Brain tumor treatment increases the number of cancer stem-like cells

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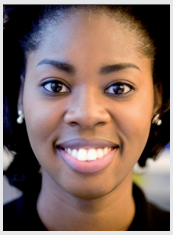

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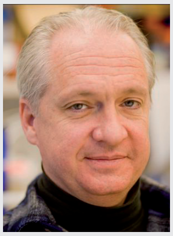

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> "...current treatment modalities employed for the treatment of brain tumors select for and induce a more stem-like population of cells that can repopulate the tumor."

Glioblastoma multiforme (GBM) is the most common primary brain tumor with a devastatingly poor prognosis, mainly attributed to its resistance to treatments and aggressive recurrence of tumors. Standard therapies used for the treatment of gliomas result in surviving cells that appear more stem-like than prior to treatment. The standard of care for brain tumors after surgical resection is ionizing radiation and chemotherapy. Recent data suggest these therapies increase the proportion of brain tumor stem-like cell populations within these tumors. These tumor stem-like populations preferentially survive these treatments by two mechanisms. The first involves enrichment of a small population of cancer stem-like cells through direct selection and the second involves enhancement of cancer stem cell-like characteristics in surviving populations of cells following enrichment.

Enrichment of brain tumor stem-like cells has been demonstrated in response to chemotherapy treatments. These therapy-induced changes in the characteristics of a heterogeneous cell population could be due to selection for subsets of cells resulting in skewing of the resultant cell population. It is also possible that the therapy directly affects the character of cells in the population. There is emerging evidence that both are occurring in brain tumors.

Enrichment of stem-like cells in treated brain tumors

Many studies have shown that brain tumors that survive therapy are more resistant to further therapy. Recently, the concept of resistant cells being those with stem-like character has prompted investigations showing that stem-like cells are enriched in treated tumors. Most of these studies, however, are not designed to specifically demonstrate selection for resistant cells within the tumor. Treatment of human GBM cells with carmustine leads to preferential survival and thus selection of a small population of GBM cells enriched for CD133, a marker of stem cell-like populations in brain tumors. Following survival and proliferation, these cells were significantly more resistant to carmustine treatment than their respective parental cell lines. These cells were multipotent and displayed a capacity for tumor initiation *in vivo*, suggesting an enrichment in stem-like cells [1]. Patient data supports this finding, the percentage of $CD133^+$ cells was found to be significantly elevated in recurrent human gliomas when compared with newly diagnosed gliomas from the same patient. Several primary cultured cell lines generated from human GBM patients were significantly more resistant to several chemotherapy reagents including temozolomide, carboplatin, paclitaxel and etoposide. The chemoresistance of these cells correlated with elevated gene expression of antiapoptotic genes, as well as O6-methylguanine-DNA-methyl transferase (MGMT) – a DNA repair enzyme that inhibits the activity of DNA methylating agents such as temozolomide [2].

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One mechanism for chemotherapy resistance and multidrug resistant phenotype, is the expression of ATP-binding

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cassette transporters (ABCs). ABC transporter function affords drug efflux capacity, some of which are stem cell markers and mediate chemoresistance and enrichment of brain tumor stem-like cells following therapy. Indeed, the chemoresistance of $CD133^+$ cells to the aforementioned therapies was associated with increased expression of *ABCG2* [2]. Furthermore, brain tumor stem cells were shown to express elevated levels of the multidrug resistant associated proteins 1 and 3 when compared with nonstem cell populations derived from the same sample [3].

> "...chemo- and radio-therapies used in the treatment of glioblastomas may prime brain tumor stem cells to enhance their stem cell-like characteristics."

Enrichment of brain tumor stem cells following radiation treatment has also been demonstrated and may be due to differential DNA damage repair capacity. $CD133^+$ brain tumor stem cells in human glioma xenografts and isolated human GBM tumor specimens were significantly enriched following radiation. Glioma tumor stem cell radioresistance and enrichment were mediated via activation of the DNA damage response. The $CD133^+$ tumor cell population repaired their DNA more efficiently and, therefore, underwent less apoptosis than their $CD133^-$ counterparts from the same glioma sample [4].

Therapy-induced introduction of stem-like character

In addition to the enrichment of brain tumor cells with stem cell-like properties by chemo- and radio-therapies, a more intriguing question is whether these therapies also induce a more stem-like cell phenotype on individual cells in the surviving/enriched tumor cell population. In medulloblastomas, radiation induces apoptosis in the bulk of the tumor while activating the PI3K/AKT survival pathway in cells located in the perivascular niche. In the process, these surviving cells significantly increase the expression of the stem cell marker nestin (potentially increased 'stemness') in surviving radioresistant medulloblastoma stem-like cells [5]. These nestin-expressing perivascular cells later re-entered the cell cycle in 72 h and repopulated the tumor [5], demonstrating that the cancer stem-like populations are resistant to conventional genotoxic therapies, further suggesting that these therapies selectively enrich this subpopulation of brain tumor cells and induce changes in them to further promote their stem-like characteristics.

In PDGF-induced gliomas the side population (SP) cells are defined as a cell population showing ABCG2-induced Hoechst dye exclusion by flow cytometry, and represent the stem cell-like population within gliomas. In these tumors, the SP is increased by PTEN loss and reversed by PI3K blockade. Following treatment with temozolomide, the current standard chemotherapeutic agent used in the treatment of GBMs, the SP is elevated particularly in the absence of PTEN [6]. This temozolomide-induced SP phenotype is associated with increased gene expression of nestin and higher expression of *MGMT*, which confers resistance to temozolomide [7]. Accordingly, increased gene expression of *MGMT* in the SP correlated with enhanced temozolomide resistance of SP cells relative to the main population cells of the tumor, and thus probably plays a role in mediating their resistance to temozolomide-induced DNA damage and apoptosis. Moreover, this increase in the number of SP cells induced by temozolomide is associated with shortened tumor latency and survival of mice, suggesting that by enhancing stem cell-like characteristics in PDGF-induced gliomas, temozolomide treatment hastened the development of recurrent disease. Collectively, these findings suggest that chemo- and radio-therapies used in the treatment of GBMs may prime brain tumor stem cells to enhance their stem cell-like characteristics. This work suggests that current treatment modalities employed for the treatment of brain tumors select for and induce a more stem-like population of cells that can repopulate the tumor. The data also suggest that treatment inadvertently selects for resistant, aggressive populations partly because of their stem-like character.

The underlying mechanisms that drive these surviving populations of brain tumor cells to a more stem cell-like phenotype remain to be fully identified. It is possible that the microenvironment of the perivascular niche contributes in some way to the stem cell characteristics of this population. Signaling pathways may also be involved, including Notch, which is known to promote a stem cell-like phenotype in brain tumors [8,9]. Furthermore, Notch signaling has recently been reported to play a critical role in mediating chemoresistance in pancreatic cancers [10]. Whether the Notch signaling pathway plays a similar role in brain tumors remains to be determined.

Clinically, gliomas initially respond to chemotherapies but later reccur with aggression. Taken together, these findings underscore the need to rethink therapy and use combinations where some component specifically targets the stem-like populations within gliomas. An important consideration would be that these approaches be designed to adversely affect glioma stem cells without affecting normal somatic stem cells.

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