Neurology Asia 2026; 31(2) : 743 – 753

Cell adhesion molecule L1-like protein regulates neuregulin-ErbB receptor signaling in glioma cells

*[1]Wei-jiang Zhao, *[2]Zhai Yang, *[3,4]Kai-ye Hua, [3]Shuai Zhang, [3]Yi Wang
*WJ Zhao, Z Yang and KY Hua contributed equally to this work and are co-first authors

[1]Cell Biology Department, Wuxi School of Medicine, Jiangnan University, Wuxi, Jiangsu, China; [2]Center for Neuroscience, Shantou University Medical College, Shantou, Guangdong, China; [3]Wuxi School of Medicine, Jiangnan University, Wuxi, Jiangsu, China; [4]Jiangnan University Medical Center (Wuxi No.2 People's Hospital), Wuxi, Jiangsu, China.

Abstract

Objective: It was previously demonstrated that the cytokine neuregulin 1 (NRG1) can promote the proliferation of glioma cells by modulating cell adhesion molecule L1-like (CHL1) protein expression in glioma development. However, the role of CHL1 in NRG signaling modulation remains to be elucidated. In the present study, the effects of CHL1 on NRG signaling in three glioma/glioblastoma cell lines were explored mainly by using small interfering RNAs (siRNAs) targeting CHL1. *Methods:* SHG44 and U251 glioma, and U-87 MG glioblastoma cells were treated with siRNA targeting CHL1 for 48 h. Then, the protein expression of NRG1-4, and their cognate receptors pErbB2-4 was evaluated by western blot in these cell lines. Immunofluorescence staining was used to evaluate the effect of CHL1 on pErbB2 and pErbB4. *Results:* siRNA targeting CHL1 significantly and differentially downregulated the expression of the main NRG subtypes, including NRG1, 2, 3 and 4. Downregulation of CHL1 expression also reduced the level of phosphorylated (p) activated NRG receptors p-ErbB2, 3 and 4. *Conclusions:* Overall, these data indicated that CHL1 may contribute to glioma malignancy by upregulating NRG signaling in glioma/glioblastoma cells.

Keywords: Glioma, SHG44, U251, U-87 MG, cell adhesion molecule L1-like protein, neuregulin; ErbB receptor

INTRODUCTION

As highly invasive glial cells-derived tumors in the central nervous system, gliomas account for ~50% of all intracranial tumors.[1] Despite improvement in surgical resection, chemotherapy and radiation treatment, the increase in average survival time remains limited.[2] High recurrence still exists in patients with a low degree of glioma and glioblastoma differentiation, resulting in low cure rates and high mortality, with a <5% 5-year survival rate.[3,4]

Cell adhesion molecule L1-like (CHL1) protein is a transmembrane adhesion molecule of the immunoglobulin superfamily. CHL1 is composed of an ectodomain, a transmembrane domain and a conserved intracellular domain functioning in skeleton protein interactions.[5-7] It mainly participates in cell adhesion, axon guidance and synaptic plasticity in the central nervous system.[8] Re-expression of CHL1 on the tumor mass edge can promote local invasive growth and metastatic spread in breast, ovary and colon cancer.[9] CHL1 functions as a potentially novel specific biomarker for identifying two major renal cancer types in their early pathogenesis.[9] Differential CHL1 expression may predict potential recurrence of pituitary adenoma.[10] Contrary to previous reports in other cancer types.[11,12] Our group previously demonstrated that CHL1 is abundantly expressed in glioma/glioblastoma tissues, where it modulates the proliferation and invasion of glioma/glioblastoma cells.[13] However, the role of CHL1 in the modulation of other molecules that play important roles in glioma development remains unclear.

Address correspondence to: Wei-Jiang Zhao, Department of Cell Biology, Wuxi School of Medicine, Jiangnan University, 1800 Lihu Dadao, Binhu District, Wuxi, Jiangsu 214122, P.R. China. E-mail: weijiangzhao@jiangnan.edu.cn; Zhai Yang, Center for Neuroscience, Shantou University Medical College, 22 Xin Ling Road, Shantou, Guangdong 515041, P.R. China. E-mail: zhaiyangnsc@126.com

Date of Submission: 22 October 2025; Date of Acceptance: 8 February 2026

https://doi.org/10.54029/2026www

CHL1 expression can be modulated by neuregulin 1 (NRG1) via activation of several ErbB receptors.[14] Neuregulins (NRGs) are cytokines associated with a variety of different malignant tumors, and they function as signaling molecules that mediate cellular interactions.[15] All NRG family members contain an ectodomain mainly composed of an immunoglobulin-like loop and an EGF-like structure, a transmembrane domain, and an intracellular domain of varying lengths.[16,17] NRGl-4 are main NRG subtypes that have been identified, among which, NRG1 is one of the most active malignant tumor-related cytokines, with >6 identified isoforms resulting from alternative splicing of the *NRG1* gene, including type I-III NRG1α/β.[18,19] A total of 4 transmembrane tyrosine kinases constitute the ErbB receptor family, including ErbB1 (EGFR), ErbB2 (Neu), ErbB3 and ErbB4.[20] Although all NRG subtypes can differentially bind to or transactivate ErbB2-4 receptors, they show no binding affinity for EGFR.[21] Binding of NRG1 to ErbB receptors can initiate intracellular responses responsible for cell proliferation, apoptosis, migration, differentiation and adhesion.[18] Ritch *et al*[22,23] demonstrated that glioma cell survival and migration could be enhanced via autocrine or paracrine NRG1/ErbB receptor signaling. Our group recently reported that NRG2 is expressed in gliomas in a grade-dependent manner, and it may play roles in the migration of glioma cells *in vitro*[24], which was also observed by our group when using NRG3 and NRG4 albeit to a lesser extent.[25]

Although it has been reported that NRG1 can modulate the expression level of CHL1, there is no report to date clarifying the role of CHL1 in NRG signaling. To address this issue, the present study used small interfering RNA (siRNA) targeting CHL1 to investigate its roles in the modulation of NRG-ErbB signaling in glioma/glioblastoma cells. The effects of CHL1 on the protein expression of NRG subtypes, including NRG1-4, as well as the phosphorylation-mediated activation of ErbB2, 3 and 4, was evaluated. Based on these results, it was concluded that CHL1 is mainly involved in regulating the NRG-ErbB receptor signaling pathway in glioma/glioblastoma cells. NRG-ErbB receptor signaling inhibition via targeting CHL1 may partly facilitate the management of glioma.

METHODS

Correlation analysis of CHL1 with the main NRG signaling molecules in both low-grade glioma (LGG) and glioblastoma multiforme (GBM)

Transcriptomic mRNA expression data of 529 low-grade glioma (LGG) and 174 glioblastoma (GBM) samples were retrieved from The Cancer Genome Atlas (TCGA) database. To assess the mRNA-level correlations between *CHL1* and genes including neuregulin subtypes (*NRG1, NRG2, NRG3, NRG4*) and ErbB receptors (*ERBB2, ERBB3, ERBB4*), single-gene co-expression analysis was conducted using Pearson correlation analysis, with statistical significance denoted as “ns” (not significant) or by asterisks (*, **, ***) for varying significance levels. Co-expression heatmaps, which were generated using the ggplot2 package in R Version 3.6.3, visualized the Z-score (standardized expression values) of each associated gene through color gradients corresponding to Z-score ranges. Above each heatmap, a curve depicted *CHL1* expression (as log2(TPM+1)), and samples were stratified into *CHL1*-low and *CHL1*-high subgroups to show the expression patterns of associated genes across these groups.

Cell culture and CHL1 siRNA transfection

The human glioblastoma U-87 MG cell line established in the University of Uppsala (cat. no. CL-0238) and the human glioma U251 cell line (cat. no. CL-0237) were purchased from Procell Life Science and Technology Co., Ltd. The human glioma cell line SHG44 (cat. no. SHG44), derived from II-III grade astrocytoma tissue, was purchased from Guangzhou Jennio Bioech Co., Ltd. All cell lines were authenticated using short tandem repeat analysis and tested for mycoplasma contamination.

Cells were cultured in DMEM (HyClone; Cytiva) containing 10% fetal bovine serum (Sijiqing Biotech Corp, Hangzhou, China) and 50 U/ml of a mixture of penicillin/streptomycin (Beijing Solarbio Science & Technology Co., Ltd.). All cells were routinely maintained in a 75-cm^2 cell culture plate (Corning, Inc.) at 37˚C with 5% CO_2 in a humidified atmosphere. Cells in logarithmic phase were collected for subsequent *in vitro* experiments.

Small interfering (si) RNA treatment

One control siRNA and siRNA against CHL1 (Table I) were purchased from Shanghai

Table I: Sequences for random control siRNA and siRNAs against CHL1

siRNA	Sequence	
	sense (5'-3')	anti-sense (5'-3')
random control	UUCUCCGAACGUGUCACGUtt	ACGUGACACGUUCGGAGAAtt
CHL1	GGAGCUAAUUUGACCAUAUtt	AUAUGGUCAAAUUAGCUCCtt

GenePharma Co., Ltd. To test the effect of CHL1 expression on NRG-ErbB signaling molecules. For western blot analysis, cells (5×10^4 cells/well) in normal culture medium were allowed to adhere overnight in 48-well plates. When cell confluence reached 80%, the medium was aspirated and replaced with serum-free DMEM, and cells were incubated overnight. Then, control siRNA or siRNA targeting CHL1 (10 nM) complexed with Entranster™ R4000 (cat. no. 4000-3; Engreen Co., Ltd, Beijing, China) was transfected into the aforementioned three cell lines. Cells in the vehicle control (VC) group were treated with the same volume of transfection reagent. For immunofluorescence staining, 2×10^4 cells/well were seeded onto an 8-well Lab-Tek Chamber Slide™ (Nunc; Thermo Fisher Scientific, Inc.). After overnight incubation, the medium was aspirated and replaced with serum-free culture medium containing control siRNA or siRNA targeting CHL1 (10 nM) (Table I) complexed with Entranster™ R4000 (cat. no. 4000-3; Engreen). Cells were further cultured at 37˚C in a humidified incubator with 5% CO_2 for 48 h before western blot analysis and immunofluorescence staining.

Western blot analysis

Cells were lysed with RIPA buffer (Beijing Solarbio Science & Technology Co., Ltd.) containing PMSF (1:200; Beijing Solarbio Science & Technology Co., Ltd.). Cell lysates were subjected to centrifugation at 14,000 x g for 15 min at 4˚C, and the supernatants were harvested for western blot analysis.[16] Equivalent quantities of the lysates from the cells were heated at 99˚C in 20% sample loading buffer (0.125 M Tris-HCl, pH 6.8; 20% glycerol; 10% SDS; 0.1% bromophenol blue; and 10% dithiothreitol), resolved using 8% SDS-PAGE and electroblotted onto a PVDF membrane (MilliporeSigma). Non-specific protein binding sites were blocked using 5% BSA (Beijing Solarbio Science & Technology Co., Ltd.) diluted in TBS (pH 7.4) buffer containing 0.1% Tween-20 (TBST). The membrane was incubated with a mouse anti-human NRG1 antibody specifically targeting the EGF-like domain of NRG1 (1:500; cat. no. Ab-1 7D5; Lab Vision Corporation), mouse anti-human NRG2 antibody (1:500; cat. no. SC-398594; Santa Cruz Biotechnology, Inc.), rabbit anti-human NRG3 antibody (1:500; cat. no. 16071-T16; Sino Biological) and rabbit anti-human NRG4 antibody (1:500; cat. no. 12183-R001; Sino Biological). Rabbit anti-human phosphorylated (p)-ErbB2 (1:500; cat. no. AP0152; ABclonal Biotech Co., Ltd., Wuhan, China), anti-human p-ErbB3 (1:500; cat. no. sc135654; Santa Cruz Biotechnology, Inc.) and anti-p-ErbB4 (1:500; cat. no. AP0034; ABclonal Biotech Co., Ltd.) antibodies were applied to identify the effect of CHL1 on NRG signaling. Mouse monoclonal antiβ-actin antibody (1:1,000; cat. no. sc8432; Santa Cruz Biotechnology, Inc.) was used as a loading control for normalization. The membranes were incubated individually with primary antibodies overnight at 4˚C. The membranes were then washed with TBST 3 times at room temperature (5 min each wash), followed by further incubation with a horseradish peroxidaseconjugated goat antimouse secondary antibody (1:1,000; cat. no. BA1051; Wuhan Boster Biological Technology, Ltd.) and an antirabbit secondary antibody (1:1,000; cat. no. BA1055; Wuhan Boster Biological Technology, Ltd.). The membranes were subsequently washed with TBST 3 times for 5 min each at room temperature. The immunoreactive bands were visualized using an enhanced chemiluminescence kit (BioRad Laboratories, Inc.) and an imaging system (Alpha Innotech Corporation). The signal intensity was quantified via average densitometry multiplied by the number of pixels using Image J software (National Institutes of Health). The relative protein expression level was indicated by the ratio of its signal intensity to that of β-actin.

Immunofluorescence analysis

At 48 h after treatment, the cells were fixed with 4% paraformaldehyde in PBS (pH 7.4) at 4˚C for 15 min, followed by treatment with 10% donkey serum (cat. no. SL050; Beijing Solarbio Science & Technology Co., Ltd.) in PBS for 10 min and overnight incubation with a mouse-anti glial fibrillary acidic protein (GFAP) antibody (1:200; cat. no. sc-58766; Santa Cruz Biotechnology, Inc.) mixed with either rabbit anti-human p-ErbB2 antibody (1:200; cat. no. AP0152; ABclonal Biotech Co., Ltd.) or rabbit anti-human p-ErbB4 antibody (1:200; cat. no. AP0034; ABclonal Biotech Co., Ltd.).

Cells were washed in PBS 3 times for 5 min each, and then incubated at room temperature for 180 min with a mixture of donkey anti-mouse secondary antibody conjugated to Dylight™ 488 (1:500; cat. no. 715−545-150, Jackson ImmunoResearch, West Grove, PA, USA) and donkey anti-rabbit secondary antibody conjugated to Dylight™ 594 (1:1,000; 711−515-152, Jackson ImmunoResearch). Subsequently, the cells were co-stained with DAPI at room temperature for 20 min and mounted using an anti-fade mounting solution (Beyotime Institute of Biotechnology). Confocal images were acquired under an Olympus confocal system (FV-1000; Olympus Corporation). DAPI, Dylight™ 488 and Dylight™ 594 were respectively excited at 405, 488 and 594 nm.

Statistical analysis

n vitro experiments were repeated ≥3 times using independent culture preparations. All data are presented as mean values with standard error of the mean. Statistical analyses were performed using Prism 6 software (GraphPad Software, Inc.). One-way ANOVA with Tukey's post hoc test for multiple comparisons were used to compare the data from the control siRNA group with the VC group, as well as data from the CHL1 siRNA group with those from the control siRNA group. $P<0.05$ was considered to indicate a statistically significant difference.

RESULTS

CHL1 is correlated with the main NRG signaling molecules in both LGG and GBM

As shown in Fig. 1A, the mRNA expression of CHL1 was significantly correlated with that of NRG1, NRG3 and NRG4 ($P<0.001$ for all three subtypes), while no significant correlation was detected between CHL1 and NRG2 in LGG. In addition, the mRNA expression of CHL1 was significantly correlated with that of ErbB2 and ErbB4 ($P<0.001$ for ErbB2 and $P<0.01$ for ErbB4), with no significant correlation detected between CHL1 and ErbB3 in LGG. As shown in Fig. 1B, the mRNA expression of CHL1 was significantly correlated with that of NRG2 ($P<0.05$), with no significant correlations detected between CHL1 and NRG1, NRG3 or NRG4 in GBM. In addition, the mRNA expression of CHL1 was significantly correlated with that of ErbB3 ($P<0.05$), with no significant correlations detected between CHL1 and ErbB2 or ErbB4.

CHL1 silencing affects the protein levels of NRG1 in glioma/glioblastoma cell lines

Western blot analysis was applied to evaluate the effects of CHL1 on the protein level of NRG1, the main NRG subtype, upon using siRNA targeting CHL1. The efficiency of siRNA was confirmed in our previous study.[13] As shown in Fig. 2A, only the protein levels of the 33 and 123-kDa NRG1 isoforms were shown to be reduced in comparison with those of the control siRNA group in SHG44 cells ($P<0.05$ for 33 and 123-kDa vs. control siRNA) (Fig. 2A). As shown in Fig. 2B, the protein levels of the 33, 36, 44, 71 and 123-kDa NRG1 isoforms were all reduced in the CHL1-siRNA group compared with those of the control siRNA group in U251 cells ($P<0.05$ for all NRG1 isoforms).

As shown in Fig. 2C, the protein levels of all NRG1 isoforms were reduced in U-87 MG cells in the CHL1-siRNA group compared with those in the control siRNA group ($P<0.05$ vs. control siRNA for the 33, 71 and 123-kDa NRG1 isoforms, and $P<0.01$ vs. control siRNA for the 36 and 44-kDa NRG1 isoforms). There was no significant difference in the protein level of NRG1 isoforms between the VC and control siRNA groups in any of the three cell lines tested (Fig. 2A-C).

CHL1 silencing affects the protein levels of NRG2, 3 and 4 in glioma/glioblastoma cell lines

The present study further explored the effects of CHL1 on the protein levels of other NRG subtypes, including NRG2, 3 and 4, in the three aforementioned glioma/glioblastoma cell lines. The western blot results demonstrated that CHL1 downregulation reduced the protein level

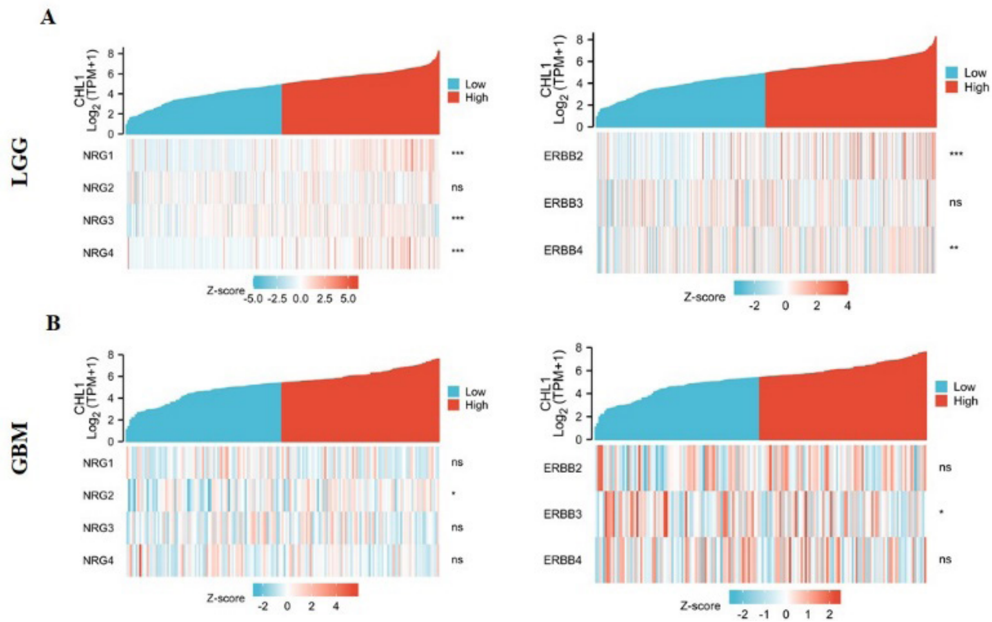

Figure 1. CHL1 expression correlates with major NRG signaling molecules in both low-grade glioma and glioblastoma multiforme. Single-gene co-expression analysis was undertaken to analyze the correlations between CHL1 and the main NRG subtypes (NRG1, NRG2, NRG3 and NRG4), and those between CHL1 and the major ErbB receptors (ErbB2, ErbB3 and ErbB4) at the mRNA level. *P<0.05, **P<0.01 vs. normal brain tissues ns, no significant vs. normal control. CHL1, cell adhesion molecule L1-like; NRG, neuregulin.

of NRG2, although no significant difference was detected in comparison with that of the control siRNA group in SHG44 cells. However, downregulation of CHL1 significantly reduced the level of NRG3 and 4 in SHG44 cells (P<0.05 vs. control siRNA for NRG3 and 4) (Figure 3A).

CHL1 silencing was able to significantly downregulate the protein levels of NRG2, 3 and 4 in U251 cells (P<0.05 vs. control siRNA for NRG2, 3 and 4) (Figure 3B). In addition, CHL1 siRNA significantly reduced the protein levels of Nrg2, 3 and 4 in U-87 MG cells (P<0.05 vs. control siRNA for Nrg3 and Nrg4, and P<0.01 vs. control siRNA for Nrg2) (Figure 3C).

CHL1 silencing affects ErbB receptor activation

The effects of CHL1 on the phosphorylation-mediated activation of the main ErbB receptors for NRGs were next studied. The levels of p-ErbB2, p-ErbB3 and p-ErbB4 were significantly reduced in SHG44 cells (P<0.05 vs. control for p-ErbB2, p-ErbB3 and p-ErbB4) (Fig. 4A). The protein levels of p-ErbB2, p-ErbB3 and p-ErbB4 were significantly downregulated by CHL1 silencing in U251 cells (P<0.01 vs. control siRNA for p-ErbB2 and p-ErbB4, and P<0.001 vs. control siRNA for p-ErbB3) (Fig. 4B). In addition, the levels of p-ErbB2, p-ErbB3 and p-ErbB4 were significantly reduced in U-87 MG cells (P<0.05 vs. control for p-ErbB2, p-ErbB3 and p-ErbB4) (Figure 4C).

Immunofluorescence staining of p-ErbB2 and p-ErbB4 in glioma/glioblastoma cell lines

Immunofluorescence staining was used to further confirm the effects of CHL1 on the activation of the main NRG receptors. The results demonstrated that the fluorescence signaling of p-ErbB2 was apparently impaired in SHG44 (Figure 5A), U251 (Figure 5B) and U-87 MG (Figure 5C) cells treated with CHL1 siRNA. Similarly, the present results demonstrated that the fluorescence signaling of p-ErbB4 was apparently impaired in SHG44 (Figure 5D), U251 (Figure 5E) and U-87 MG (Figure 5F) cells treated with CHL1 siRNA. These immunofluorescence staining results, in combination with the western blot results, clearly indicated that CHL1 could modulate the phosphorylation-mediated activation of NRG receptors in all the cell lines tested.

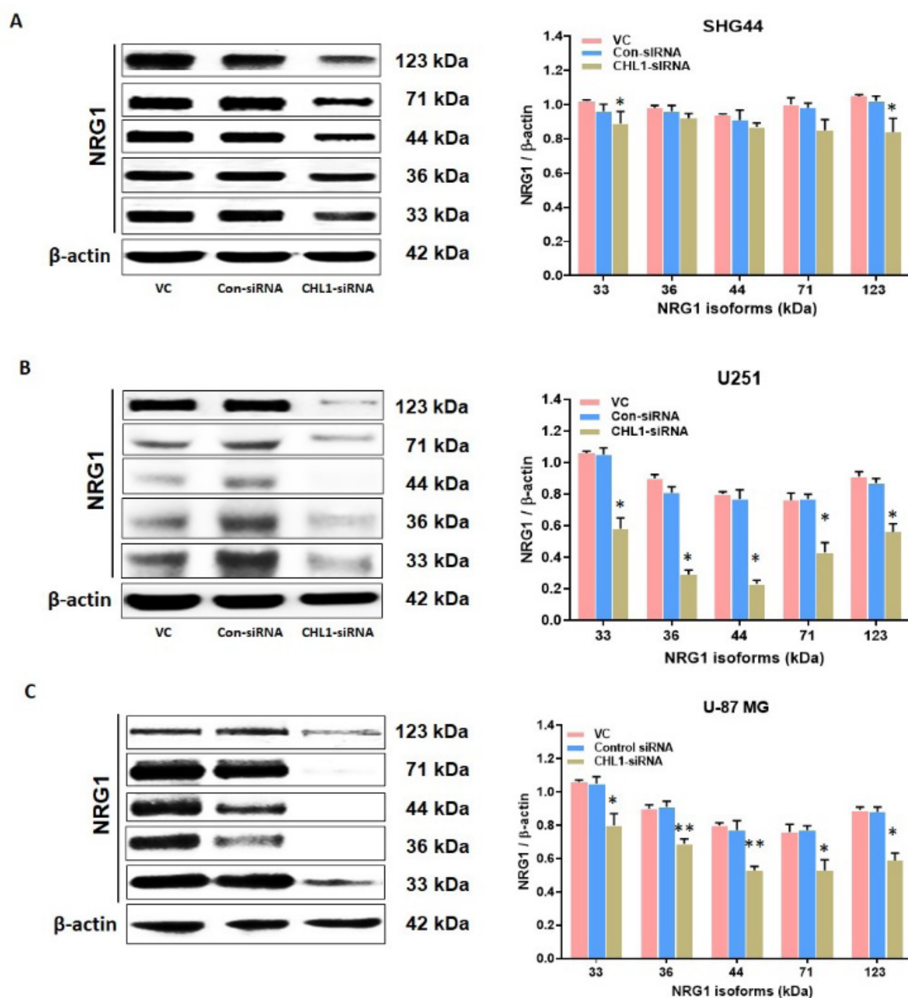

Figure 2. CHL1 silencing downregulates the protein levels of NRG1 in glioma/glioblastoma cells. (A) SHG44, (B) U251 and (C) U-87 MG cells were treated with vehicle control, control siRNA and siRNA targeting CHL1. Western blotting was performed to determine the protein expression levels of neuregulin 1 in glioma cells. β-actin was used as a loading control. *$P<0.05$, **$P<0.01$. Experiments were repeated three times, independently. CHL1, cell adhesion molecule L1-like; siRNA, small interfering RNA.

DISCUSSION

Both NRG1 and CHL1 have been reported to regulate the survival and migration of glioma cells, as well as glioma development.[13,15] Our group recently reported that NRG1 signaling contributed to the expression of CHL1.[14] The present study demonstrated that, CHL1 can modulate to varying extents the expression of the main NRG isoforms, including NRG1, 2, 3 and 4 subtypes. In addition, CHL1 regulates the phosphorylation and activation of the ErbB2, 3 and 4 receptors. These data suggest that CHL1 may function in glioma malignancy transformation in part by modulating NRG signaling.

CHL1 regulates both synapse formation and synaptic activity, thus playing vital roles in the normal development of important brain regions, as well as in the maintenance and rehabilitation of neural circuits in the adult brain.[26] A previous study suggested a tumor-suppressing role for CHL1, whose expression was decreased in low-grade malignant human breast cancer.[11] In another study, CHL1 expression downregulation enhanced human cervical cancer cell migration and invasion.[27] Contrary to these data, it was

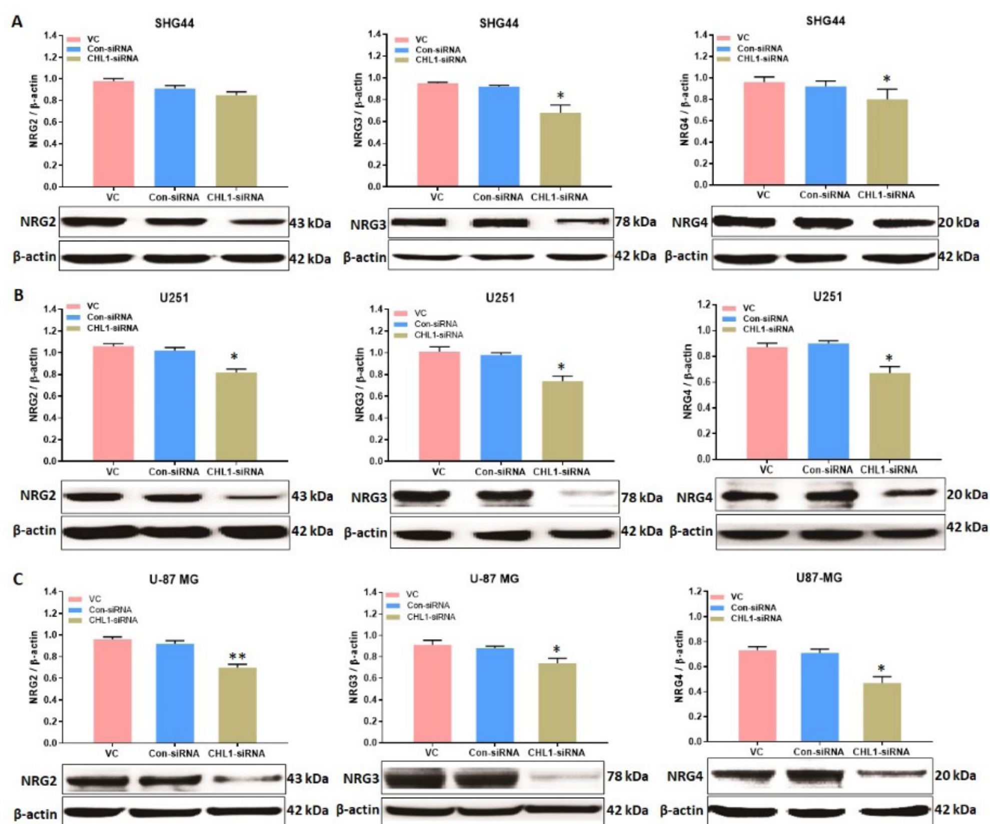

Figure 3. CHL1 silencing downregulates the protein levels of NRG2-4 in glioma/glioblastoma cells. (A) SHG44, (B) U251 and (C) U-87 MG cells were treated with vehicle control, control siRNA and siRNA targeting CHL1. Western blotting was performed to determine the protein expression levels of NRGs in glioma cells. β-actin was used as a loading control. *P<0.05, **P<0.01. Experiments were repeated three times, independently. CHL1, cell adhesion molecule L1-like; NRG, neuregulin; siRNA, small interfering RNA.

recently demonstrated that CHL1 could promote the proliferation, metastasis and migration of human glioma cells both *in vitro* and *in vivo*, thus suggesting a positive association between CHL1 level and glioma malignancy.[13,28] Enhanced co-expression of both CHL1 and GFAP were found in reactive astrocytes in a mouse spinal cord injury model.[29] In addition, CHL1 deficiency could decrease the expression of inflammatory cytokines in a dextran sodium sulfate-induced inflammatory bowel disease mouse model.[30]

Although the roles of NRG1 in glioma malignancy have been systematically investigated, no data have been reported to date about the role of other NRG subtypes, including NRG2, NRG3 and NRG4. NRG2 exhibited an expression pattern distinct from that of NRG1 in adult brain, where it mediated distinct biological processes by acting at different sites in tissues and eliciting different biochemical cellular responses.[31,32] Similar to NRG1, the EGF-like domain of NRG2 can bind to both the ErbB3 and ErbB4 receptor tyrosine kinases, and can transactivate ErbB2.[32] However, due to the lack of α/β sequence, the NRG2 isoform can inhibit angiogenesis and endothelial cell proliferation.[19] NRG2 has been reported to be expressed as a modulating target of mucin 4 in pancreatic adenocarcinoma.[33]

Both NRG2 and NRG3 have been reported to be expressed in multiple myeloma cells.[34] NRG3 is expressed in breast tumors with pre-invasive (ductal carcinoma *in situ*) and invasive potential.[18,35] NRG3 was reported to be preferentially expressed in breast tumors co-expressing both EGFR and ErbB4.[36] NRG4 could specifically stimulate the phosphorylation of ErbB4 receptor in an Akt-dependent manner, and its treatment could block colonic epithelial apoptosis induced by TNF and IFN-γ in cultured

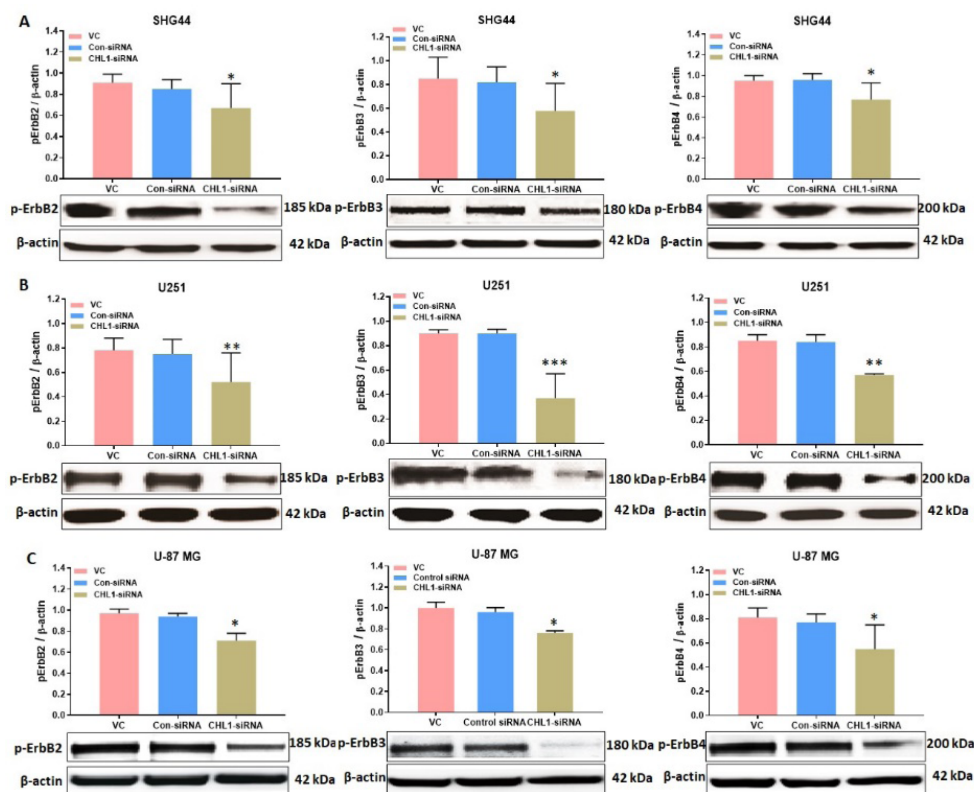

Figure 4. CHL1 silencing downregulates the protein levels of p-ErbB2, p-ErbB3 and p-ErbB4 in glioma/glioblastoma cells. (A) SHG44, (B) U251 and (C) U-87 MG cells were treated with vehicle control, control siRNA and siRNA targeting CHL1. Western blotting was performed to determine the protein expression levels of p-ErbB2, p-ErbB3 and p-ErbB4 in glioma cells. β-actin was used as a loading control. *P<0.05, **P<0.01. Experiments were repeated three times, independently. CHL1, cell adhesion molecule L1-like; p, phosphorylated; siRNA, small interfering RNA.

cells and in mice.[37] Unlike the role of the other NRGs, NRG4 mainly enhanced cell survival with no apparent effects on cell proliferation or migration.[37] In addition, NRG4 could induce the proliferation of lymphoma cell lines by activating the ErbB4 receptor, which suggested a major role of the NRG4-ErbB4 axis in the proliferation of malignant lymphoma cells in the gastrointestinal tract.[38]

NRG-based ErbB receptor activation contributes to the modulation of human astrocytic glioma development.[22] In comparison to that in U251 and U-87 MG cells, lowered NRG1-4 reduction in response to siRNA targeting CHL1 was found in SHG44 cells. However, the extent of phosphorylation-mediated activation of ErbB2-4 receptors was also markedly reduced in SHG44 cells, suggesting that the reduction of certain forms of NRGs can affect the activation of their cognate receptors.

The NRG1-ErbB receptor interaction can promote intercellular communication in a cell-cell or a cell-extracellular matrix interaction manner.[14,15] Both NRG1 isoforms positively correlated with L1 in terms of protein expression in a World Health Organization tumor grade-dependent manner. Compared with NRG1α, NRG1β enhanced L1 expression at a lower dose.[15] Although *in vitro* administration of NRG1α can significantly increase the protein expression level of CHL1 in human glioma SHG44, U251 and U-87 MG cells, NRG1β failed to increase the CHL1 expression levels in U251 cells.[14] Overall, these data indicated that Nrg1 isoforms could differentially modulate L1 or CHL1 expression in different glioma/glioblastoma cells.

CHL1 and certain growth factors, including fibroblast growth factor 3, may cooperate in the malignancy transition of tumor cells.[39] The present study revealed that downregulation of CHL1 significantly reduced the expression of the four main Nrg subtypes in all the three

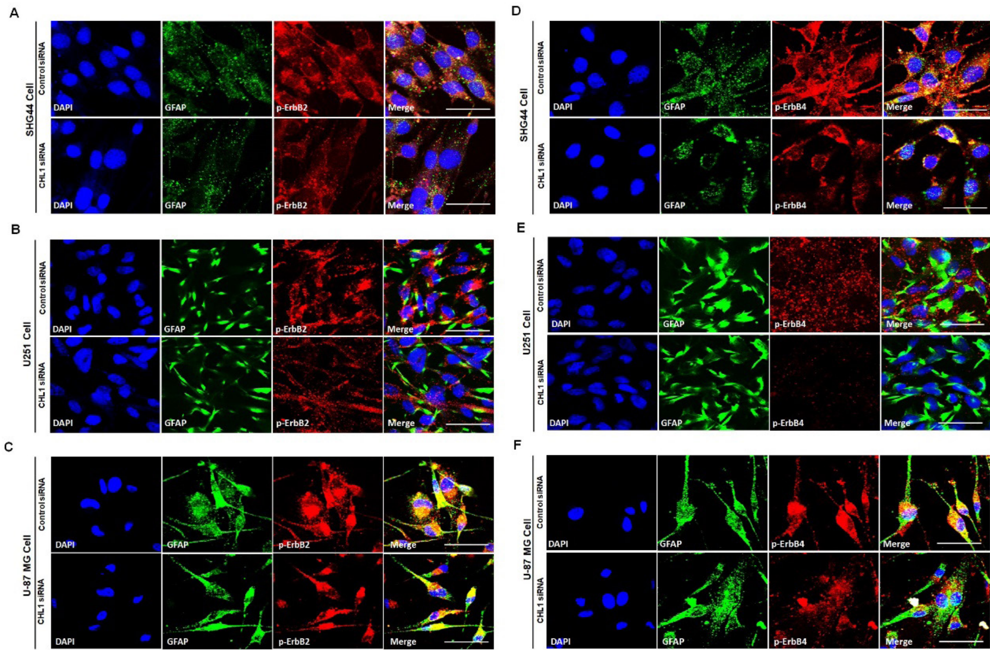

Figure 5. Immunofluorescence evaluation of the effects of CHL1 silencing on the protein levels of p-ErbB2 and p-ErbB4 in glioma/glioblastoma cells. (A) SHG44, (B) U251 and (C) U-87 MG cells were fixed, and then incubated with antibodies recognizing GFAP and p-ErbB2. The immunofluorescence analysis results indicated that the level of p-ErbB2 expression was downregulated in response to siRNA-CHL1 compared with that of the VC in all three cell lines. (D) SHG44, (E) U251 and (F) U-87 MG cells were fixed, and then incubated with antibodies recognizing GFAP and p-ErbB4. Immunofluorescence analysis indicated that the level of p-ErbB4 was downregulated in response to siRNA-CHL1 compared with that caused by exposure to VC in all three cell lines. Confocal images were obtained under ×600 magnification. Scale bars, 5 µm. CHL1, cell adhesion molecule L1-like; p, phosphorylated; GFAP, glial fibrillary acidic protein; VC, vehicle control; siRNA, small interfering RNA.

glioma/glioblastoma cell lines evaluated, which was accompanied by reduction of the phosphorylation-mediated activation level of the ErbB2, ErbB3 and ErbB4 receptors. Our group previously demonstrated that *in vivo* CHL1 silencing could modulate the growth of U-87 MG cell-derived xenograft in nude mice.[13] Overall, these data strongly supported an intermodulation loop composed of CHL1 and NRG signaling in the development of glioma, and a central role played by CHL1 in modulating the level of ErbB receptor-based signaling, at least in part by modulating NRG1-4 in gliomas, thus suggesting that CHL1 may function as a potential target in the treatment of gliomas.

Hyperactivation of the PI3K/AKT signaling pathway regulates tumor cell survival, growth and motility in GBM.[13,40] NRG1β promotes glioma cell survival, and inhibits the apoptosis of glioma cells via the PI3K/AKT signaling pathway.[22,41] CHL1 also modulates glioma behavior mainly via the AKT signaling pathway.[13] In addition, inhibition of Akt activation reduced the activation of ErbB2.[42] These data collectively indicated that CHL1 may modulate NRG-ErbB receptor signaling via the Akt signaling pathway. NRG1 could enhance CHL1 expression via the ERK1/2 signaling pathway, thus modulating the growth and proliferation of glioma cells as an upstream signaling molecule of CHL1.[14] L1CAM has been reported to bind to ErbB receptors via the extracellular Ig-like domain, thereby enhancing the response of ErbB receptor to neuregulins during nervous system development.[43] Therefore, we hypothesize that CHL1, being homologous to L1CAM, may play the same role in modulating NRG-ErbB signaling.

In conclusion, targeting CHL1-NRG signaling in the development of glioma/glioblastoma may provide a therapeutic basis for ameliorating the devastating consequences of glioma malignancy. However, further studies using a glioma xenograft nude mouse model is needed to validate this hypothesis in the future.

DISCLOSURE

Financial support: This research was funded by Jiangsu Province Shuangchuang Talent Plan (Grant No. JSSCRC 2021533), the research Start-up Fund of Jiangnan University (Grant No. 1285081903200020), and Research start-up Fund of Wuxi School of Medicine, Jiangnan University (Grant No. 1286010242190060).

Conflict of interest: None

REFERENCES

1 Gabriel A, Batey J, Capogreco J, *et al.* Adult brain cancer in the U.S. black population: a Surveillance, Epidemiology, and End Results (SEER) analysis of incidence, survival, and trends. *Med Sci Monit* 2014;20:1510-7. doi: 10.12659/msm.890762.

2 Davis ME. Glioblastoma: Overview of disease and treatment. *Clin J Oncol Nurs* 2016;20(5 Suppl):S2-8. doi: 10.1188/16.Cjon.S1.2-8.

3 Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin* 2010;60(5):277-300. doi: 10.3322/caac.20073.

4 Sathornsumetee S, Rich JN, Reardon DA. Diagnosis and treatment of high-grade astrocytoma. *Neurol Clin* 2007;25(4):1111-39, x. doi: 10.1016/j.ncl.2007.07.004.

5 Holm J, Hillenbrand R, Steuber V, *et al.* Structural features of a close homologue of L1 (CHL1) in the mouse: a new member of the L1 family of neural recognition molecules. *Eur J Neurosci* 1996;8(8):1613-29. doi: 10.1111/j.1460-9568.1996.tb01306.x.

6 Zhang X, Davis JQ, Carpenter S, Bennett V. Structural requirements for association of neurofascin with ankyrin. *J Biol Chem* 1998;273(46):30785-94. doi: 10.1074/jbc.273.46.30785.

7 Maness PF, Schachner M. Neural recognition molecules of the immunoglobulin superfamily: signaling transducers of axon guidance and neuronal migration. *Nat Neurosci* 2007;10(1):19-26. doi: 10.1038/nn1827.

8 Panicker AK, Buhusi M, Thelen K, Maness PF. Cellular signalling mechanisms of neural cell adhesion molecules. *Front Biosci* 2003;8:d900-11. doi: 10.2741/1014.

9 Senchenko VN, Krasnov GS, Dmitriev AA, *et al.* Differential expression of CHL1 gene during development of major human cancers. *PLoS One* 2011;6(3):e15612. doi: 10.1371/journal.pone.0015612.

10 Marko NF, Coughlan C, Weil RJ. Towards an integrated molecular and clinical strategy to predict early recurrence in surgically resected non-functional pituitary adenomas. *J Clin Neurosci* 2012;19(11):1535-40. doi: 10.1016/j.jocn.2012.01.038.

11 He LH, Ma Q, Shi YH, *et al.* CHL1 is involved in human breast tumorigenesis and progression. *Biochem Biophys Res Commun* 2013;438(2):433-8. doi: 10.1016/j.bbrc.2013.07.093.

12 Huang CX, Hu SX, Chen B. [Expression of the neural cell adhesion molecule in human astrocytomas]. *Hunan Yi Ke Da Xue Xue Bao* 2001;26(6):543-5.

13 Yang Z, Xie Q, Hu CL, *et al.* CHL1 Is Expressed and Functions as a Malignancy Promoter in Glioma Cells. *Front Mol Neurosci* 2017;10:324. doi: 10.3389/fnmol.2017.00324.

14 Lin WW, Ou GY, Lin JZ, *et al.* Neuregulin 1 enhances cell adhesion molecule L1 like expression levels and promotes malignancy in human glioma. *Oncol Lett* 2020;20(1):326-36. doi: 10.3892/ol.2020.11548.

15 Zhao WJ, Schachner M. Neuregulin 1 enhances cell adhesion molecule l1 expression in human glioma cells and promotes their migration as a function of malignancy. *J Neuropathol Exp Neurol* 2013;72(3):244-55. doi: 10.1097/NEN.0b013e3182863dc5.

16 Zhao W, Ren SG. Neuregulin-1 (Nrg1) is mainly expressed in rat pituitary gonadotroph cells and possibly regulates prolactin (PRL) secretion in a juxtacrine manner. *J Neuroendocrinol* 2011;23(12):1252-62. doi: 10.1111/j.1365-2826.2011.02223.x.

17 Zhao WJ, Jiang Q, Mei JP. Neurohypophyseal neuregulin 1 is derived from the hypothalamus as a potential prolactin modulator. *Neuroendocrinology* 2015;102(4):288-99. doi: 10.1159/000431377.

18 Marshall C, Blackburn E, Clark M, Humphreys S, Gullick WJ. Neuregulins 1-4 are expressed in the cytoplasm or nuclei of ductal carcinoma (in situ) of the human breast. *Breast Cancer Res Treat* 2006;96(2):163-8. doi: 10.1007/s10549-005-9073-z.

19 Nakano N, Higashiyama S, Ohmoto H, Ishiguro H, Taniguchi N, Wada Y. The N-terminal region of NTAK/neuregulin-2 isoforms has an inhibitory activity on angiogenesis. *J Biol Chem* 2004;279(12):11465-70. doi: 10.1074/jbc.M311045200.

20 Baulida J, Kraus MH, Alimandi M, Di Fiore PP, Carpenter G. All ErbB receptors other than the epidermal growth factor receptor are endocytosis impaired. *J Biol Chem* 1996;271(9):5251-7. doi: 10.1074/jbc.271.9.5251.

21 Ou GY, Lin WW, Zhao WJ. Neuregulins in neurodegenerative diseases. *Front Aging Neurosci* 2021;13:662474. doi: 10.3389/fnagi.2021.662474.

22 Ritch PS, Carroll SL, Sontheimer H. Neuregulin-1 enhances survival of human astrocytic glioma cells. *Glia* 2005;51(3):217-28. doi: 10.1002/glia.20197.

23 Ritch PA, Carroll SL, Sontheimer H. Neuregulin-1 enhances motility and migration of human astrocytic glioma cells. *J Biol Chem* 2003;278(23):20971-8. doi: 10.1074/jbc.M213074200.

24 Zhao WJ, Yi SJ, Ou GY, Qiao XY. Neuregulin 2 (NRG2) is expressed in gliomas and promotes migration of human glioma cells. *Folia Neuropathol* 2021;59(2):189-97. doi: 10.5114/fn.2021.106460.

25 Qiao XY, S.J. Y, W.J. Z. Comparison of NRG1, 3, and 4 in promoting glioma cell migration. *Chin Pharmalog Bull (Chn)* 2021;37(10):1396-401.

26 Demyanenko GP, Schachner M, Anton E, *et al.* Close homolog of L1 modulates area-specific neuronal

positioning and dendrite orientation in the cerebral cortex. *Neuron* 2004;44(3):423-37. doi: 10.1016/j.neuron.2004.10.016.
27 Long MJ, Wu FX, Li P, Liu M, Li X, Tang H. MicroRNA-10a targets CHL1 and promotes cell growth, migration and invasion in human cervical cancer cells. *Cancer Lett* 2012;324(2):186-96. doi: 10.1016/j.canlet.2012.05.022.
28 Liu Y, Yu Y, Schachner M, Zhao W. Neuregulin 1-β regulates cell adhesion molecule L1 expression in the cortex and hippocampus of mice. *Biochem Biophys Res Commun* 2013;441(1):7-12. doi: 10.1016/j.bbrc.2013.09.102.
29 Jakovcevski I, Wu J, Karl N*, et al.* Glial scar expression of CHL1, the close homolog of the adhesion molecule L1, limits recovery after spinal cord injury. *J Neurosci* 2007;27(27):7222-33. doi: 10.1523/jneurosci.0739-07.2007.
30 Yao J, Gao R, Luo M*, et al.* Close homolog of L1-deficient ameliorates inflammatory bowel disease by regulating the balance of Th17/Treg. *Gene* 2020;757:144931. doi: 10.1016/j.gene.2020.144931.
31 Crovello CS, Lai C, Cantley LC, Carraway KL, 3rd. Differential signaling by the epidermal growth factor-like growth factors neuregulin-1 and neuregulin-2. *J Biol Chem* 1998;273(41):26954-61. doi: 10.1074/jbc.273.41.26954.
32 Carraway KL, 3rd, Weber JL, Unger MJ*, et al.* Neuregulin-2, a new ligand of ErbB3/ErbB4-receptor tyrosine kinases. *Nature* 1997;387(6632):512-6. doi: 10.1038/387512a0.
33 Chaturvedi P, Singh AP, Moniaux N*, et al.* MUC4 mucin potentiates pancreatic tumor cell proliferation, survival, and invasive properties and interferes with its interaction to extracellular matrix proteins. *Mol Cancer Res* 2007;5(4):309-20. doi: 10.1158/1541-7786.Mcr-06-0353.
34 Mahtouk K, Cremer FW, Rème T*, et al.* Heparan sulphate proteoglycans are essential for the myeloma cell growth activity of EGF-family ligands in multiple myeloma. *Oncogene* 2006;25(54):7180-91. doi: 10.1038/sj.onc.1209699.
35 Dunn M, Sinha P, Campbell R*, et al.* Co-expression of neuregulins 1, 2, 3 and 4 in human breast cancer. *J Pathol* 2004;203(2):672-80. doi: 10.1002/path.1561.
36 Breuleux M. Role of heregulin in human cancer. *Cell Mol Life Sci* 2007;64(18):2358-77. doi: 10.1007/s00018-007-7120-0.
37 Bernard JK, McCann SP, Bhardwaj V, Washington MK, Frey MR. Neuregulin-4 is a survival factor for colon epithelial cells both in culture and in vivo. *J Biol Chem* 2012;287(47):39850-8. doi: 10.1074/jbc.M112.400846.
38 Ebi M, Kataoka H, Shimura T*, et al.* The role of neuregulin4 and HER4 in gastrointestinal malignant lymphoma. *Mol Med Rep* 2011;4(6):1151-5. doi: 10.3892/mmr.2011.542.
39 Kotani N, Yamaguchi A, Ohnishi T*, et al.* Proximity proteomics identifies cancer cell membrane cis-molecular complex as a potential cancer target. *Cancer Sci* 2019;110(8):2607-19. doi: 10.1111/cas.14108.
40 Asati V, Mahapatra DK, Bharti SK. PI3K/Akt/mTOR and Ras/Raf/MEK/ERK signaling pathways inhibitors as anticancer agents: Structural and pharmacological perspectives. *Eur J Med Chem* 2016;109:314-41. doi: 10.1016/j.ejmech.2016.01.012.
41 Yin F, Zhang JN, Wang SW*, et al.* MiR-125a-3p regulates glioma apoptosis and invasion by regulating Nrg1. *PloS one* 2015;10(1):e0116759. doi: 10.1371/journal.pone.0116759.
42 Lin JZ, Zhao WJ. Neuregulin 2 is highly expressed in glioma tissues to regulate glial fibrillary acidic protein expression via Akt signaling. *J South Med Univ* 2021;41(8):1171-6.
43 Donier E, Gomez-Sanchez JA, Grijota-Martinez C*, et al.* L1CAM binds ErbB receptors through Ig-like domains coupling cell adhesion and neuregulin signalling. *PLoS One* 2012;7(7):e40674. doi: 10.1371/journal.pone.0040674.