LETTER TO THE EDITOR

Magnetic Resonance T2*-Weighted Study of U87 MG Glioma Tumors and its Relationship between Tumor Hypoxia and VEGF Expression

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Received 27 November 2012; revision 06

December 2012; accepted 06 December 2012.

doi: 10.1111/cns.12055

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There are some regions with hypoxic tissue ($pO_2 < 10$ mmHg), which is a characteristic feature of many tumors [1]. Hypoxic tumor tissues may cause the resistance to radiotherapy and chemotherapy. It has been shown to be associated with malignant progression, an increased incidence of metastases and poor outcome [2]. Currently, the electrode pO_2 histograph system is regarded as the "gold standard" to measure tumor oxygenation. However, it is invasive with limited accessible tumors. A noninvasive method is attractive for measuring tumor oxygenation. Blood oxygen level-dependent (BOLD) MRI uses the paramagnetic nature of deoxyhemoglobin to noninvasively monitor changes [3]. Blood oxygenation measure by using the determination of the effective transverse relaxation rates $R2^* = 1/T2^*$ and thus of absolute MR values is made possible by BOLD sequences. Brain glioma is the most common malignant tumor in central nerve, accounting for about 60–70% of tumors in central nerve system, which is attracting more and more attention because of its high-grade malignancy, easy relapse and high death rate. Therefore, this article aims to compare MRI $R2^*/T2^*$ sequence with oxygenation status obtained by the polarographic needle electrodes within the U87 MG glioma xenograft tumor to improve prognosis. It will not only check the relationship between measures of hypoxia defined by functional imaging and pO_2 electrodes, but also measure the level of tumor angiogenesis.

Tumor models used U87 MG glioma xenografts in adult female BALB/c nude mice. All images were acquired by using a 1.5T Signa HD MR whole body scanner system (General Electric Medical Systems, Fairfield, CT, USA) with a dual coil to receive the MR

signals. BOLD imaging is acquired by using $T2^*/R2^*$ sequence for axial scanning. Based on scout images, one axial slice in the middle of the tumor was chosen for BOLD MRI data acquisition [4]. $R2^*$ maps were produced from the $T2^*$ -weighted images. Three indistinct regions of interest (ROI) were selected based on the

Figure 1 The relationships between $R2^*$ (y-axis) and pO_2 (x-axis) for the two groups (the median $pO_2 \leq 10$, >10 mmHg) are shown. The gradients, y-intercepts, and correlation coefficients are shown for the two regression lines: (●) away from the main curve may be due to necrosis; (○) measured values are selected around the curve uniform.

Figure 2 (A) Blood oxygenation level-dependent (BOLD) MRI data collection and analysis. The left map shows the native BOLD MRI image, and the right is the processed image. The positions of the three regions of interest (ROIs) shown in the graph are shown on the $R2^*$ map. Each region of interest has the same size and shape with not <5 pixels. (B) Immunohistochemical staining of the hypoxia-related markers: VEGF expression (cytoplasmic staining) of human tumor xenografts in nude mice corresponding to the three ROI. (ROI 1; ROI 2; ROI 3), $\times 400$.

high, medium, and low $R2^*$ values within the tumors which had the same size and shape. The mean $R2^*$ values were recorded, whose unit was Hz.

Tumor oxygenation was measured by using a sterile polarographic needle electrode (Oxford Optronix Ltd., Oxford, UK). The details of the tumor oxygenation measurement methods were described elsewhere [5]. Tumor size and accessibility determined the length and number of tracks. To minimize intratumor variability, at least three electrode tracks were obtained per tumor. Tumor oxygenation values and the median measurement were recorded. For histological analysis, rats were assessed under intratumor vessel density. Sections of tumor biopsies were dewaxed, rehydrated, and microwaved, and then antibodies were added to them [6]. We selected the results and judgment by using a semimeasurement method [7]. All data were analyzed using Spearman's rank correlation and curve fitting followed by using SPSS 13.0 for Windows (SPSS, Chicago, IL, USA).

The relations between pO_2 measurement and $R2^*$ values are shown in Figure 1. The curve is divided into two sections for curve estimation (the median $pO_2 \leq 10$ mmHg and >10 mmHg). Correlation coefficients of 0.98 and 0.99 were obtained for the two groups, respectively. The downtrend of median $pO_2 \leq 10$ mmHg segment changes more slowly along with the change of partial pressure of oxygen than the latter part. These differences in slope were statistically significant, and they were consistent with the oxygen dissociation curve generally. There are two points away from the main curve, which may be contributed by necrosis. In this study, tumor histopathology and immunohistochemistry (Figure 2B) were taken from all tumors in the corresponding position of the ROI (Figure 2A). A positive association was found between $R2^*$ values and vascular endothelial growth factor (VEGF) ($r = 0.42$; $P < 0.05$).

The change in the deoxyhemoglobin content of blood is the main source of signal intensity change in $T2^*$ -weighted images in

fMRI. The mechanism is the change of proportion between deoxyhemoglobin and oxyhemoglobin [8]. The deoxygenated hemoglobin carries Fe^{2+} (with four unpaired electrons which have strong paramagnetic effects), can lead to local magnetic field inhomogeneity [9]. Necrotic edema tissue and cell proliferation may also contribute to the trend. And a lot of macrophages will possibly devour the present necrotic tissue. Steap3 gene in macrophage cells has been found to have participated in the iron to release ferrous iron [10].

As we all know, the major driver for VEGF production is hypoxia by stimulating the expression of hypoxia-inducible factor 1 (HIF1)- α transcription factor. And in the above result of data, the relations between pO_2 measurement and $R2^*$ values are clear. So, we could get the positive association between $R2^*$ values and VEGF. But it should be mentioned that the expression of VEGF is regulated by the cell's genetic characteristics and many factors in the tumor microenvironment.

Our results indicate that BOLD MRI provides a noninvasive distribution map of hypoxia with high sensitivity. An alternative method is to utilize regional maps of hypoxia distribution to direct dose painting algorithms for dose escalation in intensity-modulated radiotherapy planning. The incidence of brain glioma is high with poor clinic treatment effect. So, we could use intensity-modulated radiotherapy to target relevant areas for dose modification to improve the prognosis of glioma patients.

Acknowledgments

This work was supported by China Postdoctoral Science Foundation (20080431411).

Conflict of Interest

The authors have no conflicts of interest.

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