Metabolomics reveals in vivo significant impact from iron oxide nanoparticle-labeled, grafted cells on the host organism

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Target audience: Scientist who are interested in molecular MRI and metabolic approaches.

Purpose: Cellular MRI techniques, detecting intracellular iron oxide nanoparticles (IONP) assess a variety of functional and cellular parameters of tumors. While conventional volume measurements evaluate tumor progression and responses to treatment no information is given about different cell populations, their proliferation behavior and their specific functional tasks. Labeled tumor cells in contrast can be tracked in vivo over time course [1,2] and since the iron label is diluted during cell growth, the proliferative activity of the cells can be estimated. However, potential toxic effects of IONPs were demonstrated both in vivo and in vitro, and may lead to misinterpretation [3,4]. Here, we investigated in vivo the effect of IONPs on C6 glioma cells with regards to growth behavior and metabolic pathogenesis by MRI and NMR metabolomics using statistical pattern recognition techniques.

Methods: *Cell labeling*: a cell culture of 10^7 C6 rat glioma cells expressing hrGFP (C6-SP-GFP) was labeled with 0.5 µm rhodamine coated IONPs (ScreenMAG-R) in a concentration of 70 µg/10ml. *Immunofluorescence and histochemistry:* C6-SP-GFP cells with or without rhodamine labeled IONPs were grown on glass coverslips and nuclear counterstaining (Hoechst 33258) and Prussian blue staining was performed according to standard protocols. *Animal model:* In 2µl PBS $4x10^4$ C6-SP-GFP cells labeled with or without IONPs, were transplanted into the right striatum of 24 6-weeks old female NMRI nude mice (Elevage Janvier, Le Genest-Saint-Isle, France). On day 3, 8, and 10 post transplantation MRI measurements were performed and 50 µl of urine was collected and included in the NMR metabolomics study. *MRI measurements:* MR images were acquired at 9.4 T on a Bruker BioSpec 94/20 (Ettlingen, Germany) equipped with a 0.7 T/m gradient system and a cryoprobe. Tumor contrast was obtained by a T2 weighted TurboRARE sequence carried out with the following parameters: slice thickness, 0.3 mm; number of slices, 26; matrix size, 256×256; field of view, 20×20 mm^2; RARE factor, 12; averages, 8; TE, 42 ms; TR, 4.5 s. For imaging of labeled cell cultures, T2 maps were acquired using a 3.5 cm birdcage coil. *Volumetric analysis:* Tumor regions as well as brain volume were segmented by using the Amira software (Version 5.4.0, Visage Imaging GmbH, Berlin, Germany). *NMR-based metabolic profiling:* For NMR profiling of the mouse urine, ^{1}H spectra were acquired on a 500 MHz Bruker Avance NMR spectrometer with a 5 mm BBFO probe head equipped with z-gradient using a 1D presat sequence (noesygrpr1d), using 32k points, 6000 Hz sweep width, 2.73 s acquisition time and a relaxation delay of 4 s. The metabolomic profiles (bins of 0.04 ppm) were created using Mnova (version 7, Santiago de Compostela, Spain), and peaks assignments were made using published chemical shift values and Chenomx NMR Suite (version7.9, Edmonton, Canada). After normalizing the data to minimize the diurnal variation, the profiles were used as input in the software package SIMCA-P (version 12, Umetrics AB, Umea/Malmo, Sweden) for statistical modeling.

Results and Discussion: A labeling protocol with 0.5 µm IONPs was established for C6 rat glioma cells and was validated by immunofluorescence microscopy, Prussian blue staining, viability assays and by measuring MR relaxivity. T2 measurements of growth curves showed in culture experiments that the label is transferred to daughter cells during cell proliferation, leading to a step by step dilution of the contrast agent. In culture experiments, viability and growth of labeled tumor cells did not show significant differences compared to unlabeled cells (Fig. 1). *In vivo*, MRI of labeled tumors showed a hyperintense tumor periphery, indicating that the cells may have undergone several cell division steps, while the tumor core remained hypointense (Fig. 2). This observation suggests that either the cells residing in the core are not dividing or that highly proliferating cells migrate to the tumor periphery. Tumor growth of both unlabeled (UL) and iron labeled (IL) tumor cells was monitored by MRI on day 3, 8, and 10 post transplantation and did not differ significantly between the two groups (UL: day3=0.71±0.58 mm^3, day8=2.72±2.13 mm^3, day10=5.3±4.26 mm^3; IL: day3=0.61±0.43 mm^3, day8=1.38±1.10 mm^3, day10=2.85±1.60 mm^3) but tended to a smaller volume for iron labeled tumors. In contrast ^{1}H NMR spectra from urine of mice transplanted with iron labeled C6 glioma cells differed significantly from those of untreated cells and showed significant increase in trans-aconitate, N,N-dimethylglycine and 2-phenylproprionate (Fig. 3). For tumors without iron labeled cells tumor size correlated significantly with changes in the metabolic spectra (Fig. 4) while no correlation was obtained for iron labeled tumors. These results indicate that IONPs may have significant impact on cell behavior and tumor growth in vivo which may result in metabolic changes which superpose the pathological ones.

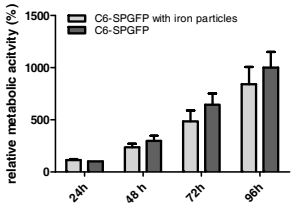

Fig.1: MTT assay of IONP labeled and untreated C6 glioma cells.

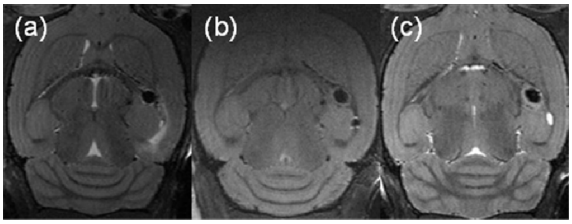

Fig. 2: T2 weighted TurboRARE images of 0.5 µm IONP labeled c6 glioma tumors on (a) day 3, (b) day 8 and (c) day 10 post transplantation.

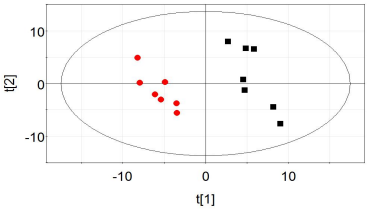

Fig. 3: Partial least squares discriminant analysis (PLS-DA) scores plots, representing urine ^{1}H NMR spectral data from NMRI mice being transplanted with iron labeled (red) and untreated (black) C6 glioma cells on day 3 post transplantation (R2: 96.5%, Q2: 74.3%).

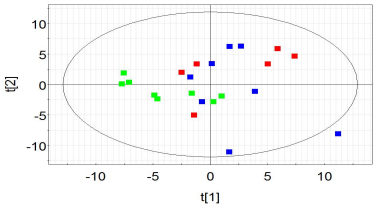

Fig. 4: Partial least squares (PLS) plot representing urine ^{1}H NMR spectral data over time course from NMRI mice being transplanted with untreated C6 glioma cells and using tumor size as y variable (R2: 94.7%, Q2: 54,6%; green: day3, blue: day8, red: day 10).

Conclusion: In cell tracking experiments interpretation of cellular behavior and proliferative activity based on iron patterns in IONP-labeled tumors should be performed with caution. Iron labeled and unlabeled tumors resulted in vivo in a significantly different pathologic and metabolic pattern, indicating a substantial impact of the iron on disease progression and cell characteristics. Even though metabolomics questions commonly accepted strategies, cellular MRI remains a powerful tool to localize and identify cells of interest.

References: [1] B.J. Nieman et al., Neuroimage. 2010, 50:456-64. [2] C. Heyn et al., Magn Reson Med. 2006, 55:23-9. [3] B.Szalay et al., J Appl Toxicol. 2012, 32:446-53. [4] S.J.H. Soenen et al., Biomaterials 2011, 32:195-205.