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Evaluation of chemotherapeutic response of temozolomide in orthotopic glioma using bioluminescence tomography

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ABSTRACT

Glioma is one of the most important leading causes of cancer-related deaths worldwide. Temozolomide (TMZ) is a DNA methylating agent that presents promising antitumor activity against high grade glioma. However, there is no effective way to assess the therapeutic response to TMZ at early stage. In this study, we evaluated the efficacy of TMZ on brain tumor through bioluminescence tomography (BLT) based on multi-modality imaging system.

Initially, the human glioma cell line U87MG-fLuc cells were cultured, and the orthotopic mouse brain tumor model was established. 10 days after the tumor cell implantation, the mice were divided into two groups including the TMZ group and the control group. The mice in the TMZ group were treated with Temozolomide with dosage of 50 mg/kg/day intraperitoneally for continuous 6 days, and the mice in the control group were treated with sterile saline at equal volume. The bioluminescence imaging (BLI) was acquired every 5 days for monitoring the therapeutic responses. A randomly enhanced adaptive subspace pursuit (REASP) algorithm is presented for bioluminescence tomography reconstruction. Basically, numerical experiments were used to validate the efficiency of the proposed method, and then the mice's CT and BLI data were acquired to reconstruct BLT using the REASP algorithm.

The results in this study showed that the growth of glioma can be monitored from very early stage, and the TMZ treatment efficacy can be reliably and objectively assessed using BLT method. Our data demonstrated TMZ can effectively inhibit the tumor growth.

Keywords: glioma, temozolomide (TMZ), randomly enhanced adaptive subspace pursuit (REASP), bioluminescence tomography.

1. INTRODUCTION

Malignant gliomas are among the most devastating cancers, commonly producing profound and progressive disability and leading to death in most cases [1]. The main cause for the high mortality rate is due to the detection of glioma at a very late stage, when potentially curative therapies are ineffective. Therefore, it urgently needed to find an effective and sensitive method to detect cancer lesions at an early stage. Simultaneously, many commonly used

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chemotherapy agents (e.g., nitrosoureas or the combination regimen procarbazine, lomustine, vincristine) have limited activity against glioma [2]. Hence, seeking effective ways for monitoring their treatment efficacy on glioma is urgently needed.

Temozolomide (3,4-dihydro-3-methyl-4-oxoimidazo-[5,1-d]-1,2,3,5-tetrazin-8-carboxamide, TMZ) is a new alkylating agent used for the chemotherapy of malignant gliomas. TMZ is a potent inhibitor on glioma cell growth and angiogenesis at non-toxic doses [3]. This agent has shown promising antitumor activity against high grade glioma. However, currently, there is still lacking effective ways for monitoring and evaluating TMZ treatment effects. Conventional anatomic imaging techniques typically detect cancers when they are a centimeter or greater in diameter, at which point there are already more than 10^9 cells in tumors (including circulating and microscopic metastatic deposits).

Molecular imaging is expected to play an important role in this setting, because it will allow sensitive and specific monitoring of key molecular targets and host responses associated with early events in carcinogenesis [4]. Molecular imaging allows the non-invasive assessment of biological and biochemical processes in living subjects, and exhibit the potential to enhance our understanding of diseases and drug activities [5]. Bioluminescence Tomography (BLT) is one of the molecular imaging methods, and it can be applied to study many physiological and pathological processes. Hence, it becomes an increasingly important tool for researchers to evaluate therapies, and facilitate drug development.

In this study, we aimed to evaluate the drug treatment efficacy of TMZ on glioma using bioluminescence tomography. In order to realize it, we established the orthotopic glioma mouse model, and administered TMZ daily for continuous 6 days. The treatment efficacy was monitored using BLI and micro-CT. Moreover, the reconstruction method based on randomly enhanced adaptive subspace pursuit (REASP) was performed to obtain the results for bioluminescence tomography reconstruction. To evaluate the performance of the proposed method, multisource heterogeneous simulation experiments with different algorithms was conducted. Then, we reconstructed bioluminescence tomography and evaluated the treatment efficacy of TMZ on glioma. Our data indicated that the glioma can be detected at early stage, and the TMZ drug treatment efficacy can be effectively assessed in a sensitive and dynamic way.

2. MATERIALS AND METHODS

2.1 Materials

A human U87MG-fLuc glioma cell line was obtained from American Tissue Type Culture Collection (ATCC, Manassas, VA, USA). The culture medium and fetal bovine serum (FBS) were purchased from HyClone (Thermo Scientific, USA). D-Luciferin was bought from Biotium (CA, Fremont, USA). Temozolomide (TMZ) was obtained from Schering-Plough Corporation (Kenilworth, NJ, USA).

2.2 Cell culture

The human U87MG-fLuc glioma cell line was cultured in Dulbecco's modified eagle medium and supplemented with 10% fetal bovine serum (FBS). They were maintained at 37°C incubator with 5% CO_2.

2.3 Animal model

The experiments with glioma were carried out on 4~5 week-old male BALB/c athymic mice. Mice were anesthetized with sodium pentobarbital. After disinfection and incision of the skin, a small hole was made in the skull through the skin overlying the cranium. 4μl of a cell suspension containing 10^6 cells was injected at an infusion rate of 1 μl/1 min. And then, the scalp was closed with sutures. All animal experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) at Peking University (Permit No: 2011-0039). All procedures were carried out in accordance with the approved guidelines.

2.4 TMZ treatment

10 days after tumor cell inoculation, the mice were divided into two groups (n = 8 for control group and n = 8 for TMZ group). The TMZ group and control group were intraperitoneally injected with TMZ (50 mg/kg/day) and an equal amount of 0.9% saline for 6 days, respectively.

2.5 Micro-CT/BLI System

Our Micro-CT/BLI system was shown in Fig. 1. The multi-modality imaging system was located in a dark lead room that can block both external lights and X-rays.

The Micro-CT system consists of a micro-focus X-ray source (UltraBright, Oxford Instruments, USA). The maximum output power of the X-ray tube was 80 W. The X-ray flat panel detector (C7942CA-02, Hamamatsu, Japan) had a 120 mm×120 mm photodiode area. The CT data acquisition and processing were performed using the Windows Molecular Imaging System (WINMI) [6].

In addition, there is the optical detector on the turntable. This device is a scientific charge-coupled device (CCD) camera (VersArray, Princeton Instruments, Trenton, New Jersey) with the temperature cooled to -110°C to reduce dark current noise. The BLI measurements were acquired by this CCD camera directly. It is worth mentioning that they are all mounted on a vertical electric turntable rotating at a uniform speed.

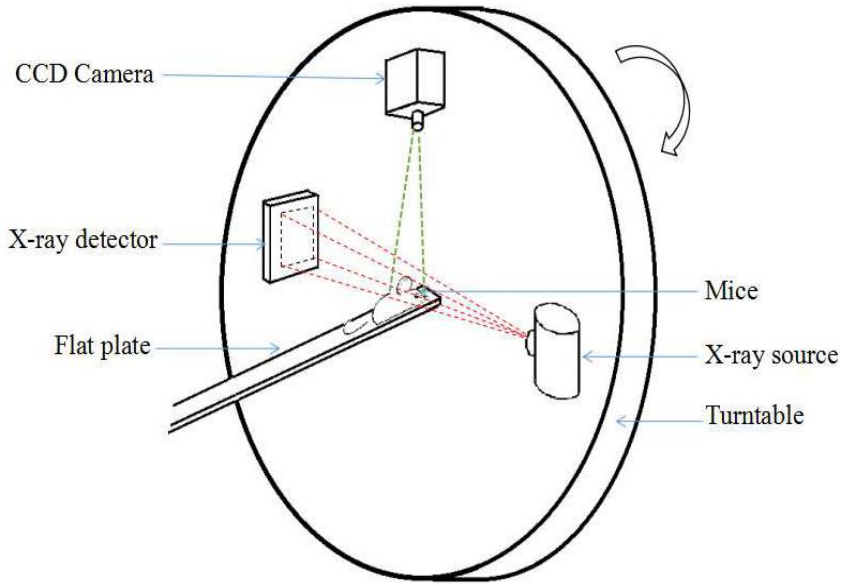

Figure 1. The scheme of the experimental setup. The Micro-CT system consists of the X-ray source and the X-ray detector. the BLI measurements were acquired by this CCD camera directly. In order to obtain image data, the anesthetized mice were placed on the plate perpendicular to the turntable.

2.6 *In vivo* BLI imaging of mouse glioma

To evaluate the antitumor efficacy of TMZ, BLI of the glioma mice was acquired every 5 days with an IVIS Spectrum Imaging System (PerkinElmer, Germany) during the TMZ treatment.

2.7 *In vivo* BLT imaging of mouse glioma

The BLT was implemented to the orthotopic tumor mouse models on the 7th day after completion of drug treatment. The anesthetized mice were placed on a flat plate perpendicular to the turntable. The BLI signal was obtained first, and then 3D anatomical data was acquired using the Micro-CT system.

2.8 Reconstruction method

From the perspective of the compressed sensing, the tumor reconstruction was conducted as a problem of sparse signal recovery. So a randomly enhanced adaptive subspace pursuit (REASP) method is presented for BLT reconstruction. REASP adds more irrelative atoms using a random strategy, compared to the general subspace pursuit algorithms, which has superior performance [7].

The main process of this reconstruction algorithm was as follows:

Algorithm1: Randomly Enhanced Adaptive Subspace Pursuit (REASP)

Input: A-M × N matrix, Φ-measurement vector, K-sparsity level, μ-preset atom-addition parameter threshold, N_{max}-the number of maximum iterations allowed, q-preset upper bound of the estimated set's cardinality

Initialization: I={indices of the K largest absolute value in $A^{\mathrm{T}}\Phi$}(estimated support set), $x^0 = A_I^{\dagger}\Phi$, $r^0 = \Phi - Ax^0$, E ={1,2, ⋯ ,N} (whole coordinate set), $S_0 = \phi$, $D_0 = \phi$

Iteration ($n \geq 1$):

compute the current residual correlation $c_n = A^T r^{n-1}$

form the index set $S_n = \{s : |c_n[s]| > \mu \cdot \|c_n\|_\infty\}$

If $s > q - K$ then

compress S_n to its subset of $q - K$ indices randomly, set $D_n = \emptyset$

else

generate random set D_n, a subset of $E\backslash(I_{n-1} \cup S_n)$ with $q - K - s$ random indices

End if

form the new supporting index set $I_n = I_{n-1} \cup S_n \cup D_n$

compute $\hat{x}_{I_n} = A_{I_n}^{\dagger}\Phi$

update $I_n$with indices of the K largest absolute coefficients in $\hat{x}_{I_n}$

If halting condition true $n \geq N_{max}$ then

quit the iteration

End if

End iteration

Output: $x = \hat{x}_{I_n}$

3. RESULTS

3.1 Monitoring of the anti-tumor efficacy of TMZ by BLI

BLI of the glioma bearing mice was acquired for continuous 15 days during the TMZ treatment and the bioluminescent intensity was calculated to evaluate anti-tumor efficacy of TMZ. In Fig. 2, the data show that the BLI intensity in the control group increased rapidly and the BLI intensity in TMZ group was obviously suppressed during the observation period. The result suggested that TMZ can effectively inhibit glioma growth, and the BLI imaging can provide early and dynamic information for the evaluation of drug treatment efficacy.

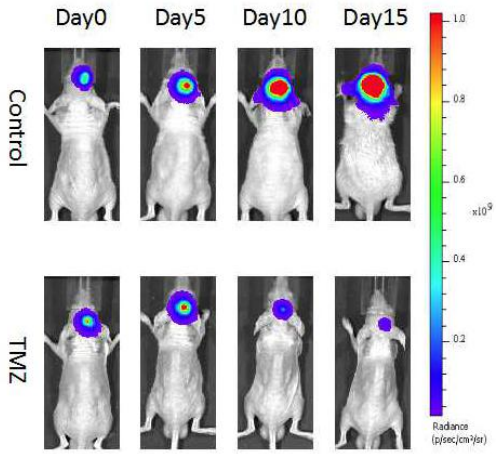

Figure 2. BLI of orthotopic glioma mice on the 0th, 5th, 10th and 15th day after treatment began. BLI images of control group (the upper row), BLI images of TMZ group (the lower row).

3.2 Numerical Experiments

A heterogeneous phantom was used to validate the efficiency of the REASP algorithm. Fig. 3 shows the phantom, which is 20 mm in diameter and 20 mm in height. This phantom consists of four kinds of materials, muscle (M), lungs (L), heart (H), and brain (B). Fig. 3a shows a 3D view of the phantom and Fig. 3b demonstrates the slice image of the phantom in a z = 0 plane. The bioluminescent sources are represented by three spheres (S) with a diameter of 2 mm and are centered in the z = 0 plane.

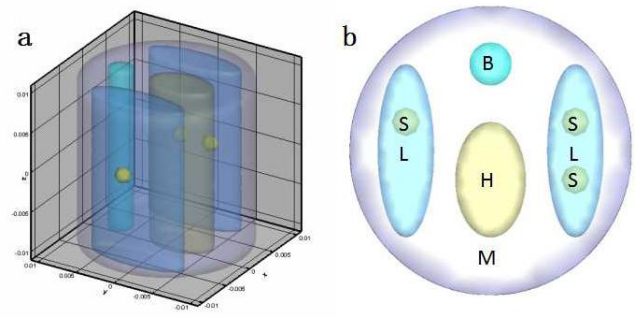

Figure 3. The phantom with regions resembling muscle (M), lungs (L), heart (H), and brain (B). **a** 3D view of the phantom. **b** Slice image of the phantom in z = 0 plane.

To better evaluate the proposed method, we compared it to the IS-L1 method, which can be regarded as the main stream method for BLT. Here, μ was set to 0.7, N_{max}=M, q= $0.8\lceil M \rceil$. In Fig. 4a, b, there were artifacts for the results obtained by the IS-L1 method. In contrast, the proposed method best preserved the sparsity of the bioluminescent sources and there were fewer artifacts.

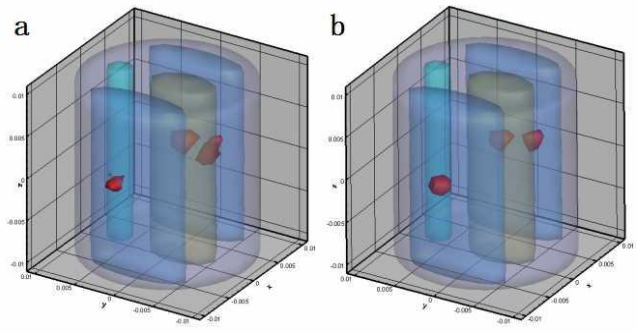

Figure 4. the 3D views of the reconstructed sources in the phantom. Reconstruction results from **a** the IS-L1 method and **b** the proposed method.

Besides, to quantitatively assess the outcome of the performance, the position error (PE) indice was defined as the touchstone of judgment results:

$$\mathrm{PE} = \|P_r - P_0\|_2 \tag{1}$$

where P_0 is the true location of the source center and P_r is the location of the finite element node with the maximum reconstructed. As shown in Table 1, the PE indice of the proposed method is lower than IS_L1 method. Overall, the REASP algorithm, as the reconstruction method in this study is potential for BLT applications.

Table 1. The PE indice of two methods

Method / Source NO.	IS_L1	REASP
S1	1.254	0.171
S2	0.128	0.125
S3	0.195	0.196

3.3 Evaluation of chemotherapeutic response using BLT after TMZ treatment

BLT was required for tumor localization and distribution inside a small animal to produce accurate tomographic reconstruction and visualization in 3D mode. We segmented the brain, bone, lung and heart through CT data. The reconstruction results were shown in Fig. 5. Comparing Fig. 5a with Fig. 5b, we found that the tumor volume of glioma in the TMZ group was smaller than control group. The results confirmed that the growth of orthotopic glioma in the TMZ group was significantly inhibited compared to the control group. And the drug treatment efficacy of TMZ can be reliably assessed on glioma through BLT imaging method.

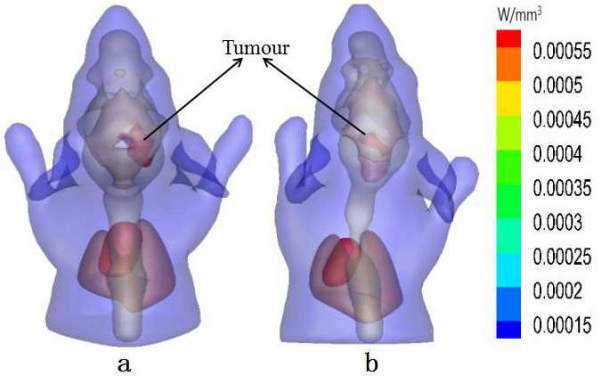

Figure 5. The BLT volume rendering of mouse glioma model. **a** and **b** are the control group and the TMZ group of BLT images of glioma, respectively.

4. DISCUSSION

Optical molecular imaging is an ideal imaging method *in vivo* to observe the biological behavior of tumor and assess the drug therapeutic efficacy at cellular levels. BLT, as a novel modality of optical molecular imaging, furnishes the accurate location and quantitative analysis of glioma in deep tissue, which could provide 3D tumor volume information.

In this paper, BLT imaging method was utilized to make an assessment of TMZ on glioma treatment. We reconstructed the bioluminescence distribution of the orthotopic glioma, which could provide 3D tumor volume information. Hence, we could evaluate the drug treatment efficacy of TMZ more accurately and comprehensively with BLT. The results demonstrated that TMZ can effectively inhibit the growth of tumor and BLT is an ideal method for the evaluation of drug treatment efficacy.

Our future work direction is to combine multiple imaging modalities, such as fluorescence molecular tomography (FMT), magnetic resonance imaging (MRI) and positron emission tomography (PET), to assess the anti-tumor efficacy of TMZ or other drugs in glioma. Multi-modality imaging can be used from multi-angle to verify the effects of drug treatment in brain cancer.

5. CONCLUSION

In this study, we evaluated the chemotherapeutic efficacy of TMZ on glioma using BLT based on Micro-CT/BLI multi-modality imaging system. TMZ was demonstrated to inhibit tumor growth effectively in our study. BLT is a reliable and dynamic way for the assessment of therapeutic efficacy of TMZ on glioma. The information from this study is considered to have a far-reaching significance on cancer detection, pharmaceutical and clinical research.

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