Case Report

Case report of pulmonary metastasis in a male Wistar rat glioblastoma model

Jing Zhou1–3, Xuejing Shi1,3, Yaocheng Li1,3, Shulan Hao1,3, Zhi Guo1,3, Fupeng Zhang3, Yu Gao1,3, Hao Guo4*, and Likun Liu1,3*

1 Department of Oncology, Shanxi Province Academy of Traditional Chinese Medicine, Shanxi Province Hospital of Traditional Chinese Medicine, Taiyuan, Shanxi 030012, China
2 Shanxi University of Chinese Medicine, Taiyuan, Shanxi 030619, China
3 Department of National Traditional Chinese Medicine Clinical Research Base, Shanxi Province Hospital of Traditional Chinese Medicine, Taiyuan, Shanxi 030012, China
4 Department of Anesthesiology, Shanxi Provincial People’s Hospital, Affiliate of Shanxi Medical University, Taiyuan, Shanxi 030000, China

Abstract: Glioblastoma (GBM) is a highly aggressive central nervous system cancer. Its extracranial metastases have rarely been reported in the past few decades. Moreover, the pathogenesis of extracranial GBM metastases remains unclear. Here, we report a case of pulmonary metastasis in a male Wistar rat of C6 GBM model. This reported Wistar male rat was one of the experimental control group without any other intervention except for C6 GBM cells orthotopic implantation. On postoperative day 15, the animal which was reported in this study showed highly cellular, pleomorphic, tumor with nuclear atypia in the brain (Ki67, approximately 65.7%) and lungs (Ki67, 49.5%). Tumor cells in the lung showed immunoreactivity for glial fibrillary acidic protein. Inflammatory CD68+ cell infiltration, weakly positive E-cadherin, and strongly positive staining for vimentin were observed both in tumors in the brain and lungs. Based on further morphological analysis, we speculate that the potential metastatic route into the lung might be hematogenous metastasis. (DOI: 10.1293/tox.2020-0034; J Toxicol Pathol 2021; 34: 95–99)

Key words: glioblastoma, extracranial metastases, pulmonary metastasis, Wistar rat

Background

Glioblastoma (GBM) is a highly aggressive central nervous system (CNS) tumor, accounting for 14.9% of primary tumors and 46.6% of primary malignant tumors of the CNS1, 2. Although chemotherapy moderately increasing survival span which provides hope for therapy to GBM patients, however, lack of bioavailability of the drug from the blood to intracranial tumor cells due to the impermeability of blood-brain barrier (BBB) limits the application of some chemotherapies3. Hence, pre-clinical tests in orthotopic animal models that mimic the influence of the BBB seem relatively more suitable than subcutaneous transplantation tumor models for developing better therapeutic strategies for intracranial GBM. Clinically, extracranial metastases of GBM have rarely been reported in the past few decades4, 5, let alone in orthotopic GBM rats. More disturbingly, the pathogenesis of extracranial GBM metastases is still unclear. In the present study, we report a case of pulmonary metastasis in a male Wistar C6 GBM model rat. This rat was one of the experimental control group and did not receive any other treatments except for C6 GBM cell orthotopic implantation. Several large, round, and unevenly sized masses in the lung were discovered. Pathologic specimens of the intracranial neoplasm and pulmonary nodules were consistent with GBM.

Case Presentation

The adult male Wistar rat was one of an experimental group for GBM induction. Animals (220–250 g) were obtained from SPF (Beijing) Biotechnology Co., Ltd. (Beijing, China) They were housed in the animal room controlled at 22–25°C, 12-h light/dark cycles, and 50 ± 10% humidity. The rats were fed a normal standard chow diet (Beijing Huafukang Bioscience Co., Inc., Beijing, China) and tap water ad libitum. The experimental protocol was established, performed in accordance with the guidelines, and was ap-
proven by the Institutional Animal Care and Use Commiss-
ion of the Shanxi Province Academy of Traditional Chinese
Medicine. Animals were allowed to adapt to the housing
environment before the experiments. They were then anes-
thesized with intraperitoneally administered pentobarbital
sodium (50 mg/kg). Once rats were confirmed to be uncon-
scious by toe-pinch, the cranial region was shaved and the
animals were positioned in a stereotaxic frame (Stoelting
Co., Chicago, IL, USA). After generating a hole in the cra-
nial bone, 5 x 10^6 C6 cells were implanted into the brain.
The coordinates of the injection were 1 mm anterior, 3 mm
lateral to the bregma, and 5 mm ventral to the cortical sur-
face. The injection lasted over 10 min, with the needle kept
in position for an additional 10 min. All surgical procedures
were conducted using sterile instruments in a clean environ-
ment. Bone wax was applied to seal the cranial bone after
surgery. Rats recovered from anesthesia, were returned to
the animal care facility, and had free access to pelleted food
and water.

On postoperative day 15, paraformaldehyde perfusion
was performed for further morphological analysis. Surpris-
ingly, during the process of opening the chest cavity, the
animal reported in this report showed several large, round
masses of uneven sizes in the lungs. To identify pathological
features of pulmonary nodules, hematoxylin and eosin
(H&E) staining and immunohistochemistry of glial fibril-
lary acidic protein (GFAP) were performed. Light micros-
copy showed a highly cellular, pleomorphic, tumor with
nuclear atypia in the brain (Fig. 1A). Immunohistochemical
staining against GFAP confirmed the astrocytic nature of
the orthotopic tumor (Fig. 1B). Figure 2A showed the histo-
logical examination of the pulmonary lobe, in which meta-
static lesions with uneven size were present. Similarly, high
cellular, pleomorphic, and nuclear atypia tumor cells were
observed in the Lung tissue (Fig. 2B). Moreover, tumor cells
in the lung (Fig. 2C and 2D) showed immunoreactivity for
the GFAP, demonstrating the same histological character-
istics as intracranial GBM. Immunohistochemical analysis
showed that the Ki67-positive index was about 65.7% in the
brain and 49.5% in the lung (Fig. 3). Moreover, infiltration
of inflammatory CD68+ cells, weak immunohistochemi-
cally positive E-cadherin, and strongly positive staining for
vimentin were observed both in the tumors in the brain and
lung (Fig. 3). By further morphological analysis, it revealed
big nuclei, hyperchromatism and heteromorphism in cells
that were morphologically similar to C6 cancer cells in the
microvessels of the brain, suggesting the possibility of
hematogenous metastasis. However, the specific molecu-
lar mechanisms underlying the passage through anatomical
hurdles is unclear. Conventional knowledge assumes that
the absence of a true lymphatic system may also prevent
tumor cells from forming extracranial metastases. However,
recently, accumulating evidence has suggested the existence
of intradural lymphatic vessels in the brain13. Hence, extra-
cranial metastases of GBM through the lymphatic system
need further study.

In experimental studies, C6 cells transplanted in rats
generate putative models of human GBM. In our study, C6
GBM cells were orthotopically implanted in Wistar rats.
In our animal experiment, thoracotomy was performed for
perfusion. One rat among hundreds showed several large
round masses of uneven size in the lung, which was proven
to be pulmonary metastasis focus. The pathological speci-
men obtained from this animal will be beneficial for explor-
ing the mechanism of GBM incidence and metastasis in fu-

Discussion

GBM is the most common and malignant adult brain
tumor. The 5-year survival rate is still very low. Current
therapeutic strategies for GBM, such as surgery followed
by radiation or chemotherapy, usually turn out to be invalid
owing to low response or resistance6. In a short period of
time, GBM cells are able to migrate and invade the sur-
rrounding normal brain tissue.

Extracranial metastases of GBM have been rarely re-
ported in past decades. In 1928, the first case of extracranial
GBM metastases was reported8. It is worth noting that there
has been an increase in the number of extracranial dissemi-
nation cases reported in recent years. The development of
diagnostic methods perhaps partly contributed to this in-
crease. Moreover, it has been shown that patients with ex-
tracranial GBM metastases tend to be younger and healthier,
which may be attributed to the longer overall survival.
The relatively longer overall survival in these patients make
them potential developing extracranial metastases8. Nota-
ably, even multiple metastatic cases have been reported. For
instance, in 2018, Rosen et al. reported a case of metastases
in the bones, lung, pleura, liver, mesentery, and subcutane-
ous soft tissue in a patient with GBM9.

Because of the low probability of occurrence, rela-
tively little is known about the underlying pathogenesis
of extracranial GBM metastases. Although the reported
patients with extracranial metastasis tended to be younger
and healthier, but actually the incidence is still low in these
two groups of patients8. This reality reminds us that other
factors rather than time are more related to the rare inci-
dence of extracranial metastases of GBM. Previously, it was
presumed that the anatomical hurdles inherent to the cere-
bral environment help confine systemic dissemination of
GBM10. The compositions of the BBB form a highly selec-
tive microfilter. However, previous study revealed that ves-
sels in high-grade glioma show morphological alterations
compared with the normal ones11. In addition, circulating
tumor cells have been identified in the peripheral blood of
patients with GBM12. Hence, hematogenous spread has been
proposed as a route for extracranial GBM metastases. In the
pulmonary metastasis case reported in the present study, we
found large nuclei, hyperchromatism, and heteromorphism
in cells that were morphologically similar to C6 cancer cells
in the microvessels of the brain, suggesting the possibility
of hematogenous metastasis. However, the specific molecu-
lar mechanisms underlying the passage through anatomical
hurdles is unclear. Conventional knowledge assumes that
the absence of a true lymphatic system may also prevent
tumor cells from forming extracranial metastases. However,
recently, accumulating evidence has suggested the existence
of intradural lymphatic vessels in the brain13. Hence, extra-
cranial metastases of GBM through the lymphatic system
need further study.

In experimental studies, C6 cells transplanted in rats
generate putative models of human GBM. In our study, C6
GBM cells were orthotopically implanted in Wistar rats.
In our animal experiment, thoracotomy was performed for
perfusion. One rat among hundreds showed several large
round masses of uneven size in the lung, which was proven
to be pulmonary metastasis focus. The pathological speci-
men obtained from this animal will be beneficial for explor-
ing the mechanism of GBM incidence and metastasis in fu-
Fig. 1. Hematoxylin and eosin (H&E) staining (A, ×400, bar = 50 μm) and immunohistochemical staining of glial fibrillary acidic protein (GFAP) (B, ×400, bar = 50 μm) exhibit the histological characteristics of glioblastoma (GBM) in the brain.

Fig. 2. The histological examinations of the pulmonary lobe, in which many metastatic lesions with uneven size are presented (A, ×100, bar = 200 μm). Hematoxylin and eosin (H&E) staining showed a highly cellular, pleomorphic tumor with nuclear atypia in the lung (B, ×400, bar = 50 μm). Immunohistochemically, tumor cells in the lungs show immunoreactivity for glial fibrillary acidic protein (GFAP) (C, ×200, bar = 100 μm; D, ×400, bar = 50 μm).
Fig. 3. Immunohistochemical analysis showed that the Ki67-positive index is about 65.7% in the brain and about 49.5% in the lung. Moreover, infiltration of inflammatory CD68+ cells, weak immunohistochemical positive E-cadherin and strong positive staining for vimentin were observed both in the tumors in the brain and lung (×200, bar = 100 μm).
Fig. 4. Hematoxylin and eosin (H&E) staining demonstrated big nuclei, hyperchromatism and heteromorphism cells that the morphology of which is consistent with the C6 cancer cells were present in the vascular structure in both the brain (A, ×400, bar = 50 μm) and lungs (C, ×400, bar = 50 μm). The immunohistochemical analysis further demonstrated that cancer cells were present in the CD31+ microvessel (B, brain; and D, lung, ×400, bar = 50 μm).

Medicine of Shanxi Province (2020ZYYC022).

References

1. Ostrom QT, Gittleman H, Xu J, Kromer C, Wolinsky Y, Kruchko C, and Barnholtz-Sloan JS. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2009–2013. Neuro-oncol. 18(suppl_5): v1–v75. 2016.

2. Wirsching HG, Galanis E, and Weller M. Glioblastoma. Handb Clin Neurol. 134: 381–397. 2016.

3. Dréan A, Goldwirt L, Verreault M, Canney M, Schmitt C, Guehennec J, Delattre JY, Carpentier A, and Idbaih A. Blood-brain barrier, cytotoxic chemotherapies and glioblastoma. Expert Rev Neurother. 16: 1285–1300. 2016.

4. Waite KJ, Wharton SB, Old SE, and Burnet NG. Systemic metastases of glioblastoma multiforme. Clin Oncol (R Coll Radiol). 11: 205–207. 1999.

5. Piccirilli M, Brunetto GM, Rocchi G, Giangaspero F, and Salvati M. Extra central nervous system metastases from cerebral glioblastoma multiforme in elderly patients. Clinico-pathological remarks on our series of seven cases and critical review of the literature. Tumori. 94: 40–51. 2008.

6. Sorribes IC, Handelman SK, and Jain HV. Mitigating temozolomide resistance in glioblastoma via DNA damage-repair inhibition. J R Soc Interface. 17: 20190722. 2020.

7. Davis L. Spongioblastoma multiforme of the brain. Ann Surg. 87: 8–14. 1928.

8. Blume C, von Lehe M, van Landeghem F, Greschus S, and Boström J. Extracranial glioblastoma with synchronous metastases in the lung, pulmonary lymph nodes, vertebrae, cervical muscles and epidural space in a young patient - case report and review of literature. BMC Res Notes. 6: 290. 2013.

9. Rosen J, Blau T, Grau SJ, Barbe MT, Fink GR, and Gall-diks N. Extracranial metastases of a cerebral glioblastoma: a case report and review of the literature. Case Rep Oncol. 11: 591–600. 2018.

10. Ray A, Manjila S, Hdeib AM, Radhakrishnan A, Nock CJ, Cohen ML, and Sloan AE. Extracranial metastasis of glioblastoma: Three illustrative cases and current review of the molecular pathology and management strategies. Mol Clin Oncol. 3: 479–486. 2015.

11. Coomber BL, Stewart PA, Hayakawa K, Farrell CL, and Del Maestro RF. Quantitative morphology of human glioblastoma multiforme microvessels: structural basis of blood-brain barrier defect. J Neurooncol. 5: 299–307. 1987.

12. Müller C, Holtschmidt J, Auer M, Heitzer E, Lamszus K, Schulte A, Matschke J, Langer-Freitag S, Gasch C, Stoupiec M, Mauermann O, Peine S, Glatzel M, Speicher MR, Geigl JB, Westphal M, Pantel K, and Riethdorf S. Hematogenous dissemination of glioblastoma multiforme. Sci Transl Med. 6: 247ra101. 2014.

13. Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, Derecki NC, Castle D, Mandell JW, Lee KS, Harris TH, and Kipnis J. Structural and functional features of central nervous system lymphatic vessels. Nature. 523: 337–341. 2015.