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Brain tumors disrupt the resting-state connectome☆

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ABSTRACT

Brain tumor patients often experience functional deficits that extend beyond the tumor site. While resting-state functional MRI (rsfMRI) has been used to map such functional connectivity changes in brain tumor patients, the interplay between abnormal tumor vasculature and the rsfMRI signal is still not well understood. Therefore, there is an exigent need for new tools to elucidate how the blood-oxygenation-level-dependent (BOLD) rsfMRI signal is modulated in brain cancer. In this initial study, we explore the utility of a preclinical model for quantifying brain tumor-induced changes on the rsfMRI signal and resting-state brain connectivity. We demonstrate that brain tumors induce brain-wide alterations of resting-state networks that extend to the contralateral hemisphere, accompanied by global attenuation of the rsfMRI signal. Preliminary histology suggests that some of these alterations in brain connectivity may be attributable to tumor-related remodeling of the neurovasculature. Moreover, this work recapitulates clinical rsfMRI findings from brain tumor patients in terms of the effects of tumor size on the neurovascular microenvironment. Collectively, these results lay the foundation of a preclinical platform for exploring the usefulness of rsfMRI as a potential new biomarker in patients with brain cancer.

1. Introduction

The devastating consequences of a brain tumor on a patient's quality of life have sparked widespread research into approaches capable of early detection of tumor-induced alterations in brain function. Noninvasive techniques such as magnetoencephalography (MEG) (Bartolomei et al., 2006), and task-based fMRI have been at the forefront of such efforts (Holodny et al., 1999). However, spatial localization of the MEG signal is challenging (Hillman, 2014) because one needs to employ computational approaches to localize neural activity within the brain from the induced magnetic fields measured externally (Hämäläinen et al., 1993). In contrast, task-based fMRI has proven immensely useful for pre-surgical mapping of eloquent cortex prior to surgical resection. However, this method can be challenging to conduct on brain tumor patients due to issues such as task noncompliance, the constraint of long imaging times, and false-negatives (Zaca et al., 2014) arising from neurovascular uncoupling (Ulmer et al., 2003). Although one could circumvent the first two issues by using task-independent resting-state fMRI (rsfMRI) approaches, little is known about how the blood-oxygen-level-dependent (BOLD) rsfMRI signal is modulated by the presence of a brain tumor (Pak et al., 2017). Therefore, the objective of this study was to systematically quantify brain tumor-induced changes on the BOLD rsfMRI signal and on resting-state brain connectivity in a preclinical model.

Resting-state functional MRI is based on the premise that distinct brain regions exhibit temporally correlated spontaneous fluctuations in blood flow within a frequency range of 0.01–0.1 Hz, to meet the energy demands necessary for healthy brain function (Biswal et al., 1995). As a result, rsfMRI has been successfully used to map changes in 'connectivity' of spatially distinct brain regions in an array of disease models using a stimulus-independent paradigm. These include mapping alterations in neuronal connectivity in patients with stroke (Golestani et al., 2013), schizophrenia (Lynall et al., 2010), bipolar disorder (Mamah et al., 2013), multiple sclerosis (Filippi et al., 2013), and Alzheimer's disease (Agosta et al., 2012). Recently, work by Hillman and colleagues elegantly demonstrated that resting-state

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hemodynamics are spatiotemporally coupled to synchronized neural activity in excitatory neurons (Ma et al., 2016) thereby validating some of the biophysical mechanisms underpinning the rsfMRI signal. However, rsfMRI studies in patients with brain tumors have been limited (Agarwal et al., 2016; Chow et al., 2016). This is attributable to several factors. The first is that the abnormal vascular architecture (Kim et al., 2011) and anomalous blood flow characteristics (Jain et al., 2007) modulate the BOLD signal within the tumor region (Chow et al., 2016). Next, there is the phenomenon of neurovascular uncoupling (NVU) in which brain tumor cells perturb the integrity of the blood-brain-barrier (BBB), disrupt the homeostatic coupling between neurons and astrocytes, and the mechanism underlying cerebral blood flow regulation (Watkins et al., 2014). This NVU can confound the interpretation of resting-state connectivity in brain tumor patients (Agarwal et al., 2016). Therefore, there is an exigent need for new tools to elucidate how the blood-oxygenation-level-dependent (BOLD) rsfMRI signal is modulated in brain cancer.

Early BOLD signal fluctuation-based MRI studies of cancer focused on elucidating the functional status or 'maturity' of the tumor vasculature in preclinical tumor models (Baudelet et al., 2006). More recent studies have employed spontaneous BOLD fluctuations to generate maps of 'active' tumor regions using independent component analysis, and showed that heterogeneous tumor vessel functionality can result in uniquely correlated tumor regions (Goncalves et al., 2015). However, none of these studies employed an orthotopic (i.e. occurring at the normal place in the body) brain tumor model to investigate the relationship between the brain tumor microenvironment (TME) and the BOLD rsfMRI signal. While rsfMRI studies in rodents have only been reported recently (Pan et al., 2015), they have demonstrated that certain resting-state networks can be observed across mammalian species (Pawela et al., 2008). For example, rsfMRI studies have shown that the functional connectivity in the limbic, motor, visual, and somatosensory networks can be successfully mapped in rodents (Bergonzi et al., 2015; Jonckers et al., 2011; White et al., 2011). Collectively, these developments afford us the opportunity to elucidate the effect of brain tumors on resting-state connectivity in rodent models and then potentially translate these findings into patients.

In this study, we characterized the collective effects of the abnormal brain tumor vasculature and tumor-induced neurovascular uncoupling on rsfMRI dynamics. Specifically, we quantified and compared resting-state BOLD signal dynamics in healthy murine brains relative to tumor-bearing brains. We then examined tumor-induced alterations in resting-state connectivity between multiple brain regions across both cerebral hemispheres. Furthermore, we demonstrated global tumor-induced modulations in resting-state BOLD signal fluctuations. The effect of brain tumor volume on resting-state connectivity between brain regions, as well as between the tumor and cortex were also investigated. Preliminary histology suggests that some of the observed alterations in brain connectivity may be attributable to tumor related remodeling of the neurovasculature, amongst other mechanisms.

2. Methods

2.1. Animal preparation

9L-GFP brain tumor cells were orthotopically inoculated into the cortices of SCID mice (n = 8) as described in (Pathak et al., 2001). Brains of healthy SCID mice (n = 8) served as the control group. Briefly, severe combined immune deficient (SCID) mice weighing approximately 30 g (Charles River/NCI, Frederick, MD), were anesthetized with a xylazine/ketamine cocktail for tumor cell inoculation. Their heads were immobilized in a stereotactic frame and a 1 mm burr hole drilled in the skull 1 mm anterior and 2 mm lateral to the bregma on the right side using an aseptic technique. A 10 µl gas-tight syringe (Hamilton Comp, Reno, Nevada) was used to inject 10^5 cells of the 9L gliosarcoma cell line, into the right frontal lobe at a depth of 3 mm relative to the dural surface. Starting with 10^5 cells, the tumor cell inoculum was diluted to yield tumors of varying volumes. The 9L cell line was obtained from the Brain Tumor Biology Laboratory, and grown in DMEM (Gibco, Gaithesburg, MD) with 10% FBS. Cells were expanded prior to inoculation. The injection time was 5 min, after which the needle was retracted slowly for an additional 5 min. The skin was then closed with surgical staples that were removed prior to MRI. All animal studies were performed according to institutional guidelines and the NIH "Guide for the Use and Care of Laboratory Animals".

2.2. In vivo MRI protocol

In vivo MRI was conducted at two weeks post-inoculation on a 400 MHz vertical bore Bruker spectrometer under isoflurane anesthesia (1–1.5% mixed with air and oxygen at a 3:1 ratio) using the following sequences after localized shimming: (i) T2w rapid acquisition with relaxation enhancement (RARE), RARE-factor = 8, TE = 15.0 ms, TR = 3.5 s, NA = 8, in-plane resolution = 0.1 mm × 0.1 mm, 16–24 coronal slices, slice thickness = 0.3 mm; (ii) 16-segment gradient-echo EPI, TE = 8.4 ms, TR = 400 ms/segment (resulting in an effective TR = 6.4 s), 110 repetitions (i.e. ~12 min imaging session), in-plane resolution = 0.2 mm × 0.2 mm, 16–24 coronal slices, slice thickness = 0.3 mm. Body temperature of the animals was maintained at 37 °C and respiration rate at 40–60 bpm.

2.3. Histology and immunofluorescence protocol

After in vivo MRI, animals were perfused with the intravascular tracer dextran-TRITC (70 kDa, Sigma-Aldrich, St. Louis, MO) via the tail vein, euthanized, brains excised, fixed in 10% buffered formalin and frozen in liquid nitrogen for cryosectioning. Adjacent 12 µm frozen brain sections were cut onto silanized slides and immunofluorescent labeling of the neurovascular unit components carried out. Astrocytes were labeled with anti-glial fibrillary acidic protein (GFAP) antibody (Cell Signaling, Danvers, MA), vasculature associated smooth muscle with α-smooth muscle actin antibody (Sigma-Aldrich, St. Louis, MO), and blood vessel endothelium detected on the same tissue with anti-mouse laminin antibody (Sigma-Aldrich, St. Louis, MO). Slides were counterstained with DAPI (Molecular Probes Inc., Eugene, OR) and cover-slipped. Slides were imaged on a Nikon ECLIPSE-TS100 microscope (Nikon Instruments Inc., NY) with the appropriate filters for detecting immunofluorescence. Regions-of-interest (ROI) were digitized at 20× and 40× using a SPOT INSGHT™ CCD camera (Diagnostic Instruments Inc., MI).

2.4. Image processing

2.4.1. Region of interest segmentation and preprocessing

A 3D MRI-based mouse atlas (Aggarwal et al., 2009) was used as a reference for segmenting anatomical regions-of-interest (ROI) using the segmentation editor in Amira® (FEI Software, OR). ROI included: left/right (L/R) hippocampus (Hi), L/R neocortex (Neo), L/R olfactory bulb (OB), L/R thalamus (Th), L/R striatum (Str), L/R hypothalamus (Hy), brainstem (Stem), and tumor (TUM) where applicable.

The Analysis of Functional Neuroimages software (AFNI; http://afni.nimh.nih.gov/afni/) was utilized for all image processing (Cox, 1996). Resting-state fMRI data was co-registered to the anatomical MRI data using a two-step method in which an initial alignment using linear interpolation to smoothed variants of the functional and anatomical datasets was followed by a final alignment step utilizing Fourier interpolation. The rsfMRI BOLD time course was then filtered using a high-pass filter with a 0.01 Hz cut-off, and spatially smoothed using a Gaussian filter with a full width half maximum (FWHM) of 0.5 mm. Data from the normal and tumor-bearing brains underwent identical image processing steps.