Biomaterials 32 (2011) 5402–5410

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Non-viral gene delivery nanoparticles based on Poly(β-amino esters) for treatment of glioblastoma

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ARTICLE INFO

Article history:
Received 23 March 2011
Accepted 5 April 2011
Available online 4 May 2011

Keywords:
Drug delivery
Gene therapy
Nanoparticle
Stem cell
DNA
Cancer

ABSTRACT

Glioblastoma (GB) is currently characterized by low survival rates and therapies with insufficient efficacy. Here, we describe biodegradable polymers that can deliver genes to primary GB cells as well as GB tumor stem cells *in vitro* with low non-specific toxicity and transfection efficiencies of up to 60.6 ± 5% in normal (10%) serum conditions. We developed polymer-DNA nanoparticles that remained more stable in normal serum and could also be stored for at least 3 months in ready-to-use form with no measurable decrease in efficacy, expanding their potential in a practical or clinical setting. A subset of polymers was identified that shows a high degree of specificity to tumor cells compared with healthy astrocytes and human neural stem cells when cultured (separately or in co-culture), yielding higher transfection in GB cells while having little to no apparent effect on healthy cells.

1. Introduction

Approximately 184,300 new central nervous system (CNS) tumors are diagnosed annually, with 50,000 of those originating in the CNS and the rest being metastases from other tumors [1–5]. Malignant gliomas are the most common primary brain tumor and are refractory to combinatorial surgical resection, radiation, and chemotherapy [6–8]. These tumors possess the ability to extensively invade surrounding tissue, making curative resection impossible [9–11]. As a result, experimental treatments like gene therapy are attractive for GB patients. Despite the vast potential of gene therapy, viral methods are plagued with safety concerns [12], while non-viral delivery vehicles often lack efficiency [13]. A high-throughput screening approach based on a synthetic library of cationic poly(β-amino esters) (PBAEs) has led to high transfection efficiency and low toxicity in many cell types [14,15], though they have not previously been tested in brain tumors. Hundreds of these polymers can be synthesized by combinatorial chemistry, and many cause superior transfection compared to leading commercial agents like Lipofectamine™ 2000 (Invitrogen), while also being less toxic to cells [16].

Also important for eventual clinical use is the stability of the polymer-DNA nanoparticles, as well as their ease of preparation. Because PBAEs are electrostatically complexed with DNA in slightly acidic, aqueous suspension, the particles have rapid release properties due to hydrolytic degradation of the polymers. While these release properties are advantageous for intracellular gene delivery, they are not conducive for practical use by clinicians or large-scale production. It is therefore necessary to develop a way of preparing a dried form of the polymer-DNA nanoparticles that can be stored for long periods of time and easily reconstituted just before use. However, increased aggregation is a complication for colloidal suspensions that are dried without a lyoprotectant [17], and an effective way of lyophilizing and storing these particles is essential for eventual translation of this technology.

We sought to apply and improve upon previously validated methods of non-viral gene delivery for the treatment of primary human brain tumor cells, including the identification of efficacious reagents and the development of more practical and easily-used formulations. Aside from the malignant astrocytes that comprise the bulk of a GB tumor, we are also interested in brain tumor stem cells (BTSCs). BTSCs are hypothesized to be the source of GB tumors

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doi:10.1016/j.biomaterials.2011.04.016

and to play a role in the inevitable tumor recurrence following surgery, chemotherapy, and radiotherapy [18–21]. Aside from being resistant to more types of therapy than astrocytes [22], BTSCs can cause regrowth of an entire tumor at low cell concentrations [23], which the malignant astrocytes alone cannot do [20,21]. The persistence of BTSCs despite therapy, as well as their tumorigenic capacity, make them a particularly good target of experimental therapies like gene delivery. A comparison of the transfection efficiencies in GB astrocytes, BTSCs, and healthy (non-cancerous) versions of both is of interest, since we have recently found that cell-type specificity can be determined simply by the polymer structure itself [15].

2. Materials and methods

2.1. Materials

Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA), FuGENE® HD (Roche), Opti-MEM I (Invitrogen), pEGFP-N1 DNA (Elim Biopharmaceuticals, Hayward, CA), pDsRed-Max-N1 [24] and FUGW [25] (Addgene DNA plasmids 21718 and 14883, respectively, Cambridge, MA), and cell culture media components were used as received. *Label* IT® Tracker Cy3 Kit was purchased from Mirus Bio. 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) was purchased from Sigma (Saint Louis, MO) and used as a 750 nM solution in PBS.

2.2. Cell culture

All intraoperative samples were obtained as described previously under institutionally approved protocols. Human BTSCs (BTSC line 551) was isolated from a GB tumor sample of an adult patient (69 years old) diagnosed with GB, as previously described [26]. Briefly, necrotic tissue and blood vessels were removed from the tumor, which was dissociated mechanically and with trypsin/EDTA. After inhibition of trypsin with 1:1 DMEM/F-12 (Invitrogen) with 10% heat-inactivated FBS and 1% antibiotic–antimycotic (anti–anti, final concentration 1X, Invitrogen), the cells were collected by centrifugation, and the serum-containing medium was replaced with BTSC neurosphere medium [1:1 DMEM/F-12, 2% B-27 serum-free supplement (B-27, final concentration 1X, Gibco, Bethesda, MD), 1% anti–anti, 20 ng/mL basic fibroblast growth factor (bFGF, Invitrogen), and 20 ng/mL epidermal growth factor (EGF, Sigma, Saint Louis, MO)]. A GB astrocyte cell line (GB 319) was derived from BTSCs isolated as above (from a 79-year-old adult GB patient). After isolation, they were maintained in astrocyte medium with serum (1:1 DMEM/F-12, 10% FBS, 1% anti–anti) as adherent cultures.

Human fetal neural stem cells (NSCs) (F34) were derived after 17 weeks of gestation, obtained from elective abortion. Brain cortical tissue was mechanically dissociated and cells were maintained in neurosphere medium consisting of 2:1 high-glucose DMEM with L-glutamine (Invitrogen)/Ham's F-12 (Cellgro), 1× B-27, 1% anti–anti, 20 ng/mL bFGF, 20 ng/mL EGF, 20 ng/mL leukemia inhibitory factor (LIF, Millipore, Billerica, MA), and 5 μg/mL heparin (Sigma)]. BTSC 551 was stably transduced with EGFP using the lentiviral vector FUGW. Cells were incubated with the virus overnight in the presence of 8 μg/mL polybrene, then sorted by FACS. The GFP^+ 551 BTSCs were maintained in neurosphere medium in nonadherent flasks and passaged approximately every 10 days by mechanical dissociation. For

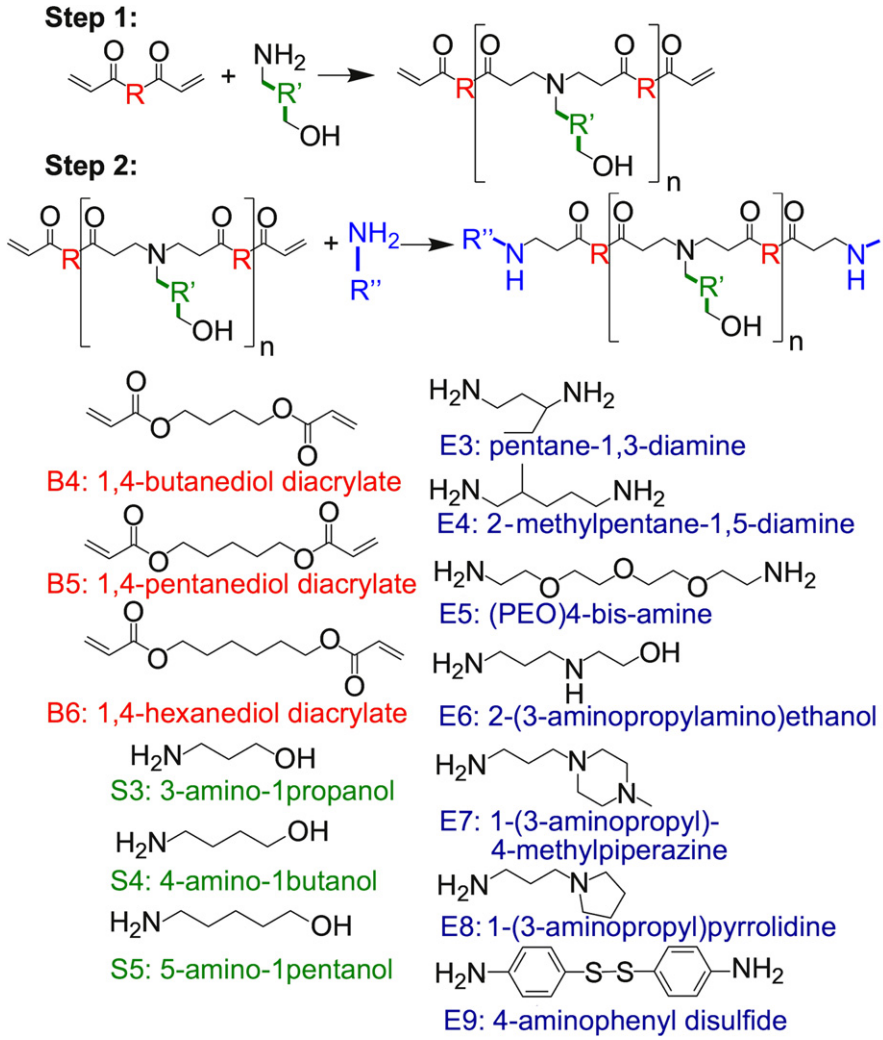

Fig. 1. Monomer structures used to synthesize PBAEs. Backbone (B) monomers were polymerized with sidechain (S) monomers. The B–S base polymers were then endcapped with small molecules (E).