A dual Fluorescent/Paramagnetic Chemical Exchange-Based MRI Probe for Cell Death Imaging

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Target Audience: Scientists and clinicians interested in a targeted MRI contrast agent of programmed cell death.

Purpose: Programmed cell death (apoptosis) is responsible for homeostasis in normal tissues (1). Too little may contribute to tumour development, too much could lead to loss of tissue function. The artificial induction of increased apoptosis in tumours is an effective cancer therapy approach, and the reduction of apoptosis may improve prognosis of ischemia and neurological pathologies (1). To study and optimize approaches to manipulate the evolution of apoptosis it is desirable to have a non-invasive imaging method to serially detect this process. The purpose of this study is to determine whether a novel targeted molecular imaging PARACEST (2) MRI contrast agent (CA) can be used to detect apoptosis in immortalized cells following ionizing radiation.

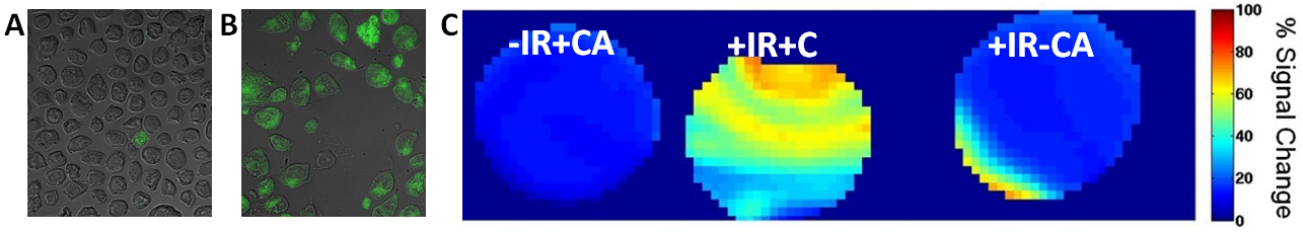
Methods: Recently, we have synthesized a novel CA containing a cleavage site specific to Caspase 3 (a central mediator of the apoptosis pathway) which contains components for both MRI (Tm^{3+}-DOTAM) and fluorescent (Oregon Green ® dye) detection. The CA has also been conjugated to a cell penetrating peptide (CPP) facilitating transport into, and out of a cell. Active Caspase 3 within cells irreversibly removes the CPP segment from an internalized CA, in effect, reducing the transport kinetics of the cleaved CA out of the respective cells, increasing retention.

The first step in the synthesis of the probe was a peptide coupling between the DOTAM-derived carboxylic acid and the caspase 3 targeting peptide attached to the resin. The DOTAM-peptide conjugate was purified by semi-preparative HPLC and was characterized by high resolution mass spectrometry. Subsequent conjugation with Oregon Green ® and metallation with $TmCl_3 \cdot 6H_2O$ furnished the desired probe in 10% overall yield. The probe was purified by size exclusion chromatography and dialysis and was characterized by high resolution mass spectrometry.

Prostate cancer cells (PC3M) were either irradiated with ultraviolet radiation (UVB; 40 W/m^2 312 nm) or maintained without any insult. Immediately after irradiation, cells were incubated overnight (24 hours) with the CA (concentration of 0.68 µM for confocal microscopy and 3.1 µM for PARACEST MRI).

Confocal microscopy of centrifuged cells (LSM 510 META NLO; Carl Zeiss, Inc) provided bright field visualization of the cells and fluorescent localization of the internalized CA (N=2). MRI detection of cell lysates (2×10^6 cells per ml for all samples) was achieved using a 9.4 Tesla, 31 cm diameter bore Agilent (Palo Alto, CA) small animal MRI scanner (N=1). Two images were acquired using a fast low angle shot (FLASH) pulse sequence (TE/TR=2.5/5.3 ms, 25.6 mm x 25.6 mm field of view, 128×128 matrix, 3 mm thick slice, 5 s pre-delay), where the second image was preceded by a WALTZ-16 preparation pulse (480 ms, 6 µT) centered on the bulk water frequency to generate contrast (3) using the on-resonance paramagnetic chemical exchange effect (OPARACHEE).

Results: Green fluorescence was expressed diffusely within the cells (Figures A and B). However, fluorescent imaging clearly demonstrated a preferential uptake/retention (75 % vs. 6 %) by the irradiated cells (Figure B) compared to the control cells (Figure A). Furthermore, the PARACEST OPARACHEE contrast (% change of signal in Figure C) showed a difference in saturation between irradiated cells incubated with CA (+IR+CA, average percent change of signal of 50 %), non-irradiated cells with CA (-IR+CA, average percent change of signal of 13 %), and irradiated cells without CA (+IR-CA, average percent change of signal of 15 %).



Discussion & Conclusion: This study demonstrates preferential uptake of a novel MRI contrast agent targeted to Caspase 3 in cells irradiated with UVB light to induce apoptosis. Detection of the agent by MRI using OPARACHEE suggests in-vivo detection is possible. In the future, the contrast agent will be utilized in an *in-vivo* murine model of brain cancer to study the spatial and temporal distribution of apoptotic cells prior to and following radiation treatment.

References:

1. Blankenberg FG. J Nucl Med. 49:81S–95S 2008
2. Zhang S, Merritt M, Woessner DE, Lenkinski RE, Sherry AD. Acc Chem Res. 36(10):783-90. Oct 2003
3. Vinogradov E, Zhang SR, Lubag A, Balschi JA, Sherry AD, Lenkinski RE. J Magn Reson 176 (1): 54-63. SEP 2005