In vivo Targeted Detection and Imaging of Ovarian Cancer by SPMR and MRI using Anti-folate Receptor-α Functionalized Iron Oxide Nanoparticles

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Introduction

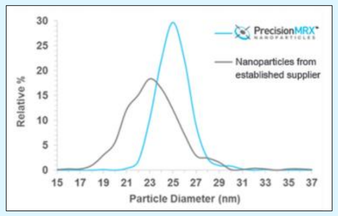
To achieve true diagnostic utility as an *in vivo* targeted imaging agent, it must be able to provide both specificity and sensitivity. Iron oxide nanoparticles (NPs) have been used in a variety of preclinical and clinical cancer detection/imaging applications. Here we show that targeted superparamagnetic iron oxide nanoparticles (SPION) can be used for *in vivo* detection by two different magnetic imaging methods. Superparamagnetic Relaxometry (SPMR) is a highly sensitive *in vivo* detection technology that can differentiate the magnetic signature of nanoparticles by their Néel relaxation when bound to tumor cells. Unbound nanoparticles such as those freely circulating in the bloodstream are not detected due to their rapid Brownian relaxation. The same targeted SPIONs accumulated in tumor provide molecular magnetic resonance imaging (MRI) contrast as well. Recently, anti-folate receptor-α targeted intraoperative tumor–specific fluorescence imaging of ovarian cancer has been reported to be clinically effective in detecting metastatic ovarian cancers *in situ*. Here we show a non-radioactive and non-invasive SPION-based targeted imaging agent for detection using SPMR and MRI to detect ovarian cancer in preclinical animal models.

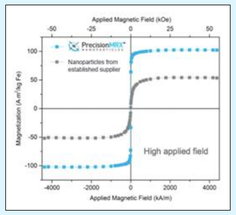
Study Objective

Previously, we have reported the success of developing an *in vivo* anti-HER2 targeted SPION-based imaging agent for SPMR and MRI detection. This program is currently in clinical testing for the detection of nodal metastases in HER2-positive breast cancer. Leveraging the same targeted SPION platform, the purpose of this current study is to demonstrate anti-folate NP targeting folate receptor-α in ovarian cancer as magnetic contrast agent for detection using SPMR and MRI.

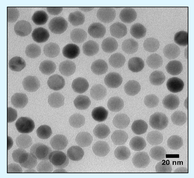
Anti-folate NP

Nanoparticle (NP) cores of 25nm superparamagnetic magnetite (Fe_3O_4) are made with high magnetic relaxivity providing excellent Néel relaxation and T2 contrast. Particles are monodispersed with narrow size distribution and exhibit high magnetic saturation.



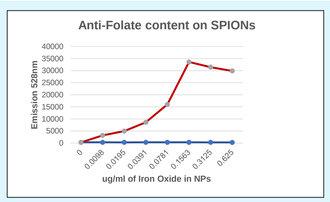


PrecisionMRX® NANOPARTICLES



To bio-functionalize the NP, cores were encapsulated by a layer of polymer and then functionalized with carboxylate (COO^-) surface. PEG + Folate were subsequently conjugated onto the polymer surface. Size of resulting NPs were measured by DLS. Folate content on the nanoparticles were measured using a plate-based ELISA. PEG-only NPs served as a control.

Surface	DLS (size)	PDI
PEG + Folate NP	72.1 nm	0.15
PEG NP	58.3 nm	0.06



Folate NP

Non-targeted PEG NP control

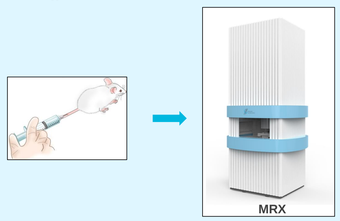
Method - SPMR

In vitro specific binding and uptake assay

- Both KB (Ovarian cancer cell line with high folate receptor-α expression) and A549 (Lung cancer cell line with no or minimum folate receptor-α expression) were incubated with 100 µg of Folate NP per million of cells in 6-well plate as well as the non-targeted PEG NP control overnight.
- Cells were washed, harvested, centrifuged, and pellets were subsequently measured for SPMR signal on a Magnetic Relaxometry (MRX) instrument.
- Folate receptor-α (FRα) expression levels were confirmed by flow cytometry for all the cell lines used in the study.

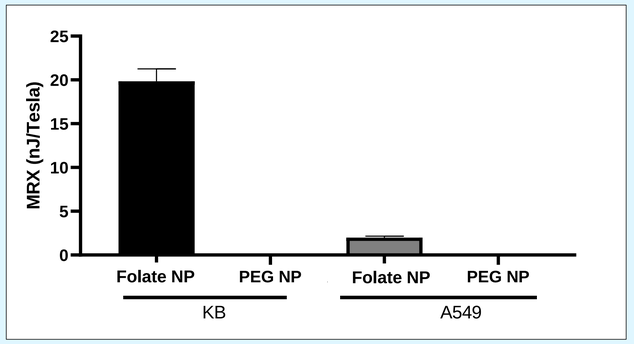
In vivo targeted delivery in ovarian xenograft tumor model

- Folate NPs or PEG NPs (20mg/kg) were injected into KB (FRα +ve and A549 (FRα -ve) tumor bearing mice by tail vein (IV) delivery.
- After 24 hr post NP injection, mice were euthanized, and tumors and organs were excised for *ex vivo* SPMR measurement.



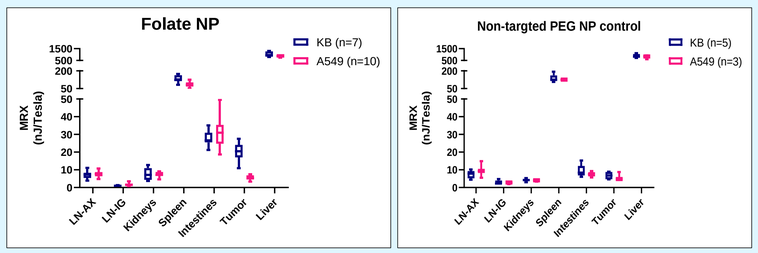
Results - SPMR

In vitro cell binding and uptake assay



- The SPMR signal is proportionate to the Folate receptor-α expression level of the cell line showing Folate NPs can clearly distinguish high, and low FRα expressing cells *in vitro* while non-targeted NPs (PEG NP) do not produce an SPMR signal indicating Anti-folate receptor-α NPs function with targeting specificity *in vitro*.

In vivo tumor xenograft mice study



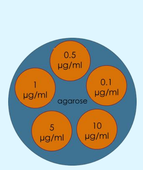
- *In vivo* models show Folate NPs generate higher binding signal than PEG NPs in FRα +ve expressing tumors as well as in organs such as intestine and kidney where there is a high level of endogenous FRα expression indicating targeting is predominately a function of the targeting ligand and not EPR or other non-specific mechanisms *in vivo*.

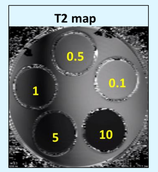
Method - MRI

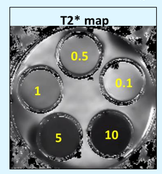
- All MRI measurements were conducted using a 11.7 T horizontal Bruker Biospec MRI at the Molecular Imaging Center at Sanford Stem Cell Consortium, San Diego, CA.
- Different concentrations of NP Phantoms samples were prepared and imaged to establish MRI measurement parameters.
- Folate NPs (20mg/kg) were injected into KB tumor bearing mice (tumor size were between 300-500mm^3) by tail vein (IV) delivery. Folate NPs dose used in current mouse study is human equivalent dose of 1.6mg/kg which is well below the clinical dose used for iron oxide nanoparticles.
- MRI images of the tumor mice were taken pre- and 24-hour post dosing of the animal and analyzed.

Results - MRI

Nanoparticle Phantom Study



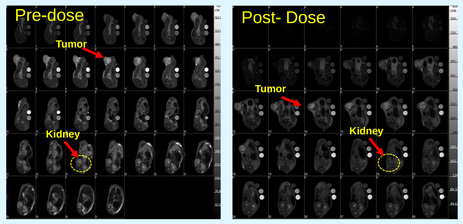




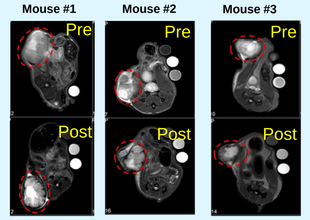
- NP produce negative T2 and T2* contrast
- Decay increases (shorter T2 and T2*) with an increase in NP concentration
- *r2* = 323 mM^{-1} s^{-1} *r2** = 460 mM^{-1} s^{-1}

In vivo T2 MRI imaging of Folate NP uptake in KB implanted tumor in mice (n=3)

MRI images of a representative tumor mice



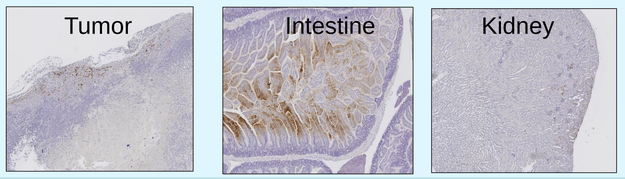
Post- dose tumor images showing heterogenous hypo-intensity, compared to pre-dose



-25% intensity | -29% intensity | -27% intensity

- Monochrome colored images showed hypo-intensity in implanted FRα +ve KB tumor post-dose compared to pre-dose, demonstrating that Folate NP accumulated in tumor and kidney tissue specifically and were observable by MRI.

Tissue Fe Staining Study



- **Folate NP presence in the tumor, intestine and kidney were confirmed by enhanced Fe Prussian Blue staining**

Conclusions – Future Work

Together, these results suggest that our Folate nanoparticles can provide target-specific delivery to cancerous tissue and generate measurable signal by SPMR and provide molecular T2 contrast in MRI, showing that SPIONs can provide both non-radioactive and non-invasive approach for detection of ovarian cancer. Future studies will include orthotopic ovarian tumor model which is more clinically relevant. Thus, these preliminary studies provides groundwork for translational research on clinical ovarian cancer tumor detection and as well as monitoring therapy response.