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14. ABSTRACT

This work is aimed to study MR guided high intensity focused ultrasound (MRgHIFU) enhancement of gene therapy for Prostate Cancer. The first statement of work is to determine if high intensity focused ultrasound (HIFU) increases the cellular uptake of AS-MDM2, AS-bcl-2 and AS-PKA, thereby suppressing MDM2, bcl-2 and PKA, respectively, in vivo. The tasks include (a) Characterization of the output of the focused ultrasound unit using MR guidance; (b) Determination of the uptake of AS-bcl-2 in orthotopically grown LNCaP tumors; (c) Determination of the timing of the reduction in MDM2 in orthotopically grown LNCaP tumors;(d) Statistical assessment of targeted antisense therapy on gene expression in tumor bearing mice. We performed phantom studies on characterization of the output of the focused ultrasound unit using MR guidance. The IACUC protocol has just been approved by the USAMRMC Animal Care and Use Review Office (ACURO) for the use of mice. Now we are able to perform the animal studies.

15. SUBJECT TERMS

MRgHIFU, Gene Therapy

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Introduction

This project is aimed at exploring MR guided pulsed high intensity focused ultrasound (HIFU) enhancement of gene therapy combined with androgen deprivation and radiotherapy for prostate cancer treatment. We have proposed to work on the first task for the first research year. The tasks include (a) Characterization of the output of the focused ultrasound unit using MR guidance; (b) Determination of the uptake of AS-bcl-2 in orthotopically grown LNCaP tumors; (c) Determination of the timing of the reduction in MDM2 in orthotopically grown LNCaP tumors; (d) Statistical assessment of targeted antisense therapy on gene expression in tumor bearing mice. In the following we describe our research progress for the first year.

Body

Our research project has been delayed due to several unforeseeable reasons: 1) the animal protocol being used in this study was requested to be resubmitted for approval due to Dr. Pollack's departure. The protocol (IACUC protocol number 02-7) associated with this project was originally approved by both our IACUC and the USAMRMC Animal Care and Use Review Office on April 11 2008 with Dr. Alan Pollack as a Principal Investigator. When Dr. Pollack relocated from Fox Chase Cancer Center to University of Miami in July 2008 the approved protocol was terminated in October 2008. A new protocol was submitted with Dr. Lili Chen as Principal Investigator. This protocol has been approved by our IACUC effective September 23, 2009. The protocol was approved by the USAMRMC ACURO on September 24, 2009. 2) Due to Dr. Pollack's departure my co-investigator, Dr. Zhaomei Mu, working on Dr. Pollack's project was transferred to the lab of another researcher here at Fox Chase, Dr. Brian Lally, in February 2008 due to the lack of salary support from Dr. Pollack before my DOD funds were released. It took me several months to recruit a person with certain skills required by the project. Since Dr. Brian Lally left for Miami in March 2009 I was able to hire Dr. Zhaomei Mu for my research project and 3) originally, my studies would be carried out in Dr. Pollack's lab. His lab was closed when he left and it took several months for me to establish my own lab space with the support of our institution.

In summary, due to personnel relocation, IACUC transition and change of lab space my research project has been delayed. However, with the institutional support we are able to move forward with our research project smoothly, as demonstrated by our other research projects funded by focused ultrasound surgery foundation (FUSF) (1).

In this annual report we report on the research progress associated with the tasks outlined in the approved "Statement of Work" task 1 between Sept. 1, 2008 and Aug. 31, 2009. We will provide detailed information below for the results in the first year without animal studies due to the reasons explained above.

<u>Characterization of the output of the focused ultrasound unit using MR guidance</u>

Study of ultrasound output parameters on phantom

A series of pilot experiments were performed on an MRgHIFU system (ExAblate 2000 HIFU system, InSightec, Inc. and 1.5 T MR unit GE) with an acoustic phantom provided by InSightec. The purpose of these studies were to determine the ultrasound output including frequency, acoustic power and pulse width that are adequate for the enhancement of gene therapy for treatment of prostate cancer in mice, without damage to tissues permanently. The MR proton resonance frequency shift sequence was used

for temperature monitoring during the treatment. We assumed that tissue would not be damaged below 42°C. In order to avoid perm anent tissue damage the temperature elevation should be below 5 °C with animal am bient tem perature. W e summarize our resu lts from the phantom studi es that with 1 MHz, pulse width 0.1 s, duty cycle: 50%, acoustic power: 5 W the tem perature elevation is approxim ately 4 °C. W ith th ese ultra sonic trea tment param eters, we have further dem onstrated tha t the re we re no ultrasonic lesions seen in ex-vivo tissue. The 1 MHz frequency was chosen based on the cavitation mechanism, which has been discussed in the literature (i.e., refs 2-8).

We perform ed studies on the relationship between acoustic energ y (acous tic power x ultrasonic exposure time) and the tem perature elevation with a given acoustic pow er of 4 W. Results showed that 4 °C te mperature elevation is m aintained from 40 joule (exposure tim e 10 s) to 240 joule (exposure time 60 s) due to the therm al equ ilibrium. W e t hought that although th e tem perature elevation is the same for different acous tic en ergy th e biolog ical effects in anim al (*in vivo)* m ay be different. T hese phantom measurements provided basic ultrasonic parameters for the *in vivo* studies.

Key Research Accomplishments

We have accomplished the following tasks:

- We have completed co-investigator' training for ne w techniques required for the project including MR scan and operating the MRgHIFU treatment unit.

- We have derived "optim al" ultrasonic treatment parameters from phantom studies. The param eters will be verified *in vivo* animal studies.

- We generated the protocol for the use of ani mals. The protocol is approved by both the IACUC a nd the USAMRMC Animal Care and Use Review Office.

Reportable Outcomes

Peer-reviewed abstracts and proceedings resulting from or supported in part by this grant:

L Chen, Z Mu, P Hachem , C-M Ma, A Pollack. Enhan cement of Drug Delivery in Prostate Tumor *in vivo* Using MR Guided Focused Ultrasound (MRg HIFU). WC, IFMBE Proceedings 25: pp341-344, 2009

L Chen, C Ma, T Richardson, G Freedm an, A Konski. Treatment of Bone Metastasis Using MR Guided Focused Ultrasound. *Medical Physics*: 36: 2486, 2009.

L Chen, Z Mu, P Hachem , C-M Ma and A Pollack Enhancem ent of ^{3}H-Docetaxel Delivery in Prostate Tumor *in vivo* using MR Guided Focused Ultrasound (MRgHIFU) 2009 astro

C Ma, **L Chen,** A Pollack, A Konski, G Freedm an, M Buyyounouski. Quality Assurance f or MR Guided Focused Ultrasound as a Multi-Moda lity Platform for Cancer T herapy. *Medical Physics*: 36: 2631, 2009

Non peer-reviewed papers resulting from or supported in part by this grant: none
Funding applied for based on work resulting from or supported in part by this grant: none

Conclusions

We have made progress during our first-year investigation despite the delay of our animal protocols and the re-establishment of lab space and personnel. We have performed machine calibration and established the procedures for daily quality assurance (DQA). We have derived "optimal" ultrasonic treatment parameters for animal studies. Now we are able to do animal studies under the approval from USAMRMC ACURO.

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Appendices

L Chen, Z Mu, P Hachem, C-M Ma, A Pollack. Enhancement of Drug Delivery in Prostate Tumor *in vivo* Using MR Guided Focused Ultrasound (MRgHIFU). WC, IFMBE Proceedings 25: pp341-344, 2009

Enhancement of Drug Delivery in Prostate Tumor *in vivo* Using MR Guided Focused Ultrasound (MRgHIFU)

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Abstract—**The purpose of this work is to investigate the effects of pulsed focused ultrasound on the enhancement of ^{3}H-Docetaxel delivery in prostate tumors *in vivo*. Human prostate cancer LNCaP cells (5 x 10^{5}) in 24 ml medium were injected into the prostates of male nude mice. When tumor reached the size of 160 ± 10 mm^{3} on MRI, HIFU treatment was performed using an InSightec ExAblate 2000 system with a 1.5 T GE MR scanner. The animals were randomly divided into 3 groups (n=8 per group): Group 1, HIFU treatment + ^{3}H-Docetaxel; Group 2, HIFU treatment only and Group 3, as control. For group 1, each mouse was treated with pulsed HIFU under general anesthesia using MR guidance. Immediately after, HIFU treated animals received a single dose of i.v injection of Docetaxel at 15 mg/kg mixed with ^{3}H-Docetaxel at 50 uCi/kg in total volume of 150 µl. Animals in group 2 were treated the same as in group one with the exception of HIFU treatment. Animals were sacrificed 30 minutes after i.v injections and tumors were removed and processed. The radioactivity of ^{3}H-docetaxel in the tumor tissue was quantitatively measured by a liquid scintillation counter. Results showed that all animals tolerated the MRgHIFU treatment well. There were no treatment-related adverse events including skin toxicity. Our data show increased ^{3}H-docetaxel concentration in tumor in the MRgHIFU treated group (1079 ± 132 cmp/75 mg) vs. those without MRgHIFU treatment (524 ± 201 cmp/75 mg) with P = 0.037. We have demonstrated the enhancement of ^{3}H-Docetaxel uptake in implanted prostate tumors with MRgHIFU *in vivo*. Future studies will be carried out on the efficacy of Docetaxel combined with radiotherapy (RT) to inhibit prostate cancer growth *in vivo*.**

Keywords— **MRgHIFU, Prostate Cancer, Mouse Model, In Vivo, Drug Delivery**

I. Introduction

Current leading therapies for local prostate cancer include radical prostatectomy and radiation therapy (external beam, seed implants or a combination), and additional hormonal therapy (androgen deprivation, AD) and chemotherapy for locally advanced or disseminated prostate cancer. Radiation therapy (RT) is one of the most effective treatment modalities for prostate cancer. External beam RT of prostate cancer depends on accurate targeting and delivery of an optimal dose distribution to the desired treatment volume. Biochemical failure occurs in 30-50% over the long term, even when radiation doses are escalated [1]. Failure after RT with/without AD appears to be related to both local persistence of disease and metastasis. Docetaxel, either as a single agent or combined with others, has shown a survival benefit in prostate cancer patients. It has been used for the treatment of advanced hormone refractory prostate cancer [2, 3]. Docetaxel is also a potent radio-sensitizer [4]. If the delivery of Docetaxel is enhanced, increased tumor inhibition is expected.

The research proposed for this project is based on the following technical advances and findings: (1) ultrasound emitted in short, high-energy pulses, will result in focused regional shock waves, which alter vascular permeability without permanently damaging the tissue, (2) MR imaging can be used to place the ultrasound beam in the target area and to monitor the effect of the treatment, and (3) the increased vascular permeability will increase macromolecular pharmaceutical agents in the treatment target. In the model system proposed, pulsed high intensity focused ultrasound (HIFU) is hypothesized to improve the delivery of Docetaxel into human prostate tumors grown orthotopically in the prostates of nude mice. Our previous studies demonstrated that it is feasible to enhance drug delivery using HIFU in animal models *in vivo* [5, 6]. Our hypothesis is that enhancement of the Docetaxel delivery using HIFU under MR guidance combined with RT will improve the tumor response in vivo. The purpose of this work was to perform animal experiments to evaluate the effects of pulsed focused ultrasound on the enhancement of ^{3}H-Docetaxel delivery in prostate tumors *in vivo.*

II. Materials and methods

A. Tumor models and MR protocols

In vivo studies were carried out in compliance with guidelines and approval of the institutional animal care committee. Human prostate cancer LNCaP cells were obtained from the American Type Culture Collection and cultured in Dulbecco's modified Eagle's medium (DMEM)-F12 medium, containing 10% fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin-streptomycin as described

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previously [1]. In order to use MR guidance for the focused ultrasound treatment, we developed an optimal MR imaging protocol, which allows us to obtain a higher quality image to visualize the prostate tumor while the scan time remains acceptable for the whole HIFU treatment procedure to be within approximately 1 h anesthesia time. Figure 1 shows an example of the high quality images that were used for HIFU treatment planning. The MR parameters were: T2-weighted fast spin echo (FSE) sequence; TR/TE=2150/102 ms; Bandwidth: 10.4 kHz; FOV=9 x 9 cm; Matrix: 384 x 384 NEX: 4; slice thickness: 2.0 mm/0.0 sp; frequency direction: SI and the spatial resolution: 0.23 mm.

B. Experimental setup

The MRgHIFU treatments were performed on an In-Sightec ExAblate 2000 HIFU system together with a 1.5T GE MR scanner (Figure 2). The system was installed in the Department of Radiation Oncology, FCCC in 2006. The HIFU treatment system is FDA-approved for the treatment of uterine fibroids clinically and is being investigated at FCCC for treating bone metastases, prostate and breast cancers under local IRB approval. The phased array transducer is housed in a sealed bath and connected to a motion system. The focal region is cigar-shaped, approximately 3mm in diameter and 10mm in focal length. Quality control (QC) was performed according to the procedures provided by the vendor to check the transducer output and the automatic electronic motion system prior to treatment. Figure 3 shows the animal setup for the MRgHIFU treatment. A gel phantom was placed on the treatment table in line with the transducer. Degassed water was used for the interface between the treatment table and the gel phantom for the acoustic coupling. Care has been taken to eliminate any air bubbles between the interfaces. The gel phantom was warmed to approximately 37 °C and a shallow hole measured about 2cm x 3cm with 8mm in depth was made. The hole was located in the center on the top of the gel and filled with warm degassed water. The animal was carefully placed on

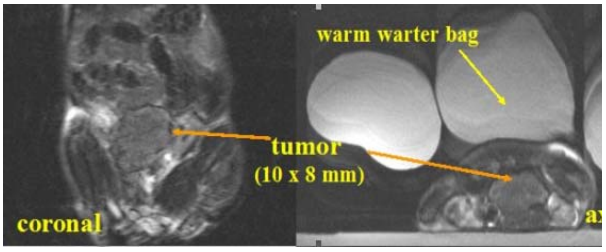


Figure 1. MR images showing the prostate tumor in the coronal view and axial view.

Figure 2. The high intensity focused ultrasound unit with a 1.5 Tesla MR scanner. The workstation is also shown.

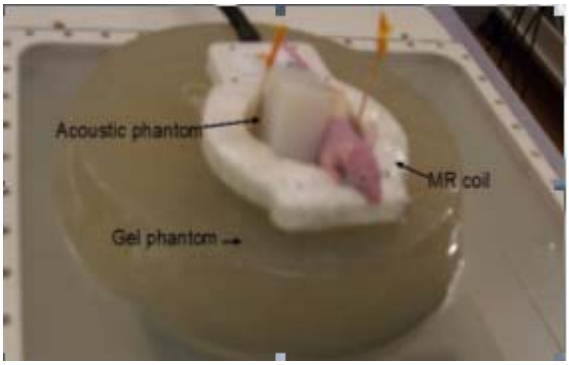


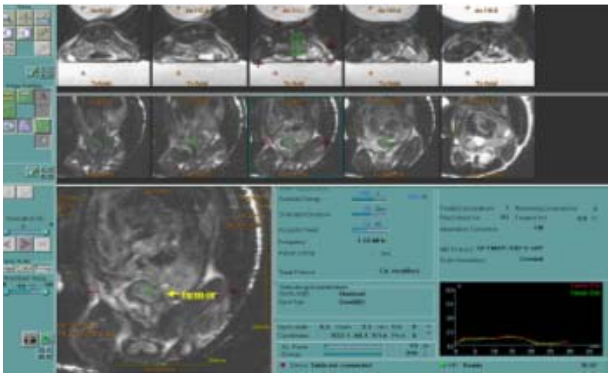
Figure 3. The animal set up for MR-guided HIFU treatment for this study.

the gel phantom in contact with the degassed water in a prone position. A 3-inch surface coil was placed around the animal to receive the MR signals. A small acoustic phantom was placed beside the animal for the purpose of the beam focus verification. A small warm water bag was placed on the animal to maintain its body temperature. A treatment plan was generated in real time based on both coronal and axial MR images. Figure 4 shows the treatment planning window on the MRgHIFU workstation.

C. MRgHIFU treatment

When tumor reached the size of 160 ± 10 mm^3 on MRI, HIFU treatment was performed. The animals were randomly divided into 3 groups (n=8 per group): Group 1, HIFU treatment + ^{3}H-Docetaxel; Group 2, HIFU treatment only and Group 3, as control. For group 1, each mouse was treated with HIFU under general anesthesia. MR images were used for target delineation, treatment planning and monitoring of temperature elevation during the HIFU

treatment. Each animal was treated with pulsed ultrasound using 1 MHz; 4 W acoustic power and the 81 mode setting (5 Hz



Figur 4. Real-time treatment planning for MR guided HIFU.

frequency with 0.1s power on, 0.1s power off) for 60 seconds for one sonication. The ultrasound treatment parameters were used based on our pilot studies with acoustic phantom. A total of 8-10 sonications were used to cover the whole tumor volume. Immediately after, HIFU treated animals received a single dose of i.v injection of Docetaxel (Taxotere; sanofi-aventis U.S. LLC, Bridgewater, NJ) at 15 mg/kg mixed with ^{3}H-Docetaxel (American radiolabled Chemicals, Inc) at 50 uCi/kg in total volume of 150 µl. Animals in group 2 were treated the same as in group one with the exception of HIFU treatment.

Animals were sacrificed 30 minutes after i.v injections and tumors were removed. Tumor samples (100mg) were digested in 1.2ml of Solvable (PerkinEkmer, Boston, MA) for 1 h at 55^{o}C, and decolorized by adding 0.2 ml of 30% hydrogen peroxide for 30 min at 55 ^{o}C. Scintillation cocktail (10 ml) was added. The radioactivity of ^{3}H-docetaxel in the tumor tissue was quantitatively measured by a liquid scintillation counter.

III. Results

All animals tolerated the MRgHIFU treatment well. There were no treatment-related adverse events including skin toxicity (figure 5). Our data show increased ^{3}H-docetacel concentration in tumor in the MRgHIFU treated group (1079 + 132 cmp/75 mg vs. those without MRgHIFU treatment 9524 + 201 cmp/75mg) with p=0.037 (figure 6).

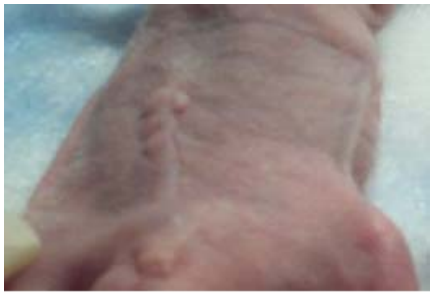


Figure 5. MRgFU treated mouse showing that there is no skin toxicity after the treatment.

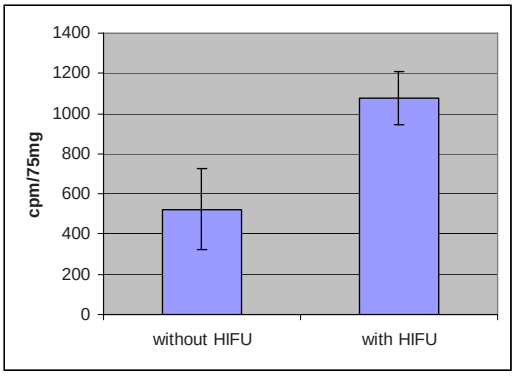


Figure 6. Comparison of 3H-Docetaxel between with HIFU treatment and without HIFU treatment group.(n=8)

IV. Conclusions

We have demonstrated the enhancement of ^{3}H-Docetaxel uptake in implanted prostate tumors with MRgHIFU *in vivo.* Future studies will be carried out on the efficacy of Docetaxel combined with radiotherapy (RT) to inhibit prostate cancer growth *in vivo.*

V. Acknowledgements

This work is supported by FUSF award and partially supported by DOD PC073127

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