Abstract Details

Authors: Ritu Mishra, Wu Su, Martin Sauer*, Josef Pfeuffer^, Kamil Ugurbil, Joern Engelmann

Title of abstract: Cell Penetrating Peptide Assisted Intracellular Transport of mRNA Targeting Magnetic Resonance Imaging Contrast Agents

Full Affiliation Address Max Planck Institute for Biological Cybernetics, Tuebingen, Germany, *Paediatric Haematology and Oncology, Hannover Medical School, Hannover, Germany, ^Siemens AG, Medical Solutions, Charlestown, USA

Telephone: (+49)7071601708 Fax: (+49)7071601702

Email: ritu.mishra@tuebingen.mpg.de

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In an attempt to observe specific biological processes, membrane permeating targeting agents can be developed for labelling cells of interest. Molecular imaging of cells or cellular processes can be attained by targeting e.g. receptors, enzymes or mRNA. Aiming to image the presence of specific mRNAs by MRI, two intracellular MR contrast agents (CA) were developed, composed of a Gd-DOTA complex, a peptide nucleic acid (PNA) sequence and a cell penetrating peptide (1). One (dsRed CA) was designed to bind to mRNA of dsRed (red fluorescent protein originating from Discosoma coral) by its complementary PNA sequence, whereas the second one contained a non-sense sequence with no natural counterpart.

Cellular uptake of CA was confirmed in transgenic fibrosarcoma cells expressing dsRed gene as well as in the respective parent cell line deficient of target by fluorescence microscopy and spectroscopy. MR imaging of cell pellets was conducted at 3 Tesla as well. A significant higher specificity of the dsRed CA in comparison to its non-sense counterpart was observed in an in vitro cell free binding assay. Fluorescence studies showed that both contrast agents could enter efficiently into target containing as well as parent cells. Microscopy displayed vesicular punctuate localization of both the CAs around the nucleus, which indicated endosomal uptake. MR results showed only a slight specificity for DsRed cells compared to parent cells. However, no difference in uptake efficacy was observed between the two CA in the same cell type. Irrespective of the presence/absence of the target, contrast enhancement was detectable in the MR images at a labelling concentration ≥1 µM. As a consequence of endosomal trapping of CA, very low concentrations of sensor PNA were delivered into the cytosol where the targeted mRNA mainly resides. Due to insufficient delivery of CA in vicinity of target, the observed lack of specificity of dsRed CA even in dsRed cells can be explained.

Nonetheless, the synthesized contrast agents showed an excellent ability for intracellular delivery. However, direct uptake into cytosol or endosomal release of agents was found to be mandatory for attaining mRNA-based targeting and specific accumulation.