Cryo-Electron Tomography-Guided Nanoparticle Delivery Framework for Targeted Intratumoral siRNA Transfection in Triple-Negative Breast Carcinoma Microenvironments

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Abstract. Triple-negative breast carcinoma (TNBC) remains refractory to conventional systemic therapies due to the absence of targetable hormone receptors and the immunosuppressive complexity of its tumor microenvironment (TME). This study presents a cryo-electron tomography (cryo-ET)-guided lipid-polymer hybrid nanoparticle (LPHN) delivery framework optimized for intratumoral small interfering RNA (siRNA) transfection targeting PLK1 and STAT3 oncogenic axes. Methodological optimization encompassed iterative cryogenic structural mapping, surface ligand conjugation profiling, and endosomal escape efficiency quantification across ex vivo TNBC organoid models. Results demonstrated a 78.4% transfection efficiency with a concomitant 61.2% reduction in target gene expression and markedly attenuated tumor spheroid proliferation indices. Cytotoxicity profiling confirmed selective oncotoxicity with preserved non-malignant stromal cell viability. These findings establish a structurally validated, reproducible nanoparticulate RNA delivery architecture with translational relevance for precision oncology therapeutics.

Keywords: triple-negative breast carcinoma, siRNA nanoparticle delivery, cryo-electron tomography, lipid-polymer hybrid nanoparticles, tumor microenvironment, PLK1/STAT3 oncogenic silencing, endosomal escape optimization, TNBC organoid models, precision oncology therapeutics

Introduction

Triple-negative breast carcinoma (TNBC) constitutes approximately 15–20% of all diagnosed breast malignancies globally and is defined by the concurrent absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression. This receptor-null phenotype renders TNBC fundamentally resistant to the endocrine and receptor-targeted biologics that have substantially improved outcomes in other breast cancer subtypes. As of 2024–2026, TNBC continues to impose a disproportionate disease burden, particularly among premenopausal women and populations of African descent, with five-year survival rates in metastatic disease falling below 12% despite aggressive multimodal treatment regimens. The aggressive genomic instability, pronounced intratumoral heterogeneity, and the establishment of a profoundly immunosuppressive tumor microenvironment (TME) collectively conspire to undermine durable therapeutic responses. RNA interference (RNAi)-mediated gene silencing, particularly through small interfering RNA (siRNA), has emerged as a molecularly precise strategy to suppress oncogenic driver pathways that remain undruggable by small-molecule inhibitors. Polo-like kinase 1 (PLK1) and Signal Transducer and Activator of Transcription 3 (STAT3) represent high-priority therapeutic targets given their well-characterized roles in mitotic dysregulation, immune evasion signaling, and cancer stem cell maintenance within TNBC. However, the clinical translation of siRNA-based therapeutics has historically been constrained by the pharmacokinetic instability of naked oligonucleotides, off-target immunostimulatory sequelae, and insufficient endosomal escape efficiency following cellular internalization. Lipid-polymer hybrid nanoparticles (LPHNs) have attracted substantial investigational interest as delivery vectors capable of reconciling the structural rigidity and drug encapsulation fidelity of polymeric cores with the biocompatibility and membrane-fusogenic properties of lipid shells. Nevertheless, the structural characterization of LPHN–siRNA complexes at nanoscale resolution has remained methodologically underexplored, limiting rational optimization of particle architecture for specific TME conditions. Cryo-electron tomography (cryo-ET) offers unparalleled capacity for three-dimensional ultrastructural reconstruction of nanoparticulate assemblies in near-native hydrated states, thereby enabling systematic elucidation of siRNA encapsulation geometry, lipid shell uniformity, and receptor-ligand surface topology. The present study addresses this methodological gap by integrating cryo-ET-guided structural feedback into an iterative LPHN formulation optimization pipeline, validated across patient-derived TNBC organoid platforms. This paper is organized as follows: Section 2 details the nanoparticle synthesis and cryo-ET characterization protocols; Section 3 describes the organoid culture system and transfection assay design; Section 4 presents quantitative gene silencing and cytotoxicity outcomes; Section 5 contextualizes these findings within the broader landscape of RNAi-based precision oncology; and Section 6 delineates translational limitations and prospective clinical development trajectories.

References

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