Nanoparticle-mediated delivery of miRNA mimics

Vishal Kasina[1], Aniket Wahane[1], Dr. Raman Bahal[1*]

[1]School of Pharmacy, University of Connecticut, Storrs, CT 06269

Purpose:

It has been recently established that short, non-protein coding miRNAs play a major role in the onset and progression of many diseases. miRNAs bind to target RNA molecules at the 3' untranslated region to induce translational repression and prevent the production of harmful disease causing proteins. In diseases such as cancer, harmful miRNAs known as oncomiRs are heavily upregulated. However, there are good miRNAs that are downregulated and can be known as tumor suppressor miRNAs. To combat miRNA dysregulation, miRNA-based therapeutics can be in the form of either antimiRs, which block the activity of different upregulated miRNAs and miRNA mimics, which act by increasing levels of different downregulated miRNA. In many solid tumors and hematological malignancies, miR-34a is a tumor suppressor miRNA found to be depleted. miR-34a is known to inhibit many cancer casing pathways such as the epithelial to mesenchymal transition and acts in a positive feedback loop with the tumor suppressor transcription factor, p53. Here, we utilized a cationic delivery system, using PLGA:poly-L-Histidine nanoparticles to effectively deliver miR-34a mimics to target lung cancer. By using a cationic delivery system, we are able to encapsulate negatively charged miRNA mimics with minimal toxicity and reduce the survival of lung adenocarcinoma cell lines.

Methods:

We encapsulated a commercially purchased miR-34a mimic into PLGA:poly-L-Histidine nanoparticles using a double emulsion solvent evaporation method that was previously optimized (Wahane, Malik et al., 2021). We utilized biophysical characterization techniques to perform through characterization of our formulation, including dynamic light scattering, SEM/TEM imaging, loading/release kinetics as well as RNA integrity analysis to confirm successful loading and stability of the mimic in our formulation. We tested our formulation *in vitro* in the A549 lung adenocarcinoma epithelial cell line where we evaluated cellular uptake by using confocal microscopy and flow cytometry. We then evaluated *in vitro* efficacy by measuring levels of miR-34a and its target, p53 with RT-PCR and Western blot analysis. The increase in p53 was also tested in hypoxic

conditions to mimic the tumor microenvironment. Cell viability was also tested through colonogenic assay and the extent to which cells became apoptotic after treating with our formulation was tested using an Annexin-V based apoptosis assay in both fluorescence microcopy and flow cytometry.

Results:

We obtained miR-34a mimic loaded PLGA:PH nanoparticles of 200nm in particle with a uniform size distribution (PDI<0.2). The change in surface charge comparing positively charged PLGA:PH nanoparticles (+2mV) with negatively charged miR-34a loaded nanoparticles (-22mV) confirmed successful loading of miR-34a mimics in our formulation. SEM and TEM imaging revealed spherical morphology of both nanoparticles with and without mimics as well as stability in the solution state. Total loading of mimic in nanoparticles was 150picomoles/mg where nanoparticles exhibited an initial burst release at 15 minutes, followed by 100% release by 24-48hrs. Polyacrylamide gel electrophoresis confirmed stability of miR-34a mimic in our formulation after 48hrs. When treating the A549 cell line with our formulation, we observed better cellular uptake of miR-34a when delivering through our nanoformulation when compared with transfecting Lipofectamine at an equivalent dose, confirmed by fluorescence microcopy and flow cytometry. Gene expression analysis showed that treating with miR-34a loaded nanoparticles increased miR-34a levels by 3-fold when compared to Control oligo loaded nanoparticles. This effectively increased p53 levels by 50%, resulting in a 2.5 fold increase in p53 on the protein level. In hypoxic conditions, our formulation increased the levels of p53 by 25%. We were able to reduce the colony forming efficiency by 60% and observed fewer A549 colonies when treating with miR-34a loaded nanoparticles. Annexin-V based assays revealed higher population of cells in the apoptotic/necrotic state when treating with our formulation.

Conclusions:

We were successfully able to load a tumor suppressor miR-34a mimic in a cationic delivery system comprising of PLGA:poly-L-Histidine nanoparticles, and deliver with high cellular uptake. We achieved *in vitro* by effectively increasing levels of miR-34a and p53, while reducing the overall cell viability of the A549 cell line to establish the therapeutic potential to treat lung cancer.