Optimizing siRNA delivery for liver cancer therapy: an interdisciplinary approach using lipid nanoparticles decorated with nucleic acid aptamers, including photochemical internalization

Shayan Ahmed*[1], Julia Richert[1], Sandrine Pelet[1], Ines Barahona[2], Celia Sequera[2], Flavio Maina[2], Halina Anton[1], Maria Vittoria Spanedda[3], and Laurence Choulier[1]

[1]Laboratoire de Bioimagerie et Pathologies – université de Strasbourg, Centre National de la Recherche Scientifique – France

[2]Centre de Recherche en Cancérologie de Marseille – Aix Marseille Université, Institut Paoli-Calmettes, Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique, Centre National de la Recherche Scientifique : UMR7258, Institut National de la Santé et de la Recherche Médicale : U1068, Institut Paoli-Calmettes : UMR7258, Aix Marseille Université : UM105 – France

[3]Laboratoire d'Innovation Thérapeutique – université de Strasbourg, Institut de Chimie - CNRS Chimie, Centre National de la Recherche Scientifique – France

Résumé

The tumor microenvironment significantly influences cancer development, progression, and therapeutic resistance. We are interested in targeting a secreted protein found highly overexpressed in hepatocellular carcinoma (HCC). Our project aims to inhibit its expression, using small interfering RNAs (siRNAs). They are a powerful molecule for gene silencing, but their clinical application is limited by challenges such as instability in biological fluids and poor cellular uptake. To overcome these barriers, this study focuses on developing a targeted delivery strategy using aptamers. Aptamers are short, single-stranded DNA or RNA molecules that can fold into defined three-dimensional structures to bind specific targets with high affinity. Aptamers targeting cell-surface receptors can be internalized, making them promising carriers for the delivery of therapeutic molecules like siRNAs. Our objective is to evaluate the potential of siRNA encapsulated in lipid nanoparticles (LNP) and decorated with nucleic acid aptamers to target HCC.
Lipid nanoparticles (LNPs) decorated with aptamers were formulated using a lipid composition of DLin-MC3-DMA (50%), cholesterol (_˜38.5%), DSPC (10%), PEG-2000-C-DMG (0.75%), and PEG-2000-N3 (0.75%). These LNPs exhibited an average size of approximately 100 nm, a zeta potential near neutrality, and low cytotoxicity, as confirmed by MTT assays. High encapsulation efficiency (> 90%) for siRNA and conjugation efficiency (> 86%) for aptamers were achieved. The siRNA demonstrated effective gene silencing in HCC13 and HUH1 cell lines.

To further enhance cytosolic internalization, photochemical internalization (PCI) was employed. Two photosensitizers, PS1 (A1PcS2a)1 and PS2 (BODIPY), were tested to trigger

*Intervenant

light-induced endosomal disruption. Incubation with PS1 and PS2 led to a two-fold improvement in gene silencing efficiency, as quantified by RT-qPCR.

Overall, LNPs emerge as a promising delivery platform to enhance targeted internalization and bioavailability of aptamer-guided siRNA toward HCC cell-surface receptors. Furthermore, PCI facilitates endosomal escape, thereby improving cytosolic siRNA release and therapeutic efficacy. We next plan to encapsulate the photosensitizers (PS) within LNPs. In parallel, a pharmacological approach will be employed to evaluate siRNA cytosolic delivery using drug-based strategies. Additionally, confocal microscopy and flow cytometry experiments are scheduled to assess intracellular localization and binding efficiency of our formulation. Based on the outcomes of these experiments, we will design and initiate *in vivo* studies.

References

1. Figliola C, Anton H, Sutter C, Chériaux C, Sutter A, Mazan V, et al. Lysosomes Targeting pH Activable Imaging-Guided Photodynamic Agents. ChemBioChem. 2023;24(12):e202300139. doi:10.1002/cbic.202300139

Mots-Clés: Hepatocellular carcinoma, Aptamer, siRNA, LNP, Photochemical Internalization and Photosensitizer.