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Manipulation of the Composition and Physico-chemical Properties of Combo Lipid Nanoparticles for Highly-selective Chemo-gene Therapy of Hepatocellular Carcinoma *In Vivo*

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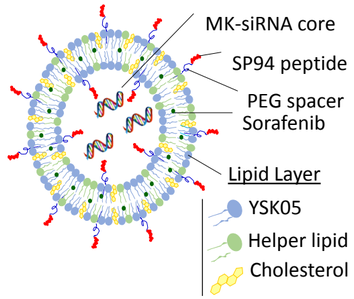
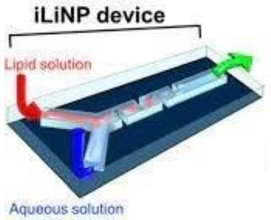
PURPOSE

Design and optimization of combo lipid nanoparticles (cLNPs) for the selective co-delivery of the cytotoxic drug, sorafenib (SOR), and siRNA targeting the Midkine gene (MK-siRNA) to hepatocellular carcinoma (HCC) *in vivo*.

METHODS

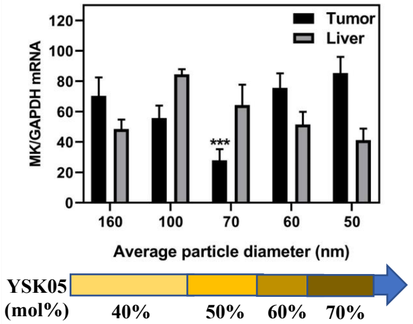




- ❖ cLNPs were designed based on YSK05, a novel pH-sensitive cationic lipid designed in our laboratory, helper phospholipids, PEGylated lipid moieties and a powerful targeting peptide, SP94.
- ❖ Lipid composition was extensively tweaked to control the *in vivo* performance of cLNPs.
- ❖ A novel microfluidic device, iLiNP was recruited to manipulate the physico-chemical properties of cLNPs.

RESULTS

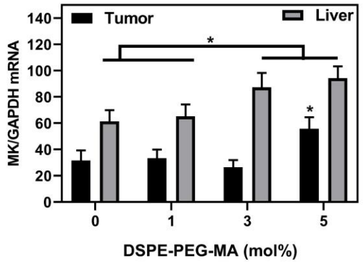
Impact of the particle size



*siRNA dose= 0.5 mg/Kg. ANOVA followed by Bonferroni test, ***P<0.001 versus tumor in case of other particle diameters.*

Particle size and main lipid ratio should be optimized to balance between tumor penetration and selectivity.

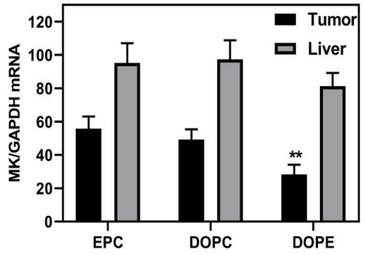
Improving tumor-selective gene silencing



*siRNA dose=0.5 mg/Kg. ANOVA followed by Bonferroni, *P<0.05 for tumor at 5 mol% versus other ratios and for livers at 3 and 5 mol% versus other ratios.*

Insertion of PEG moiety at optimum ratio improved tumor selectivity via masking recognition by the healthy liver.

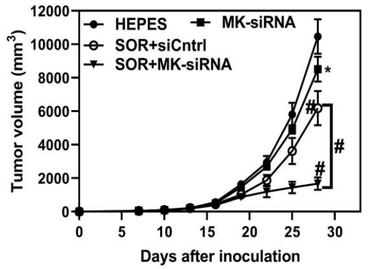
Optimizing the endosomolytic properties



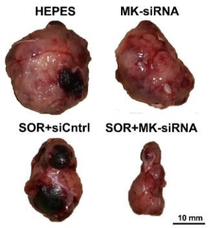
*siRNA dose=0.3 mg/Kg. ANOVA followed by Bonferroni test, **P<0.01 versus other helper lipids.*

Proper selection of the helper lipid significantly impacted endosomolytic properties with subsequent effect on the gene knockdown efficiency.

Therapeutic output



*SOR dose=2.5 mg/Kg, siRNA dose= 0.5 mg/Kg. ANOVA followed by Bonferroni test, *P<0.05, #P<0.0001 versus HEPES.*



Novel chemo-gene combination synergistically-eradicated HCC from mice.

CONCLUSIONS

- ✓ Tweaking the lipid composition and physico-chemical properties of cLNPs dramatically affected their *in vivo* performance.
- ✓ Our DDS demonstrated highly-selective and potent gene silencing in the tumor (ED_{50}=0.1 mg/Kg).
- ✓ The novel combination presented in this study demonstrated synergistic anticancer effect on HCC with promising potential for clinical application.

DECLARATION

JPO JAPAN PATENT OFFICE

A patent application for the intellectual property associated with this work has been submitted to Japan Patent Office, Tokyo, Japan (No. 2020-075619).