Evaluation of the anti-neoplastic effect of sorafenib on liver cancer through bioluminescence tomography

Qian Liang[a,1], Jinzuo Ye[a,1], Yang Du[a,*], Chongwei Chi[a], Jie Tian[a,*]

[a]Key Laboratory of Molecular Imaging of Chinese Academy of Sciences, Institute of Automation, Chinese Academy of Sciences, Beijing, China, 100190
[1] Qian Liang and Jinzuo Ye contributed equally to this paper.

ABSTRACT

Hepatocellular carcinoma (HCC) is one of the most important leading causes of cancer-related deaths worldwide. In this study, we evaluated the efficacy of sorafenib on hepatocellular carcinoma through bioluminescence tomography (BLT) based on Micro-CT/BLT multi-modal system.

Initially, the human hepatocellular carcinoma cell line HepG2-Red-FLuc, which was transfected with luciferase gene, was cultured. And then, the orthotopic liver tumor mouse model was established on 4~5 weeks old athymic male Balb/c nude mice by inoculating the HepG2-Red-FLuc cell suspension into the liver lobe under isoflurane anesthesia. 15~20 days after tumor cells implantation, the mice were divided into two groups including the sorafenib treatment group and the control group. The mice in the treatment group were treated with sorafenib with dosage of 62 mg/kg/day by oral gavage for continuous 14 days, and the mice in the control group were treated with sterile water at equal volume. The tumor growth and drug treatment efficacy were dynamically monitored through BLT.

The results in this study showed that the growth of liver cancer can be dynamically monitored from very early stage, and also the sorafenib treatment efficacy can be reliably and objectively assessed using BLT imaging method. Our experimental result demonstrated sorafenib can inhibit the tumor growth effectively. BLT enabled the non-invasive and reliable assessment of anti-neoplastic drug efficacy on liver cancer.

Keywords: bioluminescence tomography, hepatocellular carcinoma, sorafenib

1. INTRODUCTION

Liver cancer is one of the highly fatal cancers. During 2012, about 782,500 new liver cancer cases and 745,500 deaths occurred worldwide. China accounted for about 50% of the total number of cases and deaths[1]. The main cause for the high death rate is due to the detection of liver cancer at a very late stage, when potentially curative therapies are ineffective. Therefore, it is urgently needed to find an effective and sensitive method to detect cancer lesions at an early stage. Simultaneously, seeking new drugs and effective ways for monitoring their treatment efficacy on liver cancer is quite important.

Sorafenib is a multikinase inhibitor that has shown therapeutic efficacy against a wide variety of liver tumors in preclinical models and also clinical researches[2]. It is a tyrosine kinase inhibitor of several receptors, such as vascular

* Correspondence to Jie Tian, Email: tian@ieee.org; Yang Du, Email: yang.du@ia.ac.cn

Medical Imaging 2017: Biomedical Applications in Molecular, Structural, and Functional Imaging
edited by Andrzej Krol, Barjor Gimi, Proc. of SPIE Vol. 10137, 1013702 · © 2017 SPIE
CCC code: 1605-7422/17/$18 · doi: 10.1117/12.2254323

endothelial growth factor-2,3 (VEGFR-2, VEGFR-3), which plays important roles in tumor progression[3]. In preclinical studies, sorafenib was found to inhibit tumor angiogenesis and to induce tumor cell apoptosis in HCC models[2]. However, currently, there is still lacking effective ways to monitor and evaluate sorafenib treatment effects.

As for the evaluation of liver cancer treatment, the traditional biological method, such as tumor volume measurement, is not accurate enough for the study of liver cancer progression and cannot truly and objectively reflect the progression of tumor growth[4]. Histopathological analysis is accurate, but cannot provide dynamic and three-dimensional structural information. Therefore, it is an urgent need to develop a more effective and sensitive detection method for this aggressive disease.

Molecular imaging is the visualization, characterization, and measurement of biological processes at molecular and cellular levels in humans and other living systems[5]. Optical molecular imaging technology plays a major role on cancer detection, individualized treatment, and drug development, as well as our understanding of how cancer arises[6]. Bioluminescence imaging (BLI) as a novel modality of optical molecular imaging is considered as an ideal *in vivo* imaging pattern for small animals, because it possesses the advantages of high specificity, high sensitivity, non-radioactive pollution, low cost and non-invasive. It can realize the observation of biological behavior of tumors and also assessment of drug treatment efficacy at molecular and cellular levels.

In this study, we aimed to evaluate the drug treatment efficacy of sorafenib on hepatocellular carcinoma using bioluminescence tomography (BLT). In order to realize that, we established the orthotopic liver tumor mouse model, and administered the sorafenib drug daily for 14 days. The treatment efficacy was monitored by using bioluminescence imaging. Then we reconstructed three-dimensional bioluminescence yields of tumor and evaluated the efficacy of sorafenib on hepatocellular carcinoma through BLT imaging method. The BLT system allowed early detection of tumor progression and therapeutic responses, and we presented a comprehensive evaluation of the anti-tumor effects of Sorafenib.

2. METERIALS AND METHODS

2.1 Materials

The human hepatocellular carcinoma cell line HepG2-Red-FLuc was purchased from PerkinElmer (Waltham, MA, USA). Sorafenib was bought from the corporation of meilunbio (Dalian meilunbio company, China). The culturing medium and fetal bovine serum (FBS) were got from Hyclone (Thermo Scientific, USA).

2.2 Cell culture

HepG2-Red-FLuc cells were cultured in Eagle's MEM supplemented with 10% fetal bovine serum (FBS) at 37℃ incubator with 5% CO_2.

2.3 Animal model

4~5 weeks old athymic male BALB/c nude mice were purchased from Vital River Laboratory Animal Technology corporation (Beijing, China). 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.

The orthotopic liver tumor mouse model was established by injecting the 3×10^6 HepG2-Red-FLuc cells into the liver of BALB/c nude mice through laparotomy under isoflurane anesthesia.

2.4 Sorafenib treatment

15~20 days after the liver tumor cells were implanted, the orthotopic tumor-bearing mice were randomly divided into two groups, the experiment group (n = 3) and the control group (n = 3). The mice in the experiment group were treated with sorafenib at a dosage of 62 mg/kg/day by daily oral gavage for continuous 14 days and the control group was treated with sterile water at equal volume.

2.5 Micro-CT/BLT system

The Micro-CT/BLT system is a fusional system which was shown in Fig.1. The Micro-CT system and the BLT system share the same imaging platform, and the two systems were placed vertically.

The Micro-CT system consists of a microfocus X-ray source (UltraBright, Oxford Instruments, USA), a three-dimensional imaging stage with mouse holder and a flat-panel X-ray detector (C7942CA-02, Hamamatsu, Japan), which was designed to provide high-resolution anatomic information. The X-ray source is continuously adjustable from 13 μm to 40 μm. The target voltage of the X-ray tube is 20 to 90 kVp, with maximum output power 80 W. The X-ray flat panel detector, based on CMOS technology with a column CsI scintillator plate, has a 120 mm×120 mm photodiode area with 50 μm pixel size[7].

The BLT system consists of an optical detector and a three-dimensional imaging stage with mouse holder, which is responsible for capturing the light signals emitted from the surface of the organism. The optical detector is a charge-coupled device (CCD) camera (VersArray, Princeton Instruments, Trenton, New Jersey) with the temperature cooled to -110℃ to reduce dark current noise. The bioluminescence signals on the external surface of the small animal can be directly collected by the CCD camera[8].

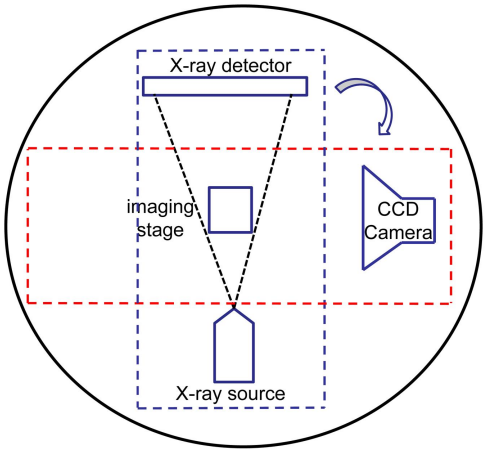

Figure 1. The schematic of the Micro-CT/BLT multi-modal system. The red dashed line represents the BLT system and the blue dashed line represents the Micro-CT system.

2.6 *In vivo* BLI of mouse orthotopic HCC model during antitumor sorafenib treatment

We acquired the bioluminescent images of the orthotopic liver tumor mice for continuous 14 days after daily treatment began and calculated the bioluminescent intensity to evaluate antitumor efficacy of sorafenib. Before bioluminescent images was acquired, the mouse was anesthetized with 2% isoflurane and injected intraperitoneally D-luciferin (150 mg/kg) for 8 minutes. The exposure time of the CCD camera was set to be 10 seconds.

2.7 *In vivo* BLT of mouse orthotopic HCC model during antitumor sorafenib treatment

We acquired the BLT data on the 0^{th} and 14^{th} day after the daily treatment began. The mouse was fixed on the three-dimensional imaging stage. We gave the mouse 2% isoflurane through a respiratory mask, which was attached to the imaging stage. Firstly, we captured the bioluminescent signals emitted from the surface of the mouse by the CCD camera. We acquired four bioluminescent images at 0° , 90° , 180° and 270° from mice for reconstruction of the three-dimensional bioluminescent images. The exposure time of the CCD camera was set to be 10 seconds. Then, we obtained the 3D anatomical data using the Micro-CT system.

2.8 Reconstruction method

The reconstruction method based on the sparsity adaptive subspace pursuit (SASP) was used to reconstruct the bioluminescence yields of tumor. This method adopts a subspace projection and correlation maximization approach to simplify the BLT problem with sparsity-promoting L_1-norm regularization and to treat it as the basis pursuit problem, which enhances the reconstruction accuracy and robustness[9]. The process of SASP reconstruction algorithm was as follows:

Firstly, the current iteration (for example, the *n*-th iteration) applies matched filtering to the current residual vector r^{n-1} , getting a vector of residual correlation to be:

$$c^{n-1} = A^T r^{n-1} \tag{1}$$

Secondly, the observation vector Φ is projected onto the subspace spanned by the columns of A_{J^n} , where A_{J^n} denote the submatrix of A with indices from the candidate set J^n .

Thirdly, the true support set of the proposed SASP method was established:

$$I_n = \{ K \text{ indices with the largest magnitude entries of } A_{J^n}^{+}\Phi \} \tag{2}$$

where $A_{J^n}^{+}$ denotes the pseudo-inverse of matrix A_{J^n} .

Fourthly, the updated residual is computed as follows:

$$r = \Phi - proj(\Phi, A_I) \tag{3}$$

Finally, the unknown bioluminescence yields were updated:

$$x = \{ \hat{x} \mid \hat{x}_{\{1,2,3\cdots,N\}-I^n} = 0 \text{ and } \hat{x}_{I^n} = A_{I^n}^{+}\Phi . \} \tag{4}$$

3. RESULTS

3.1 Anti-neoplastic effect of sorafenib was monitored by BLI during treatment

In order to track tumor progression accurately during sorafenib treatment, we acquired the bioluminescence images for the orthotopic liver tumor mice and record the dynamic light intensity changes for continuous 14 days after daily treatment began. These bioluminescence images were shown in Fig. 2, and the bioluminescence intensity changes were

shown in Fig. 3. The results showed that the BLI signal of the treatment group was significantly inhibited during the treatment and maintained the inhibition effect throughout the treatment course. While the light intensity of the control group increased steadily.

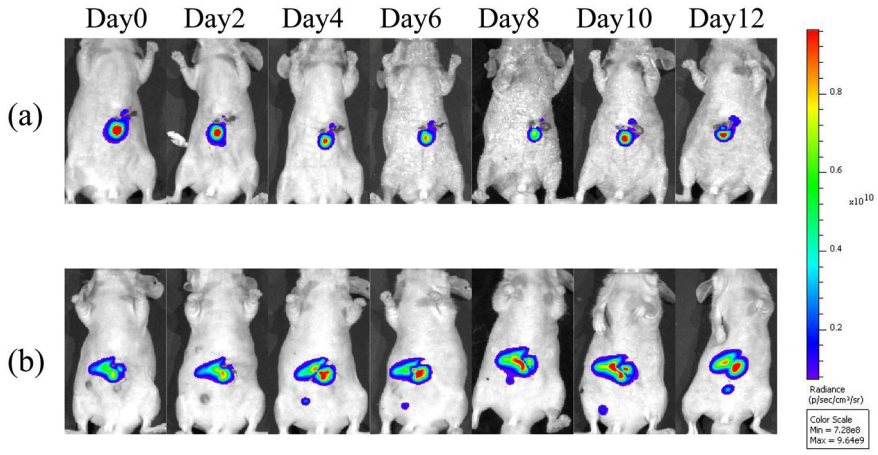

Figure 2. Bioluminescence images (BLI) of orthotopic liver tumor mice on day 0, 2, 4, 6, 8, 10 and day 12 post-treatment. (a). BLI images of experiment group; (b). BLI images of control group.

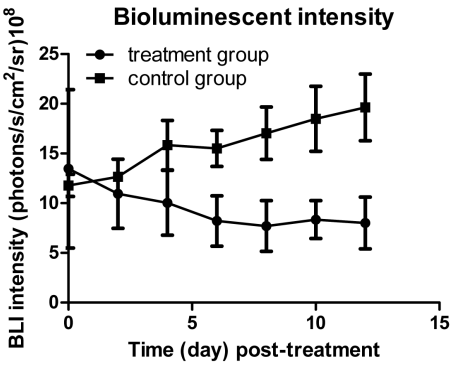

Figure 3. The quantified bioluminescent intensity of the orthotopic liver tumor during 12-day observation.

3.2 Monitoring of neoplastic inhibition using BLT during sorafenib treatment

For the comprehensive evaluation of drug treatment on orthotopic liver cancer, two-dimensional plane bioluminescence imaging information was not enough, because it cannot access the bioluminescence bio-distribution and tumor volume deeper in the tissues [4]. BLT is to reconstruct three-dimensional bioluminescence yields of tumor inside living animals, which can provide the tumor location and distribution information. Therefore, we further used Micro-CT/BLT system to observe the three-dimensional bioluminescence changes of orthotopic liver tumors dynamically during sorafenib treatment. The reconstruction results were shown in Fig. 4.

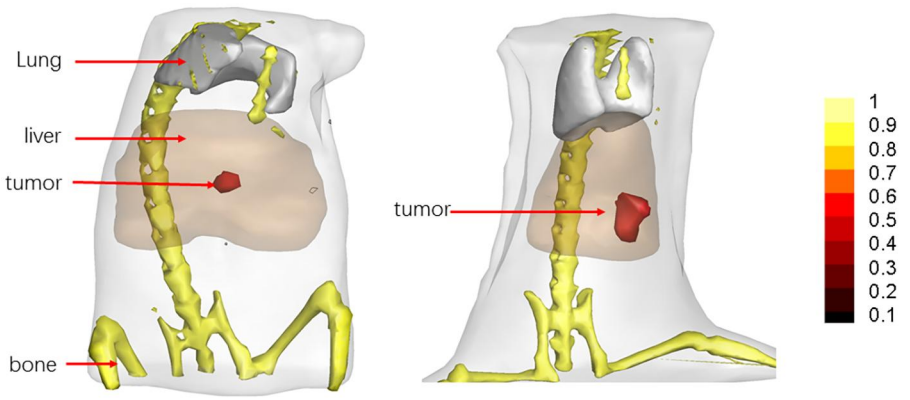

Figure 4. The 3D BLT reconstruction of the orthotopic tumor model. (a). The BLT reconstruction result of the sorafenib treatment group. (b). The BLT reconstruction result of the control group.

The reconstruction result of the sorafenib treatment group was shown in Fig. 4(a) and the control group was shown in Fig. 4(b) through BLT reconstruction method. Both images showed that BLT can show the location of tumors in the liver lobe. Comparing Fig. 4(a) with Fig. 4(b), we found that the liver tumor volume in the treatment group was smaller than that of the control group, which was consistent with BLI data. Besides, the reconstruction processes of the liver tumor were conducted under the same experimental conditions for both control and sorafenib treated mice. These results confirmed that the growth of orthotopic liver tumor in the sorafenib treatment group was significantly inhibited compared to the control group. So we can reliably assess the drug efficacy of sorafenib on liver cancer through BLT imaging method.

4. DISCUSSION

Optical molecular imaging technology is an ideal imaging pattern *in vivo* for small animals to observe the biological behavior of the tumor and assess the drug therapeutic efficacy at molecular and cellular levels. BLT, as a novel modality of optical molecular imaging, is an effective and sensitive method to detect cancer lesions at the early stage.

In this paper, we used BLT imaging method to make an assessment of sorafenib on liver cancer treatment. Firstly, the bioluminescent light intensity reflects the number of living cells of the tumor area, which is a reliable and sensitive method to evaluate the anti-neoplastic effect of sorafenib on liver cancer. Moreover, we reconstruct the 3D bioluminescence distribution of the orthotopic liver tumor, which can provide three-dimensional tumor volume information. So we can evaluate the drug treatment efficacy of sorafenib on hepatocellular carcinoma more accurately and comprehensively with BLT method. Our experimental results demonstrated that sorafenib can effectively inhibit the growth of the tumor and BLT is a reliable imaging method for the assessment of the drug treatment efficacy of sorafenib on liver cancer.

Our future work will focus on combining multiple imaging modalities, such as fluorescence molecular tomography (FMT), magnetic resonance imaging (MRI) and positron emission tomography (PET), to make a more comprehensive, systematic and accurate assessment of the anti-tumor efficacy of sorafenib or other drugs in liver cancer as well as other human cancers. Multi-modality imaging can be used from multi-angle to verify the effects of drug treatment in liver cancer. Moreover we will monitor the vital signs of mice, blood pressure, metabolism and weight for assessing the side effects of drugs.

5. CONCLUSION

In this study, we evaluated the therapeutic efficacy of sorafenib on hepatocellular carcinoma using bioluminescence tomography based on Micro-CT/BLT multi-modal system. The results showed that we can reliably and dynamically assess the drug therapeutic efficacy of sorafenib on liver cancer through BLT imaging. Moreover, sorafenib was demonstrated to inhibit the hepatocellular growth effectively in our study. Our study provides an experimental basis for the clinical study, and the development of further optical imaging may facilitate our more comprehensive observation of cancer progression and accelerates the discovery of new therapeutic regimen.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (81227901, 81470083), the Research and Development Program of China (973) under Grant (2014CB748600, 2015CB755500) and Beijing Natural Science Foundation (Z16110200010000).

REFERENCES

[1] Torre L A, Bray F, Siegel R L, et al, "Global cancer statistics, 2012," CA: a cancer journal for clinicians 65(2): 87-108 (2015).

[2] Liu L, Cao Y, Chen C, et al, "Sorafenib blocks the RAF/MEK/ERK pathway, inhibits tumor angiogenesis, and induces tumor cell apoptosis in hepatocellular carcinoma model PLC/PRF/5," Cancer Res 66(24): 11851-11858 (2006).

[3] Frenette C, Gish R, "Targeted systemic therapies for hepatocellular carcinoma: clinical perspectived, challenges and implications," World J Gastroenterol 18(6): 498-506 (2012).

[4] Zhang Q, Du Y, Xue Z, et al, "Comprehensive evaluation of the anti-angiogenic and anti-neoplastic effects of endostar on liver cancer through optical molecular imaging," PLoS ONE 9(1): e85559 (2014).

[5] Mankoff D A, et al, "A definition of molecular imaging," Nuclear Medicine 48(6): 18N-21N (2007).

[6] Weissleder R, "Molecular imaging in cancer," SCIENCE 312(5777): 1168-1171 (2006).

[7] Zhu S, Tian J, Yan G, et al, "Cone beam micro-CT system for small animal imaging and performance evaluation," Journal of Biomedical Imaging 2009: 16 (2009).

[8] Qin C, Zhu S, Tian J, "New optical molecular imaging systems," Current Pharmaceutical Biotechnology 11(6): 620-627 (2010).

[9] Ye J, Chi C, Xue Z, et al, "Fast and robust reconstruction for fluorescence molecular tomography via a sparsity adaptive subspace pursuit method," Biomedical Optics Express 5(2): 387-406 (2014).