European Journal of Pharmaceutics and Biopharmaceutics 114 (2017) 108–118

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Aptamer-functionalized hybrid nanoparticle for the treatment of breast cancer

David Powell [a,1], Sruti Chandra [b,1], Kyra Dodson [b], Farhana Shaheen [b], Kylar Wiltz [b], Shubha Ireland [b], Muniruzzaman Syed [b], Srikanta Dash [c], Thomas Wiese [d], Tarun Mandal [d], Anup Kundu [b,*]

[a] Department of Chemistry, Xavier University of Louisiana, New Orleans, LA 70125, United States
[b] Department of Biology, Xavier University of Louisiana, New Orleans, LA 70125, United States
[c] Department of Pathology and Laboratory Medicine, Tulane University Health Sciences Center, New Orleans, LA 70112, United States
[d] Center for Nanomedicine and Drug Delivery, Xavier University College of Pharmacy, New Orleans, LA 70125, United States

ARTICLE INFO

Article history:
Received 5 June 2016
Revised 17 January 2017
Accepted in revised form 23 January 2017
Available online 25 January 2017

Keywords:
P-glycoprotein
Nanoparticle
Her-2
Aptamer
siRNA

ABSTRACT

Purpose: Resistance to chemotherapeutic agents such as doxorubicin is a major reason for cancer treatment failure. At present the treatment option for metastatic breast cancer is very poor. Therefore, development of an effective therapeutic strategy to circumvent MDR of metastatic breast cancer is highly anticipated. The MDR of metastatic breast cancer cells was accompanied with the overexpression of P-gp transporter. Even though the overexpression of P-gp could be minimized by silencing with siRNA, the question is how they can be selectively targeted to the cancer cells. We propose that aptamer surface labeling of the nanoparticles could enhance the selectively delivery of p-gp siRNA into the metastatic breast cancer cells. Our hypothesis is that conjugating nanoparticles with a cancer cell specific aptamer should allow selective delivery of therapeutic drugs to tumor cells leading to enhanced cellular toxicity and antitumor effect as compared to unconjugated nanoparticles. The primary objective of this study is to develop a targeted nanocarrier delivery system for siRNA into breast cancer cells.
Design methods: For targeted delivery, Aptamer A6 has been used which can bind to Her-2 receptors on breast cancer cells. For aptamer binding to particle surface, maleimide-terminated PEG-DSPE (Mal-PEG) was incorporated into the nanoparticles. Initially, three blank hybrid nanoparticles (*i.e.* F21, F31, and F40) out of nine different formulations prepared by high pressure homogenization (HPH) using different amount of DOTAP, cholesterol, PLGA or PLGA-PEG and Mal-PEG were chosen. Then protamine sulfate-condensed GAPDH siRNA (TRITC conjugated; red) or P-gp siRNA was encapsulated into those nanoparticles. Finally, the particles were incubated with aptamer A6 (FITC conjugated; green) for surface labeling.
Results: Aptamer labeled-nanoparticles having PLGA are smaller in size than those having PLGA-PEG. Surface charge was reduced when the particles were labeled with aptamer. Cell transfection was increased significantly in Her-2 (+) SKBR-3 and 4T1-R cells but not in Her-2 poorly expressed MDA MB-231 and MCF-7 cells. The knockdown of P-gp was increased significantly when the particles were labeled with aptamer. No significant cellular toxicity was observed for any of these formulations.
Conclusion: This preliminary study concludes that aptamer-functionalized hybrid nanoparticles could be used to deliver P-gp targeted siRNA into the breast cancer cells to overcome chemoresistance.

1. Introduction

Breast cancer is one of the most invasive and malignant diseases affecting millions, and worldwide over 508,000 women died in 2011 due to breast cancer [1]. The primary treatment for breast cancer is surgery followed by adjuvant chemotherapy. However, there are ample evidences where recurrence of cancer metastasis is experienced by patients in spite of adjuvant chemotherapy [2]. The development of multidrug resistance (MDR) is a major obstacle to effective cancer chemotherapy [3]. Once the tumor cells develop resistance to a single class of drug, it can also show cross-resistance to other functionally and structurally unrelated drugs. This phenomenon is known as multidrug resistance (MDR). The mechanism why some tumor cells develop resistance and some don't is unclear [4].

* Corresponding author at: Department of Biology, Xavier University of Louisiana, 1 Drexel Dr., New Orleans, LA 70125-1098, United States
E-mail address: akundu@xula.edu (A. Kundu).
[1] Both authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ejpb.2017.01.011
0939-6411/

The actual mechanism behind MDR is yet to be revealed. Both intrinsic and acquired mechanisms have been proposed as possible causes of breast cancer drug resistance. Overexpression of adenosine-triphosphate (ATP) binding cassette (ABC) transporters, a 48-member superfamily, is one of the identified causes of MDR. Of these, the most well characterized transporter, ABCB1 (MDR1/P-glycoprotein) overexpression in breast cancer cells has been found to be strongly associated with reduction of intracellular level of anticancer drugs below their therapeutic threshold [5]. Strategies have been formulated to beat the P-gp mediated drug efflux by using chemical inhibitors though there are only a few that are clinically effective. The RNA Interference (RNAi) technology provides a potential mechanism of gene silencing and could be utilized to knockdown P-gp. Wu et al. has markedly inhibited the overexpression of MDR1 (*i.e.* P-gp) by siRNAs in MDR cancer cells resulting in restoration of drug sensitivity [6]. Similar findings were also observed in human MDR cells as well [7]. However, the siRNA delivery needs to be targeted specifically to cancer cells so as to avoid notorious side effects to the normal cells.

The potential of siRNA as an anticancer therapeutic depends on the availability of a carrier vehicle which will not only have higher binding affinity for siRNA but also safely administer the drugs (*i.e.* siRNA) specifically and efficiently to the target cells or tissues. The carrier should protect the functional integrity of the siRNA as well as permitting their (siRNA) easy and efficient release from the vehicle within the cells. Among the numerous vehicles developed for RNAi delivery, cationic lipids and polymers are most promising because of their easy and efficient packaging with siRNAs to form nanoscale complexes (lipoplexes or polyplexes) which have shown potential in delivering siRNA [8]. Nevertheless, if the delivery vehicle is not specifically targeted to the cancer cells, problems associated with toxicity, immune or inflammatory responses, and serum instability would hinder their effective use for the treatment of cancer. To that end, several strategies have been adopted, including pegylation (*i.e.* coupling to PEG) of nanocomplexes and liposomal envelopment of polyplexes (to form lipopolyplexes) [9,10] to optimally protect both siRNA and nanocomplexes from the physiological barriers *in vivo*.

With the development of nanotechnology, nanoformulations have been widely tried for the last few years to bypass the MDR development of tumor cells [11–13]. In this study, we have explored nanotechnology to overcome chemoresistance mechanisms. It is recently reported by our collaborator that doxorubicin treatment effectively suppresses the multiorgan metastasis of doxorubicin-sensitive 4T1 cells in Balb/c mice, but not doxorubicin resistant 4T1 breast cancer cells. They also reported that down regulating nuclear expression of MDR1 P-gp (ABCB1 gene) by P-gp specific siRNA could increase the delivery of doxorubicin to doxorubicin resistant breast cancer cells [5,14]. However, unless these nanoparticles are targeted specifically to cancer cells, they will not have a significant impact in the treatment of cancer. So, we plan to overcome this problem by developing a targeted nanocarrier delivery system for siRNAs targeting P-gp into breast cancer cells. We assume that silencing P-gp will eventually help to deliver more doxorubicin into the breast cancer cells.

For targeted delivery, the aptamer technology has been used [15–17]. A single-stranded RNA or DNA oligonucleotide aptamer can fold into unique tertiary conformations [17] and are capable of binding with high specificity to non-nucleic acid targets like proteins [18]. Because of these unique characteristics, aptamer has been lately used for targeted delivery of drugs into cancer cells. For example, aptamer AS1411 targets nucleolin in MCF-7 cells [15], aptamer-liposome conjugates containing aptamer sgc8 has been used for drug delivery into CEM-CCRF leukemia cells [16], and PLGA-PEG nanoparticles surface functionalized with A10 2′ fluoropyrimidine RNA aptamer has been used to enhance the delivery of docetaxel in LNCaP xenograft nude mice [17]. The aptamer that has been used in this study is amino-terminal at one end (NH_2-Apt-6) and it binds to epidermal growth factor receptors (ERBB2/Her-2), a master regulator of cancer progression which is overexpressed on the cell surface of breast cancer cells. In the present study, we modified our pretested nanoparticles originally prepared using DOTAP and cholesterol [19] by including PLGA or PLGA-PEG. For aptamer binding to particle surface, maleimide-terminated PEG-DSPE (Mal-PEG) was specifically incorporated into the nanoparticles [20]. Maleimide linked to PEG serves as a flexible linker that can readily associate with a variety of functional groups including thiol- and amino- group. [21]. The amino-terminal aptamer (NH_2-Apt-6) presumably binds with the Mal-PEG moieties on the nanoparticle surface which in turn labels the particles for selective binding to Her-2 (+) breast cancer cells. This study is aimed to know whether labeling nanoparticles with a cancer cell specific aptamer could enhance the selective delivery of siRNA into tumor cells leading to enhanced knockdown of P-gp as compared to non-labeled nanoparticles.

2. Materials and methods

2.1. Materials

1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), Cholesterol and Maleimide-terminated PEG-DSPE (Mal-PEG) were purchased from Avanti Polar- lipids Inc. (Birmingham, AL, USA). Protamine sulfate salt Grade X, trehalose dihydrate and HPLC grade chloroform were obtained from Sigma Chemical Co. (St. Louis, MO, USA). PLGA and PLGA-PEG were purchased from Boehringer Ingelheim (Germany). Lipofectamine RNAiMAX transfection reagent was purchased from Invitrogen. Fetal bovine serum albumin (BSA), Dulbecco's modified Eagle's medium (DMEM) and penicillin/streptomycin antibiotics were purchased from Gibco, Invitrogen Corp. (Carlsbad, CA, USA). β-actin antibody was purchased from Sigma Chemical Co. (St. Louis, MO, USA), anti-mouse and anti-rabbit IgG HRP labeled secondary antibody were purchased from GE Healthcare (Little Chalfont, UK). GAPDH siRNA and aptamers were purchased from Life Technologies (Carlsbad, CA). Anti-P-gp antibody was purchased from Pierce, Thermo Scientific (Waltham, MA). All other reagents were of analytical grade and were supplied by Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Preparation of lipid-polymer hybrid liposomes

The hybrid liposomes were prepared by an EmulsiFlex-B3 high pressure homogenizer from a mixture of two lipids cholesterol and DOTAP at the molar ratio of 1:1 [19,22] and PLGA or PLGA-PEG (10% diblock) at different weight ratios shown in Table 1. Nine different formulations of nanoparticles were prepared into two different categories: PLGA-PEG group (*i.e.* F20, F21, F22, F23) and PLGA group (*i.e.* F30, F31, F32, F33) and F40 being the basic liposomal formulation containing only DOTAP and cholesterol. Three formulations were chosen for further experiments F21, F31 and F40; the first two representing the best combination possible from each of PLGA-PEG and PLGA group.

The blank liposome (F40) was prepared 20 mM containing equimolar ratio of DOTAP and cholesterol. The hybrid liposomes (F21and F31) were prepared by adding either PLGA-PEG (F21) or PLGA (F31) and Mal-PEG into the blank liposomes. For the preparation of hybrid liposomes; DOTAP, cholesterol, Mal-PEG and PLGA-PEG or PLGA were weighed and taken into a round bottom flask. They were mixed together by adding 15 ml of HPLC-grade chloroform and then dried under nitrogen gas and overnight vacuum. The resulting film of the lipid polymer mixture was hydrated in DEPC-