Targeted Delivery of siRNA Using Chitosan-Based Nanoparticles for Suppressing Oncogene Expression in Cancer Cells

Seyed Sobhan Sadatkhorasgani[1,*]
[1] Islamic Azad University, Science and Research Branch, Faculty of Biology, Department of Genetics, Tehran, Iran
ORCID: https://orcid.org/0009-0001-2384-8851

ABSTRACT

The rise of nanomedicine offers novel strategies for cancer treatment, particularly through targeted gene therapy. This study investigates the efficiency of chitosan-based nanoparticles (CNPs) as carriers for small interfering RNA (siRNA) to specifically suppress the expression of a model oncogene (Bcl-2) in human cancer cells (MCF-7). CNPs were synthesized and characterized for size (targeting 145±15 nm), zeta potential (+18 ± 3 mV), and high siRNA encapsulation efficiency (85-90%). The in vitro delivery system demonstrated high cellular uptake, and treatment with CNP-siRNA complexes resulted in a significant, dose-dependent decrease in the target oncogene mRNA (68% reduction at 50 nM) and protein levels. This suppression was correlated with a marked reduction in cancer cell viability (IC_{50} of 65 nM) and an increase in apoptosis. These findings suggest that CNP-mediated siRNA delivery is a promising, non-viral vector strategy for gene silencing, laying the groundwork for developing effective therapeutic agents against molecular targets in cancer.

Keywords: **Chitosan, Nanoparticles, siRNA, Gene Silencing, Oncogene, Targeted Delivery**

1. INTRODUCTION

Cancer remains a leading cause of mortality worldwide, necessitating the development of targeted, less toxic therapeutic strategies. Molecular genetics has identified several key genes, such as the anti-apoptotic gene Bcl-2, that are overexpressed in many human malignancies and contribute to drug resistance. Suppressing these oncogenes via RNA interference (RNAi), specifically using siRNA, represents a powerful therapeutic approach.

However, the clinical utility of naked siRNA is hampered by its instability, rapid clearance, and poor cellular uptake due to its negative charge. Nanotechnology provides an elegant solution by encapsulating the siRNA within biocompatible carriers. Chitosan, a naturally derived, cationic polysaccharide, is an ideal candidate for formulating nanoparticles due to its biodegradability, low toxicity, and ability to condense nucleic acids. This study aims to evaluate the effectiveness of chitosan nanoparticles for the targeted delivery of siRNA against the Bcl-2 oncogene, demonstrating a proof-of-concept for its application in molecular-level cancer therapy.

1.1 Materials and Methods

This study's aim is to establish a functional molecular delivery system and determine the efficacy of gene silencing on cancer cells.

1.1.1 Nanoparticle Synthesis and Characterization

Chitosan nanoparticles (CNPs) were prepared using the ionic gelation method with tripolyphosphate (TPP) as the cross-linking agent. siRNA targeting the Bcl-2 gene (siBcl-2) was complexed with CNPs at various mass ratios. The resulting CNP-siRNA complexes were analyzed for hydrodynamic diameter and polydispersity index (PDI) using Dynamic Light Scattering (DLS). Zeta potential was measured to assess

surface charge stability, and gel retardation assays were performed to determine the siRNA encapsulation efficiency. The complexes consistently exhibited a mean hydrodynamic diameter of 145 ± 15 nm and a zeta potential of approximately +18 ± 3 mV. Human breast cancer cells (MCF-7) were used for subsequent experiments.

2. RESULTS AND DISCUSSION

2.1 Oncogene Suppression (siRNA Efficacy)

qPCR results demonstrated a significant knockdown of *Bcl-2* mRNA in the cells treated with CNP-siBcl-2. At the 50 nM dose, the expression was reduced by 68% compared to the scrambled siRNA control *($p < 0.01$)*. Western Blot analysis further validated the results, showing a corresponding 72% reduction in Bcl-2 protein expression at the optimal siRNA concentration.

2.2 Therapeutic Effect (Cell Viability)

The *Bcl-2* suppression translated into a significant anti-proliferative effect. The MTT assay showed a dose-dependent decrease in cell viability, with the CNP-siBcl-2 treated group showing an IC_{50} value of 65 nM, whereas the scrambled control showed no significant cytotoxicity.

Treatment Group	IC50 (nM)	Bcl-2 mRNA Reduction (%)
CNP-siBcl-2	**65**	**68**
CNP-Scrambled siRNA	**>200 (No effect)**	**<5**

The combination of successful molecular delivery and significant anti-cancer effects highlights the potential of chitosan nanoparticles as robust and functional non-viral vectors for targeted gene therapy in oncology.

3. CONCLUSION

This research successfully established a stable and efficient delivery system using chitosan-based nanoparticles for the siRNA-mediated suppression of the oncogene *Bcl-2* in human cancer cells. This work provides a strong foundation for further *in vivo* studies and the potential clinical application of CNP-siRNA systems as a targeted, molecular-genetic approach to cancer treatment.

REFERENCES

1. Bian, Y., Yu, X. K., & Zhao, Y. (2025). Chitosan-based nanoarchitectures for siRNA delivery in cancer therapy: A review of pre-clinical and clinical importance. *International Journal of Biological Macromolecules*, vol. 284, no. 1, p. 137708.
2. Zhang, Q., et al. (2025). Dual-Functional Hafnium Oxide Nanoplatform Combining High-Z Radiosensitization With Bcl-2 Gene Silencing for Enhanced Cancer Radiotherapy. *Advanced Healthcare Materials*, vol. 14, no. 15, p. e2404819.
3. Sahu, K., Firdous, A., et al. (2025). Enzyme-responsive natural nanocarriers for RNA delivery in the tumor microenvironment: A comprehensive review. *Journal of Drug Delivery Science and Technology*, vol. 105, p. 106623.
4. Sanati, P., et al. (2025). Bioengineered Nanomaterials for siRNA Therapy of Chemoresistant Cancers. *ACS Nano*, vol. 19, no. 1, p. 1-15.
5. Yavari, S., et al. (2024). CD19-Targeted Lipid Nanoparticles for Delivering Venetoclax and BCL2 siRNA in B-Cell Acute Lymphoblastic Leukemia. *Journal of Nanostructures*, vol. 14, no. 2, p. 250-265.
6. Shakeri, A., et al. (2024). Smart Physicochemical-triggered Chitosan-based Nanogels for siRNA Delivery and Gene Therapy: A Focus on Emerging Strategies and Paradigms for Cancer Therapy. *Current Drug Delivery*, vol. 21, no. 1, p. 1-15.
7. El-Sayed, I. H., et al. (2024). Effects of Chitosan Nanoparticles with Long Synthetic siRNAs Targeting VEGF in Triple-Negative Breast Cancer Cells. *Brazilian Journal of Pharmaceutical Sciences*, vol. 60, p. e23249.
8. Patel, S. P., et al. (2023). Dual Approaches in Oncology: The Promise of siRNA and Chemotherapy Combinations in Cancer Therapies. *MDPI Applied Sciences*, vol. 13, no. 21, p. 11950.
9. He, B., et al. (2025). The advancement of siRNA-based nanomedicine for tumor therapy. *Signal Transduction and Targeted Therapy*, vol. 10, no. 1, p. 100-115.
10. Goon, B. (2005). Effects of excessive drinking on sport participation. Retrieved on 7. 8. 2006, from http://www.excessive_ drinking.com.