



## Human Glioma Nude Mouse Xenograft Model *in situ*

Zhijie Wang<sup>1,\*</sup>, Jianglong Kong<sup>1,\*</sup>, Ziteng Chen<sup>1</sup>, Meiru Mao<sup>1</sup>, Jiacheng Li<sup>1</sup>, Hui Yuan<sup>1</sup>, Ya-nan Chang<sup>1,#</sup>, Kui Chen<sup>1,#</sup>, Juan Li<sup>1,#</sup>

<sup>1</sup>CAS Key Laboratory for Biomedical Effects of Nanomaterial & Nanosafety, Institute of High Energy Physics, Chinese Academy of Science (CAS), Beijing 100049, China

**Backgrounds:** Surgery and chemotherapy are difficult because of the specific location of the glioma. The establishment of a suitable *in situ* model of glioma is the premise of the treatment of glioma. 8 week-old female BALB/c nude mice were chose to establish the glioma model.

**Methods:** For the orthotopic glioma mice model,  $1 \times 10^5$  cells/5  $\mu$ L U87-MG-Luc or U87-MG cells which were trypsinized and resuspended in sterile PBS were slowly injected into the right corpus striatum (1.8 mm lateral, 0.6 mm anterior to the bregma and 3.0 mm in depth) by a stereotactic fixation device using a mouse adaptor.

**Results:** The orthotopic U87 glioma mice model identified by imaging on IVIS Spectrum and magnetic resonance imaging after 2 weeks from surgery. H&E-stained tumor sections in brain of the mice model were also observed.

**Conclusions:** After identification, the glioma mouse xenograft *in situ* model obtained could be used in the evaluation system of therapeutic drugs or methods.

**Keywords:** U87-MG cells; Glioma; Xenograft model; *in situ*.

### Introduction

Journal of the National Cancer Center published its first online article entitled "Statistics of Cancer Incidence and death in China in 2015" by Academician Hejie's team<sup>[1]</sup>. Based on the data of 368 high-quality surveillance sites selected from 501 cancer surveillance sites in China, covering a population of 310 million, accounting for 22.52% of the total population in 2015. The article comprehensively reflects the epidemic characteristics and regional differences of cancer spectrum in China, including the incidence rate of brain tumor was 4.9 cases per 100, 000, and the mortality rate was 2.9 cases per 100, 000. In 2020, 56, 000 people died, ranking among the top 10 cancer deaths in China<sup>[2]</sup>. Among all kinds of brain malignancies, glioma is the most malignant glioma among astrocytomas. It is a tumor produced by glial cells or their precursors in the central nervous system (CNS)<sup>[3]</sup>. It is extremely invasive and the most common CNS malignant tumor, accounting for 47% of such tumors<sup>[4-6]</sup>. The median survival for this type of cancer is just 15–17 months, and less than 5% of patients with glioblastoma who receive standard treatment survive five years. Glioma occurs frequently in teenagers and has a great

impact on society and family<sup>[4]</sup>. On March 4, 2021, the topic "Six-year-old Girl Lin Nuo died and donated organs to save 5 people in Fuzhou, Fujian province" was listed in the hot search list on Weibo news, which was read 210 million times, liked 290, 000 times and triggered 33, 000 discussions within half a day. Lin Nuo was a glioma patient.

Due to the special location of glioma, surgery and chemotherapy are very difficult. The premise of studying glioma therapy is to establish a suitable *in situ* glioma model. The *in situ* glioma model mice were suitable for the treatment study for drugs crosses the blood-brain barrier (BBB). Proliferating tumor cells invade local tissue, travel through the circulatory system and implant in a foreign tissue. Metastasis leads to high death rates in cancer patients. Cell line derived xenograft (CDX) mouse models are highly utilized in preclinical assessment of novel cancer therapeutics and are a crucial link between initial high-throughput *in vitro* screening data and anti-tumor efficacy. Metastatic mouse models are utilized to understand the interactions of the anti-metastatic therapeutic and tumor in regards to all organs, bioavailability (e.g. half-life), clearance, immune response and tumor efficacy. Due to the inability to palpate metastatic tumors, the insertion of a luciferase (bioluminescence) or GFP (fluorescence) gene into the genome of the cell line of interest enables the researcher to visually track and quantitate internal tumor progression throughout the inlife portion of the animal study.

Researchers are extensively carrying out basic research on glioma animal model<sup>[7-9]</sup>. Hara et al. analyzed and established the glioma model based on human genetic abnormalities and the human glioma model based on cells, especially the of the tumor model *in situ*<sup>[10]</sup>. Due to the easy, economical and efficient culture of cell lines, tumor models such as U251<sup>[11]</sup> and 9L<sup>[12]</sup> have been established at present. However, as the cell lines were con-

\* The authors contributed equally to this work

# Correspondence: Ya-nan Chang. E-mail: [changyn@ihep.ac.cn](mailto:changyn@ihep.ac.cn); Kui Chen. E-mail: [chenkui@ihep.ac.cn](mailto:chenkui@ihep.ac.cn); Juan Li. E-mail: [lijuan@ihep.ac.cn](mailto:lijuan@ihep.ac.cn). Address: CAS Key Laboratory for Biomedical Effects of Nanomaterial & Nanosafety, Institute of High Energy Physics, Chinese Academy of Science (CAS), No. 19, Yuquan Road, Shijingshan District, Beijing 100049, China.

Received 9 September 2021; Revised 18 October 2021; Accepted 30 October 2021; Available Online 5 November 2021

DOI: [10.54457/DR.202101003](https://doi.org/10.54457/DR.202101003)

© The Author(s) 2021. This is an open access article under the CC BY 4.0 license (<https://creativecommons.org/licenses/by/4.0/>).

tinuously propagated *in vitro*, the cells continuously propagated adapted to the environment of external petri dish. The success rate of tumorigenesis *in vivo* is greatly reduced<sup>[13,14]</sup>. Moreover, the tumor model with stable expression of green fluorescent protein (GFP) was established based on inbred immunodeficient mice<sup>[15]</sup>, the complex relationship between tumor and host can be intuitively distinguished. The method of establishing green fluorescent BALB/C nude mouse model and its model application were used widely<sup>[16-18]</sup>.

Based on the above, we have carried out relevant work. The surgery process for U87-MG cells expressing luciferase (U87-MG-Luc) implanted *in situ* in the normal nude mice was profiled in the present articles.

## Methods

### Materials

The U87-MG cell line was bought from the National Infrastructure of Cell Line Resource. The U87-MG-Luc cell line were purchased from Shanghai Sciencelight Biology Science & Technology Co. Ltd. (China). DMEM medium, penicillin-streptomycin and fetal bovine serum (FBS) were obtained from Thermal Fisher Scientific (China). The nude BALB/c mice were purchased from Huafukang Bioscience Company (China).

### Methods

All the animal experiments were performed in accordance with the National Institutes of Health regulations for the care and use of animals in research and were approved by the Medical Ethics Committee of Peking Union Medical College (No. YZS20191004). The bioluminescence imaging was performed using IVIS Spectrum (PerkinElmer, USA) to monitor tumor growth status. The brain locator (SANS, China) was used to transplant operation.

### U87-Luc basic tumor xenograft study design and description of establishing the glioma model process

Female nude mice aged 6–8 weeks (about 18 g) were selected. The mice were anesthetized with 2% pentobarbital sodium on the dosage of 80 mg/kg. The U87-MG or U87-MG-Luc cells were digested by trypsin (digestion time was about 30 s) centrifugated and re-suspended by sterile PBS. The number of cells was adjusted to  $1 \times 10^8$  /mL.

The mouse head skin was sterilized by a cotton swab in 75% alcohol or iodine solution. The scissors and tweezers were sterilized. The skin and the fascia of mouse were cut and opened, the surface of the skull was blow dry. The two points of Bregma and lambda should be find as showing in the Fig. 2. The skull should be drill in the same height on the two points of Bregma and lambda with the head fixed on the brain locator. The red dot in the Fig. 1 was the drill position. Then, the mice were fixed on the microinjector. The needle of the microinjector with U87-MG or U87-Luc MG cells was inserted the skull. The needle enter the brain for 3.5 mm and slowly inject 5  $\mu$ L by the speed of 0.5  $\mu$ L/30s. Then the needle should be stopped for 3–5 min after the injection. At last, the needle was removed slowly. The wound was sealed with bone wax and suture.

Then anesthetized the mice with isoflurane and used IVIS Spectrum Live Imaging System (PerkinElmer, USA) to achieve whole body fluorescent distribution. The magnetic resonance imaging (MRI) (4.7 T /30 cm Bruker Biospec scanner, Germany) was also used to observe tumor xenograft status.

### Histopathological Evaluation

For histological analysis, the brain of the mice model in the different groups were fixed in 4% paraformaldehyde for 24 h. Paraffin-embedded sections were stained with H&E and observed on a microscopy.

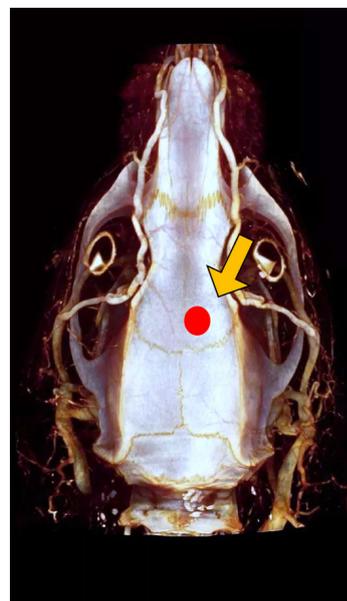


Fig. 1. The drill position on the head of the mice model.

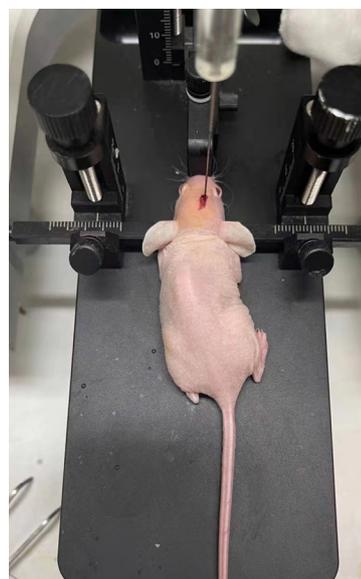


Fig. 2. The Mice awaiting surgery on the stereotaxic apparatus. *In vivo* imaging of the mice model by Spectrum and MRI

Results and Discussion

8–12 weeks old NOD/SCID or Nude (Nu/Nu) mice could be chose and firstly acclimated to the facility for 5–7 days prior to tumor cell injection/implantation. The surgical procedure for glioma nude mouse xenograft model *in situ* was profiled in the Methods Section. For the orthotopic glioma mice model,  $1 \times 10^5$  cells/5  $\mu$ L U87-Luc cells which were trypsinized and resuspended in sterile PBS was slowly injected into the right corpus striatum (1.8 mm lateral, 0.6 mm anterior to the bregma and 3.0 mm in depth) by a stereotactic fixation device using a mouse adaptor. The glioma tumors were allowed to grow and tumor growth was monitored throughout the study after an intraperitoneal (IP) injection of luciferin for imaging. After 5 min post-intraperitoneal injection of D-luciferin, the images were obtained. The orthotopic U87 glioma mice model identified in Fig. 3. The tumor location and the ability to capture a quantifiable value for orthotopic or metastatic tumor progression is the main strength of the luciferase expression (emitted photons) in the U87 MG-Luc model. The orthotopic glioma mice models were imaged and evaluated using IVIS Spectrum Live Imaging System (PerkinElmer, USA). Signals in the region of interest were quantified in units of mean photons, per second, per square cm and per steradian.

To identify the location of the tumor, the 3D imaging for the orthotopic glioma mouse model of U87-MG-Luc cells was observed by IVIS Spectrum as shown in Fig. 4. The fluorescence of bioluminescence from U87-MG-Luc cells were shown in the mouse brain and the tumor site were consistent with the surgical site. The virtual skeleton from Spectrum software system helped us determine the tumor location further.

Further, the MRI imaging was also used to observe tumor xenograft status *in situ* as shown in Fig. 5. The gliomas show more contrast enhancement (white on the outside) as shown in the red circle in Fig. 5. In clinic, a biopsy to confirm diagnosis of

glioma was the gold standard. A biopsy also provides information to help guide treatment decisions. The cells in the tissue will be stained by hematoxylin and eosin (HE) and observed under a microscope to confirm that the sample tissue is cancerous. The hematoxylin stains cell nuclei a purplish blue, and eosin stains the extracellular matrix and cytoplasm pink, with other structures taking on different shades, hues, and combinations of these colors. Histological features of gliomas in Fig. 6 marked hypercellularity, nuclear atypia, microvascular proliferation, and necrosis. The tumor shows palisading of tumor cells around necrotic foci.

If the subcutaneous tumor model were tried to be achieve, the operation process will be simple. Each mouse receive one subcutaneous injection in the flank of the hind or the front leg<sup>[19, 20]</sup>. Each site will receive one million tumorigenic cells in a volume of 100  $\mu$ L containing 50% matrigel (use of matrigel matrix improves tumor cell survival and tumor formation). The injection sites were palpated three times weekly until tumors were established. Once palpable tumors are observed, tumors are measured and mouse weights recorded 2 times a week. The tumors sizes should be measured and record until they reach an average size of 150–250 mm<sup>3</sup>. Injected animals should be monitored daily with cage-side observations, and body weight measurements<sup>[19]</sup>. Then the animals model could be randomized into treatment groups and treated according to the treatment schedule.

It should be noted that animals should be euthanized when tumor size was over 2,000 mm<sup>3</sup> limit. Gross necropsy and tissue collection should be performed as defined for termination of experiment. Animals showing signs of morbidity due to tumor bur-

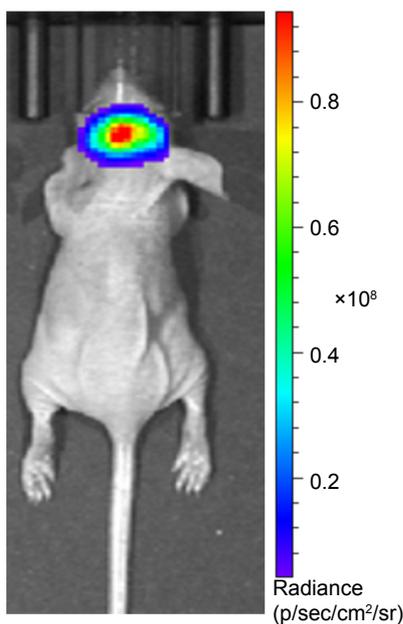


Fig. 3. Expression of luciferase in U87- MG-Luc orthotopic model. The implanted glioma mouse model fluorescence was captured after intraperitoneal luciferin injection 5 min.

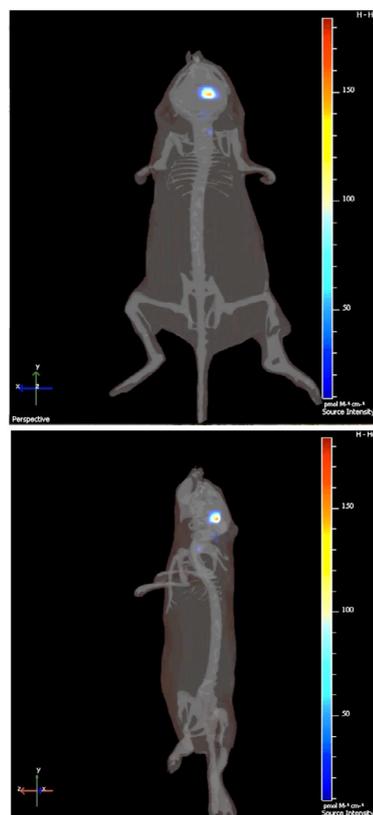


Fig. 4. The 3D imaging for the orthotopic glioma mouse model of U87-MG-Luc cells by IVIS Spectrum.



Fig. 5. The imaging by brain MRI scanning *in vivo*.

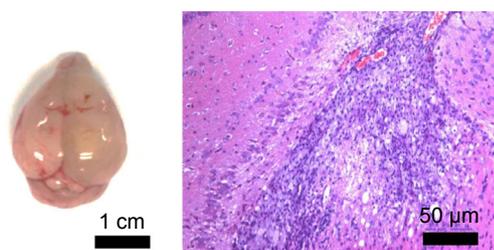


Fig. 6. The microscopic image of H&E-stained tumor sections in brain of the mouse model.

den or treatment toxicity are euthanized and processed as defined for termination of experiment.

## Conclusions

For the orthotopic glioma mouse *in situ* model, the vibrant U87-MG-Luc or U87-MG cells were slowly injected into the right corpus striatum in the selected immunodeficient nude mice by a stereotactic fixation device. The orthotopic U87 glioma mice model was successfully established by imaging on IVIS Spectrum, MRI and H&E-stained brain pathological sections. The glioma mouse xenograft *in situ* model obtained could be used in the evaluation system of therapeutic drugs or methods.

## Abbreviations

BBB, blood-brain barrier; CDX, Cell line derived xenograft; CNS, central nervous system; FBS, fetal bovine serum; HE, hematoxylin and eosin; IP, intraperitoneal; MRI, magnetic resonance imaging.

## Funding

The National Key Research and Development Program of China (No. 2019YFB1300303). Science and Technology Innovation Project of IHEP and the National Natural Science Foundation of China (No. 31971294).

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' contributions

ZJW and JLK: Wrote the manuscript, analyzed all data, prepared all tables and figures, and conducted the most of the experimental work. ZTC and MRM: Assisted in conducting animal experiments and the manuscript revision. JCL: Assisted in conducting animal experiments and the manuscript revision. HY: prepared and observed the HE tissue slides. YNC and KC: Assisted in conducting animal experiments. YNC, KC and JL: Designed experiments, contributed to the writing of manuscript and supported data interpretation. All authors read and approved the final manuscript.

## References

- [1] Chen WQ, Zheng RS, Baade PD, et al. Cancer Statistics in China, 2015. *CA: Cancer J Clin*, 2016, 66(2): 115-132.
- [2] He YT, Liang D, Li DJ, et al. Incidence and mortality of laryngeal cancer in China, 2015. *Chinese J Cancer Res*, 2020, 32(1): 10-17.
- [3] Ji JQ, Luo Z, Chen YC, et al. Characteristics and trends of childhood cancer in Pudong, China, 2002-2015. *Bmc Public Health*, 2020, 20(1).
- [4] Li YQ, Li Q, Mo HN, et al. Incidence, risk factors and survival of patients with brain metastases at initial metastatic breast cancer diagnosis in China. *Breast*, 2021, 55: 30-36.
- [5] Wang D, Chen C, Ge XX, et al. Factors Prognostic for Brain Metastases from Colorectal Cancer: A Single-Center Experience in China. *Cancer Manag Res*, 2021, 13: 6767-6774.
- [6] Qiu Z, Wang K, Huang M, et al. Clinical data from the real world: Efficacy analysis of ceritinib (450mg) in ALK-positive non-small cell lung cancer patients with brain metastases in China. *Ann Oncol*, 2020, 31: S1395-S1395.
- [7] Hicks WH, Bird CE, Traylor JI, et al. Contemporary Mouse Models in Glioma Research. *Cells*, 2021, 10(3).
- [8] Chen ZR, Peng P, Zhang XL, et al. Advanced Pediatric Diffuse Pontine Glioma Murine Models Pave the Way towards Precision Medicine. *Cancers*, 2021, 13(5).
- [9] Hetze S, Sure U, Schedlowski M, et al. Rodent Models to Analyze the Glioma Microenvironment. *Asn Neuro*, 2021, 13.
- [10] Hara T. Exploring phenotypic mosaicism in a mouse model of glioma towards understanding intratumoral heterogeneity. *Cancer Res*, 2019, 79(13).
- [11] Liu PD, Jin HZ, Guo ZR, et al. Silver nanoparticles outperform gold nanoparticles in radiosensitizing U251 cells in vitro and in an intracranial mouse model of glioma. *Int J Nanomed*, 2016, 11: 5003-5013.
- [12] Zhang J, Shin MC, Yang VC. Magnetic Targeting of Novel Heparinized Iron Oxide Nanoparticles Evaluated in a 9L-glioma Mouse Model. *Pharm Res*, 2014, 31(3): 579-592.
- [13] Fabiani MG, Haase S, Kadiyala P, et al. Characterization of the Tumor Immune Microenvironment in a Pediatric High Grade Glioma Mouse Model Harboring the H3.3-G34r Mutation. *Neuro-Oncology*, 2019, 21: 121-121.
- [14] Patel S, Hartley R, Bear H, et al. Development and Characterization of Diffuse Intrinsic Pontine Glioma Mouse Models Generated by Brainstem in Utero Electroporation. *Neuro-Oncology*, 2019, 21: 124-

124.

- [15] Saini M, Bellinzona M, Meyer F, et al. Morphometrical characterization of two glioma models in the brain of immunocompetent and immunodeficient rats. *J Neurooncol*, 1999, 42(1): 59-67.
- [16] Sreedharan S, Maturi NP, Xie Y, et al. Mouse Models of Pediatric Supratentorial High-grade Glioma Reveal How Cell-of-Origin Influences Tumor Development and Phenotype. *Cancer Res*, 2017, 77(3): 802-812.
- [17] Irvin DM, McNeill RS, Bash RE, et al. Intrinsic Astrocyte Heterogeneity Influences Tumor Growth in Glioma Mouse Models. *Brain Pathol*, 2017, 27(1): 36-50.
- [18] Zhang JL, Wang SN, Liu H, et al. Quantitative MRI assessment of glioma response to bevacizumab in a mouse model. *Int J Clin Exp Med*, 2017, 10(10): 14232-14243.
- [19] Inoue E, Ito Y, Kobuchi S, et al. Detection of circulating tumor cells in orthotopic vs. subcutaneous GFP-SUIT2 pancreatic cancer xenograft models. *Cancer Science*, 2021, 112: 878-878.
- [20] Tachibana T, Oyama TG, Yoshii Y, et al. Establishment of an In Vivo Xenograft Mouse Model of a Subcutaneous Submillimeter HT-29 Tumor Formed from a Single Spheroid Transplanted Using Radiation-Crosslinked Gelatin Hydrogel Microwell. *Appl Sci*, 2021, 11(15).