Exosomes loaded with siRNA targeting the RRM2 gene trigger apoptosis in the SKOV3 Ovarian Cancer cell-line

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Abstract

Introduction: Using exosomes as vehicles to deliver therapeutic cargos such as small RNAs has been a novel approach in addressing specific cancers. To load a high concentration of the siRNA therapeutic, exogenous methods of loading have been investigated. Here we show that electroporation as a method of loading, yields in high loading efficiency with great functional efficacy, in-vitro. .

Methods: 293F cell derived exosomes were loaded via electroporation with CY3 tagged siRNA targeting the Ribonucleotide Reductase Regulatory Subunit M2 (RRM2) gene. Loading efficiencies (LE) were determined via flow cytometry and RT-qPCR. In-vitro cargo delivery (IVCD) was performed on the SKOV3 Ovarian Cancer cell-line. A set number of loaded exosomes loaded with a known concentration of siRNA were added to a set number of SKOV3 cells. Electroporated RRM2 siRNA, and RRM2 siRNA (un-electroporated) were also included as negative controls. Replicate wells (n=3 per method) underwent the following characterizations: cell imaging and viability determination, RRM2 gene expression analysis via RT-qPCR, RRM2 Protein expression analysis via Western blot, and Caspase 3/7 apoptosis assay using a luminometer.

Results: Loading efficiencies of >%98 is determined via both flow cytometry and RT-qPCR analyses. IVCD onto the SKOV3 cell line resulted in 70% suppression of the RRM2 gene, resulting in 26% suppression of the RRM2 protein, causing 80% of the cell population to undergo apoptosis. The respective siRNA controls had a negligible effect on the cells.

Methods

RRM2 siRNA Loading, figure 1. HEK293F cell-derived exosomes were loaded with CY3 tagged siRNA targeting the RRM2 gene. Two separate methods were used to determine loading efficiencies of the siRNA cargo within the exosomes. **A.** Flow cytometry analysis exhibiting the ungated population of samples wherein the exosome only and loaded exosomes overlap, and the PBS, siRNA only and EP siRNA samples overlap one another. **B.** Gated population yields >98% loading efficiency of the CY3 tagged siRNA within the loaded exosomes. **C.** RT-qPCR analysis of loaded exosomes and respective controls; the standard curve method was utilized for quantification showing >98% loading efficiency is obtained. The % loading efficiency is also used to determine dosing.

Disclosure

This work is proprietary of Capricor Therapeutics, Inc. (NASDAQ: CAPR)

Methods and Results

Figure 1. siRNA loading

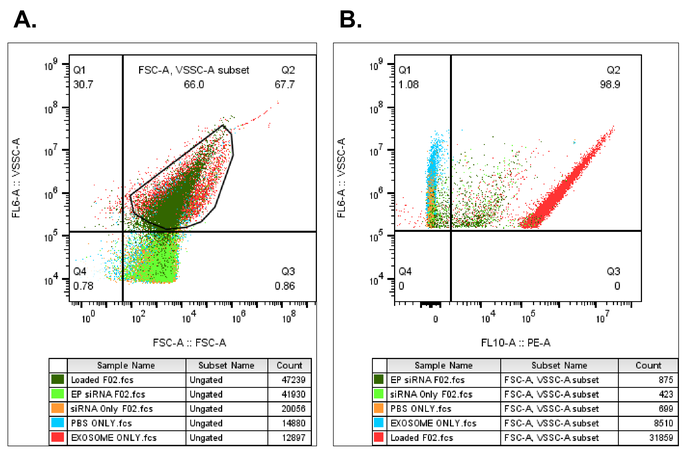


Figure 2. In-Vitro Cargo Delivery

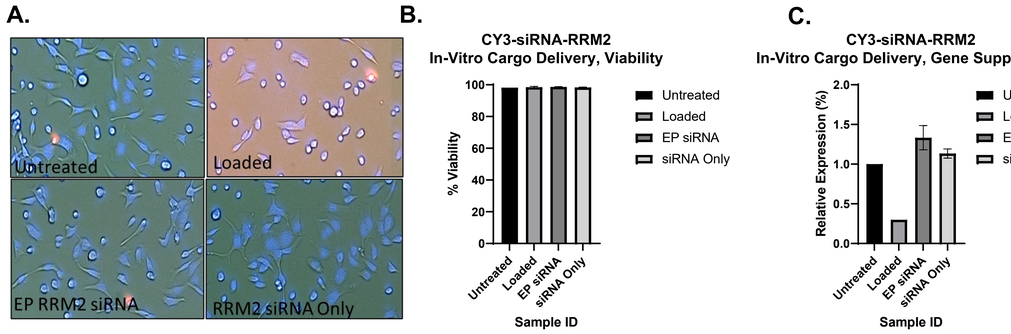


Figure 3. Western Blot Analysis

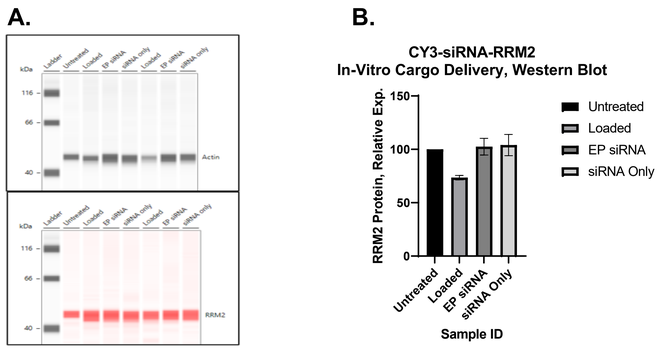
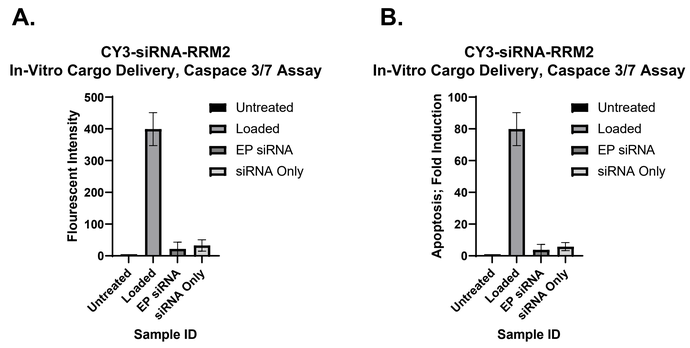


Figure 4. Apoptosis Assay



In-Vitro Cargo Delivery, figure 2: Loaded exosomes and respective controls were added to the SKOV-3 ovarian cancer cell-line at the same dose in triplicate per characterization assay, for treatment overnight. The following day, the cells were characterized. **A.** Image analysis, and viabilities using Hoechst/Propidium Iodide. The wells with the CY3-siRNA loaded exosomes are red in color and the wells with the respective controls are normal (Blue: Hoechst nuclear stain, Red: PI dead cell stain). **B**. Cell viabilities were >98% post overnight treatment. The wells treated with the loaded exosomes have many rounded cells as they are undergoing apoptosis, their membrane is not yet compromised, and PI is not up-taken within the cells. **C.** Total RNA was isolated from the cells undergoing in-vitro cargo delivery for RRM2 gene expression analysis. Post RT-qPCR, double dCT analysis was performed relative to the untreated cells. Only the cells treated with loaded exosomes resulted in 70% RRM2 gene suppression.

Western Blot Analysis, figure 3: A. Whole cell protein lysates were analyzed for both b-Actin (~43KDa) and RRM2 (~45KDa) protein expression levels using the Jess. **B.** The area under the curve generated for both proteins per sample was used to normalize RRM2 protein levels, which in-turn were normalized relative to the untreated control population. The cells treated with loaded exosomes have a 26% reduction in RRM2 protein expression relative to the untreated cells.

Apoptosis Assay, figure 4: A. Caspase 3/7 fluorescent assay kit was utilized to measure apoptosis. **B.** The fluorescent intensity of the cells treated with loaded exosomes was used to determine the induction of apoptosis relative to the untreated cell population. Relative to the untreated control population, 80% of the cells treated by the loaded exosomes are undergoing apoptosis.

Conclusions

293F cell derived exosomes loaded with CY3-RRM2-siRNA have a potent effect on eradicating the SKOV3 ovarian cancer cell line. Using electroporation to load the exosomes, loading efficiencies of >98% were obtained. Within an in-vitro cargo delivery model, SKOV3 cells treated with the loaded exosomes resulted in 70% reduction in the expression of the RRM2 gene, yielding in 26% reduction in the RRM2 protein which in-turn caused 80% of the cells to undergo apoptosis. Using exosomes loaded with siRNA to target the silencing of the RRM2 gene would be a promising therapeutic / co-therapeutic in combating Ovarian Cancer.