Journal of Colloid and Interface Science 527 (2018) 141–150

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

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

Regular Article

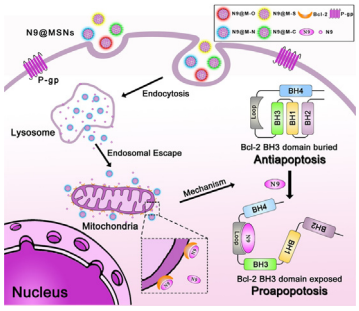
Macroporous silica nanoparticles for delivering Bcl2-function converting peptide to treat multidrug resistant-cancer cells

Weixia Xu, Pengjin Ge, Boning Niu, Xiaokun Zhang, Jie Liu, Jingjing Xie *

School of Pharmaceutical Sciences, and Fujian Provincial Key Laboratory of Innovative Drug Target Research, Xiamen University, Xiang'an South Road, Xiamen, Fujian 361102, China

GRAPHICAL ABSTRACT

Conceptual illustration of how surface functionality-modulated macroporous silica nanoparticles delivering Bcl-2-function converting peptide to combat the therapeutic resistant cancer cells.



ARTICLE INFO

Article history:
Received 19 February 2018
Revised 13 May 2018
Accepted 14 May 2018
Available online 15 May 2018

Keywords:
Macroporous silica nanoparticles
Surface functionality
Bcl-2-converting peptide
Intracellular delivery
Multidrug resistance

ABSTRACT

The abundance of B cell lymphoma gene 2 (Bcl-2) is closely correlated with the resistance of cancer cells to chemotherapeutic agents, and a peptide derived from orphan nuclear receptor Nur77 can convert Bcl-2 from a protector to a killer of cancer cells. However, successful application of the Bcl-2-converting peptide to treat drug-resistant cancer cells depends on an efficient delivery carrier. Mesoporous silica nanoparticles (MSNs) have been extensively studied as promising candidates for small molecule drug delivery. However, the effective encapsulation and intracellular delivery of peptides using small pore-sized MSNs still remain a great technical challenge. In this paper, an effective delivery platform for Bcl-2-converting peptide was fabricated by us to treat multidrug resistant-cancer cells via tuning the surface functionality of macroporous silica nanoparticles. The resulting large-sized pore silica nanoparticles, especially those modified with thiol group, exhibited the high Bcl-2-converting peptide-loading efficiency of over 40%. Moreover, the peptide induced MCF7/DOX cells into apoptotic status by penetrating cytomembrane into mitochondria and being bound with Bcl-2 to expose the BH3 domain with the aid of various surface functionalities-decorated MSNs. In particular, amine-modified surface of MSNs caused the greater influence on the cell apoptosis-inducing effects of peptide in comparison with other functionalities-modified ones. Taken together, our study, for the first time, demonstrates a special

* Corresponding author at: School of Pharmaceutical Sciences, and Fujian Provincial Key Laboratory of Innovative Drug Target Research, Xiamen University, Xiang' an South Road, Xiamen, Fujian 361102, China.

E-mail addresses: 827436396@qq.com (W. Xu), 792256253@qq.com (P. Ge), 568554637@qq.com (B. Niu), xkzhang@xmu.edu.cn (X. Zhang), jieliu@xmu.edu.cn (J. Liu), xiejj@xmu.edu.cn (J. Xie).

https://doi.org/10.1016/j.jcis.2018.05.033
0021-9797/

approach towards pore size and surface functionality-collectively modulated silica-based nanostructural material for effective delivery of bio-macromolecules (e.g., Bcl-2-converting peptide) to treat the multidrug resistant-cancer cells with elevated Bcl-2 levels.

1. Introduction

With repeated treatment, tumors often become resistant not only to the specific chemotherapeutics being employed, but cross-resistant to both similar and structurally unrelated classes of cytotoxic drugs, which was called multidrug resistance (MDR). Nowadays, MDR is currently one of the biggest challenges to cancer chemotherapy. The termed MDR mechanisms mainly include efflux pump-mediated MDR and efflux pump-independent MDR [1]. The latter usually refers to decreasing drug influx, activating DNA repair, and altering expression of apoptosis-associated proteins and tumor suppressors. B cell lymphoma gene 2 (Bcl-2) family proteins are a component of the anti-apoptotic machinery, which comprise the sentinel network that regulates the mitochondria or intrinsic apoptotic response. Given the overexpression properties of Bcl-2 in different malignancies [2], great efforts have been devoted to developing novel drugs that directly target anti-apoptotic Bcl-2 family proteins. Several drugs targeting the Bcl-2 family such as Apogossypol, HA-14, Antimycin A, Oblimersen sodium, Gossypol (AT-101), ABT-737 (ABT-263), GX15-070, VENCLEXTA are in clinical application or trials by FDA approval [3]. Though many Bcl-2-targeting agents were developed or undergoing, they mainly focused on inhibiting the Bcl-2 anti-apoptotic function by deregulating Bcl-2 gene expression as Bcl-2 inhibitors [4]. Different from those, an orphan nuclear receptor Nur77-based peptide was developed by our team to show the potential use as cancer therapeutics by binding Bcl-2 and converting Bcl-2 from a protector to a killer [5]. Given the fact that the abundance of Bcl-2 family proteins is closely associated with chemotherapy resistance in various human cancers [6], Bcl-2-converting peptide may be employed to overcome MDR for cancer chemotherapy.

Silica is generally recognized as safe by FDA and used as an excipient in tablet-form drug formulation. Silica nanoparticles (Cornell dot) have recently received FDA approval for stage I human clinical trials [7]. Mesoporous silica nanomaterials (MSNs) as one of the most promising drug delivery carriers possess many unique advantages [8], such as the ordered mesoporous structure accompanied with the high surface areas and large pore volumes, the tunable particle diameter (10–1000 nm) and pore size (2–20 nm), the flexible morphology and facile surface functionalization, the excellent biocompatibility and biodegradability [9,10]. MSNs also exhibit a distinctively high loading capacity (up to 50 wt%) of small-molecule drugs and the controllable release feature for guest molecules, and the easily modified surface with targeting and "stealth" molecules [11]. Therefore, MSN nanomedicines have shown great potentials in cancer therapy [12]. However, delivering Bcl-2-functional conversion peptide to the mitochondria of drug-resistant cancer cells using MSNs with large pore size hasn't been reported to date. That's because it is currently still a big technical challenge to synthesize MSNs both of small sizes (e.g., < 50 nm) and being fabricated with well-defined and large enough pore channels (>10 nm), while the diffusion of large molecule through the shells of NPs with pores <10 nm is usually a slow process or even impossible to realize [13]. However, the small size at less than 50 nm [14] and the pore size at more than 5 nm [15] were reported to be the key factors for MSNs loading anticancer drugs to the drug-resistant cancer cells for efficient MDR-overcoming effect. Therefore, it is badly urgent to prepare MSNs with both small particle size and enough large pore size to encapsulate and deliver large entities such as bio-macromolecules (e.g., siRNA, DNA, peptide or protein) for satisfying our varied prerequisites [16].

In this study, we fabricated the MSNs with large pore size (>5 nm) for the high loading efficiency of Bcl-2-converting peptide and small particle size (<50 nm) for the enhanced cell-penetrating and intracellular-delivering capabilities, aiming at achieving the precisely mitochondrial localization and the improved therapy efficacy for multidrug-resistant breast cancer cells. We also, for the first time, investigated how the surface functionality of MSNs would affect the MDR-reversal capacity and explored the potential mechanism related to MSN-based nanomedicine. The present study will provide the strategy for designing novel silica-based nanomaterials with large pore size to deliver Bcl-2-targeted peptide for treating tumor MDR.

2. Experimental section

2.1. Materials

Triethanolamine (TEA, ≥98%) and 3-Mercaptopropyltrimethoxy silane (MPTES, ≥95%) were purchased from Aladdin. Hexadecyl trimethyl ammonium chloride (CTAC, ≥98%) and succinic anhydride (SA, ≥99%) were obtained from Sinopharm Chemical Reagent Co. 3-Aminopropyltriethoxysilane (APTES, ≥98%), Tetraethyl orthosilicate (TEOS, 98%), Rhodamine 123, and [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrasodium bromide] tetrazolium salt (MTT) were obtained from Sigma-Aldrich. Bcl-2-converting peptide (FSRSLHSLL, NuBCP9, i.e., N9, ≥98%) with or without Rhodamine B (i.e., RB) labeling was synthesized by GL Biochem (Shanghai) Ltd. PARP, caspase-3, cleaved caspase-3, P-gp, Bcl-2 and Bcl-2 BH3 antibodies, and horseradish peroxidase (HRP)-labeled secondary antibodies were provided by Cell Signaling Technology (CST). LysoTracker (Deep Red) and MitoTracker (Green) were purchased from Life Company. Deionized water was used in all experiments. All other chemicals, unless otherwise specified, were all purchased from Sinopharm Chemical Reagent Co., Ltd and used without further purification.

2.2. Preparation of MSNs with different surface functionalities

To make the MSNs for the effective loading of macromolecules, the MSNs with small particle and large pore sizes were successfully synthesized for the first time by taking what previously reported with minor modification [14,17]. The hydroxyl-decorated MSNs (MSNs-OH, i.e., M-O) were prepared above all. Briefly, CTAC (2g) and TEA (0.18 g) were dissolved in 80 ml water at 95 °C for 1 h of stirring, followed by the dropwise addition of TEOS (1.5 ml) for another 2 h. The products were then collected by centrifugation at 18000 rpm for 30 min and washing with ethanol for several times to remove the residual reactants. The collected product was finally extracted with a 1 wt% solution of NaCl in methanol at 3 * 12 h to completely remove the CTAC template and suspended in pure water for subsequent experiments. Following M-O, the amine, thiol, and carboxyl-decorated MSNs were synthesized via different surface functionalization. The surface of MSNs was functionalized with amine groups by treatment with APTES. M-O