Caveolae mediated labeling of fibroblasts for tracking by MRI

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Introduction

MRI studies of cell trafficking involves paramagnetic and superparamagnetic cell labeling. The use of paramagnetic metal cation compounds which are membrane impermeable has been limited and so far they were mostly utilized by microinjection (1) or receptor mediated endocytosis of Gd chelates covalently attached to peptides (2). However, most work in the field has been focused on detection of cells belonging to the hematopoietic system and none of the studies addressed cell tracking during vascular remodeling and angiogenesis. Since fibroblasts at the tumor-host interface are suggested to differentiate into myofibroblasts and then into pericyte-like cells, which are proposed to guide endothelial sprouts (3) we labeled fibroblasts in vitro with biotin-BSA-$GdDTPA_{33}$. Here, we show the feasibility of cellular labeling by magnetic contrast material that may allow in-vivo monitoring of fibroblasts.

Material and Methods

Contrast material: Biotin-BSA-$GdDTPA_{33}$ was synthesized as described (4) and was further labeled with 5(6)-carboxyfluorescein, succinimidyl ester.
Cell culture: Fibroblasts from normal skin, normal breast and breast tumor, were isolated from biopsies.
In vitro cell labeling: Confluent cultures were incubated with 10 mg/ml contrast material for 48 h and labeling was terminated by 4 washes in serum free medium. Inhibition of labeling was tested by addition of nystatin. Retention of the contrast material was assayed using labeled cells that were subsequently washed and cultured in fresh medium prior to R_1 measurement. Fluorescence imaging of cells labeled with the fluorescent conjugated contrast material and counter stained with propidium iodide (PI) was performed by confocal microscopy. Cell survival was determined by neutral red accumulation.
MRI studies: Data was acquired on a 400 MHz wide-bore DMX Bruker spectrometer. Labeled and control cells were washed, harvested, suspended in agarose and placed in a 5 mm NMR tube. R_1 relaxation was determined from spin echo images acquired with TR 100-2000 ms; TE 8.8 ms; 128 X 128 pixels; 2 averages; slice thickness of 1mm; FOV 1.5 X 0.6 cm; in-plane resolution 117 µm; SW =50,000 Hz.
Analysis of MR data: Images acquired with 10 different TR values were used for generation of R_1 maps. ΔR_1 was calculated from the difference in R1 between labeled and unlabeled cells (Eq 1):

$$\Delta R_1 = R_1 \text{ (labeled cells)} - R_1 \text{ (unlabeled cells)} \qquad [1]$$

Results:

Fluorescence microscopy of labeled fibroblasts demonstrated internalization of biotin-BSA-$GdDTPA_{33}$ into intracellular granules. R_1 of fibroblasts was significantly increased due to labeling with contrast material, and remained significantly elevated after two weeks in culture. Internalization of biotin-BSA-$GdDTPA_{33}$ could be significantly suppressed by the addition of nystatin, suggesting a role for caveolae mediated endocytosis. Efficiency of labeling was determined after 48 h of incubation, and revealed internalization of the contrast material into the cytoplasm of all cells. In addition, the cells remained viable as was shown by neutral red viability assay.

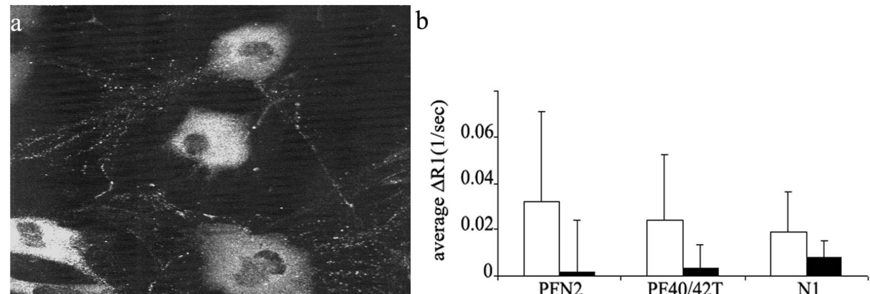

Figure 1: a) Representative confocal microscopy image of PFN2 fibroblasts labeled with biotin-BSA-$GdDTPA_{33}$-FAM. b) average ΔR_1 (=R1 labeled cells-R1 unlabeled cells) was obtained from ROI analysis of the data. Fibroblasts were labeled with biotin-BSA-$GdDTPA_{33}$ in the presence of nystatin (close bars) or without (open bars). Note that ΔR_1 was significantly larger than zero for all fibroblast types. $p<0.05$

Conclusions:

- MRI measurement showed a significant increase in R_1 of fibroblasts labeled with biotin-BSA-$GdDTPA_{33}$, indicating both uptake and MR visibility of the intracellular contrast material.
- Contrast enhancement was retained by cells for at least two weeks in culture.
- The contrast material did not alter the viability of the fibroblasts.
- We suggest that biotin-BSA-$GdDTPA_{33}$ uptake is carried out by caveolae mediated endocytosis.

Reference:

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