

Molecular imaging of stem cell therapy in brain tumors: a step towards personalized medicine

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Advances in gene-based medicine since the 1990s have ushered in a new therapeutic strategy of gene therapy for inborn error genetic diseases and also for cancer [1-6]. Personalized treatment strategies using stem, modified or also genetically engineered cells are becoming a reality in clinical medicine. Allogenic or autologous cells can be used for treatment and possibly for early diagnosis of diseases. Hematopoietic, stromal and organ specific stem cells are under evaluation for cell-based therapies, not only for neurological, but also cardiac, pneumological, gynecological, autoimmune and other disorders. Such cell replacement therapy and gene transfer have provided the basis for the development of potentially powerful new therapeutic strategies for a broad spectrum of neurological diseases. However, currently available treatment modalities for brain tumors, including radical surgical resection followed by radiation and chemotherapy, have substantially improved the survival rates [7, 8]; however, a large proportion of patients with brain tumors remain incurable [9]. Therefore, there is substantial need for more effective therapies for patients with malignant brain tumors, and gene therapy targeting brain tumors should fulfill this requirement. Gene therapy for brain tumors includes many therapeutic strategies and these strategies can be subgrouped in two major categories: (i) molecular and (ii) immunologic. The widely used molecular gene therapy approach is suicide gene therapy based on the conversion of non-toxic prodrugs into active anticancer agents via introduction of enzymes, and genetic immunotherapy involves the gene transfer of immune-stimulating cytokines including interleukin (IL)-4, IL-12 and tumor necrosis factor related apoptosis inducing ligand (TRAIL) [10, 11]. For both molecular and immune gene therapy, stem cells can be used as a delivery vehicle of therapeutic genes [12]. Stem cells possess an inherent tumor tropism and have the capacity to target therapeutic genes to tumors, which supports their use as a reliable delivery vehicle to target therapeutic gene products to primary brain tumors and metastatic cancers throughout the brain [13, 14]. The main promise of this emerging technology centers on the potent migratory tropism exhibited by stem cells for disseminated foci of intracranial pathologic findings. This important characteristic, which has been validated in a wide set of preclinical studies, forms a foundation for the use of transplanted stem cell populations as vehicles for the delivery of tumor-toxic molecules to sites of intracranial tumor [9, 14]. The significance of neuronal stem cell (NSC)-based gene therapy for brain tumor is that it is possible to exploit the tumor-tropic

property of stem cells to mediate effective, tumor-selective therapy for primary and metastatic cancers in the brain and outside, for which no tolerated curative treatments are currently available. Before widely using stem cells in clinical trials, translational research in experimental animal models is needed, with a critical emphasis on developing non-invasive methods for tracking the temporal and spatial homing of these cells to target tissues.

The current development of novel treatment strategies for brain tumors based on the transplantation or infusion of cells that can seek out invading tumor cells demands thorough *in vivo* monitoring [7]. Molecular imaging may allow modification of treatment to maximize treatment efficacy and minimize late effects on chemotherapy and radiotherapy. Especially, stem cells attract great interest as they show a tropism to tumor cells and will even migrate long distances to track down single tumor cells [15, 16]. It is thought that the migration of stem cells to neoplastic cells is mediated by the secretion of chemical factors, such as vascular endothelial growth factor (VEGF) [7, 17], that are involved in the proliferation, growth, and maintenance of tumors. Transplantation of unaltered stem cells has resulted in a prolonged survival of animals with experimental tumors [18], but the insertion of anti-tumor cytokine genes (e.g., IL-12) or proapoptotic genes (e.g., TRAIL) further improves the efficiency of this approach [19]. Little is known about how these cells exert their beneficial effects *in vivo*. Despite contrary evidence from preclinical studies, there is some concern that transplantation of stem cells could further exacerbate tumor formation as there is mounting evidence that brain tumors are potentially caused by a single stem cell that did not differentiate [20]. Targeting oncogenic pathways that are essential to the survival and growth of brain tumor stem cells represents a promising area for developing therapeutics. However, due to the multiple oncogenic pathways involved in brain tumors, it is necessary to determine which pathways are the essential targets for therapy.

The identification of genetic and biochemical mechanisms underlying tumor growth and progression along with the unraveling of the human genome provided a plethora of new targets for cancer detection, treatment and monitoring. Simultaneously, the extraordinary development of a number of imaging technologies, including hybrid systems, allowed the visualization of biochemical, molecular and physiological aberrations linked to underlying mutations in a given tumor. *In vivo* evaluation of complex biological processes such as proliferation, apoptosis, angiogenesis, metastasis, gene expression, receptor-ligand interactions, transport of substrates and metabolism of nutrients in human cancers is feasible by molecular imaging using

positron emission tomography/computed tomography (PET/CT) and radiolabeled molecular probes. Some of these compounds are in preclinical phases of evaluation whereas others have already been applied in clinical settings. Molecular imaging of brain tumors provides a prominent example of how some biological processes and target expression can be visualized by PET/CT in animal tumor models and also in patients with various cancers – such as lung, breast or even brain tumors – for the non-invasive detection of well-known markers of tumor aggressiveness, invasiveness and resistance to treatment and for the evaluation of tumor response to therapy while reducing side effects or even for evaluation of prognosis. Such ability to monitor cell therapy *in vivo* is therefore desirable to potentially provide more control over the activity of stem cells [21, 22]. This strategy is relatively new for brain tumors, but is already well established for other cancers [23]. One possibility is that stem cells will be engineered with a suicide gene [24] that could be activated if transplanted cells do not behave in a therapeutic manner. However, the ability to control the activity of transplanted cells presupposes that it is possible to visualize the presence, location, and activity of stem cells *in vivo*. Although magnetic resonance imaging (MRI) has been extensively used to track cells repeatedly *in vivo* [25], it is the higher specificity of positron emission tomography (PET) ligands and its ability to detect reporter genes that are attractive features to develop long-term *in vivo* monitoring of transplanted cells [26]. This new “image and treat” strategy, involving assessment of target presence and distribution in an individual patient followed by optimized, target-specific drug delivery, may potentially improve efficacy of brain tumors while reducing side effects. However, this strategy is a great step towards a personalized medicine.

The reporter gene approach has also been used with MRI to facilitate iron uptake [27, 28]. The contrast agents used to detect transplanted cells from the background of the brain are incorporated into the cell *in vitro* before transplantation [22, 29]. The presence of the contrast agent inside the cell therefore allows the prolonged visualization of grafted cells on MRI scans. However, the continued presence of the contrast agent inside the cell can also affect cellular functions [25, 30]. Although this approach has been demonstrated with PET/single photon emission computed tomography (SPECT) ligands and allowed monitoring for up to 14 days [31], it is the short half-life of PET ligands that compromises the long-term visualization of cells. A more promising method is to engineer reporter genes inside the cells before grafting and to systemically inject the PET ligands to detect transplanted cells [32]. The use of reporter genes avoids

most issues pertaining to the long-term effects the contrast agent might exert on cellular functions. The repeated application of a contrast agent complements a flexible imaging approach that can target various relevant targets, such as fludeoxyglucose (FDG)-PET to investigate tumor metabolism [29, 33, 34]. Especially for the continued assessment of tumor evaluation in response to treatment, it is important to be able to assess the malignancy of the tumor noninvasively at various time points. The main concerns with this approach regard the potential immunogenic properties of reporter genes that might lead to graft rejection or the down-regulation of the reporter gene abolishing graft detection. Unfortunately, at present little research is dedicated to the development of PET techniques that would allow the continued assessment of cell therapy. The use of radioligands to a reporter gene will provide maximum flexibility and further integration into a wider molecular imaging strategy of brain tumors. Currently, many studies utilize the combination of two or more reporter genes that would enable the use of different imaging modalities to overcome the drawbacks associated with a single reporter gene and/or associated detection system [35].

Apart from *in vivo* monitoring, PET will also be instrumental to gain a greater mechanistic understanding of how cell therapy exerts its therapeutic effects. As stem cells can be engineered to express particular genes and serve as Bsmart delivery vehicles [36], their effects on tumor cells could be through inhibition of angiogenesis, induction of apoptosis in tumor cells, or induction of differentiation of tumor cells [37, 38]. Being able to study how stem cells will alter the molecular composition of the tumor will allow *in vivo* monitoring of the therapeutic efficacy of cell therapy. Additionally, PET imaging allows the serial *in vivo* assessment of the inflammatory and immunological response by visualizing, for instance, a T-cell response [39]. Modulation of the immune response could provide additional benefit to combat tumor cells. The PET imaging is at present the only *in vivo* technology that can assess these various molecular aspects of stem cell efficacy and lead to a mechanistic understanding of how stem cells attack tumor cells.

Molecular imaging by PET is already used clinically to evaluate stem cell therapy for different neurological diseases besides the classical brain tumors [40]. Interesting in this context are patients with primary central nerve syndrome (CNS) lymphoma under the age of 60 years: high dose chemotherapy with autologous stem cell therapy represents one of the first-line treatment options in this subpopulation [41]. Additionally, simple FDG-PET leads to a 92% positive and 88% negative prediction of treatment outcome and correlates more strongly with disease-free survival in cerebral lymphoma

than computed tomography [42]. The FDG-PET can therefore already serve an exquisite role in patient selection and help to determine which conditions are most suitable for stem cell therapy [43, 44]. Serial quantitative PET will provide a means to monitor treatment progression [45] and afford a refinement of dosage and integration with other therapies.

Imaging of brain tumors already commonly uses FDG-PET. Translation of cell therapy for brain tumors will be best suited in conditions where imaging technology can already provide a robust baseline assessment that would allow patient selection. As the clinical translation of stem cell therapy for brain tumors progresses [45], preclinical validation studies will need to rely more on molecular imaging to ensure the safety and efficacy of this promising therapy. Growing sophistication of molecular imaging will allow increasingly sophisticated therapeutic approaches to be envisaged and monitored *in vivo* [46-49]. The multitude of therapeutic effects that can be exerted by unaltered and genetically engineered stem cells will need to be determined in terms of their efficacy in relation to the molecular composition of the tumor. Being able to select patients at an early stage based on a molecular signature of the entire tumor will allow clinicians to determine what strategy might be best suited to remove the neoplastic cells.

With increasing focus on the advance towards (more) curative solutions in brain tumor therapies, it is hard not to be excited by the potential of stem cell-based therapies. However, the efficacy of stem cell therapy continues to remain in question. Initial clinical trials have focused on evaluation of multiple adult stem cell phenotypes in their unaltered, native state as a "first generation" resource for repair. Though significant strides in perfecting delivery of these biologics to the diseased brain have been achieved, the benefits with regard to cerebral functional recovery have been modest at best. There is a clear need for robust genetic characterization before widely using the above described clinical stem cell therapy approaches. We have to be certain that the genome of the starting material is stable and normal, but the limited resolution of conventional karyotyping is unable to give us such assurance. Advanced molecular cytogenetic technologies and single nucleotide polymorphism analysis should be introduced [50]. As seen with pharmacotherapeutics in the last century, successful translation of "second generation" biotherapeutics in the 21st century will require close integration of a community of practice and research to ensure broad application of this emerging technology in the treatment of brain tumors.

Developments in molecular imaging will therefore provide the diagnostic framework of a molec-

ular medicine of brain tumors [12]. This is of special importance as, despite exciting initial reports, clinical potency of stem cell therapy in animal brain tumor models has to date proven disappointing. Attempts to extrapolate the animal study results to humans are stymied by the fact that stem cells are heterogeneous, resulting in differences in their efficacy. Indeed, therapeutic success relies on an effective strategy to select for a stem cell sub-population within some particular stage of the development at which they are competitive and capable of targeting brain tumors. Molecular imaging may help to better understand the specific tropic mechanisms that govern stem cell migration toward invasive tumors and the need to identify appropriate tissue sources and culture processes for the generation of adequate therapeutic stem cell populations. Despite all these current limitations, the use of stem cells as vectors for the treatment of brain tumors holds significant promise and may prove to be an important therapeutic modality for patients with malignant brain tumors. In combination with stem cells, molecular imaging may help to revolutionize a personalized, molecularly targeted medicine.

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