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The Role of Cancer Stem Cells (CD133$^+$) in Malignant Gliomas

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Malignant gliomas, particularly glioblastoma multiforme (GBM) tumors, are very difficult to treat by conventional approaches. Although most of the tumor mass can be removed by surgical resection, radiotherapy, and chemotherapy, it eventually recurs. There is growing evidence that cancer stem cells (CSCs) play an important role in tumor recurrence. These stem cells are radioresistant and chemoresistant. The most commonly used tumor marker for CSCs is CD133. The amount of CSC component is closely correlated with tumor malignancy grading. Isolating, identifying, and treating CSCs as the target is crucial for treating malignant gliomas. CSC-associated vascular endothelial growth factor (VEGF) promotes tumor angiogenesis, tumor hemorrhage, and tumor infiltration. Micro-RNA (miRNA) plays a role in CSC gene expression, which may regulate oncogenesis or suppression to influence tumor development or progression. The antigenesis of CSCs and normal stem cells may be different. The CSCs may escape the T-cell immune response. Identifying a new specific antigen from CSCs for vaccine treatment is a key point for immunotherapy. On the other hand, augmented treatment with radiosensitizer or chemosensitizer may lead to reduction of CSCs and lead to CSCs being vulnerable to radiotherapy and chemotherapy. The control of signaling pathway and cell differentiation to CSC growth is another new hope for treatment of malignant gliomas. Although the many physiological behavioral differences between CSCs and normal stem cells are unclear, the more we know about these differences the better we will be able to treat CSCs effectively.

Key words: Cancer stem cells (CSCs); CD133$^+$; Glioblastoma multiforme (GBM); Malignant gliomas; Micro-RNA (miRNA); Signaling pathway

INTRODUCTION

Malignant gliomas are the most common malignant primary intracranial tumors. Their incidence is 5.4/100,000 (11). Glioblastoma multiforme (GBM) (WHO IV) tumors are the most common highly invasive malignant gliomas. Their prognosis is still dismal even though aggressive surgery, radiotherapy, and chemotherapy are used for treatment (40). The median survival is only 12–15 months for GBM (22,37). It seems to be an incurable disease due to its highly infiltrative phenotype. There is growing evidence that GBM harbors small cell populations that may sustain tumor formation and growth. These cells are called cancer stem cells (CSCs) (31,32). Cancer stem cells are not only found in leukemia, multiple myeloma, and breast cancer but also in GBM. The CSCs share many properties with normal stem cells including self-renewal and multipotency (14). They have also been shown to express various specific neural progenitor proteins such as nestin (8), Sox2, Oct 4, and Musashi (4).

Various stem cell markers are found in different malignant tumors. The CD133 tumor marker is specific and commonly used for CSCs of malignant gliomas (30). Recent studies showed that biological behaviors of CD133$^+$ were like the CSC subpopulation that confers glioma radio- and chemoresistance (21,34). These stem cells may be the source of tumor recurrence after radiation (25). Oka et al. (29) reported that CD133$^+$ CSCs were identified in the peripheral brain regions adjacent to the tumor. They were frequently localized around vascular structures (the vascular niche). Singh et al. (36) reported that CD133$^+$ CSCs in GBM might be transplantable to xenograft tumors in SCID (severe combined immunodeficiency disease) mice brain, which recaptured the features of the original tumor regarding morphology lineage and marker expression, and generated both CD133$^+$ and CD133$^-$ cells. However, in the same

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patient from whom the CD133 cells were obtained, the cells could not form neurospheres in culture medium and could not form tumor growth in SCID mice (18).

The CD133 CSC marker is analyzed by immunohistochemistry staining and Western blotting. The component amount of $CD133^+$ cells closely correlates with glioma malignancy. The quantities of $CD133^+$ CSCs within the tumor mass might show a clear quantitative correlation with glioma grading (WHO II, III, and IV). These findings support the concept of $CD133^+$ CSC-dependent gliomagenesis (38).

ISOLATION AND IDENTIFICATION OF CSCs IN GLIOMAS

The definition of CSCs must meet the following four criteria: 1) generate clonally derived cells that form neurospheres; 2) possess properties of cell renewal and proliferation; 3) differentiate and express typical CSC markers of tumor cells; 4) generate tumors after in vivo transplantation in animal models that resemble the original donor tumors (39).

CSCs of brain tumors were first identified in primary brain tumors (e.g., GBM, medulloblastoma, pilocytic astrocytoma, and ganglioglioma). They formed clonogenic spheres (neurospheres) in serum-free culture media containing epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF). The CD133 marker is a 120-kDa transmembrane cell-surface protein. In the course of enzymatic digestion of tumor tissue, an excessively long incubation period easily damages CSCs and breaks the structure of cell-surface antigens. The CD133 tumor marker can be also present in other tumors including prostate tumors and colon cancers. We may identify CSCs in cell culture by neurosphere formation with positive $CD133^+$ and nestin neural stem cell markers (42). When the CSCs of brain tumor cells are transferred into the nude or SCID mice brain they may induce tumor formation (26). Singh et al. (36) demonstrated that as few as 100 $CD133^+$ cells derived from human GBM could form GBM in SCID mice; however, the injection of 5×10^4 to 1×10^5 $CD133^-$ cells did not. The CSCs from gliomas are concentrated by cell sorting or magnetic columns using specific immunoreactivities. The CSCs are thought to maintain their drug (Hoechst 33342 dye) efflux ability, which makes it possible to separate CSCs in unstained cell fractions (17). Kang et al. (19) reported radiation might increase CSCs from 2–3% initially to 5–7% in recurrent tumor. Low-dose radiation or hypoxic challenge might be used in the future for experimental study to increase the concentration of CSCs in cell culture (20).

THE COMPONENT AMOUNT OF CSCs IN GLIOMAS

$CD133^+$ cell markers are positive in 60–70% of glioma tissue. Oka et al. (29) reported that the percentage of tumor CSCs varied from 1.02% to 2.32% based on flow cytometric analysis. Singh et al. (36) reported less than 20% of $CD133^+$ in total tumor mass, but wide ranging variations in the $CD133^+$ cell ratio (0.1–50% in GBM) were reported. Low-grade gliomas also contain glioma stem cells, but in relatively low numbers. Thon et al. (38) also reported that the number of $CD133^+$ cells quantitatively correlated with tumor grade.

NEOANGIOGENESIS IN CSCs

The $CD133^+$ cells from gliomas of different grades might contribute to intratumoral neoangiogenesis. $CD133^+$ cells frequently accumulate within highly vascularized regions, particularly in the case of GBM. Bao et al. (2) reported evidence that the mutual influence of stem cells and endothelium account for neoangiogenesis. Thon et al. (38) also reported that $CD133^+$ cells exhibit expression of endothelial progenitor and display a typical endothelial "cobblestone" growing pattern. Vascular endothelial growth factor (VEGF) promotes tumorigenesis via angiogenesis (28). VEGF-repressing CSCs lead to the massive expansion of vascular-rich GBM and tumor-associated hemorrhage, thereby causing high morbidity. VEGF provides a vascular-rich tumor environment stem cell niche.

MICRO-RNA REGULATION OF CSCs

Micro-RNAs (miRNAs) are a novel group of short RNAs, about 22 nucleotides in length, that regulate gene expression in a posttranscriptional manner by paring with complementary nucleotide sequences in the 3′-untranslatable region of target mRNA. The miRNAs regulate cell functions, either oncogenesis or tumor suppression, which influence cancer development and progression. Recently, miRNA-7, miRNA-21, and miRNA-451 were found to be involved in regulation of brain differentiation (16). MiRNA-451 may disperse neurospheres and inhibit the growth of GBM cells. MiRNAs may drive $CD133^+$ cells to differentiate and lose their stem cell character. When the miRNA levels in $CD133^+$ cells are raised by transfection, there may be inhibition of cell growth and proliferation from loss of cell renewal potential. MiRNA-124 and miRNA-137 may also inhibit proliferation of GBM cells and induce differentiation of brain tumor stem cells (35).

IMMUNE RESPONSE TO CSCs

The brain is not an immunologically exempt organ without elicitation of inflammation and immune response (1). According to tumor immunosurveillance theory, tumor can be recognized and eliminated as a result of the natural antitumor immune response (9). But in tumor development, tumor cells may influence immunity either from the tumor environment, rendering a tumor invisible to the host immune system, or resistant to

the antitumor response. Most cancers are composed of a mixture of normal stem cell and CSCs. The antigenesis of normal stem cells and CSCs may be different. The CSCs frequently escape the T-cell immune response. It is critical to fully characterize the immunological features of CSCs and to develop immunotherapeutic approaches to eliminate CSCs without excessive toxicity to normal stem cells (41).

CHEMOSENSITIZER AND RADIOSENSITIZER FOR CSCs

Liu et al. (24) reported that $CD133^+$ CSCs play an important role in tumor resistance to chemotherapy. This resistance is probably contributed to by $CD133^+$ cells with greater expression of breast cancer resistance protein (BCRP1) and methyl-guanine methyltransferase (MGMT) as well as the antiapoptosis proteins and inhibitors of apoptosis protein families. Miqueli et al. (27) reported that the monoclonal antibodies for anti-epidermal growth factor receptors can increase radiosensitization to GMB CSCs. Chang et al. (6) reported that the mean survival rate of GBM with $CD133^+$ mice treated with radiation was significantly improved by knockdown of silencing information regulator (SirT1), a member of the sirtuin family, which is an NAD-dependent histone deacetylase and essential mediator of longevity in normal cells. Ehtesham et al. (12) showed that cell surface chemokine receptor (CCR4), a mediator of cancer proliferation and invasion, was overexpressed in GBM CSCs. Administration of CXCL12, the only known ligand for CXCR4, stimulates a specific and significant proliferative response in progenitors but not in differentiated tumor cells.

Kang and Kang (20) reported pharmacological blockage of chloride channel synergistically enhanced apoptosis in drug-resistant CSCs. Casper et al. (5) reported that acetaminophen, acetylsalicylate, and ibuprofen also increased radiosensitivity in culture and reduced glioma cell growth. Similarly, other nonsteroidal anti-inflammatory drugs (NSAIDs) also directly act on tumor cell growth by the inhibition of prostaglandin synthesis. NSAIDs increase the concentration of arachidonic acid, a precursor of prostaglandins, which induces ceramide formation from sphingomyelin. Subsequently, ceramide-induced apoptosis ensues (7). The inhibition of prostaglandin synthesis also decreases tumor size directly by inhibiting angiogenesis (43). Whether these radiosensitizers and chemosensitizers are effective for CSCs too needs to be further evaluated.

IMPLICATION OF CSCs FOR GLIOMA TREATMENT

As with normal tissue stem cells, CSCs are mostly quiescent and resistant to conventional treatment. Signaling pathways involving activation of normal stem cells also involve tumor stem cell proliferation. Knowing how to identify such tumor-specific signaling pathways and their inactivation mechanism will provide information on key targets for glioma treatment. In comparison to $CD133^-$ tumor cells, $CD133^+$ CSCs have higher DNA repair capacity, which results in a selective postirradiation increase in the $CD133^+$ population (15). CSCs express multidrug resistance genes (e.g., ABCG2 and BCRPI) that aide in the efflux of drugs and in the selective promotion of CSC survival (15). The sonic hedgehog (shh) (10) and the Notch pathway are very important for brain development and for maintaining cell "stemness." An shh pathway inhibitor, cyclopamine, depletes CSCs (3). In the Notch pathway, γ-secretase inhibitors also decrease the $CD133^+$ fraction (13). Promoting the differentiation of stem cells to normal cells is another possible approach for CSC treatment. Piccirillo et al. (33) reported that bone morphogenetic protein (BMP4) might reduce the CSCs in GBM by differentiating the CSCs to astrocytes.

CONCLUSIONS

Cancer stem cell investigation is a good starting point for controlling tumor growth and recurrence (23). Although many differences in physiological behavior between CSCs and normal stem cells are unclear, the more we know about CSCs the more we can treat them effectively.

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