19F MRI for Evaluating ERK1 as a Factor Regulating Dendritic Cell Migration in High Grade Glioma

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Target audience: This study is intended for MR researchers interested in in vivo cell tracking, and for scientists interested in identifying new therapeutic options for the treatment of brain tumors.

Introduction/Purpose: Glioblastoma multiforme (GBM) remains the least curable and most common, aggressive subtype of primary brain tumors despite continuous advancements in radio- and chemotherapy. Immunotherapy with dendritic cell (DC)-based vaccines for treating brain tumors has become one attractive approach not only because of its anti-glioma effect but also for exploiting the natural immune system to target and eradicate residual tumor tissue. In order to access the anti-glioma activity of DC vaccines, it is necessary to monitor the spatio-temporal distribution of DCs in the CNS or lymphoid organs. Our goal is to target molecules that are involved in regulating the migratory capacity of DCs within the GBM context. Our earlier work has shown that extracellular signal-regulated kinase 1 (ERK1) is a prerequisite for regulating the ability of DCs to prime an immune response in vivo. In this study we hypothesized that ERK1 regulates DC migration. In parallel to in vivo migration assays, we implemented fluorine (19F) proton (1H) MRI to study the distribution of DCs in vivo following application in a mouse GBM model.

Methods: In vitro DC migration assay: Day 9 cultured bone marrow derived DCs from wild type (WT) or ERK1−/− C57BL/6 mice were plated onto 3.5 mm plates (MatTek, Ashland, USA) containing both PBS and CCL21 agarose spots. The migration of DCs into the spots was monitored 4 hours after plating. DC labeling and application: For the in vivo migration assay, WT and ERK1−/− DCs were labeled with 19F-enriched (emulsified perfluoro-15-crown-5-ether; PFCE) and fluorophore-tagged multimodal nanoparticles. Labeled DCs were matured with 0.5 mg/ml lipopolysaccharide for 18 hours and finally 5x10⁶ DCs were injected intradermally into the footpad of C57BL/6 mice (WT: right foot; ERK1−/−: left foot). For tracking DCs in the glioma model, DCs were pulsed with glioma lysate (from GL261; 50 μg/ml) following 19F nanoparticle labeling. 1x10⁶ DCs were injected intracranially (lateral ventricle). Mouse glioma model: 2x10⁶ GL261 cells were stereotactically injected into WT or ERK1−/− mouse brain. In vivo MRI: All MRI was performed using a 9.4 Tesla animal scanner (Biospec 94/20, Bruker Biospin, Germany). For measuring glioma size anesthetized mice were placed below a cryogenically-cooled quadrature RF coil (CryoProbe, Bruker Biospin, Germany) and brain T2-weighted images (TE=60ms) of gliomas in WT mouse brains. For the in vivo migration assay the mouse legs were imaged using a 1H/19F dual-tunable volume birdcage resonator (Rapid Biomed, Germany). Gradient echo images were acquired using a 3D-RARE sequence with an isotropic resolution of 250 μm³ for 19F (TR/TE = 8/3 ms; 80 averages, flip angle = 10°) and for 1H (TR/TE = 11/4 ms; 8 averages, flip angle = 15°). For tracking DCs in glioma and other brain/head regions an in-house built dual-tunable 19F/H head coil was employed and a protocol consisting of a 3D RARE sequence with an isotropic resolution of 250 μm³ for 19F (TR/TE = 800/5-9ms, 128 averages) and of 125 μm³ for 1H (TR/TE = 1500/47ms) MRI.

Results and discussion: In vitro (Fig. 1A) and in vivo (Fig. 1B) migration assays revealed that deletion of ERK1 in DCs enhanced cell migration. In line with this, ERK deficient glioma-bearing mice were more capable at staving off glioma growth than WT mice (Fig. 2; 8.9 and 25.6 mm³, p=0.0048). When applying 1-2 x10⁶ 19F labeled-DCs into the lateral ventricle of glioma bearing mice (n=6) the 19F signal on both, WT and ERK deficient DCs, could be visualized in the ventricular system over 3 weeks (Fig. 3A, only WT DC shown). The migration of DCs from the ventricular system to the tumor area (Fig. 3B) can be seen 9 days after DC application. Further research into the role of ERK1 for in vivo migration is ongoing. Immunofluorescent microscopy could also help to investigate the infiltration of exogenous DCs as fluorescent signal within the glioma tumor core and lymph nodes.

Conclusion: Our results indicate that ERK1 may be a crucial factor for DC migration. 19F/H MRI enabled us to achieve non-invasive monitoring of DC therapy efficiency in GBM. It could be a valuable tool for understanding the migratory behavior of DC vaccines and developing novel anti-glioma treatments.

References: