In Vivo Monitoring of Tumor Response to Anti-cancer Therapy Using MRI and a Novel Caspase-3 Activatable Gadolinium Contrast Agent for Imaging Apoptosis

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INTRODUCTION: Apoptosis is the natural suicide mechanism built into cells to maintain normal physiology. One of the hallmarks of cancer is the breakdown of this process, resulting in uncontrolled cell proliferation. Non-surgical cancer treatments like chemotherapy, irradiation, etc rely on the re-induction of apoptosis to enable tumor regression. Apoptosis is preceded by the release of a number of caspase enzymes, one of which is caspase-3. Detection of this executor caspase enzyme signals imminent apoptosis and is an early indicator of therapy response. A number of optical imaging techniques have targeted caspase-3 for imaging apoptosis, but to the best of our knowledge, *in vivo* caspase-3 sensing by MRI has not yet been successfully demonstrated.

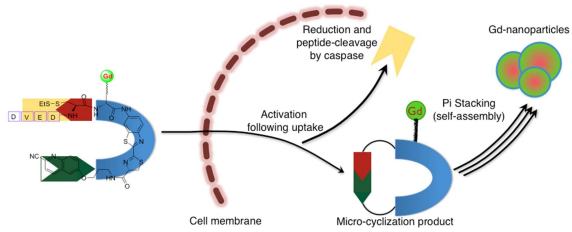

Figure 1*: Activation (reduction and enzymatic cleavage) leads to macro-cyclization reaction between aminothiol and cyano hydroxy quinoline (CHQ) of the same molecule, followed by self-assembly for intracellular Gd-nanoparticles formation.*

Previously, our group reported on a novel generalized platform for activatable contrast agents based on intracellular biocompatible condensation[1]. These agents begin as small molecules that upon activation at the target site self-assemble to form nanoparticles. This work reports on a new generation of these agents. Here the condensation reaction is *intra-molecular cyclization*[2] as opposed to *inter-molecular polymerization*[3] in the previous generation. The new agents have the following two advantages making them more suitable for *in vivo* imaging for therapy response monitoring: (1) they are less susceptible to interaction with intracellular free cysteine, and (2) their activation is not concentration dependent. Here, we use a subcutaneous mouse model with doxorubicin treatment to demonstrate the *in vivo* efficacy of these agents for the first time.

METHODS: All animal procedures were approved by the Stanford Institutional Animal Care and Use Committee. Figure 1(a) shows the experiment design. A cancer mouse model was established by subcutaneously injecting 1.5 million HeLa cells in the shoulders of athymic female nude mice. Pre-treatment MR images were acquired 10-14 days post-inoculation when the longest dimension in the tumor was ~ 0.8mm. This was followed by intra-tumoral doxorubicin treatment, which consisted of 2 injections (10mg/mL and 5 mg/mL, each in a 20µL volume) administered across an interval of 2 days. Post-treatment MR imaging was carried out 2 days after the second doxorubicin injection. All procedures were carried out under isofluorane anesthesia and the animals were euthanized after the final imaging session. A total of 8 mice were used in this study.

MR imaging was performed on an Aspect M2 1T permanent magnet (Aspect Imaging, Shoham, Israel). Intravenous contrast injection at 0.1mmol/kg concentration was administered in a 100µL volume for every imaging session. Multi-slice (312um in-plane, 1mm slice) T1-weighted spin echo images (TE/TR = 8.9/250 ms) were acquired, one before contrast injection and then every 4 minutes up to 4 hours after contrast injection. Image analysis was carried out in ImageJ and tumor volume data were used to calculate % signal enhancement relative to pre-contrast injection image. Matlab was used to fit the contrast kinetics curves to a Weibull model: $f(t) = \gamma \left(\frac{t}{\alpha}\right)^{(\beta-1)} e^{-(t/\alpha)^{\beta}}$, where α scales the curve along the horizontal axis, β defines the shape of the curve and γ scales the curve along the vertical axis.

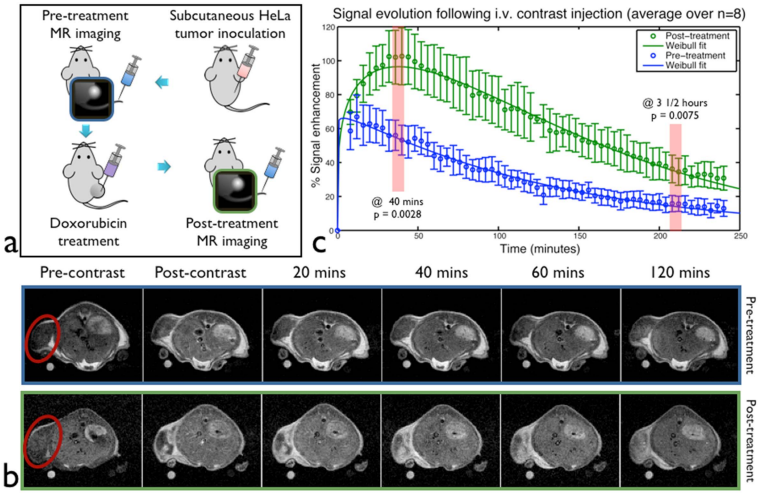

Figure 2*: (a) Experimental design. (b) Pre and post treatment T1-weighted time series images of the same mouse. (c) Signal evolution over tumor volume.*

RESULTS and DISCUSSION: Figure 1(b) shows a representative time series of T1-weighted images before and after treatment for one mouse. As is evident from the images, post-treatment tumor stays enhanced longer and the absolute intensity is also higher as compared to the same tumor pre-treatment. This is also reflected in Fig. 1(c), which shows percentage signal enhancement over the tumor volume, taken relative to the pre-contrast image. Data are averaged over 8 mice, and error bars represent standard error for each time point. Solid line shows the Weibull model fit for the two cohorts; pre (α=126.6, β=1.03, γ=74.6) and post (α=142.0, β=1.24, γ=160.8) treatment. Paired t-tests for the fit parameters yielded p-values of 0.055, 0.001, and 0.002 respectively, emphasizing clear differences between the shape and peak of the two curves.

In the pre-treatment cohort the contrast agent does not get activated. The early peak and washout seen here is similar to that observed in small-molecular weight contrast agents. In the post-treatment cohort, the presence of caspase-3 causes activation of the contrast agent. This results in nanoparticle formation, which we believe causes the following two effects: (i) higher relaxivity due to slower rotational correlation time and thus enhanced signal intensity, and (ii) prolonged retention at the tumor site due to larger particle size. This can be interpreted from the statistically significant differences seen between the contrast kinetics of the two cohorts during peak post-treatment enhancement (p-value = 0.003), and at 3½ hours post contrast injection (p-value = 0.008), respectively.

CONCLUSION: We have developed a Gd-based caspase-activatable contrast agent that can self-assemble into nanoparticles and provide signal enhancement at apoptotic sites *in vivo*. We have tested these novel agents in a doxorubicin-treated mouse model of cancer and have shown statistically significant differences in enhancement in the same tumor before and after treatment. MRI is already a preferred modality for a variety of oncologic application due to its advantages over competing modalities, i.e. its noninvasive, nonionizing nature and excellent spatial and contrast resolution. With our probe it can now also achieve improved sensitivity and specificity, making MRI even more attractive for cancer theranostics.

REFERENCES: [1]*Pandit, et al, Proc. 20th ISMRM (2012) 0482* [2]*Ye, et al, Angew Chem. Int. Ed. (2011) 50:2275-2279* [3]*Liang, et al, Nature Chemistry (2010) 2:54-60*

This research is supported by the National Cancer Institute (NCI) Center for Cancer Nanotechnology Excellence (1U54A151459-01) and the NCI In vivo Cellular and Molecular Imaging Center (1P50A114747-06) at Stanford University, and the Stanford Molecular Imaging Scholars Program (NIH R25 CA118681).