Acoustically-Active LHRHa-targeted microbubbles mediating RNA interference and inducing apoptosis in A2780/DDP human ovarian carcinoma cells

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**Background:** Survivin is a member of the inhibitor of apoptosis protein (IAP) family that is involved in drug resistance in ovarian cancer and inhibits cellular apoptosis. Recently, ultrasound mediated microbubbles delivery system carrying RNA interference (RNAi) has been reported in ovarian cancer cells. Untargeted microbubbles delivery systems, however, have a major limitation of poor gene silence efficiency due to lack of the native receptor, Luteinizing Hormone-Releasing Hormone Receptor (LHRHR) on the surface of ovarian cancer cells. We hypothesize that the gene transfection efficiency of the pshRNA-survivin would be enhanced by adding targeting microbubbles, thus increasing the knockdown effect of survivin expression in ovarian carcinoma cells.

**Methods:** Two microbubbles (pshRNA-survivin loaded lipid microbubbles and LHRHa-targeted pshRNA-survivin loaded lipid microbubbles) were created using biotin-streptavidin bridge recombination. A2780/DDP cell lines were used to perform experiments. The cells were divided into seven groups: control group, pshRNA-survivin group, pshRNA-survivinloaded lipid microbubbles group, US+ pshRNA-survivin group, US+ pshRNA-survivin loaded lipid microbubbles group, US+ LHRHa-targeted pshRNA-survivin loaded lipid microbubbles group and were treated respectively. The RNAi knockdown efficiency was determined by Q-PCR and Western blot. The cellular proliferation was detected by MTT assay and apoptosis was detected by FACS analysis and Hoechst staining.

**Results:** QPCR at 48 hours revealed survivin mRNA knockdown induced by US+ pshRNA-survivin loaded lipid microbubbles and US+ LHRHa-targeted pshRNA-survivin loaded lipid microbubbles was 56% and 64% respectively compared to negative control. Western blot revealed translational inhibition induced by both groups. MTT assay indicated increased cell death in US+LHRHa-targetedpshRNA-survivin loaded lipid microbubbles, the ratio of inhibition was 42.08%, 54.60%, and 74.25%. After transfection 24h, 48h, 72h, respectively, which were higher than other groups (p<0.05), FACS analysis revealed increased Annexin V positivity cell and Hoechst 33258 staining revealed increased hyperchromatic and compact at condensed or granular state of apoptotic nuclei cells in US+LHRHa-targetedpshRNA-survivin loaded lipid microbubbles group was more significant than other groups, which suggest higher rate of apoptosis. Western blotting revealed enhanced caspase-9 and caspase-3 expression in US+LHRHa-targetedpshRNA-survivin loaded lipid microbubbles than in other groups.

**Conclusions:** Ultrasound mediate LHRHa targeted pshRNA-survivin loaded lipid microbubbles resulted in survivin expression knockdown and apoptosis in A2780/DDP cells in vitro and shows that LHRHa targeted lipid microbubbles as a potential vehicle application in future gene therapy models of RNAi in ovarian cancer.

**Biography**

Shufang has completed her Ph.D at the age of 27 years from Chongqing. She is a Gynecological oncologist of the Second Affiliated Hospital of Chongqing Medical University. She has published more than 40 papers in journals.

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