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Polyamine metabolism-based dual functional gene delivery system to synergistically inhibit the proliferation of cancer

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ABSTRACT

Polyamine content, which is associated with tumor growth, can be regulated by ornithine decarboxylase (ODC) and *S*-adenosyl methionine decarboxylase (SAMDC), two key enzymes in polyamine biosynthesis. Here we aim to develop a pH-responsive cationic poly(agmatine) based on a polyamine analogue–agmatine that can dually function as a gene delivery vector as well as an anticancer agent by inhibiting ODC after intracellular degradation. The core-shell nanoparticles, formed by poly(agmatine)/SAMDC siRNA complex as a core, were coated with bovine serum albumin for better *in vivo* circulation stability and tumor targeting. When the nanoparticles were taken up by tumor cells *via* endocytosis and degraded in endosome, the released agmatine and SAMDC siRNA can synergistically inhibit polyamines biosynthesis, inducing inhibition of tumor proliferation. Our study offered a potential way in tumor therapy based on polyamine metabolism.

1. Introduction

Cancer is a disease of unregulated cell growth (Schulze and Harris, 2012). It can invade to other parts of the body if the abnormal proliferation cannot be early suppressed. It was reported that the proliferation of tumor cells have a close relationship correlation with the content of polyamines such as putrescine (Put), spermidine (Spd) and spermine (Spe) (Igarashi and Kashiwagi, 2010; Pegg, 2009). Usually, the occurrence of cancer is accompanied by dysregulated polyamine metabolism (Andersson and Heby, 1972; Russell and Snyder, 1968; Seiler, 2003), including higher content of polyamine in tumor tissue and higher activity of ornithine decarboxylase (ODC) and S-adenosyl methionine decarboxylase (SAMDC) (two key enzymes in polyamine biosynthesis) in tumor cells, which suggested polyamine metabolism is a potential target for antineoplastic therapy.

On the other hand, gene/drug co-delivery system is becoming increasingly important due to the molecular complexity of cancer. The nanoscale particles consist of therapeutic gene and drug have been proposed to achieve a synergistic effect. Yang's group reported the cationic core-shell nanoparticles that were self-assembled from a biodegradable amphiphilic copolymer and suppressed cancer growth more efficiently than the delivery of either paclitaxel or the gene (Wang et al., 2006). Moreover, Oupicky et al. described a novel class of polycations that can deliver plasmid DNA as well as function as CXCR4 antagonists to inhibit cancer cell invasion and possibly limit metastasis (Li et al., 2012). However the co-delivery system cannot release prototype drug. To better exert therapeutic effect of the drugs, it is necessary to design polymeric prodrug to release prototype drug (Delplace et al., 2014).

Agmatine (Agm), as an anticancer agent based on polyamine metabolism interference (Gardini et al., 2003; Wang et al., 2005; Wolf et al., 2007), not only reduce polyamine synthesis *via* inhibition of ODC, but also compete at the polyamine transport system, thus reducing cellular polyamine content (Satriano, 2004). And Agm has been successfully developed as genetic vector for gene therapy in several cancer cells (Yang et al., 2012). However, Agm cannot exert its effect due to the unbroken carbon-nitrogen single bond used in these NPs. Therefore, in this study, we have

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designed and synthesized a pH-responsive cationic poly(agmatine), which was a poly(4-vinyl benzaldehyde) main chain coupled with Agm by Schiff base. The poly(agmatine) can condense SAMDC siRNA (siSAMDC) to form NPs as a core, and then coated with bovine serum albumin (BSA) for better *in vivo* circulation stability and tumor targeting (denoted by PA/siSAMDC/BSA, the formation is shown in Fig. 1a). This nano-co-delivery system can achieve combinational therapeutic effects based on pH-responsive polymeric prodrug carrier and the therapeutic gene in carcinoma *via* polyamine metabolism interference (Scheme 1).

2. Materials and methods

2.1. Materials

Terephthalaldehyde, methyltriphenyl phosphonium bromide, potassium carbonate, 2,2′-azoisobutyronitrile (AIBN) were purchased from commercial supplier (J&K, China). SAMDC siRNA and Bcl-2 siRNA were purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). The primers for quantitative polymerase chain reaction were purchased from with Genewiz, Inc. (Suzhou, China). All other chemicals and reagents were obtained from commercial sources with the highest purity available unless otherwise noted.

2.2. Synthesis of 4-vinyl benzaldehyde (St-CHO)

A mixture of anhydrous tetrahydrofuran (40 mL) containing terephthalaldehyde (27.5 mmol), methyltriphenyl phosphonium bromide (30 mmol), and potassium carbonate (34.4 mmol) was heated at reflux overnight and cooled to room temperature (r.t.). The reaction mixture was poured onto water, and the crude product was extracted with ethyl acetate. The collected organic layer was dried over anhydrous $MgSO_4$, and was purified by column chromatography (SiO_2, 6 × 40 cm, the ratio of petroleum ether to ethyl acetate was 9–1) to give St-CHO as a pale yellow oil.

2.3. Synthesis of poly(4-vinyl benzaldehyde) (p-St-CHO)

A DMSO solution (4 mL) of St-CHO (7.6 mmol) and AIBN (2.28 mmol) was degassed with Ar at r.t. for 5 min. After degassing, the reaction mixture was stirred at 65 °C for 16 h. The reaction mixture was exposed in air to quench the polymerization. The polymer was purified by re-precipitation ($CHCl_3$/MeOH) to produce white powder. The polymer was analyzed by a Shimadzu GPC system equipped with a refractive index detector (RID-10A), and a Shodex KF-803 column. 10 mM LiBr in DMF was used as the mobile phase at the flow rate of 1.0 mL/min at 40 °C. Calibration with polystyrene standards (American Polymer Standard Corp., USA) was performed on the GPC for estimation of the relative molecular weight of the polymer.

2.4. Synthesis of poly(agmatine) (PA)

A mixture of 2 mL DMSO and 0.7 mL triethylamine (5 mmol) containing agmatine sulphate (2 mmol) was degassed with Ar at r.t. for 5 min and then the mixture was stirred at 30 °C overnight. After that, the reaction mixture was dropped into DMSO solution (2 mL) of p-St-CHO (1 mmol of CHO) and was kept stirred at 30 °C for 12 h. The polymer was purified by re-precipitation (DMSO/ether) to produce yellow powder.

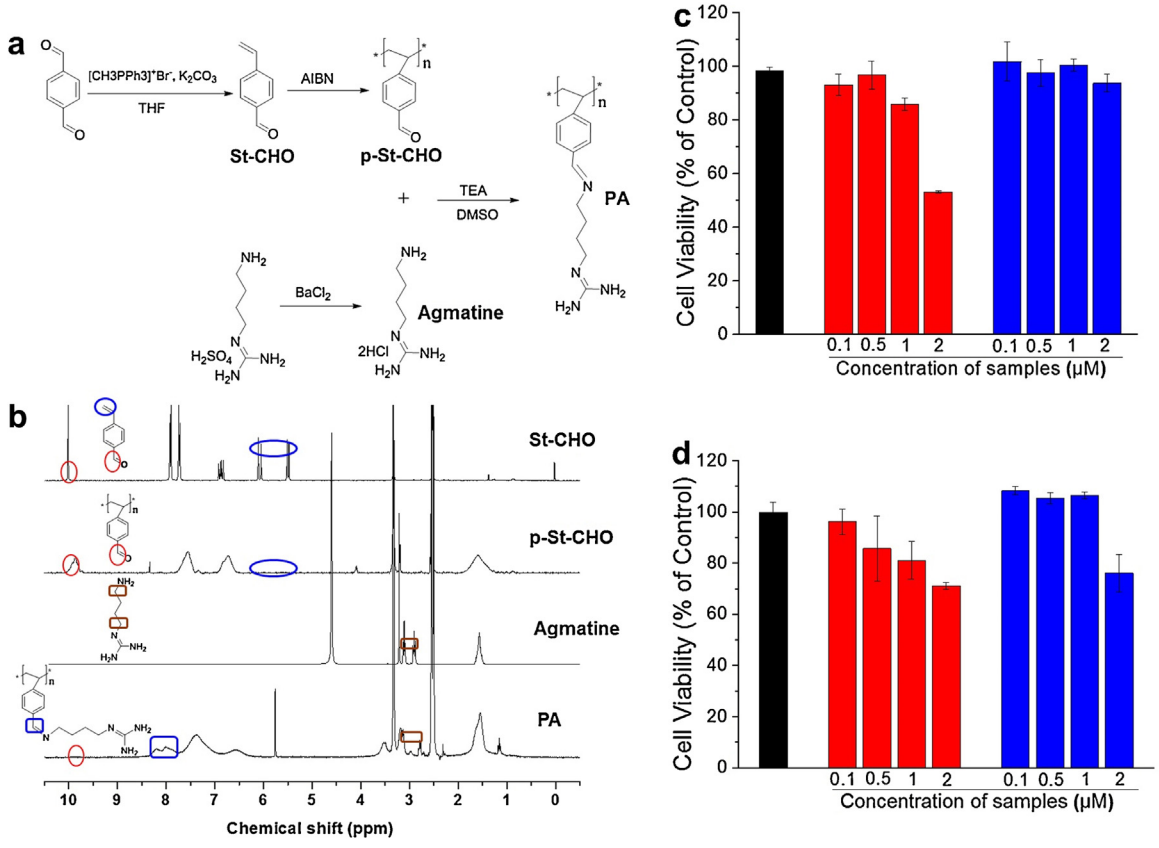

Fig. 1. Synthesis and characterization of gene carrier poly (agmatine) (PA). (a) Synthesis scheme of of St-CHO, p-St-CHO and PA. (b) ^{1}H NMR spectra of St-CHO, p-St-CHO and PA. Cytotoxicity of St-CHO and p-St-CHO in MCF-7 (c) HepG2 (d) treated with St-CHO (red bar), p-St-CHO (blue bar), and 2% DMSO containing DMEM media (black bar) for 48 h. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)