Investigation on Changes of Blood-brain-barriers in Lesions of Brain Metastasis with Immunohistochemical and Electron Microscope

Ruilin Wang, Wenguang Song, Zhanzhao Fu, Zhiyong Zhang, Yu Wu, Yanfeng Zhong① Shenglan Wang①

Tangshan Worker´s Hospital of He bei Medical University, Tangshan 063000, China
① Department of Pathology, Medical School, Beijing University

Abstract **Objective** To investigate the immunohistochemical and ultramicroscopic features of brain metastasis and illustrate the changes of blood--brain-barrier in the metastasis tumor tissue and its clinical meaning. **Methods** With immunohistochemistry staining, CD34、F-Ⅷ、S-100、GFAP、NF、NSE、SYN and MBP were detected in tumor tissue which were resected from 16 patients with brain metastasis and 16 with glioma. Meanwhile fresh specimens of 4 metastatic tumors and 2 gliomas were observed under the electron microscope. **Results** CD34 and F-Ⅷ were richly expressed both in the metastatic tumor and glioma tissues, which indicated the foundation of new blood vessels; on the other hand, nervous marker tissues such as S-100、GFAP、NF、NSE、SYN and MBP, were rarely expressed in metastatic tumor tissue and strongly expressed in glioma tissues, which indicated that there was no fundamental structure of blood-brain-barrier-neuralgia membrane. Just the same as those in their primary lesions, the ultramicroscopic characters of metastatic tumors included that endothelium cells were vacuolate, the conjunction of endothelium cells was not always tight and the basilar membrane was slightly thinner than that of glioma, besides there was no neuralgia foots around the blood capillary. **Conclusion** Neuroglia, the basic integrant of blood-brain-barrier is defective in the metastatic tumor tissue, and the endothelium cells of the blood vessels are not neatly and tightly connected, thus there is no complete blood–brain–barrier existing in the brain metastatic tumor lesions.

Key Words Brain tumor; Blood–brain–barrier; Immunohistochemistry; Electron microscope; chemotherapy

Almost 25% -35% of the malignancy patients have brain metastasis during the illness and finally die of it. Compared with the brain primary tumor, the brain metastatic tumor has different reaction to the therapy especially the chemical therapy so that the water–soluble medicine is difficult to be effective in the glioma, yet in the metastatic tumor the similar curative effect as that in the primary lesion can be gained. In order to investigate the intrinsic discrepancy, we analyze the two microstructures respectively by means of immunohistochemistry and electron microscope as is reported below.

MATERIALS AND METHODS

Clinical Data

Immunohistochemical specimen From January 1989 to December 2002, 16 specimen with brain metastasis were collected in our hospital, 14 of which had the primary affection of lung cancer (pathological types are 4 glandular cancer, 2 squamous cancer, 7 small cell carcinoma and one glandular squamous cancer), one came from the hepatocellular carcinoma and one was mammary gland aggressive duct cancer. At the same time, we randomly chose 16 specimens after the glioma tissues operation to make a comparison. The pathological grades were 4 specimens in grade Ⅰ－Ⅱ, 10 in grade Ⅱ－Ⅲ and 2 glioblastoma multiform (Ⅳ grade).

Electron microscope specimen We collecte 12 fresh brain tumor specimens resected in the operation by our neurosurgery from January 2002 to December 2003. There were four brain metastasis, two lung cancer, one low– differentiated glandular cancer, one low– differentiated squamous cancer, one

* This subject is supported by the Scientific Technology Research and Developing and Planning Program of the city of Tangshan(Serial Number: 021460 13A–9)

hepatocellular carcinoma, one mammary gland aggressive duct cancer and two glioma tissues (Ⅱ-Ⅲ grade).

Main Reagent

We bought the rat antibody GFAP、 S-100、 NSE、 NF、 CD34 and the rabbit antibody SYN、 MBP、 FV-Ⅲ、 SP kit and so on all from the Fujian Maixin Biotechnology Company.

Methods

Immunohistochemical SP method Paraffin sections were dewaxed by the xylene and hydrated by the gradient ethanol. Antigen was exposed: put the sections into the 10 mmol/L sodium citrate buffer (PH6.0) and repaired the antigen for 2 minutes under the high temperature and pressure, then added the antibody treatment fluid and stayed warmly cared over night at 4℃. DAB was coloring and the hematoxylin contrast stained. We replaced PBS with the antibody to make a negative comparison and used the pictures provided by the Maixin Company for the positive comparison.

Making the electron microscope specimen Put the fresh specimen resected in the operation into the 3% OHC (CH2) 3CHO fixed and sealed in the 4℃ fridge. Fixed in 1% osmium tetroxide for 1.5 hour, hydrated with 50%-100% alcohol stage by stage, embed the epoxy resin, thinly slices (50-70mm) were made, stained with uranium and lead and observed with the JEM-100 electron microscope.

Results Judgment Under the electron microscope, 100 target structure cells were chosen in every slice randomly with 400-time-view, according to the staining strength and the percentage of the positive cells, the specimen may be classified into negative and positive. When stainless cells or positive cells≤10% were negative and ones >10% were positive.

RESULTS

Immunohistochemistry

The expression of CD34 and F-Ⅷ (Fig. 1) The positive numbers of the metastasis tumor tissue CD34 and F-Ⅷ were 10 (62.5%) and 13 (81.3%). While the positive numbers of the glioma tissues CD34 and F-Ⅷ were 9 (56.3%) and 15 (93.8%). There were no remarkable differences between the two tissues.

The expression of the S-100、 GFAP、 NF、 NSE、 SYN and MBP In the metastasis tumor tissues, three were positive (from the small cell lung cancer) except NSE and the rest were all negative (Table 1).

Electron Microscope Observation

In the glioma tissues, small blood vessels had obvious hyperplasia, endothelium cells were thick and partly vacuolate, vascular walls got thick, basilar membrane substances under the endothelium cells increased, a little collagenic fiber was visible and there were some neurogia foots around the blood vessels. The density of the capillary in the metastasis tumor tissue was smaller than that in the glioma tissue, but the endothelium cells were almost vacuolate, gaps between part area got enlarged, basilar membrane was thin and asymmetrical, between the tumor cells lymphocytes were visibly wetted and there were no neurogia foots

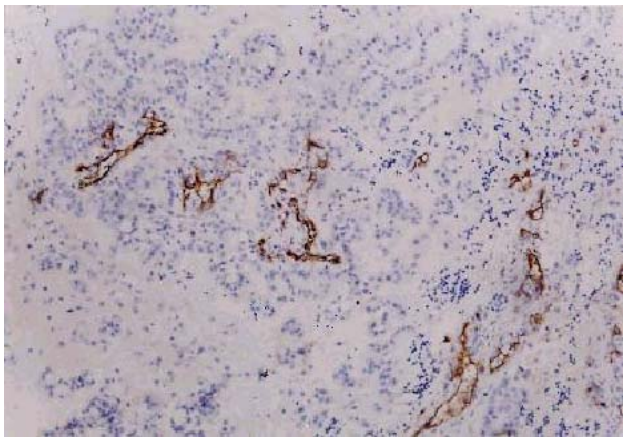

Fig.1 The positive expression of CD34 in the brain metastasis Immunohistochemistry, SP×100

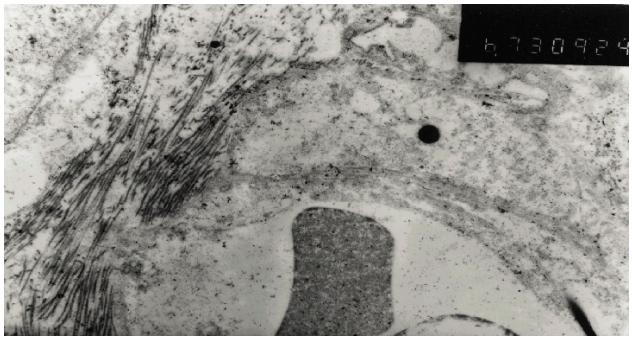

Fig. 2 Parts of the endothelium is loosely connected in the blood vessels of the metastasis tumor tissue

Table 1 The expression of nervous tissue relating marks in the two tissues

Types	Numbers	S-100	GFAP	NF	NSE	SYN	MBP
Metastasis tumor tissue	16	0(0.0)	0(0.0)	0(0.0)	3(18.8)	0(0.0)	0(0.0)
Glioma tissues	16	12(75.0)	14(87.5)	14(87.5)	12(75.0)	11(68.8)	11(68.8)

around the periphery of the blood capillary (**Fig. 2**). Moreover, the case from the hepatoma had a few extremely small blood vessels in the structure of hemal sinus.

DISCUSSION

Among the metastasis of the malignant tumor, the brain metastasis ranks the third, only next to the lung metastasis and the liver metastasis. Because the symptoms of the brain metastasis are more serious and the living quality will be badly affected, most patients will die in about 4 weeks without active treatment. Many years because of the bound of the blood-brain-barrier theory, the position of the chemical therapy in the treatment of the metastasis tumor tissue was not established. Some scientists have ever found that the general chemical therapy has the similar ultramicroscopic characters as that in the primary lesion and predicted that the blood-brain-barrier of the metastasis tumor tissue has been partly destroyed, but there was no further research on it.

The structural basis of the blood-brain-barrier is:

(1) Tight endothelium cells of the capillary;

(2) Capillary basilar membrane;

(3) Glioma membrane formed of the surrounding star-shaped neuralgia foots outside the capillary basilar membrane.

Our research used the endothelium markers of the blood vessel and the relating markers of many nerve tissues to immunohistochemistrical stain the metastasis tumor tissue and the glioma tissues respectively. CD34 and F-Ⅷ were the membrane protein located in the endothelium membrane of the blood vessel, whose existence marked the establishment of the capillary in the tissues. S-100、GFAP、NF、NSE、SYN and MBP reflected the markers of the neurogliocyte filum terminale or the minute fibers structure especially the gelatine fiber acid protein (GFAP), which was related to the formation and the maintaining of the star-shaped neurogia apophysis. The existence of the GFAP determined whether the glioma membrane in the structural basis of the blood-brain-barrier existed or not.

This result showed that the blood vessel endothelium markers such as CD34 and F-Ⅷ were richly expressed both in the metastatic tumor and glioma tissues, which indicated that both the metastatic tumor and glioma tissues had their own supplying blood vessels. Nervous marker tissues such as S-100、GFAP、NF、NSE、SYN and MBP, were rarely expressed in metastatic tumor tissues and strongly expressed in glioma tissues, which indicated that there was no fundamental structure of blood-brain-barrier-neuralgia membrane, which meant that the Glioma membrane formed by the surrounding star-shaped neuralgia foots was defective around the vascular walls in the metastatic tumor mesenchyma. But the expression of the NSE in parts of the brain metastasis was in concordance with its original small cell lung cancer and had no connection with the blood-brain-barrier. The electron microscope observation offered the further illustration that the endothelium cells were not neatly connected in the capillary of the metastatic tumor, frequently with the vacuolation or other inflammation changes, basilar membrane around the blood vessels were very thin, neurogia foots did not exist and there only were tumor interstitial cells or tumor cells. The structure of hemal sinus of the extremely small blood vessels in the liver cancer brain metastasis gave a further explanation that the blood vessels of the metastatic tumor had the characters of its primary lesion, not the characters of the central nervous system just because it was metastasized into the brain. Therefore, we hold that during the process of the metastatic tumor forming the metastasis lesion, there is no complete blood-brain-barrier all the way. This conclusion will provide valuable theoretical evidence for the clinical medication of the metastatic tumor.

REFERENCE

1. Yan Sun. Internal Medicine Tumor Studies. Beijing: Peoples Medical Publishing House, 2001. 504
2. Hoskin P, Crow J, Ford HT. The influence of extent and local management on the outcome of the radiotherapy for brain metastasis. Int J radiat Oncol Biol Phys, 1990, 19 (1): 111–115.
3. Kleisbauer JP, Vesco D, Orehek J, et al. Treatment of brain metastasis of lung cancer with high dose of etopo side (VP16–213): Cooperative study from the Group Francais Pheumocancerologie. Eur J Cancer Clin Oncol, 1988, 24(2): 131–135.
4. Tummarello D, Lippe P, Bracci R, et al. First line chemotherapy in patients with brain metastasis from non–small and small cell lung cancer. Oncol Rep, 1998, 5(4): 897–900.
5. Franciosi V, Coccino G, Michiara M et al. Front–line chemotherapy with cisplatin and etoposide for patients with brain metastasis from breast carcinoma, non–small cell lung carcinoma or malignant melanoma. Cancer, 1999, 85(7): 1599–1605.
6. Zhaoyou Tang. Modern Tumor Studies. 2nd. Shanhai: Shanghai Medicine University Publishing House, 2000, 88–90.
7. Kantanrjian H, Farha PA, Spitzer G, et al. Systemic combination chemotherapy as primary treatment of brain metastasis from lung cancer. South Med J, 1984, 77(4): 426–430.
8. Patcbell RA. Brain metastasis and carcinomatous meningi–tis. In: Abbeloft MD, Armitage JO, Lichter AS, et al. Clinical Oncology. 2nd. Churchill Living-stone, New York. 2000, 820–835.
9. Greig N, Jones H, Cavanagh J. Blood brain barrier integrity and host responses in experimental metastatic brain tumor. Clin exp Metastasis, 1983, 1(3): 229–246
10. Lingzhong Cheng. Histology and embryology. 4th. Beijing: Peoples Medical Publishing House, 1996, 101–105.
11. Stephen S, Sternburg. Translator: Hui Yunzhong. Diagnostic Surgical Patholgy. 3rd. Beijing University Medicine Press, 2003, 139–140.
12. Qingzhong Xu. Central nervous system tumor diagnosing pathological tomoscan. 3rd. Beijing: Beijing Science and Technology Literature Publishing House, 2000, 11–15.