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Multimodal imaging enables early detection and characterization of changes in tumor permeability of brain metastases ☆

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ABSTRACT

Our goal was to develop strategies to quantify the accumulation of model therapeutics in small brain metastases using multimodal imaging, in order to enhance the potential for successful treatment. Human melanoma cells were injected into the left cardiac ventricle of immunodeficient mice. Bioluminescent, MR and PET imaging were applied to evaluate the limits of detection and potential for contrast agent extravasation in small brain metastases. A pharmacokinetic model was applied to estimate vascular permeability. Bioluminescent imaging after injecting D-luciferin (molecular weight (MW) 320 D) suggested that tumor cell extravasation had already occurred at week 1, which was confirmed by histology. 7 T T1w MRI at week 4 was able to detect non-leaky 100 μm sized lesions and leaky tumors with diameters down to 200 μm after contrast injection at week 5. PET imaging showed that ^{18}F-FLT (MW 244 Da) accumulated in the brain at week 4. Gadolinium-based MRI tracers (MW 559 Da and 2.066 kDa) extravasated after 5 weeks (tumor diameter 600 μm), and the lower MW agent cleared more rapidly from the tumor (mean apparent permeabilities 2.27×10^{-5} cm/s *versus* 1.12×10^{-5} cm/s). PET imaging further demonstrated tumor permeability to ^{64}Cu-BSA (MW 65.55 kDa) at week 6 (tumor diameter 700 μm). In conclusion, high field T1w MRI without contrast may improve the detection limit of small brain metastases, allowing for earlier diagnosis of patients, although the smallest lesions detected with T1w MRI were permeable only to D-luciferin and the amphipathic small molecule ^{18}F-FLT. Different-sized MR and PET contrast agents demonstrated the gradual increase in leakiness of the blood tumor barrier during metastatic progression, which could guide clinicians in choosing tailored treatment strategies.

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1. Introduction

Brain metastasis is the most frequent neoplasm of the central nervous system (CNS), and is ten times more common than primary malignant tumors [1,2]. The majority of brain metastases originate from lung cancer, breast cancer or melanoma [3]. Among these, melanoma has the highest propensity to metastasize to the brain, occurring in over 50% of all melanoma patients with advanced disease [4,5]. The incidence of brain metastases appears to be rising, due to improved detection by increased use of refined imaging modalities, and improved systemic treatments that prolong survival and control tumor burden in other organs [6–8].

Brain metastases are diagnosed late in the clinic, when they are sufficiently large to be detected by imaging, and magnetic resonance imaging (MRI) using passive gadolinium contrast enhancement is currently the method of choice [9]. At this time the prognosis is poor, as in a large study of melanoma patients with brain metastases, the median survival was 3.8 months [10].

Patients with brain metastases are treated with surgery, radiation therapy and chemotherapy. Surgery is performed on patients with single brain metastases and controllable systemic disease [10], in order to relieve pressure effects and achieve local tumor control [11]. Palliative,

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whole brain radiation therapy (WBRT) is the preferred treatment for most patients, since more than 70% of the patients have multiple metastases at the time of diagnosis [11]. Control of neurological symptoms may be achieved in 70–90% of the patients after WBRT. Stereotactic radiosurgery (SRS) is a method for delivering focused single high-dose irradiation, using a Gamma Knife or a dedicated linear accelerator. Local growth control of single brain metastases is high, and SRS is also effective on tumors that are regarded resistant to WBRT, such as brain metastases from melanoma [11]. SRS can safely be performed repeatedly on new brain metastases, due to low radiation doses to nearby brain tissue. The role of chemotherapy for brain metastases is limited [12]. Temozolomide has been the most promising drug, either used alone, in combination with other cytotoxic or biological drugs, or combined with WBRT [12]. Most of the chemotherapeutic agents used today are too large or hydrophilic to cross an intact (non-leaky) blood–brain barrier (BBB), making the brain a "sanctuary" site for metastasis [6,13].

Medical treatment of brain metastases is to date controversial, as survival is only modestly improved [12]. The dismal prognosis after treatment strongly suggests that early detection is needed for improved therapy and hence better patient outcome.

Also, the efficacy of treatment has been shown to diminish in larger lesions as tumor cells create an altered local environment *via* their interactions with astrocytes. Thus, treatment strategies that are highly effective *in vitro* often fail once this altered microenvironment is established [14,15]. Here, in order to enable early detection and characterize the BBB to improve treatment of small metastases, we set out to evaluate accumulation and transport kinetics of contrast agents of varied size and lipophilicity in serial studies of brain metastasis development.

In the presence of brain tumors, the BBB in the tumor area is commonly referred to as the blood–tumor barrier (BTB). There is a heterogeneous permeability (leakiness) to different-sized molecules through the BTB, with many metastases showing little or no permeability at all [16–20]. Thus, efficient drug delivery to brain metastases may be compromised by an intact BTB around small lesions [14]. Experimental studies of BTB permeability have been restricted to employing fluorescence imaging, and recently a few high field MRI studies have been performed. Here, we provide for the first time a detailed characterization of changes in BTB permeability by performing longitudinal molecular imaging studies using a wide range of contrast agents, by incorporating a novel MRI probe and also by comparisons with PET imaging.

Very few relevant experimental brain metastatic models are available to study biological mechanisms and therapeutic limitations and potentials in small brain metastases in relation to the BTB [17,21]. Intracardial injections of human tumor cell lines followed by intravenous injections of sodium fluorescein (MW 376 Da) showed that lesions had an intact BTB until they reached a diameter larger than 0.25 mm [5,22]. In a permeability study using a mouse model of breast cancer metastasis, Percy and collaborators injected Dextran (MW 3 kDa) and Gd-DTPA (MW 938 Da), and showed by MRI that a significant number of brain metastases were non-permeable, and that permeability developed late in tumor development [17]. Others administered Texas Red Dextran and radiolabeled chemotherapies to tumor bearing mice to demonstrate partial BTB permeability varying in magnitude within and between metastases [16].

It is evident that still very little is known about changes in BTB permeability during brain metastasis formation. Moreover, there is little data on the ability of high field MRI to improve detection of smaller brain lesions, which likely are impermeable to contrast agents. We address these issues by performing multimodal imaging studies following administration of contrast agents with various molecular weights (Suppl. Fig. 2) and thus different abilities to cross the BTB, into a well-established melanoma brain metastasis model [23]. By combining these contrast agents with bioluminescent imaging, MRI and PET imaging, we show that impermeable brain metastases as small as 100 μm may be detected by high field MRI, and the tumors become permeable to small molecular weight contrast agents upon reaching a diameter of 200 μm. However, accumulation and transport kinetics of agents in these small lesions are dependent on the agent properties. This is likely to be important as improved diagnosis and treatment of brain metastases are critical and will likely require a personalized treatment approach.

2. Materials and methods

2.1. Cell lines and cell culture

The H1 cell line was developed in our laboratory from a patient biopsy of a human melanoma brain metastasis as previously described [24] (Suppl. Fig. 1a i–iv). Written consent was obtained from the patient before tumor material was collected. The Regional Ethical Committee (REC number 013.09) and the Norwegian Directorate of Health (NSD number 9634) approved tissue collection and biobank storage of tumor biopsies and derived cell lines. The cells were authenticated in February 2013 using the AmpF⁄STR Profiler Plus PCR Amplification Kit (Applied Biosystems) and short tandem repeat (STR) profiles were matched to the parent tumor and cross-checked with cell line profiles at www.dsmz.de.

The H1 cells were transduced with two lentiviral vectors, encoding Dendra (a GFP variant) and Luciferase to obtain the H1_DL2 cell line (Suppl. Fig. 1a v, and Supplementary material). Flow cytometric isolation of cells by GFP expression was performed (BD FACSAria, Becton Dickinson, Franklin Lakes, NJ, USA) (Suppl. Fig. 1a vi and vii). The transduced H1_DL2 cell line was proven positive for Luciferase activity by *in vitro* bioluminescence imaging (BLI), and then used in all experiments.

The cells were grown in DMEM supplemented with 10% heat-inactivated newborn calf serum, four times the prescribed concentration of non-essential amino acids, 2% L-glutamine, penicillin (100 IU/ml), and streptomycin (100 μl/ml) (BioWhittaker, Verviers, Belgium). The cells were kept in a standard tissue culture incubator at 37 °C (100% humidity, 5% CO_2). The growth medium was exchanged twice a week.

2.2. Animal model

All animal studies were conducted under a protocol approved by the University of California Davis, Institutional Animal Care and Use Committee. Eight week old female NOD/SCID mice (NOD.CB17-*Prkdc*^{scid}/NcrCrl) weighing 19–22 g were purchased from Charles River Laboratories International (Wilmington, MA, USA). During all experiments, the mice were anesthetized with 3% isoflurane (in oxygen, flow 2 l/min) and maintained at 1.5% isoflurane (in oxygen, flow 2 l/min).

2.3. Tumor cell injections

The mice received a subcutaneous injection of 0.05 ml buprenorphine hydrochloride (Buprenex, 0.05–0.1 mg/kg; Cardinal Health, Elk Grove, CA) for prolonged pain relief post injection. 5×10^5 H1_DL2 cells suspended in 0.1 ml PBS were slowly injected into the left cardiac ventricle by freehand using a 30G insulin syringe (Omnican50, B. Braun Melsungen AG, Melsungen, Germany), as described previously [25] (Suppl. Fig. 1a viii). Tumor development was then followed by multimodal imaging weekly for 6 weeks, as described below (Suppl. Figs. 1 a ix and 1b).

2.4. Bioluminescence imaging (BLI)

BLI was performed 10 min after tumor cell injections to determine possible inoculation failures, and weekly over 6 weeks to study systemic

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