Development of a specific targeting system for pancreatic tumors utilizing antibody conjugated Pt-loaded micelles

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ABSTRACT

Specific delivery of anticancer drugs to targeted sites is one of the principle goals in designing drug delivery system to increase the therapeutic efficacy and decrease the side effects to normal tissues. In this research, the tissue factor (TF) targeting antibodies were conjugated with maleimide-surface functionalized dichloro(1,2-diaminocyclohexane)platinum(II)-loaded micelles (DACHPt/m) to selectively deliver cytotoxic drugs while overcoming the limitations of antibody-drug conjugates (ADC), including low drug capacity and stability in the body. Through the high affinity between antibodies and TF antigens known to be highly expressed on various human cancer cells, the antibody-conjugated micelles (immunomicelles) strongly associated with diverse cancer cells, subsequent faster cellular internalization which resulted in enhancement of cytotoxicity. Furthermore, the in vivo antitumor efficacy evaluation using a pancreatic BxPC3 xenograft tumor model demonstrated immunomicelles could significantly suppress the tumor growth compared to control micelles due to the targeting of the TF that exist on the solid tumor.

INTRODUCTION

The major goal of anticancer drug delivery system is to selectively deliver the cytotoxic molecules to tumor sites with minimal side effects to healthy tissues\textsuperscript{1}. Although ADC systems have been shown to make steady progress for specific delivery of drugs, limitation of payloads, stability under physiological systems, and conjugation to drugs without disturbing binding characteristics of antibodies are still difficulties that need to be improved in order to fully accomplish targeted drug delivery system\textsuperscript{2}. Integration of antibodies into the polymeric nanocarriers that incorporate anticancer drugs is one of the more suitable solutions to overcome these difficulties. DACHPt-incorporating micelle (DACHPt/m), which consists of oxaliplatin derivatives and block copolymers of PEG and poly amino acid, was modified to have the maleimide functionalities on the surface for the antibody conjugation to exploit their advantages including long blood circulation, low toxicity, controlled drug release behavior and superior antitumor efficacy\textsuperscript{3,4}. (Figure 1)

Tissue factor (TF), cell-membrane associated proteins that are up-regulated on various human cancer cells, are selected as an antigen\textsuperscript{5}. The anti-TF antibodies were introduced on the surface of DACHPt/m to specifically target TF expressing tumors. Furthermore, we extend the types of antibodies to anti-human (AH)-TF and anti-mouse (AM)-TF antibodies to investigate the targeting effect of the immunomicelles.

EXPERIMENTAL METHODS

The poly(ethylene glycol)-b-poly(glutamic acid) block copolymers having different chain end 1) MeO-PEG-b-P(Glu) and 2) maleimide-PEG-b-P(Glu) were synthesized by anionic polymerization of ethylene oxide, followed by NCA polymerization of glutamic acid. By the incubation of polymer 1, 2 and DACHPt in aqueous condition, maleimide group surface-functionalized micelle incorporating DACHPt (Mal-DACHPt/m) was self-assembled. (Figure 1) Then DTT treated fab’ of AH-TF and AM-TF antibodies were reacted with Mal-DACHPt/m to obtain immunomicelles (IM/m). The formation of immunomicelles was confirmed by gel permeation chromatography (GPC) and fluorescence imaging.

Figure 1. Schematic illustration of immunomicelle preparation.
correlation spectroscopy (FCS). The cellular binding and uptake were observed using flow cytometry (FCM) and confocal laser scanning microscope (CLSM). The BxPC3 subcutaneous tumor model was established by inoculating $5 \times 10^6$ cells to balb/c nude mice (female, 6 weeks). The mice were i.v. administrated thrice every four day by groups (n=5) after 4 weeks inoculation, and the tumor volume was calculated by the following equation: \[ \text{Volume}_{\text{tumor}} = 0.5 \times (\text{Major diameter}) \times (\text{Minor diameter})^2 \]

RESULTS AND DISCUSSION

(1) Preparation of IM/m
The AH and AM immunomicelles were prepared by conjugating Mal-DACHPt/m based on PEG-b-P(Glu) copolymers to AH-TF and AM-TF antibodies, as shown in Figure 1. The results from GPC and FCS from Figure 2 illustrate the successful formation of immunomicelles. Antibodies and Mal-DACHPt/m were labeled with Alexa 488 and Alexa 647, respectively, and the fluorescence peaks in the chromatograms were compared to confirm the conjugation of antibody to micelle. Conjugation was also confirmed by the autocorrelation peaks obtained from FCS using Alexa 488-labeled antibodies.

(2) In vitro analysis of cell binding, uptake, and proliferation
The enhanced targeting efficacy of immunomicelle to tumor sites was investigated in vitro by flow cytometry to observe specific binding behaviors of micelles. Alexa 647-labeled DACHPt/m, AH IM/m, and AM IM/m were incubated with TF-overexpressing cell lines such as DLD1 (human colorectal cancer), BxPC3 and PANC1 (human pancreatic cancer) for 1 h at 4 °C.

CONCLUSION
Novel polymeric micelles conjugated to antibodies were developed to enhance drug targeting to cancer cells. The IM/m exhibited strong affinity to various cell lines, faster intracellular uptake, and improved cytotoxicity compared to DACHPt/m, suggesting the conjugation of the antibody onto micelles is promising strategies for improved treatment of pancreatic human carcinoma.

REFERENCES