Dual drug-loaded solid lipid nanoparticles for enhanced cancer stem cell therapy

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ABSTRACT SUMMARY
A new approach for the effective treatment of cancer stem cells (CSCs) by using a co-delivery system for loading both PTX and miR-34a was developed, which could enhance cellular uptake and increase the drug location in the cancerous lung followed by repressing the CSC properties. This drug delivery strategy is raising the hope for the synergistic cancer stem cell suppression.

INTRODUCTION
The satisfactory effect on CSC treatment is still limited with the risk of therapeutic relapse, which may be attribute to that tumor development and tumorigenicity originating from CSC is extremely multiple[1]. Consequently, further studies still need to be done to achieve successful CSC therapy, miR-34a, regulating tumor-related genes (including CD44 molecule) that occur in tumors[2], is valid against the CSC tumorigenicity induced by other mechanisms. That is, it is necessary to develop a combination approach to assist miR-34a to achieve CSC therapy. With this in mind, the combination therapy using two therapeutic approaches which can respectively target cancer cells and cancer stem cells may provide a new therapeutic strategy to eliminate the tumor relapse. Therefore, miR-34a could target cancer stem cells and achieve a synergistic effect on CSC with other chemotherapeutic agent such as paclitaxel (PTX).

Herein, we developed an approach for the effective treatment of CSCs by using a co-delivery system for loading paclitaxel and miR-34a.

EXPERIMENTAL METHODS
First of all, PTX-loaded solid lipid nanoparticles (SLNs) were prepared using a film-ultrasonic method, which composed of 15 mg glyceryl monostearate (GMS), 15 mg soy phosphatidylcholine (SPC), 10 mg cholesterol (Chol), and 10 mg dimethyldioctadecylammonium bromide (DDAB). For preparation of drug-loaded SLNs/PTX (miSLNs-34a/PTX), isovolumetric RNA solution was added to 50 μL of SLNs/PTX colloidal solution for 30-min incubation. The characteristics of miSLNs-34a/PTX were evaluated in forms of average diameter, morphology, encapsulation efficiency and stability.

Then the influence of the miR-34a formulations on cell apoptosis was assessed. After B16F10-CD44+ cells were treated with the miR-34a/PTX formulations at a concentration of 150 nM of RNA and 10 μg/ml of PTX, the cell nuclei were stained with a highly specific fluorescing DNA dye, 4′-diamidino-2-phenylindole (DAPI) for 10 min[3]. The nuclear morphology of cells was checked under a Zeiss fluorescence microscope (ex 358nm, em 461 nm). Cells were judged to be apoptotic or not based on the nuclear morphology changes including chromatin condensation, fragmentation and apoptotic body formation.

In vivo evaluation of miSLNs was employed on C57BL/6 mice. Coumarin/Cy3-labeled miSLNs-34a(Cy3)/PTX(coumarin-6) were prepared as mentioned above, then the mice were given i.v. injections of SLNs. The fluorescence signals of mice were recorded using an IVIS® Spectrum system (Caliper, Hopkington, MA).

Aiming to verify whether miSLNs-34a/PTX had antitumor efficacy in the CSC-bearing mice, their survival rates, along with the weight and histological staining of lungs and CD44 expression, were analyzed respectively after i.v. injections with miR-34a/PTX formulations via tail vein at a rate of 0.5 mg of miR-34a per kg of body weight and 1 mg of PTX per kg of body weight from day 9 to 15.

RESULTS AND DISCUSSION
The particle size of miSLNs-34a/PTX was approximately 220 nm with spherical morphology (Figure 1). The entrapment efficiency of miSLNs was 95.13% for miR-34a and 93.05% for PTX, and the PTX and miRNA loading yields were 1.82% and 1.79 nmols per mg lipids, respectively.

To facilitate detecting, coumarin-6 and Cy5 labeled RNA were loaded into SLNs (miSLNs-RNA(Cy5)/PTX(coumarin-6)) by the method of miSLNs-34a/PTX preparation. Confocal microscopy results demonstrated that the coumarin-6 and Cy5 fluorescent signals were mainly located into the cytoplasm (Figure 2), indicating miSLNs-34a/PTX could achieve the cytosolic delivery of these two drugs. The positive charge and pertinent particle size (~ 200 nm) contributed to the endocytosis of miSLNs. As shown in Figure 3, compared with the negative control groups, it was readily evident that substantially greater numbers of cells produced extreme chromatin condensation, nuclear fragmentation and exhibited apoptotic body formation in both miSLNs-34a and miSLNs-34a/PTX groups.

More signals from the fluorescein-labeled SLNs accumulated in the lung and tumor (Figure 4). The
ex vivo tissues were collected for image monitoring of Cy3-labeled miR-34a and coumarin-labeled SLNs (Figure 4), showing that miSLNs could efficiently mediate miR-34a and PTX delivery into the lung and tumor.

As expected in in vivo therapeutic experiments, the control groups including saline, mock, SLNs/PTX and miSLNs-34a, exhibited relatively rapid death as a function of time, while miSLNs-34a/PTX significantly prolonged the survival time of mice (Figure 5). Lung morphology displayed that the growth of the tumor nodules in the lung was significantly inhibited by miSLNs-34a/PTX (Figure 6B), which showed enhanced CSC therapy compared with the miSLNs-34a group. In immunohistochemistry test, the vast majority of cells in the tumor-bearing lungs were strongly CD44 positive when treated with PBS, mock, PTX and SLNs/PTX, while CD44 immunohistochemical patterns in the miSLNs-34a and miSLNs-34a/PTX group were opposite (low CD44 level) compared with them (Figure 5), indicating that miR-34a could inhibit CSC growth by attenuating CD44 expression with a synergistic effect by using of PTX.

**CONCLUSIONS**

In conclusion, the results indicate that miSLNs-34a/PTX is a strong candidate for impeding CD44-positive CSC growth and migration, a key stage in the development of a CSC-based lung cancer therapy.

**REFERENCES**


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