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The Role of Laminin on Cultured Human Glioma Cell Proliferation and Metastasis in Nude Mice and Rats

Xue-Yun Zhong,* Masachika Senba,* Yun-Xian Chen,** Takashi Nishigami*** and Hideki Miyaji***

*Department of Pathology, Institute of Tropical Medicine, Nagasaki University, 12-4 Sakamoto-machi, Nagasaki 852, Japan
**Department of Internal Medicine, Atomic Disease Institute, Nagasaki University School of Medicine, 12-4 Sakamoto-machi, Nagasaki 852, Japan
***Department of Pathology, Hyogo College of Medicine, Mukogawa-cho Nishinomiya City, Hyogo 663, Japan

It is difficult to propose simple idea in which cell adhesion molecules can be related to tumor growth and metastasis. The integrins function as extracellular matrix receptors binding to such proteins as collagen, laminin, and fibronectin. In this study, the cell culture of human glioma cells were xenografted to nude mice and rats associated with laminin injection, resulting in tumor growth and metastasis in the large amount of laminin group. In nude mouse experiment, cultured glioma cells were xenografted in the back of cutaneous with 10μg and 100μg laminin. The tumor lump of the 100μg laminin group grew faster and larger than the 10μg laminin group. In nude rat experiment, cultured glioma cells were xenografted intravascularly in the tail with 10μg and 100μg laminin. Of the 6 cases, 4 (66.7%) showed metastasis in the lungs after 2 weeks in the 100μg laminin group. However, metastasis was not detected in 10μg laminin group. These results suggest that quantities of laminin may play an important role in tumor growth and metastasis.

Introduction

Malignant tumor cell attachment and migration is thought to play a crucial role in the complex multistep process of tumor invasion and metastasis. It was found that basement membranes are important extracellular matrix structures that play key roles in morphogenesis, mitogenesis, maintenance of normal tissue architecture, tumor invasion and metastasis.[8,9,15-17] This metastasis formation by way of complex series of sequential steps involving specific tumor cell and host properties is now widely accepted.[1] In our previous report,[18] when a low grade glioma (grade I-II) was passed through glioma cell culture at 19th passage, these tumor cells were malignantly transformed (grade IV). By histochemical and immunohistochemical analysis, malignantly transformed glioma cells can produce laminin. However, relationships among laminin quantities, tumor proliferation, and metastasis is unknown.

To understand the role of integrins in tumor growth and metastasis, it is important to investigate their action in a variety of human malignancies, because they are somewhat cell-type specific. To the best of our knowledge, the cell culture of human glioma was not yet studied for integrins in tumor growth and metastasis. Therefore, the authors established a human glioma cell line and it was used in this experiment. To examine tumor growth and metastasis, cultured human glioma cells were xenografted to nude mice and rats associated with small or large amount of laminin.

All correspondence to: Dr. Masachika Senba, Department of Pathology, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852, Japan

Materials and Methods

1. Cell cultures:

The human SWO-38 glioma cell line was provided by Dr. Rui (Department of Pathology, Jinan University, Guangzhou, China). This cell line was established from tissue culture of neurosurgical specimen. The patient was a 12-year-old Chinese male. This tumor grew on the cerebellaris medius. The tumor was diagnosed as glioma (grade 1-11) with glial fibrillary acidic protein (GFAP) positive by histopathological procedure. The cell line was maintained in PRMI 1640 medium with 10% FCS (fetal calf serum), penicillin (100Iu/ml), and streptomycin (100μg/ml). They were kept in a standard tissue culture in the incubator with 5% CO_2. After cultivation, the cell line was cloned at limiting dilution.

2. Subcutaneous injection of cells with laminin into nude mouse:

Nude 5-week-old, male, BALB/c-nu/nu mice were divided into 3 groups of 10 mice per groups: Group 1 was given laminin 100μg/mouse in 0.1ml of PBS with 2 X 10^5 of culture cells injected subcutaneously in the back, Group 2 was given laminin 10μg/mouse in 0.1ml of PBS with 2 X 10^5 of culture cells injected subcutaneously in the back, and Group 3 for control. Subcutaneous tumors were observed 3

to 20 days after injection. They were examined by histochemical and immunohistochemical studies.

3. Tail intravascular injection of culture cells with laminin into SD rat:

Six-week-old male SD rats were divided into 2 groups (6 rats/group): in the first group, laminin 100μg/rat in 0.5ml of PBS with 2 X 10^5 of culture cells was injected intravascularly in the tail. The second group (control group), laminin 10μg/rat in 0.5ml of PBS with 2 X 10^5 of culture cells was injected intravascularly in the tail. The rats were killed after 14 days with examination of the following organs: the lungs, liver, pancreas, heart, kidneys and spleen. The specimens were stained with hematoxylin-and-eosin, and immunohistochemistry.

4. Histological and immunohistochemical analysis:

The specimens were fixed in 10% formalin, and embedded in paraffin for histopathological and immunohistochemical studies, sections were cut at 4 micron and stained with hematoxylin and eosin (H & E) stain for histological examination. These specimens were stained by the avidin-biotin-peroxidase complex (ABC) methods for glial fibrillary acidic protein (GFAP) (polyclonal antibody), vimentin (monoclonal antibody), laminin (monoclonal antibody), fibronectin (monoclonal antibody), and keratin (polyclonal antibody). The primary antibodies used were DAKO, Copenhagen, Denmark.

The steps involved in the ABC immunohistochemical procedure (Vector Laboratories; U. S. A.) are as follows: (1) Deparaffinize and hydrate tissue sections through xylenes and graded alcohol series. (2) Rinse for 5 minutes in distilled water. (3) Treat with hydrogen peroxidase for 5 minutes. (4) Wash slides in Tris buffer pH 7.4 using three cycle changes of 5 minutes. (5) Treat with normal serum for 20 minutes. (6) Treat with blot excess normal serum from sections. (7) Incubate sections for 30 minutes with primary antiserum diluted in Tris buffer pH7.4. (8) Wash slides Tris buffer pH7.4 using three cycle changes of 5 minutes. (9) Incubate sections for 30 minutes with diluted biotinylated antibody solution. (10) Wash slides in Tris buffer pH7.4 using three cycle changes of 5 minutes. (11) Incubate sections for 30 minutes with ABC reagent. (12) Wash slides in Tris buffer pH7.4 using three cycle changes of 5 minutes. (13) Incubate sections for 5 minutes in peroxidase substrate solution. (14) Wash slides in running water for 5 minutes. (15) Treat with nuclei stain in Mayer's hematoxylin for 2 minutes. (16) Wash slides in running water for 5 minutes. (17) Dehydrate, clear, and mount.

Results

1. Xenograft of cultured glioma cells to nude mouse:

As shown in Fig. 1, nude mice were inoculated subcutaneously with 2 X 10^5 cultured glioma cells. The tumor appeared in the laminin 100μg group, the laminin 10μg group, and control group. A visible tumor nodules have been observed in the laminin 100μg group, and is about twice as big in size than the other groups (Fig. 2). In the laminin 100μg group, subcutaneous transplanted glioma cells, tumor nodules appeared first on the 5th day after injection. On the 20th day tumor nodules grew 30mm in diameter, with central necrosis and hemorrhage.

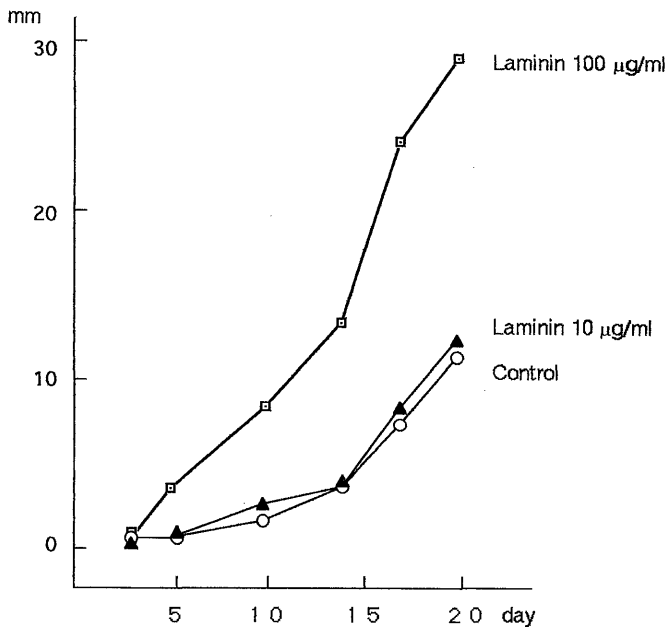

Fig. 1. Glioma cell proliferation of laminin 100μg group was more rapid than laminin 10μg group.

2. Xenograft of cultured glioma cells to SD rat:

The 2 X 10^5 glioma cells were injected intravascularly into the tail vein of SD rats, and the rats were killed on the 14th day after tail intravascular injection. In the laminin 100μg group, metastasis to the lungs appeared in four (66.7%) of six rats. Cut surface of the lungs showed multiple metastatic nodules which were reddish, 0.1 to 0.2mm in diameter with congestion and edema. Histologically, metastatic pulmonary nodule showed epithelial-like cells, oval to round nuclei, cellular polymorphism, mitosis, and necrosis (Fig. 3). Other organs showed no metastasis. On the other hand, control group did not show any metastatic nodule.

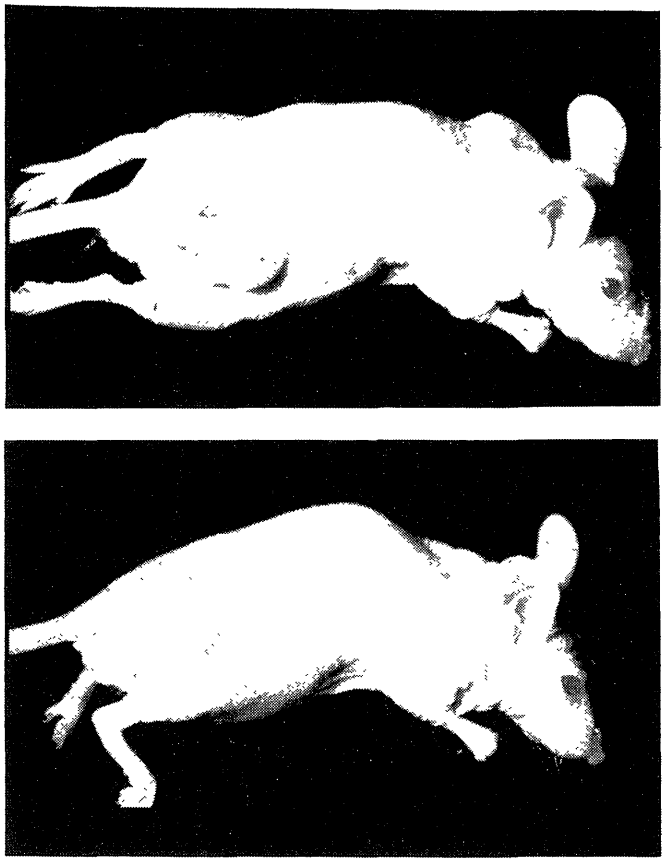

Fig. 2. Xenograft of glioma cells were seen sulface of back of nude mouse. The tumor cell proliferation of laminin 100 μg group (upper position) area was wilder than laminin 10 μg group (lower position).

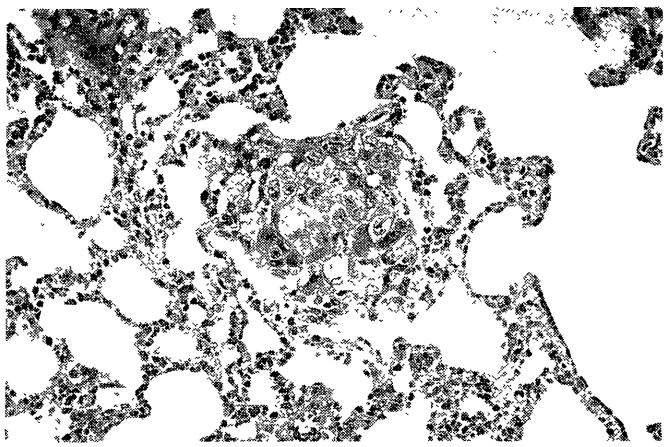

Fig. 3. Glioma cells were injected into the tail vein of SD rat. The laminin 100 μg group was appeared metastasis nodule in the lung. Hematoxylin-and-eosin stain, X 200.

3. Immunohistochemical examination of results:

All xenograft tumor cells were positively stained with laminin, fibronectin, and vimentin, on the other hand, GFAP, and keratin were negatively stained. Laminin, fibronectin, and vimentin were diffusely distributed in the cytoplasm of the tumor cells (Fig. 4).

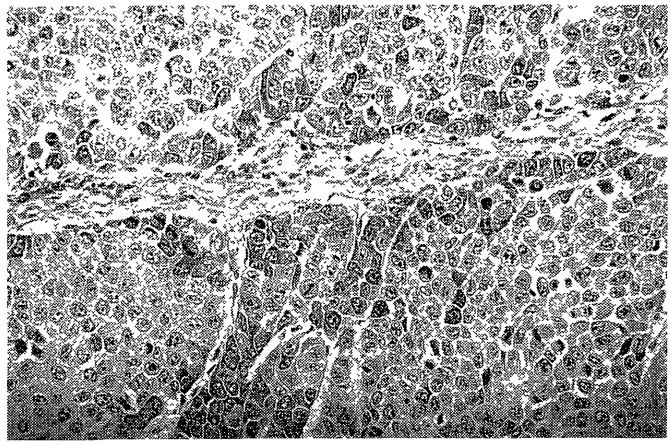

Fig. 4. Invasive growth of glioma cells were detected in the back of subcutaneous of nude mouse. These tumor cells were stained with laminin antibody. Immunoreaction for laminin, X 200.

Discussion

Proliferation, invasion, and metastasis of malignant tumor cell is the major cause of death for cancer patients. Recently, Dr. Albelda reviewed[1] that the process of tumor growth and metastasis is a complex cascade of events that includes the following steps: 1) Tumor growth, invasion, and release of neoplastic cells from the primary tumor. 2) Movement of tumor cells into the lymphatics or vasculature. 3) Survival of the tumor cells within the circulation and interaction of cells with platelets and with the clotting system. 4) Arrest of the tumor cells in distant sites via interactions with vascular or lymphatic endothelium and/or subendothelial basement membrane. 5) Migration of tumor cells into tissue parenchyma. 6) Growth of the tumor at the metastatic site. Extracellular matrices are composed of macromolecules that include fibronectin, laminin, collagen, and proteoglycans. It is found that extracellular matrix has focused on the interaction of tumor proliferation, invasion, and metastasis. The metastasizing tumor cells must interact with the matrix at many stages of tumor proliferation, invasion, and metastasis.[7,8,16] The interaction of cells with the extracellular matrix is mediated by the surface receptors. The receptors for fibronectin, type I collagen, and laminin have been identified. Of these proteins at least fibronectin, type I collagen, and laminin have the three amino-acid sequence, arginin-glycine-aspartic acid as the core structure recognized by the receptors, thus, these peptides have become valuable tools for the analysis of cell adhesion phenomena.[4] The action of the peptides was not inhibition of the initial cell attachment. The peptides did not

substantially inhibit the attachment of the tumor cells to laminin or type IV collagen which are the two main adhesive proteins in basement membranes. Therefore, the basement membranes are important extracellular matrix structures that play key roles in morphogenesis, mitogenesis, maintenance of normal tissue architecture, and tumor proliferation, invasion, and metastasis.[14-16]

In our previous report, we observed that the glioma cell line can produce laminin by immunohistochemical investigation.[18] Other researchers also described secretion of laminin from the glioma cells by using biochemical and immunohistochemical methods.[2, 3, 5, 6, 10, 11] In this paper, we investigated effect of quantities of laminin on the tumor cell proliferation and metastasis. In this animal experiment, the authors compare the 10μg with the 100μg laminin injection groups, and we found that the 100μg laminin injection group showed multiple pulmonary metastasis, however, the other groups did not show any metastasis. The laminin 100μg group grew faster and larger than the laminin 10μg and control groups. These data suggested that laminin plays an important role in the proliferation of tumor cells and in the tumor cell metastasis to the lungs in this model. The cultured glioma cell line can secrete laminin from the culture medium which may help in promotion dedifferentiation of the tumor.[13-16] However, some possible insights into the biological process and mechanisms of laminin have been suggested by malignant tumor growth-associated and growth-inhibitory studies. Laminin on the tumor cell surface usually stimulate hematogenous metastasis. Treating the cells with the receptor-binding fragments of laminin markedly inhibits or abolishes lung metastasis.[11] Laminin antagonist substances may be useful for prevention of metastasis in human malignant tumors.

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