Pan-cancer MicroRNA Delivery Design using Green Synthesis-based Nanotechnology for Cancer treatment

Balarohitha Sundaram, Mythili V. S. Akella, Lekhana Akula, Ravikiran S. Yedidi*

The Center for Advanced-Applied Biological Sciences and Entrepreneurship (TCABS-E), Rajamahendravaram & Visakhapatnam, A.P. India.
Department of Biotechnology, Andhra University, Visakhapatnam, A.P. India. *Correspondence to R.S.Y.: tcabse.india@gmail.com

INTRODUCTION

Cancer is caused by mutations in gene sequence or aberrations in chromosomes that control cell division (errors that can occur during cell division), proliferation and function. Genetic changes that can cause cancer could be due to the damage to DNA caused by harmful substances (mutagens) in the environment, such as chemicals in consumables and ultraviolet rays from the sun or alternately, inherited from parents.

Three primary gene types are typically impacted by the genetic alterations that lead to cancer: DNA repair genes, tumor suppressor genes and proto-oncogenes. DNA repair genes are capable of fixing nucleotide alterations in DNA. Mutations in these genes frequently lead to further mutations in other genes and chromosomal abnormalities. These alterations have the potential to make the cells malignant (e.g. BRCA1). Tumor suppressor genes regulate the division and development of cells. Mutations in these genes can cause uncontrollable cell proliferation (e.g. TP53). Proto-oncogenes play a major role in the proper division and development of cells. Upon mutation, these genes may become oncogenes allowing cells to proliferate and survive when they shouldn't be able to be active (e.g. KRAS).

Among the several anticancer treatment options, usage of therapeutic RNA such as microRNA (miR) molecules is gaining importance in the recent years. These miRs are non-coding sequences that are complementary to coding sequences in mRNA. However, considering the stability issues of RNA therapeutics, their delivery is one of the challenges. In this study, we designed nanoparticles that are crosslinked with miRs that can regulate the above mentioned 3 types of genes responsible for cancer.

HYPOTHESIS

In this study, we hypothesize that using specific miRs (non-coding microRNA transcripts) crosslinked to nanoparticles will bind to the coding region of the complementary mRNA thereby inducing the silencing of the transcriptomic segment thus stopping the cell from further division and growth.

EXPERIMENTAL DESIGN

To understand the overall organization of the miRs, the 2D structures of the miR34A, miR21, miR29A, miR494, miR127 and miR130A were generated using the RNAfold software. These miRs were reported to be effective against Ovarian cancer, Gastric cancer, Breast cancer, Colorectal cancer, Hepatocellular carcinoma and Leukemia, respectively. The RefSeq transcript sequence of the miRs were downloaded from the NCBI. The final miR nucleotide sequences were taken from the mirdb.org and using RNAfold software the 2D structures of these miRs were generated. Minimum free energy of each structure was taken from the RNAfold calculations.

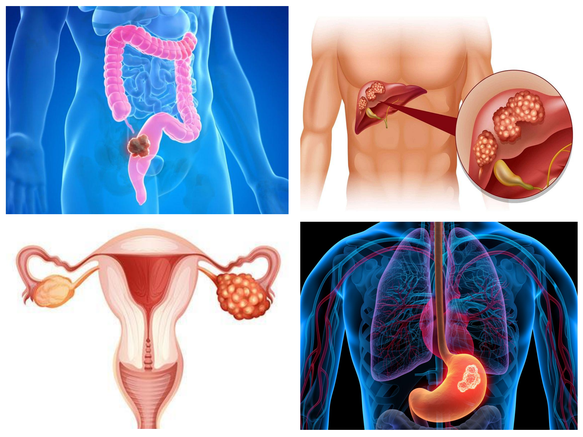
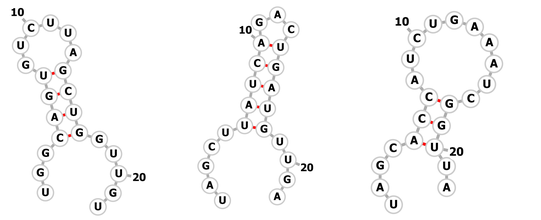


Figure 1. Cancers: Colorectal cancer (top left panel), Hepatocellular carcinoma (top right panel), Ovarian cancer (bottom left panel) and Gastric cancer (bottom right panel).

1. miR34A: 5'-UGGCAGUGUCUUAGCUGGUUGU-3'
2. miR21: 5'-UAGCUUAUCAGACUGAUGUUGA-3'
3. miR29A: 5'-UAGCACCAUCUGAAAUCGGUUA-3'
4. miR130A: 5'-CAGUGCAAUGUUAAAAGGGCAU-3'
5. miR127: 5'-UCGGAUCCGUCUGAGCUUGGCU-3'
6. miR494: 5'-AGGUUGUCCGUGUUGUCUUCUCU-3'

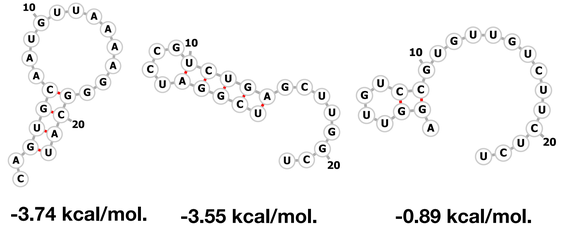
Figure 2. Nucleotide sequences of 6 miRs used in this study design.

EXPERIMENTAL DESIGN



-3.74 kcal/mol. -2.04 kcal/mol. -1.14 kcal/mol.

Figure 3. The secondary stem-loop structures of miR34A (left panel), miR21 (middle panel) and miR29A (right panel).



-3.74 kcal/mol. -3.55 kcal/mol. -0.89 kcal/mol.

Figure 4. The secondary stem-loop structures of miR130A (left panel), miR127 (middle panel) and miR494 (right panel).

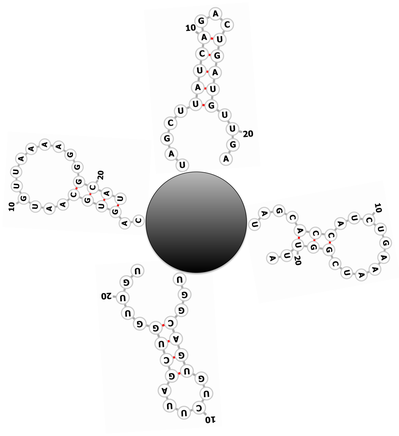


Figure 5. A model of the nanoparticle crosslinked with miRs for different cancer related genes.

EXPECTED RESULTS

The nanoparticles loaded with miRs against different oncogenes must be tested in appropriate animal models for different cancers. Especially the miR secondary structures as shown in Figures 3, 4 and 5 must be considered whether it may affect the binding of miR to the mRNA of the oncogene in the target cells. Due to high entropy these secondary structures might not be stable hence one can expect the miRs to bind the target mRNA. However, the thermodynamic stability of miRs loaded onto the nanoparticles must be extensively tested before using animal models.

FUTURE DIRECTIONS

1. The designed nanoparticle has to be synthesized using green synthesis.
2. The designed nanoparticle has to be characterized for quality analysis using SEM, etc.
3. The fully characterized nanoparticle has to be evaluated *in vitro* using biochemical and biophysical techniques for binding affinity analysis followed by testing the *in vivo* potency.

REFERENCES

1. Szczepanek J, Skorupa M, Tretyn A. MicroRNA as a Potential Therapeutic Molecule in Cancer. Cells. 2022 Mar 16;11(6):1008. doi: 10.3390/cells11061008. PMID: 35326459; PMCID: PMC8947269.
2. Menon A, Abd-Aziz N, Khalid K, Poh CL, Naidu R. miRNA: A Promising Therapeutic Target in Cancer. Int J Mol Sci. 2022 Sep 29;23(19):11502. doi: 10.3390/ijms231911502. PMID: 36232799; PMCID: PMC9569513.
3. "Defining Cancer". National Cancer Institute. 17 September 2007. Retrieved 28 March 2018.
4. "What is Cancer?" National Cancer Institute. 17 September 2007. Retrieved 28 March 2018.

ACKNOWLEDGEMENTS

We thank The Yedidi Institute of Discovery and Education (TyiDE), Toronto for funding this project idea. We thank Andhra University for giving us this opportunity to present our project idea.

Poster presented at the VLSE2024, Visakhapatnam.