Early Detection of Ovarian Cancer through Nanomagnetic Relaxometry

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Introduction

Early detection remains one of the greatest opportunities to increase survival of women with ovarian cancer. We hypothesize that a novel technology known as nanomagnetic relaxometry could be used to accomplish this task by providing an alternative to transvaginal ultrasound (TVU) as a second-line diagnostic tool in patients with rising levels of cancer antigen 125 (CA125). The MRX instrument (Senior Scientific LLC, Albuquerque, NM) uses an array of ultra-sensitive superconducting quantum interference devices (SQUIDs) to detect tumor-bound superparamagnetic nanoparticles (NPs). Tumor detection is accomplished by injecting biologically-targeted NPs, applying a brief magnetic pulse, and then detecting the resulting signal with the SQUID sensors.

Technology Overview

Nanomagnetic relaxometry was first described in the literature in 2005.[1] Since 2005, this technology has been considered for a wide range of applications, including early detection of ovarian cancer. The MRX instrument uses an array of SQUIDs to detect iron oxide NPs *in vivo* and *in vitro* (see Fig. 1); Figure 2 diagrams the complete process. A second-generation MRX instrument was recently installed at The University of Texas MD Anderson Cancer Center within the Small Animal Imaging Facility (Fig. 3).

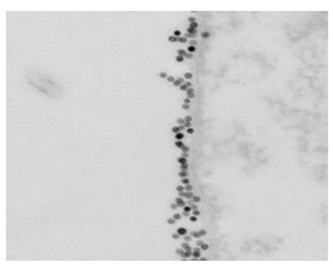

Figure 1. Transmission electron microscopy photographs of an ovarian cancer cell covered with NPs targeted using CA125 antibodies.

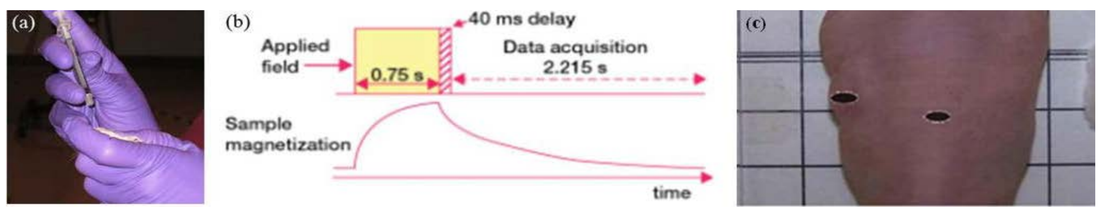

Figure 2. Nanomagnetic relaxometry is an *in vivo* magnetic detection technology that relies on the use of targeting agents (antibodies) linked to superparamagnetic NPs. (a) The targeted NPs are injected into a mouse model and then (b) a small magnetizing field (50 Gauss) is applied for 0.75 seconds before the field is turned off. The application of a brief magnetic field allows NPs specifically bound to biomarker-expressing cells to be measured using SQUIDs – sensors that are ultrasensitive for detection of low magnetic fields. Only bound NPs are detected by the SQUID sensors because, once the magnetic field is turned off, unbound NPs decay by Brownian motion within less than 1 millisecond, while biomarker-bound NPs decay at a much slower rate (0.1 to 2 seconds) by the Néel mechanism. The signal detected by the SQUID sensors is then used to determine where the bound NPs were located. Shown here (c) is the location of biomarker-bound NP sources (as 95% confidence limit ellipses) in a nude mouse with an ovarian tumor (cell line: NIH:OVCAR3) superimposed on a photograph of the mouse; note that one of the sources in this image represents the tumor and the other represents the mouse's liver.

Figure 3. The second-generation MRX instrument located within the Small Animal Imaging Facility at The University of Texas MD Anderson Cancer Center (Houston, TX).

Unlike current second-line ovarian cancer screening methods (e.g., TVU), which are imaging-based and rely on visualization of the shape and other physical properties of the tumor, MRX is similar to a blood test in that it detects the presence of cancer cells (along with their approximate location) using targeted NPs. The advantage of this technology is that (based on unpublished data) it is up to 200 times more sensitive than current imaging technologies. In addition to the sensitivity advantages, it is also believed to be unparalleled in terms of specificity. Because the technology relies on NPs binding to specific cancer cells and is detecting molecular markers, only cancer will presumably be detected with this technology. Another advantage of nanomagnetic relaxometry compared to some imaging-based technologies is that it does not expose patients to potentially harmful ionizing radiation and, because this technology is considered to be safe for human use, we expect that human studies could begin within the next five years. Thus, nanomagnetic relaxometry holds promise for detecting ovarian cancer much earlier, which is crucial to increasing survivorship.

Methods

To test our hypothesis that nanomagnetic relaxometry is capable of detecting early-stage ovarian cancer, we performed both cell tiration and mouse xenograft studies. For the cell titration assays, human ovarian cancer cells (cell line: SKOV3) were diluted to concentrations ranging from 10^6 to $1.2x10^4$ cells. NPs were then added to the cells and the magnetic moments were measured at each dilution step with the MRX instrument. The NPs used in this study were conjugated with a CA125 antibody using a carbodiimide method; both OC125 (produced in Dr. Robert Bast's lab at MD Anderson) and X75 (QED Bioscience Inc., San Diego, CA) clones were tested. In addition to the cell studies, xenograft tumors were grown from SKOV3 ovarian cancer cells on the flank of athymic nude mice. Once tumor diameters reached approximately 8 mm, the mice were injected via tail vein with NPs labeled with CA125 antibodies at a dose of 5mg [Fe]/kg. After 15 minutes, the mice were placed in the MRX device, and the magnetic moments were measured.

Results

The *in vitro* detection limits determined through our cell titration studies indicated that nanomagnetic relaxometry was capable of detecting on the order of 10^4 ovarian cancer cells (see Fig. 4). Additionally, the technology was able to successfully detect ovarian tumors *in vivo* (Fig. 5). It is important to note that, in addition to detecting the tumors, signals from the mice livers were also detected by the MRX instrument. This is believed to be due to the agglomeration of NPs within the liver, which has been shown to produce a similar signal to that resulting from NP-cell binding. However, to verify that only tumor-bound NPs are detected through nanomagnetic relaxometry, CA125-labeled NPs were also injected into the normal (non-cancerous) tissue of mice and this was found not to produce a signal.

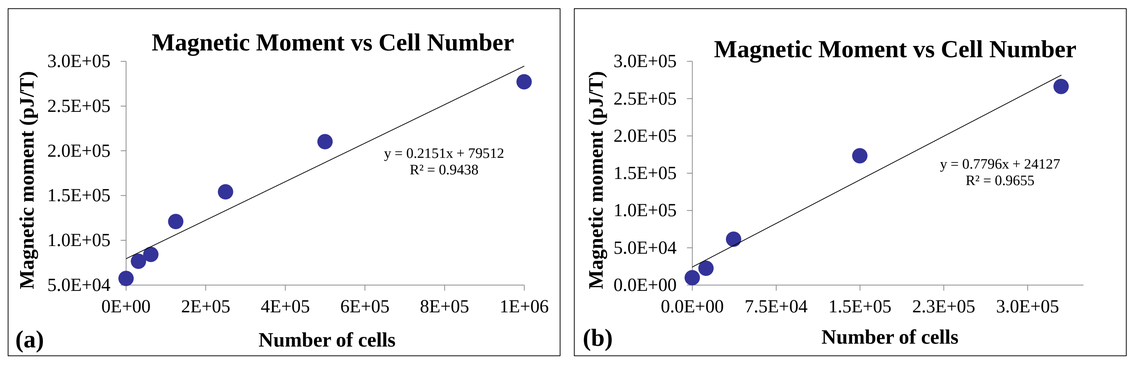

Figure 4. Magnetic moment, as measured by the MRX instrument, for ovarian cancer cell line SKOV3 as a function of cell number. Two CA125 antibodies were used, including **(a)** OC125 and **(b)** X75, to target the cells. For both of these clones, the *in vitro* detection limit was on the order of 10^4. The magnetic moments shown in these graphs for 0 cells represent the detected moments of the labeled NPs before any cells were added.

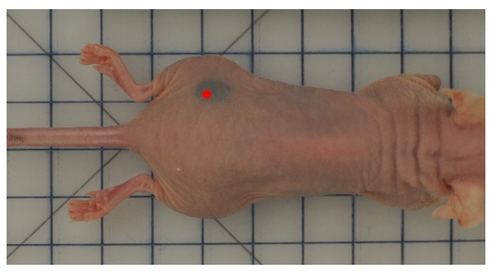

Figure 5. Image overlay of injected NPs with MRX localization signal. A female nude mouse injected subcutaneously with 15 pmol of CA125 conjugated nanoparticles and $3x10^6$ SKOV3 cells. Nanoparticles are recognizable in the image as a dark spot on the left hind flank. The red dot represents the signal localization obtained from MRX analysis, the magnetic moment was $1.15x10^6$ pJ/T.

Conclusion

Nanomagnetic relaxometry is unparalleled in terms of both sensitivity and specificity compared to imaging-based ovarian cancer detection methods, such as TVU; thus, it holds promise for detecting ovarian cancer much earlier.

Reference

[1]Flynn ER, Bryant HC. A biomagnetic system for in vivo cancer imaging. Phys Med Biol. 2005;50:1273-93.