High resolution MRI of tumors in the Smo/Smo mouse medulloblastoma model

D. Lee[1], S. Hansen[2], R. Ellenbogen[1], M. Zhang[1], and J. Olson[2]
[1]University of Washington, Seattle, WA, United States, [2]Fred Hutchinson Cancer Research Center, Seattle, WA, United States

Introduction

A genetically engineered mouse (GEM) model of medulloblastoma, the most common malignant brain tumor in children, have been generated through manipulation of sonic hedgehog signaling (1,2). GEM models of human cancer allow toxicity and *in vivo* effectiveness, the most important issues in cancer drug development, to be addressed pre-clinically. However, to fully exploit the potential of GEMs, sensitive and non-invasive methods for tumor diagnosis and measuring treatment response are required. Noninvasive monitoring is important because longitudinal observations allow each animal to serve as its own control. Early diagnosis is important because signs of brain cancer in mice may not be obvious until the tumor has reached an advanced stage.

Here we use high resolution MRI to image early stage medulloblastoma of Smo/Smo (Smo) mice and radiologically characterize tumor growth and changes in the blood brain barrier (BBB). Using relaxation- and diffusion-weighted imaging we were able to detect tumors weeks before the animals showed overt clinical symptoms. The Smo/Smo mice tolerated multiple imaging demonstrating that serial MRI may be used to follow tumor development and treatment response in this model.

Methods

Animals. Mouse husbandry and procedures were performed in accord with the NIH Guide for the Care and Use of Experimental Animals and with approvals from the Fred Hutchinson Cancer Research Center's Institutional Animal Care and Use Committee.

Magnetic resonance imaging. Magnetic resonance imaging was conducted using a 4.7 Tesla magnetic resonance scanner with a Bruker magnet (Bruker Medical Systems, Karlsruhe, Germany), equipped with an INOVA 200 spectrometer (Varian, Inc., Palo Alto, CA). A stereotactic device was fabricated to hold the head still and minimize motion during imaging. T1, T2, and diffusion weighted imaging were conducted with the following parameters of spin echo sequence: TR (recycle delay)/TE (echo time) = 500/12 ms, number of averaging = 1, matrix = 256 x 128 for T1 weighted (T1w) imaging; TR/TE = 2s/60ms, number of averaging = 1 and matrix = 256 x 128 for T2 weighted (T2w) imaging; TR/TE = 2s/50ms, matrix = 256 x 128 and b values of 0, 206, 404 and 998 s/mm^2 for diffusion imaging.

Gd-DTPA experiment. To examine the blood brain barrier in wild type and Smo/Smo mice baseline pre-contrast imaging signal intensities were averaged from three T1weighted images acquired immediately prior to intravenous Gd-DTPA injection (0.1 mM/kg gadopentetate dimeglumine; 5 x diluted Magnevist; Berlex Laboratories, Wayne, NJ).

Histopathology. Animals were euthanized using CO_2 inhalation and their brains removed. Whole brains were fixed in 10% buffered formalin, paraffin embedded, cut into 4 micron sections, and stained with hematoxylin and eosin (H&E) using standard methods.

Results and Discussion

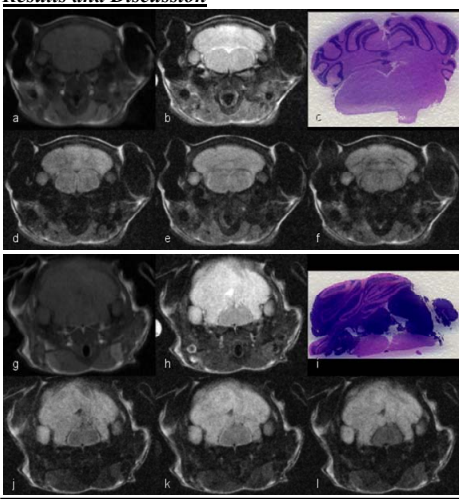

Figure 1. Directional effect of diffusion gradient fields on *in vivo* MR images acquired for wild type (a-f) and Smo tumor (g-l) mice. For the wild type mouse, T1 weighted (a) T2 weighted (b) axial images for a wild type mouse. The similar slice was H&E stained as shown in (c). Diffusion weighted images (TR/TE=2s/60ms, b=1123 s/mm^2) acquired with diffusion gradients applied along the vertical (readout), horizontal (phase encoding) and through-plane directions (slice selection gradient) in (d), (e) and (f), respectively. The same parameters were used for the Smo tumor mouse (g-l).

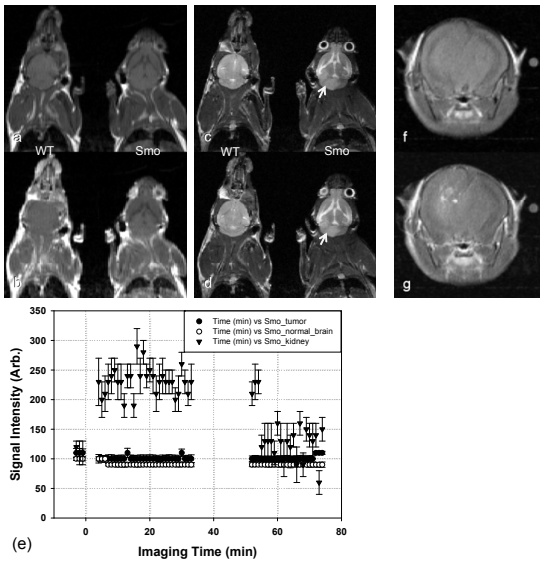

Figure 2. Blood brain barrier (BBB) examined by Gd-DTPA for a wild type (WT), early stage Smo (a-e) and late stage Smo Smo (f,g) mice. Pre (a, c, f) and post (b, d, g) contrast images compared among the wild type, early stage Smo mice and late stage Smo mice. T1 weighted MR images in (a,b,f,g) and T2 weighted images in (c, d). (e) Variations of signal intensities were measured from serially acquired T1 weighted MR images acquired for the early stage Smo mouse. The signal measurements were conducted for three different regions: tumor area in cerebellum, normal brain and kidney.

Using T2 weighted imaging, tumors could be detected as hyperintense areas in the cerebellum. The presence of tumor was confirmed by histological examination following MRI. The limit of detection by MRI was in the sub-millimeter range and an early stage tumor with calculated volume of approximately 0.3 microliters. Mice with later stage tumors show enlarged ventricles, plus the entire brain shows some expansion. Visible cancer signs, such as domed skull would be expected to follow. Tumor volumes and apparent diffusion coefficient (ADC) values were also measured in individual animals once per month for four months. As expected, tumor volume in individual mice increased with age. ADC values measured at 2 months of age showed potential sign of early cancer death for one of the mice, which cannot be explained only by tumor volume changes.

The diffusion gradients were separately applied along three perpendicular planes consisting of the readout, phase encoding, and slice selection gradient field axes (Fig. 1). Two b values of 0 and 1123 s/mm^2 were used to generate diffusion maps. Better tumor contrast was obtained when a diffusion gradient was oriented along the slice selection direction.

The normal function of the blood brain barrier (BBB) is to protect the central nervous system, unfortunately it can also hinder the delivery of therapeutic agents to brain cancers. We therefore investigated BBB status in different stages of Smo tumors. Figure 2 shows T1 and T2 weighted images acquired from wild type and Smo mice before and after Gd-DTPA injections. In young mice with small tumors, immediately after Gd-DTPA injection no signal increase was observed on any region of brain while enhancement was easily detected in the kidney (Fig. 2e). In later stage Smo tumors, however, Gd-DTPA leakage in the tumor is clearly seen as spots of hyper-intense MR signal (Fig. 2g). Thus, similar to observations in human medulloblastoma the integrity of the BBB in Smo/Smo tumors can become disrupted as disease progresses.

References

1. Hallahan, A.R., et al. Cancer Res. **64**, 7794-800 (2004)
2. Hatton, B.A., et al. Cancer Res. **68**, 1768-76 (2008).

Acknowledgements

This work was supported by NIH R01CA112350, R01CA119408, R01CA134213 and R21EB008166.