Can neural stem cells be used to track down and destroy migratory brain tumor cells while also providing a means of repairing tumor-associated damage?

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Successful eradication of central nervous system (CNS) tumors is an exceptionally complex problem, in part because of the devastating effects that injury to the brain or spinal cord can have on normal function. Such injury is associated with brain tumors for multiple reasons. In part, it is well established that both reperfusion and compressive forces, which are unavoidable consequences of tumor growth, are injurious to the CNS. It is likely that other mechanisms also contribute to tumor-related damage, such as abnormal ion fluxes and triggering of glutamate release from damaged cells, possibly even from the tumor cells themselves. Moreover, it is clear that therapeutic regimes used to treat CNS tumors may themselves cause injury. Such a conclusion is based on the increasing recognition that treatment with chemotherapeutic agents and/or radiation often is associated with significant cognitive impairment (1–3). The physiological basis for this cognitive impairment is not wholly known, although a number of studies have demonstrated that radiation kills oligodendrocytes, stem cells of the subventricular zone, and precursor cells of the dentate gyrus of the hippocampus in rodent models (1, 4–7).

Taking the above considerations as a starting point, the obvious conclusion emerges that a useful advance in brain tumor therapy would be to develop therapeutic approaches that would both kill tumor cells and repair injury to the damaged CNS. Repairing CNS damage requires the recruitment of endogenous stem cells (or lineage-restricted precursor cells) or the transplantation of cells with the capacity to carry out repair, thus making of particular interest attempts to use neural stem cells as therapeutic delivery vehicles.

Recently, Benedetti *et al.* (8) reported that neural stem cells genetically modified to produce IL-4 could promote tumor regression and prolonged survival in mice that have been injected intracranially with the GL261 mouse glioma cell line. These intriguing results did, however, leave unanswered a number of questions of relevance to construction of clinical trials (9). For example, it is necessary to determine whether any particular CNS stem cell or lineage-restricted precursor cell offers advantages as a therapeutic delivery vehicle and whether the therapeutic agent that these cells are modified to produce will damage normal CNS tissue. In addition, it is problematic that many of the models used to study gliomas in mice and rats do not reproduce all of the important characteristics of malignant gliomas in the human, including variability of phenotypes within individual tumors and the expression of radioresistance and chemoresistance. In particular, as the GL261 glioma cell line does not exhibit the migratory characteristics *in vivo* that are such an important feature of human CNS neural tumors, the studies of Benedetti *et al.* (8) did not shed light on the value of neural stem cells as delivery vehicles when confronted with a tumor in which cells have disseminated large distances from the original tumor mass.

This issue of PNAS provides an important new contribution from Aboody and colleagues (10) that suggests that neural stem cells might prove an effective therapeutic vehicle even when a migratory tumor cell population is the target for treatment. In these studies, transplanted neural stem cells were shown to have the ability to migrate toward an intracranial tumor cell mass. This ability of stem cells to migrate toward a tumor mass was seen when stem cells were injected at intracranial sites distant from the tumor, and even after somatic injection into the tail vein. Moreover, some neural stem cells migrated so as to be juxtaposed with tumor cells that had themselves become distributed away from the primary tumor mass. Neural stem cells engineered to produce cytosine deaminase, which converts 5-fluorocytosine to the oncolytic drug 5-fluorouracil, were able to kill tumor cells *in vitro* and cause objective reductions in tumor mass *in vivo*. Thus, it appears that the extensive migratory capacity of neural stem cells is retained in the tumor environment and may be advantageously applied both to delivering these novel drug delivery vehicles to the primary tumor and at distances removed from the central tumor mass.

The possibility that transplanted neural stem cells also might repair damage associated with the occurrence and/or treatment of a brain tumor can only be assessed at the moment in the context of other efforts to use stem cell transplantation in the repair of CNS injury. Of particular interest are recent studies on the ability of hypoxic injury to alter the behavior of endogenous stem cells of the CNS. In these studies, it was found that experimental induction of ischemic lesions was associated with increases in the division of stem cells of the subventricular zone and a preferential migration of newly generated cells into the region of injury. Moreover, this recruitment of endogenous cells with the capacity for division is associated with the generation of new neurons and oligodendrocytes that become incorporated into the CNS parenchyma in an apparently normal manner (Evan Snyder, personal communication). Whether similar behavior will be seen with neural stem cells transplanted into tumor-bearing brain is not yet known, but numerous studies showing that transplanted CNS stem cells and lineage-restricted precursor cells will readily generate new neurons, oligodendrocytes, and astrocytes *in vivo* gives one reason to be hopeful in this regard.

The studies of Aboody and colleagues bring us closer to the point where clinical trials will be initiated on the use of neural stem cells to treat CNS tumors, thus making it essential to consider what additional

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preclinical evidence is required to reach this point and how such clinical trials themselves might be structured. A number of relevant questions have been raised previously, including the need to examine tumor cell killing in animal models that mimic the human condition more closely than is generally the case (9). In addition, a particularly critical issue derives from the fact that this is a treatment that might confer two wholly different kinds of benefits, one on survival and one on repair of CNS damage. Structuring clinical trials to gain useful insights into these distinct possibilities is particularly challenging, particularly if—as has generally been the case—obvious therapeutic success is not achieved in the first attempts.

Among the questions that need to be assessed in preclinical studies, some of the most important are those related to determining in more detail whether stem cells can really preferentially migrate toward tumor cells present in small numbers, and perhaps even distributed as individual cells. The information provided in the studies of Aboody *et al.* (10) is insufficient to distinguish unambiguously between the hypothesis that stem cells are able to ''track down'' individual tumor cells and the possibility that the instances in which these cells are colocalized instead represent utilization of identical migratory substrates by tumor cells and stem cells. Both of these influences may be relevant, as stem cells might be expected to migrate toward a source of growth factor production (as occurs in a tumor mass), but it also is possible that tumor cells migrate along identical substrate pathways to those used by normal migratory CNS cells. If migration along identical substrate pathways is the predominant reason transplanted stem cells and tumor cells are juxtaposed, this would introduce a strong element of chance in the occurrence of this event. If so, the great majority of disseminated tumor cells might be expected to escape killing. As tumor cells that have migrated away from the central tumor mass play a major role in brain tumor recurrences, it is essential to determine with greater accuracy the extent to which neural stem cells might be guided to small numbers of tumor cells. Precise quantitative experiments in which small tumors are established and then labeled neural stem cells are transplanted into and/or around the tumor bed, followed by determination of the frequency with which migratory tumor cells and migratory neural stem cells are in contact with each other are required to address these questions.

Closely correlated with the question of whether transplanted stem cells can successfully home to the location of dispersed tumor cells are the issues of division and differentiation of the stem cells, and the number of tumor cells that can be killed by a single stem cell. These are finely graded balances that are closely interrelated. If the stem cells continue to divide after transplantation, then it is possible that they will themselves create an inappropriate cell mass. If, in contrast, they do not divide (as appears to be the case in the present studies), then as tumor cells continue their own division they eventually will become too numerous for the therapeutically modified stem cells to kill directly. Thus, if the mode of tumor cell killing requires close proximity to the transplanted stem cells, as might be expected in the studies of Aboody *et al.*, then the action of the stem cells would be expected to only be temporarily effective. Moreover, if the transplanted stem cells differentiate into neurons or oligodendrocytes, then their migratory capacity will be compromised. It is possible, for these reasons, that killing of tumor cells and repair of CNS damage might require transplantation of two different stem cell populations, only one of which has been modified to kill the tumor cells. Still further considerations of importance are whether the therapeutic agent produced by the transplanted stem cells causes injury to normal brain cells, how to engineer the stem cells to cease producing the therapeutic protein when it is no longer necessary to do so, and whether the use of nonautologous stem cells eventually will trigger an immune reaction against the cells they produce (as discussed previously, ref. 9).

For the patient with a malignant glioma, concern over a number of the above questions is an unrealistic luxury. In these very needy patients, it is likely that trials will go ahead before all relevant information is obtained in experimental animals, and it is critical that such clinical trials are designed so that knowledge is gained even in failure. Thus, if one does not see prolonged regression of tumor mass, or enhancement of neurological function, in clinical trials, how will it be possible to determine the reasons for this apparent failure? How can one identify variables that might be manipulated to obtain more successful outcomes? Such questions can only be answered by transplanting cells that are permanently labeled and making certain that a thorough analysis will be conducted on the brains of patients who die at various time points after receiving stem cell transplants. Such a label could be intrinsic to the cell, as would be the case if neural stem cells from a male were transplanted into the CNS of a female patient (thus enabling recognition of transplanted cells with Y chromosome-specific probes), or could be expressed because of genetic modification of the transplanted cells. It also will be important to make certain that patients who seem likely to die receive injections of BrdUrd, so as to determine whether the transplanted stem cells continue to partake in DNA synthesis. BrdUrd administration to glioma patients has been used to label tumor cells before biopsy or surgery and also has been used as a radiosensitizing agent (as described, for example, in refs. 11–14). BrdUrd also is taken up by normal human brainstem cells or precursor cells that are engaged in DNA synthesis *in situ* in the CNS of patients with brain tumors (15). Thus, it is well established that BrdUrd can be used to label dividing stem cells in the human CNS. In case division of transplanted stem cells continues for long periods after transplantation into the human CNS, the BrdUrd injection will need to be as close in time to the point of death as is possible in some patients to prevent dilution of label to undetectable levels. To allow for the possibility that cell cycle times are long, it would be advantageous if BrdUrd delivery could be continuous over a period of 8–24 h. Appropriate informed consent would need to be obtained for all such experiments, but their importance warrants examination of this possibility.

By combining the use of labeled stem cells with BrdUrd administration, it will be possible to obtain a great deal of information that will not be observable through standard neurological and radiological examination. The ability to unambiguously recognize transplanted cells will allow determination of whether they form aberrant growths, whether they become incorporated into the subventricular zone or other germinal zones, whether a population of dividing cells is retained for long periods, and whether the transplanted cells generate new neurons, oligodendrocytes, and astrocytes. It also would be advantageous to determine whether the tumors of patients treated with this approach have any molecular characteristics (e.g., expression of a truncated or amplified receptor for epidermal growth factor) that would allow tumor cells to be identified with immunohistochemical markers. Although it will not be possible in the human to quantify the apposition between stem cells and tumor cells with the accuracy that is possible in preclinical models, it is nonetheless important to use any means available to at least estimate the extent to which this hoped-for association actually occurs.

As discussed, the problems that remain to be resolved in obtaining benefit from this novel therapeutic approach are formidable ones. Nonetheless, the consistent failure, year after year, to significantly increase the survival of patients with malignant brain tumors makes it clear that obtaining dramatic improvements in outcome in this devastating disease requires the development of fundamentally new

treatment strategies. A number of such treatments have been proposed, including vaccination, gene therapy, pharmacological blockade of specific receptors involved in glioma growth, and the application of angiogenesis inhibitors. All of these various treatments have been applied successfully in rodent models, but it is not yet clear whether any will prove of clinical value. As, thus far, none of the treatments of brain tumors that have been successful in preclinical models have worked in human patients, the need for continued research in this arena remains as great as it has ever been.

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