

Efficient siRNA delivery and tumor accumulation mediated by ionically cross-linked folic acid-poly(ethylene glycol)-chitosan oligosaccharide lactate nanoparticles: For the potential targeted ovarian cancer gene therapy

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## **Background**

Ovarian cancer has the highest mortality rate and is among the most common of female malignancies. Ovarian cancer cells are known to develop resistance to standard chemotherapeutic treatments. Gene silencing via RNA interferences (RNAi) mediated by short interfering RNA (siRNA) has enormous therapeutic potential for the treatment of cancer.

Hypoxic inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) is often overexpressed in cancers including ovarian cancer and it is associated with tumor aggressiveness, angiogenesis, cell migration, proliferation, survival, glucose metabolism, metastasis and drug resistance. It is predicted that HIF-1 $\alpha$  suppression via siRNA technique would provide effective tumoricidal outcome in human ovarian cancer cells.

The siRNA gene therapy is hindered because achieving sufficient concentration of siRNA at the tumor site(s) is difficult. siRNA has a high degradation rate in serum due to its physical characteristics, a rapid elimination by the renal pathway and a low permeability across cellular membranes. One way to enhance the delivery of siRNA to the site of action is a development of a suitable delivery platform with characteristics that enables biocompatibility, a high loading capacity, protection of siRNA during transport and a high targeting ability.

Recently the delivery system for gene therapy has moved from viral vectors to synthetic and natural cationic polymers because viral vectors have potential to evoke immunogenic responses and can be hazardous during preparation. A representative cationic polysaccharide is a natural substance, chitosan. Nano-metric particles are easily formed by crosslinking chitosan with a counter ion such as tripolyphosphates (TPP), which particle provides a protection against degradation of loaded siRNA.

There are problems in the use of chitosan for gene delivery. First, excessive positive charge that remains on the surface of the nanoparticles after formulation brings about interaction with red blood cells (RBCs), opsonization and activation of immune system resulting in elimination of them. Second, achieving a sufficient concentration of siRNA at the tumor site in a timely manner is difficult, as systemically administered chitosan nanoparticles are only passively delivered there via the enhanced permeability and retention (EPR) effect. Third, uptake of chitosan nanoparticles by nonspecific endocytosis in tumor cells results in a low siRNA transfection efficiency. To address these problems, an active targeting system that can also aid the uptake of nanoparticles is required.

In the present study we have employed folic acid (FA) as a targeting ligand because FA is harmless on normal cells, little immunogenic, inexpensive and stable under both *in-vitro* and *in-vivo* conditions. The expression level of the FA receptor is high in ovarian cancer cells, which promotes the receptor-mediated endocytosis of nanoparticles. The present study shows the potential utility of an siRNA delivery system with FA-PEG-COL nanoparticles encapsulating an HIF-1 $\alpha$  siRNA for a targeted ovarian cancer gene therapy.

## **Methods**

The chemical structure of FA-PEG-COL was verified with MALDI-TOF-MS (Applied Biosystems SCIEX TOF/TOF 5800), Fourier transform infrared spectroscopy (FT/IR-460, JASCO Corporation, Tokyo, Japan), and 400 MHz NMR spectrometer (JNM-LA 400 (Lambda 400) JEOL, Tokyo, Japan). The siRNA/FA-PEG-COL nanoparticles were obtained spontaneously via a modified ionic gelation method using TPP. Particle size and polydispersity index (PDI) of the different formulations were determined using a dynamic

light scattering analyzer (ELS-8000, Photal, Osaka, Japan). The zeta potential of the different formulations were determined by a laser Doppler microelectrophoresis Zetasizer (ELS 21, Photal, Osaka, Japan). Cellular uptake of formulations by OVK18#2 human ovarian cancer cells was measured using a FACS Calibur flow cytometer (Becton-Dickinson, San Jose, CA, USA). The HIF1- $\alpha$  gene knockdown by siRNA was assessed at two levels, at the protein level by Western blotting and at the mRNA level using real time quantitative-PCR (RT-qPCR). In-vivo ovarian cancer xeno-graft accumulation efficiency of nanoparticles was examined with an IVIS<sup>®</sup> Spectrum imaging system (Caliper LifeScience, Hopkinton, MA, USA).

## **Results**

- 1) We have confirmed the chemical structure of the synthesized FA-PEG-COL.
- 2) The particle size of siRNA/FA-PEG-COL is about 200 nm and the zeta potential is +8.4 mV. The positive charge of COL was lowered by conjugation with FA-PEG as well as by loading siRNA.
- 3) In serum, rapid degradation of the unformulated siRNA was observed after 1 hour, while some siRNA remained intact with FA-PEG-COL nanoparticles over 36 hours.
- 4) The RBC aggregation and haemolysis with FA-PEG-COL nanoparticles were markedly reduced compared with COL nanoparticles. FA-PEG-COL nanoparticles were significantly less toxic compared with COL nanoparticles.
- 5) The FA receptor-mediated uptake of the FITC-FA-PEG-COL nanoparticles peaked at 9 hours. The uptake of FITC-FA-PEG-COL nanoparticles was enhanced than that of FITC-COL nanoparticles.
- 6) Effect of HIF-1 $\alpha$  knockdown by siRNA on proliferation of OVK18#2 ovarian cancer cells was more remarkable with FA-PEG-COL nanoparticles than COL nanoparticles.
- 7) In nude mice bearing OVK18#2 ovarian cancer cells, FA-PEG-COL nanoparticles were significantly accumulated in tumor than COL nanoparticles.

## **Discussion**

The effectiveness of using a FA-PEG-COL nanoparticles system for delivery of siRNA is shown for the potential anti-HIF-1 $\alpha$  treatment of ovarian cancer. High encapsulating efficiency and good protection of siRNA from serum degradation with appropriate particle size were obtained with this system. Moreover, FA-PEG-COL nanoparticles have excellent tumor targeting ability with minimum uptake by the liver, implying that it has great potential not only for targeting the primary cancer site but also metastatic sites. The results shown here indicate that FA-PEG-COL nanoparticles containing siRNA may have good potential in use along with traditional chemotherapy and radiotherapy to achieve maximum tumoricidal activities.