Abstract #1864: Feasibility of magnetic relaxometry for early ovarian cancer detection: Preliminary evaluation of sensitivity and specificity in cell culture and in mice

Kelsey B. Mathieu, Zhen Lu, Hailing Yang, Lan Pang, Adam Kulp, John D. Hazle, Robert C. Bast, Jr.

The University of Texas MD Anderson Cancer Center, Houston, TX

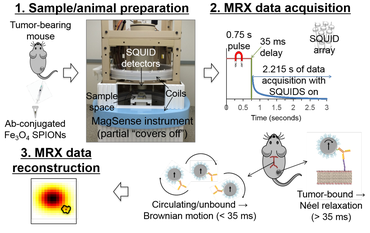
THE UNIVERSITY OF TEXAS MD Anderson Cancer Center
Making Cancer History®

Introduction

Most ovarian cancers are diagnosed today at a late, incurable stage. This leads to continued emphasis for earlier detection and the development of effective screening strategies. To be considered effective, early detection technologies must have sufficient sensitivity and specificity to impact patient mortality, while minimizing false positives. Magnetic relaxometry (MRX) can differentiate between superparamagnetic iron oxide nanoparticles (SPIONs) bound to cancer cells and those free floating in the vascular space with high sensitivity and specificity, promising to improve early detection.

Background on Magnetic Relaxometry

MRX uses an array of ultra-sensitive superconducting quantum interference detectors (SQUIDs) to measure the relaxation properties of SPIONs in response to a brief magnetizing pulse [1-3]. SPIONs are targeted to selectively bind to cancer cells using antibodies. Characteristic temporal differences in relaxation behavior are used to distinguish free SPIONs, which relax relatively quickly (< 35ms) by Brownian motion after the magnetic field is turned off, from bound (or sufficiently immobilized) SPIONs, which relax at a much slower rate (> 35ms) by the Néel mechanism.



Methods

We investigated the sensitivity and specificity of MRX by scanning ovarian cancer cell samples containing 10^5, 10^6, and 10^7 cells incubated with a fixed amount (57 µg of Fe_3O_4) of anti-HER2 antibody-conjugated, PEG-coated SPIONs or unconjugated, PEG-coated SPIONs (MagSense; Imagion Biosystems). To further evaluate specificity, we used cell lines with both high and low expression of HER2 (SKOV3 and HEY, respectively). To assess MRX under *in vivo* conditions, we subcutaneously injected 10^5, 10^6, and 10^7 anti-HER2 SPION-labeled SKOV3 cells into nude mice (n = 9) and immediately performed MRX scanning.

ELISA on SPION pellets

We verified the reported concentration of anti-HER antibody in the SPION solutions using an ELISA assay. Additionally, we verified successful antibody-SPION conjugation through an ELISA performed on the SPION pellets after high-speed centrifugation, confirming the presence of anti-HER2 antibody in the pellets compared with the supernatant, which had a much lower concentration of antibody.

Flow Cytometry

Flow cytometry was performed to confirm a high level of specific binding between SKOV3 cells and anti-HER2 antibody, as well as to confirm similar levels of binding between SKOV3 cells and anti-HER2-conjugated SPIONs and SKOV3 cells and free anti-HER2 antibody. After performing our initial study using fixed cells (Fig. 1), we repeated the study with live cells (Fig. 2) to establish relevance to our MRX studies, which used live cells.

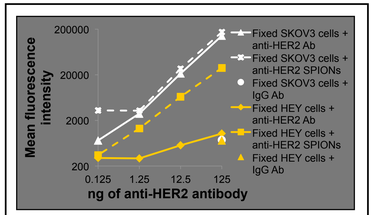


Fig. 1 Flow cytometry was performed to confirm specific binding between high-HER2-expressing ovarian cancer cell line SKOV3 and anti-HER2 antibody-conjugated SPIONs. Fixed SKOV3 and Hey cells were incubated overnight at 4°C with free anti-HER2 antibody and anti-HER2 SPIONs, washed twice, and then incubated for 1 hour with a secondary antibody tagged with Alexa 488. Note that the above plots use a logarithmic scale.

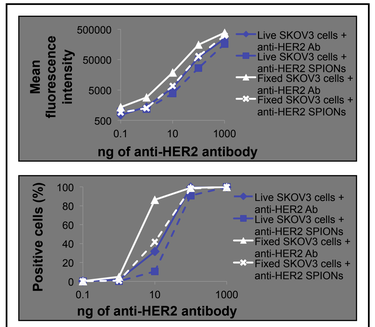


Fig. 2 Flow cytometry was repeated with live cells since all MRX studies were performed using live cells. Live SKOV3 cells were incubated for 2 hours at room temperature with free anti-HER2 antibody and anti-HER2 SPIONs, washed twice, and then incubated for 1 hour with a secondary antibody tagged with Alexa 488. Note that the above plots use a logarithmic scale.

Results

Our *in vitro* MRX data (Fig. 3) revealed strong linearity between cell number and MRX signal for both SKOV3 and HEY cells incubated with anti-HER2 SPIONs (R^2 = 0.99 and 1, respectively). Furthermore, there was little to no MRX signal for all cell samples incubated with unconjugated, PEG SPIONs regardless of cell number. The highest MRX signal was observed when 10^7 SKOV3 cells were incubated with anti-HER2 SPIONs, which was 2.1 ± 0.3 and 15.7 ± 1.4 times higher than when 10^7 Hey cells were incubated with anti-HER2 SPIONs or when 10^7 SKOV3 cells were incubated with unconjugated PEG SPIONs, respectively; significantly higher MRX signals (relative to the controls; $p < .01$) were also noted for samples containing 10^6 SKOV3 cells incubated with anti-HER2 SPIONs.

When scanning live mice injected with 10^7 anti-HER2 SPION-labeled SKOV3 cells (Fig. 4), the MRX signal was significantly higher than the signal from the mice prior to the injection ($p < .001$). Furthermore, MRX signal versus cell number in the injected mice was highly correlated ($r = 0.99$) with the MRX data from the corresponding cell sample scans. When the *in vitro* portion of the study was repeated several months later, similar MRX data was obtained (Fig. 5). An iron assay based on inductively coupled plasma mass spectrometry (ICP-MS) performed on the anti-HER2-SPION-labeled cell pellet for the sample containing 10^7 SKOV3 cells revealed a concentration of 4.2 µg of Fe_3O_4 in the cell pellet; the MRX signal produced by this sample ($5.1 \cdot 10^4 \pm 1.5 \cdot 10^4$) was consistent with the MRX signal generated by phantoms containing a similar concentration of SPIONs.

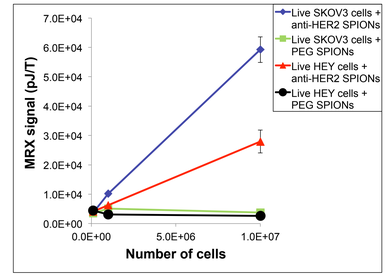


Fig. 3 MRX signal from scanning anti-HER2 and PEG SPION-labeled cell pellets containing 10^5, 10^6, and 10^7 SKOV3 and HEY cells.

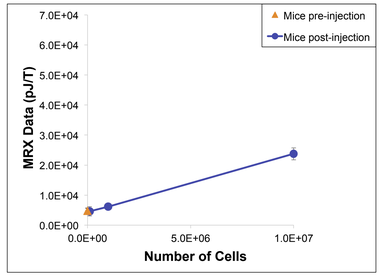


Fig. 4 SPION-labeled SKOV3 cell pellets were re-suspended in 50% Matrigel/PBS and subcutaneously injected in the hind flank of nude mice. Although the MRX signal from scanning the injected mice was lower than the MRX signal from the cell pellets (Fig. 3), presumably due to some loss of the injected dose in the syringe, the signals were highly correlated.

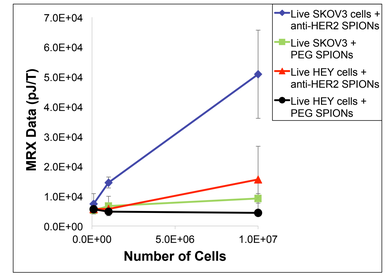


Fig. 5 The experiment performed in Fig. 3 was repeated several months later and similar results were obtained.

Conclusion

MRX appears sufficiently sensitive to detect clusters of 1 million cells in culture or 10 million cells in mice with a high level of specificity. Sensitivity may be improved using nanoparticles coated with antibodies against antigens that are overexpressed by a larger fraction of ovarian cancers and will be the focus of future work. Efforts towards such evaluation have already begun with the conjugation of anti-CA125 antibodies to SPIONS and verification of binding to ovarian cancer cells through flow cytometry. Our next step will be to perform MRX studies evaluating sensitivity and specificity of the anti-CA125 SPIONS in cell culture and in mice.

References

1) E.R. Flynn, H.C. Bryant, "A biomagnetic system for in vivo cancer imaging," Phys Med Biol **50**, 1273-1293 (2005).
2) J. Lange, R. Kötitz, A. Haller, L. Trahms, W. Semmler, W. Weitschies, "Magnetorelaxometry—a new binding specific detection method based on magnetic nanoparticles," Journal of Magnetism and Magnetic Materials **252**, 381-383 (2002).
3) L.P. De Haro, T. Karaulanov, E.C. Vreeland, B. Anderson, H.J. Hathaway, D.L. Huber, A.N. Matlashov, C.P. Nettles, A.D. Price, T.C. Monson, "Magnetic relaxometry as applied to sensitive cancer detection and localization," Biomedical Engineering/Biomedizinische Technik **60**, 445-455 (2015).

Contact

Kelsey B. Mathieu, Ph.D.
Instructor, Magnetic Relaxometry Research Lab
T: 713-792-6216
E: kmathieu@mdanderson.org