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Research papers

Quantitative *in vivo* imaging of tissue factor expression in glioma using dynamic contrast-enhanced MRI derived parameters

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ABSTRACT

Objective: Tissue Factor (TF) has been well established in angiogenesis, invasion, metastasis, and prognosis in glioma. A noninvasive assessment of TF expression status in glioma is therefore of obvious clinical relevance. Dynamic contrast-enhanced (DCE) MRI parameters have been used to evaluate microvascular characteristics and predict molecular expression status in tumors. Our aim is to investigate whether quantitative DCE-MRI parameters could assess TF expression in glioma.

Materials and methods: Thirty-two patients with histopathologically diagnosed supratentorial glioma who underwent DCE-MRI were retrospectively recruited. Extended Tofts linear model was used for DCE-MRI post-processing. Hot-spot, whole tumor cross-sectional approaches, and histogram were used for analysis of model based parameters. Four serial paraffin sections of each case were stained with TF, CD105, CD34 and α-Sooth Muscle Actin, respectively for evaluating the association of TF and microvascular properties. Pearson correlation was performed between percentage of TF expression area and DCE-MRI parameters, multiple microvascular indexes.

Results: Volume transfer constant (K^{trans}) hot-spot value best correlated with TF ($r = 0.886$, $p < 0.001$), followed by 90th percentile K^{trans} value ($r = 0.801$, $p < 0.001$). Moreover, histogram analysis of K^{trans} value demonstrated that weak TF expression was associated with less heterogeneous and positively skewed distribution. Finally, pathology analysis revealed TF was associated with glioma grade and significantly correlated with these two dynamic angiogenic indexes which could be used to explain the strong correlation between K^{trans} and TF expression.

Conclusion: Our results indicate that K^{trans} may serve as a potential clinical imaging biomarker to predict TF expression status preoperatively in gliomas.

1. Introduction

Tissue factor (TF), a 47 kDa cell membrane intercalated protein, is the key inducer of the extrinsic coagulation cascade and functions as the receptor for coagulation factor VII/VIIa [1]. However, apart from its well-known role in hemostasis, TF plays a central role in cancer progression, angiogenesis, invasion, and hematogenous metastatic dissemination [2]. Accumulating evidence showed that TF is aberrantly overexpressed in several solid tumors including glioma, breast cancer, colorectal cancer, and pancreatic cancer, *etc*. Within glioma it has been

Abbreviations: TF, tissue factor; DCE, dynamic contrast-enhanced; K^{trans}, volume transfer constant; MRI, magnetic resonance imaging; PET, positron emission tomography; ROI, region of interest; AIF, arterial input function; *K*ep, ratio constant of tracer refluxing from tissue to plasma; Ve, extravascular extracellular space per unit volume of tissue; Vp, blood plasma volume; SD, standard deviation; PCI, proliferating capillary index; MPI, microvessel pericyte coverage index; HGG, high-grade gliomas; LGG, low-grade gliomas; mRF, maximum relative frequency; rCBV, relative cerebral blood volume

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shown that TF, measured by immunohistochemistry, correlates with histological malignancy grade and is a prognostic marker of overall survival [3]. TF expression contributes to oncogene-driven gliomagenesis [4], provokes escape from glioma dormancy [5], enhances glioma neovascularization [6], proliferation and metastasis [7]. The key role of TF in tumor progression makes it a favorable target for glioma imaging and therapy.

Clinically, the method for assessment of TF status in glioma relies on histologic analysis using surgical specimens. This process requires an invasive procedure and inherently prone to sampling error due to a spatially heterogeneous glioma [8]. In addition, it is difficult to monitor therapeutic response timely during treatment and as companion diagnostics for emerging TF-targeting therapy. Hence, quantitively *in vivo* imaging of TF expression in glioma is a high priority. Emerging studies tried to develop a noninvasive method to image the TF expression in tumors. Optical imaging with use of EGFP or dye-, magnetic resonance imaging (MRI) with iron oxide nanoparticle-, positron emission tomography (PET) imaging with radionuclide-conjugated with peptide/ligand/antibody specifically targeting TF have been used to image the TF expression in tumors [9–11]. However, these existing *in vivo* methods for assessing tumor TF status are all applied in preclinical research. Therefore, effective clinical imaging approaches need to be settled to non-invasively predict TF expression in glioma.

Advanced MRI techniques have been utilized to provide estimation of various biological features in tumors, including molecular characteristics [12,13]. Among these techniques, dynamic contrast-enhanced (DCE) MRI is capable of quantitatively measuring tissue blood flow, vascularity, and parenchymal contrast uptake via kinetic modeling methods and has been used in the assessment of gliomas [14]. It provides us both absolute measurement of quantitative metrics and spatial variation of vascular leakiness, which has been recognized as a sensitive tool to evaluate structure and function of neovascularization [15]. In various clinical studies, DCE-MRI parameters have been reported to be serve as a potential imaging biomarker to predict molecular expression status in tumors, like VEGF, EGFR, MGMT methylation, HIF-1α, *etc*. [12,14,16,17]. However, no clinical study has evaluated the association between DCE-MRI parameters and TF expression status in glioma. Therefore, the aim of this study was to identify *in vivo* imaging biomarkers for TF expression in glioma using quantitively parameters from DCE-MRI.

2. Materials and methods

2.1. Patients

In this retrospective study, from January 2013 to September 2014, fifty-three consecutive patients who were pathologically diagnosed suprationtorial glioma were enrolled. Twenty-one patients were excluded, for either poor imaging quality or insufficient volume of tumor tissue block acquired from pathological specimen bank of our hospital. All patients underwent conventional intracranial MRI and DCE-MRI. Surgical resection was performed without any other form of treatment before pathological diagnosis. Use of archival surgical pathology specimens for immunohistochemical studies was approved by the Institutional Review Board at our institution (IRB #201216), and all patients underwent MRI scanning completed a written consent form.

2.2. MRI protocol

All participants underwent MR scanning on 3T (Verio, Siemens, Erlangen, Germany), with a 16-element head matrix coil. Conventional imaging included turbo spin-echo T2-weighted images (TR/TE 4900/92 ms, number of excitations, NEX 1), T2-weighted FLAIR (TR/TE 8000/94 ms, NEX 1), T1-weighted FLAIR (TR/TE 250/2.7 ms, NEX 1), and post-contrast T1-FLAIR with identical parameters as that of pre-contrast T1-FLAIR imaging. All images were acquired with a field of view (FOV) of 230 × 230 mm^2 and 26 axial slices of 5 mm with intersection gap of 1 mm.

DCE-MRI included two pre-contrast T1-weighted scanning (3D volumetric interpolated breath-hold examinations, TR/TE 5.1/1.8 ms, FOV 260 × 260 mm^2, matrix 192 × 138, 26 axial slices of 5 mm with intersection gap of 1 mm) with two flip angles (2°,15°) for obtaining voxel-wise tissue longitudinal relaxation time (T10). These sequences were aimed at converting MR intensity to the contrast agent concentration of DCE-MRI data. Then the DCE-T1 sequence was examined every 5.32 s as a phase for 80 repetitions in total, employing the same parameters above and a 15° flip angle. The contrast agent Gd-DTPA (Omniscan, GE Healthcare, Ireland) was injected at the fifth phase of the DCE-T1 sequence with a dose of 0.1 mmol/kg of body weight and rate of 4 ml/s.

2.3. Imaging post-processing

DCE-MRI data were processed by DCE post-processing software (Omni Kinetics software, GE HealthCare, China). Extended Tofts linear model (two compartments model) was used, with the region of interest (ROI) placed in the middle cerebral artery to seek for the fittest time-signal curve as the patient-specific arterial input function (AIF) according to previous research [18]. Then four pharmacokinetic parameters: volume transfer constant from plasma to tissue (K^{trans}), ratio constant of tracer refluxing from tissue to plasma (*K*ep), extravascular extracellular space per unit volume of tissue (Ve) and blood plasma volume (Vp) were automatically calculated.

2.4. Imaging analysis

Imaging analysis was performed by two experienced, board-certified neuro-radiologists with more than 7-year working seniority. Average value of measurements by the two neuro-radiologists was employed as the final value. Two kinds of ROIs were contoured, ROI 1: areas with high K^{trans} value visually on color map, *i.e.* hot-spot areas; ROI 2: whole tumor area on axial image. For ROI 1, we selected at least five of them on multiple slices, each containing 9–11 pixels. The other 3 parameters (*K*ep, Ve, Vp) were generated simultaneously. For ROI 2, tumor boundary was initially identified as the contrast enhanced components of lesion, while necrosis, cyst and giant vessels could be easily excluded on T1-weighted post contrast enhanced images. Next step, to distinguish non-enhancing low-grade gliomas and non-enhancing components in peripheries of high-grade ones from edema, we managed to identify subtle differences on T2 weighted images. For instance, the T2 weighted signal of edema approaches cerebrospinal fluid and much higher than grey matter. Conversely, non-enhancing tumor is subtly brighter than grey matter. Additionally, edema was usually confined in white matter, resulting in increased conspicuity of the gray matter-white matter junction. While, non-enhancing tumor blurs the gray matter-white matter junction as this interface becomes infiltrated by the tumor. From ROI 2, mean value, standard deviation (SD) and 90th percentile were calculated by the software. Histograms were generated by classifying K^{trans} values of ROI 2 into calculated number of bins by Sturges' equation (bins number = 1 + lgN/lg2, N is pixel number of ROI 2). The data from histogram were assessed by the maximum relative frequency (*i.e.* the ratio between maximum frequency number and total pixel numbers) of K^{trans}, in selected number of bins. Skewness of histogram, which represents asymmetry of probability distribution, was also calculated.

2.5. Immunohistochemistry

For each case, all paraffin blocks obtained from surgery were used for immunohistochemistry. Four serial sections (4 μm) were obtained from each block, followed by standard immunohistochemistry, using different primary anti-bodies: anti-TF (Santa Cruz Biotechnology, Santa