International Journal of Pharmaceutics 515 (2016) 186–200

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Research article

Dipeptide-functionalized polyamidoamine dendrimer-mediated apoptin gene delivery facilitates apoptosis of human primary glioma cells

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ARTICLE INFO

Article history:
Received 25 March 2016
Received in revised form 23 September 2016
Accepted 29 September 2016
Available online 11 October 2016

Keywords:
Apoptin
Gene therapy
PAMAM dendrimer
Human primary glioma cells
Apoptosis

ABSTRACT

Glioblastoma multiform (GBM) is the most frequent and aggressive form of brain tumors in adults. However, the development of more efficient and safe nonviral vector gene therapy represents a promising therapeutic approach, using a tumor-specific killer gene, named apoptin. In this study, we describe the efficacy of non-viral gene delivery vectors, the amino acid-conjugated PAMAM derivatives (PAMAM-H-R and PAMAM-H-K) in delivering a therapeutic gene, displaying affinity toward human primary glioma cells (GBL-14 cells) and dermal fibroblasts. We analyzed transfection efficiency, using luciferase (Luci) and a pDNA encoding for enhanced fluorescent protein (EGFP), and cytotoxicity in both cells. The results show that transfection efficiency of PAMAM-H-R improved compared to native PAMAM dendrimer, but cytotoxicity of PAMAM-H-R and PAMAM-H-K were very low. We treated both cells with a polyplex formation of PAMAM-H-R or PAMAM-H-K/apoptin, and analyzed their cellular uptake and localization by flow cytometry and confocal microscopy. Furthermore, we analyzed the endosomal escape effect using TEM images, and found that PAMAM-H-R showed very fast escape from endosome to the cytosol. Caspase 3 activity assay, cell cycle distribution, and JC-1 analysis showed apoptosis induced by apoptin in GBL-14 cells. This indicates that PAMAM-H-R can be a potential nonviral vector gene delivery carrier for brain tumor therapy. The present study demonstrates that PAMAM-H-R/apoptin gene polyplex can be used as an effective therapeutic candidate for GBM due to its selective induction of apoptosis in primary glioma cells as a potential nonviral gene delivery carrier for brain tumor therapy.

1. Introduction

Glioblastoma multiform (GBM) is a highly lethal malignant brain tumor, comprising 50% of all glioma. GBM displays uncontrolled cellular proliferation, invasion, angiogenesis, and high genomic instability (Assi et al., 2012; Juratli et al., 2013). There have been recent advances in surgery and adjuvant therapy for GBM, including chemotherapy, radiotherapy, and cytotoxic therapy. However, due to drawbacks of these tumor therapies there is invariably tumor recurrence (Jia et al., 2012; Kanu et al., 2009; Okura et al., 2014). Therefore, there is hope to improve brain tumors therapies, using targeted gene therapy, which is the stable

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http://dx.doi.org/10.1016/j.ijpharm.2016.09.083
0378-5173/

expression by efficiently transported genetic material to target cells or tissue, without immune response (Engelhard, 2000; Wegscheid et al., 2014). This could then apply to clinical trials in brain tumors with several types of gene (Nano et al., 2012; Sheridan, 2011). These systems can be divided into viral and nonviral systems. The benefit of viral vectors is very high transgene transfection efficiency, but virus vectors have drawbacks such as cytotoxicity, high immunogenicity, inflammatory reactions, and tumorigenicity. To overcome the limitations of viral vectors there is increased interest in the field of nonviral vectors systems (Kane et al., 2015; Pathak et al., 2009). The commonly used nonviral vectors are cationic lipids, polymers, and cationic polypeptides (Lv et al., 2006; Paleos et al., 2009). Nonviral vectors can deliver diverse genetic materials into cells, and they have lower immunogenicity, increased biocompatibility, and decreased cytotoxicity. To date, nonviral vectors have not been used successfully to deliver a therapeutic gene to brain tumors in clinical trials. Among nonviral vectors, biopolymer-based gene carriers are important for therapeutic applications because of their flexibility of structure, non-toxicity, biocompatibility, and biodegradability (Al-Dosari and Gao, 2009; Jones et al., 2013; Lammers et al., 2008).

In particular, the PAMAM dendrimer (lower generations like generation 4) is one of the well-known synthetic cationic polymers used as a nonviral gene delivery vector, because of higher transfection efficiency, lower cytotoxicity, and lower immunogenicity compared to other polycationic and nondegradable polymers (Cloninger, 2002; Lee et al., 2005). PAMAM dendrimers form complexes with DNA through electrostatic interactions between the negatively charged phosphate DNA backbone and the protonated primary and tertiary amine groups of the PAMAM dendrimer, and the polyplexes effectively promote intracellular uptake via endocytosis (Bielinska et al., 1997; Santos et al., 2010). In our previous study, we performed histidine and arginine grafting to PAMAM dendrimers, and the resulting PAMAM-H-R gave higher proton buffering capacity and gene transfection efficiency compared to the native PAMAM dendrimer (Yu et al., 2011). Very recently, we have shown that histidine and lysine grafted PAMAM dendrimers, PAMAM-H-K show low cytotoxicity and improved effective gene delivery potency, because the primary amino groups on the surface participate in nucleic acid binding, and promote cellular uptake, and because tertiary amino groups support a proton buffer effect which gives enhanced endosomal escape (Park et al., 2014).

It was attempted to develop a therapeutic application using a tumor-selective killer gene such as apoptin, and the PAMAM dendrimer derivatives as novel delivery systems for loading of patient-derived human primary glioma cells, GBL-14 cells.

Apoptin, derived from the chicken anemia virus (CAV), is a 13.6 kDa serine-threonine rich protein of 121 amino acids. Apoptin selectively induces apoptosis in a variety of cancer cells, but not in normal cells (Los et al., 2009; Rollano Penaloza et al., 2014). The cancer-specific toxicity of apoptin is relative to its cellular localization. Apoptin is localized in the nucleus of tumor cells, whereas in normal cells it remains in the cytoplasm. It is hypothesized that nuclear trafficking and the Thr-108 phosphorylation of apoptin in cancer cells lead to induction of apoptosis, but the mechanisms involved in apoptin-induced cell death are not clearly understood. Several studies showed that apoptin is shuttled from the nucleus to the cytoplasm by Nur77, a member of the steroid receptor family, which activates by interaction with the anti-apoptotic Bcl-2, leading to the p53-independent mitochondrial death pathway of caspase activation (Chaabane et al., 2014; Danen-Van Oorschot et al., 1997; Tavassoli et al., 2005). Apoptin has potential as an tumor-specific antitumor agent, but therapeutic application has many challenges including lack of efficient delivery systems (Alvisi et al., 2006; Argiris et al., 2011).

We therefore have the aim of developing conjugated PAMAM derivatives (PAMAM-H-R and PAMAM-H-K) as efficient apoptin gene delivery carriers. In this study, we performed assays of polyplex formation, cytotoxicity, and transfection efficiency of the polymers including the native PAMAM dendrimer and PEI25KD as control vectors to demonstrate the outstanding features of conjugated PAMAM derivatives. The cellular uptake and intracellular distribution of conjugated PAMAM derivatives were analyzed by TEM imaging, flow cytometry, and confocal microscopy. Furthermore, we examined GBL-14 cells and dermal fibroblasts for antitumor activity, cell cycle properties, caspase 3 activity, JC-1 analysis, and GSH assays upon apoptin gene transfer with PAMAM derivatives. Here, we showed that among conjugated PAMAM derivatives, PAMAM-H-R/pJDK-apoptin shows low cytotoxicity and improved transfection efficiency and cellular uptake, and can induce apoptosis through the mitochondrial pathway in GBL-14 cells. The results suggest that PAMAM-H-R/pJDK-apoptin has excellent potential to be used in brain tumor therapy.

2. Materials and methods

2.1. Materials

PAMAM dendrimer (ethylenediamine core, generation 4.0, 10 wt.% in methanol, MW 14214.17), PEI25KD (polyethylenimine, branched, 25KD), *N,N*-diisopropylethylamine (DIPEA), *N,N*-dimethylformamide (DMF), and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich (Seoul, South Korea). *N*-Hydroxybenzotriazole (HOBt), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetra-methyluronium (HBTU), and Fmoc-His(trt)-OH were purchased from Anaspec (San Jose, CA, USA), and Fmoc-Arg(pbf)-OH was obtained from Novabiochem (San Diego, CA, USA). The luciferase assay kit and 5 x Reporter Lysis Buffer were purchased from Promega (Madison, WI, USA). The Micro Protein Assay Kit was obtained from Pierce (Rockford, IL, USA). EZ-Cytox and EZ-LDH reagent were purchased from Daeil Lab Service (Seoul, South Korea). PicoGreen reagent, Mitoprobe JC-1 assay kit, Nucleic acid labeling kit, Alexa fluor 488 5-SDP ester, Trizol, DMEM medium supplemented with 10% fetal bovine serum (FBS), streptomycin (100 μg/ml), and penicillin (100 U/ml) were purchased from Invitrogen (Seoul, South Korea). Glutathione Colorimetric and Caspase-3/CPP32 Colorimetric Assay kit were purchased from BioVision (Seoul, South Korea). Transcriptor first strand cDNA synthesis kit was purchased from Roche (Seoul, South Korea). Polyclonal antibodies for GFP were purchased from Santa Cruz Biotechnology. β-Actin antibodies were from Sigma –Aldrich (Seoul, South Korea).

2.2. Synthesis of PAMAM-H-R and PAMAM-H-K

The dipeptide-functionalized PAMAM dendrimer derivatives, PAMAM-H-R and PAMAM-H-K, were synthesized as reported previously (Yu et al., 2011; Park et al., 2014).

2.3. Plasmids and cell culture

The luciferase reporter plasmid DNA, pJDK/Luci, pEGFP-C2, pEGFP-C2/apoptin, pJDK vector, and pJDK-apoptin constructs were prepared as reported (An et al., 2013). GBL-14 cells, GBL-37 cells, and dermal fibroblasts were maintained in DMEM medium supplemented with 10% fetal bovine serum (FBS), streptomycin (100 μg/ml), and penicillin (100 U/ml) (Invitrogen, USA).