Ultrasound enhanced miRNA sponge delivery using sponge-loaded magnetic nanodroplets for Hepatocellular carcinoma therapy

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Background, Motivation, and Objective:

MicroRNA (miRNA) sponges are common approaches to suppress oncogenic miRNAs for cancer therapy. Ultrasound (US) mediated delivery can avoid toxicity, immune response and limitation of time, space and quantity, which shows great application prospects for sponge delivery. We attempted to utilize magnetic nanodroplets as a carrier loading sponges inhibiting oncogenic miRNAs for hepatocellular carcinoma (HCC) therapy, and enhance tumor accumulation and transfection of sponges after US activation under magnetic field.

Statement of Contribution/Methods:

We performed miRNA profiling in 48 benign and malignant hepatocellular tumors, and synthesized sponges with six repeat miRNAs binding sites (MBS), including sponge 1-5 targeting miR-515 family members and sponge 6 inhibiting miRNA-449 family members. The sponges-loaded magnetic nanodroplets (SLMNDs) were constructed with hydrated lipid, polyethylenimine (PEI), and perfluorocarbon with Fe_3O_4 nanoparticles dispersed in. The *in vitro* sponge delivery efficiency of SLMNDs under 5 MHz focused US exposure was detected based on Green Fluorescent Protein using flow cytometry. The *in vivo* therapeutic effect of SLMNDs was evaluated on mouse xenograft HCC model (HepG-2 and SMMC-7721) for two month with 20 treatments. During US exposure, passive cavitation detect was used to monitor and quantify the cavitation dose.

Results/Discussion:

There 4 major miRNA families were found among 94 up-regulated miRNAs. Herein most members of miR-515 and -449 families were certificated that up-regulated in HepG-2 and SMMC-7721 cells. Sponge 1 and 2 with six repeat MBS targeting miR-515 family had significant probability to inhibit cancer cells proliferation (Fig. 1A, B). The SLMNDs had a mean size of 260 ± 48 nm and good magnetic targeted delivery ability. Aagarose gel electrophoresis showed that each SLMND could carry 15 sponges (Fig. 1C). The amount of PEI used to protect sponges depended on the molecular weight. The sponge delivery efficiency was up to 70% (Fig. 1D). The tumor accumulative and therapeutic effect of SLMNDs was further validated on mouse xenograft model.

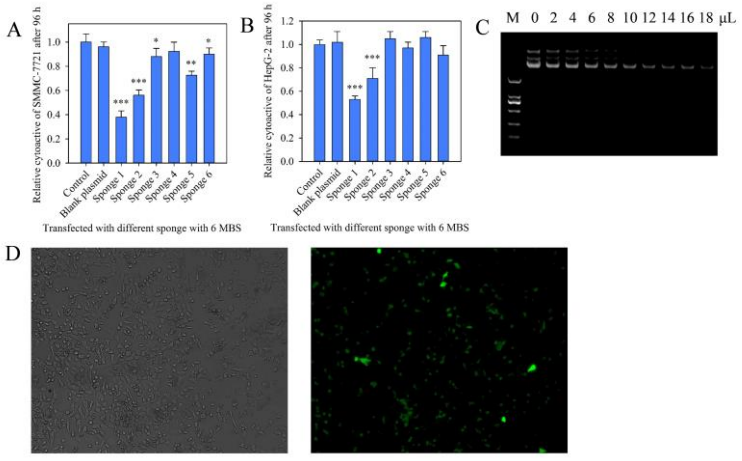

Fig. 1
A and **B:** Relative cytoactive of SMMC-7721 and HepG-2 after transfected with sponges.
C: Agarose gel electrophoresis presented the unloaded sponges in the supernatant decreased with adding more magnetic NDs.
D: GFP fluorescence of cells showed the sponges were delivered into tumor cell with SLMNDs under US treatment.