Long Noncoding RNA FGD5-AS1 Promotes Glioma Cell Proliferation, Migration and Invasion by Regulating wnt/β-Catenin Pathway

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Purpose: To investigate the specific function of long noncoding RNA FGD5 antisense RNA 1 (IncRNA FGD5-AS1) in glioma.

Materials and Methods: The level of FGD5-AS1 was detected in clinical samples and cell lines by qRT-PCR. Small interfering RNA (siRNA) of FGD5-AS1 or scramble siRNA was transfected into U87 cell lines to examine the role of FGD5-AS1 on glioma development. The proliferation of glioma cells was tested by Cell Counting Kit-8 (CCK-8), the migration and invasion of glioma cells were tested by transwell assay without matrigel or with matrigel. Western blot was used to detect the protein expression, and XAV-939 was used to inhibit wnt/β-catenin pathway. The effect of FGD3-AS1 on tumorigenesis of glioma was confirmed by xenograft nude mice model.

Results: FGD5-AS1 was significantly increased in glioma tissues and cells. Loss of FGD5-AS1 inhibited the proliferation, migration and invasion of U87 cells. Furthermore, over-expression of FGD5-AS1 increased the mRNA and protein levels of β-catenin and cyclin D1. Blocking of wnt/β-catenin using XAV-939 reversed the promotion role of FGD3-AS1 on glioma cells’ migration and invasion. The in vivo tumor growth assay showed that FGD3-AS1 accelerated glioma tumorigenesis with activating wnt/β-catenin pathway.

Conclusion: Our research emphasized FGD5-AS1 acting as an oncogene by regulating wnt/β-catenin signaling pathway, thus providing some novel experimental basis for clinical treatment of glioma.

Keywords: IncRNA FGD5-AS1, glioma, cell proliferation, migration, wnt/β-catenin pathway

Introduction

Glioma is the most common malignant tumor of the central nervous system, accounting for about half of all intracranial primary tumors.1,2 Because of the limitations of treatment and high recurrence rate, glioma becomes one of the deadliest tumors of the nervous system.3 At present, there are little studies to elucidate the specific pathogenesis and molecular mechanism of glioma. Thus, the priority is to explore the underlying mechanisms and develop effective treatment.

Recently, noncoding RNAs (ncRNAs) have attracted a lot of attention.4 Originally, ncRNAs were considered as waste products in the process of cell metabolism. With the deepening of scientific research, it has been gradually found that ncRNAs are involved in multiple cellular processes, including cell growth, proliferation, differentiation, apoptosis and autophagy.5,6 Specially, long

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noncoding RNAs (lncRNAs) have been found to be differentially expressed in various diseases and play a pivotal role in tumorigenesis and tumor progression. Silencing lncRNA SNHG12 inhibited gastric cancer cell proliferation, migration and invasion, but promoted cell apoptosis and cell cycle retardation. And the function of SNHG12 was achieved by regulating the PI3K/Akt pathway.

LncRNA FGD5-AS1 has been reported to expressed in colorectal cancer and acted as a tumor promoter by sponging miR-302e, and promoted non-small cell lung cancer cell proliferation through sponging hsa-miR-107 to upregulate FGFRL1. However, the function and molecular mechanism of FGD5-AS1 in glioma remain unknown.

Abnormal proliferation and differentiation are the main features of tumor cells, which are mediated by cellular and molecular signaling pathways. Especially, wnt/β-catenin pathway plays a critical role in the early development of animal embryos, organ formation, tissue regeneration and other physiological processes. And abnormal activation of wnt/β-catenin signaling can cause excessive proliferation and differentiation of tumor cells, eventually leads to tumorigenesis. In the past years, mounting evidences have verified that disruption of wnt/β-catenin is able to inhibit tumor progression. Studies showed that lncRNA UCA1 promoted tumorigenesis by activating wnt/β-catenin in oral squamous cell carcinoma and melanoma. LncRNA PART1 regulated miR-150-5p/miR-520h/CTNNB1 axis and activated wnt/β-catenin pathway in colorectal cancer. In addition, wnt/β-catenin pathway was involved in glioma formation. However, whether FGD5-AS1 interacts with wnt/β-catenin pathway in glioma remains elusive. The purpose of our study was to clarify the specific function of lncRNA FGD5-AS1 in glioma and to further clarify regulation of FGD5-AS1 on wnt/β-catenin pathway.

Materials and Methods

Human Glioma Tissues Collection

We collected 20 glioma patients and got their tissue specimens and adjacent tissues when they underwent surgical resection from January 2017 to January 2019 with their consent. All tissues were saved in −80°C before we did related experiments. All of the patients or their guardians (for those who are too poorly educated to write) provided written consent, and the Ethics Committee of the First Affiliated Hospital of Zhengzhou University approved all aspects of this study.

Cell Culture and Transfection

The normal human astrocytes (HA) and glioma cell lines including U251, SHG139 and U87 were purchased from the Science Cell Laboratory. Cells were cultured in Dulbecco’s modified Eagle’s medium (Waltham, USA) supplemented with 10% fetal bovine serum (Cromwell, USA) and 100 μL/mL penicillin and streptomycin (Sigma-Aldrich, USA) and placed at 37°C with 5% CO2. 2 μg FGD5-AS1 plasmid or si-FGD5-AS1 was transfected into cells, the transfection buffer was purchased from Invitrogen.

Quantitative Real-Time PCR

RNA isolation, reverse transcription and quantitative expression were carried according to manufacturer’s instructions. All the kits were purchased from Vazyme, and gene expression was calculated using $2^{-\Delta\Delta C_{t}}$ method.

Protein Isolation and Western Blotting

Anti-body of β-catenin, cyclin D1 and GAPDH were purchased from Abcam (Cambridge, UK). Cells were collected, total protein was extracted using RIPA lysis buffer and separated by SDS-PAGE before transferring to a PVDF membrane (Millipore, USA). Blocked with 5% BSA and washed in TBST for three times. Incubate with primary antibody overnight and then the corresponding secondary antibody. ECL regents were used to visualize the protein. The density of the protein bands was analyzed by Image J software.

CCK8 Assay

Cells were seeded in 96-well cell plates, and added CCK-8 solution (Vazyme, China) at 0, 24, 48 and 72 h. 2 hours later, measure the OD value at 450 nm.

Transwell Assay

Cells in logarithmic growth phase were adjusted to $3 \times 10^4$ cells/well of medium (without serum) and plated into the upper chamber. Lower chamber was added with 500 μL of medium (with 10% FBS), and then incubate the chamber at 37°C for 48 h. Then, the migrated cells were visualized by the crystal violet and inverted microscope.

Wound Healing Assay

$5 \times 10^5$ cells were planted in a 6-well plate, and when the cells grew to fuse, two vertical parallel lines were drawn with 10 μL suction head against the ruler. The floating
cells were washed with PBS and cultured in serum-free medium for 24 hours. Images were taken at 0 and 24 hours of cell culture, respectively.

**In vivo Tumor Growth Assay**

Nude mice were purchased from Guangdong provincial experimental animal center for medicine. U251 cells (5 x 10⁵) transfected with FGD5-AS1 plasmid or NC were subcutaneously injected in right lower limb of the nude mice (n = 10 for each group). Tumor size was measured every five days. After 30 days of injection, mice were intraperitoneally injected with 3% pentobarbital sodium and were killed by excessive anesthesia with a dose of 90 mL/kg, and the tumors were removed for follow-up study. This study was reviewed and approved by the NIH Animal Welfare Guidelines and Institutional animal care and use committee of the First Affiliated Hospital of Zhengzhou University. The animal testing was conducted in laboratory of the First Affiliated Hospital of Zhengzhou University.

**Statistical Analysis**

ANOVA was performed to compare results among the groups. All experiments were repeated three times. Statistical analysis was performed using SPSS19.0 statistical software (SPSS Inc, Chicago, IL).

**Results**

**Increase of FGD5-AS1 in Glioma Tissues and Cells**

The expression changes of lncRNAs are basis for exploring the regulation of lncRNAs, thus we firstly examined the level of lncRNA FGD5-AS1 in glioma clinical samples. In 20 samples of patient diagnosed glioma, FGD5-AS1 was increased than that in normal tissues (Figure 1A). In addition, qRT-PCR revealed that FGD5-AS1 level was dramatically higher in glioma cell lines than that in normal HA (Figure 1B). These data exhibited an apparent difference of FGD5-AS1 expression in glioma, which urged us to further explore whether FGD5-AS1 regulating glioma development.

**Knockdown of FGD5-AS1 Inhibited Proliferative, Migratory and Invasive Ability of Glioma Cells**

To further identify FGD5-AS1 function in glioma progression, we constructed siRNA of FGD5-AS1 to silencing the expression of FGD5-AS1 in glioma cells. As FGD5-AS1 was increased in glioma cell lines and has the highest expression in U87 cells (Figure 1B), we chose the U87 for later studies. As showed in Figure 2A, transfection of si FGD5-AS1 significantly blocked FGD5-AS1 expression in U87 cells compared with scrambled siRNA transfection group. CCK-8 assay showed that loss of FGD5-AS1 significantly inhibited growth rate at 48 h and 72 h than cells transfected with scrambled siRNA (Figure 2B). The migration and invasion of tumor cells were conducive to tumor metastasis and poor prognosis. In the present study, we carried transwell assay with or without matrigel and wound healing assay to detected migration and invasion. The results showed that FGD5-AS1 siRNA significantly reduced the cell migratory and invasive ability in U87 cells (Figure 2C-E).

**FGD5-AS1 Induced Glioma Cell Invasion and Migration by Regulating wnt/β-Catenin Pathway**

Wnt/β-catenin signaling pathway plays an essential role in various types of cancer development, and we speculated that FGD5-AS1 regulated glioma progression via wnt/β-catenin pathway. To test our hypothesis, we first detect the mRNA and protein expression of the key molecules in the wnt/β-catenin pathway. As shown in Figure 3A, knockdown of FGD5-AS1 significantly decreases β-catenin and Cyclin D1 protein level, which can be reversed by wnt/β-catenin pathway activator LiCl in U87 cells. In addition, we constructed FGD5-AS1 plasmid to force expression of FGD5-AS1 in glioma cells (Figure 3B), and we treated U251 cells...
with FGD5-AS1 plasmid and XAV-939 (wnt/β-catenin signaling inhibitor). Overexpression of FGD5-AS1 distinctly increased the mRNA and protein expression of β-catenin and cyclin D1 (Figure 3C and D). Furthermore, we detected β-catenin and Cyclin D1 expression in glioma cell lines and normal HAs. As shown in Figure 3E and F, the mRNA and protein levels were increased in glioma cell lines than that in HAs. Followed functional analysis showed that forced expression of FGD5-AS1 facilitated the migration and invasion of glioma, while treatment with XAV-939 suppressed migration and invasion (Figure 3G–I). These data suggested that FGD5-AS1 motivated migration and invasion by activating wnt/β-catenin pathway in glioma.

FGD5-AS1 Promoted Tumorigenesis of Glioma in vivo
To determine the effect of FGD5-AS1 on tumorigenesis of glioma, we set up xenograft nude mice model. U251 cells transfected with FGD5-AS1 or NC plasmid were subcutaneously injected into nude mice, and we measured tumor volume. The mice with FGD5-AS1 plasmid cells showed a large tumor volume, and tumors grew faster when forcing expression of FGD5-AS1 (Figure 4A). The tumors were isolated at 30 days after injection, FGD5-AS1 significantly increased tumors weight than that in NC mice (Figure 4B). In addition, we isolated these tumor tissues and found that the expression of FGD5-AS1 was increased in FGD5-AS1 overexpression mice (Figure 4C). Moreover, FGD5-AS1 increased the mRNA level of β-catenin and cyclin D1 in tumor tissues (Figure 4D).

Discussion
The annual incidence of glioma is 3 to 8 per 100 000 population, and potential risk factors may include heredity, viral infection, exposure to carcinogens, and radiation. Studies indicated that the onset age of glioma is mostly between 21 and 50 years old, and the peak is between 31 and 40 years old. However, with the change of life habits and the increase of life pressure, the incidence of glioma in recent years has become younger population, which caused widespread concerns. At present, the difficulties in the treatment of glioma lie are rapid progression, poor prognosis and high postoperative recurrence. Therefore, it is necessary to start from the cellular and molecular level to target tumor growth, so as to assist clinical surgical resection and reduce postoperative recurrence rate.
Recent studies indicated that lncRNAs acted as a key mediator in various diseases, such as myocardial infarction, pulmonary fibrosis, breast cancer.21–23 Interestingly, lncRNAs were involved in glioma progression. LncRNA PAXIP1-AS1 enhanced migration, invasion, and angiogenesis of human umbilical vein endothelial cells in glioma by recruiting the transcription factor ETS1 to upregulate the expression of KIF14.24 Knockdown of lncRNA NEAT1 inhibited glioma cell migration and invasion via modulation of SOX2 by increasing miR-132.25 SNHG12 facilitated the tumorigenesis of glioma through miR-101-3p/FOXP1 axis,26 and promoted the proliferation and migration of glioma cells by binding to HuR.27

And in our study, we explored the expression of lncRNA FGD5-AS1 in glioma to clarify its function. Surprisingly, FGD5-AS1 was significantly increased in clinical glioma tissues, and we also found a remarkable elevation of FGD5-AS1 in glioma cell lines compared with normal human astrocytes. These data indicated that FGD5-AS1 might be involved in the development of glioma, which was similar to the high expression of FGD5-AS1 in colorectal cancer.28 To further investigate the role of FGD5-AS1 in glioma, we constructed siRNA to silence FGD5-AS1 expression. In accordance with expectation, loss of FGD5-AS1 inhibited the proliferation, migration and invasion of U87 cells.

Wnt/β-catenin signaling controls kinds of biological processes throughout development and adult life of vertebrates and invertebrates.28 And growing researches suggested that wnt/β-catenin was involved in the growth and metastasis of tumor cells.29–31 As well, activation of wnt/β-catenin pathway was found in glioma cells, and blocking of wnt/β-catenin pathway suppressed multiple oncogenic targets.32 In our study, forced expression of FGD5-AS1 activated wnt/β-catenin pathway with a dramatic change of β-catenin and cyclin D1. Moreover, disruption of wnt/β-catenin signaling removed the inhibitory effect of si-FGD5-AS1 on glioma cell migration and invasion. The in vivo tumorigenesis of glioma assay got the same results, FGD5-AS1 exerted its oncogenic function via modulating the activity of Wnt/β-catenin pathway in glioma.

In the present study, there are several limitations. First, the present study only the expression of FGD5-AS1 detected 20 glioma patients, and further study may explore the correlation between FGD5-AS1 level and glioma grades. Second, the orthotopic glioma models will be used to further confirm our findings. Third, the present...
study only proved that FGD5-AS1 regulated glioma via Wnt/β-catenin pathway, and future studies will explore how does FGD5-AS1 activate Wnt/β-catenin pathway and whether FGD5-AS1 regulates other pathways in glioma.

Conclusions

In conclusion, our results showed that FGD5-AS1 acted as a pivotal factor in the regulation of glioma cell proliferation, invasion and migration via Wnt/β-catenin signaling pathway. In the future clinical treatment, drugs can be designed based on FGD5-AS1, and other experiments are needed to further explore detail molecular mechanisms.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Jiang J, Wang X, Gao G, et al. Silencing of lncRNA HOXA11-AS inhibits cell migration, invasion, proliferation, and promotes apoptosis in human glioma cells via upregulating microRNA-125a: in vitro and in vivo studies. Am J Transl Res. 2019;11(10):6382–6392.

2. Carlsson SK, Brothers SP, Wahlestedt C. Emerging treatment strategies for glioblastoma multiforme. EMBO Mol Med. 2014;6 (11):1359–1370. doi:10.15252/emmm.201302627

3. Chen J, Li Y, Yu TS, et al. A restricted cell population propagates glioblastoma growth after chemotherapy. Nature. 2012;488(7412):522–526. doi:10.1038/nature11287

4. Ramnarine VR, Kobelev M, Gibb EA, et al. The evolution of long noncoding RNA acceptance in prostate cancer initiation, progression, and its clinical utility in disease management. Eur Urol. 2019;76 (5):546–559. doi:10.1016/j.euro.2019.07.040

5. Yang Z, Jiang S, Shang J, et al. LncRNA: shedding light on mechanisms and opportunities in fibrosis and aging. Ageing Res Rev. 2019;52:17–31. doi:10.1016/j.arr.2019.04.001

6. Zhao Z, Lin X, Tong Y, Li W. Silencing lncRNA ZFAS1 or elevated microRNA-135a represses proliferation, migration, invasion and resistance to apoptosis of osteosarcoma cells. Cancer Cell Int. 2019;19(1):326. doi:10.1186/s12935-019-1049-x

7. de Andres-pablo A, Movillion A, Wery M. LncRNAs, lost in translation or licence to regulate? Curr Genet. 2017;63(1):29–33. doi:10.1007/s00294-016-0615-1

8. Zhang R, Liu Y, Liu H, et al. The long non-coding RNA SNHG12 promotes gastric cancer by activating the phosphatidylinositol 3-kinase/AKT pathway. Aging (Albany NY). 2019;11(23):10902.

9. Li D, Jiang X, Zhang X, Cao G, Wang D, Chen Z. Long non-coding RNA FGD5-AS1 promotes colorectal cancer cell proliferation, migration, and invasion through upregulating CDC42 via sponging miR-302e. In Vitro Cell Dev Biol Anim. 2019;55(8):326–339. doi:10.1007/s11626-019-00376-x

10. Fan Y, Li H, Yu Z, et al. Long non-coding RNA FGD5-AS1 promotes non-small cell lung cancer cell proliferation through sponging hsa-miR-107 to up-regulate FGFR1. Biosci Rep. 2020;40(1). doi:10.1042/BSR20193009

11. Liu L, Michowicz W, Kołodziejczyk A, Sicinski P. The cell cycle in stem cell proliferation, pluripotency and differentiation. Nat Cell Biol. 2019;21(9):1060–1067. doi:10.1038/s41561-019-0384-4
12. Chen D, Xie R, Shu B, et al. Wnt signaling in bone, kidney, intestine, and adipose tissue and interorgan interaction in aging. *Ann N Y Acad Sci*. 2019;1442(1):48–60. doi:10.1111/nyas.13945

13. Liu HW, Su YK, Bamodu OA, et al. The disruption of the beta-catenin/TCF-1/STAT3 signaling axis by 4-acetylantranilquinolinol B inhibits the tumorigenesis and cancer stem-cell-like properties of glioblastoma cells, in vitro and in vivo. *Cancers (Basel)*. 2018;10(12). doi:10.3390/cancers10120491

14. Yang YT, Wang YF, Lai JY, et al. Long non-coding RNA UCA1 contributes to the progression of oral squamous cell carcinoma by regulating the WNT/beta-catenin signaling pathway. *Cancer Sci*. 2016;107(11):1581–1589. doi:10.1111/cas.13058

15. Chen X, Gao J, Yu Y, Zhao Z, Pan Y. Long non-coding RNA UCA1 targets miR-185-5p and regulates cell mobility by affecting epithelial-mesenchymal transition in melanoma via wnt/beta-catenin signaling pathway. *Gene*. 2018;676:298–305. doi:10.1016/j.gene.2018.08.065

16. Zhou T, Wu L, Ma N, Tang F, Zong Z, Chen S. LncRNA PART1 regulates colorectal cancer via targeting miR-150-5p/miR-520h/CTNNB1 and activating wnt/beta-catenin pathway. *Int J Biochem Cell Biol*. 2019;118:105637. doi:10.1016/j.biocel.2019.105637

17. Zhang B, Fang S, Cheng Y, Zhou C, Deng F. The long non-coding RNA, uterine cervical carcinoma associated 1, promotes cell growth, invasion, migration, and chemo-resistance in glioma through wnt/beta-catenin signaling pathway. *Aging (Albany NY)*. 2019;11(19):8239–8253. doi:10.18632/aging.102317

18. Bi J, Chowdhry S, Wu S, Zhang W, Masui K, Mischel PS. Altered beta-catenin/TCF-1/STAT3 signaling axis by 4-acetylantroquinonol inhibits the tumorigenesis and cancer stem-cell-like properties of glioma cells, in vitro and in vivo. *Biosci Rep*. 2018;38(1):486. doi:10.1103/s12943-018-0147-7

19. Anssar TM, Leitzmann MF, Linker RA, et al. Autoimmune diseases co-dependency targets. *J Immunol*. 2018;107(11):1581–1589. doi:10.1111/cas.13058

20. Neamati F, Asemi Z. The effects of melatonin on signaling pathways and immunosuppressive therapy in relation to the risk of glioma. *Pathology*. 2004;36(2):120–128. doi:10.1080/00313020410001671957

21. Li K, Zhang J, Tian Y, et al. The wnt/beta-catenin/VASP positive feedback loop drives cell proliferation and migration in breast cancer. *Oncogene*. 2020;39(11):2258–2274.

22. Sun J, Su W, Zhao X, et al. LncRNA PPAR interacts with fibrogenic factors to promote survival of fibroblasts through competitively binding to miR-15a. *Biosci Rep*. 2019;39(7). doi:10.1042/BSR20190280

23. Wang Y, Wu S, Zhu X, et al. LncRNA-encoded polypeptide ASRPS inhibits triple-negative breast cancer angiogenesis. *J Exp Med*. 2020;217(3). doi:10.1084/jem.20190950

24. Xu H, Zhao G, Zhang Y, et al. Long non-coding RNA PAXIP1-AS1 facilitates cell invasion and angiogenesis of glioma by recruiting transcription factor ETS1 to upregulate KIF14 expression. *J Exp Clin Cancer Res*. 2019;38(1):486. doi:10.1186/s13046-019-1474-7

25. Zhou K, Zhang C, Yao H, et al. Knockdown of long non-coding RNA NEAT1 inhibits glioma cell migration and invasion via modulation of SOX2 targeted by miR-132. *Mol Cancer*. 2018;17(1):105. doi:10.1186/s12943-018-0849-2

26. Sun Y, Liu J, Chu L, et al. Long noncoding RNA SNHG12 facilitates the tumorigenesis of glioma through miR-101-3p/FOXP1 axis. *Gene*. 2018;676:315–321. doi:10.1016/j.gene.2018.08.034

27. Lei W, Wang ZL, Feng HJ, Lin XD, Li CZ, Fan D. Long non-coding RNA SNHG12 promotes the proliferation and migration of glioma cells by binding to HuR. *Int J Oncol*. 2018;53(3):1374–1384. doi:10.3892/ijo.2018.4478

28. Karim R, Tse G, Putti T, Scolyer R, Lee S. The significance of the wnt pathway in the pathology of human cancers. *Pathology*. 2004;36(2):120–128. doi:10.1080/00313020410001671957

29. Li K, Zhang J, Tian Y, et al. The wnt/beta-catenin/VASP positive feedback loop drives cell proliferation and migration in breast cancer. *Oncogene*. 2020;39(11):2258–2274.

30. Salmeron-Barcenas EG, Illades-Aguirar B, Del Moral-Hernandez O, Ortega-Soto A, Hernandez-Sotelo D. HOTAIR knockdown decreased the activity wnt/beta-catenin signaling pathway and increased the mRNA levels of its negative regulators in HeLa cells. *Cell Physiol Biochem*. 2019;53(6):948–960.

31. Predes D, Oliveira LFS, Ferreira LSS, et al. The chalcone loncho-carpin inhibits wnt/beta-catenin signaling and suppresses colorectal cancer proliferation. *Cancers (Basel)*. 2019;11(12). doi:10.3390/cancers11121968

32. Yue X, Lan F, Yang W, et al. Interruption of beta-catenin suppresses the EGFR pathway by blocking multiple oncogenic targets in human glioma cells. *Brain Res*. 2010;1366:27–37. doi:10.1016/j.brainres.2010.10.032