Hindawi
Journal of Nanomaterials
Volume 2021, Article ID 9942260, 7 pages
https://doi.org/10.1155/2021/9942260

Research Article

High Expressions of Notch and Survivin in Elderly Patients with Glioma Contribute to an Unfavorable Prognosis

Yuguo Liao, Kangsheng Zhou, Haikun Li, and Lin Yang

Neurosurgery Department, Huidong People's Hospital, China

Correspondence should be addressed to Yuguo Liao; liaoyuguo1892368@126.com

Received 26 March 2021; Accepted 26 April 2021; Published 11 May 2021

Academic Editor: Songwen Tan

Gliomas account for 24% of all primary brain and central nervous system tumors. To date, elderly patients constitute 10-25% of patients with a diagnosis of glioblastoma multiforme, but limited attention has been put on their optimal treatment, largely due to a very poor expected survival (only 4-6 months). Unraveling the molecular mechanism of gliomas provides an opportunity to develop novel biomarkers and therapeutic targets. In this study, we collected fasting blood samples from elderly patients diagnosed with glioma and who received treatment in our hospital between May 2016 and May 2019 and determined the expression levels of Notch and Survivin proteins in different clinical stages and their relationship with patient survival. A total of 68 healthy volunteers in this hospital during the same period served as healthy controls. Compared with the healthy controls, the expressions of Notch 1, Notch 2, Notch 3, and Survivin protein in the serum of elderly glioma patients were remarkably increased ($P < 0.05$), but the expression of caspase-3 protein declined ($P < 0.05$). As the clinical stage of the patient advanced, the expressions of Notch 1, Notch 2, Notch 3, and Survivin increased, and this increase was statistically significant ($P < 0.05$). It was observed that high expressions of serum Notch 1, Notch 2, Notch 3, and Survivin were associated with poor overall survival of elderly patients with glioma. We used γ-secretase inhibitor MRK-003 and specific ligand Jagged1 to alter the Notch pathway in U251 cells. It was revealed that MRK-003 incubation effectively suppressed the mRNA expression of Survivin in U251 cells, but Jagged1 stimulation significantly promoted the mRNA expression of Survivin in U251 cells. Results of MTT and transwell migration assays revealed reduced U251 cell viability and migration following MRK-003 treatment and enhanced cell viability and migration following Jagged1 stimulation. In conclusion, the finding obtained from these results supports that Notch and Survivin proteins contribute to the development of glioma in elderly patients and could serve as prognostic factors.

1. Introduction

Gliomas represent the most common primary brain and central nervous system tumors worldwide and occur almost exclusively in the following four lobes of the brain: frontal (23.6%), temporal (17.4%), parietal (10.6%), and occipital (2.8%), leading to significant morbidity and mortality in adults, especially elderly people [1, 2]. To date, elderly patients already account for 10-25% of all patients with a diagnosis of glioblastoma multiforme, but limited attention has been put on their optimal treatment, largely due to a very poor expected survival ranging only from 4 to 6 months [3]. In this context, the increasing aging of the population will contribute to at least twofold increase in the number of glioma diagnoses in elderly patients in the following two decades [4, 5]. Because of the inability to tolerate the treatment, elderly patients with gliomas have far less chance to undergo resection and receive adjuvant therapies than their younger counterparts and typically only receive palliative care [6]. Therefore, the management of gliomas in elderly patients has received much attention and becomes an important and challenging topic in neurooncology.

Unraveling the molecular mechanism of gliomas provides an opportunity to develop novel biomarkers and therapeutic targets. Notch is a crucial evolutionary conserved pathway that engages in the modulation of central cellular processes during embryonic and postnatal development, and it encodes for four paralogs, Notch 1-4 [7]. The Notch pathway inhibits neuronal differentiation in the central nervous system through maintenance of neural stem cells and

commitment of neural progenitor cells into the glial lineage. Notch is therefore often implicated in the development of brain tumors, as tumor cells share various characteristics with neural stem and progenitor cells [8]. Survivin is known as a member of the inhibitor of apoptosis family and is nearly undetectable in most normal tissues in adults. It is highly expressed in almost all human malignancies [9]. Recent studies focus on nanoparticles based on Survivin-targeting treatments as brain cancer therapies [8]. Caspase-3 is a member of cysteinyl aspartate-specific proteases that are highly conserved in multicellular organisms and function as a key regulatory molecule in neurogenesis and synaptic activity [10]. In this study, we collected fasting blood samples from elderly patients diagnosed with glioma and who received treatment in our hospital between May 2016 and May 2019 and determined the expression levels of Notch and Survivin proteins in different clinical stages and their relationship with patient survival.

2. Materials and Methods

2.1. Patient Information. The study recruited elderly patients with glioma according to predefined inclusion and exclusion criteria. Inclusion criteria are age ≥ 60 years, initial diagnosis of glioma according to the 2007 WHO classification of tumor of the central nervous system [11], and patients' or their family members' signed informed content. Exclusion criteria are patients with other malignant tumors; patients with severe metabolic system diseases such as diabetes; patients with immune system diseases; patients with severe heart, kidney, and lung dysfunction; and patients with severe cognitive dysfunction. Finally, a total of 100 elderly patients with glioma diagnosed and treated in our hospital from May 2016 to May 2019 were recruited into the study. Among these 100 patients, 65 were males and 35 were females, aged 62-77 years old, with an average of 66.6 ± 8.6 years; there were 29 patients in clinical phase I, 21 patients in clinical phase II, 30 patients in clinical phase III, and 20 patients in clinical phase IV. The 68 healthy persons undergoing physical examination in this hospital during the same period were selected as the control group. There was no statistical difference in the average age and gender ratio between the two groups ($P > 0.05$). A 6-year follow-up was performed on each patient. The study protocol was approved by the Ethics Committee of our hospital.

2.2. Blood Sample Collection and Enzyme-Linked Immunosorbent Assay (ELISA). Fasting venous blood from each elderly patient was sampled and centrifuged at low speed to separate the upper serum for testing. The serum levels of Notch 1, Notch 2, Notch 3, Survivin, and caspase-3 were measured by ELISA methods using commercial available kits (Santa Cruz Company, Elabscience Company, and Omega Company) following the instructions provided by the manufacturers.

2.3. Classification of High, Medium, and Low Expressions. Elderly patients with high, medium, and low serum expressions of Notch 1, Notch 2, Notch 3, Survivin, and caspase-3 were classified according to the following threshold values: Notch 1 (low expression: ratio to β-actin < 0.45, medium expression: $0.45 \leq$ ratio to β-actin ≤ 0.50, and high expression: ratio to β-actin > 0.50), Notch 2 (low expression: ratio to β-actin < 0.40, medium expression: $0.40 \leq$ ratio to β-actin ≤ 0.55, and high expression: ratio to β-actin > 0.55), Notch 3 (low expression: ratio to β-actin < 0.45, medium expression: $0.45 \leq$ ratio to β-actin ≤ 0.55, and high expression: ratio to β-actin > 0.55), and Survivin (low expression: ratio to β-actin < 0.35, medium expression: $0.35 \leq$ ratio to β-actin ≤ 0.40, and high expression: ratio to β-actin > 0.40). Besides, the serum levels of caspase-3 in patients with different stages were detected.

2.4. Cell Culture. Human glioma cell line U251 (Shanghai North Connaught Biotechnology Co., Ltd., China) was maintained in DMEM with 10% fetal bovine serum (FBS, Gibco, USA), 100 μg/mL streptomycin, and 100 IU/mL penicillin in a humidified environment in the presence of 5% CO_2. U251 cells were seeded into the 6-well plate and allowed to settle overnight. Afterwards, the cultured cells were treated with 10 μM γ-secretase inhibitor (GSI) MRK-003 for 48 h to block the Notch signaling pathway [12].

2.5. Soluble Jagged1 Ligand Immobilization. The recombinant rat Jagged1-Fc fusion chimera (R&D Systems, Minneapolis, MN) was dissolved in phosphate-buffered saline and immobilized in flat-bottom 96-well plates for 20 hours at 4°C at 10 μg/mL (100 μL/well). Human IgG-Fc (R&D Systems) was used and served as the control for Jagged1. U251 cells were seeded into the plates coated with Jagged1 or IgG-Fc at 10^5 cells/well.

2.6. Survivin Promoter Luciferase Assay. U251 cells were cotransfected with reporter plasmids for Survivin promoter (pLuc-surP-596, Invitrogen, USA) and pN3-N1ICD using Lipofectamine 2000 (Invitrogen) as per the manufacturer's manual. Dual-luciferase assays were performed using GeneCopoeia's dual-luciferase assay kit (D0010, Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) on a GloMax 20/20 Luminometer (Promega, USA) using pRL-TK (Renilla luciferase) as the loading control.

2.7. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR). Total RNA was extracted from U251 cells using the TRIzol reagent. The extracted RNA was then reverse transcribed into cDNA using the PrimeScript RT kit (Takara, Japan). qRT-PCR was performed as per the instructions of the SYBR® Premix Ex Taq™ II Kit (Takara) in an ABI 7300 thermal cycler (Applied Biosystems, Foster City, CA, USA). The relative mRNA level of Survivin to β-actin was calculated by relative quantification ($2^{-\Delta\Delta CT}$ method). The primer sequences for Survivin are forward: 5′-CTTTCTCAAGG ACCACCG-3′ and reverse: 5′-CACGACGACCATTGTC ACC-3′. The primer sequences for β-actin are 5′-CTCCAT CCTGGCCTCGCTGT-3′ and reverse: 5′-GCTGTCACC TTCACCGTTCC-3′.

Table 1: Expression levels of Notch proteins in elderly patients with glioma at different clinical stages.

Group no.			Notch 1/β-actin	Notch 2/β-actin	Notch 3/β-actin
1	Healthy control ($n = 68$)		0.33 ± 0.07	0.29 ± 0.04	0.31 ± 0.05
2	Elderly glioma ($n = 100$)		0.46 ± 0.09	0.40 ± 0.09	0.45 ± 0.10
3		Stage I ($n = 29$)	0.39 ± 0.08	0.32 ± 0.04	0.36 ± 0.05
4		Stage II ($n = 21$)	0.42 ± 0.06	0.37 ± 0.08	0.41 ± 0.09
5		Stage III ($n = 30$)	0.49 ± 0.11	0.45 ± 0.08	0.50 ± 0.13
6		Stage IV ($n = 20$)	0.57 ± 0.12	0.51 ± 0.12	0.61 ± 0.13
Statistical comparison	t1-2		2.369	3.754	2.584
	P1-2		0.025	0.033	0.025
	F3-6		45.15	34.87	36.94
	P3-6		0.017	0.029	0.014

2.8. MTT Assay. Following a 48 h period of cell treatment, U251 cell viability was determined using the MTT assay. In brief, U251 cells (5×10^3 cells/well) were plated into the 96-well plate, and the plate was added with 10 μL of MTT solution (R&D Systems, USA), followed by 4 h of further incubation. The absorbance at 450 nm wavelength (OD450) was measured at indicated time points (24^{th} and 48^{th}) using a plate reader (Thermo Scientific, Watertown, USA).

2.9. Transwell Migration Assay. U251 cell migration was assessed using a transwell (8 μm pore size), and 5×10^4 cells were plated into each well of the upper chamber uncoated with the membrane. The lower chamber was added with the medium containing 10% FBS. After cells were incubated for 24 hours, those that migrated from the upper chamber into the lower chamber were fixed with methanol and stained with 0.1% crystal violet dye. A microscopic view was captured under an inverted microscope.

2.10. Statistical Analysis. Data statistics were completed using SPSS 21.0 statistical software (IBM Corp., Armonk, NY, USA). The measurement data were expressed by the mean $\pm$ standard deviation. The t-test was used to compare the two groups. One-way analysis of variance was used for multiple-group comparisons. If the overall comparison was different, the F test was used for pairwise comparison. The possibility of a difference less than 0.05 was held to be statistically significant.

3. Results

3.1. Expression Levels of Notch Proteins in Elderly Patients with Glioma according to Different Clinical Stages. First, we collected blood samples from 100 elderly patients with glioma and performed ELISA methods to examine expression levels of Notch 1, Notch 2, and Notch 3. Results showed that compared with healthy controls, the expression levels of Notch 1, Notch 2, and Notch 3 in elderly patients with glioma were evidently increased ($P < 0.05$, Table 1). Next, we classified 100 elderly patients with glioma based on their clinical stage. It was revealed that the expression levels of Notch 1, Notch 2, and Notch 3 were associated with the clinical stage of glioma ($P < 0.05$, Table 1), suggesting that high expression of Notch proteins may contribute to the progression of glioma.

3.2. Expression Levels of Survivin and Caspase-3 Proteins in Elderly Patients with Glioma according to Different Clinical Stages. Subsequently, we examined expression levels of Survivin and caspase-3 in blood samples from 100 elderly patients with glioma by ELISA methods. Results of ELISA revealed that elderly patients with glioma presented an elevated expression level of Survivin concomitant with a declined expression level of caspase-3 compared with healthy controls ($P < 0.05$, Table 2). In addition, higher Survivin expression levels and lower caspase-3 expression levels reflected the advanced clinical stage of glioma ($P < 0.05$, Table 2), indicating that Survivin and caspase-3 proteins may be associated with the progression of glioma.

3.3. High Expressions of Notch and Survivin Proteins Were Associated with Poor Overall Survival of Elderly Glioma. The overall survivals of elderly patients with high expression levels of Notch 1, Notch 2, Notch 3, and Survivin were shorter than those with medium expressions ($P < 0.05$). Elderly patients with medium expression levels of Notch 1, Notch 2, Notch 3, and Survivin were longer than those with low expressions ($P < 0.05$). The Kaplan-Meier curve was plotted to depict the overall survival of elderly patients with glioma. As shown in Figure 1, expression levels of Notch 1, Notch 2, Notch 3, and Survivin were correlated with the prognosis of elderly patients with glioma. As expression levels of Notch and Survivin proteins increased, the patient's prognosis became worse.

3.4. Alternation of Notch Pathway Affected Survivin Expression in Glioma Cells. Previous evidence demonstrated the relationship between Notch activation and Survivin expression in human cancers. Considering these given facts, we asked if Notch mediates Survivin in glioma. To test this hypothesis, we used γ-secretase inhibitor MRK-003, which blocks the activation of Notch receptors by inhibiting γ-secretase activity, to examine the effect of Notch inhibition on Survivin expression in U251 cells. Results of qRT-PCR

Table 2: Expression levels of Notch proteins in elderly patients with glioma at different clinical stages.

Group no.			Survivin/β-actin	Caspase-3/β-actin
1	Healthy control ($n = 68$)		0.30 ± 0.05	0.50 ± 0.12
2	Elderly glioma ($n = 100$)		0.40 ± 0.07	0.42 ± 0.08
3		Stage I ($n = 29$)	0.34 ± 0.05	0.55 ± 0.11
4		Stage II ($n = 21$)	0.38 ± 0.09	0.49 ± 0.10
5		Stage III ($n = 30$)	0.41 ± 0.10	0.40 ± 0.08
6		Stage IV ($n = 20$)	0.48 ± 0.11	0.36 ± 0.07
Statistical comparison	t1-2		2.551	3.174
	P1-2		0.036	0.022
	F3-6		49.21	36.47
	P3-6		0.014	0.021

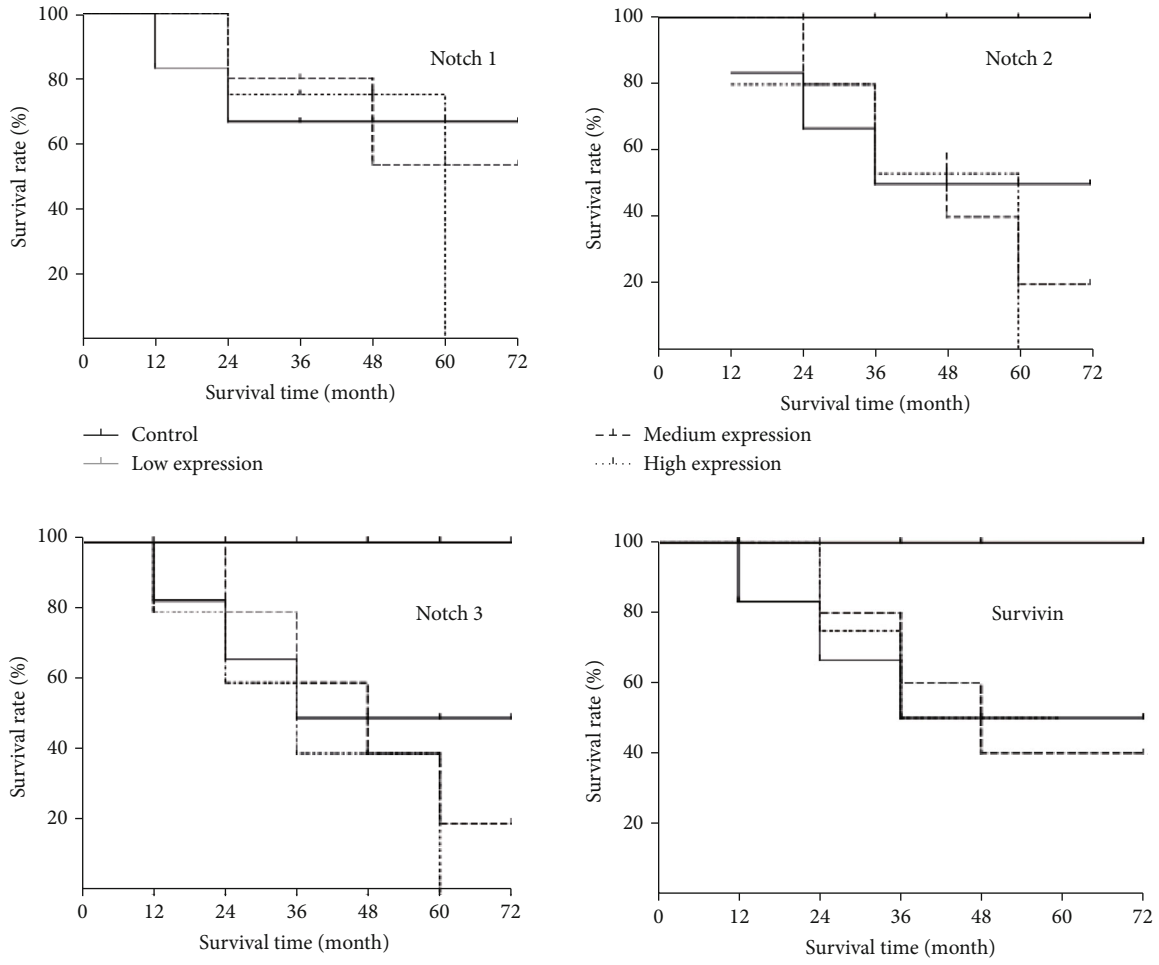

Figure 1: The Kaplan-Meier curve was plotted to depict the overall survival of elderly patients with glioma according to high, medium, and low expression levels of Notch 1, Notch 2, Notch 3, and Survivin.

displayed that the incubation of cells with MRK-003 effectively suppressed the mRNA expression of Survivin in U251 cells (Figure 2(a)). Next, we are also wondering the effect of Notch activation on Survivin expression in U251 cells. Since the Notch pathway is activated upon specific ligands like Jagged1 binding to the related transmembrane receptors, U251 cells were cultured for 48 h on immobilized Jagged1 ligand and then analyzed for Survivin expression. As expected, an increased mRNA expression of Survivin was detected in U251 cells treated with Jagged1 compared with those treated with IgG-Fc (Figure 2(b)). Notably, the delivery of pN3-N1ICD in combination with the Survivin

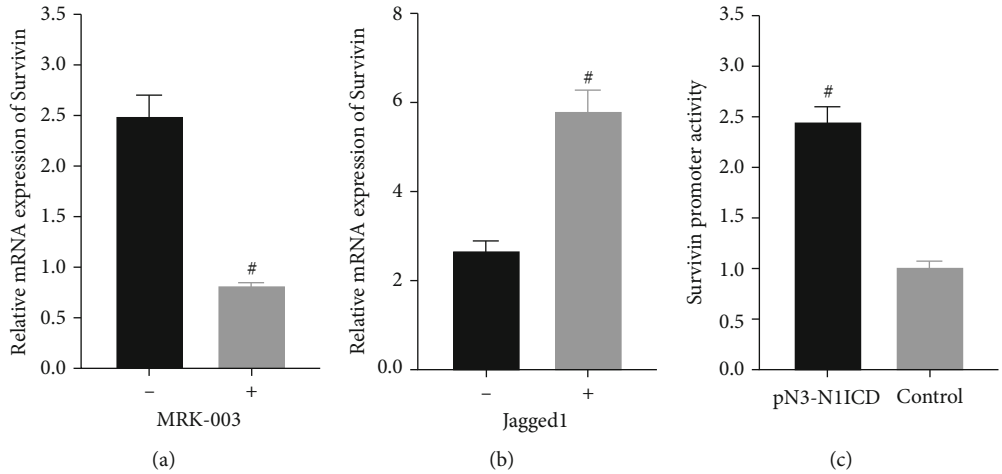

Figure 2: Alternation of the Notch pathway affects Survivin expression in glioma cells. The mRNA expression of Survivin was determined by qRT-PCR in the cultured U251 cells treated with 10 μM γ-secretase inhibitor (GSI) MRK-003 for 48 h to block the Notch signaling pathway (a) and stimulated with 10 μg/mL Jagged1 to activate the Notch signaling pathway (b). (c) The delivery of pN3-N1ICD in combination with Survivin promoter into U251 cells yielded a 2.4-fold increase in Survivin promoter activity. # indicates $P < 0.05$ compared with the corresponding control.

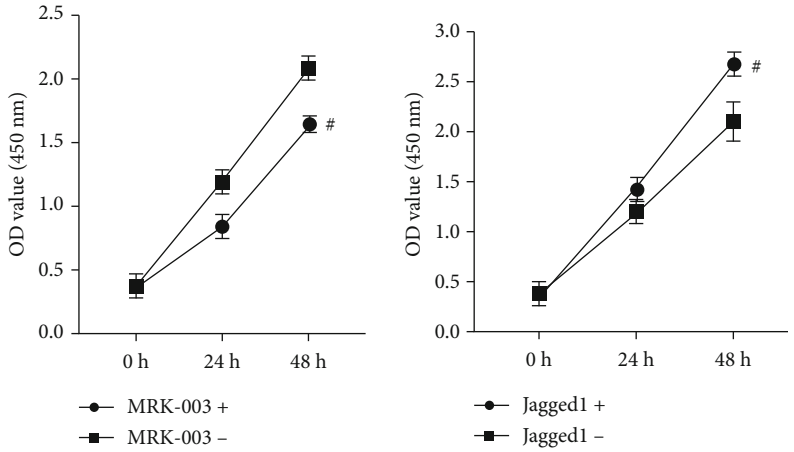

Figure 3: Alternation of the Notch pathway affects glioma cell viability. Reduced cell viability was detected by MTT assays in the cultured U251 cells treated with 10 μM γ-secretase inhibitor (GSI) MRK-003 for 48 h, and increased cell viability was detected in the cultured U251 cells stimulated with 10 μg/mL Jagged1 to activate the Notch signaling pathway. # indicates $P < 0.05$ compared with the corresponding control.

promoter into U251 cells yielded a 2.4-fold increase in Survivin promoter activity (Figure 2(c)). These findings indicated that Notch activation was associated with Survivin overexpression in glioma.

3.5. Alternation of Notch Pathway Affected Glioma Cell Viability and Migration. Next, we set out to ascertain the effects of the Notch pathway on U251 cell viability and migration. Results of MTT assays revealed reduced U251 cell viability following MRK-003 treatment and enhanced cell viability following Jagged1 stimulation (Figure 3). Results of transwell migration assays demonstrated fewer U251 cells migrating from the upper chamber into the lower one upon MRK-003 treatment and more U251 cells migrating from the upper chamber into the lower one upon Jagged1 stimulation (Figure 4). These findings indicated that activation of the Notch pathway may contribute to the development of glioma.

4. Discussion

Gliomas are the most common malignant tumors in clinical practice, common in the elderly population. Glioma patients are usually in the middle and advanced stages when they are clinically diagnosed, and the prognosis is poor. Analyzing the related biological macromolecules of the onset and clinical stage of glioma and exploring the relationship between it and the prognosis of patients are of great value to clinical diagnosis and treatment, as well as the improvement of the prognosis of patients. As revealed by the analysis on the

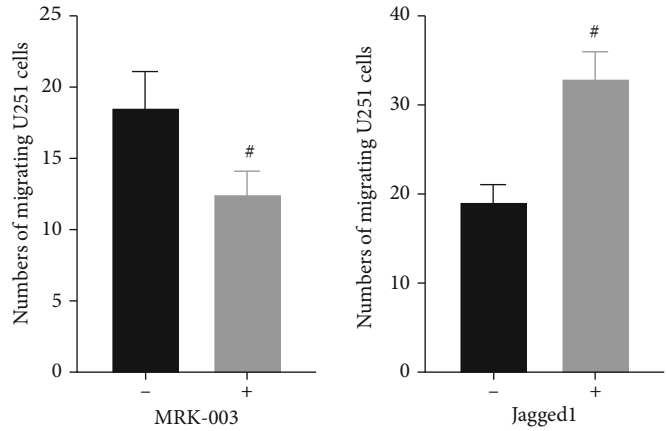

Figure 4: Alternation of the Notch pathway affects glioma cell migration. Transwell assays detected fewer U251 cells migrating from the upper chamber into the lower one upon MRK-003 treatment and more U251 cells migrating from the upper chamber into the lower one upon Jagged1 stimulation. # indicates $P < 0.05$ compared with the corresponding control.

clinical value of high expression of Notch and Survivin proteins in elderly patients with glioma and its relationship with the prognosis of patients, compared with healthy controls, the expression of Notch 1, Notch 2, Notch 3, and Survivin proteins in the elderly glioma group significantly increased while the expression of caspase-3 significantly decreased. Besides, with the increase in the clinical stages of patients, the abovementioned protein changes became more apparent, with significant differences between the clinical stages. Therefore, the expression of Notch and Survivin in elderly patients with glioma is closely related to the clinical stage and prognosis of patients.

The abnormal expression of Notch protein is closely related to the pathogenesis of various malignant tumors [13, 14] and is a potential target of drug therapy [15]. In the investigation of the inhibitory effect of Notch 1 blocker on tumor growth of the osteosarcoma mouse model and related molecular mechanisms, it was discovered that Notch 1 blocker can inhibit the tumor growth of the osteosarcoma mouse model. The molecular mechanisms involved in this process include reducing the production of proproliferation molecules and proinvasion molecules and increasing the production of proapoptosis and invasion-inhibiting molecules [16]. The analysis of the effect of RNAi interference with the expression of Notch 1 gene in melanoma cells on the immune mechanism of mouse tumors also confirmed that siNotch 1 can inhibit the Notch pathway of melanoma cells and then reduce its secretion of TGF-β; as a result, its inhibitory effect on lymphocytes is reduced, the infiltration of gp100 antigen-specific $CD8^+$ T cells in tumor tissues is increased, the secretion of IFN-γ is promoted, the proportion of Treg cells is lowered, and the body's antitumor immune function is enhanced [17]. Previous studies have also proposed that in renal clear cell carcinoma cell lines, overactivated Notch 1 can promote cancer by regulating the PTEN/PI3K/AKT signaling pathway; this provides a new treatment strategy for the treatment of renal clear cell carcinoma [18]. In our study, compared with the control group, the expression of Notch 1, Notch 2, and Notch 3 proteins in the elderly glioma group significantly increased; with the increase in the clinical stages of patients, the abovementioned protein changes became more apparent, with significant differences between the clinical stages. This indicates that the expression of Notch in elderly patients with glioma is closely related to the clinical stage and prognosis of the patients.

Survivin is a member of the inhibitor of apoptosis protein family. It is expressed in a cell cycle-dependent manner during mitosis. It is located in distinct parts of the mitotic apparatus and plays an essential role in cell division and inhibition of apoptosis. Survivin is expressed in most human cancers, but not in normal adult tissues [19, 20]. Inhibition of Survivin has been well-studied as a cancer therapeutic. For example, Carrasco and his team found specific inhibition of Survivin expression by LY2181308-sensitized tumor cells to chemotherapeutic-induced apoptosis. Most importantly, they found that LY2181308 yielded antitumor activity in an *in vivo* human xenograft tumor model, and this model was sensitized to gemcitabine, paclitaxel, and docetaxel [21]. With the increase of age, the mechanism of neurogenesis decrease is still unclear, but it has been reported that it involves the changes of the microenvironment of neural progenitor cells. Astrocytes regulate the cycle of neural progenitor cells by acting on the expression level of Survivin, a known mitotic regulator. Among the cell cycle genes found in the elderly neural progenitor cells, Survivin is the only one that can restore the proliferation of the elderly brain neural progenitor cells. Miranda et al. provide a mechanism for the gradual loss of neurogenesis in the brain in relation to aging and reveal that targeted silencing of Survivin expression directly or through Wnt signaling could stimulate adult neurogenesis [22]. Survivin exhibits its antiapoptotic function in part by inhibiting caspase-3 activity [23]. The results in our study also demonstrate that compared with the healthy control, the expression of Survivin protein in the elderly glioma group was significantly increased while the expression of caspase-3 significantly was reduced; with the increase in the clinical stages of patients, the abovementioned protein changes became more apparent, with significant differences between the clinical stages. It suggests that Survivin and Survivin-dependent caspase-3 proteins are also closely

related to the clinical stage and prognosis of elderly patients with glioma.

In summary, the present study showed that Notch and Survivin proteins were overexpressed in elderly glioma and their upregulation may contribute to the development of glioma in elderly patients and could serve as prognostic factors. We also demonstrate that activation of the Notch pathway mediates upregulation of Survivin expression and leads to glioma cell viability and migration. Finally, these data might be helpful in identifying the molecular mechanism involved in the elevated level of Survivin in glioma cells, and targeting these factors might be an important implication in the development of new therapeutic strategies aimed at blocking Notch activation in glioma cells.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] R. Chen, M. Smith-Cohn, A. L. Cohen, and H. Colman, "Glioma subclassifications and their clinical significance," *Neurotherapeutics*, vol. 14, no. 2, pp. 284–297, 2017.

[2] Q. T. Ostrom, H. Gittleman, L. Stetson, S. M. Virk, and J. S. Barnholtz-Sloan, "Epidemiology of gliomas," *Cancer Treatment and Research*, vol. 163, pp. 1–14, 2015.

[3] F. Laigle-Donadey and J. Y. Delattre, "Glioma in the elderly," *Current Opinion in Oncology*, vol. 18, no. 6, pp. 644–647, 2006.

[4] G. Minniti, G. Lombardi, and S. Paolini, "Glioblastoma in elderly patients: current management and future perspectives," *Cancers (Basel)*, vol. 11, no. 3, p. 336, 2019.

[5] N. Laperriere, M. Weller, R. Stupp et al., "Optimal management of elderly patients with glioblastoma," *Cancer Treatment Reviews*, vol. 39, no. 4, pp. 350–357, 2013.

[6] J. Gallego Perez-Larraya and J. Y. Delattre, "Management of elderly patients with gliomas," *The Oncologist*, vol. 19, no. 12, pp. 1258–1267, 2014.

[7] G. Monticone and L. Miele, "Notch pathway: a journey from notching phenotypes to cancer immunotherapy," *Advances in Experimental Medicine and Biology*, vol. 1287, pp. 201–222, 2021.

[8] M. Teodorczyk and M. H. H. Schmidt, "Notching on cancer's door: notch signaling in brain tumors," *Frontiers in Oncology*, vol. 4, p. 341, 2014.

[9] F. Shojaei, F. Yazdani-Nafchi, M. Banitalebi-Dehkordi, M. Chehelgerdi, and M. Khorramian-Ghahfarokhi, "Trace of survivin in cancer," *European Journal of Cancer Prevention*, vol. 28, no. 4, pp. 365–372, 2019.

[10] M. D'Amelio, V. Cavallucci, and F. Cecconi, "Neuronal caspase-3 signaling: not only cell death," *Cell Death and Differentiation*, vol. 17, no. 7, pp. 1104–1114, 2010.

[11] D. N. Louis, H. Ohgaki, O. D. Wiestler et al., "The 2007 WHO classification of tumours of the central nervous system," *Acta Neuropathologica*, vol. 114, no. 2, pp. 97–109, 2007.

[12] H. D. Lewis, M. Leveridge, P. R. Strack et al., "Apoptosis in T cell acute lymphoblastic leukemia cells after cell cycle arrest induced by pharmacological inhibition of notch signaling," *Chemistry & Biology*, vol. 14, no. 2, pp. 209–219, 2007.

[13] J. Guo, P. Li, X. Liu, and Y. Li, "NOTCH signaling pathway and non-coding RNAs in cancer," *Pathology - Research and Practice*, vol. 215, no. 11, p. 152620, 2019.

[14] Y. Li, J. Ma, X. Qian et al., "Regulation of EMT by Notch signaling pathway in tumor progression," *Current Cancer Drug Targets*, vol. 13, no. 9, pp. 957–962, 2013.

[15] L. Wang, F. Jin, A. Qin et al., "Targeting Notch1 signaling pathway positively affects the sensitivity of osteosarcoma to cisplatin by regulating the expression and/or activity of caspase family," *Molecular Cancer*, vol. 13, no. 1, p. 139, 2014.

[16] P. Liu, Y. Man, Y. Wang, and Y. Bao, "Mechanism of BMP9 promotes growth of osteosarcoma mediated by the Notch signaling pathway," *Oncology Letters*, vol. 11, no. 2, pp. 1367–1370, 2016.

[17] Z. Yang, Y. Qi, N. Lai et al., "Notch1 signaling in melanoma cells promoted tumor-induced immunosuppression via upregulation of TGF-β1," *Journal of Experimental & Clinical Cancer Research*, vol. 37, no. 1, p. 1, 2018.

[18] S. Liu, X. Ma, Q. Ai et al., "NOTCH1 functions as an oncogene by regulating the PTEN/PI3K/AKT pathway in clear cell renal cell carcinoma," *Urologic Oncology*, vol. 31, no. 6, pp. 938–948, 2013.

[19] J. Zhou, X. Guo, W. Chen, L. Wang, and Y. Jin, "Targeting survivin sensitizes cervical cancer cells to radiation treatment," *Bioengineered*, vol. 11, no. 1, pp. 130–140, 2020.

[20] D. C. Altieri, "Targeting survivin in cancer," *Cancer Letters*, vol. 332, no. 2, pp. 225–228, 2013.

[21] R. A. Carrasco, N. B. Stamm, E. Marcusson, G. Sandusky, P. Iversen, and B. K. R. Patel, "Antisense inhibition of survivin expression as a cancer therapeutic," *Molecular Cancer Therapeutics*, vol. 10, no. 2, pp. 221–232, 2011.

[22] C. J. Miranda, L. Braun, Y. Jiang et al., "Aging brain microenvironment decreases hippocampal neurogenesis through Wnt-mediated survivin signaling," *Aging Cell*, vol. 11, no. 3, pp. 542–552, 2012.

[23] A. Lebelt, R. Rutkowski, W. Och et al., "Survivin, caspase-3 and MIB-1 expression in astrocytic tumors of various grades," *Advances in Medical Sciences*, vol. 61, no. 2, pp. 237–243, 2016.