

Abstract

Breast cancer remains the most prevalent aggressive cancer affecting women, with significant mortality rates, especially in high-income countries. Traditional cancer treatments, while effective, often cause severe non-specific cytotoxicity and lead to drug resistance with prolonged use. To address these issues, this research first focuses on developing a single-targeted nanomedicine using estrogen receptor (ER) targeted chitosan nanoparticles (NPs) loaded with Palbociclib (PLB), a chemotherapeutic agent for advanced breast cancer therapy. The ER-targeted NPs were characterized for particle size, zeta potential, polydispersity, surface morphology, drug entrapment efficiency, and *in vitro* drug release. Cytotoxicity assays, cellular uptake studies, apoptosis assays, and cell cycle analysis were conducted on ER+ breast cancer cell lines (MCF-7 and T-47D). *In vivo* studies included pharmacokinetic analysis, histopathology of vital organs, and evaluation of antitumor activity in a DMBA-induced breast cancer rat model. Results demonstrated that ER-targeted NPs showed significantly improved anticancer activity, pharmacokinetics, and tumor targeting compared to non-targeted NPs and free drugs.

Building on these findings and to further enhance therapeutic efficacy and incorporate imaging capabilities, the study further developed a dual-targeted theranostic system that targets both estrogen and folate receptors. This system used chitosan NPs loaded with both PLB and ultra-small magnesium nanoclusters (UMN), the latter serving as an imaging agent. The dual-receptor targeting employed estrone and folic acid to enhance the specificity and efficacy of the treatment. The dual-targeted NPs were characterized similarly to the single-targeted NPs, with additional evaluations of their imaging capabilities. *In vitro* and *in vivo* studies assessed the cytotoxicity, cellular uptake, pharmacokinetics, and antitumor activity of the dual-targeted NPs. Simultaneous imaging was performed using ultrasound and photoacoustic techniques to visualize tumor size,

hypoxic regions, and vascularity. Results showed that the dual-targeted NPs had significantly enhanced anticancer activity and imaging capabilities compared to non-targeted and single-targeted NPs. The dual-targeted NPs exhibited substantial accumulation in breast tumors and better visualization of tumor characteristics, indicating improved therapeutic efficacy and precise monitoring of tumor response.

Conclusively, the single-targeted ER nanomedicine and the dual-targeted theranostic system developed in this study offer promising approaches for advanced breast cancer treatment. The ER-targeted NPs demonstrated effective drug delivery and anticancer activity, while the dual-targeted system provided enhanced therapeutic efficacy combined with robust imaging capabilities, potentially leading to better management and monitoring of breast cancer.