

# Neural stem cells as novel therapeutic vehicles for genetic therapy in gliomas

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## SUMÁRIO

**Introdução:** Estudos recentes têm demonstrado, em gliomas (especialmente naqueles de alto grau), a existência de uma pequena fração de células com características de células progenitoras primitivas. Como essas células-tronco neurais são dotadas de potencial migratório significativo para a área patológica de onde se originam, teoricamente, supõe-se que possam ser utilizadas para produção de produtos geneticamente determinados com ação anti-tumoral.

**Materiais e Métodos:** Os autores, após revisão crítica da literatura, apresentam um apanhado geral acerca dos estudos recentes sobre o papel das células-tronco neurais na origem de gliomas malignos, bem como sua possível utilidade como veículos carreadores de genes com ação anti-tumoral.

**Resultados:** Além do seu provável envolvimento na gênese dos gliomas, evidências recentes sugerem que as tais células-tronco modificadas “ex-vivum” seriam capazes de integrar-se no microambiente tumoral de onde se originam, vindo a expressar genes específicos e, cumprindo, desta forma a função de vetores ideais para futuras terapias genéticas. Além disso, a pluripotência destas células-tronco as tornaria capaz de reparar tecidos danificados (como já demonstrado em modelos animais de acidente vascular cerebral isquêmico), modificando assim o microambiente peritumoral.

**Conclusões:** Uma melhor compreensão dos mecanismos pelos quais as células-tronco neurais migram para locais específicos do sistema nervoso central possivelmente possibilitará seu uso como veículos para futuras terapias genéticas para pacientes com gliomas de alto grau. Entretanto, é importante enfatizar que o uso promissor de tais células-tronco modificadas na terapia dos gliomas certamente será, pelo menos em um futuro próximo, somente parte da terapêutica adjuvante, não substituindo, o atual tratamento padrão baseado em cirurgia (com ressecção total sempre que possível), radioterapia e quimioterapia com Temozolamida.

**Palavras-chave:** Células-tronco neurais, terapia genética, gliomas

## ABSTRACT

**Introduction:** Recent studies have demonstrated the existence of a small fraction of glioma cells (specially in high-grade tumors) endowed with features of primitive neural progenitor cells and tumor-initiating function. As such neural stem cells have tremendous migratory potential for pathological areas in the central nervous system, they, theoretically, could be used to produce a desired gene product against glioma tumor cells.

**Material and Methods:** The authors perform a critical literature review in order to highlight recent studies about the role of neural stem cells in the origin of malignant gliomas as well as its possible role as novel vehicles for delivery of targeted genetic therapy approaches.

**Results:** Several recent studies have addressed the possible role of stem cells in the origin of gliomas. Furthermore, recent evidence has also suggested that engraftment of such cells would be followed by integration into the “local neural milieu” and accompanied by stable gene expression, acting, this way, as ideal genetic “vectors”. In addition, the pluripotency of such stem cells would endow them with the capability to replace diseased tissues in an appropriate manner (such as demonstrated in animal models of ischemic stroke).

**Conclusions:** A better understanding of the mechanisms by which neural stem cells migrate to specific sources of injury may allow us to harness these cells as vehicles for delivery of molecular therapies and impact survival in patients with high-grade gliomas. It is important to emphasize, however, that the promising use of neural stem cells for genetic therapy in gliomas, is adjunctive and not a replacement of current standart therapies such as surgery (with maximal possible resection, radiotherapy and chemotherapy (with Temozolamide), which are expected to continue being part of the therapeutic armamentary against gliomas, at least in the near future.

**Keywords:** Neural stem cells, genetic therapy, gliomas, genetic vectors

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## INTRODUCTION

Conventional therapeutic approaches such as surgery, radiotherapy, or chemotherapy have proved rather unsuccessful in the treatment of infiltrative growing tumors such as the malignant glioblastoma multiforme. Thus, new therapeutic strategies that are suitable for inducing cell death also in migrating tumor cells have to be developed. These new therapeutical strategies include cell and/or gene therapeutic approaches<sup>9,10,11,14</sup>.

## STEM CELLS AND THE ORIGIN OF GLIOMAS

Gliomas, in particular the high-grade anaplastic glioma and glioblastoma multiforme, are manifested by morphological, genetic and phenotypic heterogeneity. Most of the studies hitherto have been performed on bulk glioma cells, with limited understanding on the origin and the relative contribution of particular glioma cell populations to glioma growth and progression. Recent studies have demonstrated the existence of a small fraction of glioma cells endowed with features of primitive neural progenitor cells and tumor-initiating function.

Such cells have been defined as glioma stem cells. However, questions remain as to whether the currently identified glioma stem cells are the cell-of-origin for glioma initiation and progression, or the results of such processes<sup>19,7</sup>.

## NEURAL STEM AND PROGENITOR CELLS

Neural stem and progenitor cells derived from the central nervous system (CNS) of embryonic and adult mammals share three critical features: 1. Stem and progenitor cells are highly migratory, 2. Stem and progenitor cells have affinity for areas of CNS pathology, and 3. The pluripotentiality of neural stem and progenitor cells allows them to engraft and replace damaged tissues in the CNS<sup>16</sup>. These properties suggest that transplanted neural stem cells might be used to deliver molecular therapy to diseased regions of the nervous system, and to regenerate lost tissues. One of the greatest challenges and potential promises of stem cell therapy is to direct therapy to pathological tissues comprised of cells which themselves are migratory. The ability of glioma cells to migrate extensively into normal brain parenchyma in part underlies the lethal nature of these tumors.

As neural stem cells (NSCs) are capable of tremendous migratory potential to areas of pathology in the central nervous system. When implanted into a diseased or injured nervous system, NSCs can travel through great distances to and engraft within areas of discrete as well as diffuse abnormalities. Engraftment is often followed by integration into the local neural milieu, accompanied by stable gene expression from the NSCs. In addition, the pluripotentiality of NSCs endows them with the capability to replace diseased tissues in an appropriate manner<sup>1</sup>. (Figure 1) Recent evidence has also suggested that engrafted exogenous NSCs may have effects on the surrounding microenvironment, such as promoting protection and/or regeneration of host neural pathways. These characteristics of NSCs would seem to make them ideal agents for the treatment of various central nervous system pathologies, especially brain tumors<sup>8</sup>. Brain tumors are generally difficult to treat because of the unique location of the lesions. In primary gliomas, the extensive infiltrative nature of the tumor cells presents a challenge to their effective and total eradication, hence the high rate of treatment failure and disease recurrence<sup>2</sup>. In addition, normal brain structures are distorted and are often destroyed by the growing neoplasm. Even with effective therapy to surgically resect and destroy the neoplastic tissues, the brain is still injured, which often leaves the patient in a debilitated state. The unique ability of NSCs to “home in” on tumor cells followed by the delivery of a desired gene product makes the NSC a very promising agent in brain tumor therapy<sup>23</sup>. Cytolytic viruses and genes coding for anti-tumor cytokines, pro-drug converting enzymes, and various neurotrophic factors have all been engineered into engraftable NSCs for delivery to tumors<sup>2,12,13</sup>. When they are specially tagged, such injected NSCs can be visualized with the use of novel imaging techniques and tracked in vivo within living animals over real time. If the NSCs were also capable of participating in the subsequent repair and regeneration of the tumor-afflicted brain-at present a potential but as-yet-unproven aspect of this intervention-then its role in abetting anti-tumor therapy would be complete.

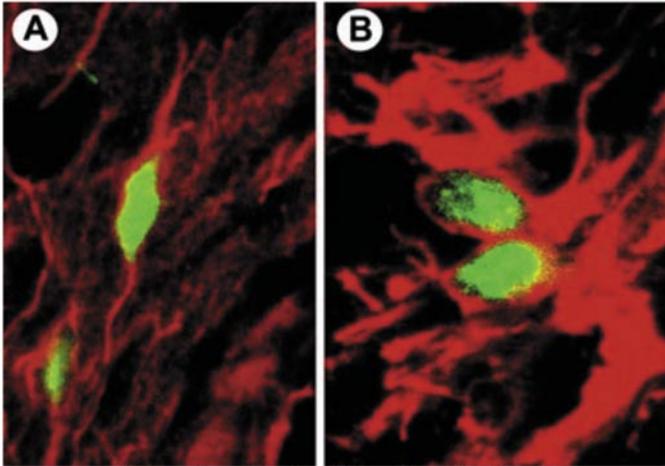


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**Figure 1:** After transplantation into the brains of young mice, the neural precursor cells give rise to functioning neurons (red in A) and astrocytes (red in B).

## STEM CELL AND CENTRAL NERVOUS SYSTEM REPAIR

Closely correlated with the question of whether transplanted stem cells can successfully home 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 it might be expected in the studies of Aboody *et al.*, then the action of the stem cells would be expected to be only 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 the killing of tumor cells and the 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.

## STEM CELL IMAGING TRACKING

Superparamagnetic iron oxide (SPIO) nanoparticles are being used for intracellular magnetic labeling of stem cells and other cells in order to monitor cell trafficking by magnetic resonance imaging (MRI) as part of cellular-based repair, replacement and treatment strategies. Using MRI, new methods for noninvasively tracking grafted neural progenitor cells and bone marrow stromal cells (MSCs) in brain tumor of the rat have been proposed<sup>17,20,21</sup>.

## BONE MARROW STEM CELLS

Bone marrow is an alternative source of stem cells. Human bone marrow-derived stem cells are well suited for clinical application because they are easily obtained from patients and because autologous transplantation, which obviates immunologic incompatibilities, is possible of the various progenitor cells that exist within bone marrow, human mesenchymal stem cells (hMSC) are particularly attractive for clinical use because they are easily isolated, can be expanded into culture, and can be genetically manipulated using currently available molecular techniques. hMSCs are precursors that cause bone marrow stroma by differentiating them into adipocytes, chondrocytes, and osteoblasts. However, MSCs have also proved capable of being differentiated into nonmesodermal tissues, including neurons and astrocytes<sup>5</sup>.

The rationale for using bone marrow-derived stem cells for delivering therapies to brain tumors is based on the developing current concept that bone marrow is a source of circulating stem cells that are recruited from the blood into peripheral solid organs in times of tissue stress or injury. Because microenvironments of solid tumors are similar to the environment of injured/stressed tissue, it is logical to hypothesize that solid tumors may provide a permissive environment for the engraftment of exogenously given hMSCs. In this context, some authors have previously demonstrated that systemically delivered hMSCs are capable of integrating into human tumors grown within the lungs of nude mice<sup>18</sup>. However, the unique features of the microenvironment of the brain and gliomas, including their highly specialized vasculature and glia-derived stroma, led them to evaluate whether brain tumors would also provide a

permissive environment for the selective engraftment of hMSC. Using an intracranial model of gliomas, the same authors have recently shown that hMSCs have a tropism for human gliomas after intravascular and local delivery and that this tropism can be exploited therapeutically by engineering hMSCs to release a soluble antiglioma factor<sup>18</sup>.

The finding that hMSCs localize to human gliomas is of interest because it suggests that the capacity for integration into tumors is an intrinsic property of these stem cells. This observation is consistent with the hypothesis that the intratumoral integration of exogenously delivered hMSCs is a recapitulation of the natural recruitment of endogenous, circulating hMSCs to aid in the process of stroma formation and tissue remodeling and suggests that hMSCs may contribute to the stroma of tumors<sup>3,22</sup>. Human gliomas grown in the brain of nude mice also support the engraftment of hMSCs delivered by an intravascular route. This finding in brain tumors is surprising because the stroma of primary brain tumors is composed of glial/astrocytic cells (ectodermal origin) and is thus distinct from the fibroblast-based (mesenchymal) stroma of most systemic (extracerebral) cancers. However, it has been shown that MSCs are capable of being differentiated into glial cells including astrocytes, and it is thus possible that this property may explain the intrinsic capacity of hMSCs to integrate into the stroma of gliomas<sup>6</sup>.

Alternatively, human gliomas, similar to other cancers, require the elaboration of mesodermal elements, specifically endothelial cells and pericytes. It has been suggested that MSCs are a main source of pericytes within the bone marrow stroma thus, hMSCs may integrate into gliomas to contribute to the mesenchymal elements of the tumor<sup>4</sup>. In support to this concept is the observation that animals bearing U87 xenografts that received hMSC- $\beta$ -gal (i.e., nonsecreting hMSCs) survived for shorter times than did animals who received saline treatments<sup>18</sup>. Thus, hMSCs may localize to tumor under physiologic conditions to assist with tissue repair and in so doing provide a conducive microenvironment to improved tumor growth. Regardless of their physiologic role within tumors these works suggest that hMSCs seem to have the capacity of engrafting themselves into a variety of histologically disparate tumors, including gliomas, and thus may be a cellular vehicle that is universally applicable for delivery of therapeutic agents to most tumor types<sup>15</sup>.

## CONCLUSION

A better understanding of the mechanisms by which neural stem cells migrate to specific sources of injury may allow us to harness these cells as vehicles for delivery of molecular therapies to impact survival in patients with recalcitrant gliomas.

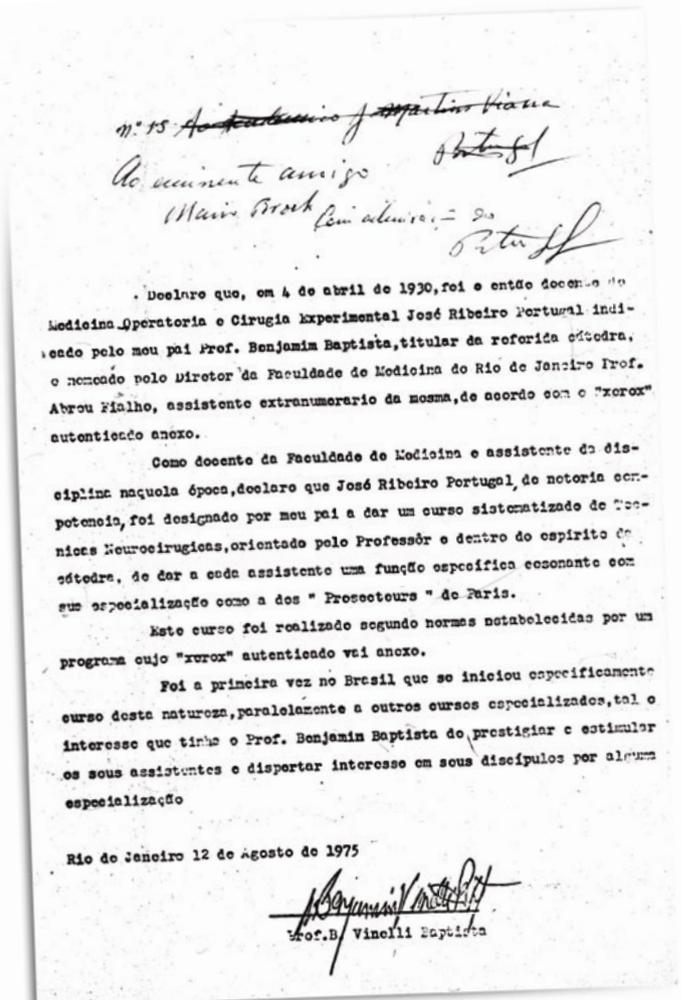
It is important to emphasize, however, that the promising use of neural stem cells for genetic therapy in gliomas, is adjunctive and not a replacement of standard current therapies such as surgery, radiotherapy and chemotherapy, which are expected to remain the most important therapeutic tools in the armamentarium of glioma therapy.

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### Destaque desta edição



**Declaração do Professor Vinelli Baptista** sobre as atividades de José Ribeiro Portugal como docente da Faculdade de Medicina do Rio de Janeiro.

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