In Vivo Monitoring of Caspase-3 Activity with MRI in Response to Different Treatment Modalities

Kimberly Brewer[1], Adam J Shuhendler[1], Deju Ye[1], Prachi Pandit[1], Magdalena Bazalova[2], Edward Graves[2], Jianghong Rao[1], and Brian K Rutt[1]

[1]*Radiology, Molecular Imaging Program, Stanford University, Stanford, California, United States,* [2]*Radiation Oncology, Stanford University, Stanford, California, United States*

Target Audience: Researchers and clinicians interested in MR molecular imaging for evaluation of cancer therapies.

Purpose: Apoptosis, otherwise known as programmed cell death, is essential for maintaining tissue integrity through the control of cell number and removal of abnormal cell types. Apoptosis is also one of the mechanisms (albeit not the only) through which anti-cancer treatments such as chemotherapy or irradiation induce tumor regression[1]. One of the key executor enzymes signalling a commitment to apoptosis is caspase-3, therefore the detection of caspase-3 activity *in situ* can report on apoptosis as an indicator of early therapy response.

Previously our group has reported on the development of a novel MRI caspase-3 activatable contrast agent based on intramolecular cyclization[2-4]. This agent is introduced into the system as small molecules, but cyclizes and self-assembles into Gd-nanoparticles inside target cells using both reduction and enzymatic cleavage reactions. In this study we used this probe technology to investigate caspase-3 activity (and thus apoptosis) in two different but common treatment modalities that rely on the induction of apoptosis in cancer cells. By studying the differences in caspase activity and localization we can explore the efficiency of these clinically relevant cancer treatments.

Methods: All animal procedures were approved by Stanford's IACUC. A cancer mouse model was established by injecting 1.5 million HeLa cells subcutaneously in the right shoulders of athymic female nude mice. Two treatment methods were studied and four mice were implanted with tumors and imaged, but not treated. *Chemotherapy* (Fig 1 - top): Eight mice received intravenous 8mg/kg Doxorubicin treatment 3 times beginning approximately 10-15 days post-implantation (once tumors were approximately 0.8 mm in diameter). Treatments were given 4 days apart and mice were imaged ~48 hours after the final treatment. *Radiation therapy* (Fig 1 - bottom): Mice received a single gamma radiation dose of 10Gy over approximately 5 minutes with the beam positioned directly over the tumor (while the rest of the mouse was shielded). Four mice were imaged between 24 and 72 hours post-therapy. All mice were also evaluated for treatment effect prior to MR imaging using a fluorescent analog (DJ290) of the MRI contrast agent that has been exhaustively evaluated for its mechanism of caspase-3 activity sensing.

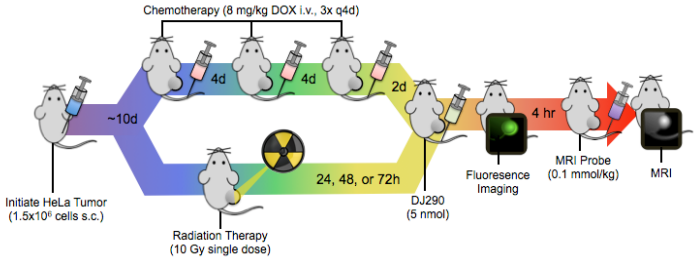

Figure 1 – Experimental design for Chemotherapy treatment (top) and Radiation therapy (bottom)

MR imaging was performed on a 1T Bruker ICON scanner. Mice received 0.1mmol/kg of contrast agent in 100ul (90ul for negative control probe) intravenously. The control probe can be reduced and is an indicator of perfusion and probe delivery to the tumor cells, but cannot undergo caspase cleavage, abrogating self-assembly and preventing retention. All images were acquired using a T_1-weighted fast spin echo sequence (TR/TE = 466/15ms) ETL=2, ~150um in-plane resolution, twelve 1mm thick slices, and 16 averages. Images were acquired every 10 minutes, including one pre-contrast image. Image analysis was performed using Inveon software to select the tumor ROI for all individual slices (Fig 2). Histograms of voxel image intensity were generated for each time point and tumor-averaged image intensity changes as well as "hot spots" (defined as voxels above the 75th percentile image intensity) were evaluated over time.

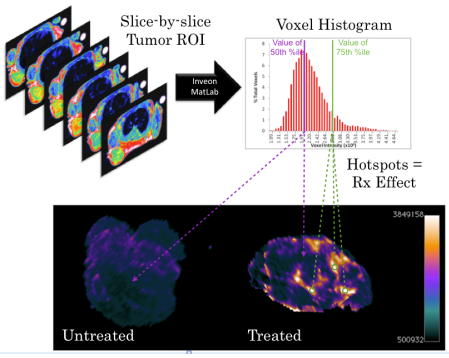

Figure 2 – Example tumor ROI and histogram analysis for sample chemotherapy treated mouse.

Results & Discussion: *Chemotherapy:* Fluorescence results showed that DOX-treated mice receiving active probe DJ290 had significantly higher tumor probe retention than non-treated mice (results not shown). MR imaging (Figs. 2 & 3) also demonstrated that treated mice receiving active probe had significantly higher signal enhancement in the tumor than untreated mice. The signal enhancement with active probe was localized to "hot spots", demonstrating that DOX chemotherapy as applied results in localized regions of tumor cell apoptosis. Mean signal enhancement (SE) was 40% in treated vs 20% in untreated tumors, and SE in hot spots was 50% vs 20% for treated vs untreated (all at 50min). The fact that significant signal enhancement remained only in treated tumors following active probe administration is indicative of probe self-assembly and enhanced retention of *in situ* formed nanoaggregates. *Radiation Therapy*: There was significantly greater SE in treated tumors at all three time points compared to untreated tumors. The signal enhancement and probe retention appeared to peak at 48 hours post-therapy, which correlated with fluorescence results as well. While hot spot signal enhancement was elevated relative to DOX-treated mice (67% SE vs 50% for DOX-treatment), the spatial distribution of probe retention was qualitatively different. Hot spots occurred within the tumor core and on the edge of the tumor most proximal to the radiation source (Fig. 3).

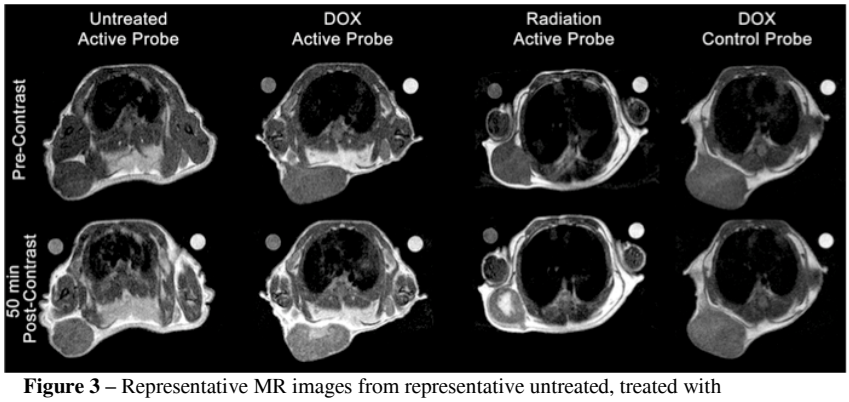

Figure 3 – Representative MR images from representative untreated, treated with chemotherapy or radiation treated mice and imaged 48 hours post-therapy. Top row images are pre-contrast injection, bottom row is 50min post-contrast injection.

Conclusions: This work demonstrates (1) that our caspase-3 specific MRI probe can be used to explore apoptosis independent of treatment modality; (2) even at a modest probe dose, the probe demonstrates strong activation-induced signal enhancement, enabling longitudinal monitoring of response to common anti-cancer treatments administered at clinically relevant doses. These promising results open the door to clinical translation and evaluation of cancer treatments.

References: [1] *Brown & Attardi, Nat. Rev. Canc. (2005) 5:231-237.* [2] *Pandit, et al, Proc. 21st ISMRM (2013) 0440.* [3] *Pandit, et al, Proc. 20th ISMRM (2012) 0482.* [4] *Ye, et al, Angew Chem. Int. Ed. (2011) 50:2275-2279.*

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