J Toxicol Pathol 2021; 34: 95–99

Case Report

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

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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 CNS[1, 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 chemotherapies[3]. 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 decades[4, 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-

Received: 15 May 2020, Accepted: 25 September 2020
Published online in J-STAGE: 19 December 2020
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proved by the Institutional Animal Care and Use Committee of the Shanxi Province Academy of Traditional Chinese Medicine. Animals were allowed to adapt to the housing environment before the experiments. They were then anesthetized with intraperitoneally administered pentobarbital sodium (50 mg/kg). Once rats were confirmed to be unconscious 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 cranial bone, 5×10^6 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 surface. 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 environment. 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. Surprisingly, 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 fibrillary acidic protein (GFAP) were performed. Light microscopy 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 histological examination of the pulmonary lobe, in which metastatic 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 characteristics 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 immunohistochemically 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 cells that the morphology of which is consistent with the C6 cancer cells (Fig. 4A and 4C, H&E staining) were present in the $CD31^+$ microvessels (Fig. 4B and 4D).

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 resistance[6]. In a short period of time, GBM cells are able to migrate and invade the surrounding normal brain tissue.

Extracranial metastases of GBM have been rarely reported in past decades. In 1928, the first case of extracranial GBM metastases was reported[7]. It is worth noting that there has been an increase in the number of extracranial dissemination cases reported in recent years. The development of diagnostic methods perhaps partly contributed to this increase. Moreover, it has been shown that patients with extracranial 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 metastases[8]. Notably, 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 subcutaneous soft tissue in a patient with GBM[9].

Because of the low probability of occurrence, relatively 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 patients[8]. This reality reminds us that other factors rather than time are more related to the rare incidence of extracranial metastases of GBM. Previously, it was presumed that the anatomical hurdles inherent to the cerebral environment help confine systemic dissemination of GBM[10]. The compositions of the BBB form a highly selective microfilter. However, previous study revealed that vessels in high-grade glioma show morphological alterations compared with the normal ones[11]. In addition, circulating tumor cells have been identified in the peripheral blood of patients with GBM[12]. 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 molecular 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 brain[13]. Hence, extracranial 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 specimen obtained from this animal will be beneficial for exploring the mechanism of GBM incidence and metastasis in fu-

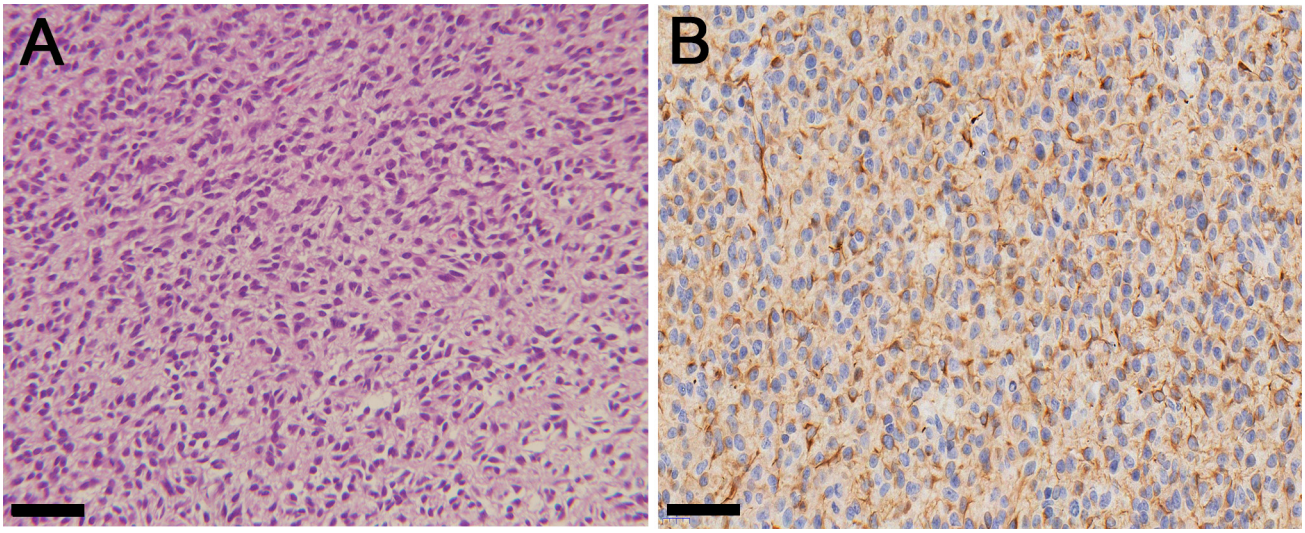

Fig. 1. Hematoxylin and eosin (H&E) staining (A, ×400, bar = 50 μm) and immunohistochemical staining of glial fibrillary acidic protein (GFAP) (B, ×400, bar = 50 μm) exhibit the histological characteristics of glioblastoma (GBM) in the brain.

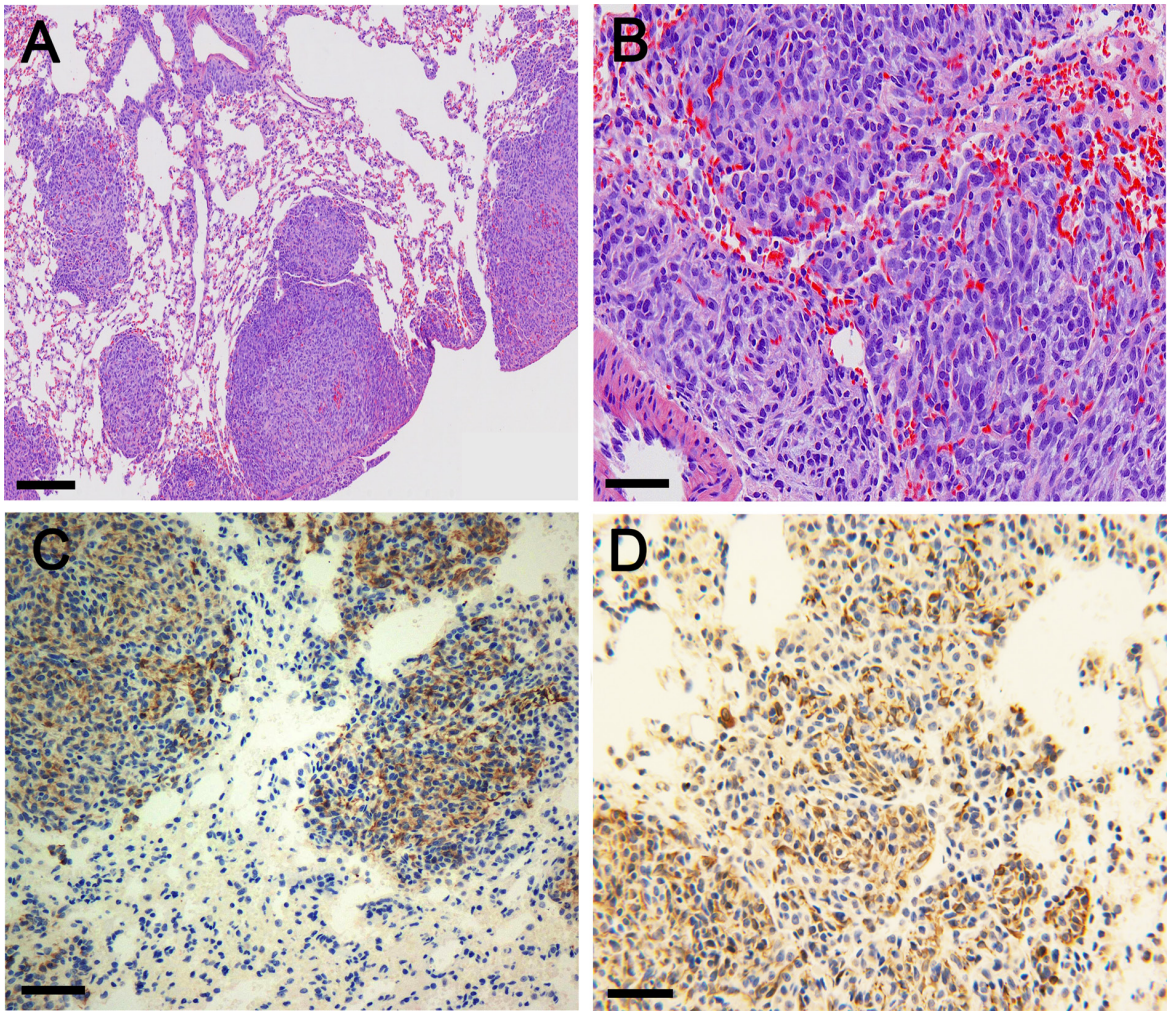

Fig. 2. The histological examinations of the pulmonary lobe, in which many metastatic lesions with uneven size are presented (A, ×100, bar = 200 μm). Hematoxylin and eosin (H&E) staining showed a highly cellular, pleomorphic tumor with nuclear atypia in the lung (B, ×400, bar = 50 μm). Immunohistochemically, tumor cells in the lungs show immunoreactivity for glial fibrillary acidic protein (GFAP) (C, ×200, bar = 100 μm; D, ×400, bar = 50 μm).

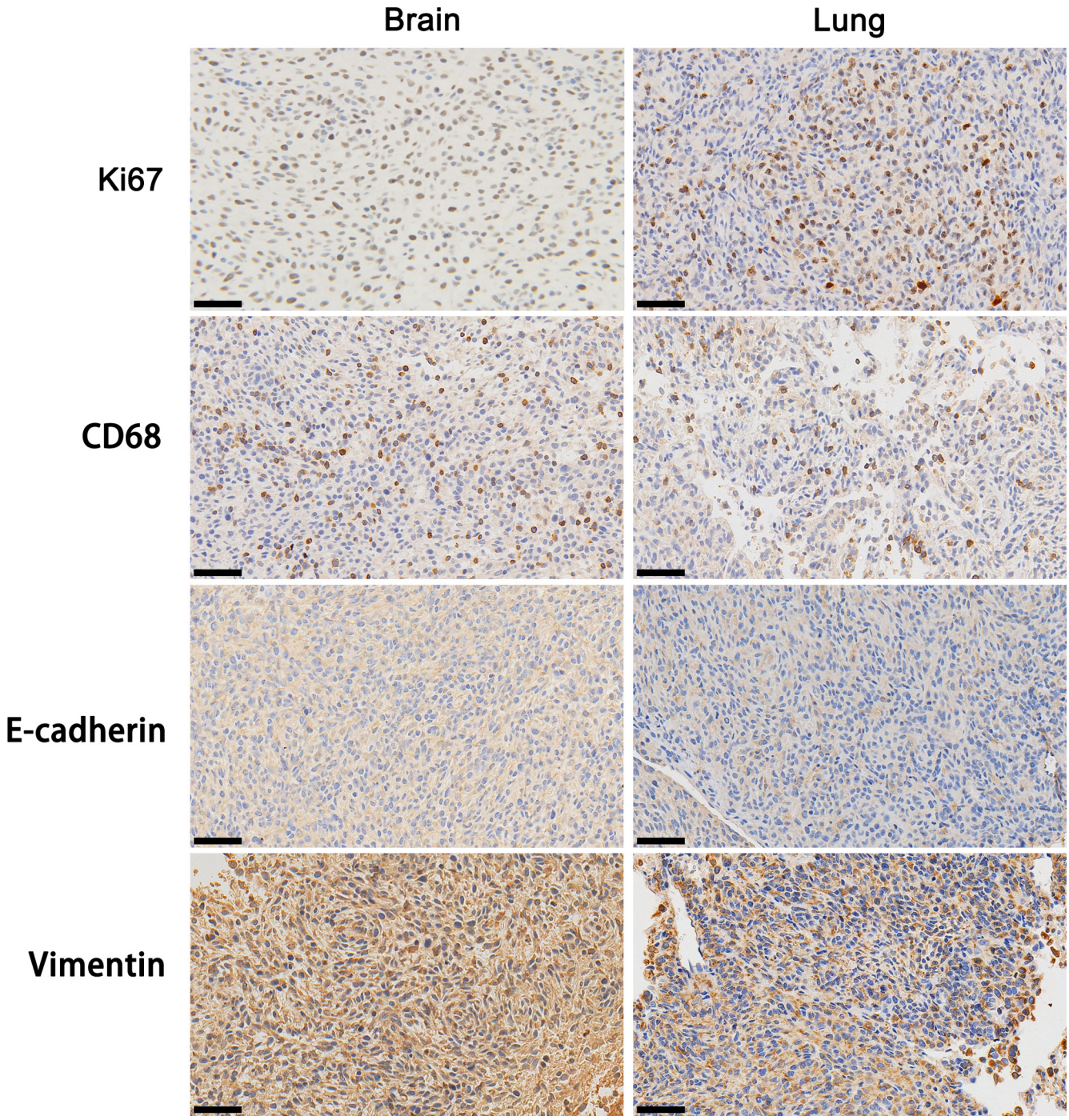

Fig. 3. Immunohistochemical analysis showed that the Ki67-positive index is about 65.7% in the brain and about 49.5% in the lung. Moreover, infiltration of inflammatory $CD68^+$ cells, weak immunohistochemical positive E-cadherin and strong positive staining for vimentin were observed both in the tumors in the brain and lung (×200, bar = 100 μm).

ture studies.

Disclosure of Potential Conflicts of Interest: The authors have no conflicts of interest in connection with this study.

Acknowledgment: This study was supported by the National Natural Science Foundation of China (No. 81904183), the Project of Health Commission of Shanxi Province (No. 2018078), the Natural Science Foundation of Shanxi Province (No. 201801D121300), the cultivation project of Science and Technology Innovation Ability of Shanxi University of Chinese Medicine (2019PY-017), the Project of Shanxi Province Academy of Traditional Chinese Medicine (201901), and the project of Administration of Traditional Chinese

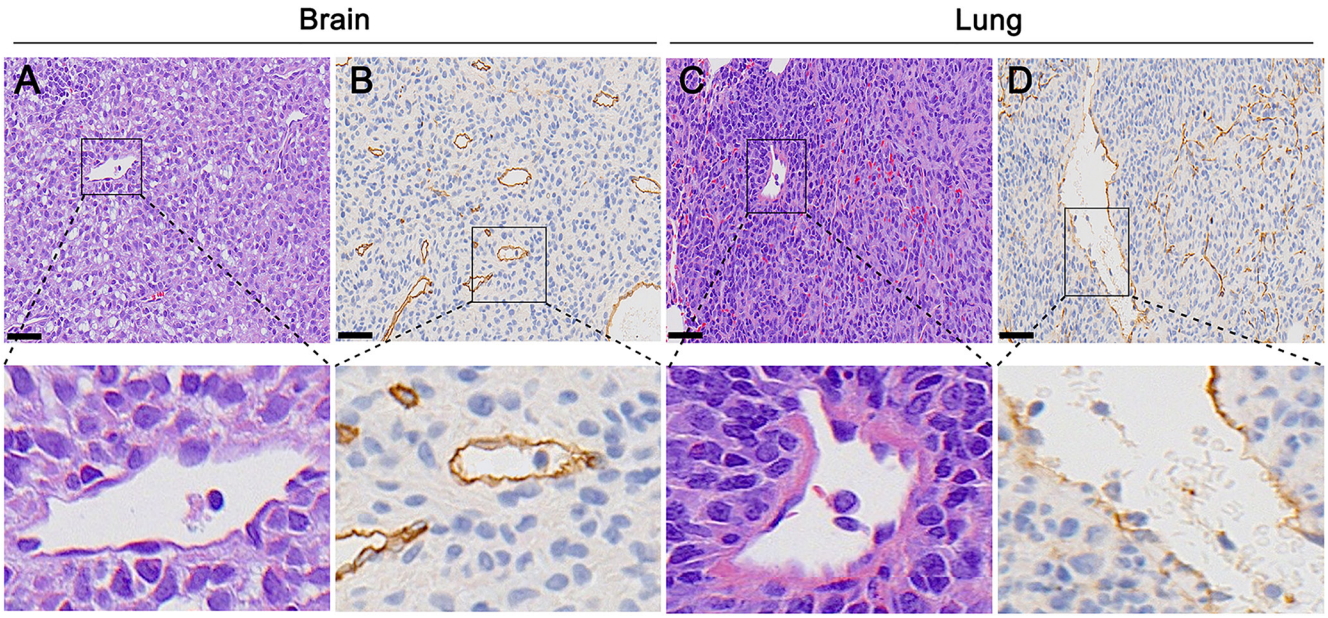

Fig. 4. Hematoxylin and eosin (H&E) staining demonstrated big nuclei, hyperchromatism and heteromorphism cells that the morphology of which is consistent with the C6 cancer cells were present in the vascular structure in both the brain (A, ×400, bar = 50 μm) and lungs (C, ×400, bar = 50 μm). The immunohistochemical 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).

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