MRI - imaging, histological analysis and gene expression of a squamous cell carcinoma model treated with Focused ultrasound

W. Hundt[1], E. Yuh[1], M. Bednarski[1]

[1]Stanford University School of Medicine, Stanford, CA, United States

Introduction

Imaging methods are being developed to follow the course of thermal therapies for tumor ablation in cancer. MR-temperature monitoring is a new method to follow thermal therapies [1], but it is difficult to perform clinically and doesn't give information about the molecular changes occurring in treated regions. The search for molecular targets and the development of new molecular imaging agents is necessary to elucidate these biological tissue changes at the molecular level. Focused ultrasound (FUS) treatment of a mouse squamous cell carcinoma provides a model system to study gene expression patterns at different levels of energy deposition [2]. Like rf ablation, radiotherapy and cryotherapy, FUS causes physical changes in tissues through energy deposition, and is undergoing development for diverse therapeutic applications including thermal ablation [3], and gene and protein delivery [4]. In this study, our aim was to use functional genomics on tumor tissue treated with various energy deposition modes using FUS in order to define potential targets for the development of molecular imaging agents. These targets can be useful in understanding and in following therapy.

Materials and Methods

C3H/Km mice aged 10 weeks were implanted with 2 x 10^5 mouse squamous cell carcinoma (SCC VII) cells intradermally in each flank. Two weeks were required for tumors to grow to 1 cm size.

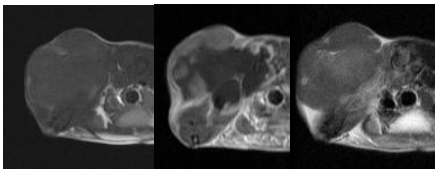
Focused ultrasound was applied to the tumors using a dual imaging/therapeutic ultrasound system (Focus Surgery; Indianapolis, IN). The system employs a compound transducer consisting of a 1 MHz spherical therapeutic transducer, combined with a 6 MHz imaging transducer to monitor the progress of therapy. Two different dose regimes of FUS were investigated. The pulsed wave mode consisted of 50 msec pulses of FUS and a pulse frequency of 0.5 Hz, applied for 10 minutes. The continuous mode consisted of continuous-wave FUS applied for 20 seconds. The resulting average intensity at the focal point was in the pulsed wave mode 217.27 Ws/cm^2 and in the continuous wave mode approximately 3500 Ws/cm^2. The total energy deposition was in both modes nearly the same; in the pulsed wave mode 130208 W/cm^2 and in the continuous wave mode 134613 W/cm^2.

Pre- and post- contrast T1-weighted images and T2-weighted images of the FUS-treated tumors and their ipsolateral untreated controls were obtained on a 1.5 GE scanner using a multislice spin echo sequence. For T1-weighted images, TR and TE were 400 and 30 msec. For T2-weighted images, TR and TE were 3000 and 90 msec. All images had a 50 x 50 mm FOV and a 256 x 256 matrix**.**

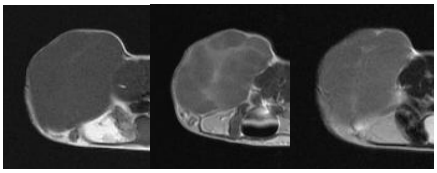
After FUS treatment and MR imaging were completed, total RNA was isolated from tumors using TRIzol Reagent® (GibcoBRL Life Technol., Rockville, MD). A set of labeled cRNA probes was synthesized from the original total RNA and hybridized to oligonucleotide microarrays (mouse genome chip Affymetrix MgU74Av2). Each gene was then analyzed for fold change (FC), or factor by which a gene's mRNA product was elevated in treated tumors over their untreated controls. We analyzed 10 pairs of FUS-treated tumors and their untreated controls. Genes upregulated by a factor over 2 in all tumors receiving the same FUS dose were further investigated.

Results

To see if there are obvious MR-changes due to the FUS treatment we obtained MR images as shown in Figure 1. In the continuous wave mode no KM uptake in the T1-weighted post-contrast images were seen due to the devascularisation of the tumor tissue. In the pulsed wave mode no signs of devascularisation were seen.



Continuous wave mode



Pulsed wave mode

Figure 1 MRI of continuous and pulsed wave treated SCC tumors

Gene expression analysis revealed profound changes in expression levels of 4 genes in the continuous wave treated tumors relative to their untreated controls. Three of these four genes encode heat shock (HSP 70, HSP 40) or related (MHC Class III) proteins. A fourth highly upregulated gene (fold change = 7.0) encoded an insulin-like growth factor binding protein. The largest degree of upregulation occurred for HSP 70 and its related MHC Class III gene (fold change = 25 and 14, respectively). In the pulsed wave mode transcription factor related genes like the ribosomal protein L41 (fold change=6) and the RNA polymerase II (fold change = 4) were upregulated.

Conclusion

Using FUS treatment of SCC tumors as a model system, we showed that dramatic changes in gene expression occur upon the initiation of thermal therapy. Functional genomic analyses of treated tumors revealed potential targets for developing molecular imaging probes depending on the applied energy intensity. Further studies are in progress to correlate temperature mapping techniques to these gene expression patterns and the development of MR probes utilizing these targets.

References

[1] JN McDannold *et al.* Mag Res Imaging **11**, 191 (2000)
[2] M.D. Bednarski *et al., Radiology* 204, 263 (1997)
[3] K.R. Kendell *et al., Radiology* **209**, 856 (1998)
[4] S.P. Bao *et al. Cancer Research* **58**, 219 (1998