

LncRNA DCST1-AS1 downregulates miR-29b through methylation in glioblastoma (GBM) to promote cancer cell proliferation

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Abstract

Background: Glioblastoma (GBM) is the most malignant form of brain cancer, owing to the high mortality rate. We in this study analyzed the role of DCST1-AS1 in glioblastoma (GBM).

Methods: QuantiTect Reverse Transcription Kit (QIAGEN) was used, with RNA samples as template to synthesize cDNA.

Results: It is observed that upregulation of DCST1-AS1 in GBM predicted poor survival. MiR-29b was downregulated in GBM and inversely correlated with the expression of DCST1-AS1. In GBM cells, DCST1-AS1 overexpression led to the downregulation of miR-29b and the increased methylation level of miR-29b gene. Cell proliferation analysis showed that DCST1-AS1 overexpression led to increased cell proliferation rate. Moreover, DCST1-AS1 overexpression significantly reversed the inhibitory effects of miR-29b on cancer cell proliferation.

Conclusions: DCST1-AS1 may downregulate miR-29b through methylation in GBM to promote cancer cell proliferation.

Background

Glioblastoma (GBM), also refers to glioblastoma multiforme, is the most malignant form of brain cancer[1]. GBM only affects about 3 per 100,000 adults per year[2]. In spite of the low incidence rate, GBM is considered as a major cause of cancer deaths, owing to the high mortality rate[3, 4]. It has been estimated that only 25% GBM patients can survive longer than 1 year after the initial diagnosis even after active treatment[3, 4]. Therefore, novel therapeutic approaches are needed to improve the survival of GBM patients. However, molecular mechanism of GBM remains hardly known, which challenge the development of more effective therapy [5, 6].

It has been well established that the occurrence and development of GBM requires the involvement of multiple signaling pathways[7, 8]. Importantly, increased understanding of the functions of molecular players in GBM provided novel targets for the development of targeted therapy, which aim to suppress cancer development by regulating cancer-related gene expression[9, 10]. Non-coding RNAs (ncRNAs), such as miRNAs and long (> 200nt) ncRNAs (lncRNAs), have no protein-coding capacity but they can regulate gene expression at multiple levels to participate in cancer biology[11]. In effect, regulating lncRNA expression is a potential target for cancer therapy [12]. However, function of most lncRNAs in cancer biology remains unclear. DCST1-AS1 has been characterized as an oncogenic lncRNA in liver cancer and breast cancer[13–15], while its role in GBM is unknown. Our preliminary RNA-seq analysis revealed the dysregulation of DCST1-AS1 in GBM and its inverse correlation with miR-29b, which plays tumor suppressive roles[16]. This study was therefore carried out to explore the interaction between DCST1-AS1 and miR-29b in GBM.

Methods

Specimen collection

A total of 62 GBM patients including 38 males and 24 females (46 to 67 years, 55.7 ± 5.7 years) were enrolled at xx hospital between July 2016 and July 2018. Ethics Committee of this hospital approved this study before the admission of patients. All GBM patients were newly diagnosed cases and the diagnosis was made based on histopathological examination results. Other severe clinical disorders were excluded from the patients. No therapy was initiated before this study. All patients signed informed consent. Biopsy was performed on all patients to collect paired GBM and non-tumor tissues. All tissue samples were confirmed by histopathological exam. Fresh tissue samples were stored in a liquid nitrogen tank before use.

Treatment and follow-up

All patients were treated with Temozolomide, while the dosage varies according to patients' health and disease conditions. From the day of admission, all patients were followed-up for 12 month through a monthly manner. Survival condition of each patient was recorded. All 62 patients completed the follow-up.

GBM cells

U87 and U251 human GBM cell lines (ATCC) were used in this study. Cell culture medium was composed of 90% DMEM and 10% FBS. Cells were cultivated in a 5% CO₂ incubator at 37°C with 95% humidity.

Transient transfection

Expression vector of DCST1-AS1 was constructed using pcDNA3.1 vector as backbone. Negative control (NC) miRNA and the mimic of miR-29b were the product of Sigma-Aldrich (USA). U87 and U251 cells were transfected with expression vector (10 nM) and/or miRNA (50 nM) using lipofectamine 2000 (Invitrogen). Empty vector- or NC miRNA-transfected cells were NC cells. Control (C) cells were un-transfected cells. Cells were cultivated for 48h before subsequent experiments.

RNA preparations

Paired tissue samples and cells of U87 and U251 cell lines were subjected to total RNA extraction using Ribozol reagent (VWR). RNA precipitation was performed using 85% ethanol to harvest miRNA. Genomic DNA was removed from RNA samples using gDNA eraser (Takara).

RT-qPCR assay

Reverse transcriptions were performed using QuantiTect Reverse Transcription Kit (QIAGEN) with RNA samples as template to synthesize cDNA. With cDNA samples as template, SYBR Green Master Mix (Bio-Rad) was used to perform qPCR reactions with 18S rRNA as endogenous control to measure the levels of

DCST1-AS1 expression. To analyze the expression levels of mature miR-29b, addition of poly (A), miRNA reverse transcriptions and qPCR reactions were performed using All-in-One™ miRNA qRT-PCR reagent kit (GeneCopoeia). Three replicate reactions were included in each experiment and data were normalized using $2^{-\Delta\Delta CT}$ method.

Methylation-specific PCR (MSP)

U87 and U251 cells were subjected to genomic DNA extraction using PureColumn DNA Extraction Kit (BIORON GmbH). DNA Methylation-Gold™ kit (ZYMO RESEARCH) was used to convert DNA samples. Taq 2X Master Mix (NEB) was used to perform MSP to analyze the methylation of miR-29b gene.

CCK-8 assay

U87 and U251 cells were subjected to cell proliferation assay after transfection. A 96-well cell culture plate (3000 cells in 0.1ml culture medium per well) was used to cultivate cells at 37°C. OD values were measured at 450 nm every 24h for a total of 96h. At 4h before measurement of OD values, CCK-8 solution was added into each well to reach the final concentration of 10%.

Statistical analysis

Three replicate reactions were included in each experiment and data were expressed as mean±SD values. Paired t test was used to compare paired tissues. ANOVA Tukey's test was used to compare multiple groups. The 62 GBM patients were divided into high and low DCST1-AS1 level groups (n=31) with the median level of DCST1-AS1 in GBM tissues as cutoff value. Survival curves of both groups were plotted based on follow-up data. Log-rank test was used to compare survival curves. Correlations were analyzed by linear regression. $p < 0.05$ was statistically significant.

Results

Upregulation of DCST1-AS1 in GBM predicted poor survival

To analyze the differential expression of DCST1-AS1 in GBM, the expression levels of DCST1-AS1 in paired GBM and non-tumor tissue samples were measured by performing RT-qPCR. Compared with non-tumor tissues, levels of DCST1-AS1 were significantly higher in GBM tissues (Fig.1A, $p < 0.001$). Survival curves were plotted for both high and low DCST1-AS1 level groups. Compared with low DCST1-AS1 level groups, patients in high DCST1-AS1 level group showed lower overall survival rate (Fig.1B).

MiR-29b was downregulated in GBM and inversely correlated with the expression of DCST1-AS1

To analyze the differential expression of miR-29b in GBM, the expression levels of miR-29b in paired GBM and non-tumor tissue samples were measured by performing RT-qPCR. Compared with non-tumor tissues, levels of miR-29b were significantly lower in GBM tissues (Fig.2A, $p < 0.001$). Correlation analysis showed

that expression levels of DCST1-AS1 and miR-29b were inversely and significantly correlated with each other across GBM tissues (Fig.2B), but not non-tumor tissues (Fig.2C).

DCST1-AS1 overexpression led to the downregulation of miR-29b and the increased methylation level of miR-29b gene

To analyze the interaction between DCST1-AS1 and miR-29b, U87 and U251 cells were transfected with either DCST1-AS1 expression vector or miR-29b mimic, and the overexpression of DCST1-AS1 and miR-29b was confirmed by RT-qPCR (Fig.3A, $p < 0.05$). Compared with C and NC groups, DCST1-AS1 overexpression led to downregulated miR-29b (Fig.3B, $p < 0.05$), while miR-29b overexpression failed to significantly affect DCST1-AS1 (Fig.3C). MSP was performed to analyze the effects of DCST1-AS1 overexpression on the methylation of miR-29b. Compared with cell transfected with empty pcDNA3.1 vector, cells transfected with DCST1-AS1 expression vector showed significantly increased methylation of miR-29b (Fig.3D).

DCST1-AS1 promoted GBM cell proliferation through miR-29b

The effects of DCST1-AS1 and miR-29b on the proliferation of U87 and U251 cells were analyzed by performing cell proliferation assay. Compared with C group, DCST1-AS1 overexpression led to increased cell proliferation rate. Moreover, DCST1-AS1 overexpression significantly reversed the inhibitory effects of miR-29b on cancer cell proliferation (Fig.4, $P < 0.05$).

Discussion

This study analyzed the interaction between DCST1-AS1 and miR-29b in GBM. We found that DCST1-AS1 was upregulated in GBM and predicted poor survival. In addition, DCST1-AS1 may downregulate miR-29b through methylation to promote the proliferation of GBM cells.

The function of DCST1-AS1 has been analyzed in multiple types of cancers, such as liver cancer and breast cancer [13–15]. DCST1-AS1 is overexpressed in liver cancer and it interacts with AKT/mTOR signaling pathway and the axis of miR-1254/FAIM2 to promote the migration and proliferation of GBM cells [13, 14]. In breast cancer DCST1-AS1 is also overexpressed and forms a positive regulation loop with miR-873-5p and MYC to promote cell migration and proliferation [15]. This study is the first to report the upregulation of DCST1-AS1 in GBM. In addition, DCST1-AS1 overexpression led to increased proliferation rate of GBM cells. Therefore, DCST1-AS1 may promote GBM by enhancing cell proliferation.

Compared with other types of cancers, prognosis of GBM is generally poor [3, 4]. In this study we showed that the high level of DCST1-AS1 expression before therapy is closely correlated with the poor survival of GBM patients. Therefore, measuring the expression level of DCST1-AS1 before treatment may assist the prognosis of GBM patients, thereby guiding the determination of treatment approaches and improving patients' survival.

MiR-29b plays tumor suppressive roles in different types of cancers including GBM [16, 17]. In effect, the downregulation of miR-19b may serve as a diagnostic and prognostic marker for GBM[17]. Consistently, our study also reported the downregulation of miR-29b in GBM. In addition, miR-29b overexpression led to decreased proliferation rate of GBM[17]. Interestingly, DCST1-AS1 overexpression led to increased methylation of miR-29b, thereby downregulating miR-29b expression. It is worth noting that DCST1-AS1 and miR-29b were only inversely correlated with each other across GBM tissues not non-tumor tissues. Therefore, the interaction between DCST1-AS1 and miR-29b is likely GBM-specific and certain pathological factors may mediate the interaction between them.

Conclusion

In conclusion, DCST1-AS1 is upregulated in GBM and miR-29b is downregulated in GBM. In addition, DCST1-AS1 may downregulate miR-29b through methylation to promote the proliferation of GBM cells.

Declarations

Authorship change form

Authorship responsibilities we attest that:

1. The manuscript is not currently under consideration, in press, or published elsewhere and the research reported will not be submitted for publication elsewhere until a final decision has been made as to its acceptability by the journal.
2. The manuscript is truthful original work without fabrication, fraud, or plagiarism.
3. I have made an important scientific contribution to the study and am thoroughly familiar with the primary data.

Authors' contributions

Yongjian Zhu: manuscript writing and literature search; Yiqun Yao: data analysis and statistical analysis; Xiao Hu: data collection and study design. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Ethical approval was obtained from the Ethics Committee of Huangshi Central Hospital. All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All patients signed the informed consent.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Figures

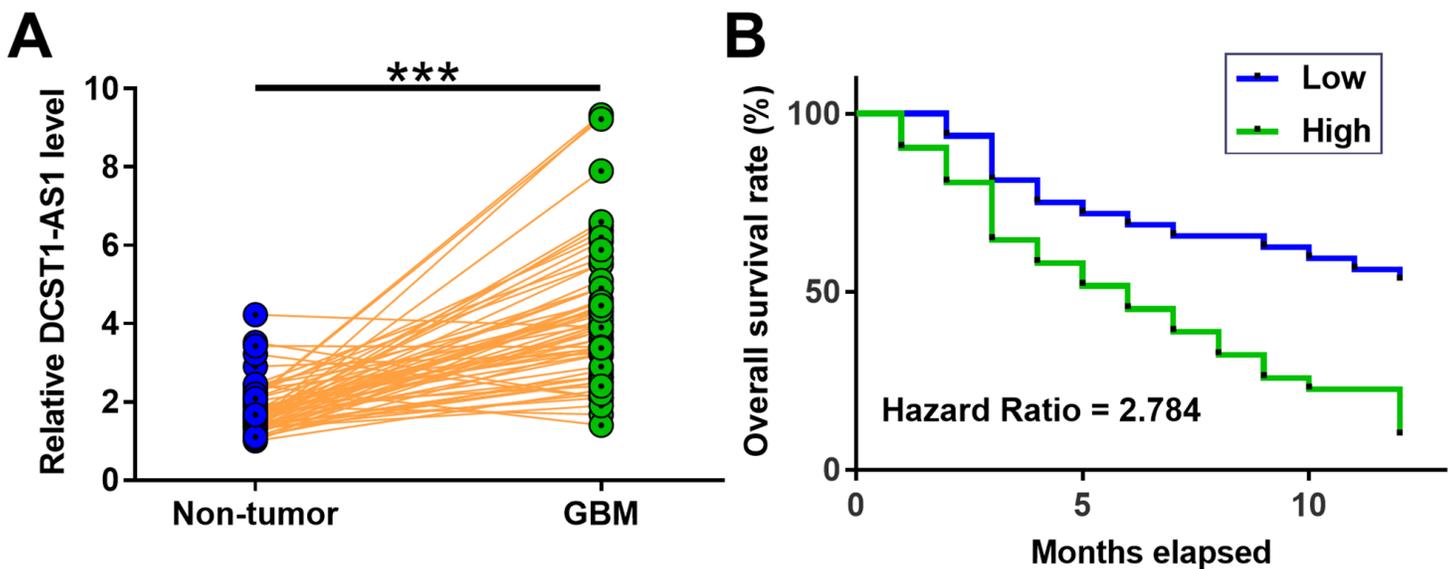


Figure 1

Upregulation of DCST1-AS1 in GBM predicted poor survival To analyze the differential expression of DCST1-AS1 in GBM, the expression levels of DCST1-AS1 in paired GBM and non-tumor tissue samples were measured by performing RT-qPCR. PCR reactions were repeated 3 times and mean values were presented (A), *******, $p < 0.001$. The 62 GBM patients were divided into high and low DCST1-AS1 level groups

(n=31) with the median level of DCST1-AS1 in GBM tissues as cutoff value. Survival curves of both groups were plotted based on follow-up data. Log-rank test was used to compare survival curves (B).

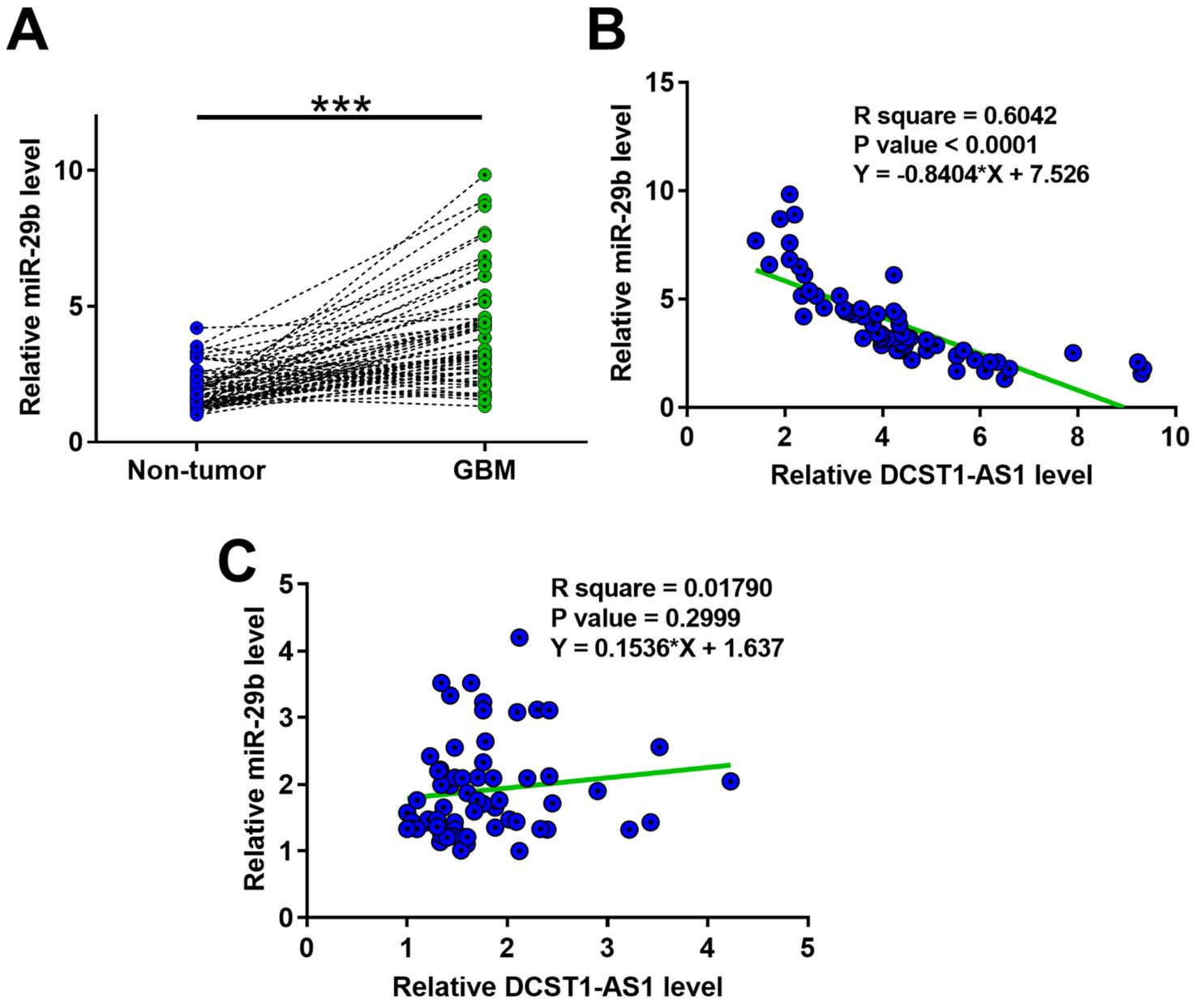


Figure 2

MiR-29b was downregulated in GBM and inversely correlated with the expression of DCST1-AS1. To analyze the differential expression of miR-29b in GBM, the expression levels of miR-29b in paired GBM and non-tumor tissue samples were measured by performing RT-qPCR. PCR reactions were repeated 3 times and mean values were presented (A), $***, p < 0.001$. Linear regression was used to analyze the correlations between expression levels of DCST1-AS1 and miR-29b across GBM tissues (B), but not non-tumor tissues (C).

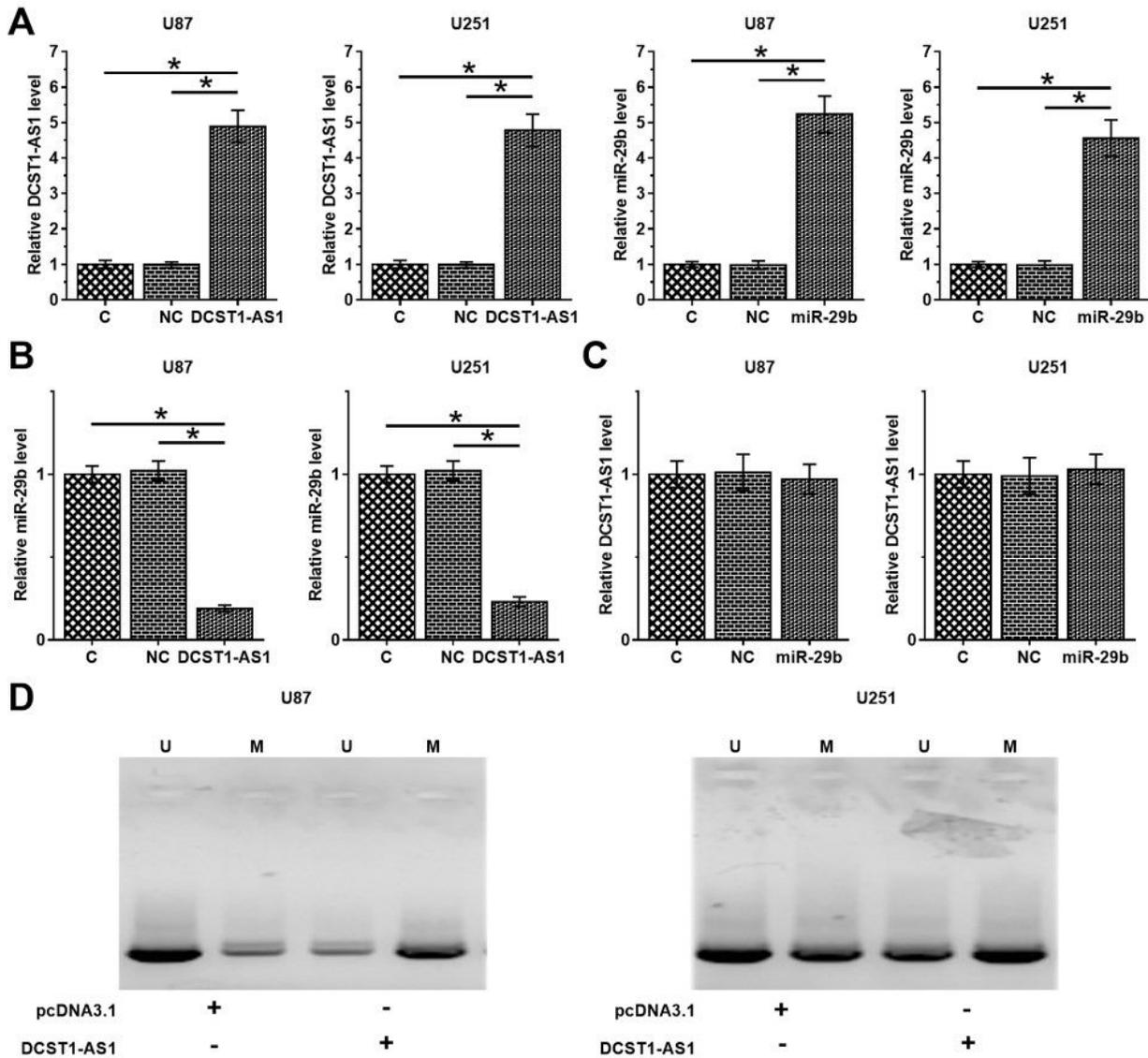


Figure 3

DCST1-AS1 overexpression led to the downregulation of miR-29b and the increased methylation level of miR-29b gene. To analyze the interaction between DCST1-AS1 and miR-29b, U87 and U251 cells were transfected with either DCST1-AS1 expression vector or miR-29b mimic, and the overexpression of DCST1-AS1 and miR-29b was confirmed by RT-qPCR (A). The effect of DCST1-AS1 overexpression on miR-29b (B) and the effects of miR-29b overexpression on DCST1-AS1 (C) were also analyzed by RT-qPCR. MSP was performed to analyze the effects of DCST1-AS1 overexpression on the methylation of miR-29b (D). Experiments were repeated 3 times and mean±SD values were presented. U, unmethylation; M, methylation; *, $p < 0.05$.

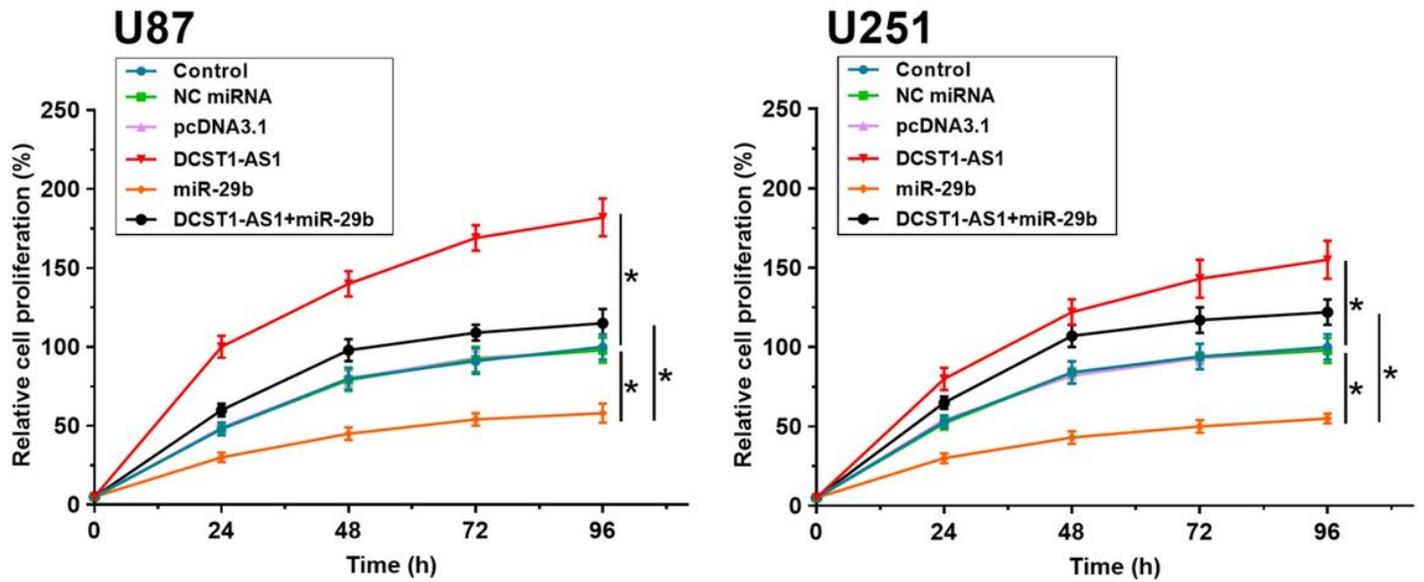


Figure 4

DCST1-AS1 promoted GBM cell proliferation through miR-29b. The effects of DCST1-AS1 and miR-29b on the proliferation of U87 and U251 cells were analyzed by performing cell proliferation assay. Experiments were repeated 3 times and mean±SD values were presented. *, p<0.05.

Supplementary Files

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