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# Original article

## Activated vascular endothelia regulate invasion of glioma cells through expression of fibronectin

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***Keywords:*** *glioma; fibronectin; neoplasm invasiveness; tumor-host microecology; cell-to-cell communication*

**Background** Previous researches have indicated that glioma invasion may occur within a tumor-host microecology, and that fibronectin may be involved in glioma invasion as an important component of the extracellular matrix. However, how the interaction between tumor cells and vascular endothelial cells affects glioma invasion is poorly understood. The aim of this study was to investigate the effects of the interaction between tumor cells and vascular endothelial cells on glioma invasion, and the relationship of this interaction to fibronectin.
**Methods** The localization of fibronectin in different brain astrocytoma tissues was determined by immunohistochemistry. Then, vascular endothelial cells and glioma cells were co-cultured in a Transwell co-culturing system. Fibronectin expression was detected by reverse transcriptase-polymerase chain reaction, immunocytochemistry, and enzyme-linked immunosorbent assay. Additionally, the influence of the interaction between tumor cells and vascular endothelial cells on glioma cell invasion was determined by an *in vitro* rapid invasion test.
**Results** In brain astrocytoma tissues, fibronectin was present on the endothelial cells, in the extracellular matrix. Fibronectin expression was greater in higher grade tumors than in lower grade tumors. The interaction of glioma cells and vascular endothelial cells *in vitro* induced fibronectin release from vascular endothelial cells, which in turn stimulated glioma cell migration. This effect was inhibited by fibronectin blocking antibody.
**Conclusion** Glioma cells may induce vascular epithelial cells to express fibronectin, and in turn fibronectin could promote glioma cell invasion.

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Glioma is the most common tumor in the central nervous system and it continues to be a major therapeutic challenge. The prognosis for malignant glioma is poor, with the best 5-year survival being only 9.8% despite patients having undergone surgery, cranial radiation treatment, and intensive chemotherapy.[1] One of the most difficult problems in treatment is that although gliomas are rarely found to metastasize outside the central nervous system, diffusive infiltration tumor growth within the brain makes surgical removal impossible. Excessive proliferation and diffusive infiltration of surrounding brain tissues are key biological features of the malignant phenotype of glioma.[2] Previous research has indicated that invasion occurs within a tumor-host microecology.[3] In a tumor-host microecology, tumor cells recruit vasculature and stroma through production and secretion of stimulatory growth factors and cytokines.[4] The locally activated host microenvironment (including both cellular and extracellular elements) in turn modifies the proliferative and invasive behavior of the tumor cells.[5,6] Host cells include immune cells, inflammatory cells, muscle cells and myofibroblasts, vascular cells, etc.[3,7] We have previously performed studies on the relationship between immunohistochemistry of laminin (LN), fibronectin (FN), P53, and the tumor invasion microecosystem (TIMES) in human brain glioma, and found that micrangium endothelial cells may play a role in regulating the TIMES,[8] The role of the microenvironment in glioma invasion was recently revealed by time-lapse microscopy of the invasion of rat glioblastoma multiforme into a vital rat brain slice.[9] In this model, glioblastoma multiforme cells were observed to travel along blood vessels and to pause at selected vascular branch points where they proliferated, supporting the hypothesis that glioblastoma multiforme cells may respond to vascular endothelial-derived cues. However, how the effect of the interaction between tumor cells and vascular endothelial cells (VECs) promotes glioma invasion is poorly understood.

Glioma cells primarily invade individually anywhere within the brain, showing some tendency to infiltrate the

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extracellular matrix (ECM).[10] The roles of the ECM in glioma neoplastic transformation are complex and only recently have begun to be understood. Accumulating evidence shows that changes in ECM components modulate brain tumor growth, proliferation, and invasion through a variety of mechanisms. Fibronectin, an extracellular matrix molecule, has been identified as a potent mediator for cell adhesion and motility in various cells, and plays a key role in cell differentiation and tumor invasion.[11] Fibronectin was initially found in the stroma of brain tumors[12] and the expression of fibronectin in glial tumor tissue is still controversial. Schachner[13] reported that fibronectin was present at the gliomesenchymal junction of tumors, although absent in normal glial cells and glial tumor cells. However, recent studies have shown that fibronectin is expressed in tumor cells, vessel basement membrane, and the ECM of glioblastoma sections,[8,14,15] indicating that fibronectin might facilitate communication between tumor cells and their microenvironment.

In the present study, we investigated the cross-talk between tumor cells and VECs on glioma invasion.

## METHODS

### Patient samples

Thirty-two astrocytoma samples were obtained intraoperatively from patients undergoing resection of intracranial lesions in the Department of Neurosurgery, the First Affiliated Hospital, Fujian Medical University. Tumor tissues were embedded in paraffin for subsequent histological and immunohistochemical analyses. The tumor diagnoses were confirmed by histopathology according to the 2007 World Health Organization (WHO) criteria:[16] pilocytic astrocytoma (grade I), astrocytoma (grade II), anaplastic astrocytoma (grade III), glioblastoma multiforme (grade IV). All specimens were obtained from patients who did not undergo preoperative radiation, chemotherapy, or immunotherapy treatments. Prior to this study, patients provided informed consent pursuant to the protocol approved by the Review Board of the First Affiliated Hospital, Fujian Medical University.

### Cell culture

*Independent cell culture for RNA extraction*

Human umbilical vein endothelial cells (HUVECs) and Suzhou Human Glioma Cell Line-44 (SHG-44) subpopulation cells were gifts from Dr. QIANG Huang (Suzhou Medical University, Suzhou, China). HUVECs and SHG-44 cells were seeded in DMEM culture medium (GIBCO-BRL, Gaithersburg, USA) containing 10% fetal bovine serum and incubated at 37°C in 5% $CO_2$ and 95% air with standard humidity. When 80%–90% confluence was achieved, the cells were washed with DMEM media twice and cultured in serum-free DMEM media for 24 hours. Then the HUVECs were harvested for extraction of total RNA.

*Independent cell culture for immunocytochemistry*

HUVECs and SHG-44 cells were first seeded on glass slides in a culture dish, and then cultured under the conditions as described above for 12 hours, washed with DMEM media twice, then cultured in serum-free DMEM media for 24 hours. The cells were washed and fixed for immunohistochemistry. For each cell line, five culture slides were prepared for immunocytochemistry.

*Co-culture for supernatants and total RNA extraction*

HUVECs ($5\times10^5$) were pre-placed on the lower chambers of the Transwell co-culture device and inserted into the Transwell device after HUVECs attached to the well; SHG-44 cells ($5\times10^5$) were then added into the upper chamber. The device (a polycarbonate membrane with a diameter of 75 mm and pore diameter of 3 μm; Costar, Cambridge, MA, USA) prevents the direct contact of SHG-44 cells and HUVECs, but allows the soluble cytokines from them to interact with each other. When 80%–90% confluence was achieved, the cells were washed with DMEM media twice and cultured in serum-free DMEM media for 24 hours. Then the supernatant was collected by suction, centrifuged, and stored at 4°C for future use as an inducer of invasion and for HUVEC activation. The HUVECs were harvested for total RNA extraction.

*Co-culture for immunocytochemistry*

The SHG-44 cells and HUVECs were separately seeded on the glass slides in culture dishes, and the cultures were maintained under the same conditions as described above (for details, see the paragraph on independent cultures for RNA extraction) for 12 hours. After being washed with DMEM media twice, one glass slide of SHG-44 cells and one slide of HUVECs were placed in the same culture dish and co-cultured with serum-free DMEM media for 24 hours. Then the cell cultures were washed and fixed for immunohistochemistry. Three cell culture slides of HUVECs were prepared for immunocytochemistry.

*Semi-quantitative analysis of reverse transcriptase-polymerase chain reaction (RT-PCR) products*

Total RNA was extracted from the HUVECs cultured alone or co-cultured with SHG-44 cells by using TRIzol® (Invitrogen, USA) by following the instructions of the manufacturer. The primers were designed according to the coding sequence of FN on Gene Bank (Genbank accession number: NM133514), by using the VectorNTI primer design software from the Shanghai Boya company (Shanghai, China). The FN primer sequences were the following: forward: 5′-TGCTGGGACTTCCTACGT-CG-3′; reverse: 5′-CGTTTGAGTTGCCACCGTAAG-3′ (the length of the PCR product was 497 bp). β-actin primer sequences (Genbank accession number: NM031144) were the following: forward: 5′-GAGGCA-TCCTGACCCTGAAG-3′; reverse: 5′-CATCACAAT-GCCAGTGGTACG-3′ (the length of the PCR product was 275 bp). RT-PCR was carried out by using the Titan one tube RT-PCR system (Roche, Indianapolis, IN, USA).

The volume of the PCR reaction was 50 µl, total RNA was 1 µg, and the final concentration of each component was as follows: 1× RT-PCR buffer, 200 mmol/L dTT, 200 mmol/L dNTP, 1.5 mmol/L $MgCl_2$, 1 µl enzyme-mix (AMV reverse transcriptase and Taq polymerase). The RT-PCR reaction program for both FN and β-actin was the following: 55°C for 30 minutes, 94°C for 3 minutes, 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, 30 cycles, with extension at 72°C for 7 minutes.

The RT-PCR products of FN and β-actin were analyzed using electrophoresis in 1.5% agarose gel. β-actin, a housekeeping gene, was used to normalize the total RNA samples between different groups. Density scanning analysis of the PCR bands on the PCR gel pictures was carried out with GeneSnap and GeneTools systems (Syngene bioimaging system, Synoptics, Maryland, USA). For each sample, the density of the FN PCR band was divided by the density of the β-actin PCR band and the ratio was used for semi-quantitative comparison of FN gene expression among different samples.

**Immunostaining**

For patient tumor samples, 4-µm serial sections were sliced using a vibratone (Leica, Wetzlar, Germany). Slices were dewaxed, rehydrated, and treated with citric acid solution to prepare for immunohistochemistry. For cell culture, the culture glass slides were washed with PBS and fixed with methanol for 30 minutes to prepare for immunocytochemistry.

The immunoreaction was performed as previously described.[17] Briefly, slices were washed with PBS and incubated in 0.3% hydrogen peroxide solution at room temperature for 10 minutes to block endogenous peroxidase. After washing with PBS, the slides were blocked by 3% goat serum, and incubated with rabbit anti-human fibronectin polyclonal antibodies (1:150, Santa Cruz Biotechnology, USA) overnight at 4°C. Slides were washed in PBS, and FN was detected by using the Elivision™ plus kit (Maixin Bio Company, China) following the manufacturer's instructions. The DAB reaction was conducted on the slides. The DAB reaction was monitored by checking for the presence of brown reactive products under a microscope. After washing with tap water, the culture was counterstained with hematoxylin. After washing, the culture slides were mounted with neutral mounting medium. For the negative control, the primary antibodies were replaced with normal rabbit serum during the incubation. FN immunoreactivity was examined under a microscope (Nikon, Japan).

**Matrigel preparation**

The stock solution basement membrane matrix Matrigel™ (BD Biosciences, USA) was diluted with pre-cooled DMEM medium to a final concentration of 3.6 mg/ml. Then 20 µl of Matrigel was added to pre-cooled multipore filter film (diameter: 6.5 mm, pore size: 8 µm, polyvinylpyrrolidone-free polycarbonate filters; Costar, Cambridge, MA) and then evenly distributed on the film in the Transwell device. Transwell plates were incubated in a 37°C incubator for 30 minutes until the Matrigel was attached to the film and then stored at 4°C until used.

***In vitro* cell migration analysis**

The *in vitro* invasion study was carried out with the *in vitro* rapid invasion assay.[18] First, $1\times10^5$ SHG-44 cells were placed on the Matrigel-coated Transwell device and co-cultured with or without HUVECs in a 6-well plate. The cells were treated with the following media: serum-free condition medium harvested from co-cultured SHG-44/HUVEC cells (SHG44/HUVEC condition media), or 0.1% bovine serum albumin (BSA) DMEM and those media preabsorbed by 10 µg/ml of anti-fibronectin antibody, respectively. After 18-hour incubation at 37°C in 5% $CO_2$, the incubation medium was discarded and the membrane was washed with PBS twice. The cells on the upper layer of the multipore filtering film were wiped away with cotton. The filtering film was carefully cut out and fixed by methanol for 2 hours at room temperature. After being washed, the film was stained with hematoxylin and eosin (HE). The tumor cells on the back of the filtering film were examined under a microscope (Nikon). The 6.5-mm diameter film was divided into 9 squares under the microscope using a 40× objective. The invading cells in the 4 corners and the center square were counted, and the average cell number of three samples in each group was calculated.

**Enzyme-linked immunosorbent assay (ELISA)**

The culture media were collected and debris was removed by centrifugation at 2000 ×*g* for 5 minutes. The supernatant was collected for ELISA assay. Serial dilutions of samples with the highest and the lowest expected values were performed to determine the fibronectin level using a commercial fibronectin ELISA kit (R&D Systems, USA) following the manufacturer's instructions. Fibronectin expression levels were calculated by a standard curve which was available from the manufacturer. All experiments were performed in triplicate reactions.

**Statistical analysis**

Data were expressed as mean ± standard deviation (SD), analyzed by one-way analysis of variance (ANOVA) followed by Tukey *post hoc* test or one-way ANOVA followed by Bonferroni *post hoc* test for multiple comparisons with the control groups using SPSS 12.0 softuare (SPSS Inc., USA). Differences were considered statistically significant if $P<0.05$.

## RESULTS

**Localization of FN immunoreactivity in brain astrocytoma tissues and the surrounding tissue of the glioma, especially the edematous region**

FN is expressed mainly in the ECM of tumor tissues and immunolocalized in the tumor vessel basement membranes of almost all malignant gliomas.[11] The data

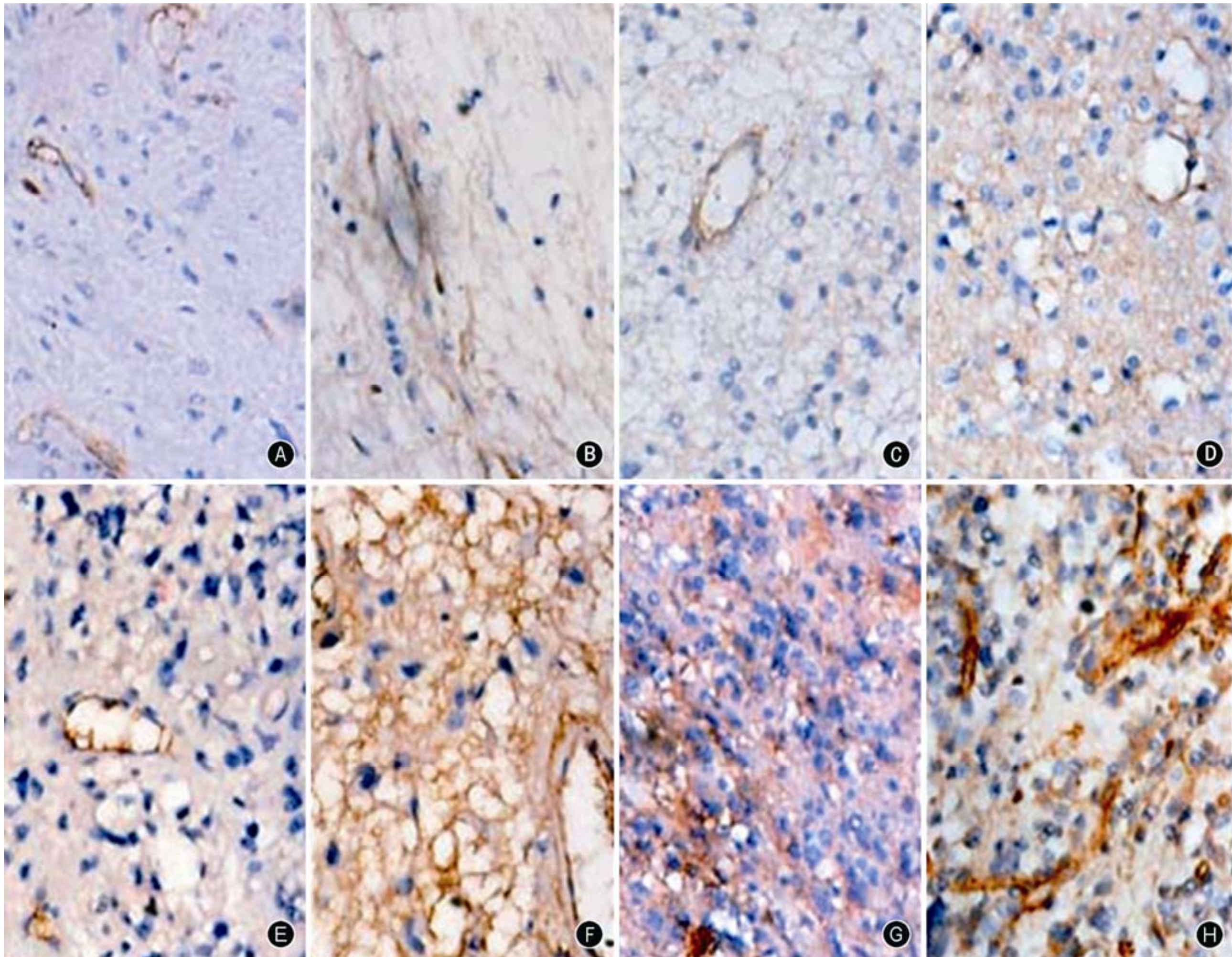

**Figure 1.** Micrographs of the localization of fibronectin in human glioma tumor tissues. Immunohistochemistry of fibronectin (brown) in tumor region (**A**, **C**, **E**, **G**) and peritumoral edematous region (**B**, **D**, **F**, **H**) of pilocytic astrocytoma (**A**, **B**), astrocytoma (**C**, **D**), anaplastic astrocytoma (**E**, **F**), and glioblastoma multiforme (**G**, **H**) (Immunohistochemistry staining, original magnification ×400).

have indicated that areas of perifocal edema include not only the tumor invasion zone but also the inflammatory tumor microenvironment.[19] To investigate the effect of FN on tumor progression, we studied FN by immunohistochemistry in brain astrocytoma tissues and surrounding tissue of the glioma, especially the perifocal edema region. In normal brain tissues, FN immunoreactivity was mainly located on the vessel basement membrane, but not on the neurons and matrix. In the astrocytoma, FN was also present on the endothelial cells, the basement membrane, and ECM. In some regions, FN immune products were dispersed in the tumor matrix with a microvillus-like or lamellar staining pattern. In the surrounding tissue of the glioma, especially in the edematous region, FN immunoreactive products might form a reticulate or microvillus-like staining pattern in the matrix and intercellular space, with tumor cells inside the grid. The more intensive the vessel reaction and the higher degree of edema, the stronger the expression of FN immunoreactive products. Some FN expression in tumor cells in malignant glioma appeared as brown granules dispersed within plasma. However, FN was not expressed in the tumor cell mass of the brain gliomas. The expression patterns in astrocytoma tissues were related to the pathological grade. In lower grade astrocytomas, the FN positive staining on the microvessel wall was thin and even. In pilocytic astrocytoma tissue, FN staining was light in tumor tissue (Figure 1A) and in the surrounding edematous tissues along with vessel tubes (Figure 1B). Notably, the levels of FN expression were elevated in both tumor tissues and the surrounding edematous tissues of astrocytoma (Figure 1C and 1D), anaplastic astrocytoma (Figure 1E and 1F), and glioblastoma multiforme (Figure 1G and 1H). Immunoreaction studies revealed that the level of FN expression correlated to the pathological grade of astrocytoma and the degree of tumor edema, suggesting that FN produced from vascular endothelia and ECM might participate in tumor progression and invasion.

**FN expression in vascular endothelia induced by glioma cells**

Previous research has indicated that glioma cells influence invading endothelial cells to develop a permeable phenotype radically different from host tissue endothelium, and that host vessel phenotype does not

influence tumor vessel morphology.[20] To investigate whether FN expression in vascular endothelia was induced by glioma cells, we placed human SHG-44 glioma cells on the Transwell device co-cultured with HUVECs. After 24-hour incubation in serum-free medium, expression of FN, detected by immunocytochemistry (Figure 2A and 2B) and RT-PCR (Figure 2C), increased significantly in HUVECs co-cultured with human SHG-44 cells (SHG-44/HUVEC) compared with that in HUVECs cultured alone (HUVEC). Simultaneously, ELISA analysis showed that FN released from co-cultured cells was markedly higher than that from HUVECs cultured alone (Figure 2D).

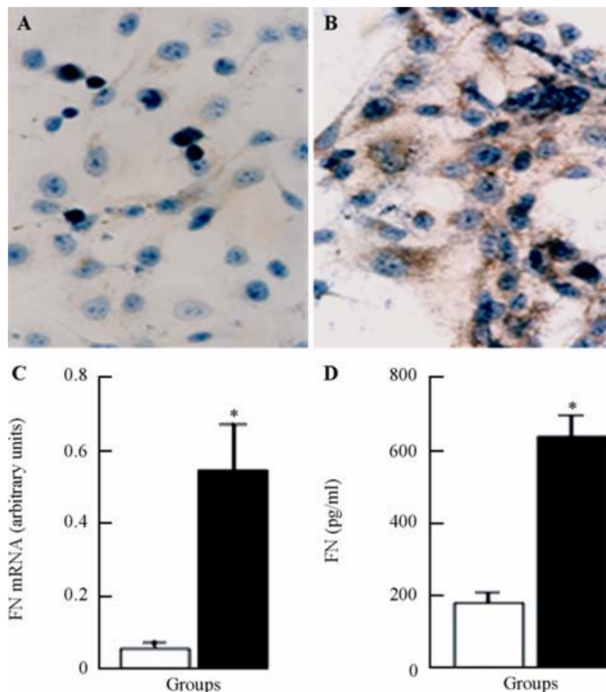


**Figure 2.** Interaction of tumor and vascular endothelial cells induced fibronectin (FN) release. Immunoreactive staining of fibronectin (brown) in HUVECs that were cultured alone (**A**), co-cultured with SHG-44 cells (**B**) for 24 hours in serum-free medium (Immunostaining, original magnification ×400). The levels of FN assessed by semi-quantitative PCR (**C**) and ELISA analysis (**D**) in HUVECs that were (i) cultured alone (open bar), (ii) co-cultured with SHG-44 cells for 24 hours in serum-free medium (black bar). $^{*}P$ <0.01; $n$=5.

To test whether soluble factors, which induced vascular endothelia to express fibronectin released from glioma cells, retain bioactivity, we treated HUVECs for 18 hours with serum-free condition medium harvested from HUVECs alone (HUVEC condition media) and human SHG-44/HUVECs co-cultured cells alone (SHG44/HUVEC co-cultured condition media). Immunocytochemistry showed significant increase of FN expression in the HUVEC cells treated with SHG44/HUVEC co-cultured condition medium compared with those treated with HUVEC condition medium (Figure 3A and 3B). Furthermore, we examined FN mRNA expression levels and the results showed that SHG44 condition medium increased FN mRNA levels (Figure 3C) in these HUVECs. FN

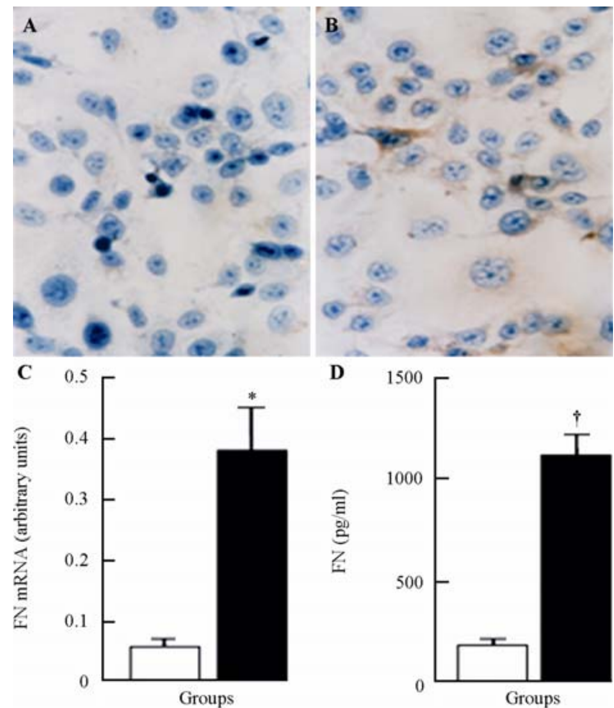


**Figure 3.** Condition media extended fibronectin (FN) expression in HUVECs. Immunostaining of FN (brown) in HUVECs cultured in serum-free condition media harvested from HUVECs cultured alone (**A**), co-cultured SHG-44/HUVEC cells alone (**B**) (Immunostaining, original magnification ×400). **C:** Levels of FN mRNA in HUVECs treated with serum-free condition media. **D:** ELISA analysis of FN levels in culture media from HUVECs after treatment with serum-free condition media. Open bars, condition medium harvested from HUVECs cultured alone; black bars, condition medium harvested from co-cultured SHG-44/HUVEC cells. $^{*}P$ <0.01, $^{\dagger}P$ <0.001, $n$=5.

released into media was markedly higher than that in human SHG-44 condition medium (Figure 3D), implying that these condition media can stimulate VECs to produce FN. FN neosynthesis in VECs can be explained as a specific host response to environmental changes. The tumor cells may use this protein either as stimulus or guiding molecules for their migration into the host tissue.

### Activated vascular endothelial cells induced glioma migration

A critical aspect during the course of invasion is the interaction of neoplastic cells with their microenvironment. The relationship between a cancer cell and its corresponding vascular niches, into which endothelial cells secrete soluble factor, regulates cancer cell behavior and progression.[21,22] We first sought to determine whether glioma migration was induced by co-culture of human SHG-44/HUVEC cells. We co-cultured human SHG-44 cells with or without HUVECs, and then treated them with either SHG44/HUVEC condition medium or 0.1% BSA DMEM media. After 18-hour incubation, we found that SHG-44/HUVEC condition medium triggered SHG-44 cells to move to the reverse side of Matrigel (Figure 4A) and this migration was enhanced when co-cultured with HUVECs (Figure 4B). In contrast, although SHG-44 cell migration was enhanced when co-cultured with HUVECs

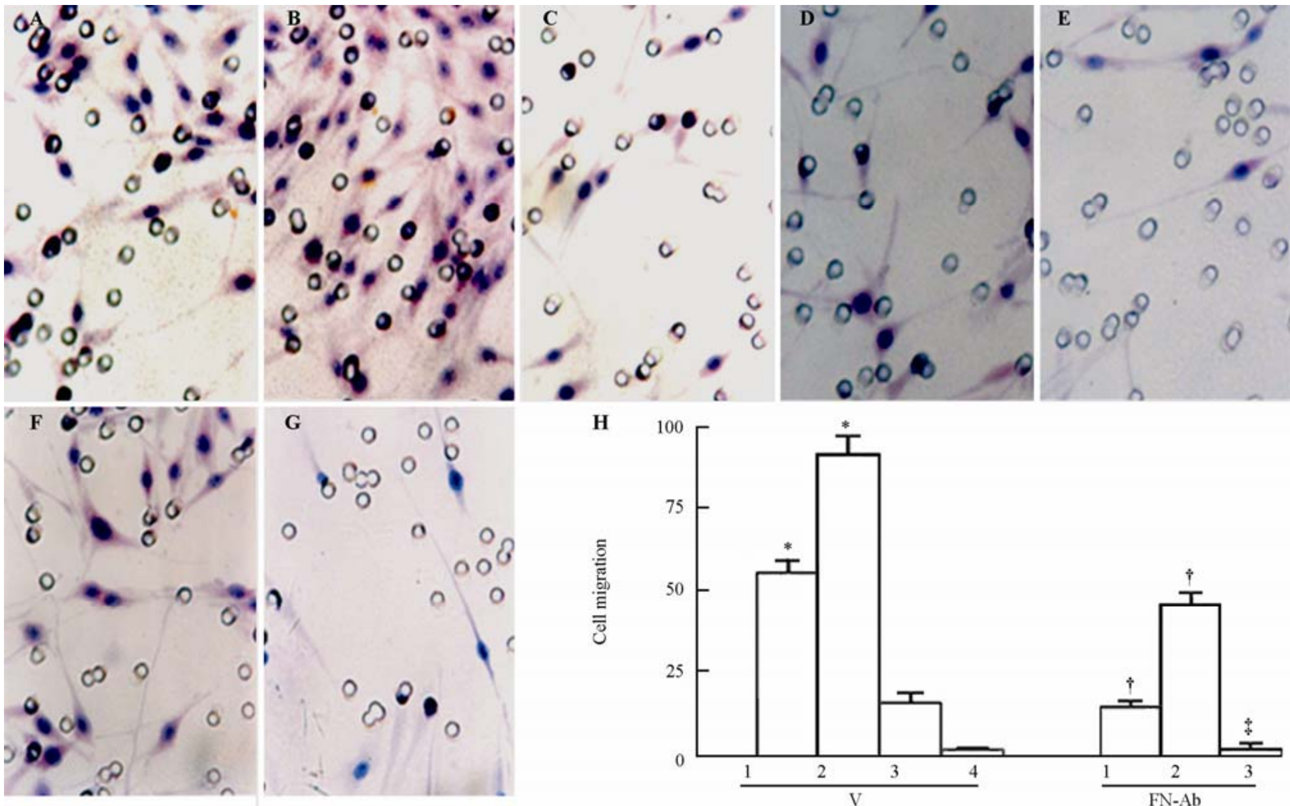


**Figure 4.** FN antibody blocked SHG-44 cell migration induced by condition media (HE, original magnification ×400). **A–D:** Staining analysis of SHG-44 cells traveled to the reverse side of Matrigel after treatment with serum-free condition medium harvested from co-culture of SHG-44/HUVEC cells (**A, B**) or 0.1% BSA DMEM medium (**C, D**), with (**B**, **C**) or without (**A**, **D**) the presence of HUVECs. **E–G:** HE staining was performed to identify cells. Effect of blocking FN on C6 cell migration. Except that the condition media were preabsorbed by FN antibody, conditions in E, F, and G were the same as that in A, B, and C, respectively. **H:** Quantitate SHG-44 cell migration by cell count. V, in condition media; FN-Ab, in condition media pre-absorbed by 10-μg fibronectin antibody; 1, SHG-44 cells cultured in serum-free condition medium harvested from co-culture of SHG-44/HUVEC cells; 2, SHG-44 cells co-cultured with HUVECs in serum-free condition medium harvested from co-culture of SHG-44/HUVEC cells; 3, SHG-44 cells co-cultured with HUVECs in 0.1% BSA DMEM medium; 4, SHG-44 cells cultured in 0.1% BSA DMEM medium. $^{*}P$ <0.001 vs. 1; $^{\dagger}P$ <0.05; $^{\ddagger}P$ <0.01 vs. V; $n$=5.

(Figure 4C), it was rare when treated with 0.1% BSA DMEM media (Figure 4D). In primary tumors of the central nervous system, the contribution of FN to the invasion behavior of glioma cells has been extensively studied.[23-25] We further determined whether release of FN from VECs contributed to glioma migration. With the same conditions, we treated the HUVECs and co-cultured SHG-44/HUVEC cells with media that were pre-absorbed by FN antibody. We found that SHG-44 cell migration induced by condition media was blocked (Figure 4E–4G), suggesting that FN released from VECs was involved in SHG-44 cell migration triggered by cell-to-cell interaction. The extent of SHG-44 cell migration was different when SHG-44 cells were cultured alone in SHG-44/HUVEC condition medium (56.3±4.05), co-cultured with HUVEC in SHG-44/HUVEC condition medium (92.7±5.78), co-cultured with HUVECs in 0.1% BSA DMEM medium (16.3±2.9), or cultured alone in 0.1% BSA DMEM media (2.00±0.05) (Figure 4H). The increase of SHG-44 cell migration was significantly inhibited by FN blocking antibody (Figure 4H). Together, our data suggest that neoplastic cells induce VECs to produce FN, which facilitates the migration of neoplastic cells toward the surrounding area.

## DISCUSSION

Our major findings in this study suggest that the cross-talk between neoplastic cells and VECs promoted tumor invasion mediated by FN released from VECs. Both condition media and co-culture glioma/HUVEC cells induced glioma cell migration that could be inhibited by FN blocking antibody. These results identified a previously unrecognized role of neovascularization during tumor invasion.[10,26] During the normal reparative process and primary brain neoplasia, ECM increases and undergoes remodeling. Accumulating evidence demonstrates that changes in ECM components modulate brain tumor growth, proliferation, and invasion through various mechanisms. The relationship between the cancer cell and its corresponding vascular niches, into which endothelial cells secrete factors, regulates cancer cell behavior and progression.[21,22] Here we reported a new *in vitro* model that interpreted the relationship between the brain cancer cell and microvascular niche, and identified vascular endothelial cells as a critical regulator of the brain cancer cell, as these cells secreted soluble factors that promoted glioma migration and invasion. There is much evidence that has led to invasion

and angiogenesis being the newest therapeutic targets for brain tumors, as they involve cell proliferation and migration in response to many molecular substances released by brain tumors. However, limited information is available concerning cell-cell communication between brain tumors and their microenvironment. We showed here for the first time that interaction between the tumor cell and vascular endothelial cells induced the release of the ECM component FN into culture, indicating that the feedback signal between the brain cancer cell and its microenvironment regulated tumor proliferation and progression.

FN is a member of a family of glycoproteins that have many biological functions, including cellular adhesion, migration, and invasion. As a peripheral protein, FN mainly acts as a bridge to link the cell surface and ECM. Therefore, the absence or reduction of FN in tumor cells may reduce the adhesion between tumor cells and matrix components, and decrease the matrix's control of cell differentiation, proliferation, and migration. Not only is intact FN chemotactic for cell migration, the splicing segments of FN can also promote cell migration and angiogenesis.[27,28] FN connects ECM components not only to the cell surface, but also to collagen fibers, which are polysaccharide molecules in the matrix, through a series of dispersed binding sites. The combined effects of FN play an important role in the development of cancer. FN may also combine with the fibrinogens which are exuded due to the vessel reactions with the surrounding cancer, to promote the formation of new matrix in tumor providing a condition for the seeding and proliferation of the invading tumor cells. Meanwhile, FN interacting with collagen may displace and release the metalloproteinase-2 bound to collagens, which may play a critical role in tumor invasion.[29] Moreover, it has been demonstrated recently that the matrix environment composed of FN is an ecology suitable for the growth of migrating cells.[30]

Migration along FN-positive mesenchymal cells may lead to glioma cells gathering in the perivascular region and around the tumor. FN is an important component in the ECM of gliomas, largely in the vessel wall.[8,14] Expression of the ECM FN component has been characterized in human glioblastomas and in a number of astrocytoma and glioblastoma cell lines.[31] *In vitro* experiments have proven that components of ECM, LN and FN, can strongly stimulate the migration of glioma cells, which occurs after glioma cells express the relevant surface receptors.[32] Knott et al[15] found that when normal brain tissues were invaded by glioma, ECM components such as LN, FN, and collagen type IV may emerge and the tumor cells may express specific integrins, depending on the change of the inner environment, and interact with these ECM components, which is essential for tumor cell invasion. Although there are many *in vitro* and *in vivo* models for FN-promoted invasion and transmigration,[24,32] the uniqueness of our findings may be attributed to interaction between tumor cells and VECs, as changes of tumoral microenvironment molecules can affect tumor status and progression. Moreover, we showed that FN expression correlated with glioma migration and with glioma malignancy. Based on the available information regarding the influence of ECM proteins on function and behavior of tumor cells, we believe that more new physiological *in vitro* models are necessary to study the functional mechanisms of migration that are applicable to *in vivo* situations. Though more work is needed to clarify how glioma cells stimulate VECs to produce fibronectin, this pilot study should provide insightful information with regard to the connection between tumoral micro-environment and tumor invasion.

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