

Heat Shock Cognate Protein 70 Enhanced Integrin β 1 Mediated Invasion in Cancer Cells

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Purpose: Glioblastoma is one of the most common malignant cancers worldwide. In our previous work, we have shown that heat shock cognate protein 70 (Hsc70) functions as a positive growth regulator in glioma. We investigated the role of Hsc70 in integrin β 1 mediated invasion of glioma cells.

Methods: In order to investigate whether the down-regulation of Hsc70 would affect the expression of integrin β 1 subunit, HeLa cells were transiently transfected with Hsc70-AS or pcDNA3.0 vectors and the down-regulation of Hsc70 was confirmed by Western blotting. Human brain glioma U87 cells were stably transfected with Hsc70-AS or pcDNA3.0 vectors to further elucidate the relationship between Hsc70 and integrin β 1 in human glioma cells. Cellular localization of integrin β 1 was detected using immunofluorescence confocal microscopy analysis.

Results: Here we reported that down-regulation of the expression of Hsc70 in U87 cells by transfection with antisense cDNA specifically increased the expression of cell surface integrin β 1 without changing its mRNA. Meanwhile, the integrin β 1 125-kD mature form increased while 105-kD precursor form decreased when Hsc70 was down-regulated. Mechanically, the U87 cells transfected with antisense cDNA of Hsc70 decreased the Golgi localization of integrin β 1, strengthened its interaction with integrin α 5 subunit, and enhanced the adhesion ability to fibronectin (FN) and the phosphorylation level of focal adhesion kinase (FAK).

Conclusion: Overall, these results suggested that the down-regulation of Hsc70 expression could promote the expression of cell surface integrin β 1 and subsequently inhibit glioma invasion phenotype.

Keywords: Hsc70, integrin β 1, glioma, focal adhesion kinase, invasion

Introduction

Glioblastoma is the most common and malignant primary central nervous system tumor, which is characterized by a high degree of invasion.¹ Therapy approaches including irradiation and surgery, with chemotherapy constitute an important strategy to glioblastoma. However, most patients with glioblastoma still have a poor prognosis, largely due to a high rate of post-surgical recurrence and metastasis.² In cancer clinical practice, invasion and metastasis is an important prognostic factor of glioblastoma, and elucidation of the mechanism of invasion and metastasis at the molecular level is desirable.³

Cancer invasion and metastasis is a complex process and is influenced by many biological factors.⁴ Integrins are transmembrane receptors that facilitate cell-extracellular matrix (ECM) adhesion. The presence of integrins allows rapid and flexible responses to

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events at the cell surface.⁵ Integrins are obligate heterodimers, which consist of α - and β -subunits, and 24 α -subunits and nine β -subunits assemble into at least 24 different integrins. Among these, 12 integrins contain the $\beta 1$ subunit.⁶ In recent years, changes in carbohydrates on integrin $\beta 1$ have been reported to regulate the activity of integrin, resulting in altered cell adhesion and metastasis.⁷ β -1,4-Galactosyltransferase (β -1,4-GalT) V is a constitutively expressed enzyme that can effectively express enzyme galactosylating the GlcNAc β 1-6Man group of the highly branched *N*-glycans which are characteristic of tumor cells.⁸ Overexpression of β -1,4-GalT has been reported to increase lactosamine glycans on integrin $\beta 1$, resulting in enhanced migration and invasion of cancer cells.⁹ The invasion of SHG44 human glioma cells has been shown to be suppressed by transfection with antisense β -1,4-GalT V cDNA, indicating that the expression of the β -1,4-GalT V is associated with the tumorigenic and invasive potentials of glioma cancer cells.¹⁰

Quite interestingly, in our previous studies, Hsc70 was found to interact with β -1,4-GalT V and played an important role in tumor metastasis and invasion. However, the effects of Hsc70 on the glycosylation and integrin $\beta 1$ mediated invasion of human glioma cells have not been reported. Hsc70 is a constitutively expressed molecular chaperone which belongs to the heat shock protein 70 (HSP70) family. Studies have displayed that Hsc70 is an important factor in kinds of tumors.¹¹ It has been found that the expression of Hsc70 is higher in some cancer cell lines than in normal cell lines, including esophageal cancer, lung adenocarcinoma, colon cancer cell, renal cell carcinoma, leukemia, lymphoma and so on.¹² The increased expression of Hsc70 is often closely related to tumor grade and tumor metastasis.¹³ These findings implied that Hsc70 may enhance malignant properties and invasion phenotype of glioma cells via modifying glycosylation and signaling of integrin $\beta 1$.

In this study, we investigated the role of Hsc70 in integrin $\beta 1$ mediated invasion of HeLa and glioma cells. We found that down-regulation of Hsc70 expression could promote the expression of cell surface integrin $\beta 1$ and subsequently inhibit glioma invasion phenotype.

Materials and Methods

Materials

Anti-mouse IgG, anti-rabbit IgG, fibronectin, DMEM, Golgi specific dye NBD were purchased from Sigma (St. Louis, MO, USA). Rhodamine or FITC-conjugated goat anti-mouse

secondary antibody were purchased from Molecular Probe (Eugene, OR, USA). Monoclonal antibody against integrin $\beta 1$ and Endoglycosidase H (Endo H) were obtained from BD Pharmingen (San Jose, CA, USA). Trizol reagent was purchased from Tiangen Biotech (Beijing, China). PrimeScript RT Master Mix was purchased from Takara (Shiga, Japan). The pcDNA3.0 vector and Transfection Reagent Sofast were purchased from Invitrogen (Carlsbad, CA, USA). The plasmid antisense cDNA of Hsc70 (Hsc70-AS) was purchased from Santa Cruz (Santa Cruz, CA, USA). Monoclonal antibody against β -actin, FAK, integrin $\alpha 5$ were purchased from Santa Cruz (Santa Cruz, CA, USA).

Cell Culture and Transfection

Human glioma cell line U87 and cervical cell line HeLa were purchased from Shanghai Institutes for Biological Sciences (SIBS) of the Chinese Academy of Sciences (CAS) (Shanghai, China). Cells were cultured in DMEM/F-12 media supplemented with 10% fetal bovine serum and PenStrep [penicillin (100 U/mL) and streptomycin (100 μ g/mL)] (Gibco, Grand Island, NY, USA) in a humidified atmosphere in 5% CO₂ at 37°C. The U87 cells stably transfected with Hsc70-As and pcDNA3.0 vector plasmids were named as Hsc70-AS/U87 and Vector/U87 cells, respectively. Transient transfections were performed with SofastTM reagent according to the manufacturer's instruction.

Subcellular Localization of Integrin $\beta 1$ and Golgi Co-Localization Study

U87 cells in 60-mm plates were fixed with 4% paraformaldehyde for 30 min at 4°C, permeabilized with 0.1% TritonX100 in PBS for 30 min at 4°C, and washed three times with cold PBS. After blocked with 1% BSA, cells were stained with anti-integrin $\beta 1$ mouse monoclonal antibody, followed by incubation with rhodamine-conjugated anti-mouse IgG and incubated at 4°C for 0.5 h. Then, cells were stained with 100-nm Golgi specific dye NBD for 30 min at for 0.5 h. After washed with PBS, cells were viewed using a Zeiss LSM780 CLSM (Carl Zeiss, Gottingen, Germany).

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted using the Trizol reagent (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. RNA was reverse transcribed into cDNA by PrimeScript RT Master Mix (Takara, Shiga, Japan) and

Q-PCR was subsequently performed using SYBR Premix Ex Taq II (Takara, Shiga, Japan) according to the manufacturer's protocol. The primers used were: for integrin $\beta 1$ forward: 5'-AACTTGATCCCTAAGTCAGCAGTAG-3', reverse: 5'-ATCAGCAGTAATGCAAGGCC-3'; for integrin $\alpha 5$ forward: 5'-ACCAAGGCCCCAGCTCCATTAG-3', reverse: 5'-GCC TCACACTGCAGGCTAAATG-3'; for β -actin forward, 5'-GCGCGGCTACAGCTTCAC-3'; reverse, 5'-GGGGCCGG ACTCGTCATA-3'. Relative band intensities were determined using Image J software (NIH, Maryland, USA).

Detection of Integrin on Cell Surface with Fluorescence-Activated Cell Sorter (FACS)

Cells were grown to subconfluence and detached with 2 mM EDTA in PBS. Cells (10^6) were washed, resuspended in 100 μ L of FACS buffer (PBS containing 1% BSA and 0.01% sodium azide), and then incubated with antibody against $\alpha 5$ or $\beta 1$ integrin (1:100) at 4°C for 30 min, followed by washing three times with FACS buffer. Cells were then labeled with FITC-conjugated secondary antibodies or FITC-streptavidin (10 μ L) at 4°C for 30 min. Analyses were then performed on FACScan (BD Biosciences, San Jose, CA, USA).

Immunoprecipitation

Immunoprecipitation of focal adhesion kinase (FAK) experiment was performed according to the manufacturer's instructions. The cells were harvested and lysed by IP lysis buffer (250 mmol/L HEPES, 50 mmol/L NaCl, 1 mmol/L EDTA, 0.1 mmol/L neocuproine, 1% NP-40, Protease Inhibitor Cocktail). Antibodies specific to Integrin $\alpha 5$ or FAK (Santa Cruz, CA, USA) were added to supernatants followed by an incubation. Immune complexes were then precipitated with protein A agarose beads. Bound proteins were eluted by boiling with loading buffer and analyzed by Western blotting with anti-Integrin β or PY-20 antibodies.

Cell Adhesion Assays

Ninety-six-well microtiter plates were coated with 0.1 mL of human plasma fibronectin in PBS, incubated at 37 °C for 1 h, and blocked by 1% BSA at 37 °C for 30 min after washing. Cells, 3×10^4 , suspended in 100 μ L of serum-free DMEM were added to each coated well and incubated at 37 °C for 30 min. Wells were gently washed three times with 100 μ L of ice-cold PBS to remove unbound cells, followed

by fixation of adherent cells using 3.5% formaldehyde for 15 min. Cells were then stained with a 0.5% crystal violet solution. After the wells were washed twice with PBS, the absorbance of each well at 595 nm was measured using an automated microtiter plate spectrophotometer.

Endoglycosidase Digestion

Cells were harvested and lysates in lysis buffer. One milligrams per milliliter of diluted cell lysates proteins, determined using BCA protein assay procedure, were added with Endo H (30 milliunits/mL) and incubated at 37°C for 24 h, followed by addition of acetone and incubated at 4°C for 1 h. The lysates were centrifuged at 12,000 g for 10 min at 4°C. The precipitate was dissolved in lysis buffer and then boiled in 2× SDS sample buffer for 10 min. Then, the samples were subjected to immunoblotting analysis with an anti-integrin $\beta 1$ antibody.

Wound-Healing Assay

Cells, 1.2×10^6 , were seeded on six-well plates coated with fibronectin with DMEM containing 10% FBS and grown to confluence. The cells were scratched with a sterile 1000- μ L pipette tip to create artificial wounds. At 0, 24 and 48 h after wounding, phase-contrast images of the wound-healing process were photographed digitally using an inverted Olympus IX50 microscope with a 10× objective lens. Eight images per treatment were analyzed to determine averaging parameters of positioning of the migrating cells at the wound edges by digitally drawing lines using the Image J software (NIH, Maryland, USA).

Western Blot Analysis

Protein samples (20 μ g/lane) were separated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes. The membrane was blocked with 5% bovine serum albumin and 0.1% Tween 20 in Tris-buffered saline for 1 h at room temperature. The membranes were incubated with antibodies against overnight at 4°C and then incubated with peroxidase-conjugated secondary antibodies for 2 h at room temperature. The bands were visualized with an enhanced chemiluminescence detection kit (BeyoECL plus, Beyotime, China), and the band densities were analyzed using Image J software. All fold changes in band densities were normalized to that of the control group. Each experiment was carried out in three biological replicates and average fold changes are reported.

Statistical Analysis

Quantitative data were presented as means \pm SEM. Student's *t* test was used to determine differences between two groups. And one-way ANOVA was adopted to compare differences among multiple groups. A value of $P < 0.05$ was considered statistically significant. Data were analyzed using SPSS software (SPSS version 17.0) (SPSS, Chicago, IL, USA).

Results

Down-Regulation of Hsc70 Promoted the Expression of Integrin $\beta 1$ in HeLa Cell Surface

In order to investigate whether the down-regulation of Hsc70 would affect the expression of integrin $\beta 1$ subunit, human cervical carcinoma HeLa cells were transiently transfected with Hsc70-AS or pcDNA3.0 vectors and the down-regulation of Hsc70 was confirmed by Western blotting (Figure 1A). Then, an equal amount of cellular protein from these transfected cells was subjected to immunoblot analysis with anti-integrin $\beta 1$ and anti- β -actin antibodies. The down-regulation of Hsc70 significantly promoted the expression of both integrin $\beta 1$ 125-kD mature form and 105-kD precursor form compared with the vector (Figure 1B and C). Furthermore, increased expression of integrin $\beta 1$ on the cell surface of HeLa cells transiently transfected with Hsc70-AS

was also verified by FACS. As shown in Figure 1D, the fluorescence intensity of cells transfected with Hsc70-AS was about 2.1-fold higher than that of control cells. Interestingly, the mRNA level of integrin $\beta 1$ was not affected (data not shown). The above results provided us the initial evidence that Hsc70 could affect the expression of integrin $\beta 1$.

Down-Regulation of Hsc70 in U87 Cells Promoted the Expression of Integrin $\beta 1$ Specifically

In order to further elucidate the relationship between Hsc70 and integrin $\beta 1$ in human glioma cells, human brain glioma U87 cells were stably transfected with Hsc70-AS or pcDNA3.0 vectors, which were called Hsc70-AS/U87 and Vector/U87 cells, respectively, and the down-regulation of Hsc70 was confirmed by Western blotting (Figure 2A). Consistent with the results in HeLa cells, down-regulation of Hsc70 promoted the expression of integrin $\beta 1$ proteins on Hsc70-AS/U87 cell surface, while its mRNA level was not affected (Figure 2B and C). Integrins are obligate heterodimers, which consist of α - and β -subunits, in order to investigate whether Hsc70 affected integrin $\beta 1$ specifically, the expression of the most common integrin $\alpha 5$ subunit was investigated.¹⁴ As shown in Figure 2D and E, the results showed that both the mRNA level and protein expression on

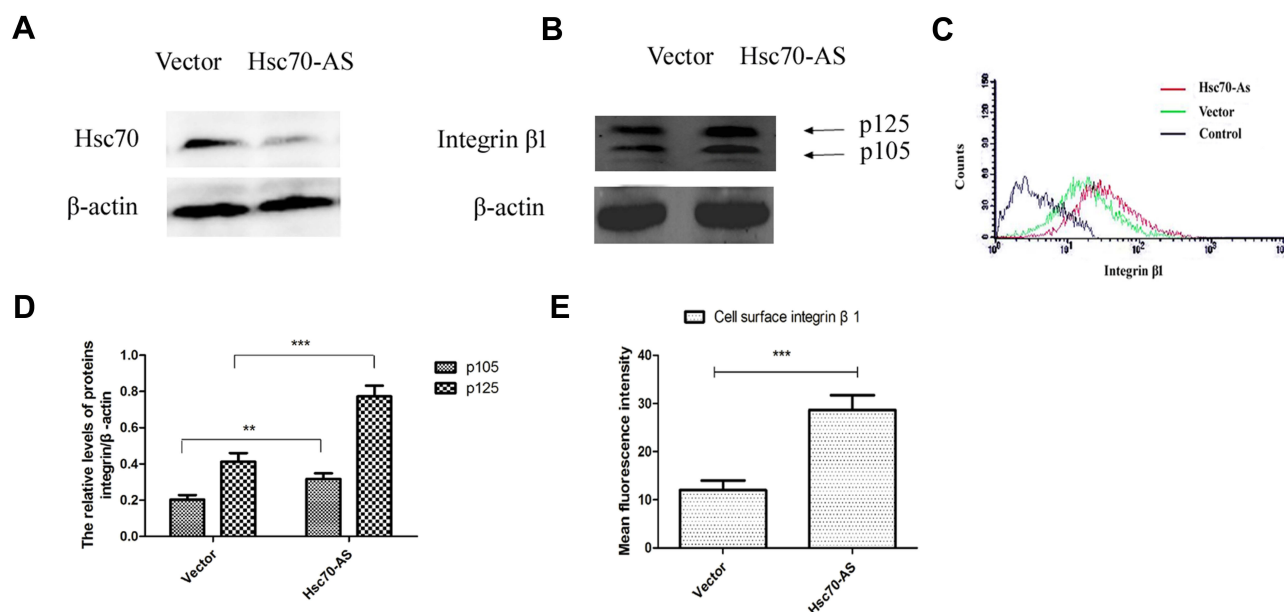


Figure 1 The expression of the cell surface integrin $\beta 1$ subunit was increased by the down-regulation of Hsc70. **(A)** The down-regulation of Hsc70 was confirmed by immunoblotting. **(B)** Equal amounts of cellular protein from HeLa cells transiently transfected with Hsc70-AS or scramble vectors were subjected to immunoblot analysis with anti-integrin $\beta 1$ and anti- β -actin antibodies. **(C)** HeLa cells transiently transfected with Hsc70-AS or vectors were subjected to analysis with FACS of cell surface integrin $\beta 1$. **(D)** Quantitative results of B. **(E)** Mean fluorescence intensity of cell surface integrin $\beta 1$, data were expressed as the mean \pm SEM from three independent experiments. ** $P < 0.01$, *** $P < 0.001$.

Hsc70-AS/U87 cell surface were not affected, suggesting the specificity of Hsc70 to integrin $\beta 1$ subunit.

Down-Regulating the Expression of Hsc70 Increased PI25 and Decreased PI05 of Integrin $\beta 1$

Integrin $\beta 1$ is a transmembrane glycoprotein, after synthesized as an 85-kDa polypeptide, it undergoes glycosylation in the endoplasmic reticulum (ER), and then in the Golgi apparatus.¹⁵ The incompletely glycosylated form of integrin $\beta 1$ has a mass of 105 kDa and is referred to as p105. The completely glycosylated form of integrin $\beta 1$ has a mass of 125 kDa (p125). The mature p125 is transferred to the cell membrane and exerts its biological effects.¹⁶ The above results have demonstrated the stable expression of integrin $\beta 1$ at the transcriptional level. We hypothesized that the increased expression of integrin $\beta 1$ on Hsc70-AS/U87 cell surface might be attributed to the transportation of the mature form of integrin $\beta 1$. Western blot was performed to investigate the protein levels of integrin $\beta 1$ in Hsc70-AS/U87 and Vector/U87 cells (Figure 3A and C). There was no obvious difference in total integrin $\beta 1$ proteins. However, the ratio of mature form p125 to precursor p105 was much

higher in Hsc70-AS/U87 cells than that in Vector/U87 cells. Endoglycosidase H (Endo H) cleaves *N*-linked glycoproteins to remove the chitobiose core of mannose and some hybrid oligosaccharides. Endo H digestion completely converted p105 to a band of 85 kDa (the size of the core protein), whereas p125, which is glycosylated by medial- and trans-Golgi enzymes, was Endo H resistant.¹⁷ As shown in Figure 3B, lysates of U87 cells transiently transfected with Hsc70-AS were digested with Endo H before Western blot analyses. The 85-kDa band of integrin $\beta 1$ was seen after Endo H digestion.

Integrin $\beta 1$ Was Localized Near the Cell Surface in Hsc70-AS/U87 Cells

The above results indicated that down-regulation of Hsc70 in glioma cells might prompt the processing of integrin $\beta 1$ in Golgi and its transportation to the cell surface. Thus, cellular localization of integrin $\beta 1$ was detected using immunofluorescence confocal microscopy analysis. Vector/U87 cells or Hsc70-AS/U87 cells were reacted with anti-integrin $\beta 1$ mouse monoclonal antibody followed by incubation with rhodamine-conjugated goat anti-mouse IgG (red) and C6-NBD (Golgi specific dye, green). As shown in Figure 4,

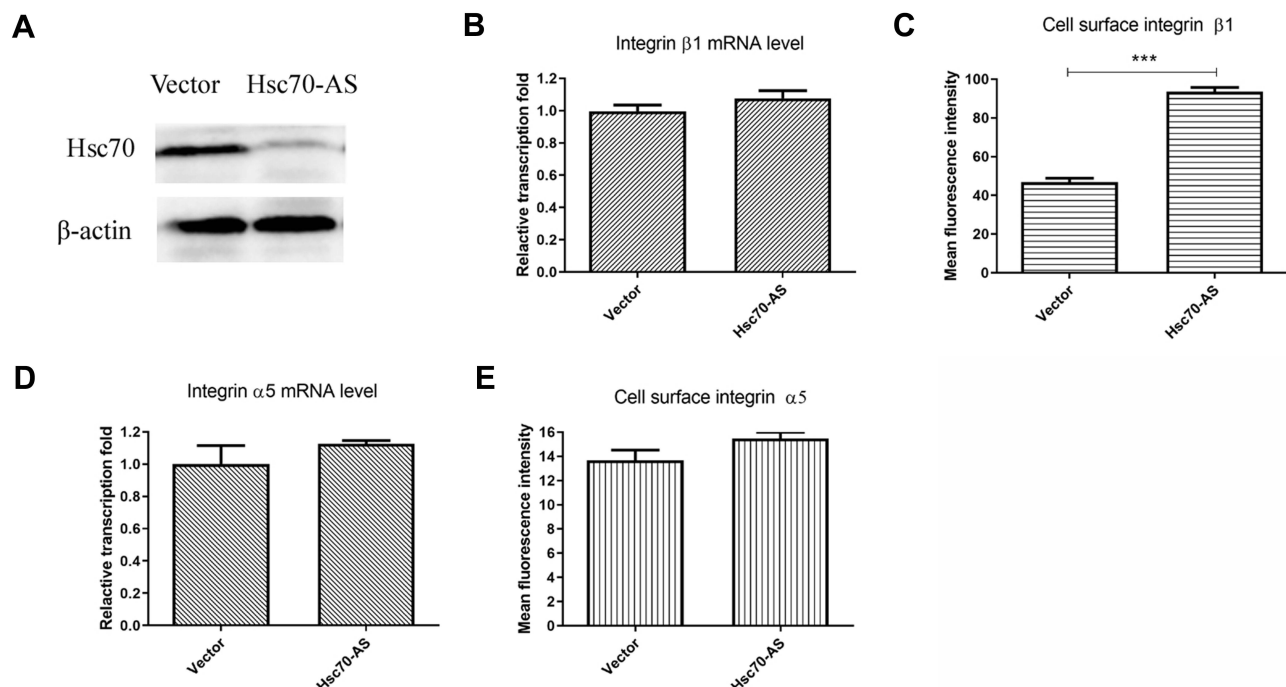


Figure 2 Analysis of the cell surface expression and transcription of integrin $\beta 1$ and $\alpha 5$ subunits in Hsc70-AS/U87 and Vector/U87 cells. (A) The down-regulation of Hsc70 was confirmed by immunoblotting. (B) RT-PCR analysis of the transcription of integrin $\beta 1$ in Hsc70-AS/U87 and Vector/U87 cells. (C) Hsc70-AS/U87 and Vector/U87 cells were subjected to analysis with FACS of cell surface integrin $\beta 1$ subunit. (D) RT-PCR analysis of the transcription of integrin $\alpha 5$ in Hsc70-AS/U87 and Vector/U87 cells. (E) Hsc70-AS/U87 and Vector/U87 cells were subjected to analysis with FACS of cell surface integrin $\alpha 5$ subunit. Data were expressed as the mean \pm SEM from three independent experiments, *** $P < 0.001$.

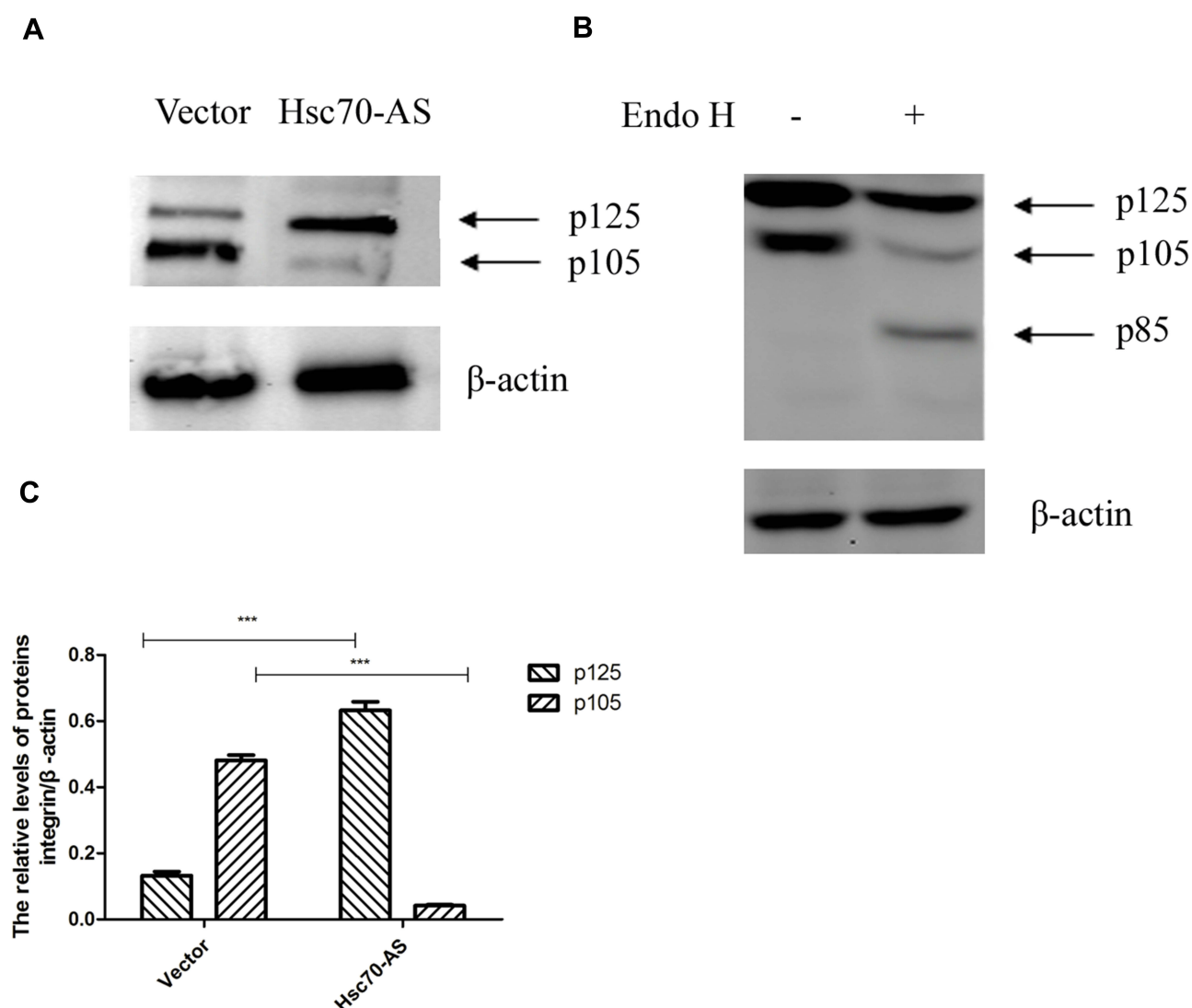


Figure 3 Down-regulating the expression of Hsc70 increased p125 and decreased p105. **(A)** Equal amounts of cellular protein from Hsc70-AS/U87 and Vector/U87 cells were subjected to immunoblot analysis with anti-integrin $\beta 1$ or anti- β -actin antibodies. **(B)** Equal amounts of cellular protein from whole-cell extracts of U87 cells transiently transfected with Hsc70-AS were treated with Endo H (+) or vehicle (-) for 24 h at 37°C. The samples were subjected to Western blot analysis with an anti-integrin $\beta 1$ antibody. **(C)** Quantitative results of A. Data were expressed as the mean \pm SEM from three independent experiments, *** $P < 0.001$.

integrin $\beta 1$ is in red and the Golgi marker is in green. The yellow image is a red/green merge to show colocalization. In Vector/U87 cell, the colocalization was much higher than that in Hsc70-AS/U87 cells. In Hsc70-AS/U87 cells, the integrin $\beta 1$ was localized near the cell surface (Lower panel, white arrow), suggesting down-regulation of Hsc70 could promote the transportation of integrin $\beta 1$ from Golgi to the cell surface.

The Interaction Between $\beta 1$ and $\alpha 5$ Subunits Was Strengthened in Hsc70-AS/U87 Cells

Integrin $\beta 1$ interacts with many alpha integrins to exert biological functions.¹⁸ It has been reported that the expression of β -

1,4-GalT regulated glycosylation and function of $\alpha 5\beta 1$ integrin.¹⁹ Thus, whether down-regulation of Hsc70 in glioma cells could affect the function of integrin $\beta 1$ through regulating the interaction between integrins $\alpha 5$ and $\beta 1$ subunits was investigated. As shown in Figure 5A, the interaction between integrins $\alpha 5$ and $\beta 1$ subunits was strengthened in Hsc70-AS/U87 cells demonstrated by immunoprecipitation assay. FAK is a key mediator for cell migration and invasion.²⁰ We further investigated the expression of FAK and phosphorylated FAK in Hsc70-AS/U87 cells by immunoprecipitation and the results showed that the phosphorylation of FAK was increased, suggesting the activation of integrin-FAK signaling axis (Figure 5B).

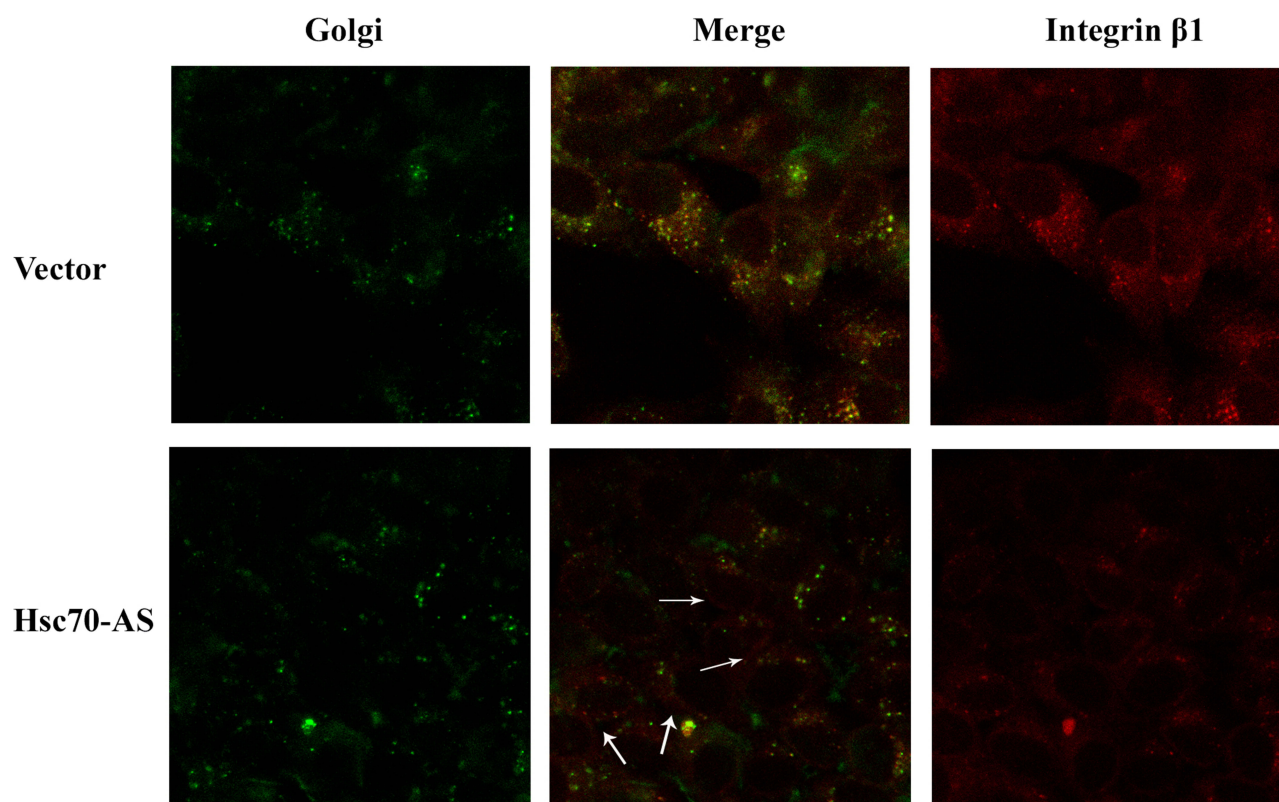


Figure 4 Reducing the expression of Hsc70 in U87 cells affected the subcellular localization of integrin β 1 subunit. After fixed and permeabilized, Vector/U87 cells (upper panel) or Hsc70-AS/U87 cells (lower panel) reacted with anti-integrin β 1 mouse monoclonal antibody followed by incubation with rhodamine-conjugated goat anti-mouse IgG and C6-NBD (Golgi specific dye). Images were captured and analyzed with a Zeiss confocal microscope (40x).

Cell Adhesion Was Increased in Hsc70-AS/U87 Cells

Fibronectin (FN) is a ~440-kDa glycoprotein of the extracellular matrix that binds to integrins, which plays a major role in cell adhesion, growth, migration, and differentiation.²¹ As shown in Figure 6A, in FN-coated plates, the adhesive ability of Hsc70-AS/U87 cells was increased compared with Vector/U87 cells. Furthermore, a wound-healing experiment was performed to evaluate the migration ability of Hsc70-AS/U87 cells. As shown in Figure 6B and C, the result was consistent with that in cell adhesion assay in Figure 6A, the migration ability of Hsc70-AS/U87 cells was significantly attenuated.

Discussion

Glioblastoma is one of the most common malignant tumors in the human central nervous system. Until now, the pathogenesis of glioblastoma has not been studied clearly. The significance of β -1,4-GalT V in invasion and metastasis of cancer cells has been verified.^{22,23} Research has indicated the increased galactosylation in astrocytomas, which was caused

by alterations of expression of β -1,4-GalT V, and the malignant degree of astrocytoma is correlated with the expression of β -1,4-GalT V.²⁴ In our previous study, we have shown that β -1,4-GalT V is over-expressed in glioma tissues and cells, and is positive correlated with the grade of gliomas. Interestingly, Hsc70 was found to interact with β -1,4-GalT V and played an important role in tumor metastasis and invasion in our previous studies. However, the detailed mechanism elucidating the function of Hsc70 in the invasion of glioma cells has not been uncovered. In this manuscript, we found that the down-regulation of Hsc70 significantly promoted the expression of integrin β 1 125-kDa mature form, indicating the involvement of integrin β 1 in Hsc70-mediated invasion of glioma cells.

Integrins are transmembrane receptors that facilitate cell extracellular matrix (ECM) adhesion. Changes in the expression of cell surface integrins are important in the genesis and development of cancer, impacting on various aspects of cancer metastasis and invasion.²⁵ Glycosylation of integrin β 1 in the Golgi complex has been related to its function in multiple cell processes, e.g., invasion, matrix adhesion, and migration.²⁶ The mature integrin β 1 subunit pairs with at least 12 different

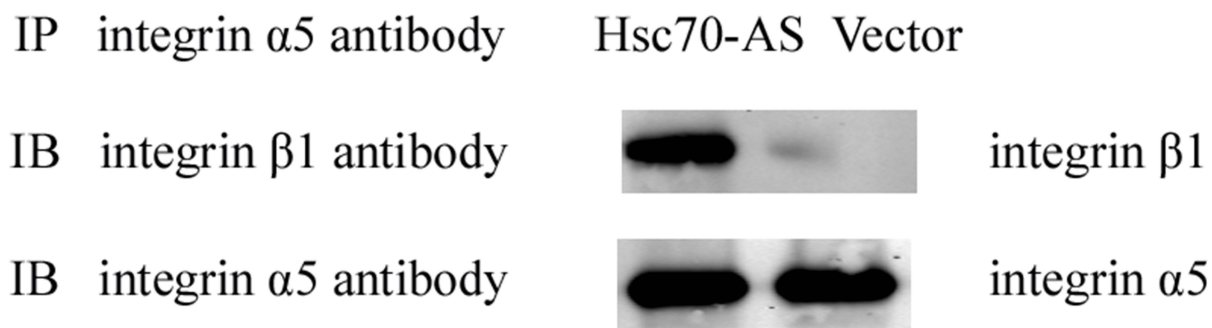
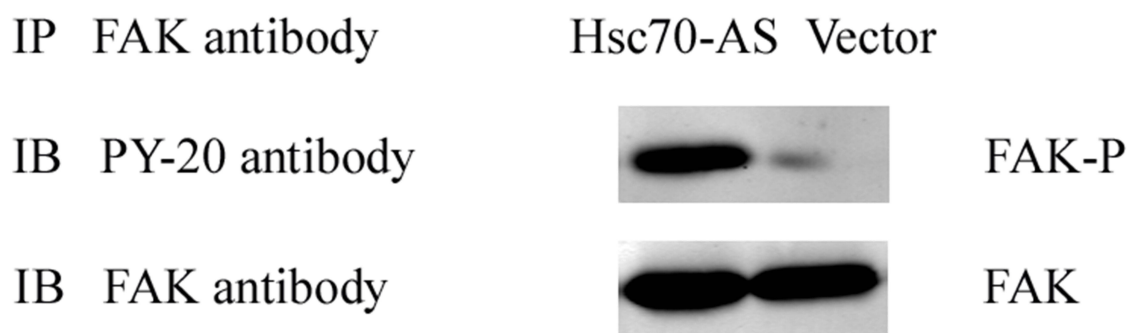
A**B**

Figure 5 Reduction in the Hsc70 expression strengthened the interaction between integrin $\beta 1$ and $\alpha 5$ subunits and increased the level of FAK phosphorylation. **(A)** Endogenous integrin $\beta 1$ subunit interacts with integrin $\alpha 5$ subunit in Hsc70-AS/U87 and Vector/U87 cells. immunoprecipitation was performed with monoclonal anti-integrin $\alpha 5$ antibody. Co-immunoprecipitation protein was probed with indicated antibodies. **(B)** After incubation on FN (15 $\mu\text{g/mL}$) for 30 min, the cell lysates were immunoprecipitated by the monoclonal anti-FAK antibody. The level of FAK and phosphor-FAK was detected by indicated antibodies.

α -subunits to form transmembrane adhesion receptors for ECM proteins, e.g., collagen, FN, and laminin. The function of $\beta 1$ depends on its accurate glycosylation catalyzed by enzymes located in morphologically and biochemically distinct cisternae of the Golgi system. Mature integrin $\beta 1$ is transported to the cell surface, where it mechanically links plasma membrane adhesion complexes to the actin cytoskeleton for bidirectional transmembrane as well as intracellular signaling.²⁷ Many factors can affect the maturation of integrin $\beta 1$ and its transport to the cell surface. In this study, we found that down-regulating the expression of Hsc70 in U87 cells specifically up-regulated the expression of cell surface integrin $\beta 1$ without the change of its mRNA. Meanwhile, the integrin $\beta 1$ 125-kDa mature form increased and 105-kDa precursor form decreased when Hsc70 was down-regulated. Furthermore, the U87 cells transfected with antisense cDNA of Hsc70 decreased the Golgi localization of integrin $\beta 1$ and increased the transportation of integrin $\beta 1$ from Golgi to the

cell surface. From these results, we can speculate the possibility that the enhancement of cell surface integrin $\beta 1$ in Hsc70-AS/U87 cells is not caused by increment of mRNA transcription of integrin $\beta 1$, but may be caused by the accelerated transformation of the precursor form of 105 kDa to mature form of 125 kDa.

FAK, also known as protein tyrosine kinase 2, is a focal adhesion-associated protein kinase involved in cellular adhesion and spreading processes. This cytosolic kinase has been implicated in diverse cellular roles including cell locomotion, mitogen response and cell survival.²⁸ It has been shown that when FAK was blocked, breast cancer cells became less metastatic due to decreased mobility.²⁹ FAK is phosphorylated in response to integrin engagement and recruited to focal adhesions. In Hsc70-AS/U87 cells, down-regulation of Hsc70 increased the function of integrin $\beta 1$ through regulating the interaction between integrins $\alpha 5$ and $\beta 1$ subunits, increasing the

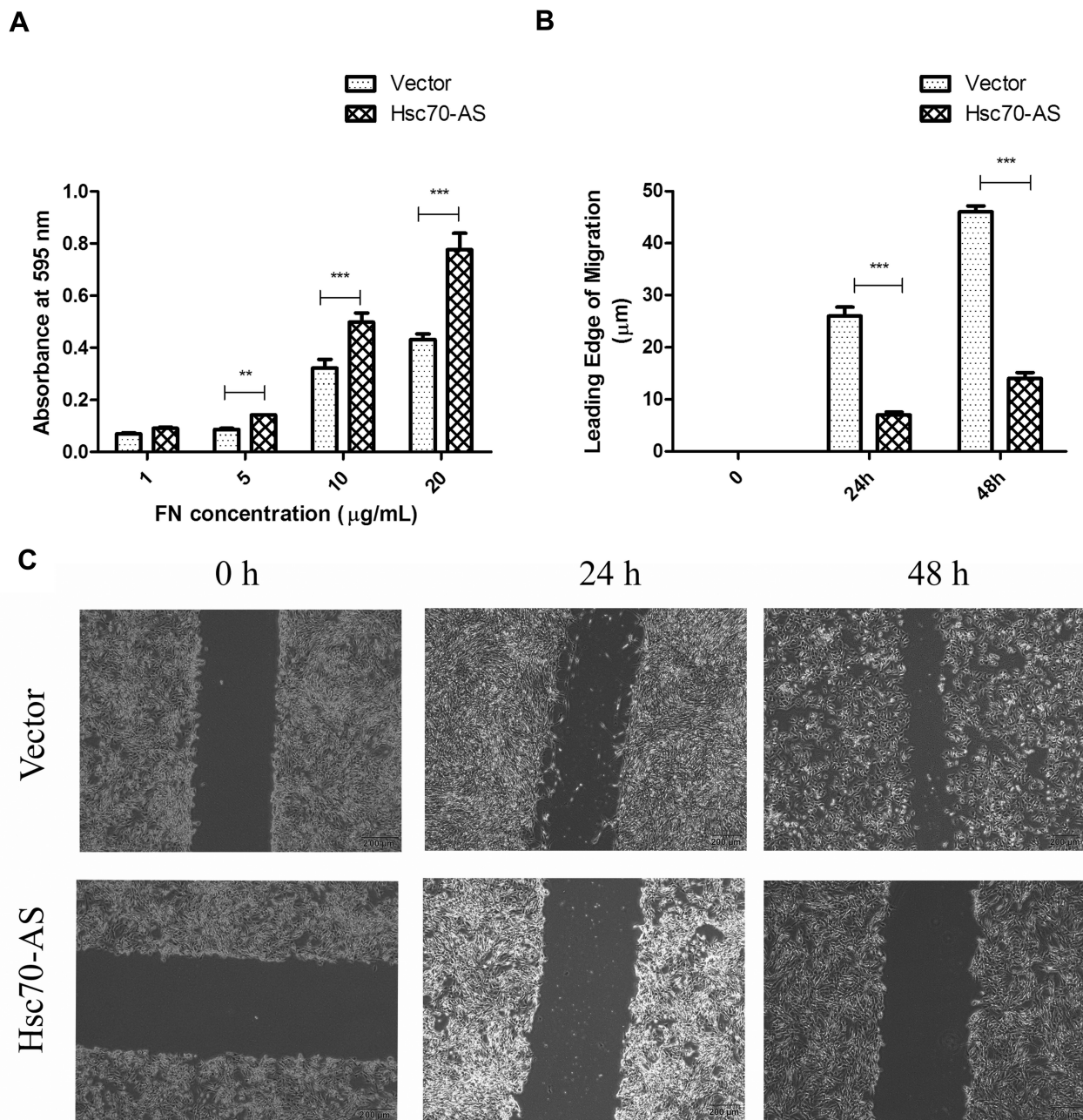


Figure 6 Increased cell adhesion in Hsc70-AS/U87 cells. **(A)** Cells were applied to FN-coated 96-well plates and incubated at 37°C for 30 min. Adherent cells were crystal violet and absorbance of each well was determined at 595 nm. **(B)** The corresponding histograms of migration in **(C)**. Each bar represents the mean±SEM from three independent experiments, ** $P<0.01$, *** $P<0.001$. **(C)** Cells were seeded onto an FN-coated (10 μg/mL) 6-well plate in a serum-free medium for 24 h and the monolayer was scratched with a plastic pipette tip. The plate was then incubated for indicated times at 37°C in serum-containing media and the wound-induced migration of cells was measured.

phosphorylation of FAK and activating integrin-FAK signaling pathway.

Conclusion

In our previous studies, the expression of Hsc70 was found to increase in the process of human cancer cells, and the overexpression of Hsc70 promoted the growth and invasion of human glioma cells. The Hsc70-AS/U87 cells in

this study demonstrate the suppression of tumor development and migration, suggesting that Hsc70 could represent a novel target in glioma therapy.

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Disclosure

The authors have no conflicts of interest in this work.

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