

# A head holder for magnetic resonance imaging that allows the stereotaxic alignment of spontaneously occurring intracranial mouse tumors

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## Abstract

The use of stereotaxic neurosurgery in rodent models of human disease requires the alignment of central nervous system (CNS) structures that can be identified and surgically approached with great accuracy. Current technologies make possible development of mouse lines with enhanced predispositions for the development of various diseases including tumors. When such tumors arise in the brain their location is unpredictable. Obtaining a biopsy or stereotaxically delivering local therapy requires that the site of such tumors be known with great precision. We devised a method to correlate images of mouse brain tumors acquired by magnetic resonance imaging (MRI) with stereotaxic coordinates that can be used for obtaining biopsies or administering local therapy. We constructed a head holder containing a pair of tubes filled with a substance that could be imaged by MR and which were separated by varying distances. This allowed the precise localization of the tumor in all three dimensions. The strategy we employed is adaptable to other imaging modalities and to other body sites. © 2002 Elsevier Science B.V. All rights reserved.

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## 1. Introduction

The use of stereotaxic neurosurgery in rodent models of human disease requires the alignment of central nervous system (CNS) structures at locations that can be identified by non-invasive imaging and surgically approached with great accuracy. Using xenografts of implanted tumor cells, it is usually possible to predict with confidence that the tumor will be located at the same site the cells were injected. Preclinical evaluations of various approaches to local therapy for brain tumors using such models routinely demonstrate the accuracy of this assumption (Pilkington et al., 1997; Herrlinger et al., 2000; Namba et al., 2000; Niranjana et al., 2000). Indeed, the predictable location of such tumor models greatly facilitates orthotopic evaluations of such thera-

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pies. With few exceptions, such tumor models are typically characterized by the *in situ* growth of spherical tumors, although they are not infiltrative. This pattern of tumor occurrence and growth contrasts sharply with that seen in emerging models of spontaneously occurring tumors in genetically manipulated animals which mimic more closely the infiltrative nature and other pathological properties of the corresponding human tumors (Weiss and Israel, unpublished data; Holland, 2000; Reilly et al., 2000).

Modern methods make it possible to develop mouse lines that have greatly enhanced predispositions for the development of specific tumor types. These models are generally developed by tissue specific expression of an oncogene. Recently, experimental strategies have been developed to inactivate tumor suppressor genes in a tissue specific manner, and technologies to regulate the tissue specificity and the temporal expression of oncogenes are widely available (Cohen, 1999; Clarke, 2000). In the case of brain tumor models, a number of tissue specific promoters, including one from the gene encoding S100 $\beta$  have been used successfully to target cell types in which oligodendroglioma arises (Weiss and Israel, unpublished data). Other experimental strategies involve the injection of recombinant viruses that encode oncogenes (Swenberg, 1977; Bilzer et al., 1989). When such manipulations result in mouse lines with an increased tumor incidence, these tumors arise largely in unpredictable locations of the CNS and at unpredictable times.

The challenge to target such tumors for biopsy or the administration of local therapies is particularly significant when such animals are used for preclinical treatment evaluations. These manipulations require that the tumor site be known with great precision. We have devised a method to localize in 3D spontaneously occurring tumors on magnetic resonance imaging (MRI) for stereotaxic manipulation. This method employs techniques similar to those used for the stereotaxic localization of human CNS disease (Brown et al., 1980; Alker and Kelly, 1984; Leksell et al., 1985). Stereotaxic surgery requires that the precise location of a therapeutic target be known in three dimensions. Furthermore, it is necessary to translate the location of the target as identified by an imaging modality into a location that can be surgically accessed in a precise manner. This requires that an investigator be able to image and identify landmarks in each of three dimensions when the animals are placed in a stereotaxic instrument. Accomplishing this seemingly straightforward task required the development and construction of a head holder containing a pair of bars that could be imaged by MR and recognized as corresponding to rostral–caudal locations within the brain with an accuracy of approximately 1 mm. The availability of such an apparatus and technical approach to stereotaxic surgery should greatly

facilitate future studies in which it is necessary to surgically manipulate or inoculate sites in the CNS that vary from animal to animal.

## 2. Materials and methods

### 2.1. Mice

We have used animals derived from lines of mice transgenic for S100 $\beta$ -v-erbB crossed to mice deleted for *ink4a/arf* (Weiss, De Pinho, Israel, unpublished data). These S100 $\beta$ -v-erbB/*Ink4a*<sup>-/-</sup> transgenic mice develop anaplastic oligodendroglioma in the first 6 months of life with a high penetrance. Animals between 3 and 6 months of age that displayed evidence of a neurologic defect were chosen for study. When not being examined or treated animals were housed in plastic cages, maintained on a 12 h light/12 h dark schedule and given food and water *ad libitum*.

### 2.2. Imaging

Animals were imaged when they had neurologic evidence of likely tumor development. Following anesthesia with Avertin (12.5 mg/ml, 0.2 ml/10 g body weight, injected intraperitoneally) mice were immobilized in a supine position on a polypropylene platform in a non-magnetic head holder as described below (see Section 3), and the entire holder placed in a home-built 3.8 cm diameter ‘birdcage’ radio frequency (rf) coil. The rf coil was then positioned in a 2.0 T magnet with a 33 cm bore, connected to a Bruker Omega CSI console (Broker Instruments, Inc., Fremont, CA) equipped with actively shielded imaging gradients ( $\pm 20$  Gauss/cm). A distinctive feature of the head holder is the incorporation of tubes containing 0.5% gadopentetate (GdDTPA) (Magnevist<sup>®</sup> Schering AG, Berlin, Germany) in the field of view (FOV). This revision allows monitoring of the rostral–caudal position of individual MR T1-weighted images were acquired from eight contiguous slices (2 mm thickness) with TR/TE = 500/12 ms, FOV = 35 mm, and data matrix = 128  $\times$  128 points (pixel dimension = 0.27 mm). For T2-weighted MRI, TR/TE was 2500/80 ms, while other settings remained unchanged. Tumors were localized for stereotaxic injection as described below.

To enhance images of high-grade primary brain tumors we inoculated animals with 200  $\mu$ l of 10% GdDTPA (500 mM) intraperitoneally 1–2 h before the examination, and we obtained T1- and T2-weighted images. Images obtained within 1 h of GdDTPA inoculation or more than 3 h after intraperitoneal inoculation were of poorer quality than those obtained during the interval of 1–3 h after injection. To visualize sites of inoculation, we stereotaxically inoculated 6  $\mu$ l of

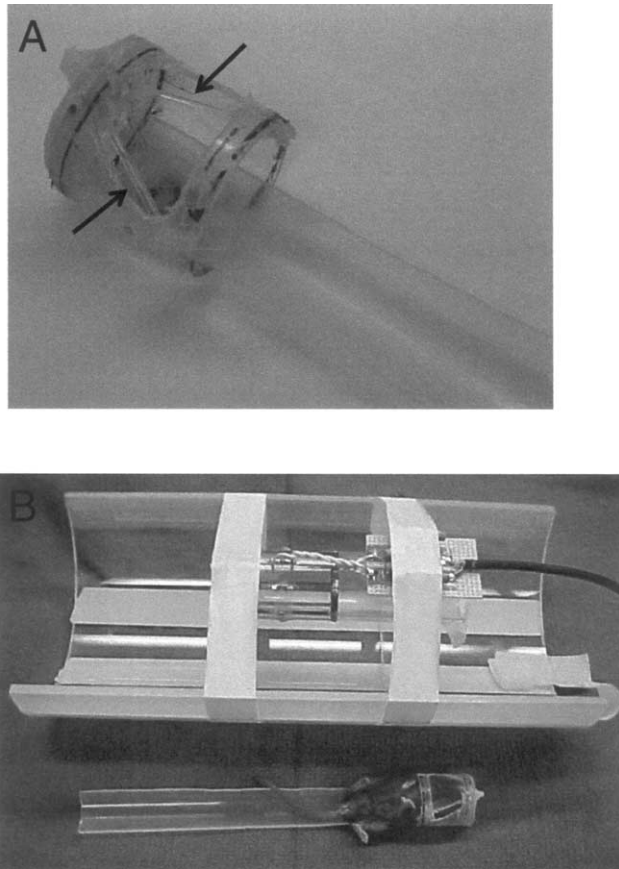


Fig. 1. Apparatus for MRI of a mouse head for subsequent alignment in a stereotaxic device. Panel A: The head holder was constructed from a polypropylene tube cut at the base to allow the nose of the mouse to rest comfortably beyond a tooth bar that was used for fixation of the mouse head. Sealed glass tubes filled with 0.5% GdDTPA were fixed with epoxy into position forming an acute angle with the apex centered at the base of the tooth bar and extending towards the body of the head holder (black arrows). Panel B: This panel shows an anesthetized mouse in position in the head holder and a homemade 'birdcage' rf coil.

GdDTPA, which resulted in a local signal increase (positive enhancement), and imaged with the T1-weighted sequence. In other experiments we stereotaxically inoculated 1 or 6  $\mu$ l of SHU555 (prepared at a concentration of 50 mM Fe) (Resovist<sup>®</sup> Schering AG, Berlin, Germany), an iron oxide particulate compound, which provides 'negative' contrast. Imaging of SHU555 was performed using a T2-weighted spin-echo sequence with an echo time of TE = 50 ms.

The head holder was constructed in the laboratory from the sidewall of a 100 ml polypropylene graduated cylinder (Greiner, supplied by Applied Scientific) cut to a length of approximately 20 cm (see Fig. 1, panels A and B). This curved length served as both the platform

on which the mouse was placed for imaging and a handle for inserting the mouse into the rf coil. The head holder that was attached to this platform consisted of two key parts, a tooth bar to immobilize the animal reproducibly and two bars that could be imaged by MR in a manner that allowed one to know precisely the distance of the MR image from the tooth bar. Initially, most of the sidewalls of a 50 ml conical polypropylene tube and both ends were removed as pictured in Fig. 1. The platform described above was glued with epoxy cement to the inner surface of the remaining part of the tube with the end of the platform extending to the end of the tube. At the end of the tube, a sandwich of three 3 cm discs, each with a vertical slot cut into it were glued together. The slots are approximately  $2.5 \times 0.7$  cm. The slot in the inner circle is slightly wider than the slots in the two outer circles, allowing the placement of a tooth bar that could be moved up and down to fix the mouse in place. Glass tubes were obtained from the ends of Pasteur pipettes. These were scored and broken at 3 cm, filled with 0.5% GdDTPA (effective concentration =  $5\% \times 500 \text{ mM} = 25 \text{ mM}$ ) and sealed at each end with epoxy. The tubes were then glued into position forming an acute angle with the apex centered at the base of the discs holding the tooth bar and extending towards the end of the platform opposite the head holder (Fig. 1, panel A).

### 2.3. Stereotaxic injections

A miniaturized stereotaxic instrument (Stoelting Co., Wood Dale, IL) was mounted onto a Kopf small animal stereotaxic instrument (Stoelting Co., Wood Dale, IL). Avertin or hypothermia, as described above, was used for anesthesia. Others have previously described hypothermia for anesthesia, and we followed precisely the procedure described by Cunningham and McKay (1993). Following the induction of anesthesia, the animal was immobilized in the apparatus as previously described (Kuriyama et al., 2000). The head was then briefly cleaned with 70% alcohol and a midline incision of the scalp was made from the eyes to the back of cranium. The skin was reflected downward to see the external auditory meati (Cunningham and McKay, 1993). A small burr hole was made with a diamond drill at coordinates determined by evaluation of the MR images (see below). A 10  $\mu$ l syringe (Hamilton Company, Reno, Nevada) was inserted into the brain to a depth determined from the MR images (see below), and various solutions were administered as described in Section 3. Following injection, the needle was withdrawn, and the skin sutured with 4-0 nylon thread.

### 3. Results

#### 3.1. Alignment of the stereotaxic injection with MR images

We designed a head holder for mice of non-magnetic material containing two non-parallel gadolinium filled glass tubes (Fig. 1). Initially, we prepared a standard curve that would allow us to predict the distance of tumors from the upper front incisors. We measured the distance between the two gadolinium containing tubes at 1 mm intervals with a caliper over a range of different distances between 5 and 20 mm from the incisor bar. We plotted the distance from the incisor bar against the distance between the two bars that appeared in the scan as two intense dots. An example of such a standard curve is shown (Fig. 2, panel A). This graph demonstrates the relationship of the distance between the two tubes ( $Z'$ ) at varying distances from the tooth bar ( $Z$ ). The standard curve is specific for each head holder, and a different standard curve must be prepared for each head holder constructed.

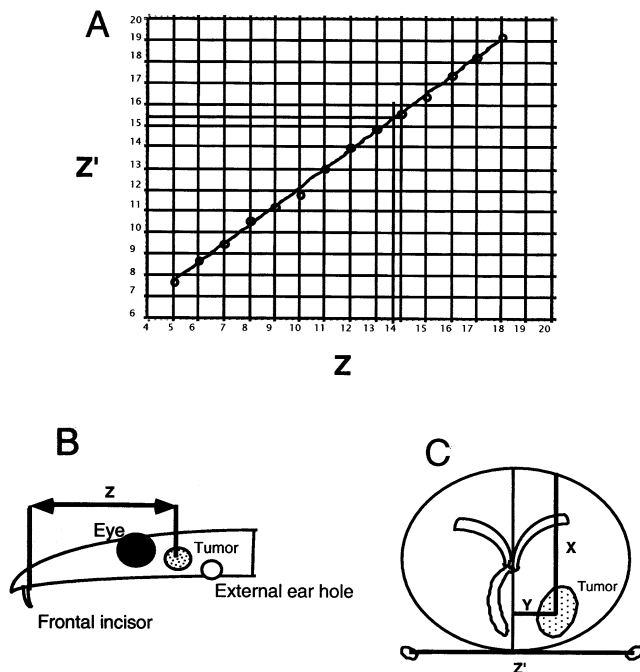


Fig. 2. Strategy for determining stereotaxic coordinates of an intracranial tumor from an MR image. Panel A: A standard curve in which the distance between the two GdDTPA-containing bars, designated as  $Z'$  and the distance of the bars from the tooth bar, designated as  $Z$ , are related. Panel B: Knowing  $Z'$  as determined from an MR image of a mouse tumor, the  $Z$  stereotaxic coordinate can be determined from a standard curve such as the one shown in panel A. Panel C: Identification of the  $Z'$  distance used to determine the  $Z$  stereotaxic coordinate is shown. The method for determining the  $X$  coordinate, the distance from the brain surface to the tumor and the  $Y$  coordinate, the distance of the tumor from the midline, as identified by the coronal suture also are demonstrated.

The standard curve allows one to determine with  $\sim 1$  mm precision the distance of a particular MR image from the front incisors, the  $Z$  distance (Fig. 2, panel B), by measuring on the image the distance between the two enhancing bars (Fig. 2, panel C) located under the supine mouse (Fig. 2, panel C). This was confirmed in preliminary experiments by obtaining images at very small interval distances and determining that the measured distance between the GdDTPA filled tubes changed in a manner that could be predicted by directly measuring the distances between the tubes at corresponding sites along the head holder (data not shown). On that same scan, one can identify the midline of the brain, and measure the exact distance from the midline to the tumor, the  $Y$  distance (Fig. 2, panel C). This establishes the distance from the midline of the skull that a stereotaxic injection would have to be made to strike the tumor (Fig. 2, panel C). Similarly, one can measure on the scan the distance from the skull to the lesion, the  $X$  distance, thereby determining the depth at which an injection into the tumor would have to be made (Fig. 2, panel C). Modifying this apparatus by including in its construction additional bars or an 'N' configuration similar to that used in human stereotaxic frames may be a useful enhancement for a variety of applications.

#### 3.2. Stereotaxic injection of spontaneously occurring mouse brain tumors with iron oxide

The inoculation into tissue of an iron oxide containing particle, Resovist, causes a loss of signal (a dark area) on T2-weighted images in regions where the material is concentrated. Fig. 3 shows the injection of such material into two different mice with spontaneously occurring brain tumors. Approximately 3 h prior to imaging, these animals had been injected intraperitoneally with 200  $\mu$ l of 10% GdDTPA. The left hand panels (Fig. 3, panels A and C) are T2-weighted images demonstrating enhancing lesions located in the brain stem (upper panel) and the ventrolateral diencephalon (lower panel). The arrows in these two panels indicate the GdDTPA-filled tubes. The appearance of these cross-sections as elliptical shapes of similar appearance may be served to indicate that the slice is not tilted with respect to the frame. Elliptical 'dots' of variable shapes should alert investigators to the possibility of such tilt. The following day the animals were again injected with GdDTPA and after 2 h they were anesthetized and placed in a stereotaxic apparatus. Six  $\mu$ l Resovist was inoculated stereotaxically into the tumors at coordinates determined from the preoperative MR images as described above (see Fig. 2). Immediately following the injection, the animals were evaluated by MRI again. These postoperative MRI evaluations are shown (Fig. 3, panels B and D). As can be seen in the figure, the iron in Resovist leads to signal loss on T2-

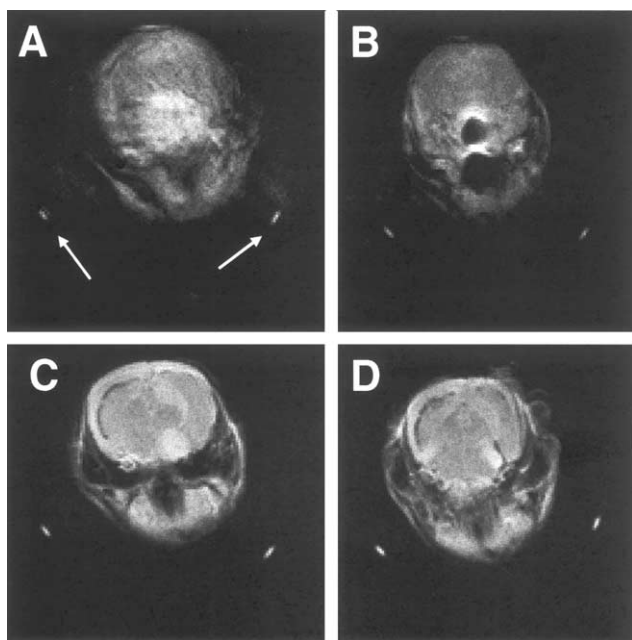


Fig. 3. Stereotaxic injection of spontaneously occurring mouse brain tumors with Resovist. The panels A and C show coronal views of MR images in which T2-weighted images of animals previously injected intraperitoneally with GdDTPA reveal the presence of enhancing tumors. The arrows indicate the location of the GdDTPA-filled tubes used for alignment of the tumors. Panels B and D show coronal views of MR images in which T2-weighted images of animals previously injected intraperitoneally with GdDTPA reveal dark images at the intratumoral Resovist injection site. We interpret panel D as being an image that is slightly posterior to the center of the injection site revealing only the contrast agent-filled needle track.

weighted images and indicates with great accuracy the location of the stereotaxic injection. As can be seen, a black area in which no signal is detectable has appeared within the tumor demonstrating the site at which the iron-containing compound had been inoculated. In panel D, the MRI slice is somewhat more posterior than the preinjection image and is through the contrast-filled needle track. We inoculated intratumorally Resovist into a total of eight animals in which a tumor had been initially identified on a T2-weighted scan. In each of these animals repeat scanning following the stereotaxic injection revealed that the tumor had been injected. Following acquisition of these postoperative scans, the animals were sacrificed and the brains examined for the presence of iron in the tumor. In most cases, we were able to identify a slight brownish/rust discoloration to tumor tissue when fresh brain tissue was dissected (data not shown).

### 3.3. Stereotaxic injection of spontaneously occurring mouse brain tumors with GdDTPA

We sought to extend these studies and to gain additional experience in the use of MRI to align these spontaneously occurring brain tumors for stereotaxic

injection. We followed the same procedures as those described above, except that in these experiments 6  $\mu$ l of GdDTPA was inoculated directly into the tumors. Two examples of intracranial brain tumors detected by MRI and subsequently injected stereotaxically *in vivo* with GdDTPA are shown in Fig. 4. In animal 13223 (Fig. 4, top row, panel A), hydrocephalus was evident on the T1-weighted MRI as hypointense, enlarged lateral ventricles and an abnormally enlarged 3rd ventricle or aqueduct. An inferior brainstem tumor was suspected based upon regional hyperintensity of the T2-weighted MRI (arrow in Fig. 4, top row, panel B), right-to-left shift in the inferior midline, and upward displacement of normal brainstem tissues. Successful administration of GdDTPA-containing infusate into the suspected mass was evident on the postoperative T1-weighted MR images obtained 2 h after the inoculation. In this scan a hyperintense region corresponding to the site where the GdDTPA was injected is easily seen (arrow in Fig. 4, top row, panel C). Following the postoperative MRI study, the animal shown here was sacrificed and the tumor tissue was histologically examined. As can be seen (Fig. 4, top row, panel D) the presence of tumor can be readily appreciated by histologic examination of a hematoxylin and eosin stained section. These sections also revealed a hole in the tissue at the location of the injected solution (arrow in Fig. 4, top row, panel D). Presumably this space is the result of tissue damage occurring in association with the injection. Comparable results are shown in a second, tumor (Fig. 4, lower panels). To date, in this study and in subsequent studies in which GdDTPA has been inoculated alone or co-inoculated with other molecules, we have studied a total of 18 animals. Of these we were able to document 17 animals in which the inoculation was delivered into the tumor. The one animal whose tumor was not injected accurately was inoculated on the wrong side of the head pointing out an important source of possible confusion, since the left–right orientation of the MR image needs to be carefully identified for each animal.

## 4. Discussion

Brain tumors of humans are routinely treated as localized diseases. The irradiation of tumors is routinely used as a component of therapy for many types of brain tumors and the intratumoral application of various therapies is more frequently being undertaken (Walter et al., 1995; Colombo et al., 1997). The development of models in which such experimental strategies can be evaluated is of considerable importance. Although numerous previous studies have reported the injection of various therapeutic molecules and viruses into intracranial rodent tumors, in virtually every case these were tumors established earlier in these very same

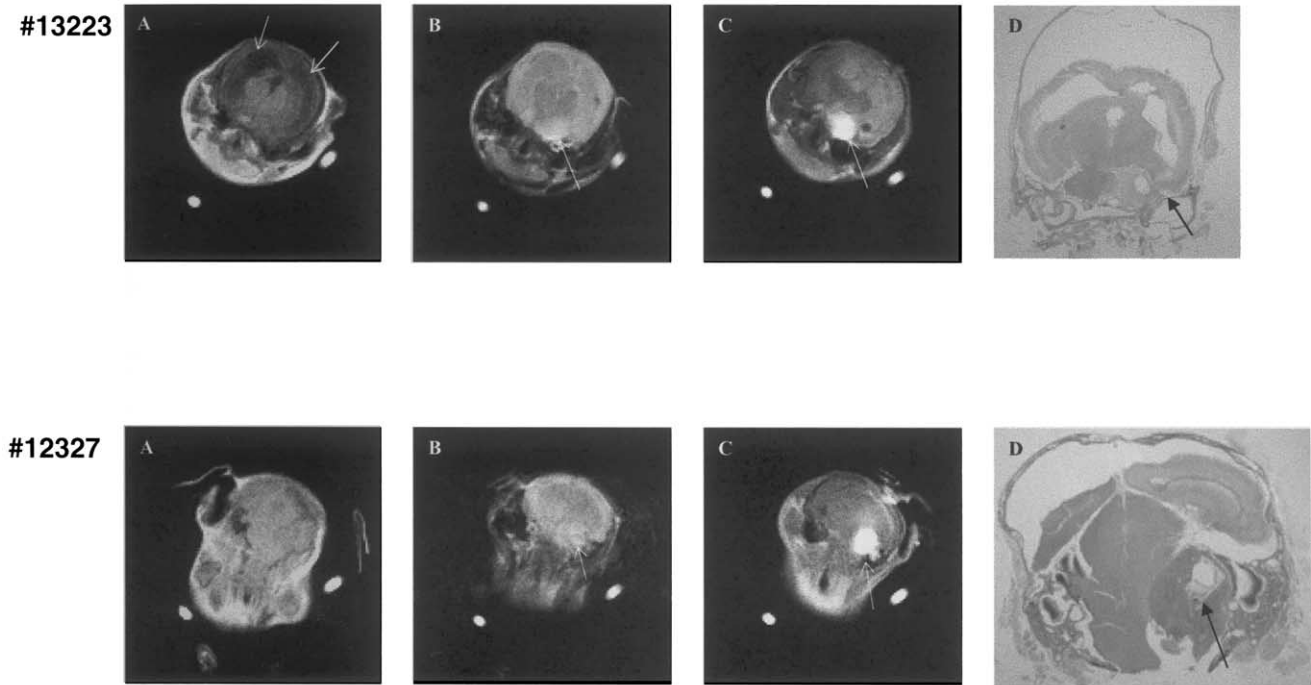


Fig. 4. Stereotaxic injection of spontaneously occurring mouse brain tumors with GdDTPA. Two examples of intracranial brain tumors, 13223 and 12327, detected by MRI and subsequently stereotaxically inoculated intratumorally with GdDTPA. Tumor 13223: hydrocephalus is evident on the preoperative T1-weighted MRI as hypointense, enlarged ventricles (panel A, white arrows) and an abnormally enlarged third ventricle. An inferior brain stem tumor (panel B, white arrow) was suspected based upon regional hyperintensity of the preoperative T2-weighted MRI, right-to-left shift in the inferior midline, and upward displacement of moral brain stem tissue, evident on preoperative images. Successful administration of GdDTPA-containing infusate to the suspected mass was evident on the postoperative T1-weighted MR images obtained within 2 h of infusion (panel C, white arrow). The presence of tumor and the site of inoculation were confirmed by the postmortem histologic examination of hematoxylin and eosin stained histologic sections (panel D), which showed a cyst-like core at the location of injected solution (panel D, black arrow). Tumor 12327: MRI examinations including a preoperative T1-weighted MRI (panel A), a preoperative T2-weighted MRI indicating the site of the tumor (panel B, white arrow), and a postoperative T1-weighted image obtained within 2 h of the inoculation indicating the inoculation site (panel C, white arrow) are shown. The presence of tumor and the site of inoculation were confirmed by the postmortem histologic examination of hematoxylin and eosin stained histologic sections (panel D, black arrow).

animals by the injection of tumor-forming cultured cells. Knowing the precise location of the inoculation of these cells made it possible to return precisely to that location at the time of tumor treatment.

We sought to develop a strategy that would allow us to stereotaxically biopsy and inject spontaneously arising tumors at locations throughout the brain. MRI identified these locations. Because there are no invariant surface landmarks that can be used to align the stereotaxic injection site with the imaged tumor, we developed a head holder that immobilized the animal's front incisors. While it is possible to determine the depth of a tumor and its distance from the midline of the brain from axial MR images, the location of the tumor in the rostral–caudal (nose–tail) axis cannot be accurately ascertained from such images. Two tubes of MRI contrast enhancing material that varied in the distance between them as they extended posteriorly from the tooth bar made it possible to determine exactly how far behind the tooth bar each MR image was. This was accomplished by comparing the rostral–caudal distance from the tooth bar and the distance between the acutely

aligned tubes. We found that rotation of the head in the coronal image did not affect our ability to accurately identify the targets for injection, although it was necessary to exercise caution that the animal's head be flat in both the imaging rig and the stereotaxic apparatus so that the Z-axis, or rostral–caudal distance from the incisors, would not vary in the two set-ups. While a truly fail-safe system would require refinement of the device's design to alert investigators of mechanical misalignment, the use of a MR-visible fiducial on one side of the frame can provide an independent confirmation of left–right orientation.

We tested the validity of the stereotaxic alignment by inoculating target tumors with MRI contrast media. In principle any marker dye could be used, with subsequent histologic verification, but MRI has the advantage that the efficacy of the injection can be assessed non-invasively and subsequent studies in the same mouse may still be performed. In this study, we tested both positive (GdDTPA) and negative (iron oxide) enhancing contrast media and both strategies confirmed our ability to stereotaxically inoculate tumors. In future studies

that might involve the co-inoculation of therapeutic pharmaceuticals with the MR contrast media it will be useful to know the postinoculation transport/diffusion characteristics of these moieties. Having a range of contrast media available, GdDTPA is a small molecule ~ 500 Da and Resovist is a relatively large particle ~ 60 nm in diameter, may allow the matching of transport kinetics. In an ideal setting these magnetic 'labels' would be transported in a similar fashion to the therapeutic moiety. In this study, imaging findings were corroborated with histological evidence of iron in the tumor; however, the precision of this relationship and the time-dependence of contrast medium localization remain to be investigated.

We believe that the general principles upon which this alignment strategy is based should be effective for the development of similar apparatus that would make possible the alignment of intra-thoracic and -abdominal organs, tumors, or other structures arising as the result of disease. Such efforts will be challenged by the less stable alignment of viscera compared to the brain within the cranium; nonetheless, the ability to target imaged structures within the mouse will become increasingly important as mouse models of human disease become more accurate and more widely used for the preclinical evaluation and development of new drugs and treatment strategies. Refinement of this approach might include the incorporation of modifications that would allow one to also identify the animal surface in regions where there were no discernable landmarks and the inclusion of telltales where the orientation of the MR images might be symmetrical and therefore confusing. Ongoing work to use this strategy for the intratumoral inoculation of spontaneously arising brain tumors with recombinant viruses suggests that it is an accurate means to deliver therapy that is efficient in treating brain tumors. The availability of strategies to effectively target spontaneously occurring animal tumors should enhance the usefulness of these models for the development of new strategies for both diagnostic imaging and therapeutic intervention.

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