

CANCER STEM CELL IN GLIOBLASTOMA MULTIFORME

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ABSTRACT

Glioblastoma multiforme (GBM) is an aggressive tumor that typically exhibits treatment failure with high mortality rates, is associated with the presence of cancer stem cells (CSCs) within the tumor. CSCs possess the ability for perpetual self-renewal and proliferation, producing downstream progenitor cells that drive tumor growth. Studies of many cancer types, have identified CSCs using specific markers, but it is still unclear as to where in the stem cell hierarchy these markers fall. This review examines the current knowledge on the CSCs markers SALL4, OCT-4, SOX2, STAT3, NANOG, c-Myc, KLF4, CD133, CD44, nestin, and glial fibrillary acidic protein, specifically focusing on their use and validity in GBM research and how they may be utilized for investigations into GBMs cancer biology.

KEYWORD: Glioblastoma multiforme, Cancer stem cells (CSCs), CSCs markers, Cancer biology.

INTRODUCTION

Glioblastoma multiforme is the most malignant and frequently occurring type of primary astrocytomas. It accounts for more than 60% of all brain tumors in adults. According to presumed cell origin, glioma is defined as a primary brain tumor. Its global incidence is 10 per 100,000 people (1-2). Its ratio is higher in men in comparison to women (2-3). It comprises astrocytic tumors (astrocytoma, anaplastic astrocytoma and glioblastoma), oligodendrogliomas, ependymomas and mixed gliomas (4-7). These are the main tumors of the central nervous system (CNS), that account for about 80% of all malignant primary tumors of the brain (6-8). A grade 4 astrocytoma, glioblastoma multiforme is a most severe form of glioma. Following treatment, it shows only median survival of 25 months. It is responsible for 60% of the brain tumors in adults (9-12). Despite several advances in research of cancer and modern therapies against GBM, it is a deadly disease, it has shown only 2% improvement in 5 year survival (13) This tumor has also shown resistance towards radiotherapy and chemotherapy (14-16). The histological features of GBM are presence of central necrosis and microvascular hyperplasia, which distinguishes it from lower grade glial tumors. Other poor prognostic characteristics of GBM are palisading cells around the area of necrosis (17-18). Some other histological features of GBM are atypical nuclei and cellular pleomorphism, increased mitosis, hypercellularity, development of lumina reminiscent of kidney glomeruli (19). The most frequent occurrence site of GBM is cerebral hemispheres, 95% of these tumors

arise in supratentorial region, whereas some percentage of tumor present in brainstem, cerebellum and spinal cord (20).

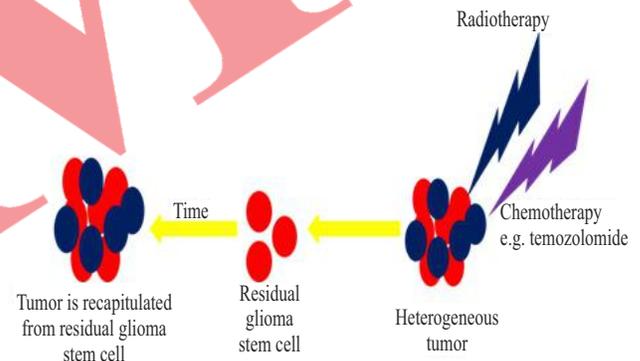
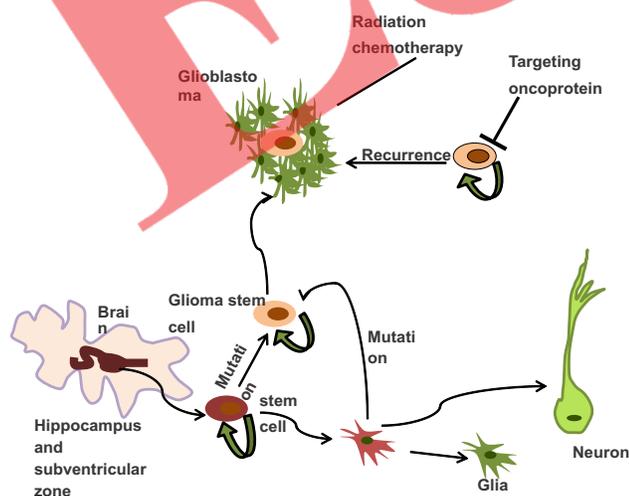


Figure 1 : Cancer stem cell theory representation in glioma tumors, illustrating the impact of clinical treatments on enriching stem cell populations of glioma. (Tracy seymour et al.)

Cancer stem cells (CSCs) are cancer cells that have the same characteristics as normal stem cells, mainly the capability to give rise to all types of cells present in a particular cancer sample. Cancer stem cells behave as tumor initiating cells or tumor propagating cells. They possess the ability of self renewal and differentiation into various kinds of cells (24). These cells show similarity with the property of stem cells like infinite cell growth, multipotency, asymmetric cell division (25). Cancer stem cells have been reported in several types of tumors like prostate cancer, colon cancer, hepatocellular cancer, brain tumors, osteosarcomas, lung cancer, and melanoma

(26). The stem cells properties in human cortical glial tumors were discovered in 2002 and these isolated precursor cells are competent to make neurospheres *in vitro* (25). Glioblastoma is the most common of all lethal brain tumors. The recent standard therapies consist of tumor resection, adjuvant chemotherapy and chemoradiotherapy (21,26). GBMs involve in the expression of multipotent neural stem cells (NSCs) that comprise of neurons, oligodendrocytes, astrocytes within the mass of tumor (27). In malignant glioma, cancer stem cells were defined as glioblastoma stem cells (GSCs) and they have the potential to differentiate into neurons, oligodendrocytes and astrocytes. The main characteristics of glioblastoma cancer stem cells contain self renewal (27), angiogenesis, invasion, proliferation, pluripotency, neurosphere formation, (26) modulation of immune response (27), multilineage differentiation and high motility (28-29) GSCs associated molecular markers express differentially in these GSCs. These markers are classified according to the site of cellular localization like cytoskeletal proteins like nestin, transcriptional factors like Sox2, Nanog, Oct-3/4, cell surface markers such as LICAM (30-31), CD133, CD15, A2B5, polycomb transcriptional suppressors like Bmi 1 and Ezh2 (32). Cell surface proteins isolation were generally used to define cancer stem cells. The detection of these cancer stem cell surface markers is an important key in the diagnosis and treatment of malignancies. The aim of this review is to define the significance of cancer stem cell markers in Glioblastoma multiforme.



Cancer stem cells

Recently, cancer stem cells become a major focus area in cancer research. Clarke et al. (33) reported about CSCs, a cancer cell have the ability of self renewal and differentiation into multiple cell lineages that play the major role in the heterogeneity and the tumor complexity (34) The clonal evolution model reveals about the randomly occurring self renewal property of the cells, whereas the CSCs hypothesis suggests a hierachial arrangement in which stem like cells are favoured (35). CSCs are characterized to be resistant to radiotherapy and chemotherapy and it possess the capability to remain in quiescent stage (36), hence its persistence results in the redevelopment of tumors. This proves that CSCs may be the cause of poor prognosis, treatment failure and disease relapse connected with many solid tumors. An intense discussion about the origin of CSCs revealed that CSCs originate from cancer cells that have been hierarchially downstream to provide undifferentiated CSCs. Likewise, cancer occur due to mutations, hence CSCs also arises from normal stem or progenitor cells. In many tissues and organs, an extensive evidence have given about the connection between cancer and normal stem cells (37). Researchers have separated stem cells from the normal brain and formed neurospheres in culture using serum free media supplemented with cytokines (38). All neurosphere is derived from a single stem cell representing their self renewal potential. Studies have also been done to isolate CSCs from grade IV gliomas. There are a lot of examples, which have reported about stem cell theory of carcinogenesis. One study proved that leukemia arises from leukemic stem like cells (LSCs) (40). The isolation and characterization of CSCs from solid brain tumors, 10 breast cancer, 37 ovarian cancer, 38 leukemia (40) have provide a unique information about the tumor initiation and maintenance abilities of CSCs gliomas (41). GBM and GSM cells have been grown on non-adherent surfaces to form tumorspheres (39). Each sphere is thought to originate from a single CSC, similar to the normal neurospheres originating from a single neural stem cell (39). There are a number of examples illustrating the stem cell theory of carcinogenesis. Evidence has indicated that leukemia originates from leukemic stem-like cells (LSCs) (40). Furthermore, Al-Hajj et al. (42) demonstrated that a small minority of cells within breast cancer express CD44 and CD24 surface markers, which distinguish and isolate tumor-initiating cells from non-tumorigenic cells (42).

CSCs have also been found in human ovarian cancers (43). The identification and isolation of CSCs from solid human brain tumors (38), leukemia (40), ovarian cancer (43), and breast cancer (42) have been achieved and provide a unique opportunity for exploring the tumor-initiating and -maintaining abilities of CSCs

Biomarkers in GBCSCs

Ignatova et al. reported first time about GBCSCs and its presence has been identified in several studies. The list of these proposed markers are CD133, cMyc, CD44, LICAM, KLF4, SOX2, STAT3, NANOG, SALL4, Olig2, Bmi 1 (44-48).

SALL4

SALL4 is a spalt like C2H2 zinc-finger transcription factor, that is found on ESCs in a same manner such as SOX2 and OCT-4 (49-50). SALL4 is a key role player in the progression of the ICM to maintain ESC pluripotency and ensure its zygotic survival (49, 51-52). SALL4 and NANOG interaction has also been confirmed by co-immunoprecipitation experiments and it has been reported that they work together in a similar manner as two ESC markers Oct-4 and SOX2 in regulation of transcription (53). SALL4 act as a main role player in several types of cancers and has been previously demonstrated as CSC marker. It has also been reported that SALL4 is overexpressed in gliomas in comparison to normal brain tissue and its higher levels correlated with poor prognosis (54). Moreover, suppression of SALL4 reduces cell proliferation in gliomas and stimulates apoptosis (55). Di Tomaso et al. (56) demonstrated that CSCs in GBM express SALL4 along with NANOG. Whereas, the utilization of SALL4 marker for CSCs in GBM is restricted to a small number of reports (54-57)

OCT-4

OCT-4 is a transcription factor play a main role with NANOG in the propagation of ESCs and they perform their work in a synergistic behavior with SOX2 to attain this regulation (58). Oct-4 is important for pluripotency and mammalian embryonic development (59). It has also been linked with cancer, which involve in the self renewal of CSCs (60, 61). Normal brain tissues do not express oct-4 whereas glioma cells express Oct-4 and it is concerned with the pathogenesis of GBM (62, 63). Indeed, GBM cells express intense staining of OCT-4 and SOX2. Moreover, majority of cells express along with SOX2 and NANOG (61). Therefore, Oct-4, NANOG and SOX2 play an important role in the regulation of CSCs.

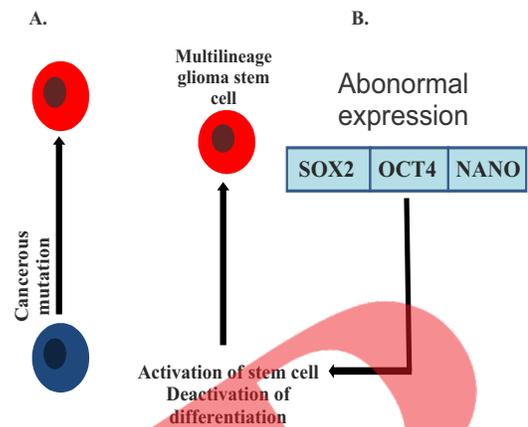


Figure II: Schematic representation of the glioma stem cells development. A. Altered over expression of pluripotent transcription factors, NANOG, OCT-4 and SOX2 involve in the promotion of multilineage potential in glioma stem cells and activates stem cell networks whereas deactivating differentiation pathways. B. Multilineage neural stem cells express SOX2, NANOG and OCT-4.

SOX2

SOX2 is a member of transcriptional co-factors, which is known to be involve in many developmental processes and is over-expressed in tumors (64-65) SOX2 is a critical factor for maintenance of stem cells and it is proposed as a neural progenitor cell marker. It play a major role in many cancers such as breast (66), rectal (67) and lung cancers (68). SOX2 shows higher expression in GBM in comparison to normal brain tissue (69). In addition to this, GBM express higher SOX2 expression than lower grade tumors (70)

pSTAT3

Cytokines activates signal transducers and activators of transcription (STAT) proteins and it involves in the regulation of several cytokines and growth factor responses (71) STAT3 plays a key role in cell cycle signaling, pluripotency, cell survival and ESC self renewal processes (72-74) Inhibition of STAT3 expression reduce self-renewal but promote cellular differentiation, that results in embryo lethality in mice (75). Abnormal STAT3 signalling pathways has known to be connected with promoting angiogenesis and cellular proliferation, weakening of immune system, inflammation in cancer (73-76) There is a plenty of studies that proves the role of STAT3 in cancer like GBM, prostate (77) thyroid, skin(melanoma), breast (73) and head and neck cancer (78) GBM express high levels of STAT3 than normal brain tissues and cells such as astrocytes and

suppression of this molecule results in apoptosis and inhibition of tumor proliferation. Many studies on STAT3 in GBM have reported the decreased expression of STAT3 leads to inhibition of tumor growth. It suggested that STAT3 is a potential target for cancer treatment (79-83).

NANOG

NANOG is an ESC transcription factor and its expression pattern has been known to be link with several types of cancer comprising of lung (84), breast (85-86) oral cavity (87) and prostate (88) It has also been seen to be involved in the regulation of GBM. Stem cell has shown higher expression of NANOG in cerebellum and medulloblastoma (89-91) NANOG alters the GBM stem cell proliferation, clonogenicity and tumorigenicity (92) NANOG suppression in GBM inhibits tumor proliferation and invasion (93) It is assumed that NANOG along with SOX2 and OCT-4 is accountable for ESCs ability to maintain their self-renewal and pluripotency (94-95). Recent data revealed about the role for NANOG in the regulation of GBCSCs.

c-Myc

c-Myc belongs to a member of the family of Myc genes, even though c-Myc, I-Myc and N-Myc have been involved in tumor growth, hence they are considered as nuclear oncogenes (96-97). Over-expression of c-myc has been associated to cellular proliferation (98-99). c-Myc is known to induce cellular dedifferentiation (100) which ensure to form iPSCs. cMyc has been found to be involve in the pathogenesis of prostate (101) breast (102-103) pancreatic (104) lung cancers (105) medulloblastoma (106) and GBM (107). Despite their role in generating iPSCs, there is an evidence indicating that c-Myc may be a marker for progenitor cells rather than ESCs (108). Recent studies have demonstrated that c-Myc increases the capacity of tumor formation in nestin expressing progenitor cells in medulloblastoma. This study suggested that c-Myc is found on progenitor cells, whereas its role as neural progenitor cell marker is not elucidated. In spite of this, c-Myc is known to be associated with GBM, CSC maintenance and self renewal and its higher expression has been associated with poor prognosis of GBM (107,109-111).

Kruppel-Like Factor 4

Kruppel-like factor-4 (KLF4) is a transcription factor that involved in the cell proliferation, differentiation and apoptosis (112). It is a member of the KLF family. It is characterized by the presence of Cys2/ His 2 zinc fingers (113-114). KLF4 is important for the self renewal of ESCs and maintenance of pluripotency

(115-116). It is one of the factors along with Oct-4 and Sox2 that re-program fibroblast to form iPSCs (117). Hence, it is not surprising that higher expression of KLF4 is linked with cancer (118-119). The first identified oncogene in 1999 was KLF4 (120) and after that its higher expression has been reported to induce cellular dysplasia and squamous cell carcinomas (121) Recent studies suggested that KLF4 is over-expressed in 70% of specimens of breast cancer (119) Whereas, there is ample evidence, showing that KLF4 inhibits formation of tumor and metastasis in different types of cancer (122-126). It is estimated that KLF4 inhibits p53, suppressing cell senescence and apoptosis and it also activate p21 induced cell cycle arrest (127-129).

There is a limited information on KLF4 expression in GBM. A study of gene expression analysis study reports about the over-expression of KLF4 in brain tumors rather than GBM (58) A recent studies on GBM cells revealed, microRNA targeting of KLF4 ,that suppress tumor growth in these cells, however the role of KLF4 in GBM is not well understood.

Neural Progenitor CSC Markers

Nestin

The nestin gene (Rat.401) is a neuroepithelial stem cell gene. It encodes a novel intermediate filament protein (130) Nestin is expressed in various types of cancers including GBM (131-139). Over-expression of nestin has been linked with higher grade gliomas with lower patient survival rate (140) In addition to this, down-regulation of nestin occurs due to inducing differentiation of GBM cells.198 It binds to a large number of cells in the embryonic brain of mammals and during the growth of the central nervous system, its presence is correlated with the cellular proliferation (141-142) These studies have suggested that nestin expressing cells can differentiate into multiple cell types, it proposed nestin as an useful stem cell marker (143). Whereas, there is an ample evidence that nestin is a neural progenitor cell marker, which is present on neuron precursor cells (142-144), and it get decreased when precursor cells differentiate into neuronal and glial cells (144).

Glial Fibrillary Acidic Protein

Glial fibrillary acidic protein is a marker of astrocytic maturation, generally used as histological marker of tumors of glial origin that involved in normal astrocytic functions (145-146) Postnatal and adult brain NSCs express GFAP, while embryonic brain NSCs do not express GFAP representing that GFAP is a marker of mature glial cells. Therefore, it have been suggested that GFAP is a progenitor rather than ESC marker. GFAP along with nestin has been found to be

co-expressed in GBM cells (147) and is known to be over-expressed in the serum and peripheral blood of patients of GBM in comparison to healthy subjects (148-149). However, both these studies have elucidated about the GFAP positivity in different proportion of GBM patients. Serum study on GBM cases have reported over-expression of GFAP in 80% cases (149) while peripheral studies on GBM have revealed over-expression of GFAP in only 20.6% cases. GFAP staining is known as a standard diagnostic marker of GBM for samples within the CNS (149-152).

CD133

CD133 (PROMININ-1) is a protein, found on the plasma membrane and HSCs (153) It is one of the cluster of differentiation (CD) antigens (154) In 2003, CD133 was found on NSCs (155) Singh et al.20 have reported that stem like cells deficient in neural differentiation markers along with expression of CD133+ in pediatric brain tumors and also represented that in the brain of immunodeficient mice, CD133+human GBM cells are able to initiate tumor formation.21. Additionally CD133 expression have been implicated in many types of cancers like colorectal and prostate cancer and a greater proportion of CD133+ have been correlated in a tumor with poor survival (156-158) Due to increased progenitor cell activation, GBM tumors recurrence after radiotherapy and chemotherapy showed amplified percentage of cells with CD133+ in comparison to original tumors (159). Additionally,CD133+ gene transcription signal can differentiate GBM from low grade tumors and its expression has been confirmed to the severity of the tumors (159). These studies have suggested that CD133 play an important role in tumor invasion and recurrence, whereas all stem cell not express CD133.

CD44

Cd44 is a transmembrane glycoprotein. It acts as a receptor for glycosaminoglycans hyaluronan (HA) (160-161). It is found in many tissues and is present on embryonic epithelia during development (162). The processes such as splicing and post-translational modifications evolved multiple forms of CD44.The most common isoform is CD44s and other variant is CD44v (163). Different types of variations on the CD44 receptor may contribute to its involvements in different pathways such as angiogenesis, cellular adhesion, cytokine release and lymphocyte activation (162). Moreover,CD44 has been seemed to be involve in head and neck cancer (164-165), Inon small cell lung cancer, breast (166-168) prostate (169-171) and colorectal cancers (172-173) . A study of xenografted

mice has reported that CD44+ cells are able to generate new tumors as like the original tumor, whereas, CD44 cells are not able to achieve this.65A study on GBM cell lines and tumors has elucidated the expression of CD44 in 100% of these cases (174).This was supported by the immunohistochemical staining of CD44 and its another variants in the comprehensive study on GBM (175) Additionally, suppression of CD44 inhibits progression of GBM, characterizing its role in tumor promotion, whereas, many GBM cell lines have shown varying expression of CD44 (176). A broad study on mouse cerebellum has shown the cell surface marker CD44 co-expression along with other marker such as brain lipid binding protein, nestin, SOX2 and astrocyte specific glutamate transporter, these all are related to neural stem/ progenitor cells (177) CD44 is also found to be co-expressed with progenitor marker Oligodendrocytes, Olig2. This evidence would suggest that CD44 is a progenitor cell marker because it is present on differentiated cells. CD44 is also co-expressed with the oligodendrocyte progenitor marker Olig2. This evidence would infer that CD44 is a progenitor cell marker, as it is present on partially differentiated cells.

CONCLUSION

GBM (Grade IV gliomas) is the severe forms of cancer. It is difficult to treat and remain incurable. There is no major recent therapeutic advances have been noticed for treatment for Grade IV gliomas, but there is the main exciting advances providing minor improvement in the survival rate. Recent research has elucidated about the CSC theory of cancer progression, presenting that grade IV gliomas contain GSCs. These GSCs possess the ability of invasion, resistance, therapeutic and tumor recapitulation post-treatment. Therefore, targeting GSCs may help improve poor prognosis and offer the possibility of a cure. In this review, we target many markers published in recent studies and proposed CSCs as a major focus area in the context of GBM. Current studies have focused on a large set of markers that help in the characterization and isolation of tumors in order to prove these markers as therapeutic target .In the light of these observation, we have discussed about the role of a variety of markers such as OCT-4, SOX-2, NANOG, CD44, CD133, GFAP, SALL4, nestin and KLF4. The further investigation is required to prove these markers in GSCs useful for early diagnosis in GBM. Hence, introduction of specific therapies against CSCs help to improve the survival rate and quality of life of patients of cancer with the main emphasis on metastatic stage (23).

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