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Integrin A3 (ITGA3) as a Prognostic Marker and Potential Therapeutic Anti-Metastasis Target in Glioma

*Tzou RD[1], Hsu YC[2], Tsai TH[1,3,4,5], Lee KT[1], Huang TY[6] and Fan WC[1]**

[1]Department of Surgery, Division of Neurosurgery, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung, Taiwan

[2]School of Medicine, I-Shou University, Kaohsiung, Taiwan

[3]Department of Surgery, Division of Neurosurgery, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan

[4]Department of Surgery, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

[5]Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

[6]Department of Neurosurgery, Changhua Christian Hospital, Changhua, Taiwan

Abstract

Objectives: Integrin α3 (ITGA3), which is located on the cell membrane and functions as a cell surface adhesion molecule, is thought to be a potential molecular marker in several cancers. Its expression has also been correlated with metastasis. This study is the first to investigate the biological function of this protein in human Glioblastoma Multiforme (GBM).

Materials and Methods: Protein expression of ITGA3 in glioma tissue samples was first measured immunohistochemically and correlated with other clinical parameters. Short interfering RNA (siRNA) was added to different cell lines to block ITGA3 activity and evaluate its effect on proliferation, invasion, adhesion, and migration by assay. Western blot analyses of these cell lines were performed to measure the expression of ITGA3, N-cadherin, and Cyclin D1 signaling molecules.

Results: We found that the expression of ITGA3 was high in glioma cells and that its high expression was significantly and independently predicted poor survival in patients with astrocytoma. While ITGA3 siRNA treatment attenuated survival/proliferation, it also inhibited locomotion of GBM8401 and U87-MG cells *in vitro*, possibly through its inhibition of N-cadherin and Cyclin D1 signaling molecules. This knockdown of ITGA3 improved TMZ-related cytotoxicity in glioma cells.

Conclusion: ITGA3 was found to be a potential prognostic biomarker. While ITGA3 may predict the glioma cell progression, it could possibly be targeted to inhibit metastasis activities in established GBM cells.

Keywords: Integrin α3; ITGA3; GBM; Cell adhesion; Cell locomotion

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Correspondence:
Fan WC, Department of Surgery, Division of Neurosurgery, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung, Taiwan, Tel: +886 921817893; E-mail: dr.wenchieh@hotmail.com

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Abbreviations

CAM: Cell Adhesion Molecules; CI: Confidence Interval; ECM: Extracellular Matrix; FAK: Focal Adhesion Kinase; GBM: Glioblastoma Multiforme; IHC: Immunohistochemistry staining; ITGA3: Integrin α3; KPS: Karnofsky Performance Score; MPP: Matrix Metalloprotein; OS: Overall Survival; SI: Staining Index; SF: Surviving Fraction; siRNA: Short interfering RNA; TMZ: Temozolomide; WHO: World Health Organization; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

Introduction

Glioblastoma (GBM) is the most common and aggressive malignant brain tumor in adults, comprising 16% of all primary brain and central nervous system neoplasms [1,2]. Current standard therapy includes maximal initial surgical resection, followed by radiation with Temozolomide (TMZ), and then adjuvant chemotherapy with TMZ [3]. However, the overall survival of glioblastoma patients remains poor with less than 5% of patients surviving five years after diagnosis [4]. Currently, the best overall median survival for these patients is 14 months [5]. Thus, it is important to identify new biomarkers in glioma for diagnosis, prognosis, and therapeutic targeting.

Cell Adhesion Molecules (CAM) mediate adhesion to the Extracellular Matrix (ECM),

and CAMs have been linked to increases in cancer cell motility, progression, and metastasis [6]. ECM proteins interact directly with cell surface receptors to initiate signal transduction pathways and modulate different processes including adhesion, migration, proliferation, cell differentiation, apoptosis, and angiogenesis [7,8]. Integrin Alpha 3 (ITGA3) genes are located on chromosome 17q21.33a, which encodes them [9,10]. ITGA3 is located on the cell membrane and functions as a cell surface adhesion molecule, and it interacts with extracellular matrix proteins and activates diverse signaling pathways that have been implicated in migration, proliferation, cell survival, and gene expression [11-13]. In addition, many studies have identified ITGA3 as a potential molecular marker in several cancers [14]. Its overexpression has previously been correlated with increased invasiveness and poor prognosis in various cancers as well as metastasis [15-17]. However, its role in glioma cells and astrocytoma is not known.

In this study, we investigate the function of ITGA3 in human Glioblastoma Multiforme (GBM). To do this, we measured its expression in glioma tissues, correlate its expression with clinicopathological parameters, measured its expression different glioma cell lines, knocked it down with siRNA to observe its effect on proliferation, invasion, migration, and adhesion, and studied changes in some signaling molecules.

Materials and Methods

Study design and participants

We selected patients diagnosed and treated for astrocytoma at the Department of Neurosurgery, Kaohsiung Medical University. We excluded those diagnosed by biopsies only or those who had incomplete medical records, no follow-up visits, low-quality pathological results, or poor immunohistochemical staining. After exclusion, we were left with 98 patients to include in our analyses. All participants have been informed of the risks and benefits involved, and all participants' questions have been answered to satisfaction. Furthermore, all participants have been assured that any future questions will also be answered by a member of the research team. All participants voluntarily agree to take part in this study. All procedures were approved by the Kaohsiung Medical University Institutional Review Board (No. KMUHIRB-G(II)-20170010).

Immunohistochemistry staining

We cut 3 μm sections from formalin-fixed, paraffin-embedded tissue blocks. The sections were deparaffinized, rehydrated, and autoclaved at 121°C for 10 min in Target Retrieval Solution, pH 9.0 to retrieve antigens. We used 3% hydrogen peroxide for 5 min at room temperature to block endogenous peroxidase. After washing with Tris buffer, the sections were incubated in a 1:200 dilution for 5 h at room temperature. After washing with Tris buffer, the sections were incubated with secondary antibody conjugated with horseradish peroxidase for 30 min at room temperature. Finally, the slides were incubated in 3,3-diaminobenzidine for 5 min followed by Mayer's hematoxylin counterstaining for 90 sec and mounted. Staining scores were used to classify into two intensity types: Low-level expression and high-level expression. Scores representing the proportion of positively stained tumor cells were graded as follows: 0 (no positive tumor cells), 1 (<10%), 2 (10% to 50%), and 3 (>50%). Cutoff values for ITGA3 were chosen based on a measurement of heterogeneity using the log-rank test with respect to overall survival. A Staining Index (SI) score of >2 was considered high ITGA3 expression and <2 low expressions of ITGA3.

Cell culture and ITGA3 siRNA transfection

All cell lines were incubated at 37°C in an atmosphere of 5% CO_2. GBM8401 and GB8901 were cultured in an RPMI medium supplemented with 10% Fetal Bovine Serum (FBS). U87 MG and SVGp12 were cultured in Modified Eagle's Medium (MEM) supplemented with 10% FBS. G5T was cultured in a Dulbecco's MEM (DMEM) medium supplemented with 10% FBS. SVGp12 was isolated from normal tissue and used as normal control. siRNA transfection of astrocytoma cells was achieved using DharmaFECT Transfection Reagents (Dharmacon, Lafayette, CO, USA) and human ITGA3 siRNA constructs. Following transfection with siRNA, cells were cultured for two days before use. ITGA3 protein expression levels were measured by western blot analysis.

GBM8401 cells and U87-MG cells were subjected to siRNA-induced knockdown of ITGA3. After 48 h incubation with ITGA3 siRNA (si-ITGA3 group) or nonsense siRNA (negative group), western blot was used to measure and compare ITGA3 protein expression levels in the control/negative control groups and the siRNA group.

Proliferation assay

A density of 3×10^4 cells was suspended in a culture medium containing 10% FBS and placed in a 6-well plate with 0.5 ml medium per well. The cells were incubated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay at 24 h and 48 h.

Cell cycle analysis

The U87-MG and GBM8401 cells were plated in 6-well plates (1×10^6) in a cell incubator and cultured overnight. The next day, the cells were centrifuged in a 10ml centrifuge tube and the supernatant was collected. They were washed twice in PBS and added with 1 × Trypsin. They were then placed in a 37°C oven for 1 min to 2 min. Once the cells fell off, they were collected, placed in a centrifuge tube, and run at 2,500 rpm for 5 min to remove the supernatant. Then, 1 ml of PBS was added to wash the remaining culture solution and the cells were centrifuged again at 2,500 rpm for five minutes. 500 μl of PBS was added to break up the cell pallet and then 500 μl of 70% ethanol was slowly added to the cells for fixation. They were placed in a refrigerator where they were left overnight. The next day those cells were centrifuged at 2,500 rpm for 5 min, and the supernatant containing ethanol was removed. The cells were washed in one ml PBS. 5 μl of RNAse A 100 mg/ml was added to PBS and placed in an oven at 37°C. After the 30 min reaction time, 20 μl of propidium iodide 2 mg/ml (final concentration 40 μg/ml) was added and the cells have placed an oven at 37°C for 15 min. After that, the cells were transferred from the centrifuge tube to the Falcon tube, and the sample was mounted on the machine (Beckman colter FC500 and FACSCalibur, BD, USA). Data were analyzed using WinMDI 2.8 free software (BD, USA).

Migration assay

U87-MG and GBM8401 cells were washed with PBS, 1× Trypsin was added, and they were placed in a 37°C oven for 1 min to 2 min. After the cells had naturally fallen off, a fresh DMEM culture medium was used to collect the cells in a 15 ml centrifuge tube. 10 μl of cell fluid was added to the cell counter used to count the number of cells of each cell in culture-inserts (U87MG: 1×10^6, GBM8401: 5×10^5). They were placed in a cell culture incubator overnight. The next

day, the culture-inserts were placed under a microscope at 0 h and observed at 12 h and 24 h.

Invasion assay

Cell invasion assays were performed in vitro in Transwell chambers (COR3452; CORNING, Corning, NY, USA). To do this, cells were seeded at 5×10^5 per insert, and 2 ml of medium was added to the lower chamber of each Transwell. After incubation for 24 h, cotton squabs were used to remove the cells remaining on the upper surfaces of the Transwell membranes. Cells that had migrated through the membranes to the bottom of the insert were fixed, stained, and photographed. The number of cells in six random high-powered fields was counted.

Adhesion assay

The U87-MG and GBM8401 cells were washed with PBS, 1× Trypsin was added, and they were placed in a 37°C oven for 1 min to 2 min. Once the cells had slid down naturally, we used a new DMEM culture medium to collect them into a 15 ml centrifuge tube. 10 μl of cell fluid was added to the cell counter, the number of cells of each cell in a 24-well plate (3×10^4) was counted, and then they were placed in the cell culture incubator. They were fixed with 37% formaldehyde for 15 min, washed with PBS, stained with 0.5% crystal violet (Sigma-Aldrich; Louis, MO, USA), rinsed with water, washed the crystal violet and observed and photographed under a microscope.

Western blotting

All samples were lysed in 200 μl of lysis buffer. A total of 50 μg of protein per sample was loaded into the wells of a Sodium Dodecyl Sulfate-Polyacrylamide Gel (SDS-PAGE) and subjected to electrophoresis at 50 V for 4 h. The separated proteins were subsequently transferred to PVDF membranes. After incubation for 1 h in blocking buffer, the membranes were incubated with primary antibodies [N-cadherin (1:1000; protein tech; 22018-1-AP), E-cadherin (1:1000; protein tech; 20874-1-AP), and β-actin (1:20000; Sigma; A5441)] for 2 h at room temperature. Subsequently, the membranes were incubated with secondary antibodies (AP132P and AP124P; Millipore, Billerica, MA, USA) or a secondary antibody (IRDye; Li-COR, USA) for 90 min. Enhanced chemiluminescence solution (Western Lightning, 205-14621; Perkin Elmer, Waltham, MA, USA), a MiniChemi™ imaging and analysis system (Beijing Sage Creation, Beijing, China), and a near-infrared imaging system (Odyssey LI-COR, USA) were used to detect specific protein bands. Data were analyzed using Odyssey 2.1 software.

Data analysis

A one-way analysis of Variance (ANOVA) was used to compare proliferation, migration, and invasion assay results. A p-value <0.05 was considered significant. All statistical operations were performed using SPSS 24.0 software (SPSS, Inc., Chicago, IL, USA).

Results

ITGA3 expression and clinic-pathological parameters

We studied the association between IHC ITGA3 staining scores, specific clinic-pathological parameters, and survival in patients with astrocytoma. ITGA3 was expressed in cytoplasm in astrocytoma tumor cells. Based on our chi-square and Kaplan-Meier survival analysis, ITGA3 expression was significantly associated with WHO grade and Overall Survival (OS). Astrocytoma patients with high ITGA3 expression had poorer prognoses and higher tumor grades than those with low ITGA3 expression. Univariate Cox analysis was performed to identify the prognostic significance of clinic-pathological factors to overall survival. We found the following to be significantly associated with survival risk: Age (p<0.001), WHO grade (p<0.001), KPS (p=0.021), and ITGA3 expression (p<0.001). Multivariate analysis revealed that the significant predictors of poor survival were high tumor grade (HR=0.500, 95% CI: 0.297-0.844, p=0.009) and high ITGA3 expression (HR=0.427, 95% CI: 0.253-0.720, p=0.001).

Glioma cells had high expression of ITGA3

We wanted to explore the expression of ITGA3 in different glioma cell lines, particularly the astrocytoma cell lines (GBM8401, U87-MG, GBM8901, and G5T) and primary normal human astrocytes (SVGp12). As shown, ITGA3 expression was upregulated in GBM8401, U87 MG, GBM8901, and G5T cells (all p<0.05).

Knock-down ITGA3 attenuated survival/proliferation of GBM 8401 and U87 MG cells.

We used a siRNA knock-down model to investigate the effect of ITGA3 on cell viability of GBM8401 and U87 cells analyzed by MTT assay after 24 h and 48 h incubation. After 24 h and 48 h, incubation with ITGA3 siRNA, cell viability was assayed *via* MTT assay. In GBM8401 cells, the results indicated reduced cell viability in the si-ITGA3 #1 and si-ITGA3 #2 group relative to the negative control on 24 h and reduced cell viability in the si-ITGA3 #1 and si-ITGA3 #2 group relative to the negative control on 48 h. In U87MG cells, the results indicated reduced cell viability in the si-ITGA3 #1 and si-ITGA3 #2 group relative to the negative control on 24 h, and reduced cell viability in the si-ITGA3 #1 and si-ITGA3 #2 group relative to the negative control on 48 h. Viability in both GBM8401 cells and U8-MG cells was significantly reduced in si-ITGA3 #1 and si-ITGA3 #2 groups, compared to controls, at 24 h and 48 h.

Knock-down ITGA3 attenuated glioma cell migration

Migration assays were performed to evaluate cell migration in untreated controls and in ITGA3 siRNA transfected groups. In GBM8401 cells, si-RNA-induced ITGA3 knockdown with si-ITGA3 #1 and si-ITGA3 #2 markedly inhibited the migratory capability at 24 h, and siRNA-induced ITGA3 knockdown with si-ITGA3 #1 and si-ITGA3 #2 inhibited the migratory capability at 48 h. In U87-MG cells, siRNA-induced ITGA3 knockdown with si-ITGA3 #1 and si-ITGA3 #2 markedly inhibited the migratory capability at 24 h, and k siRNA-induced ITGA3 knockdown with si-ITGA3 #1 and si-ITGA3 #2 inhibited the migratory capability at 48 h. In both GBM8401 cells and U87-MG cells, si-RNA-induced ITGA3 knockdown with si-ITGA3 #1 and si-ITGA3 #2 markedly inhibited the glioma cell migration.

Knock-down ITGA3 attenuated glioma cell invasion

Matrigel invasion assays were performed to assess cell invasion in two untreated control and ITGA3 siRNA transfected cell lines. In GBM8401 cells, si-RNA-induced ITGA3 knockdown with si-ITGA3 #1 and si-ITGA3 #2 markedly inhibited the invasive capability. In U87MG cells, si-RNA-induced ITGA3 knockdown with si-ITGA3 #1 and si-ITGA3 #2 inhibited the invasive capability. si-RNA-induced ITGA3 knockdown with si-ITGA3 #1 and si-ITGA3 #2 markedly inhibited the invasive capability in both GBM8401 cells and U87-MG cells.

Knock-down ITGA3 attenuated glioma cell adhesion

Adhesion assays were performed on two untreated control and ITGA3 siRNA transfected cell lines. In GBM8401 cells, si-RNA-

induced ITGA3 knockdown with si-ITGA3 #1 and si-ITGA3 #2 markedly inhibited the adhesion capability at 1 h, and siRNA-induced ITGA3 knockdown with si-ITGA3 #1 and si-ITGA3 #2 inhibited the adhesion capability at 24 h. In U87MG cells, si-RNA-induced ITGA3 knockdown with si-ITGA3 #1 and si-ITGA3 #2 markedly inhibited the adhesion capability at 1 h, and siRNA-induced ITGA3 knockdown with si-ITGA3 #1 and si-ITGA3 #2 inhibited the adhesion capability at 24 h. si-RNA-induced ITGA3 knockdown with si-ITGA3 #1 and si-ITGA3 #2 markedly inhibited the adhesion of GBM8401 cells and U87 MG cells.

Knock-down ITGA3 induced glioma cell cycle G2/M phase arrest

The cell cycle distribution of ITGA3 siRNA-treated cells was analyzed using flow cytometry. Cells were exposed to ITGA3 siRNA (si-ITGA3 #1 and si-ITGA3 #2). Exposure to ITGA3 si-RNA (si-ITGA3 #1) resulted in an increase in the number of cells in the S phase and G2/M phase; suggesting a reduction in mitosis in U87-MG and GBM 8401, though the cell cycle did not change. Furthermore, we observed that knockdown of ITGA3 with si-RNA (si-ITGA3 #2) resulted in an increase in the number of cells in the late apoptosis in U87-MG and GBM8401 cell. Therefore, glioma cell cycle G2/M phase arrest is attributed to a decrease in mitosis and increase apoptosis in both cell lines after ITGA3 siRNA transfection.

ITGA3 regulated glioma cell locomotion through the N-cadherin, Cyclin D1 signaling molecules

As shown above, we found that ITGA3 knockdown with si-ITGA3 attenuated cell locomotion in the GBM cells. Figure 7 shows the results of our Western blot analysis of cellular proteins extracted from the brain cancer cell lines treated with ITGA3 siRNA. We also measured the relative intensities of N-cadherin, Cyclin D, and ITGA3 gene expression. We found the protein expressions of N-cadherin, Cyclin D, and ITGA3 to be significantly down-regulated in ITGA3-treated GBM8401 and U87-MG cells. In the Western Blot, ITGA3 knockdown with si-ITGA3 #1 and si-ITGA3 #2 markedly decreased the relative level of N-cadherin and ITGA3 in GBM8401 cells and U87-MG cells. These results suggest that ITGA3 knockdown with siRNA attenuated glioma cell locomotion through the N-cadherin, Cyclin D signaling molecules.

Discussion

This study found high expression of ITGA3 to be a significant independent predictor of poor survival in astrocytoma and that glioma cells had high levels of ITGA3. Knocking down ITGA3 with siRNA attenuated survival/proliferation, inhibited GBM8401 and U87-MG cell locomotion *in vitro*, possibly via its inhibition of N-cadherin, Cyclin D1. Furthermore, we found that knock-down ITGA3 enhanced radiation- and TMZ-related cytotoxicities in glioma cells. These findings suggest that ITGA3 may not only be used to predict the glioma cell progression, it could potentially be targeted to reduce metastasis of established GBM cells.

Recent studies have found ITGA3 to be a potential molecular marker in many cancers as well as a useful prognostic marker [18-28]. In the current study, ITGA3 expression was upregulated in different glioma cell lines *in vitro*. ITGA3 expression was found to correlate with histological grade and to be an independent prognostic factor for the overall survival of glioma patients. To the best of our knowledge, this study the first to report that high ITGA3 expression was a significant independent predictor of poor survival and the first to explore the association between ITGA3 regulation and the clinicopathological parameters in glioma patients.

Accumulating evidence suggests that ITGA3 overexpression may accelerate the progression of cancer. It appears to act as a cell adhesion molecule for ECM proteins and plays a role in cell adhesion [29]. Its overexpression has been found to increase cell migration, metastasis, and invasiveness in many cancers [30-35]. In this study, we were interested in what role ITGA3 might play in cell locomotion in GBM. Based on our results depicted in Figures 3, 4, and 5, cell locomotion was attenuated by knock-down ITGA3 in glioma cell lines. These findings indicate that the ITGA3's promotion of glioma cell migration and invasion may be related to its effect on cell adhesion. Knockdown of ITGA3 attenuated glioma cell adhesion, migration, and invasion.

Many studies have also shown that ITGA3 regulates cell progression and metastasis through several pathways including FAK-Src signaling and PI3K-Akt signaling. It mediates cell survival and invasion through FAK-Src signaling [36-38]. Recently, it has been found that the FAK–Src complex can be activated in many tumor cells where it generates signals such as matrix Metalloprotein (MPP) promoting further growth and metastasis. Activated FAK–Src functions to promote cancer cell motility, cell cycle progression, and cell survival [38]. Both FAK and Src catalytic activities are important in promoting VEGF-associated tumor angiogenesis and protease-associated tumor metastasis [38]. In addition, it has been found that ITGA3 may accelerate cancer development by activating the PI3K-Akt signaling pathway, increasing proliferation, migration, and invasion in many cancers. Nakada et al. [26] have found a strong association between integrin α3 and ERK1/2 activation, and interaction found to be critical to the glioma cell invasion induced by ITGA3 [39,40]. In the current study, our western blots showed that Cyclin-D1 and N-cadherin expression were decreased after ITGA3 siRNA transfection. These results suggest that ITGA3 may mediate glioma cell progression and adhesion. Because little is known about how ITGA3 regulates downstream proteins of signaling pathways, further research is necessary to validate the role of ITGA3 in the process of glioma progression and invasion.

Epithelial-Mesenchymal Transition (EMT) is known to participate in the invasion and metastasis of neoplastic malignancies [41]. The EMT switching process leads to loss of E-cadherin and gain of N-cadherin [42]. One key factor involved in EMT is N-cadherin. N-cadherin can affect cell survival, facilitate the EMT process, and promote migration/invasion by recruiting signaling molecules during tumor progression [43]. Many signaling pathways are known to participate in the process of malignancy. N-cadherin/FGFR/MAPK/ERK/matrix metalloprotein-9 have been found to be involved in N-Cadherin's mediation of metastasis [43]. In our study, ITGA3 knockdown with si-ITGA3 attenuated glioma cell locomotion through the N-cadherin signaling molecules. This interplay between ITGA3 and N-cadherin can drive crosstalk to regulate the spatial distribution of these receptors as well as their signaling intermediates, the actin cytoskeleton, and intracellular forces. A fine balance between ITGA3 and N-cadherin is crucial for cell migration. Additionally, Cyclin D1 is known to be an important regulator of cell cycle progression and known to promote progression through the G1-S phase of the cell cycle [44]. Overexpression of Cyclin D1 has been linked to the development and progression of cancer and has been found to play a key role in the development of a subset of human cancers Thus, the therapeutic ablation of Cyclin D1 may be useful for the prevention

and treatment of cancer [45,46]. In this study, we found the protein expressions of Cyclin D1 to be significantly down-regulated in ITGA3-treated GBM8401 and U87-MG cells. In addition, our cell cycle analysis showed ITGA3-siRNA transfection induced glioma cell cycle G2/M phase arrest. Considered together, our findings suggest that in our ITGA3 siRNA cell lines, there was inhibition of the expression Cyclin D1 and downstream signaling molecule, hindering the progression of the cell cycle and inhibiting the cell cycle from G1 phase to S phase. This halted the cell cycle stop at the G2/M phase.

Glioma is often treated using a multidisciplinary approach, including maximal surgical resection, radiation therapy, and chemotherapy [47]. Despite all the interventions, the median Overall Survival (OS) for patients with newly diagnosed GBM is only 12 to 18 months [48]. Many scientists are actively searching looking for new molecular markers of tumor growth, apoptosis, invasion, and angiogenesis that could possibly be targeted therapeutically in glioma. In this study, we explored the therapeutic potential of ITGA3 in glioma. ITGA3 siRNA treatment enhanced TMZ-related cytotoxicities and attenuated survival and proliferation of GBM8401 and U87-MG cells. These results suggest ITGA3 may be targeted to directly treat cytotoxic cells via its effect on cell locomotion and enhance the toxicity of radiation therapy and chemotherapy.

In this article, the following new findings are reported. First, the high ITGA3 expression was significant independent predictors of poor survival in astrocytoma. High-level ITGA3 were expressed in tested glioma cells. In addition, ITGA3 siRNA treatment attenuates survival/proliferation; and inhibit cell locomotion of GBM8401 and U87-MG cells *in vitro*, possibly though its inhibition of N-cadherin, Cyclin D1 signaling molecules. Finally, knock-Down ITGA3 enhance TMZ-related cytotoxicity in glioma cells. This study has several limitations also. One limitation is that we did not focus specifically on the downstream signaling molecules of ITGA3. Although we studied Cyclin D1 and N-Cadherin in our ITGA3 siRNA treated cell lines, there are other credible molecules such as FAK-Src and PI3K-Akt signaling molecules that could also be considered. Another limitation is that, although we attempted to identify a clear mechanism underlying the anti-cancer effects of ITGA3 siRNA treatment, there is little evidence in this study verifying the relationship between cell locomotion and siRNA treatment. Therefore, further studies needed to explore the links between cell locomotion and ITGA3 siRNA treatment in glioma cells.

Conclusion

In summary, this study found that ITGA3 played a crucial role in the locomotion of glioma. Its overexpression was found in high-grade glioma cell lines and associated with a poor prognosis. ITGA3 knockdown attenuated the proliferation, migration, adhesion, and invasion of glioma cells; and down-regulated the expression of N-cadherin and Cyclin D1 signaling molecules. Considered together, these results suggest that ITGA3 can be used as a prognostic biomarker in patients with glioma and could potentially be targeted to inhibit metastasis of established GBM cells.

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