

Editorial

Finding drugs against CD133+ glioma subpopulations

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It has been more than 25 years since investigators first discussed the existence of glioma “stem” cells, but only in the last 5 years or so have scientists been able to identify such cells in tumors based on expression of specific markers or defined phenotypes and behaviors. This type of identification has led to the hypothesis that therapies aimed at such cells within tumors would eliminate the glioma’s ability to self-renew and thus be more efficacious than current treatments that target all of the neoplasm, but to which glioma stem cells are resistant. Therefore, the effort to seek new therapies that target such cells has become a hotbed of research. In this issue of the Journal of Neurosurgery, Ma et al. report that cyclooxygenase-2 (COX-2) is overexpressed in glioma cells that express the CD133 marker, previously shown to be one of the markers of “stemness.” Based on this discovery of COX-2 overexpression, they were able to demonstrate antiproliferative and proapoptotic effects against CD133+ glioma cells using the COX-2 inhibitor celecoxib in combination with radiation. They were able to demonstrate this effect both in vitro and in animal models (in vivo). Therefore, celecoxib may become a useful therapeutic agent against CD133+ glioma cells when used in combination with radiation.

There are some limitations in the study. First, clinical trials of celecoxib in human patients with gliomas do not seem to show a therapeutic effect. Although these trials are not being conducted in combination with radiotherapy, such a finding still dampens enthusiasm for future use of this particular drug. Second, the data in Fig. 5A show that COX-2 expression was only partially inhibited by celecoxib, even at the highest dose tested. This raises the concern that the observed anticancer biological effect of celecoxib may result from a yet-undiscovered “off-target” effect. Third, their procedure to obtain CD133+ “stem” cells starts off with a population of CD133− cells from resected specimens that are then radiated to obtain radioresistant CD133+ cells. All subsequent comparisons are then made between the original parental CD133− cells and the derived radioresistant CD133+ cells. This is not the usual methodology for isolation of glioma “stem” cells, raising concern that the comparison is not between glioma non–stem cells and stem cells but rather between radiosensitive glioma cells and radioresistant cells. In fact, a recent report linked CD133 expression not to “stemness,” but rather to a marker for “bioenergetic stress.”

In spite of these limitations, the study does raise awareness that drugs targeting a particular glioma subpopulation (the CD133+ subpopulation) can be discovered, raising the possibility that the future therapeutic armamentarium against these tumors will become stratified and “personalized” based on the molecular profile of the particular tumor.

References


Response

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We very much appreciate the comments of Drs. Na-
kano and Chiocca in their thoughtful letter regarding our paper. Recently, preclinical studies have shown the efficacy of selective COX-2 inhibitors in reducing viability of glioblastoma cells in vitro and in vivo through targeting COX-2. Kang et al. and Petersen et al. also demonstrated the radiosensitizing property of COX-2 inhibitors when combined with radiotherapy. Several studies in glioma cell lines have revealed that COX-2 inhibitors are synergistic with radiation therapy. Furthermore, Kang et al. found that the underlying antitumor mechanisms of COX-2 inhibitor may due to induction of DNA damage.

First, Drs. Nakano and Chiocca raised concerns regarding our omission of one recent article that reported on a trial of celecoxib in human patients with gliomas in which the treatment did not seem to show a therapeutic effect. In that article, Kesari et al. reported the first study using the combination of celecoxib and thalidomide with temozolomide to treat patients with glioblastoma. They demonstrated that this regimen was well tolerated but did not meet the primary end point of improvement of 4-month progression-free survival (as measured from study enrollment), suggesting that this combination could not significantly increase survival. However, temozolomide was only administered to patients showing stable disease after radiotherapy but not concomitantly given with radiotherapy in their study. Methylguanine-DNA methyltransferase (MGMT) promoter methylation status is a critical factor in determining the effect of temozolomide on DNA repair in glioblastomas and affecting the clinical outcome. Clinical trials have some limitations when retrospectively analyzed, and this trial failed to show the true MGMT promoter methylation status due to older specimens having poor quality DNA for testing for MGMT and samples from archival paraffin are not reliable. Therefore, MGMT status could not be confirmed. Moreover, insufficient sample size failed to correlate MGMT status with survival or therapeutic effect. Furthermore, statistical bias exists in the setting of antiangiogenic peptides demonstrated by Kesari et al., who showed a higher SD than mean value of bFGF, VEGF, TSP-1, and endostatin with outliers. Baumann et al. revealed that the addition of temozolomide to thalidomide might improve efficacy in a postradiation setting. Importantly, in our study we observed that the COX-2 inhibitor should be administrated prior to radiotherapy to obtain G2/M arrest and combined with irradiation to achieve phosphorylation of cdc-2 and thereby prevent mitosis in the CD133-expressing glioma stemlike cells. Therefore, the sequence of administering COX-2 inhibitors and radiotherapy needs to be taken into account as well. Kuipers et al. reported that COX-2 inhibitors might result in radio-protection rather than radiosensitization for human glioma cells if given after radiotherapy. Even though several Phase II clinical trails with celecoxib are ongoing, we believe that the celecoxib dosage and the timing of irradiation and pre-treatment with the COX-2 inhibitor should differ between in vitro and in vivo. Therefore, whether the concurrent use of celecoxib and radiotherapy could improve the survival for patients with glioblastoma remains unclear and needs to be further investigated.

Second, Drs. Nakano and Chiocca also raised a concern regarding COX-2 expression being only partially inhibited by celecoxib, even at the highest dose tested. The observed anticancer biological effect of celecoxib may result from a yet-undiscovered “off-target” effect. At present, the molecular events underlying the antitumor properties of COX-2 inhibitors are not fully understood. The effects and mechanism of COX-2 inhibition on the glioma stem cells also need to be further studied, especially when these agents are used in combination with radiotherapy. In our study, we observed that celecoxib could only partially inhibit the endogenous COX-2 of CD133+ glioma stem cells. Perhaps this is because the nature of CD133+ glioma stem cells themselves poses some resistance to celecoxib. But this inhibition effect by celecoxib could be enhanced with the addition of radiotherapy in our study.

Drs. Nakano and Chiocca also raised 2 other concerns regarding our methodology for isolation of glioma “stem” cells from irradiated parental GBM cells from resected specimens to obtain CD133+ “stem” cells and the comparison not being between glioma radiosensitive and radioresistant cells rather than glioma non–stem cells and stem cells. As for the methodology for isolation of glioma stem cells, several approaches have been applied to isolate brain tumor stem cells, including CD133+ magnetic bead sorting of cancer stem cells, side population, hypoxia niche to enrich and maintain the subpopulation of cancer stem cells, and serum-free stem cell culture medium to select the stem cell and dampen growth of non–stem cell populations as well as chemo- and radioselection. Bao et al. demonstrated that the fraction of CD133+ expressing tumor cells in gliomas was enriched and radioresistant after radiation. In our study, specimens of resected tumors were cultured in vitro to obtain the parental glioblastoma cells with a low percentage of CD133 expression (1.3% only). Based on the rationale of radioresistance in glioma stem cells, these parental cells (low-CD133+ cells) were then irradiated and sorted to obtain a high percentage of CD133+ expressing cells (high-CD133+ cells). The parental glioblastoma cells with a low percentage of CD133 expression were physiologically similar to human glioma cells, while the high-CD133+ glioma cells obtained after irradiation met the characteristics of cancer stem cells, including sphere formation, asymmetric proliferation, “stemness” gene expression, higher tumorigenicity, and radioresistance. All subsequent comparisons were made between the original low-CD133+ and high-CD133+ glioma cells. This is still an appropriate approach to the isolation of glioma stem cells.

Finally, Drs. Nakano and Chiocca raised concern regarding our omission of one recent report that linked CD133 expression not to “stemness” but rather to a marker for “bioenergetic stress.” Griguer et al. reported that CD133 is a marker of bioenergetic stress in human glioma, which could be regulated by environmental conditions (hypoxia) and mitochondrial dysfunction. In fact, the biology of CD133 is not well understood, and the ideal markers for cancer stem cells still remain undiscovered. Undoubtedly, CD133 is not the only marker for brain cancer stem cells. More efforts in investigating other markers and their correlation with cancer stem cells are greatly needed.

We thank Drs. Nakano and Chiocca for raising a very important issue about our study, and we believe that the fu-