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[1]. 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*[2]. In this study we hypothesized that ERK1 regulates DC migration. In parallel to *in vitro* based migration assays, we implemented fluorine (^{19}F)/ proton (^{1}H) 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 ^{19}F-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 5x10^6 DCs were injected intradermally into the footpad of C57BL/6 mice (WT: right foot; ERK1$^{-/-}$: left foot). For tacking DCs in the glioma model, DCs were pulsed with glioma lysate (from GL261; 50 µg/ml) following ^{19}F nanoparticle labeling. 1x10^6 to 2x10^6 DCs were injected intracranially (lateral ventricle). ***Mouse glioma model:*** 2x10^4 GL261 cells were stereotacticly injected into WT or ERK1$^{-/-}$ mice 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 T_2-weighted images were acquired (RARE, NEX = 12, TR/TE = 3627/60 ms, 8 averages) with an in-plane resolution of 51 mm^2. For the *in vivo* migration assay the mouse legs were imaged using a ^{1}H/^{19}F dual-tunable volume birdcage resonator (Rapid Biomed, Germany). Gradient echo images were acquired using a 3D-FLASH sequence for ^{19}F (TR/TE = 8/3 ms; 80 averages, flip angle = 10°) and for ^{1}H (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 ^{19}F/^{1}H head coil was employed[3] and a protocol consisting of a 3D RARE sequence with an isotropic resolution of 250 µm³ for ^{19}F (TR/TE = 800/5.9ms, 128 averages) and of 125 µm³ for ^{1}H (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, ERK1 deficient glioma-bearing mice were more capable at staving off glioma growth than WT mice (Fig. 2; 8.9 and 25.6 mm^3, p=0.0048). When applying 1-2 x10^6 of ^{19}F labeled-DCs into the lateral ventricle of glioma bearing mice (n=6) the ^{19}F 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. ^{19}F/^{1}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.

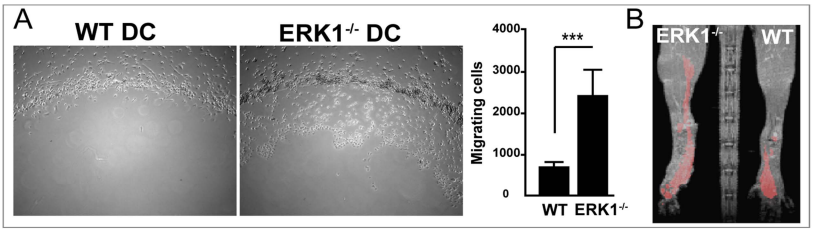

Fig. 1. *In vitro* and *in vivo* DC migration. **A)** *In vitro* DC migration into the spots was monitored 4 hours following seeding of DCs to the plates. Quantification of WT and ERK$^{-/-}$ DC migration towards chemokine gradient. **B)** *In vivo* migration was monitored for 24 hours by ^{19}F/^{1}H MRI. Red color illustrates the ^{19}F signal, which indicated the intradermally applied ^{19}F labeled DCs.

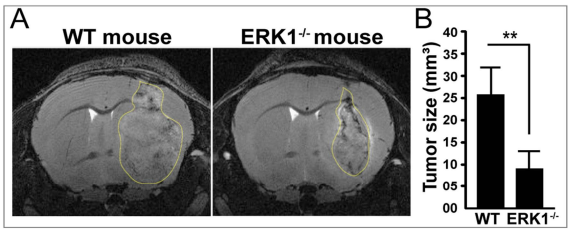

Fig 2. A) T_2-weighted images (TE=60ms) of gliomas in WT and ERK1$^{-/-}$ mouse brains. **B)** Volumetric assessment of glioma size in ERK1$^{-/-}$ mouse brains (n=6) compared with WT mouse brains (n=6).

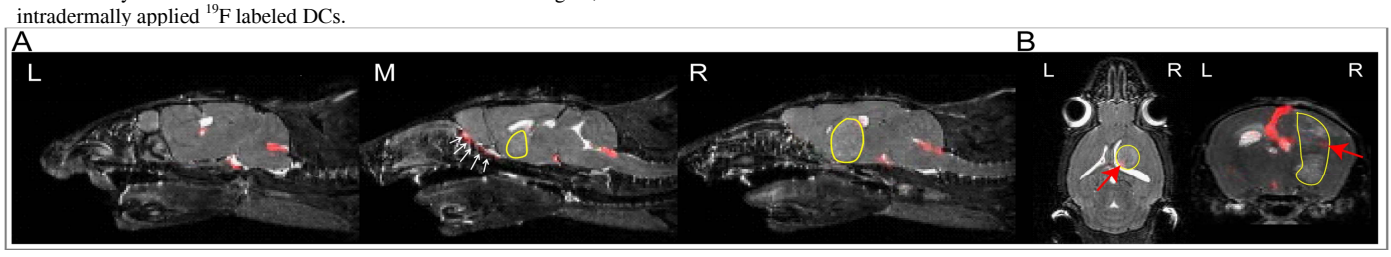

Fig 3. A) ^{19}F labeled DCs are depicted by ^{19}F/^{1}H MRI (red overlay) after stereotactic application into the left lateral ventricle. **B)** ^{19}F signal (red) was also detected in the tumor area (encircled in yellow).

References:

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