High T1-Relaxivity Nanoparticles for Target Labeling of Ovarian Cancer

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Introduction: Early diagnosis of ovarian cancer is difficult due to the absence of reliable biological markers. The design of tumor specific contrast agents will facilitate detection of ovarian cancer at an early stage. The Tumor Endothelial Marker (TEM1) is a protein selectively expressed on the surface of ovarian cancer vasculature. A high affinity antibody (scFv-CM6) specific for TEM1 has been recently purified [1, 2]. The present study was performed to develop a procedure for syntheses of scFv-CM6-carrying nanoparticles with high longitudinal relaxivity for MRI detection of ovarian cancer. We customized a preparation of Gd-DTPA-DSA and incorporated this chelate into the lipid shell of perfluorocarbon nanoparticles. The resulting particles exhibit very high longitudinal relaxivity (R1) and a strong affinity for TEM1.

Methods: *Nanoparticles preparation*: Nanoparticles were prepared as described previously [3]. Briefly: a chloroform/methanol solution of lipids (DPPC:PC:PG:Cholesterol:TxR:Ni-NTA:Gd-DTPA-DSA) was evaporated under nitrogen flow. DTPA-DSA was synthesized and purified as previously reported [4, 5]. Gd-DTPA-DSA was prepared in pyridine at 70°C from $GdCl_3 \cdot 6H_2O$ and DTPA-DSA [6]. Water and perfluoro-15-crown-5-ether were added to the dry lipids mixture at a ratio of 0.6:0.4, and the solution was extruded under pressure 10 times. The particles of different diameters were prepared by extruding the lipid-perfluorocarbon mixture through membranes of different pore sizes (200, 400, 600 nm). The stability of the nanoparticles was demonstrated by repeated size measurement. The scFv fragment of the antibody was purified from *E.Coli* strain HB2151 expressing scFv-TEM1 as described previously [1]. The emulsion was incubated with these antibodies for 1 hr and coordination binding of Ni-NTA and His_6-tag of scFv-fragment was achieved. *Cell culture*: Mouse endothelial pancreatic islet cells (MS1) were used in the experiments. The cells were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, 1% penicillin streptomycin, 4 mM L-glutamine, 4.5 g/L glucose, and 1mM HEPES. MS1 cells were infected with bicistronic lentivirus expressing hTEM1/Eme or control vector fLuc/DsR and sorted according to two fluorescent protein expressions with MoFlo.

Labeling of cells with nanoparticles: TEM1+ and control cells were incubated with the nanoparticles for 1 hr at 37°C. After incubation, the cells were washed 5 times with PBS. For optical detection the cells were labeled in chamber-slides. *Phantom preparation*: cells labeled with the nanoparticles were applied into an agar block and transferred into a 20 mm NMR tube for MRI experiments at 9.4 T magnet. The detection of labeled cells was performed with following parameters: Gradient Echo pulse sequence TR=250 ms, TE=5 ms, slice thickness=1 mm, FOV=1.6cm^2. Agar blocks with different particles dilution were imaged with an inversion-recovery sequence at 18°C [7]. The longitudinal relaxation time was measured and longitudinal relaxivity of nanoparticles per Gd^+ was calculated. The Gd-concentration was measured by the inductive coupling mass spectroscopy method.

Results: An increasing of Gd-DTPA-DSA concentrations in the lipid composition of nanoparticles decreases T1 of the particles (Fig.1); however, the stability of the nanoparticles also decreased with increasing Gd-chelate concentration. Particles containing ~10 % of Gd-DTPA-DSA in their phospholipid shell and extruded through 200 nm membranes were stable for a week and provided the best combination of stability and longitudinal relaxivity - 28.2 $mmol^{-1}sec^{-1}$at 18°C (Fig.2)

We tested the ability of nanoparticles to label cells *in vitro*. Attachment of the nanoparticles (red) to TEM1+ cells (blue) was detected (Fig.3A) while the control cells did not exhibit any specific binding (Fig.3B). MR images of agar blocks with 5x10^5 of control and TEM1+ cells are shown in Fig.4. No cells were detected in the phantom with control cells, while cells labeled with nanoparticles were clearly visible as a layer of hyperintensity.

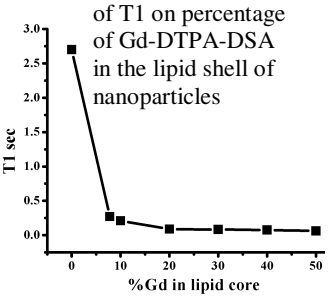

Fig.1 Dependence of T1 on percentage of Gd-DTPA-DSA in the lipid shell of nanoparticles

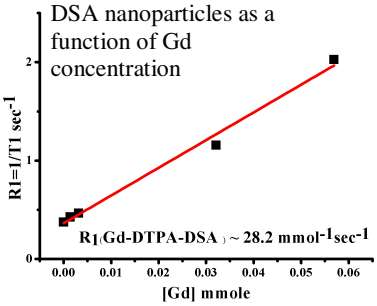

Fig.2 R1 of Gd-DTPA-DSA nanoparticles as a function of Gd concentration

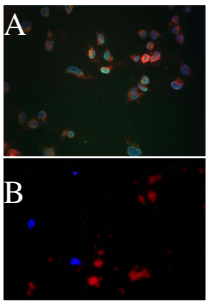

Fig.3 Fluorescent images of TEM1+ (A) and control (B) cells after 1 hr incubation with nanoparticles

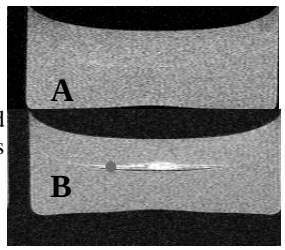

Fig.4 MR images of agar phantoms with 5x10^5 control (A) and TEM1+ (B) labeled cells

Discussion and Conclusions: Development of targeted contrast agents is a rapidly evolving field of molecular imaging. In the present study we develop ovarian cancer specific nanoparticles with high longitudinal relaxivity.

The relaxation properties of Gd-carrying nanoparticles depend on three main factors: accessibility of Gd^{3+} to water molecules and its mobility, number of Gd^{3+} in each particle and the size of the particles. In our preparation we tried to optimize all these characteristics. The rigid structure of Gd-DTPA-DSA provides optimal exposure of Gd^{3+} to water and low mobility Gd-ions. A relaxivity of 15.9/9.7$mmol^{-1}sec^{-1}$ at 4.7 T was reported for Gd-DTPA-PE/Gd-DTPA-BOA nanoparticles which have either very flexible structure (Gd-DTPA-PE) or less water exposed gadolinium (Gd-DTPA-BOA)[8]. Slower relaxivity of ~ 4.2/14.4 $mmol^{-1}sec^{-1}$ at 4.7/9.4 T was reported in Gd-DTPA-DSA-carrying nanoparticles of smaller size 20/50 nm [9, 10] We believe that our preparation provides higher water exposure and a more rigid Gd^{3+} construct which leads to a significantly higher relaxivity. Incorporation of the scFv-CM6 antibody into our system provided selective binding of this contrast agent to the targeted cells and labeled cells could be detected both by fluorescence microscopy and MRI. These preliminary results demonstrate the feasibility of implementation this contrast agent for *in vivo* detection of ovarian cancer.

Reference:

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