Molecular magnetic resonance imaging (MRI) of surface-epithelial-derived protein CA125 in human ovarian carcinoma cells using superparamagnetic immunomicelles

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Introduction: CA125 is a tumor marker that becomes elevated in ovarian cancer with most common surface-epithelial-derived ovarian carcinomas and is commonly measured through a serologic assay [1,2]. As a serologic marker, CA125 testing has only been shown to be useful for the detection of recurrent disease, following surgical resection of the primary lesion. CA125 levels have not yet been evaluated for their potential diagnostic utility in the detection of peritoneal surface tumor metastases. The evaluation for the presence of peritoneal surface tumor implants can be difficult in cases with low volume metastatic disease or in cases with low grade cytologic atypia. Thus, current approaches for ovarian cancer tumor staging fail to identify some cases that have peritoneal metastases. Conventional diagnostic imaging, such as abdominal magnetic resonance imaging (MRI), can reveal some peritoneal mass lesions but has limited specificity for carcinoma. Alternative approaches to improve the sensitivity and specificity for the detection of ovarian cancer peritoneal metastases could identify patients that are most likely to benefit from postoperative chemotherapy while also helping identify patients that will not benefit from extensive surgical staging procedures. The authors have developed molecular MRI contrast agents for the specific detection of CA125 in vitro and in vivo to detect pelvic and abdominal surface peritoneal implants in patients with serous, mucinous, endometrioid, and clear cell carcinomas.

Materials and Methods: The MRI contrast agents are superparamagnetic 10 nm magnetite (Fe_3O_4) nanoparticles and provide negative T2 contrast in T2-weighed MRI scans. The nanoparticles are encapsulated in a phospholipid monolayer covalently conjugated to fluorescein and anti-CA125 monoclonal antibodies (Biodesign, clone X306) via a maleimide-polyethylene glycol (PEG) linkage, yielding a superparamagnetic immunomicelle. The magnetite nanoparticles are well characterized and demonstrate superior properties as a T2 contrast agent [3]. Two human ovarian carcinoma cell lines from ATCC previously characterized for CA125 expression were used: OVCAR-3 (CA125 positive) and SK-OV-3 (CA125 negative). Each cell line was incubated in vitro with anti-CA125 superparamagnetic immunomicelles, washed, harvested, and subsequently analyzed in flow cytometry and MSME T2 weighted MRI in a Bruker 4.7 Tesla PharmaScan.

Results: Flow cytometry histograms in Figure 1 indicate strong staining for the OVCAR-3 cells and negligible staining for the SK-OV-3 cells. A MSME T2 weighted MRI study (Figure 2) shows noticeable darkening in the OVCAR-3 cell pellet incubated with the superparamagnetic immunomicelles compared to a control OVCAR-3 cell pellet. The measured T2 contrast change is shown in Figure 3, and quantifies the CA125 specific contrast observed in the OVCAR-3 samples compared to SK-OV-3 samples. The T2 values were decreased in OVCAR-3 cells after incubation with the nanoparticle/antibody complex (400 vs. 668 msec, $p<0.01$), but unchanged in SK-OV-3 cells (614 vs. 642 msec, n.s.). The monodispersity and micelle encapsulation of the nanoparticles are shown by phosphotungstic acid staining and transmission electron microscopy TEM of the superparamagnetic immunomicelles (Figure 4).

Discussion: The presented in vitro results demonstrate successful molecular MRI of cellular CA125 expression. The differentiable contrast observed in CA125 expressing cells indicates the viability of MRI as a modality for detecting cells and tissue expressing this cancer antigen. The slight staining in flow cytometry and MRI T2 contrast change of the SK-OV-3 cells is hypothesized to be incidental adhesion of the contrast agent to the cells, rather than non-specific binding of the antibody. Optimization of the contrast agent incubation will minimize the adhesion to cells not expressing CA125. The use of a PEGylated phospholipid monolayer supports in vivo application of the contrast agent by minimizing immunogenicity and delaying clearance by the reticuloendothelial system. The successful molecular MRI of CA125 expressing cells can potentially be applied to in vivo detection of peritoneal implants, and in vivo molecular MRI of CA125 will be the focus of subsequent xenograft and orthotopic mouse models.

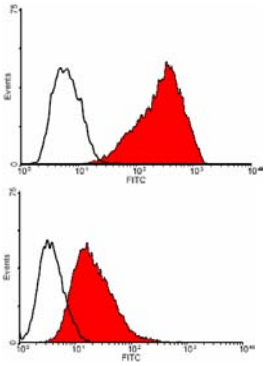

Figure 1 – Flow cytometry histograms of OVCAR-3 cells (top) and SK-OV-3 cells (bottom). OVCAR-3 cells exhibit strong staining for CA125

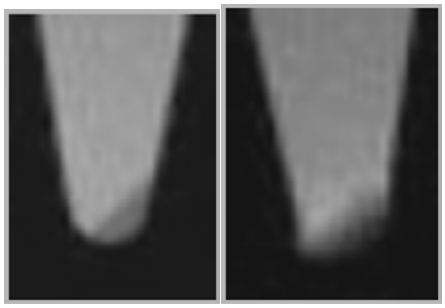

Figure 2 – MRI of OVCAR-3 cells (left) and OVCAR-3 cells incubated with superparamagnetic immunomicelles specific to CA125

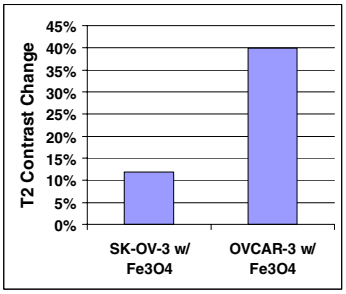

Figure 3 – T2 contrast change in SK-OV-3 cells (CA125 negative) and OVCAR-3 cells (CA125 positive). Both cell lines were incubated with superparamagnetic immunomicelles specific to CA125

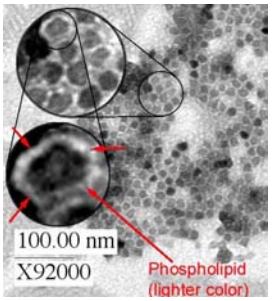

Figure 4 – TEM of superparamagnetic immunomicelles. Phospholipid monolayer is stained white [4]

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