Optimization of a Cell Labeling Strategy for Magnetic Resonance Imaging

G. Wolf[1], K. Strobel[2], S. Gruener[1], A. Koch[1], V. Hietschold[3], N. Abolmaali[1]

[1]ZIK OncoRay, TU Dresden, Dresden, Germany, [2]PET-Center, FZ Rossendorf, Dresden, Germany, [3]Department of Radiology, University Hospital, Dresden, Dresden, Germany

Introduction

With the currently available scanner technology and high field strengths, magnetic resonance imaging (MRI) allows a near cellular resolution and, thus, provides a non-invasive and repetitive means of tracking single cells or small groups of cells after their transplantation or injection in living organisms. Therefore, cells need to be labeled with a suitable MRI contrast agent (CA) in order to differentiate them from surrounding tissue *in vivo*. Using CA labeled tumor cells it would be possible to follow tumor progression over time with MRI after transplantation or injection.

Aim

In the present study we performed MRI experiments of two CA labeled human cell lines of non-small cell lung cancer (NSCLC) at field strength of 1.5 T and 7 T, respectively. We systematically determined the labeling efficiency of the tumor cells for Gadolinium- and Manganese-based CAs using five transfection agents (TAs). Our main goal was to determine a combination of CA and TA, which produces the best contrast in MRI.

Materials and Methods

Cell labeling: Human NSCLC cells A549 (p53 wildtype) and H1299 (p53 -/-) were incubated with CAs and/or TAs for 4–24 hours according to manufacturers' recommendations. As CA we used Magnevist™ (Gd; Schering, Germany) and Teslascan™ (Mn; GE Healthcare, USA) at incubation concentrations corresponding to 1.78 mg/ml Gd and 0.42 mg/ml Mn, respectively. The range of TAs covered Lipofectin™ (L; Invitrogen, USA), Lipofectamine™ (LA; Invitrogen, USA), FuGENE™ 6 (Fg; Roche, USA), Poly-L-Lysine (PLL; Sigma, Germany) and SuperFect™ (SF; Qiagen, Germany). Cells were washed three times with PBS (Invitrogen, USA) prior to any further use. Transfection of cells was checked by fluorescence imaging. For each MRI experiment approximately 10^6 cells were used.

Imaging: MRI experiments were carried out at a 1.5 T clinical scanner (Siemens Magnetom Sonata, Erlangen, Germany) using a small loop coil and a 7 T experimental scanner (Bruker BioSpec 70/30, Ettlingen, Germany) using a volume coil. T1-weighted MRI was done with a spin echo sequence (TE/TR = 8.4/400 ms) at 1.5 T and a MDEFT sequence (TE/TR/TI = 3/2400/800 ms) at 7 T. Additionally, inversion recovery TrueFISP experiments were carried out at both field strengths. From these, spin-lattice relaxation times (T1) were determined by a three-parameter fit.

Results

Fluorescence images demonstrate the uptake of TA liposomes into cells. Their distribution is visualized as bright green spots in Fig. 1 for H1299 cells incubated with 0.42 mg/ml Mn using LA (top) and SF (bottom), respectively. The corresponding uptake of CA can be estimated from MRI measurements. From T1-weighted images, one can qualitatively identify those TAs that most efficiently transfer CA into cells and, thus, yield the best contrast enhancement. In Fig. 2 the T1-weighted images (7 T) of A549 tumor cells incubated with 0.42 mg/ml Mn (top) and without CA (bottom) are given as an example. However, T1 relaxation time measurements will offer a quantitative measure of CA uptake. The relative change of T1 upon addition of 0.42 mg/ml Mn (red) and 1.78 mg/ml Gd (green) to H1299 tumor cells is presented in Fig. 3.

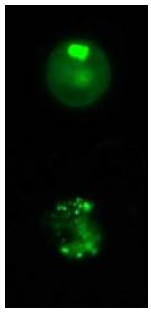

Fig. 1: Fluorescence images of H1299 cells incubated with LA (top) and SF (bottom). Note the different distribution of TA liposomes within cells.

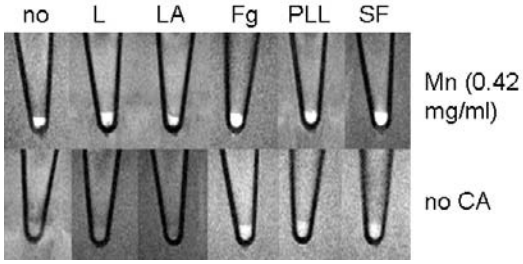

Fig. 2: T1-weighted images of A549 cells at 7 T in the presence of 0.42 mg/ml Mn (top) and in the absence of CA (bottom) for different TAs.

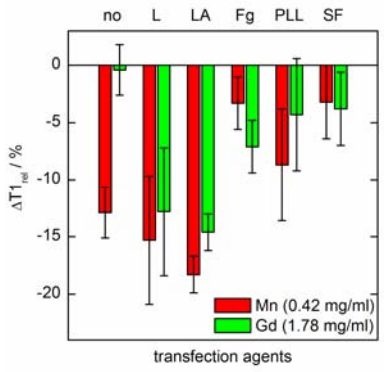

Fig. 3: Relative change of T1 at 1.5 T in H1299 cells at CA content of 0.42 mg/ml Mn (red) and 1.78 mg/ml Gd (green) for different TAs.

Discussion

In the T1-weighted images a marked signal enhancement is observed upon addition of CA, which corresponds well with the T1 decrease determined from relaxation time measurements. Clearly, L and LA are identified by MRI as the most suitable TAs for labeling NSCLC cell lines A549 and H1299 with Mn- and Gd-based CA. However, cells were noticeably labeled by Mn and to some minor extent also by Gd in the absence of TA. Fg, PLL and SF show only a small labeling efficiency in the present study. This seems to be in contrast to the large and uniform cellular uptake of TA as shown for SF in Fig. 1 (bottom). However, fluorescence imaging displays only the dye-labeled liposomes within cells, which might not necessarily be loaded with CA.

Conclusions

NSCLC cell lines A549 and H1299 are preferable labeled with Gd- and Mn-based CA using the TAs L or LA as determined from MRI experiments. The use of fluorescence imaging as an indicator for CA uptake into cells cannot be recommended. The labeling strategy presented here might easily be adopted for other cells, e.g. any kind of stem cells, which are not easily available and where destructive labeling methods like electroporation will result in a large number of dead cells.