Stem cell-derived vesicles as a gene delivery vehicle for cancer therapy

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ABSTRACT SUMMARY

We present a novel platform for targeted gene delivery based on vesicles made from membranes of naturally targeted mesenchymal stem cells. The produced vesicles were characterized, incorporated with plasmidial DNA and its interaction with cancer cells and transfection abilities were evaluated.

INTRODUCTION

Viral vectors, while being very effective in delivering genetic material into their target cells, still arouse various concerns about clinical safety and immunogenicity. In addition, they are limited in their load capacity. On the other hand, non-viral vectors are simple to use, easy to up-scale, and lack the limitation on DNA size.

We have developed a non-viral gene delivery system for cancer therapy, which is based on unique vesicles produced from the cell membrane of mesenchymal stem cells (MSC). MSC are known for their homing capability towards cancer cells and their microenvironment\(^1\). The cell derived vesicles (CDV) that resemble liposomes in their structure, will benefit the surface molecules of the MSC and thus may preserve their targeting mechanism. Into this "Trojan horse" CDV system we will incorporate therapeutic encoding DNA, which will be expressed at the tumor vicinity.

Usually, DNA containing synthetic liposomes are composed of positively charged phospholipids to trigger an interaction with the negatively charged DNA\(^2\). But in our case, and as most mammalian cell membranes are negatively charged, we have applied positive charge (using cationic agents), which enabled us to incorporate the negatively charged DNA more efficiently into CDVs, while overcoming the electrostatic repulsion. These DNA-cation complexes can also protect the DNA from nucleases. After the preparation and characterization of the CDV system and its targeting abilities, we will incorporate an EGFP encoding plasmid and then a therapeutic encoding plasmid (pPEx or pS-TRAIL) and evaluate their expression and biological activities using a prostate cancer model.

EXPERIMENTAL METHODS

CDV preparation process included hypotonic treatment, homogenization, sonication and extrusion, to produce nanoscale vesicles. PEG molecules were attached to the CDV surface to elongate their circulation time in vivo. DNA was complexed with different cationic agents- Polyethyleneimine (PEI) 25,000Da, Polylysine (PLL) 25,000Da and Ca\(^{2+}\), and the products were tested for their ability to protect the DNA from nucleases. This was done by DNase treatment followed by the addition of heparin. The products were run on 0.8% agarose gels. The complexes were then incorporated into the CDV. DNA Incorporation efficacy was evaluated using the PicoGreen™ reagent.

CDV morphology was observed after TEM imaging. Size distribution and zeta potential were evaluated using Dynamic Light Scattering. In addition, CDV were checked for MSC surface markers, to evaluate their preservation in their native form. This was done by conjugating the CDV to Dynabeads™, incubating with the MSC's markers antibodies, and analyzing by flow cytometry.

To observe the interaction between the CDV and cancer cells, PC3 cells were stained with phalloidin-FITC and were incubated with DiD stained CDV. The cells were then washed and imaged using confocal microscope.

Transfection ability of CDV incorporating different cation-DNA complexes to PC3 cells, prostate cancer cell line, was evaluated. The cells were incubated with eGFP-encoding-plasmid incorporated CDV for 6 hours. GFP expression was evaluated 24 hours post transfection, using flow cytometry.

RESULTS AND DISCUSSION

CDV and CDV-PEG were prepared and characterized for morphology, size distribution and zeta potential. The produced vesicles showed a spherical and uni-lamellar morphology, and were not affected by the PEG addition (Fig. 1A). Both CDV and PEGylated CDV exhibit a narrow size distribution with a similar average of about 180 nm. The zeta potential analysis demonstrated that the addition of PEG elevated the surface potential of the system from -16mV to -12 mV (Fig. 1B). Following a 3 hour incubation of the CDV with PC3 cells, the CDV could be observed inside the cytoplasm and the nucleus (Fig 2).
Figure 1. Characterization of CDV and CDV-PEG: (A) Cryo-TEM imaging of 1) CDV. 2) CDV-PEG. (B) Size and zeta potential of CDV and CDV-PEG.

Figure 2. Confocal imaging of the interaction between CDV and PC3 cells: PC3 cells were stained with Phalloidin-FITC (green) and incubated for 3 hrs with DiD stained CDV (red). The nuclei were counterstained with Hoechst (blue).

PEI-DNA complexes (in different N/P ratios) were treated with DNase to evaluate the PEI effect. After the digestion, heparin was added (to free the DNA from the complex) and the products were run on agarose gel. Full complexation, that protects the DNA from nucleases, occurred from N/P ratio of 4:1 and above (Fig. 3).

PEI-DNA complexes at different N/P ratios were incorporated into the CDV system, and their transfection ability was assessed. PC3 cells were incubated with CDV incorporating eGFP encoding plasmid. The cell’s GFP expression was analyzed by flow cytometry (Fig. 4).

CONCLUSION
In this work we present a novel gene delivery system based on nano-vesicles made of mesenchymal stem cells’ membrane. These vesicles can be loaded with DNA to yield a powerful transfection tool targeted to tumors and their environment. Preliminary results show the system feasibility in an in-vitro prostate cancer model. Further research will include in vitro optimization of the CDV transfection ability, and in vivo studies using a therapeutic encoding plasmid, to study the distribution and the biological effect on tumor bearing mice.

REFERENCES