Glioma Detection Maximized through Intracellular and Extracellular MR-guided Optical Fluorescence Tomography

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Background

Certain glioma tumor subtypes are not as readily apparent in contrast MR imaging, whereas delineation of other molecular features of the tumor is possible with optical reporters. In this study, two model tumor lines were examined, which are known to have significantly different detection characteristics in MR. The 9L glioma tumor and the U-251 tumor where compared for detectability with contrast MR and with two different optical reporters. The first optical reporter was protoporphyrin IX, which is produced in the mitochondria as part of the heme synthesis pathway, and is therefore an intracellular mitochondrial reporter. The second optical probe was epidermal growth factor (EGF) bound with IRDye800CW, which is taken up at the EGF receptor and is a direct measure of the cellular EGFR activity. These two reporters represent reasonable intracellular and extracellular ways to sample the tumor metabolism, and are known indicators of glioma cellular malignancy. It is shown that while some one tumor line is not readily apparent on MR that it can have considerable detectability based upon their metabolic status, as assayed by the optical reporters.

Methods

A multispectrometer system was created to allow spectroscopy at each of 16 fibers. A circular light source sequencer was used to launch laser light into the fibers one at a time. Laser light can come from a bank of 7 diode lasers or from a high power MaiTai Ti:Sapphire laser. These allow spectroscopy in the wavelength range of 680-950 nm. Luminescence spectroscopy is possible, by exciting at one wavlength, and then filtering the input of each detection spectrometer with interference filters. Custom input fiber holders were created for this filtering, combining beam collimation with a motorized filter selector, thereby allowing fluorescence detection at a number of cut off wavelengths.

The system has been tested with several fluorophores, including indocyanine green, protoporphyrin IX, lutecium texaphyrin and IRDye800CW. These are common targets for in vivo imaging of mice, allowing functional assessment of label uptake or production.

Nude mice were inoculated with either 9L tumor cells or U-251 tumor cells, injected into the cortex through a bore hole in the skull. The mice were allowed to recover and were MR imaged after 10 days to assess tumor growth. Contrast Gd-DTPA was injected prior to imaging in both cases, and T1-weighted imaging carried out to visualize the tumor extent. After MR imaging, the optical spectroscopy of these tumors was carried out with the system described above. After imaging, subsets of the mice were sacrificed to extract the brain, and slice it open for ex vivo fluorescence imaging. The protoporphyrin IX and IRDye800CW fluorescence could then be quantified by image analysis.

Results

In the case of the 9L tumors, the tumor growth is well known to have clear borders and easily enhancing regions that appear in the T1 images, and this was the case for most mice here. In most cases, the tumor grew as a solid deliniated block, which was obvious in ex vivo analysis with H&E stain. The region of enhancement on MR was apparent in all cases, and did not require difference imaging to observe the borders. For the U-251 tumors, the growth pattern is less well delinitated and involved invasion into the normal tissue in irregular patterns. The tumors enhanced substantially less in the T1 images, and in several cases tumor detection was not apparent. Contrast difference imaging provided superior delineation of the tumors, but still the U-251s showed significantly less contrast to noise in detection.

In the case of optical imaging, the U-251 tumors had substantially higher intrinsic contrast in the uptake of fluorescent EGF and in the production of PPIX. Ex vivo analysis showed contrast in the range of 3:1 up to 5:1 in PPIX, and significantly higher than that for EGF fluorescence.

Discussion

Tumor imaging based upon T1 contrast MR has known limitations, yet methods to image with diffusion or proton spectroscopy have been slow to gain widespread acceptance. A complete analysis of tools to detect, delineate and characterize glioma tumors should maximize the contrast to noise available, and ideally allow detection of a range of metabolic markers. The system used here to measure fluorescence in vivo was developed as an add-n to the standard small animal body coils. The demonstration that the 9L tumor is readily detectable in MR, whereas the U251 was significantly less so, provides a good test model. The U-251 had substantially higher ability to be detected with either PPIX fluorescence or tagged EGF uptake, showing that the cellular activity indicators may be a good way to enhance detection in some tumor subtypes. Addition of optical spectroscopy into standard MR systems is feasible, and human brain imaging is possible across regions of tissue less than 10 cm in chord length. Further analysis of multiple glioma subtypes with comparison of proton spectroscopy and optical spectroscopy should provide a better interpretation of the optimal way to detect and visualize the range of tumors that exist.

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