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Magnetic Resonance Imaging (MRI)-Guided Microdialysis Cannula Implantation in a Spontaneous High-Grade Glioma Murine Model

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Abstract

Cerebral microdialysis is used to study anti-cancer drug penetration in the central nervous system (CNS) and brain tumors in animal models. Genetically engineered mouse models (GEMMs) have been recently used to study many aspects of CNS tumors as they represent a more relevant model than orthotopic brain tumor xenograft models. However, it is challenging to implant microdialysis cannula in these animals because T2-weighted MRI imaging does not show the reference point (bregma) traditionally used to obtain stereotactic coordinates. Thus, an alternative reference point that can be visualized on MRI images is needed. In this study a novel reference point, identified as the intersection between the olfactory bulb/frontal lobe border and the midline between cerebral hemispheres on T2-weighted MRI images, was used to calculate anterior-posterior (AP) and medial-lateral (ML) coordinates of brain tumors in a GEMM. This point overlies a visible crossover between the rostral rhinal vein and the midline suture on the mouse skull, allowing for the conversion of the MRI coordinates into surgical stereotactic coordinates. Post-mortem MRI and histological examination confirmed accurate probe placement. This procedure will facilitate the accurate and precise implantation of microdialysis probes for the study of anti-cancer drug penetration in brain tumors of GEMMs.

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Keywords

microdialysis; MRI; spontaneous tumors; glioma

Introduction

Cerebral microdialysis sampling is used to monitor CNS anti-cancer drug disposition in mouse models^{1,2}. This approach provides advantages over other sampling methods (e.g., whole tissue homogenization) including sampling from discrete anatomic compartments such as the brain extracellular fluid (ECF) or ventricular cerebrospinal fluid (vCSF)³, sampling from normal brain or brain tumor tissue, and acquisition of unbound or pharmacologically active drug moieties⁴. Furthermore, microdialysis enables serial sampling from individual animals, thereby reducing the number of animals required for pharmacokinetic investigations⁵.

Microdialysis has been frequently employed in studying anti-cancer drug penetration within brain tumors, particularly high-grade gliomas⁶⁻⁹. These studies are most often performed using murine orthotopic xenograft models in which human glioma cells are stereotactically injected into a defined brain location and the microdialysis guide cannula is simultaneously implanted. Tumor tissue then develops and surrounds the cannula⁶⁻⁸. Although orthotopic tumor xenograft models are easy to use, relatively inexpensive, and reproducible, in many cases the genetics and histology of human tumors are not adequately recapitulated¹⁰. In addition, the tumor microenvironment and host immune responses are likely altered in immunodeficient mice, which diminish the ability of xenograft models to truly recapitulate the features observed in human tumors¹¹. In contrast, genetically engineered murine models (GEMMs) of brain tumors bestow many of the genetic and histological features of malignant human brain tumors¹¹. In general, these mouse models develop tumors spontaneously upon alteration of signaling pathways critical for the development of the specific tumor of interest^{11,12}. Thus, molecular and other complex processes including specific contributions of the tissue microenvironment, such as tumor angiogenesis, can appropriately mimic human disease in these spontaneous tumor models.

In GEMMs, heterogeneous tumor growth patterns and development of tumors in different brain regions pose several technical challenges to using these models for CNS pharmacokinetic investigations¹². For example, a major challenge to using cerebral microdialysis to study anti-cancer drug penetration in tumors of GEMMs is the difficulty of acquiring stereotactic coordinates to accurately place a microdialysis cannula in the tumor. Traditionally, the coordinates of the intersection of the coronal and sagittal sutures (bregma point) are used as a reference point for the placement of the microdialysis cannula^{3,13}. However, this approach is not practical in GEMMs because the bregma does not appear on images derived by MRI, which is the imaging method used to identify the size and location of the spontaneously arising tumors in the brain. The objective of the current study was to use in vivo MRI imaging to identify a reference point for implanting microdialysis cannula in spontaneously arising tumors in a GEMM for high-grade glioma. The accuracy of cannula placement was verified by post-mortem MRI and histological examination.

Materials and Methods

Animals

Transgenic mice used in this study were previously described¹⁴. Briefly, conditional deletion of *Pten*, *Tp53*, and *Rb1* in Gfap-expressing cells was induced by I.P injection of tamoxifen (Sigma, St. Louis, MO) to *GFAP-CreERTM*; *Pten*^{flox/flox}; *Tp53*^{flox/flox}; *Rb1*^{flox/flox} post-natal day 30 (P30) mice at a dosage of 9 mg/40 gm weight daily for 3 consecutive days. Mice were maintained on a 12 h light/dark cycle with free access to food and water. Mice developed tumors within 4 to 6 months. Other mouse strains (C57BL6 and CD1 athymic nude mice) were also used for validation of reference point studies. All animal studies were carried out in compliance with the Animal Care and Use Committee at St. Jude Children's Research Hospital.

Magnetic Resonance Imaging (MRI)

MRI exams were performed using a 7-Tesla Bruker Clinscan animal MRI scanner and a 4-channel phased-array surface coil (Bruker BioSpin MRI GmbH, Germany). Animals were anesthetized using isoflurane during data acquisition. Turbo Spin Echo (TSE) protocols (T_R 2000–2500 ms; T_E 40 ms) were used to produce T2 weighted transverse images with slice thickness of 0.5 mm. Turbo Gradient Spin Echo protocols (T_R 2000 ms; T_E 40 ms; Flip angle 180 degree) were used to produce fast T2-weighted images in the coronal plane with slice thicknesses of 0.7 mm. For post-mortem MRI, the mouse heads were submerged in Fluorinert FC-770 liquid (3M, Belgium) and imaged by a TSE protocol with a rat head 4-channel phased-array surface coil.

Magnetic Resonance Angiography (MRA)

MRA data were produced from a Bruker FL2D Time-of-Flight (TOF) protocol (T_R 7.6 ms; T_E 3.77 ms; Flip angle 90 degree). The raw data were acquired in the coronal plane followed by Maximum-Intensity Projection (MIP) reconstruction on a Siemens workstation using Syngo MR B15 software (Siemens, Erlangen, Germany). Image processing procedures were similar for both angiography and post-mortem MRI studies.

Stereotactic Surgery for Microdialysis Cannula Insertion

The general surgical procedure has been previously described¹⁵. Briefly, a mouse was anesthetized with 50 mg/kg ketamine (Hospira, Inc., Lake Forest, IL) and 10mg/kg xylazine (VEDCO, St. Joseph, MO). The head of the mouse was fixed in position on a KOPF stereotactic apparatus (Tujunga, CA). A small incision on the scalp was made and the rostral rhinal vein was identified. Using MRI images, tumor coordinates were calculated using View-Dimensions tool in Syngo MR B15 software. A microdialysis guide cannula was implanted in the center of a tumor. The mouse was allowed to recover for 3–5 days. On the day of the microdialysis study, a microdialysis probe (MBR-1–5, Bioanalytical Systems) was inserted through the cannula. The probe was perfused with artificial cerebrospinal fluid (aCSF¹⁵) at 0.5 μ L/min, and the probe membrane was allowed to equilibrate for 1 hour before starting the microdialysis experiment. Microdialysis experiments were performed as previously described².

Histology

At the end of the microdialysis experiment, the animal was euthanized and the brain was removed and fixed in 10% neutral buffered formalin (NBF) for at least 24 hr. Cannula and probe were removed and the brain was sliced into sagittal sections around the location of the implanted cannula, dehydrated, cleared, and embedded in a paraffin block. Sections were cut on a microtome at 4 μ m thickness and every tenth section was collected and stained with haematoxylin and eosin. All sections from a given block were examined microscopically to verify the accurate placement of the microdialysis probe via the track in the tumor.

Results and Discussion

Identification of the rostral rhinal vein as a correlative reference point

Images of blood vessels of the mouse brain acquired using angiography revealed the presence of a large vein overlying the border between the olfactory bulb and the frontal lobe (Fig. 1A and 1B). Using the mouse cerebral vasculature atlas¹⁶, this vein was identified as the rostral rhinal vein, which is readily apparent to the unaided eye and traverses both the dorsal and ventral surfaces following the olfactory bulb/frontal lobe border (Fig. 1C). The removal of skulls (n = 6 per strain used) confirmed that this vein overlies the border between the olfactory bulb and the frontal lobe. Thus, we designated our reference point as the intersection of the midline suture and the rostral rhinal vein on the mouse brain surface. This point correlated with the intersection between the midline and the olfactory bulb/frontal lobe border visualized on MRI T2-weighted images of the brain. The rostral rhinal vein was present in all mouse strains evaluated including C57BL6 and CD1 athymic nude. Anatomical dissection of these strains confirmed that this vein overlies the olfactory bulb/frontal lobe border and thus this method may be applied to target tumors in other mouse strains (data not shown).

Determination of Tumor coordinates and confirmation of probe placement

Tamoxifen injection induced Cre activity and spontaneous glioma formation in *GFAPCreERTM;Pten^{flox/flox};Tp53^{flox/flox};Rb1^{flox/flox}* mice¹⁴. Induced mice were monitored biweekly for tumor development by MRI using T2-weighted imaging beginning at P120. Tumors developed in several locations within the brain including the forebrain, midbrain, and the cerebellum. When a tumor was large enough for microdialysis cannula/probe implantation (around 2.5–4 mm in diameter), placement coordinates were determined using the View-Dimensions tool in Syngo MR B15 software. The X and Y axes were determined using the transverse plane (Fig. 2A) while the depth of implantation (Z-axis) was determined using the coronal plane (Fig. 2B). Postmortem MRI (Fig. 2C and 2D) and histochemical examination (Fig. 2E) confirmed targeted probe placement in all mice studied (n=6). Mice used in this study developed tumors that arose within several regions of the brain including the forebrain (33%), midbrain (50%), and the cerebellum (17 %). Probe recovery values for the conducted experiments were 21% \pm 4.5% (n=6).

Conclusion

It is a challenge to accurately place microdialysis cannulae in GEMMs that spontaneously develop brain tumors because the traditional reference point commonly used to derive stereotactic co-ordinates for cannula implantation, the bregma, is not visible by MRI. By using a combination of angiography and T2-weighted imaging by MRI, we identified the rostral rhinal vein as a reference point to derive coordinates for accurate placement of microdialysis cannula in tumors developing in several regions of the mouse brain.

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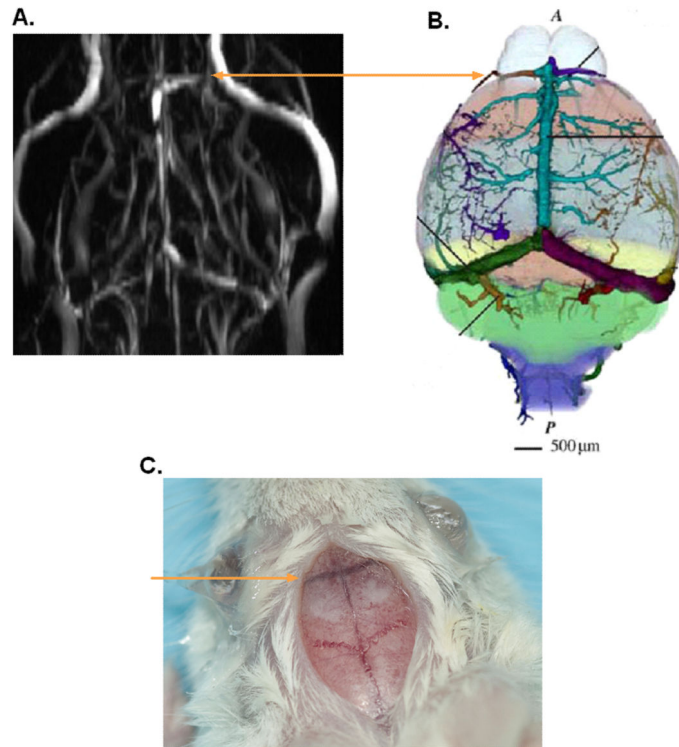


Fig. 1. Presence of the rostral rhinal vein on the surface of a mouse brain

A, Angiogram of a non-tumor bearing *GFAP-CreERTM;Pten^{flx/flx};Tp53^{flx/flx};Rb1^{flx/flx}* mouse showing a large vein across the olfactory bulb/frontal lobe border. **B**, Diagrammatic illustration of veins on mouse brain surface illustrating the rostral rhinal vein on the border between the olfactory bulb and the mouse brain frontal lobe. Reproduced with permission from¹⁶. **C**, Brain surface of a *GFAPCreERTM;Pten^{flx/flx};Tp53^{flx/flx};Rb1^{flx/flx}* mouse. Lines indicate location of the rostral rhinal vein.

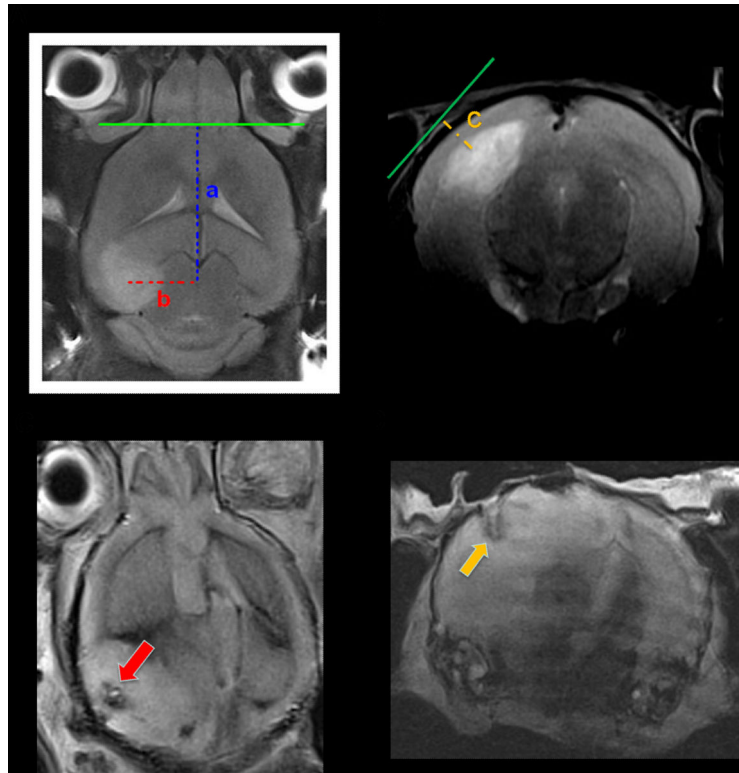


Fig. 2. Derivation of MRI-guided stereotactic coordinates for cannula implantation in a spontaneously arising brain tumors

A, Transverse section on a T2-weighted MRI demonstrating a tumor in the left hippocampus. Tumor margins are enclosed by a dashed line. The intersection between the olfactory bulb/frontal lobe border and the midbrain was used as a reference point to determine the coordinates on the Y-axis (*a*) and the X-axis (*b*). **B**, Coronal section on a T2-weighted MRI for calculation of the depth coordinates, i.e. Z-axis (*c*). In the provided example, values for *a*, *b*, and *c* were 7.6, 3, and 1.3 mm, respectively. **C–D**, Postmortem MRI showing track of the microdialysis probe in the tumor in the X and Y axes (red arrow), and on the Z-axis (yellow arrow). **E**, Hematoxylin and eosin staining confirming probe location in the targeted area (yellow dots limit tumor margins, blue dashes limit cannula track, and red dashes limit probe track; lines were drawn with Adobe Photoshop V11.0).