Advancing Bio-Inspired Rosette Nanotubes as a Novel and Effective siRNA Delivery Vehicle for the Treatment of Pancreatic Cancer

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is associated with a five-year survival rate of less than 7% and a median survival rate of merely six months following diagnosis¹. State-of-the-art therapies involve subjecting patients to a cocktail of anti-cancer drugs, many of which have a host of undesirable side effects. Implicated in 95% of PDAC patients is a mutated KRAS gene, which functions to enhance the cancer phenotype². It is demonstrated through this work that Rosette Nanotubes (RNTs), developed in the Supramolecular Nanomaterials Lab, can effectively deliver therapeutic small interfering RNA (siRNA) targeted against the KRAS gene to pancreatic cancer cells and silence the gene.

RNTs are a biocompatible and tunable nanocarrier that can effectively carry a therapeutic RNA payload to pancreatic cancer cells³. A defining characteristic and strength of the RNTs as a drug delivery vehicle is the ability to display various functional groups on their surface during self-assembly, conferring to them tunable physical (stability, dimensions), chemical (surface charge and channel properties), and biological (targeting peptides, bioactive molecules) properties. This works demonstrates the effectiveness of RNTs to silence oncogenes in an in vitro PDAC cell model. Using fluorescently tagged siRNA (si555) and siRNA targeted against the oncogene KRAS, we are able to demonstrate effective delivery and function of using the RNT delivery system.

Methods

In order to determine the efficiency of delivery, Panc1 pancreatic cancer cells were seeded in microtiter plates according to standard protocols. Groups of cells were then administered the following treatments: untreated control, naked si555, K1 + si555, and Lipofectamine + si555. The RNTs used in this study are routinely synthesized in our laboratory. Lipofectamine, a commercially available transfection reagent, functions as a positive control. Post-treatment, the cells were then separated into single cell formations and their total internal fluorescence subsequently analyzed on a benchtop flow cytometer.

Furthermore, the function of the siRNA delivered within the cells was evaluated through the assessment of the expression of the KRAS gene. In order to assess the function of the siRNA delivered within the cells, anti-KRAS siRNA (siKRAS) was purchased (Invitrogen). The Panc1 cells were then separated into replicate groups and administered the following: untreated control, naked siKRAS, K1 + siKRAS, K3 + siKRAS, or Lipofectamine + siKRAS. Once again, Lipofectamine was utilized as a positive control. The cells were incubated with the treatments for 48 hours, followed by cell lysis and harvesting of the total mRNA. The expression of the KRAS gene was then determined using quantitative reverse transcription polymerase chain reaction (RT-qPCR).
Results and Discussion

In regards to the in vitro studies, the RNTs functioned significantly more effectively than the cells treated with naked siRNA or the Lipofectamine-treated cells. For example, the cells treated with naked tagged siRNA showed a total internal fluorescence, signifying intracellular delivery of the siRNA, that is roughly 75% less than the cells treated with the RNTs (Figure 1A). This clearly illustrates the benefit of using RNTs as a gene delivery vehicle. The results in Figure 1B illustrated that the cells treated with naked siRNA exhibited little to no knockdown of the KRAS gene, as reported by RT-qPCR. However, the cells transfected using the K1 and K3 exhibited a remarkably greater knockdown levels (54% and 55% KRAS expression, respectively) as compared to the naked siRNA control (96%) sample. These results are also comparable to the cells transfected with the Lipofectamine. This result is significant because Lipofectamine, although effective at gene delivery in vitro, is well documented to have cytotoxic effects and is not viable in vivo. Overall, the results of this work demonstrate that RNTs are a viable and effective gene delivery vehicle for the treatment of pancreatic cancer.

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References: