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Crossing the Boundaries: Stem Cells and Gene Therapy

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Abstract

Oncolytic virotherapy is an emerging therapeutic modality for the treatment of cancer. It entails construction of viruses with the ability to selectively target and lyse tumor cells. This branch of therapy has significantly advanced in the past decade, heralded by the development of several novel viruses. Despite the initial success of oncolytic virotherapy in the preclinical setting, however, this modality remains hindered by several obstacles. First, failure to achieve effective viral delivery to targeted tumor beds is a well known limitation. Second, the virus-neutralizing mechanisms of the host immune system, which are in place to protect from viral pathogens, may also hinder the therapeutic potential of virotherapy. One approach to tackling these shortcomings is the use of a cell-based carriers to both help with delivery of the virus and shield it from immunosurveillance. Stem cells have recently surfaced as a potential cell-based candidate for delivery of virotherapy. Their unique migratory and immunosuppressive qualities have made them an exciting avenue of investigation. The focus of this review is to discuss the benefits of stem-cell-based delivery of oncolytic virotherapy and its role in cancer treatment.

Oncolytic Virotherapy

Cancer is a complex genetic disease that involves alteration in multiple molecular pathways including those that govern cell proliferation and programmed cell death. Increasing knowledge of the molecular mechanisms underlying oncogenesis have initiated the development of novel approaches to cancer therapy in order improve patient lifestyle and increase patient survival. Among these approaches, oncolytic virotherapy has emerged as an exciting potential therapeutic modality. Oncolytic virotherapy entails the engineering of viruses that specifically infect and lyse tumor cells, while sparing normal ones. The majority of the viruses currently under investigation are conditionally replicating in nature (Heise et al., 1999). Tumor cell infection results in viral replication and ultimately release of viral progeny; facilitating viral spread to neighboring neoplastic cells and enhancing distribution throughout the targeted tumor bed.

Achieving virus-tumor specificity is one of the hallmarks of oncolytic virotherapy. It is typically accomplished by exploiting the difference in the molecular makeup between tumor cells and their normal counterparts. For example, aberration in the p53 pathway is a well known molecular origin of tumorgenesis. One of the earliest oncolytic viruses used in clinical trials for intracranial glioma, ONYX –015 (dl1520), was engineered only to replicate in cells with a defective p53 pathway. This specificity was accomplished via deletion of a specific viral genomic region (Bischoff et al., 1996; Chiocca et al., 2004). Another approach to achieve tumor-selective replication involves expressing viral genes under promoters that are only functional in tumor cells. Survivin is an example of a tumor-specific promoter whose presence has been confirmed in a variety of cancer types. The discovery of this protein has led to the

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development of tumor-selective oncolytic adenoviruses that have shown promise in pre-clinical studies (Tyler et al., 2009).

Limitations of oncolytic virotherapy

Since its introduction, tremendous advances have been made in the field of oncolytic virotherapy. Several viruses have since been engineered and investigated in animal models and some have even advanced to clinical trials (Chiocca et al., 2004). Despite initially promising results, this treatment modality still faces limitations. First, efficient viral delivery and distribution at sites of tumor growth are significant challenges. Second, host immune-mediated virus-neutralizing mechanisms are also a barrier inhibiting viral infection of tumor cells. One potential strategy to address these obstacles proposes the use of a cell-based carrier as a method of chaperoning oncolytic viruses to targeted regions. Given their unique properties, stem cells have emerged as a cellular candidate for the task of enhancing viral delivery and ultimately maximizing the therapeutic potential of oncolytic virotherapy.

What are stem cells and how can they help?

The process of cell proliferation and differentiation into various cell lineages is a highly specific and regulated chain of events. The earliest cell type in this process is referred to as a stem cell. Stem cells have several unique properties including the ability to give rise to different cell types (multipotential) and the capacity for self-renewal (Minguell et al., 2001). There are several classes of stem cells, categorized by their origin. With regard to cell-based viral delivery systems, two classes of stem cells are most commonly described: neural and mesenchymal. Neural stem cells (NSCs) are derived from fetal, neonatal, or postnatal tissues. These multipotential cells give rise to the specialized cells of the central nervous system (CNS) such as neurons, astrocytes and oligodendrocytes (Gage, 2000). Mesenchymal stem cells (MSCs), mainly derived from bone marrow, are also mulitpotent cells. These cells differentiate into a variety of cells destined to become mesenchymal tissues, including osteoblasts, chondrocytes and adipocytes (Minguell et al., 2001).

Enhancement of viral delivery

In addition to the above mentioned characteristics, it has recently been discovered that stem cells exhibit tropism for neoplasms. The ability of stem cells to migrate to tumors is central to their utility as carriers of oncolytic viruses. The inefficiency of viral distribution is a known obstacle in virotherapy. It has been demonstrated that after injection, viral vectors spread only a short distance from initial injection sites and often do not reach sites of distant tumor spread. This relative inadequacy of viral spread may relate to several factors including large viral particle size and cell-to-cell barriers. Also, virus spread through a tumor cell mass can be limited by necrotic regions and fibrosis (Heise et al., 1999; Vile et al., 2002). Due to the infiltrative nature of malignant neoplasms this drawback is paramount.

Many of the early preclinical studies investigating the tumor-homing capability of stem cells were performed in intracranial glioma models. In their landmark study on NSCs, Aboody and colleagues demonstrated the ability of stem cells to migrate toward tumor cells *in vitro* and *vivo* (Aboody et al., 2000). In culture, NSCs migrated more rapidly to glioma cells compared to control fibroblasts. To determine the effect of intracranial pathology on NSCs activity, rats were implanted with brain tumors into their right frontal lobe. Following tumor implantation, fluorescently labeled-NSCs were injected into the normal, contralateral hemisphere. A few days following implantation, NSCs were observed migrating across the corpus callosum, toward the tumor and ultimately incorporating into the tumor bed (Aboody et al., 2000). This study was one of the earliest and most compelling to show stem cell migration toward a malignancy. These robust results were duplicated with MSCs. In a 2005 study using mice

bearing intracranial malignant glioma xenografts, MSCs that were injected intravascularly (via carotid artery) were observed to selectively target tumor following systemic administration. Furthermore, migration was independent of whether MSC injection was performed in the carotid ipsilateral or contralateral to the implanted tumor (Nakamizo et al., 2005).

The ability of stem cells to seek out tumor foci is not limited to intracranial pathologies. In fact, this phenomenon has been well described in the literature for a variety of malignancies. For example, using a murine model of breast cancer pulmonary metastases, Loebinger and authors hypothesized that MSCs labeled with iron nanoparticles could facilitate tracking of stem cell migration *in vivo*. They found that after systemic administration of iron-labeled MSCs, the authors were able to reliably track MSC homing to pulmonary metastases using magnetic resonance imaging (MRI) (Loebinger et al., 2009).

The biological mechanisms governing stem cell migration are the subject of intense research, but have yet to be fully characterized. It is speculated that stem cells are attracted to chemokines produced in a tumor mircoenviroment. Several specific factors have been investigated in the past few years. For example, Schichor and colleagues investigated the role of vascular endothelial growth factor A (VEGF-A) in stem cell migration. In *vitro*, these authors observed a 30% increase in stem cell migration after the addition of VEGF-A to a cell migration assay (Schichor et al., 2006). A recent study examined the role of platelet-derived growth factor BB (PDGF-BB) in MSC tropism. PDGF has previously been implicated in glial tumorgenesis (i.e. increased tumor cellularity and necrosis) and angiogenesis. PDGF-BB is in the PDGF family and binds both PDGFα and β. Cheng and authors demonstrated that PDGF-BB significantly increased the migration of MSCs towards C6 gliomas in their in *vitro* model (Cheng et al., 2009).

Even though the factors promoting stem cell-tumor homing are not completely deciphered, the migratory capacity of stem cells is a remarkable tool. To exploit this ability, stem cells (both neural and mesenchymal) have been used as vehicles for the delivery of targeted therapies. As mentioned above, replication competent oncolytic viruses have emerged as a potential therapeutic modality for the treatment of cancer. In this regard, stem cells, with their proven tumor-tropism, may be able to assist virus delivery.

Immune system evasion

The clinical outcome of virotherapy is not only dependent upon delivery but also the three-way interaction between the therapeutic virus, the tumor microenvironment, and the host immune system. Our immune system does not have the sophistication to distinguish between malicious pathogens and therapeutic virus. The naturally protective actions of the host immune system may potentially limit the efficacy of virotherapy. The anticipated ability of the host immune response to restrict oncolytic virus activity has been demonstrated in both preclinical (Fulci et al., 2006) and clinical studies (Chiocca et al., 2004). For example, in a rat glioma model, Fulci and authors found that the anti-tumor activity of an oncolytic herpes simplex virus (HSV) was significantly inhibited by the infiltration natural killer cells, macrophages and microglia (Fulci et al., 2006).

In order to replicate, oncolytic viruses must produce viral proteins intracellularly. These proteins can be presented on major histocompatability complex (MHC) proteins as viral antigens, attracting different antigen presenting cells and subsequently activating cytotoxic T lymphocytes to eradicate intracellular therapeutic virus. Attempts have been made to modulate host immune responses in order to enhance the oncolytic activity of the therapeutic virus. In the above mentioned study, Fulci et al. also showed that animals with brain tumors treated with a HSV-derived oncolytic virus survived significantly longer after pre-treatment with an immunosuppressive agent (cyclophosphomide) (Fulci et al., 2006).

Another attractive quality possessed by stem cells is their ability to act as an immunosuppressant. Although the precise mechanism by which stem cells suppress the immune system remains to be elucidated, the most commonly proposed pathways are: 1) direct suppressive activity toward T-cell activation and proliferation, 2) promotion of apoptosis of type 1 T-helper cells and 3) inhibition of the release of proinflammatory cytokines (Jones and McTaggart 2008). To the authors' knowledge, the earliest demonstration of stem cell-mediated immunosuppression was in a rat experimental autoimmune encephalomyelitis (EAE) model (Einstein and Ben-Hur, 2008). In this model, transplanted NSCs were shown to elicit an anti-inflammatory response thereby reducing brain inflammation and the disease's clinical severity. This was thought to be accomplished via NSC promotion of T-cell apoptosis and shifting the inflammatory process in the brain toward a more favorable type-2 T helper response. *In vitro* studies have demonstrated that MSCs are also immunosuppressive. For example, recently, Mader et al. have demonstrated that MSC cell carrier protect oncolytic measles viruses from antibody neutralization in an orthotopic ovarian cancer model (Mader et al., 2010).

This stem cell-mediated immunosuppression adds to its novelty as a cell carrier given that it will allow therapeutic viruses to be hidden from host immunosurveillance. They may also suppress the local inflammation during virotherapy, thus allowing the oncolytic virus to replicate and kill tumor cells without any immune restriction. These observations bode strongly for the use of stem cells as a platform of cell carrier to circumvent the antiviral immune response in oncolytic virotherapy.

Preclincal Studies: Stem cells and Oncolytic Virotherpay

One of the earliest studies to actually use stem cells as a vehicle for the delivery of oncolytic virotherapy was performed by Komarova et al. (2006). These authors hypothesized that MSCs could be used as a targeting strategy for oncolytic adenovirus in the treatment of disseminated ovarian cancer (Komarova et al., 2006). This innovative study demonstrated several points regarding the use of cellular carriers (in this case MSCs) in the delivery of an oncolytic adenovirus. First, they confirmed that MSCs were permissive to viral infection and supported viral replication. This property of MSCs is critical in their use as an intermediate carrier of a viral-based therapy package. Second, virus-loaded MSCs showed robust oncolysis of ovarian cancer cells *in vitro*. Third, MSCs transduced with adenovirus, maintained their ability to migrate to established ovarian tumors after systemic administration *in vivo*. Lastly, animals bearing ovarian tumors displayed improved survival compared to animal treated with virus alone (Komarova et al., 2006). This impressive data demonstrated that stem cells could be used as vehicles for targeted oncolytic virotherapy; introducing a new and exciting treatment paradigm for cancer treatment. The promising results described in the above study have been replicated in pre-clinical studies for a variety of tumors. Stoff-Khalili and authors examined MSC delivery of an oncolytic adenovirus in an animal model of breast cancer metastasis to the lung. These authors were successful in demonstrating the ability of conditionally-replicating adenovirus (CRAd)-loaded MSCs to track and kill breast cancer pulmonary metastases *in vivo*. Additionally, systemic treatment with virus-loaded MSCs ultimately led to improved survival of animals burdened with breast cancer lung metastases compared to control animals (Stoff-Khalili et al. 2007).

Intracranial gliomas, which are the focus of the authors' current research, have also been a target for stem-cell based oncolytic virotherapy. Malignant gliomas pose a unique therapeutic challenge for several reasons. First, complete surgical debulking is not feasible due to gliomas' propensity for diffuse infiltration and dissemination away from the primary tumor bed. Additionally, the presence of eloquent brain can limit the extent of surgical resection. Second, the presence of the blood brain barrier (BBB) significantly limits the effect of systemic chemotherapy due to restricted CNS penetration. There have been several notable pre-clincal

studies utilizing stem-cell based delivery of oncolytic viruses in glioma models. Sonabend and authors evaluated the ability of MSCs to deliver a conditionally replicative adenovirus (CRAd) to glioma in a murine model. The authors observed that tumors collected from animals treated with virus-loaded MSCs displayed higher adenoviral infection compared to animals treated with virus alone. The authors were able to quantify this difference by assessing viral replication. They demonstrated a 46-fold increase in viral copies within glioma tissue treated with virus-loaded MSC compared to tumors treated with virus alone. This was the first study to confirm the ability of MSCs to transport oncolytic virotherapy in the setting of malignant glioma (Sonabend et al., 2008). Similarly positive results have also been reported using viral-loaded NSC to treat intracranial bearing mice (Tyler et al., 2009). These authors found that virus-loaded NSCs also achieved enhanced viral gene throughout harvested intracranial tumors compared with virus alone. Additionally, NSCs loaded with CRAd also reduced tumor growth in mice subcutaneously with glioma cells (flank tumor model). Tumors receiving virus-loaded NSCs showed an overall reduction in mean tumor volume compared to tumors treated with virus alone (69.4 mm versus 138.1 mm) (Tyler et al., 2009).

Conclusions

Oncolytic virotherapy has made significant strides in the past decade. However, optimization of its delivery to targeted areas is needed in order for its full therapeutic potential to be met. Although this modality has its challenges; novel solutions have been proposed in order to addresses its limitations. Cell-based delivery offers an opportunity to overcome some of the hurdles encountered in oncolytic virotherapy delivery. The ideal cell carrier must: 1) possess the ability to localize to tumors, 2) be amenable to viral infection, replication and progeny production and 3) effectively avoid immune system degradation (Raykov et al., 2004). Given their confirmed tumor-tropism and their ability to help evade the protective mechanisms of the immune system, stem cells may prove to be a novel and valuable cell-based carrier for virotherapy. Despite the fact that the data is still in the pre-clinical stages, the compelling results thus far warrant further investigation. In combination with novel treatment modalities, such as oncolytic virotherapy, stem-cell based delivery provides a unique opportunity for the development of targeted cancer therapies.

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