14 Tesla MR Imaging of Mice Cholangiocarcinoma Response to Radiofrequency Heating (RFH)-enhanced Chemotherapy: towards Intrabiliary RFH-enhanced Chemotherapy for Pancreatobiliary Cancers

Feng Zhang[1], Thomas Le[1], Xia Wu[1], Tong Zhang[1], Han Wang[1], Stephanie Soriano[1], Donghoon Lee[1], and Xiaoming Yang[1]
[1]*Radiology, University of Washington, Seattle, WA, United States*

PURPOSES: Pancreatobiliary cancers have limited response to systemic chemotherapy due to inefficient drug delivery into tumor tissues and the high resistance of cancer cells to chemotherapeutics. The aim of this study was to validate the feasibility of using 14 Tesla MRI to monitor RFH-enhance chemotherapeutic effect on mice cholangiocarcinomas.

METHODS: For in vitro evaluation, green fluorescent protein (GFP)-labeled human cholangiocarcinoma cells (Mz-ChA-1) were cultured in four-chamber slides and treated by using (a) 20-min RFH at approximately 42 ℃ plus 500-mM gemcitabine; (b) 20-min RFH-only; (c) 500-mM gemcitabine-only; and (d) no treatment to serve as a control. Cell viabilities were assayed using trypan blue exclusion. For in vivo evaluation, cells were subcutaneously implanted into the backs of 24 nu/nu nude mice to create cholangiocarcinoma mice models. Six mice in each group were treated by (a) intratumor injection of gemcitabine and 5-fluouracil (5-FU) at 25mg/kg, followed by RFH at approximately 42°C for 20 min; (b) 20-min RFH alone; (c) intratumor injection of 25mg/kg gemcitabine and 5-FU; and (d) phosphate buffer solution (PBS) injection to serve as controls. 14Tesla MRI, including T2 weighted imaging and diffusion-weighted imaging (DWI) was used to image the tumors at day 1 before treatment and day 1, 7, and 14 after treatment. Tumor size and apparent diffusion coefficient (ADC) were measured. HE staining, apoptosis assay and confocal microscopy were performed to establish image/histology correlation.

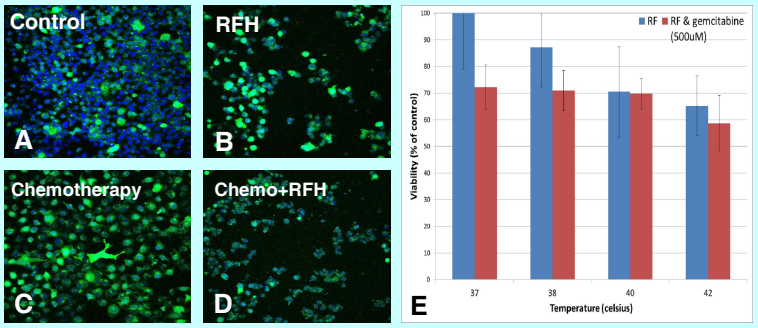

Fig.1. Qualitatively confocal microscopy shows the number of viable cells in RFH+ gemcitabine group is obviously decreased, as compared to other groups (A-D). Quantitative cells viability assay demonstrates that the relative cell viability is significantly decreased in RFH+ gemcitabine group than RFH alone and gemcitabine alone group (0.58±0.10 vs 0.65±0.11 and 0.58±0.10 vs 0.72± 0.8, $p<0.05$)(E).

RESULTS: The in vitro experiments demonstrated that RFH-enhanced chemotherapy can significantly inhibit tumor cells growth, as compared to chemotherapy alone and the RFH alone group (0.58±0.10 vs 0.72±0.8 and 0.58±0.10 vs 0.65±0.11, $p<0.05$)**(Figure 1).** The in vivo experiments show a significant decrease in relative tumor growth in the group with RFH-enhanced chemotherapy, compared with chemotherapy and RFH only group (1.6±0.2mm vs 3.7mm±0.41mm and 1.6±0.2mm vs 2.9mm±0.33mm)**(Figure 2)**. ADCs in RFH-enhanced chemotherapy group increased at day 1 and day 7 after treatment and return to the same level as that of the pre-treatment. For the chemotherapy and RFH group, there is no significant change in ADCs between and pre-and post-treatment**(Figure 3E)**. Histology confirmed that RFH can enhance the chemotherapeutic effect on tumors. (**Figure 3A-D**).

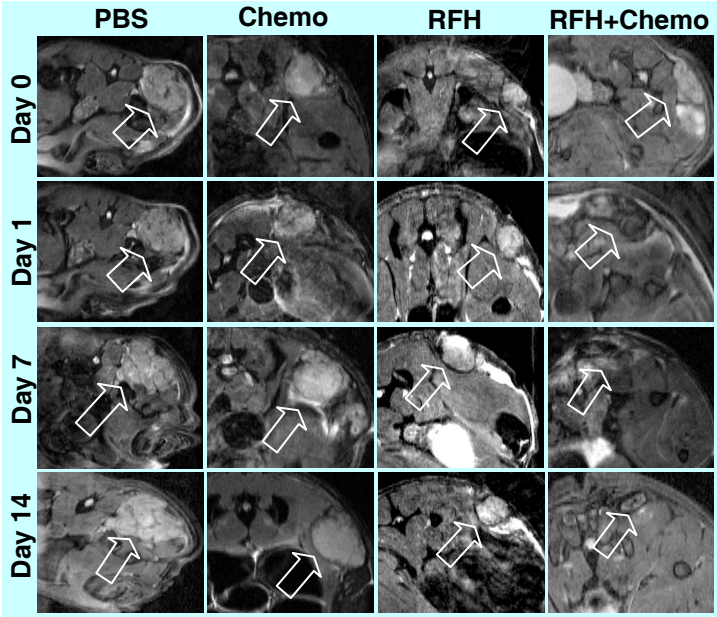

Fig.2. DW images at *b* of 25 sec/mm^2 show high signals of mice cholangiocarcinoma masses in four groups with different treatments (arrows). Compared with the PBS, chemotherapy only and RFH group, the tumor size in the group treated by RFH-enhanced chemotherapy was decreased by more than 70%.

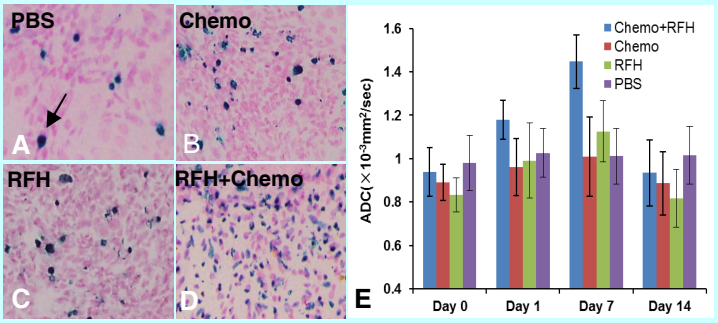

Fig.3. Histologic analyses of tumor response. *A-D,* representative TUNEL (×250) staining of Mz-ChA-1 tumors collected at day 14, after PBS, chemotherapy, RFH, and RFH plus chemotherapy were applied for different group. An apoptotic cell is stained as a blue spot (arrow). *E,* ADCs of four groups with various treatments. For the RFH-enhanced chemotherapy group, ADC increased at day 1 and day 7 and then returned to the same level as that of pretreatment.

CONCLUSIONS: This study demonstrates the feasibility of using DW MRI to monitor RFH-enhanced chemotherapeutic effect of gemcitabine and 5-fu on mice cholangiocarcinomas and shows that ADC is a useful biomarker for evaluating the therapeutic effect, which may open new avenues to effectively manage pancreatobiliary malignancies.