Solid lipid nanoparticles as a drug delivery system for cancer stem cell therapy

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ABSTRACT SUMMARY

A nanoparticulate delivery system was developed to entrap microRNA-34a to improve its stability and also the therapeutic effect of miRNA on cancer stem cells (CSCs). This delivery strategy to carry miR-34a is raising the hope for a long term lung cancer treatment.

INTRODUCTION

Numerous efforts have been made to treat the various types of cancers, however the long term and spontaneous recurrence of tumors represent a major challenge in the field, which was caused by the lack of efficiency in eliminating the cancer stem cell (CSC) population in the tumor mass. To reduce such patient relapse, CSC based therapy has been introduced as a novel concept for the treatment of malignant diseases due to its ability to eliminate tumor-initiating cells, eradicating tumor forming CSCs [1]. Recently, miR-34a was highlighted to be a regulator in inhibiting CSC growth, differentiation and metastasis and could therefore be developed as a therapeutic agent [2], raising hope for a long term lung cancer treatment. The delivery of miR-34a into the lungs for CSC-based therapy remains uncertain, with a lot of scope for investigation.

Herein, to develop a delivery system for miR-34a for CSC therapy, we used cationic solid lipid nanoparticles (SLNs) to load miR-34a to treat lung metastasis of murine B16F10-CD44+ (CSC-like) melanoma.

EXPERIMENTAL METHODS

First of all, cationic solid lipid nanoparticles (SLNs) were prepared using a film-ultrasonic method, which composed of glyceryl monostearate (GMS), soy phosphatidylcholine (SPC), cholesterol (Chol), polyoxethylene 50 stearate and dimethyldioctadecylammonium bromide (DDAB) (molar ratio, 27.9:26.5:26/1:15.9). For preparation of drug (miR-34a)-loaded SLNs (miSLNs-34a), isovolumetric RNA solution was added to 50 μL of SLNs colloidal solution for 30-min incubation. The characteristics of miSLNs-34a 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 formulations at a concentration of 150 nM, the cell nuclei were stained with a highly specific fluorescing DNA dye, 4′6-diamidino-2-phenylindole (DAPI) for 10 min. 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 addition, sphere-formation assays were carried out to evaluate CSC-like property of B16F10-CD44+ cells after miSLNs-34a treatment. Meanwhile, CD44 expression was analyzed by flow cytometry (Beckman Coulter).

In vivo evaluation of miSLNs was employed on C57BL/6 mice. FITC-labeled SLNs were prepared as mentioned above, then the mice were given i.v. injections of FITC-SLNs. The fluorescence signals of mice were recorded (ex=498 nm; em=520 nm) using an IVIS® Spectrum system (Caliper, Hopkington, MA). Then, in order to verify whether SLNs could deliver the model drug miR-34a into the lung, Cy3-miR-34a was loaded into nanoparticles for i.v. administration.

Aiming to verify whether miSLNs-34a 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 formulations via tail vein at a rate of 0.5 mg of miR-34a per kg of body weight from day 9 to 11.

RESULTS AND DISCUSSION

The particle size of miSLNs was approximately 200 nm with spherical morphology (Figure 1). The entrapment efficiency of miSLNs was 96.4±0.17%, and the RNA loading yield in miSLNs was 2.57±0.10 nmols per mg lipid.

As shown in Figure 2, 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 lipofectamine-miR-34a (lipo-miR-34a) groups.

Spheroid colony formation is an in vitro cell culture model to identify CSCs (CSC phenotype or property) [3]. As shown in Figure 3, untreated B16F10-CD44+ cells successfully produced spheroid colonies. Spheres treated with miR-34a delivered by miSLNs and lipofectamine showed a suppressive effect on the sphere colony formation. As expected, the other groups containing untreated, mock and free miR-34a did not exhibit this effect (Figure 3). These findings suggest that miSLNs-34a could greatly impact the ability of B16F10-CD44+ cells to form CSC-like spheroids in vitro. Using an anti-CD44 antibody for flow cytometry, relatively low CD44 expression was detected on the cell surface in miSLNs-34a and lipo-miR-34a treatment groups, as compared with the expression of CD44 in the control groups (Figure 4).
More signals from the carboxyfluorescein-labeled SLNs accumulated in the lung (Figure 5). The ex vivo tissues were collected for image monitoring of Cy3-labeled miR-34a (Figure 5), showing that miSLNs could efficiently mediate miR-34a delivery into the lung.

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

![Figure 1. miSLNs visualized by transmission electron microscopy (TEM), scale bar: 200 nm.](image1)

![Figure 2. DAPI staining of fragmented chromatin or apoptotic bodies in B16F10-CD44+ cells after transfection with miSLNs.](image2)

![Figure 3. Photos were taken on 72 h after administration and shown are representative spheres.](image3)

![Figure 4. Flow cytometric evaluation of CD44 expression in the B16F10-CD44+ cells after miR-34a treatments.](image4)

![Figure 5. In vivo fluorescence images of miSLNs in mice and mature miR-34a level in the lung. (A) Time-dependent intensity images of fluorescence distributed mice. (B) Fluorescent microscopy images of lung tissue sections at 2 h after i.v. injection of fluorescence-labeled SLNs. (C) In vivo fluorescence images of major organs at 2 h post-injection of miSLNs-34a. (D) Mature miR-34a level in lung tissues (n=5).](image5)

![Figure 6. CSCs originated tumor growth and metastasis inhibition by dSLN-miR-34a. (A) Survival analysis of B16F10-CD44+ bearing mice (n=8). (B) Images of the B16F10-CD44+ bearing lungs on the day 21 after three consecutive i.v. injections of dSLN-miR-34a (n=5-6). (C) Representative CD44 immunohistochemistry images of B16F10-CD44+ bearing lungs after miR-34a treatment.](image6)

CONCLUSIONS

In conclusion, the results indicate that miSLNs-34a 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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