization of CSCs. The mechanisms that drive CSCs resistance to the treatment might potentially have important clinical implications.[2,5,61,62,68] The therapies that target the CSCs population could be of great benefit, since CSCs must be eliminated to cure the cancer. However the clinical application is hindered by complexities arising from intertumoural and intratumoural heterogeneity.[78] The cell-surface immunophenotype of primary tumours, as well as the frequency of functionally defined CSCs, can vary dramatically among different patients. Moreover, different subpopulations within the tumour possess stem cell properties which may be isolated using a variety of cell-surface markers.[5,28] It is becoming evident that for the development of new therapeutic strategies it is essential to consider the heterogeneity of brain tumours like GMBs as well as different subtypes.[29,31,78] Probably, a unique marker is not enough to identify and characterize tumour-initiating population within tumour bulk.[79] Moreover, during glioma progression stem cells may be generated via accumulation of more mutations in the cells initially manifesting glioma features.[80] Tumour heterogeneity may represent inherent

instability in gene expression markers that confers undifferentiated cell character as opposed to stage in the stem cell lineage defining patterns of gene expression. Genetic alterations may make the differentiation status of the tumour cells unstable, floating up and down the lineage, so it may be impossible to assign any differentiation status at all. Thus it is important to classify gliomas into the categories according to the cell-of-origin and transformation mechanism. Such classification may provide better prognostic prediction and guidance for treatment.

Another option for designing therapeutic strategies may be the targeting of the CSCs.[81] As well as GMB CSCs are maintained by surrounding vasculature that provides niches for them, disruption of these niches by anti-VEGF therapy in a mouse xenotransplantation model resulted in CSCs depletion and tumour growth arrest.[24] Niches also have a protective role, shielding CSCs from environmental insults and enabling them to reform a tumour mass following an initial clinical responce.[25] CSCs may dictate the expansion of the normal niche as they proliferate, that may consequently lead to altered niche as cells become independent from normal regulatory signals and produce extrinsic factors that deregulate niche-forming cells.[3] Several studies suggested that the niche may play a main role in tumour initiation and progression.[24,78] The subpopulation of the cells that appeared to be non-tumourigenic might acquire tumourigenic properties in the presence of appropriate microenvironment.[78]

Although the mechanisms of GBM CSCs resistance and the role of niche were not completely clarified, elucidated and understood. And additional further studies are required to characterize the CSC niche and the mechanisms that drive tumour resistance, recent studies demonstrate the apparent synergy between anti-angiogenic therapy and conventional chemo- and radiotherapy.[82] It possible that this combination therapy disrupts the vascular niche, exposing GBM CSCs to the cytotoxic effect of conventional therapies.

CONCLUSIONS

The discovery of CSCs represented a precious tool for studying tumour biology. In particular, it offered a novel interpretation of tumour recurrence and of resistance to chemo- and radio- therapy. CSCs were identified also within brain tumours, especially GBM, and showed to possess characteristics typical of NSC. NSC characteristics suggested that these immature precursors may represent the cellular origin of brain tumours. In glioblastoma, CSCs are represented by different subpopulations and each of them contributes to the tumour growth and expansion.[29,31,78] Additional mutations may occur within the tumour causing further shift in the signalling pattern and differentiation status which in turn can result in a continuous changing of tumour phenotype. Thus, different components and signalling pathways need to be targeted in order to eradicate any given tumour. Glioblastomas have been classified according to the cell origin and gene expression profile; this fact may

provide important indications for disease progression and treatment. The tumour microenvironment was shown to be important in tumour progression, CSCs maintenance, and therapy resistance; it mimics vascular niches of normal NSCs and maintains the GBM CSC pool. It is conceivable to find molecules capable of disrupting such a niche, thereby blocking CSCs self-renewal. Further studies are needed to provide a better understanding of GMB CSCs origin and their interaction with the surrounding niche. These findings hold great promises as regards therapeutical strategies, as well as patient survival.

CONFLICT OF INTEREST

None known.

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