Hypoxia imaging-guided mesenchymal stem cell-mediated sodium iodide symporter (NIS) gene delivery in glioblastoma

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Introduction (max. 600 characters incl. spaces)
Glioblastoma (GBM) is a tumor with a poor prognosis and an urgent need for novel therapy approaches[1]. As a theranostic gene, the sodium iodide symporter (NIS) can be used for noninvasive radioiodine-based molecular imaging and therapy using distinct gene transfer systems e.g. mesenchymal stem cells (MSC)[2]. Based on their intrinsic tumor homing capacity MSC can be utilized as "Trojan horse" for the delivery of NIS to target the tumor milieu[3]. In this study, engineered MSC with a tumor-selective hypoxia-induced activation of NIS expression were investigated for a new hypoxia-guided GBM therapy.

Methods (max. 800 characters incl. spaces)
A subcutaneous xenograft GBM mouse model was established by implantation of patient-derived cells. The experimental set-up is displayed in Fig 1. To image tumor hypoxia, [^{18}F]FMISO-PET was performed 3 h after tracer application. Human bone marrow-derived MSC, stably transfected with NIS driven by a synthetic hypoxia-inducible promoter (HIF-NIS-MSC), were tested *in vitro* for functional NIS expression by ^{125}I-uptake assay. CMFDA-labelled HIF-NIS-MSC were systemically administered and tumoral iodide uptake was monitored by serial ^{124}I-PET-imaging two days later. Injection of perchlorate served as a control for NIS specificity. Resected tumors were analyzed by ^{124}I-autoradiography and immunohistochemistry for NIS and pimonidazole, which was applied 1 h prior to sacrifice, was performed.

Results/Discussion (max. 1000 characters incl. spaces)
[^{18}F]FMISO uptake indicated the presence of pronounced hypoxia within the GBM tumors prior to NIS gene delivery. ^{124}I-PET/CT scans 48 hours after systemic HIF-NIS-MSC application resulted in perchlorate-sensitive radioiodide accumulation in the tumor (Fig 2A). A strong correlation between both PET-imaging modalities was detected supporting [^{18}F]FMISO-PET to assess feasibility of hypoxia-targeted MSC-based NIS gene therapy (Fig 2B). Imaging was confirmed by ^{124}I-autoradiography displaying the spatial distribution of NIS-expressing MSC and *ex vivo* analysis of resected tumors by immunoreactivity to NIS and pimonidazole as marker for hypoxia. In addition, MSC recruitment to the tumor *per se* was evaluated by CMFDA-labelling. As a next step towards clinical application, the concept will be translated into an orthotopic model to better recapitulate the complex biological situation of GBM.

Conclusion (max. 450 characters incl. spaces)
In the present study, we have shown the potential to improve MSC-based NIS gene therapy of GBM using hypoxia-based PET imaging as a predictor for effective MSC tumor homing and hypoxia-responsive NIS transgene expression. Hypoxia-imaging provides an opportunity for improved prediction and guidance of GBM treatment.

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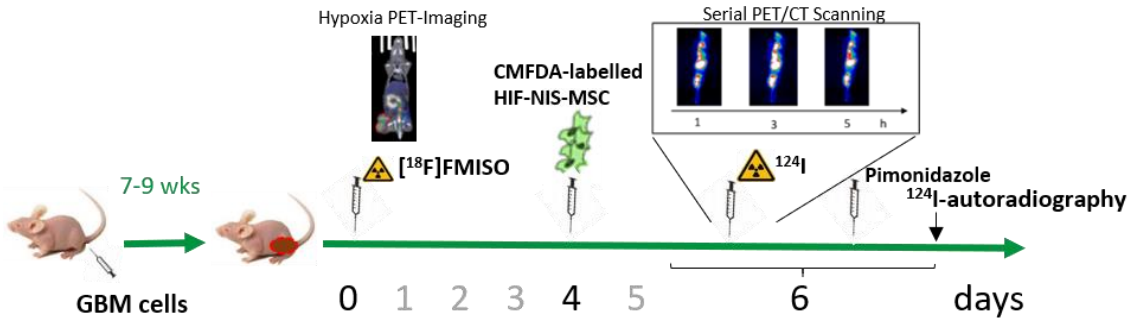

Fig.1 Experimental set-up showing the time course of PET/CT scans with applied tracers and single MSC application.

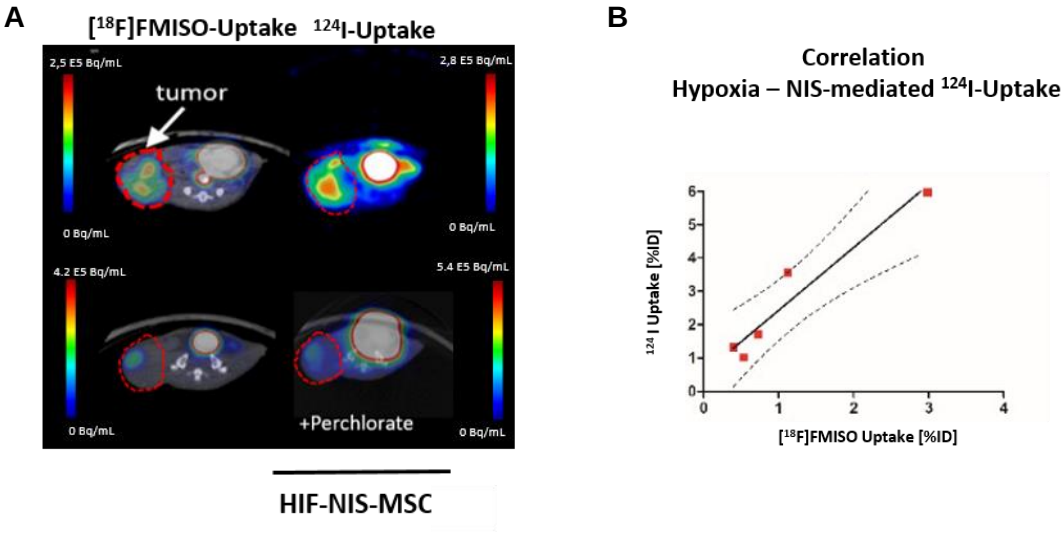

Fig.2 Correlation of hypoxia and hypoxia-responsive NIS-mediated radioiodide uptake after systemic HIF-NIS-MSC application.
(A) Axial PET/CT-images show [^{18}F]FMISO and ^{124}I-Uptake in subcutaneous GBM tumors. NIS inhibitor perchlorate was applied 30 min before radioiodine injection serving as a control for NIS-specificity.
(B) Correlation of tumoral uptake %ID of [^{18}F]FMISO and ^{124}I representing a strong correlation of r=0,96.