Original Paper

Jagged1 is Clinically Prognostic and Promotes Invasion of Glioma-Initiating Cells by Activating NF- κ B(p65) Signaling

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Key Words

Jagged1 • Glioma-initiating cells (GICs) • NF- κ B(p65) • Invasion

Abstract

Background/Aims: Jagged1 is the ligands of the Notch signaling and has been shown to promote glioma-initiating cells (GICs) in glioblastoma. The role of Jagged1 in GICs invasion and underlying molecular mechanisms remain unclear. **Methods:** Survival data from R2 genomics analysis, the Cancer Genome Atlas (TCGA), the Chinese Glioma Genome Atlas (CGGA) and visualization platform database were used to evaluate the effects of Jagged1 on overall patient survival. we investigated Jagged1 induced the GICs cells' invasion by matrix degradation assays and Transwell cell invasion assays *in vitro*, then we further explored the underlying molecular mechanisms using Co-immunoprecipitation (co-IP) analysis. **Results:** High expression of Jagged1 in human glioma was associated with poor survival. Clinical data analysis showed that the Jagged1 was positively correlated with NF- κ B(p65). Jagged1-induced invasion of GICs cells through activation of NF- κ B(p65) pathway. *In vivo*, knockdown of Jagged1 could suppress the tumorigenicity of GICs cells through NF- κ B(p65) signaling. **Conclusion:** Insights gained from these findings suggest that Jagged1 plays an important oncogenic role in GICs malignancy by activation of NF- κ B(p65) signaling, and Jagged1 could be employed as an effective therapeutic target for GICs.

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Introduction

Glioblastoma multiforme (GBM) is the most frequent and aggressive of brain malignancies [1]. Current standard therapy after initial diagnosis includes maximal safe surgical resection and a combination of radiation therapy and concurrent adjuvant temozolomide (TMZ) chemotherapy [2]. Although surgical resection aims to remove a significant portion of the visible tumor mass, it is unable to eradicate invasive and migratory cells [3]. Moreover, GBM cells respond unpredictably to radio-chemotherapy, resulting in continual observation and further relapses. Recurrent cases of GBM are common and relapsed patients unfortunately have a dismal survival of less than 15 months [4].

Recently, a subpopulation (glioma-initiating cells, GICs) with augmented tumor-initiating potential and stem cell behavior has been identified in glioblastomas [5]. It is assumed that recurrence results from GICs, GICs are inherently chemo- and radio-resistant, and are highly invasive, suggesting that therapeutic approaches targeting GICs would have enhanced antitumor efficacy [6-8].

Notch signaling, an evolutionarily conserved pathway that mediates direct cell-cell interactions, play a crucial part in maintenance and self-renewal of GICs [9, 10]. In mammals, there are five ligands (Jagged1, Jagged2, Delta-like [Dll]1, Dll3, and Dll4) and four Notch receptors (Notch1-4). Notch signaling is activated by ligand-receptor interactions between neighboring cells, releasing the Notch intracellular domain (NICD) into the nucleus, whereby the NICD binds to the transcription factor CSL. The NICD/CSL complex causes the expression of target genes, such as those of the Hairy enhancer of split (Hes) family [11].

Notch ligands are present or upregulated in several human malignancies [12]. Jagged1 has also been proven to play several vital roles in multiple aspects of cancer biology, including tumor angiogenesis, [13, 14] neoplastic cell growth, [15] cancer stem cells (CSCs), [16, 17] epithelial-mesenchymal transition (EMT) in several types of cancer [18, 19]. Jagged1 is frequently overexpressed on both glioma cells and tumor-associated endothelial cells [16, 20]. Moreover, studies have shown that down-regulation of Jagged1 induces apoptosis and inhibits proliferation in glioma cell lines [21]. These data suggest that Jagged1 may play a central role in glioma cells. As most studies have focused on the effects of inhibiting the Notch receptor and its downstream signaling, specific inhibition of the ligand Jagged1 in GICs invasion has not been fully explored.

In this study, we found that Jagged1 knockdown in GICs suppressed invasion using *in vitro* and *in vivo* models. Jagged1-mediated Notch1 signaling activation was able to increase invasion in glioma mainly through upregulation of NF- κ B(p65). Our findings indicate that Jagged1 is a promising therapeutic target for preventing GICs invasion progression.

Materials and Methods

Datasets used in this study

Gene expression profiling datasets were obtained from the Chinese Glioma Genome Atlas (CGGA, <http://www.cgga.org.cn>), including the mRNA array of 128 samples and the mRNA sequencing of 144 samples. In addition, 540 samples of GBM from the Cancer Genome Atlas (TCGA, <https://tcga-data.nci.nih.gov/tcga/>). R2:Genomics Analysis and Visualization Platform (<http://r2.amc.nl>) was used to acquire Jagged1 mRNA expression in glioma and normal brain tissues. Microarray data of GEO (<http://www.ncbi.nlm.nih.gov/geo/>), GDS3885 was enrolled in this study. GDS3885 profile included microarray data from 27 panels of GSCs and 36 panels of NSTCs. Cluster analyses were performed and visualized using Cluster/Java Treeview. ChIPBase v2.0 (<http://rna.sysu.edu.cn/chipbase/>) was used to perform Pearson's correlation tests. The Retrieval of Interacting Genes/Proteins (STRING database v10.5) resource was utilized for PPI network analysis and prediction of protein-protein interactions.

Sample collection

GBM tissues were collected from the Department of Neurosurgery, Tianjin Medical University General Hospital between July 2008 and December 2012. Collected specimens were split into three parts for 4% paraformaldehyde(PFA)fixation. All samples were histologically graded according to the 2007 World Health Organization (WHO) Classification of Nervous System Tumors. Patients did not receive radiotherapy and chemotherapy before surgery. Written informed consent was obtained from all donors and their relatives. The study was carried out in accordance with the principles of the Helsinki Declaration and approved by the ethical committee of Tianjin Medical University General Hospital.

Cell culture

Human GBM cells U87, U251 were obtained from Cell Bank Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in Dulbecco's modified Eagles medium (DMEM, Gibco, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (FBS; Gibco), 100 U/ml penicillin, and 100 µg/ml streptomycin and incubated at 37°C in 5% CO₂. The generation and identification of U251 and U87 GSCs was performed as we previously described [22]. γ-secretase inhibitor MK0752 was purchased from Selleck Chemicals. Purified Jagged1 ligand (R&D Systems Europe, Lille, France) was added to the cell medium for 7 days.

Real time-PCR analyses

RNA isolation and real-time PCR Total RNA from cells was extracted using Trizol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Then cDNA was synthesized using 2 µg of RNA via reverse transcriptional kit according to manufacturer's protocol. Real-time PCR was used to evaluate Jagged1 mRNA levels. The PCR primers were provided by Sheng Gong Company and the primer sets were as follows: forward: 5'-TCGGGTCAGTTCGAGTTGGA-3' and reverse: 5'-CGTTCACGTTCTGCATGGAC-3'.

Western blot arrays

Western blotting was carried out as previously described [23]. The primary antibodies used in this study were as follows: NICD and NF-κB(p65) antibodies were obtained from Cell Signaling Technology (dilution 1:100, CST, USA); pY421-Cortactin, MMP-2, MMP-9 and Hes1 was from Abcam (dilution 1:1000, USA). The expression of β-actin (dilution 1:2000, Abcam, USA) was used as normalization antibody and loading control.

Co-immunoprecipitation

Co-immunoprecipitation assay was performed as described previously [24]. Cells were harvested in IP lysis buffer (Thermo Fisher Scientific, USA). The cell lysates were pre-cleared with 40 µl of protein A/G-agarose beads (Santa Cruz Biotechnology, USA) and immunoprecipitated with either a primary antibody or control IgG (Santa Cruz Biotechnology) at 4 °C overnight, followed by 2 h of incubation at 4 °C with 40 µl of protein A/G-agarose beads (Santa Cruz Biotechnology) maintained with constant agitation. The beads were then washed with wash buffer, suspended in sample buffer, and boiled, and the eluted proteins were assessed using Western blot.

Invasion assay

Transwell invasion assays were performed using transwell filters (Costar, USA) coated with Matrigel (60-80 µl) on the upper surface of the polycarbonic membrane (diameter 6.5 mm, pore size 8 µm). Glioma cells (5×10^4 in 200 µl DMEM without FBS) were seeded on the top side of the Matrigel-coated chambers. The lower compartments were filled with DMEM supplemented with 10% FBS. Following 24 h incubation, the non-invading cells were removed from the upper surfaces of the invasion membranes and the cells on the lower surface were stained with crystal violet. Five visual fields were randomly selected from each membrane. All experiments were repeated three times independently.

Immunohistochemistry and Immunofluorescence

Paraffin-embedded specimens were cut into 5- μ m sections. A standard immunostaining procedure was performed using the primary antibody solution [23]. Images were acquired using an Olympus VANOX microscopy at magnifications of $\times$ 200. The immunohistochemical staining results were evaluated by two experienced pathologists. Immunofluorescence stain was performed as described previously [22]. Primary antibodies against CD133 (1:100; ab19898), Nestin (1:100; ab105389), Notch1 (1:200; ab44986) were used. Cells were observed and imaged with a fluorescence microscopy (IX73, Olympus, Tokyo, Japan). Alexa fluor-labeled anti-rabbit or anti-mouse antibody (Invitrogen, USA; 1:500) was added to the incubation. The nuclei were stained with DAPI (Sigma, USA).

Knockdown assay

A nontargeting siRNA scramble control and siRNA targeting either NF- κ B(p65) (SignalSilence® NF- κ B p65 siRNA #6261, Cell Signaling) were transfected into glioma cells using Lipofectamine 3000 (Invitrogen) according to the manufacturer's instructions. The cells were harvested for western blot assays 48 h post-transfection. For lentivirus transfections, methods used for lentivirus production and infection were performed as described previously [22]. Lentivirus containing the Jagged1 sequence and a negative control sequence was obtained from Santa Cruz. All of the cell lines were infected with lentivirus for 48 h prior to performing the assays, according to the manufacturer's instructions.

Matrix Degradation Assay

For matrix degradation assay, this protocol is adapted from Artym et al [25]. In brief, 12 mm coverslips were inverted onto FITC-conjugated gelatin (Invitrogen). A total of 20, 000 cells were seeded on each coverslip, incubated for 8 h, and processed for IF. To quantify invadopodia function condition, the area of matrix degraded round the cells was quantified with an identical signal threshold for the FITC-gelatin fluorescence for every image. The measured degraded area was the area where the fluorescence signal below the threshold as measured with Image J. The quantified area was normalized against the total number of cells. All images were taken using an Olympus FV-1000 confocal microscope. Each experiment was repeated at least three times to ensure reliability.

Nude mouse intracranial model

BALB/c-A nude mice at 4 weeks of age were purchased from the Animal Center at the Cancer Institute at the Chinese Academy of Medical Science (Beijing, PR China). A total of 5×10^5 GBM cells infected with lenti-NC, lenti-Jagged1 virus were implanted stereotactically using cranial guide screws. Bioluminescence imaging was used to detect intracranial tumor growth on days 7, 14, and 21. Two mice randomly selected from each group were sacrificed. The brains were carefully extracted and fixed in 10% formalin, then embedded in paraffin for H&E and IHC analysis. Overall survival times were closely monitored and recorded. All animal research followed the internationally recognized guidelines and national regulations.

Statistical analyses

Statistical analyses were performed using GraphPad Prism 6.0 (GraphPad, La Jolla, CA, USA) and SPSS version 19.0 (SPSS, Chicago, IL, USA). A circus plot was achieved with the circlize package of R. A one-way ANOVA was performed for comparisons between different groups. All data are presented as mean $\pm$ SD. $P < 0.05$ was considered to be statistically significant.

Results

High expression of Jagged1 in human glioma was associated with poor survival

To determine whether Jagged1 is clinically correlated with glioma progression, survival data from R2 genomics analysis and visualization platform database were used to evaluate the effects of Jagged1 on overall patient survival. Contrasting to normal tissue or lower grade glioma, Jagged1 was significantly upregulated in GBM patients according to Glioma France, Glioma Sun, CGGA and TCGA datasets (Fig. 1A-G). Accordingly, The Kaplan-Meier survival analysis revealed that patients with high levels of Jagged1 possessed shorter overall survival

Fig. 1. Jagged1 expression was increased in glioma, this elevated Jagged1 expression was a prognostic indicator of poor overall survival for glioma patients. (A, B, D, F and G) Jagged1 level was increased in WHO II, III, and IV compared with non-tumor brain tissues from the Glioma France, Glioma Sun, CGGA and TCGA datasets. NB: non-tumor brain tissue. (C, E and H) Kaplan-Meier survival curve analysis indicated that patients with Jagged1 overexpression had a significantly shorter overall survival in glioma patients. (I-K) The levels of Jagged1 were analyzed in GBM tissues from TCGA datasets and CGGA datasets. Jagged1 was significantly upregulated in classical and proneural subtypes of GBM.

(OS) time than patients with low levels of Jagged1, $P < 0.0001$ (Fig. 1C, E and F). To further confirm the Jagged1 expression results in GBM, we analyzed data in the TCGA and CGGA. The results of the analysis revealed that Jagged1 was significantly upregulated in classical and proneural subtypes of GBM. (Fig. 1I-K). All these results indicated that Jagged1 might function as an oncogene involved in the development and progression of GBM.

Notch1 pathway is preferentially expressed by GICs

We used a GEO microarray data set (GDS3885) and found that the Notch1 pathways significantly upregulated in GICs relative to the matched non-tumour stem cells (NSTCs) (Fig. 2A). Immunohistochemical staining of Notch1, Nestin, Jagged1 and CD133 were expressed in GBM cells adjacent to endothelial cells. (Fig. 2B). Consistently, elevated expression of Notch1 in GICs was validated by immunofluorescence in U87 GICs and U251 GICs (Fig. 2C, D). Co-expression of Notch1 with Nestin or CD133 was detected in GICs tumourspheres. Overall, these data demonstrate that Notch1 pathway is preferentially expressed in GICs.

Clinical data analysis showed that the Jagged1 was positively correlated with NF- κ B(p65)

To investigate whether Jagged1 expression is correlated with NF- κ B(p65) expression, we analyzed data in the TCGA and found that Jagged1 was correlated with NF- κ B(p65) in total GBM samples (Fig. S1C, D - for all supplemental material see www.karger.com/10.1159/000496044/). The PPIs network was built from the STRING resource also confirmed this finding (Fig. S1B). Pearson's correlation was performed with these factors in TCGA data set. In GBM of TCGA, Jagged1 showed high concordance with MMP2 and MMP9 (Fig. S1C).

Jagged1 regulated GICs cells invasion

In Transwell cell invasion assays, representative data showed that dramatically increased the invasive ability of GICs cells (Fig. S1E, F), indicating that Jagged1 may have a significant effect on cell invasion. Furthermore, matrix degradation assays also confirmed this result. Jagged1 significantly increased invadopodia formation and matrix degradation. Meanwhile, MK0752 (a Notch1 pathway inhibitor) notably decreased the invasiveness of glioma cells, the matrix area digested by invadopodia (Fig. 3A, B). MK0752 significantly suppressed the Hes1, NF- κ B(p65), pY421-Cortactin, MMP-2, and MMP-9 expression (Fig. 3C, D). Whereas, in Jagged1 ligand treatment GICs cells, Jagged1 increased Hes1, NF- κ B (p65), pY421-Cortactin, MMP-2 and MMP-9 expression. These data indicated that Jagged1 induced the GICs cells' invasion.

Fig. 2. Notch1 pathway contributes to stemness. (A) Expression heat map of Notch1 pathway in GSC lines (n=27) and NSTC lines (n=36) from GEO profiles (GEO: GDS3885). Jagged1 was significantly upregulated in GSCs relative to the non-tumour stem cells (NSTC). (B) Immunohistochemical staining of Notch1, Nestin, Jagged1 and CD133 were expressed in GBM cells adjacent to endothelial cells. The scale bar corresponds to 100 μ m. (C, D) Co-expression of Notch1 and Nestin, Notch1 and CD133 showing the corresponding co-localization in the U87 GICs and U251 GICs. The scale bar corresponds to 20 μ m.

Fig. 3. Jagged1 regulated invadopodia-mediated matrix degradation. (A, B) Representative fluorescence images of F-actin (red), FITC-gelatin (green) and nuclei (blue) in U87 GICs and U251 GICs cells after Jagged1 ligand and MK0752 treatment. Areas of degradation appear as the well-defined black areas beneath the cells. Scale bar: 20 μ m. (C, D) After Jagged1 ligand and MK0752 treatment, the Hes1, NF- κ B(p65), pY421-Cortactin, MMP-2, and MMP-9 expression levels were detected by Western blot. β -actin was used as a loading control. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

Jagged1-induced invasion of GICs cells through activation of NF- κ B(p65) pathway

To further investigate the effects of Jagged1, shRNA was used to knockdown Jagged1 in U87 GICs and U251 GICs cells. The efficacy of Jagged1 knockdown was confirmed by real-time PCR and western blot analysis (Fig. 4A and B). In Transwell invasion assays and matrix degradation assays, the collective representative data depicted that Jagged1-KD dramatically decreased the invasive ability of GICs cells (Fig. 4D and E). Next, we investigated the effect of NF- κ B(p65) on the invasion in GICs cells. As shown in Fig. 4E, NF- κ B(p65) interference

Fig. 4. Jagged1 regulated GICs cells invasion by NF- κ B(p65) signaling. (A, B) Western blot analysis was carried out to evaluate the expression of Jagged1 when GICs cells were transfected with negative control shRNA (shControl), Jagged1 shRNA (shJAG1). β -Actin was used as a loading control. (C) Western blot assays of Jagged1, Hes1, NF- κ B(p65), pY421-Cortactin, MMP-2 and MMP-9 expression levels in GICs cells after treatment with ShJAG1, ShJAG1+Jagged1, SiNF- κ B(p65), and SiNF- κ B(p65)+Jagged1, β -actin was used as a loading control. (D) The representative images and histograms of in vitro Transwell invasion assays. (E) Representative fluorescence images of F-actin (red), FITC-gelatin (green) and nuclei (blue) in U87 GICs and U251 GICs cells after treatment with Control, ShJAG1, SiNF- κ B(p65). (F) Immunoprecipitated by antibodies targeting either NICD or NF- κ B(p65). Both the forward and reverse immunoprecipitation actively indicated that NICD specifically bound to NF- κ B(p65).*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

strongly inhibited cell invasion, compared with the Control group in GICs cell lines. Jagged1-mediated Notch signaling activation notably enhanced the invasion abilities in both cell lines (Fig. 4F). However, in the siNF- κ B(p65)-transfected cells (U87GICs-siNF- κ B(p65) cells and U251GICs-siNF- κ B(p65) cells), after incubation with Jagged1 protein, the inhibited invasion abilities could not be restored by Jagged1-induced Notch signaling activation (Fig. 4C). The expression levels of the protein markers associated with invasion were also not restored in siNF- κ B(p65) cells after Jagged1 ligand treatment (Fig. 4C). In addition, we performed co-IP analysis and observed that NICD (cleaved Notch intracellular domain, the activated form

Fig. 5. Effect of downregulation of Jagged1 on the growth of xenograft tumors in nude mice. (A) Bioluminescent images from the ShControl, ShJagged1 animals at 7, 14 and 21 days after tumor implantation. (B) Representative photomicrographs of H&E staining and immunohistochemistry for Jagged1 and NF- κ B(p65) in sample tissues. (C) Mouse survival in the different groups was quantified with a Kaplan-Meier survival curve. (D) Bioluminescence (BLI) was performed to monitor tumor growth on days 7, 14, 21. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

of Notch) can bind to NF- κ B(p65) (Fig. 4F). Taken together, our data showed that Jagged1-induced Notch signaling activation promotes the invasion mainly through activation of NF- κ B(p65).

Silencing of Jagged1 suppressed NF- κ B(p65) signaling in vivo

We further tested whether knockdown of Jagged1 could suppress the tumorigenicity of GICs cells *in vivo*. Bioluminescence imaging showed tumor growth stasis in the Jagged1-KD-treated group compared with the Control-treated tumors, and a statistically significant difference in tumor volume on the 21 day (Fig. 5A and D). IHC analysis showed that Jagged1-KD treatment specifically downregulated Jagged1 and NF- κ B(p65). H&E staining of mouse brain tissues indicated that the boundary between the tumor and normal tissues in the Jagged1-KD groups was significantly clear compared with the control group (Fig. 5B). In addition, the Jagged1-KD groups mice exhibited significantly longer survival times (Fig. 5C). These data suggest that Jagged1 play a critical role in GICs invasion and NF- κ B(p65) signaling.

Discussion

Glioblastoma (GBM) is one of the most common and lethal primary malignant brain tumor in adults. The major reason that therapeutic treatments fail are due to the highly invasive nature of the tumor cells and the occurrence of increased angiogenesis [26]. GBM harbors a subpopulation of self-renewing, therapy-resistant cells, GICs. GICs are notably highly invasive and promote angiogenesis [27].

The abnormal activation of Notch signaling pathways augments the angiogenesis and invasiveness of GICs. The Notch ligands Dll4 and Jagged1 are upregulated in subsets of GBMs presenting an angiogenic phenotype. Zhu et al. showed ECs express Dll4 and Jagged1 that activate Notch receptors on the surface of GICs and promote GICs self-renewal [16]. Inhibition of Notch signaling with a γ -secretase inhibitor results in a decreased GIC self-renewal, which depletes ECs in the vascular niche, and promotes GIC sensitivity to radiation therapy [28, 29]. Dll4 is predominantly expressed in the endothelial cells (ECs) of tumor

blood vessels but also in a small proportion of tumour cells [30, 31]; whereas Jagged1, although also expressed in ECs, is more highly expressed in glioma cells [20]. Our data also confirm this discovery (Fig. 2B). This expression pattern suggests a pivotal role of Dll4 in the regulation of angiogenesis through the Notch receptors, whereas Jagged1 ligands may have another effect on gliomagenesis.

Previous studies reported that the Notch pathway plays an important role in normal stem cell biology. And this resulted in unacceptable levels of gastrointestinal toxicity when pan-Notch inhibitors (γ -secretase inhibitors) was attempted using [32]. Unlike the Dll1/4 ligands, Jagged1 was found to be dispensable for the homeostasis of normal intestinal stem cells, suggesting that targeting Jagged1 is less likely to have adverse side effects [33, 34]. Therefore, it might be theoretically possible that targeting Jagged1 is a more promising intervention for GBMs patients rather than targeting other Notch components. Several reports have described Jagged1 involvement in EMT and invasive potential [35, 36]. Furthermore, Jagged1 can increase tumor migratory and invasive behavior by inducing the urokinase-type plasminogen activator (uPA) [37]. Nevertheless, the effects of Jagged1 on GICs invasion were not been fully explored, and the underlying mechanism remains unclear.

In this study, we demonstrated that Jagged1 expression was significantly increased with the grade elevation in Glioma France and Glioma Sun datasets. The glioma subgroups with lower level of Jagged1 were correlated with better prognoses, suggesting that Jagged1 the specific biomarkers for the prognostic-based glioma sub-classification. Furthermore, we found that Jagged1 mRNA levels were significantly elevated in classical and proneural subtypes of GBM. Anoop P et al. showed a “hybrid” states in primary glioblastoma highly for two subtypes; most commonly classical and proneural (progenitor states) or mesenchymal and neural (differentiated states) [38]. Then we used a GEO microarray data set (GDS3885) and observed that the Jagged1 were significantly upregulated in GICs. All findings presented revealed that Jagged1 may promote the development and progression of GICs. Subsequently, downregulation of Jagged1 expression led to a reduced GICs cell invasion. Our data also showed that NF- κ B(p65) expression was dramatically downregulated by Jagged1 knockdown. Similarly, a recent report has revealed that Notch signaling pathway inhibition results in decreased NF- κ B in pancreatic cancer cells, suggesting a molecular link or cross-talk between Notch signaling and NF- κ B(p65) [39]. NF- κ B signaling pathway is constitutively activated in glioma, and has been shown to contribute to the development and progression of tumors [40, 41]. Several genes involved in invasion, such as ICAM-1, ZEB-1, uPAR, Cortactin, MMP2 and MMP9, have been identified as being regulated by NF- κ B [42-44]. Gene silencing of NF- κ B (p65) by the application of small interfering RNA significantly reduced glioma cell invasion (Fig. 4E). We further found that the inhibited invasion abilities were also not restored in siNF- κ B(p65) cells after Jagged1 ligand treatment. Finally, we found that NICD (cleaved Notch intracellular domain, activated form of Notch) can bind to NF- κ B(p65). Overall, our results indicated that inhibition of NF- κ B (p65) signaling greatly suppressed Jagged1-induced cell invasion (Fig. 4C and D), suggesting that Jagged1 mediates the GICs invasions via NF- κ B(p65) signaling.

Conclusion

In summary, our study expands the understanding of the regulatory effects of Jagged1 on GIC cells. The roles of the Jagged1-NF- κ B(p65) interactions in GICs invasion need to be explored more deeply. The findings of this present study might help to guide the development of potential therapeutic targets for the prevention of GIC cells invasion.

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Disclosure Statement

The authors declare that there is no conflict of interests in this work.

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