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Non-virally engineered human adipose mesenchymal stem cells produce BMP4, target brain tumors, and extend survival

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ABSTRACT

There is a need for enabling non-viral nanobiotechnology to allow safe and effective gene therapy and cell therapy, which can be utilized to treat devastating diseases such as brain cancer. Human adipose-derived mesenchymal stem cells (hAMSCs) display high anti-glioma tropism and represent a promising delivery vehicle for targeted brain tumor therapy. In this study, we demonstrate that non-viral, biodegradable polymeric nanoparticles (NPs) can be used to engineer hAMSCs with higher efficacy (75% of cells) than leading commercially available reagents and high cell viability. To accomplish this, we engineered a poly(beta-amino ester) (PBAE) polymer structure to transfect hAMSCs with significantly higher efficacy than Lipofectamine™ 2000. We then assessed the ability of NP-engineered hAMSCs to deliver bone morphogenetic protein 4 (BMP4), which has been shown to have a novel therapeutic effect by targeting human brain tumor initiating cells (BTIC), a source of cancer recurrence, in a human primary malignant glioma model. We demonstrated that hAMSCs genetically engineered with polymeric nanoparticles containing BMP4 plasmid DNA (BMP4/NP-hAMSCs) secrete BMP4 growth factor while maintaining their multipotency and preserving their migration and invasion capacities. We also showed that this approach can overcome a central challenge for brain therapeutics, overcoming the blood brain barrier, by demonstrating that NP-engineered hAMSCs can migrate to the brain and penetrate the brain tumor after both intranasal and systemic intravenous administration. Critically, athymic rats bearing human primary BTIC-derived tumors and treated intranasally with BMP4/NP-hAMSCs showed significantly improved survival compared to those treated with control GFP/NP-hAMCSs. This study demonstrates that synthetic polymeric nanoparticles are a safe and effective approach for stem cell-based cancer-targeting therapies.

1. Introduction

Over 20,000 new cases of malignant brain cancer are diagnosed in the United States every year, causing nearly 15,000 deaths each year [1,2]. The most common of malignant brain tumors are glioblastoma (GBM), a grade IV astrocytoma with an estimated 5% five-year survival rate. Even with the current gold standard of treatment, including surgery, chemotherapy, and radiotherapy, GBM patients have a median survival of approximately 15 months [3–5]. This dire prognosis, which has improved only incrementally in the

Abbreviations: NP, nanoparticle; hAMSC, human adipose-derived mesenchymal stem cell; PBAE, poly(beta-amino ester); BMP4, bone morphogenic protein.

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last decades, prompts the development of novel treatment strategies. Among the challenges of GBM treatment is the difficulty of drug delivery to the tumor site without significant adverse side effects, particularly when using a systemic route of administration [6]. For efficacy, a therapeutic must reach the brain in sufficient concentration after crossing the blood-brain barrier (BBB) [7], be able to diffuse [8] or otherwise move through the brain parenchyma [9,10], and ideally have an effect on cancer cells instead of healthy cells, including malignant cells that invade the tissue surrounding the main tumor site [11].

One intriguing method of targeted delivery to brain tumors is the use of adult stem cells as vehicles that can carry a therapeutic payload [12,13], with mesenchymal stem cells (MSCs) among the most attractive candidates for cell-based therapy. MSCs can cross the BBB and have been shown to have innate tumor tropism, and their low immunogenicity also enables transplantations of both autologous and heterologous MSCs [14]. After being genetically engineered, they can secrete therapeutic agents directly into the tumor [15,16], and such engineered stem cells have already produced impressive results in preclinical glioma models [17]. MSCs have been successfully used as brain tumor-targeting carriers of prodrug/drug systems [18,19] or cytokines and proteins such as IFN-beta [20], tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) alone or in combination with radiation or temozolomide [16,21,22], and bone morphogenic factor 4 (BMP4), a growth factor known to decrease the tumorigenicity of brain tumor initiating cells (BTICs) [23,24]. We have previously reported that human adipose-derived mesenchymal stem cells (hAMSCs) display brain tumor tropism, can be successfully used as brain tumor-targeting carriers, and have antiglioma effects *in vivo* when genetically engineered to deliver BMP4 to target BTICs [23,25–28]. The hAMSCs were also shown to be non-oncogenic, and they did not proliferate *in vivo*, instead decreasing in number after 2 weeks, reducing the chance of adverse effects due to the cells. Viral vectors have thus far been the most widely used method of engineering hAMSCs to carry tumor-targeting proteins and are well known to have high gene delivery efficiency. However they carry the risk of immunotoxicity related to viral proteins or replication, which can cause a decrease in the gene delivery efficiency because of containment by host antibodies [29]. Moreover, viral antigens may activate latent viruses and cause inflammatory responses [30] or facilitate autoimmunity, leading to demyelination [31] or neurodegeneration [32,33]. In addition, foreign promoters inserted into virus genomes rarely behave as they would in their natural genomic setting, and virus tropism is highly dependent on host cell permissiveness at the transcriptional level. Still, the wide use of these vectors in the human population has inspired general confidence on their relative safety [29]. However, the unsatisfactory results of the first large-scale phase III trial of gene therapy for brain tumors [34] have again raised questions about the level, tropism and consistency of virus titers in the brain [35], resurrecting safety concerns related to the use of virus at high titers and the need for a different option. In this era, when the application of nanotechnology is driving tremendous progress in medicine, research on a safer and equally effective non-viral option among nanoparticle vectors has become an appealing answer to this challenging question.

Moreover, an alternative strategy to viral transduction, such as non-viral transfection by synthetic nanoparticles (NPs), could lead to the safe application of MSC-based therapies across a broad spectrum of diseases and indications. Non-viral vectors carry low risk of insertional mutagenesis, are not restricted by plasmid size, and can be produced more quickly and easily than viral vectors. However, common non-viral materials used for gene transfer, including lipids such as commercially available Lipofectamine™ 2000 and synthetic polymers such as polyethylenimine are often limited by cytotoxicity and low efficacy [36–39]. In this study, we used poly(beta-amino ester)s (PBAEs), biodegradable cationic polymers with significant potential for non-viral gene delivery. Easily synthesized and rapidly screened [40,41], PBAEs self-assemble into NPs with nucleic acids via electrostatic interactions, enabling effective gene delivery of different types of human cell lines with high efficacy and low cytotoxicity [42,43].

We used reporter genes to demonstrate very high non-viral transfection of hAMSCs with PBAE NPs (75 ± 2% positive cells by flow cytometry). Next, we genetically engineered hAMSCs to produce BMP4 in order to target BTICs in malignant gliomas. We also evaluated the effect of non-viral transfection, as compared to viral transduction, on the migration, invasion, and multipotency of hAMSCs and found that our synthetic gene delivery devices had a relatively benign effect on cells. In addition, we provide *in vivo* evidence that NP-engineered hAMSCs administered locally and systemically in a rodent glioma model retain their intrinsic tumor-homing efficiency by migrating towards the brain and penetrating the tumor, and hAMSCs engineered to secrete BMP4 significantly increased survival in BTIC-bearing rats. To our knowledge, this is the first study showing that *in vivo* administration of hAMSCs genetically engineered with PBAE nanoparticles has a significant therapeutic effect in a human malignant glioma model. These biodegradable synthetic nanoparticles are a safe and efficient alternative to viral transduction and engineering of human stem cells that can be applied to a wide array of diseases.

2. Materials and methods

2.1. Experimental design

Poly(beta-amino ester)s (PBAEs) were complexed with DNA plasmids and used to transfect human adipose mesenchymal stem cells (hAMSCs) to identify the optimal formulations for transfection efficacy and cell viability (Scheme 1A). *In vitro* assays were used to measure the amount of target protein BMP4 that was produced by transfected cells and to assess the effect of non-viral transfection on the migration, invasion, and differentiation characteristics of hAMSCs (Scheme 1B). The ability of NP-transfected hAMCSs to migrate towards brain tumors was evaluated *in vivo* after systemic and local administration (Scheme 1C). Finally, hAMSCs engineered to express BMP4 were shown *in vivo* to have a significant effect on survival in a human primary malignant glioma model (Scheme 1D).

2.2. Polymer synthesis

PBAEs were synthesized using a two-step procedure, as previously described [44]. First, one diacrylate backbone diacrylate monomer (B3, B4, or B5) was combined with one amine sidechain (S3, S4, or S5) by conjugate addition at 90 °C for 24 h at a 1.1:1 or 1.05:1 acrylate-to-amine (B-to-S) molar ratio. Next, the resulting diacrylate-terminated neat B-S polymer was dissolved in anhydrous tetrahydrofuran (THF) and combined with end-cap amine-containing small molecules (E6 or E7) in anhydrous THF (Fig. 1). The final polymer solution was stirred for 1 h at room temperature, then precipitated into diethyl ether to remove solvents and unreacted small molecules. The polymer was collected and washed with excess ether. The ether was then decanted, and the polymer was dried under vacuum for 48 h. The neat polymer was then dissolved in anhydrous dimethyl sulfoxide (DMSO) at a concentration of 100 mg/mL and was stored at −20 °C until use.

The molecular weight of top polymers was measured by gel permeation chromatography (GPC; Waters, Milford, MA) in BHT-stabilized tetrahydrofuran with 5% DMSO and 1% piperidine.