LncRNA CRYM-AS1 Inhibits Gastric Cancer Progression via Epigenetically Regulating CRYM

Peipei Zhang
First Affiliated Hospital of Anhui Medical University

Changyu Chen
First Affiliated Hospital of Nanchang University

Jiajia Zhang
First Affiliated Hospital of Anhui Medical University

Xin Yu (✉ yuxinweiike@126.com)
First Affiliated Hospital of Anhui Medical University

Keywords: CRYM-AS1, CRYM, EZH2, gastric cancer

Posted Date: June 11th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-593172/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

**Objective:** To study the role of long non-coding RNA (lncRNA) CRYM-AS1 in human gastric cancer.

**Methods:** Expression levels of CRYM-AS1 in cell lines and clinical tissues were examined by RT-qPCR. The association between CRYM-AS1 levels and clinicopathological parameters / survival rates of gastric cancer patients was analyzed.

Cell functional experiments including MTT assay, glucose consumption / lactate production / ATP production detection were performed to examine the role of CRYM-AS1 in cell aerobic glycolysis and cell proliferation of gastric cancer cells. Subcellular fractionation location detection, western blot, RIP (RNA binding protein immunoprecipitation) assay, CHIP (Chromatin immunoprecipitation) assay and BSP (Bisulfite sequencing PCR) assay were carried out to explore the molecular mechanism of CRYM-AS1 in gastric cancer cells.

**Results:** CRYM-AS1 was low expressed in gastric cancer cells and tissues compared with normal gastric cells and tissues respectively. CRYM-AS1 was negatively correlated with TNM staging, tumor size and overall survival (OS) rate in gastric cancer patients. CRYM-AS1 inhibited gastric cancer cell aerobic glycolysis and cell proliferation. CRYM-AS1 directly bound to EZH2 and mediated the CRYM promoter methylation and consequently negatively regulated the expression of CRYM. Forced expression of CRYM rescued the decreased aerobic glycolysis and cell proliferation induced by CRYM-AS1 in gastric cancer cells.

**Conclusion:** CRYM-AS1 was an important biomarker and could be used for human gastric cancer treatment.

Background

Gastric cancer is one of the most common human malignancies and ranks third in the causes of cancer related death in the world [1, 2]. Early diagnosis developed fast in the past several years, but many patients with gastric cancer were still diagnosed at advanced stage especially in developing countries [3]. The mean survival time for advanced gastric cancer patients is no more than 1 year [4]. Up to date, the radical treatment for gastric cancer is surgical resection; other strategies such as targeted therapy with good curative effect are few [5, 6]. The mechanisms involved in initiation and development of gastric cancer were studied for many years, and many oncogenes, tumor suppressors and non-coding RNAs were revealed to play important roles in gastric cancer. However, the intrinsic mechanisms for gastric cancer still need to be further studied, and finding novel targets which could be potentially used for gastric cancer therapy is urgent.

LncRNAs (long noncoding RNAs) belong to noncoding RNA molecules and are commonly more than 200 nucleotides in length [7, 8]. Many recent studies have reported that lncRNAs participated in many human biological processes including cell proliferation, cell apoptosis, cell metastasis, cell differentiation etc. [9,
LncRNAs were also reported to play important roles in human cancers. For example, IncRNA BCRT1 were reported to promote progression in breast cancer by targeting miR-1303/PTBP3 axis [11]; IncRNA MEG3 suppressed non-small cell lung cancer by interaction with DKC1 [12] etc. For human gastric cancer, IncRNA NEAT1, IncRNA FAM230B, IncRNA HMGAI P4 etc were reported to play promoting roles [13–15], and IncRNA PCAT18, IncRNA CA3-AS1 etc were tumor suppressors in gastric cancer [16, 17]. However, the role of IncRNA CRYM-AS1 in human cancers (including gastric cancer) remains unclear.

Aerobic glycolysis played an important role in cancer cells. Not only provided energy, aerobic glycolysis also created an acidic environment to promote metastasis of cancer cells [18]. A lot of factors including KLF8, GRINA, linc00152 were reported to participate in aerobic glycolysis of gastric cancer cells [19–21]. Herein, we aimed to examine the role of IncRNA CRYM-AS1 in aerobic glycolysis of gastric cancer cells.

In this study, we have examined the expression levels of CRYM-AS1 were low expressed in gastric cancer cells and tissues compared with normal gastric cells and tissues respectively. Low expression level of CRYM-AS1 was associated with poor pathological features and OS rates in gastric cancer patients. Forced expression of CRYM-AS1 inhibited gastric cancer cell aerobic glycolysis and cell proliferation. CRYM-AS1 directly bound to EZH2 and mediated the CRYM promoter methylation and consequently negatively regulated the expression of CRYM. The suppressing role of CRYM-AS1 in cell glycolysis and proliferation of gastric cancer cells was mediated by CRYM. Therefore, IncRNA CRYM-AS1 was an important biomarker in human gastric cancer and could be used as a potential target for gastric cancer treatment.

**Methods**

**Cell culture**

Normal gastric cell line GSE-1 and gastric cancer cell lines MKN87, SUN16, SGC7901, AGS, MKN45, BGC823 used in this study were all from ATCC (the American Type Culture Collection) (Rockville, MD). Cells were cultured under 5% CO\(_2\) and 37°C as recommended.

**Tissue Samples**

For clinical tissue study, 60 normal gastric tissues and 60 gastric cancer tissues were collected from patients who underwent surgery in the First Affiliated Hospital of Anhui Medical University (Hefei, Anhui, China) during 2012–2013. Pathological features including Age, Gender, TNM staging, Tumor size, Histological differentiation and Lymphovascular invasion of the 60 gastric cancer patients were collected and these patients were followed up for more than 80 months and their OS rates were collected. Approval from the Institutional Review Boards of Anhui Medical University was got before this study was performed, and this work was carried out according to The Code of Ethics of the World Medical Association (Declaration of Helsinki). Every patient involved in this study had signed the informed consents.
Rt-qpcr

In this study, RT-qPCR was performed to evaluate expression levels of CRYM-AS1, CRYM mRNA and CRYM promoter enrichment in cells and tissues essentially as described in former study using a SYBR Premix Ex Taq kit (Takara, Dalian, China) [22]. GAPDH was detected as control. The primers used were as follows: CRYM-AS1 forward, 5’ ATAGAGCCCAAGAACAATA 3’; CRYM-AS1 reverse, 5’ AAGAAGCACAACCAGATAG 3’; CRYM forward, 5’ TAATGGCTGTAGGCCTGGAC 3’; CRYM reverse, 5’ GAACAGCTGAGTTCTTGCC 3’; GAPDH forward, 5’TTTGCTCAAGCTCATTCTG-3’; and GAPDH reverse, 5’-TGGTCCAGGGTTTCTTACTC-3’.

Plasmids Construction And Transfection

CRYM-AS1 sequence and CRYM encoding sequence were cloned into plasmid pcDNA3.1 (Invitrogen) (demonstrated as CRYM-AS1-OE and CRYM-OE respectively) for CRYM-AS1 or CRYM overexpression, and the vector plasmid pcDNA3.1 (demonstrated as Vector) was used as control. Plasmid transfection was performed using Lip3000 (Invitrogen) as recommended.

Rna Oligonucleotides And Transfection

In this study, shCRYM-AS1-1 and shCRYM-AS1-2 were used for CRYM-AS1 depletion. Negative control shRNA was designated as shCtrl. These shRNAs were synthesized from GenePharma (Shanghai, China). The RNA oligonucleotide transfection was performed using Lip3000 (Invitrogen) as recommended. The sequences of these shRNAs were as follows: shCRYM-AS1-1, F: cccg GAATATGAAGTTCCCAGTAATgatatccATTACTGGGAACCTTCATATTtttt g, R: aattcaaaaaGAATATGAAGTTCCCAGTAATgatatccATTACTGGGAACCTTCATATTCC. shCRYM-AS1-2, F: cccgCAGGGGTGTGTGATTTTGTGCggatccGCACAA AATCACAACCCCTGttttg, R: aattcaaaaaCAGGGGTGTGTGATTTTGTGCgg atccGCACAAAATCACAACCCCTG.

Western Blot

Western blot

Western blot was performed to examined protein levels of CRYM, which was carried out as described in former study [22]. CRYM Rabbit Polyclonal antibody (1:1000, 12495-1-AP, Proteintech) and Actin Mouse Monoclonal antibody (1:10000, 66009-1-lg, Proteintech) were used. Actin was examined as a negative control.

Glycolysis Analysis

We collected the supernatants of the cells for glucose consumption, lactate production and ATP production detection. D-Glucose kit, L-Lactic acid kit, ATP kit (Invitrogen) were used respectively
essentially as described in previous study [21].

**Mtt Assay**

Cell viabilities were examined by MTT assay as described in former study [23]. Briefly, 24 hours after transfection, cells were seeded into 96-well plates (5000 cells per well), and MTT detection was performed at 24, 48 and 72 hours.

**Subcellular Fractionation Location Detection**

In the study, the PARIS Kit (Life Technologies, Carlsbad, CA, USA) was used to separated nuclear and cytosolic fractions from BGC823 and MKN45 cells as described earlier [22]. The expression levels of CRYM-AS1 were determined by RT-qPCR. GAPDH and U6 were used as controls.

**Rip (Rna Binding Protein Immunoprecipitation) Assay**

According to former study, the EZ-Magna RIP kit (Millipore, China) was used for RIP assay [22]. Antibody against EZH2 (Abcam, Shanghai, China) and control IgG were used to capture immunoprecipitated RNAs. The captured RNAs were detected by RT-qPCR.

**Chip (Chromatin Immunoprecipitation) Assay**

CHIP assay was carried out using an EZ-Magna ChIP TMA Chromatin Immunoprecipitation Kit (Millipore, Billerica, MA, USA) as described in former study [22]. Antibody against EZH2 (Abcam, Shanghai, China) and control IgG were used. The captured chromatin DNAs were detected by qPCR.

**Bsp (Bisulfite Sequencing Pcr) Assay**

The EpiTect. Bisulite Kit (Qiagen) was used for BSP assay as described earlier [22]. The methylation of CRYM promoter was analyzed.

**Statistical analysis**

In this study, at least 3 independent repeats were performed for each experiment, and the figures were the average results. For RT-qPCR, Glycolysis analysis, MTT assay, RIP assay, CHIP assay and BSP assay, 2-tailed t test was used for statistical analysis. Kaplan-Meier curves were made and log-rank test was used to analyze OS in patients. The correlation analysis between CRYM-AS1 expression and patient pathological features was performed using Pearson's chi-square test. Pearson's coefficient was analyzed.
to determine the correlation between CRYM-AS1 and CRYM mRNA expression in patient tissues. \( P < 0.05 \) was considered to be statistically significant.

**Results**

**CRYM-AS1 was low expressed in gastric cancer and was associated with better pathological features and survival rates in gastric cancer patients.**

In this study, the expression levels of CRYM-AS1 in normal gastric cell line GSE-1 and gastric cancer cell lines MKN87, SUN16, SGC7901, AGS, MKN45, BGC823 were examined by RT-qPCR. As shown in Fig. 1A, the expression levels of CRYM-AS1 were extremely lower in gastric cancer cells compared with normal gastric cell GSE-1 (all \( P < 0.05 \)). For further study, 60 normal gastric tissues and 60 gastric cancer tissues from patients were collected, and the expression levels of CRYM-AS1 in these tissues were examined. Concordantly, the expression levels of CRYM-AS1 were much lower in gastric cancer tissues compared with normal gastric tissues (\( P < 0.05 \)).

Next, the 60 gastric cancer tissues were divided into CRYM-AS1 low group and CRYM-AS1 high group, each group contained 30 tissues. The pathological features (including Age, Gender, TNM staging, Tumor size, Histological differentiation and Lymphoyascular invasion) of these 60 gastric cancer patients were collected and the correlation of CRYM-AS1 levels with pathological features were analyzed. As shown in Table 1, low level of CRYM-AS1 was significantly correlated with higher TNM staging (\( P = 0.0159 \)) and bigger Tumor size (\( P = 0.0201 \)). However, there were no statistical correlations between CRYM-AS1 levels and patient Age, Gender, Histological differentiation or Lymphoyascular invasion (all \( P > 0.05 \)).
Table 1
Association between CRYM-AS1 expression and clinicopathological factors in 60 gastric cancer patients

<table>
<thead>
<tr>
<th>Expression of CRYM-AS1</th>
<th>Variables Low (%)</th>
<th>High (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.9214</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 60</td>
<td>13(44.83%)</td>
<td>16(55.17%)</td>
<td></td>
</tr>
<tr>
<td>≥ 60</td>
<td>12(46.15%)</td>
<td>14(53.85%)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>0.4321</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>16(45.71%)</td>
<td>19(54.29%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>14(56.00%)</td>
<td>11(44.00%)</td>
<td></td>
</tr>
<tr>
<td>TNM staging</td>
<td>0.0195*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>9(33.33%)</td>
<td>18(66.67%)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>21(63.64%)</td>
<td>12(36.36%)</td>
<td></td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td>0.0201*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5</td>
<td>11(35.48%)</td>
<td>20(64.52%)</td>
<td></td>
</tr>
<tr>
<td>≥ 5</td>
<td>19(65.52%)</td>
<td>10(34.48%)</td>
<td></td>
</tr>
<tr>
<td>Histological differentiation</td>
<td>0.1652</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>7(36.84%)</td>
<td>12(63.16%)</td>
<td></td>
</tr>
<tr>
<td>Moderate/Poor</td>
<td>23(56.10%)</td>
<td>18(43.90%)</td>
<td></td>
</tr>
<tr>
<td>Lymphovascular invasion</td>
<td>0.4383</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>17(54.84%)</td>
<td>14(45.16%)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>13(44.83%)</td>
<td>16 (55.17%)</td>
<td></td>
</tr>
</tbody>
</table>

For further study, these 60 gastric cancer patients were followed up for more than 80 months and their OS rates were collected. Analyzed by Kaplan-Meier curves, the OS rates were dramatically lower in gastric cancer patients with low level of CRYM-AS1 compared with patients with high level of CRYM-AS1 ($P=0.0407$).

Therefore, CRYM-AS1 was low expressed in gastric cancer cells and tissues; high level of CRYM-AS1 was associated with better pathological features and overall survival rates in gastric cancer patients.

**CRYM-AS1 inhibited gastric cancer cell aerobic glycolysis and cell proliferation.**
To examine the role of CRYM-AS1 in gastric cancer cells, two gastric cancer cell lines BGC823 and MKN45 with low base level of CRYM-AS1 were selected for CRYM-AS1 overexpression. As shown in Fig. 2A, compared with Vector control, the expression levels of CRYM-AS1 in both BGC823 and AGS cells increased dramatically after transfected with CRYM-AS1 overexpression plasmids. D-Glucose kit and L-Lactic acid kit were used to examine the concentration of glucose and lactic acid in the culture supernatant of the transfected cells respectively. Forced expression of CRYM-AS1 dramatically decreased glucose consumption in both BGC823 and AGS cells (Fig. 2B). Concordantly, remarkably decreased lactate production was observed in both BGC823 and AGS cells with forced expression of CRYM-AS1 (Fig. 2C). In addition, BGC823 and AGS cells with forced expression of CRYM-AS1 showed decreased ATP production levels compared with control respectively (Fig. 2D). To evaluate cell viabilities with forced expression of CRYM-AS1, MTT assay was performed. As shown in Fig. 2E and 2F, BGC823 and AGS cells with high level of CRYM-AS1 showed significant decreased cell viabilities during a period of 72 hours compared with control respectively. Therefore, CRYM-AS1 inhibited cell aerobic glycolysis and cell proliferation in gastric cancer cells.

**CRYM-AS1 regulated the expression of CRYM in gastric cancer.**

For further study, the mRNA levels of CRYM were examined in both BGC823 and MKN45 cells after transfected with CRYM-AS1 overexpression plasmids. As shown in Fig. 3A and 3B, the expression levels of CRYM-AS1 dramatically increased in both BGC823 and MKN45 cells after transfected with CRYM-AS1 overexpression plasmids compared with Vector control, and the mRNA levels of CRYM remarkably decreased in BGC823 and MKN45 cells with CRYM-AS1 overexpression. Concordantly, shCRYM-AS1-1 and shCRYM-AS1-2 significantly decreased the CRYM-AS1 levels in BGC823 and MKN45 cells, and the mRNA levels of CRYM increased in both BGC823 and MKN45 cells after transfected with shCRYM-AS1-1 or shCRYM-AS1-2 compared with shControl (Fig. 3C, 3D). Moreover, forced expression of CRYM-AS1 decreased the protein levels of CRYM and depletion of CRYM-AS1 increased the protein levels of CRYM in both BGC823 and MKN45 cells as determined by western blot (Fig. 3E, 3F). In addition, the mRNA levels of CRYM in the 60 normal gastric tissues and 60 gastric cancer tissues were also detected. As shown in Fig. 3G, the mRNA levels of CRYM in gastric cancer tissues were significantly higher than that in normal gastric tissues. Moreover, the correlation of CRYM-AS1 and CRYM mRNA levels in the 60 gastric cancer tissues were determined by Pearson analysis. As shown in Fig. 3H, there was a statistically negative correlation between CRYM-AS1 and CRYM mRNA ($P = 0.0342$) (Pearson’s correlation coefficient was $-0.2739$). Therefore, CRYM-AS1 negatively regulated the expression of CRYM in gastric cancer.

**CRYM-AS1 suppressed CRYM by binding to EZH2 and mediating the CRYM promoter methylation.**

For further study, the exact mechanisms of CRYM-AS1 to regulate CRYM were explored. As reported previously, lncRNAs could bind to various catalytic components (including EZH2, LSD1, etc.) of polycomb repressive complex 2 (PRC2), which catalyze the methylation of lysine residue of histone 3 [22]. As determined by subcellular fractionation location assay, in both BGC823 and MKN45 cells, CRYM-AS1 mainly located in nuclear, suggesting that CRYM-AS1 could regulate target genes at transcriptional level
rather than translational level (Fig. 4A, 4B). Next, RIP assay and CHIP assay were carried out to detect the binding activities between CRYM-AS1 and potential gene sequences. As shown in Fig. 4C and 4D, an antibody against EZH2 enriched significantly more CRYM-AS1 and CRYM promoter sequence compared with IgG control antibody in both BGC823 and MKN45 cells. Moreover, antibody against EZH2 enriched much more CRYM promoter sequence in both BGC823 and MKN45 cells after transfected with CRYM-AS1 overexpression plasmids compared with control respectively (Fig. 4E, F). Furthermore, as determined by BSP assay, the methylation levels of CRYM promoter region were much more in both BGC823 and MKN45 cells after transfected with CRYM-AS1 overexpression plasmids compared with control (Fig. 4G). Therefore, CRYM-AS1 could directly bind with EZH2, EZH2 directly bound with CRYM promoter region, and EZH2 promoted the methylation level of CRYM promoter, consequently suppressed the expression of CRYM.

**CRYM mediated the suppressing role of CRYM-AS1 in cell aerobic glycolysis and proliferation of gastric cancer cells.**

To examine whether CRYM-AS1 suppressed cell aerobic glycolysis and proliferation in gastric cancer cells was mediated by CRYM, BGC823 and MKN45 cells were co-transfected with CRYM-AS1 overexpression plasmids and CRYM overexpression plasmids. As shown in Fig. 5A and 5B, both mRNA and protein levels of CRYM decreased after transfected with CRYM-AS1 overexpression plasmids, and these decreases were rescued by co-transfection with CRYM-AS1 overexpression plasmids and CRYM overexpression plasmids in BGC823 and MKN45 cells. Concordant with former results, BGC823 and MKN45 cells with forced expression of CRYM-AS1 and decreased levels of CRYM showed decreased glucose consumption, decreased lactate production levels, decreased ATP production levels and decreased cell viabilities during a period of 72 hours. However, these decreases were abrogated by co-transfection with CRYM-AS1 overexpression plasmids and CRYM overexpression plasmids (Fig. 5C-G). Therefore, the suppressing role of CRYM-AS1 in cell aerobic glycolysis and proliferation of gastric cancer cells was mediated by CRYM.

**Discussion**

In the present study, we have examined the role of IncRNA CRYM-AS1 in human gastric cancer. CRYM-AS1 was low expressed in gastric cancer cells and tissues compared with normal gastric cells or tissues. Low level of CRYM-AS1 was associated with higher TNM staging, bigger tumor size and low OS rates in gastric cancer patients. CRYM-AS1 inhibited aerobic glycolysis and cell proliferation in gastric cancer cells as measured by glucose consumption, lactate production, ATP production examination and MTT assay. CRYM was determined to be negatively regulated by CRYM-AS1. CRYM and CRYM-AS1 were negatively correlated in 60 gastric cancer tissues as analyzed by Pearson analysis. RIP and CHIP assay demonstrated that CRYM-AS1 directly bound with EZH2, and EZH2 directly bound with CRYM promoter region. CRYM-AS1 but also promoted the methylation of CRYM promoter. Rescue experiments showed the suppressing role of CRYM-AS1 in cell aerobic glycolysis and proliferation of gastric cancer cells was mediated by CRYM. Herein we for the first time reported the role of IncRNA CRYM-AS1 in human cancers.
(gastric cancer). That would be a benefit for better understanding the mechanism involved in human gastric cancer.

CRYM was examined to be down-regulated by CRYM-AS1, and CRYM mediated the role of CRYM-AS1 in cell aerobic glycolysis and proliferation of gastric cancer cells. As reported previously, CRYM was overexpressed in leiomyosarcoma metastases [24]. CRYM was regulated by androgens, and the expression levels of CRYM was elevated in prostate cancer tissues and CRYM decreased after hormonal therapy [25]. In our study, we have identified that the expression levels of CRYM were higher in gastric cancer tissues compared with normal gastric tissues, and forced expression of CRYM rescued the decreased aerobic glycolysis and cell proliferation induced by CRYM-AS1 in gastric cancer cells. These results were concordant. Therefore, CRYM might play oncogenic roles in several human cancers especially in gastric cancer.

To explore the mechanism involved in the regulation of CRYM by CRYM-AS1, subcellular fractionation location assay was performed and CRYM-AS1 was identified to mainly located at nuclear, suggesting the regulation mechanism of CRYM-AS1 might be at transcriptional level. As known before, the main mechanisms involved in lncRNAs regulating target genes included modulating transcription, epigenetic modification, and acting as competing endogenous RNAs (ceRNAs) [22, 26]. As reported previously, PRC2 (polycomb repressive complex 2) could be recruited by lncRNAs, participating the regulation of target genes of lncRNAs [22, 27]. EZH2 was an important component of PRC2, which could suppress gene transcription by trimethylation of lysine 27 on histone H3 (H3K27) in the promoter region [22, 28]. Herein, we have examined that CRYM-AS1 directly bound to EZH2, EZH2 directly bound to CRYM promoter region, and EZH2 promoted the methylation of CRYM promoter. Therefore, EZH2 mediated the regulation of CRYM by CRYM-AS1 in gastric cancer cells through promoter methylation. As reported previously, lncRNA HOXB13-AS1 regulated HOXB13 gene methylation by interacting with EZH2 in glioma [22]; EZH2 participated the cMyc promoter methylation involved in the hepatitis B viruspositive liver cancer progression promotion by lncRNA PVT1 [29]; lncRNA SNHG14 promoted colorectal cancer metastasis through targeting EPHA7 mediated by EZH2 [30]; etc. Therefore, EZH2 was an important factor which mediated the regulation of target genes by lncRNAs and participated in tumor behaviors. In the present study, we demonstrated the mechanism of the regulation of neighboring gene CRYM by CRYM-AS1 was mediated by EZH2 and CRYM promoter methylation.

**Conclusion**

We have examined the aerobic glycolysis and cell proliferation suppressing role of lncRNA CRYM-AS1 in human gastric cancer cells. The neighboring gene CRYM was negatively regulated by CRYM-AS1 and mediated the role of CRYM-AS1 in gastric cancer cells. Promoter methylation mediated by EZH2 contributed to the CRYM-AS1/CRYM regulation. CRYM-AS1 was positively correlated with good features and OS rates in gastric cancer patients. Potential drugs based on CRYM-AS1 could be used for gastric cancer.
Declarations

Acknowledgements

Not applicable

Authors’ contributions

Xin Yu and Peipei Zhang designed experiments. Peipei Zhang and Changyu Chen performed experiments. Jiajia Zhang analyzed data. Peipei Zhang and Xin Yu wrote the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by Youth Cultivation Program of the First Affiliated Hospital of Anhui Medical University (2869)·PhD Startup Fund of the First Affiliated Hospital of Anhui Medical University (1281).

Availability of data and materials

This was not applicable to this manuscript.

Ethics approval and consent to participate

The present study was approved by Institutional Review Boards of Anhui Medical University. All participants signed informed consent before admission.

Consent for publication

Consent for publication was obtained from all participants.

Competing interests

The authors declare that they have no competing interests.

References


Figures

**Figure 1**

CRYM-AS1 was low expressed in gastric cancer and was associated with higher survival rates in gastric cancer patients. (A) Expression levels of CRYM-AS1 in normal gastric cell GSE-1 and gastric cancer cells MKN87, SUN16, SGC7901, AGS, MKN45, BGC823 were examined by RT-qPCR. (B) Expression levels of CRYM-AS1 in 60 normal gastric tissues and 60 gastric cancer tissues were examined by RT-qPCR. GAPDH
was detected as control for RT-qPCR. (C) OS rates between high CRYM-AS1 group and low CRYM-AS1 group in 60 gastric cancer patients were analyzed by Kaplan-Meier curves. *, P<0.05; **, P<0.01.

Figure 2

CRYM-AS1 inhibited gastric cancer cell aerobic glycolysis and cell proliferation. BGC823 and MKN45 cells were transfected with CRYM-AS1 overexpressing plasmid (designated as CRYM-AS1-OE) or control vector plasmid (designated as Vector). (A) Expression levels of CRYM-AS1 were examined by RT-qPCR; (B)
Glucose consumption was measured; (C) Lactate production was examined; (D) ATP production was measured; (E and F) Cell viabilities during a period of 72 hours were examined by MTT assay. *, P<0.05; **, P<0.01; ***, P<0.001.

**Figure 3**

CRYM-AS1 regulated the expression of CRYM in gastric cancer. (A and B) BGC823 and MKN45 cells were transfected with CRYM-AS1 overexpressing plasmid or control vector, the expression levels of CRYM-AS1
and CRYM mRNA were examined by RT-qPCR. (C and D) BGC823 and MKN45 cells were transfected with CRYM-AS1-shRNAs (designated as shCRYM-AS1-1 and shCRYM-AS1-2) or control-shRNA (designated as shCtrl), the expression levels of CRYM-AS1 and CRYM mRNA were examined by RT-qPCR. (E) Protein levels of CRYM were detected by western blot in BGC823 and MKN45 cells after transfected with CRYM-AS1-OE or Vector. (F) Protein levels of CRYM were detected by western blot in BGC823 and MKN45 cells after transfected with shCRYM-AS1-1, shCRYM-AS1-2 or shCtrl. For western blot, Actin was examined as control. (G) The expression levels of CRYM mRNA in the 60 normal gastric tissues and 60 gastric cancer tissues were examined by RT-qPCR. (H) Pearson correlation analysis between CRYM-AS1 and CRYM mRNA levels in the 60 gastric cancer tissues. *, P<0.05; **, P<0.01.

Figure 4

CRYM-AS1 suppressed CRYM by binding to EZH2 and mediating the CRYM promoter methylation. (A and B) Location of CRYM-AS1 in BGC823 and MKN-45 cells were determined by nuclear and cytoplasmic separation assay. CRYM-AS1 was examined by RT-qPCR. U6 and GAPDH were also detected as controls. (C) RIP assay. CRYM-AS1 enrichment by anti-EZH2 antibody or control IgG in BGC823 and MKN45 cells was examined by RT-qPCR. (D) CHIP assay. CRYM promoter enrichment by anti-EZH2 antibody or control...
IgG in BGC823 and MKN45 cells was examined by RT-qPCR. (E and F) CHIP assay. CRYM promoter enrichment by anti-EZH2 antibody or control IgG in BGC823 and MKN45 cells transfected with CRYM-AS1-OE or Vector was examined by RT-qPCR. (G) Methylation of CRYM promoter in BGC823 and MKN45 cells transfected with CRYM-AS1-OE or Vector was detected by BSP assay. *, P<0.05; **, P<0.01.

Figure 5

CRYM mediated the suppressing role of CRYM-AS1 in cell aerobic glycolysis and proliferation of gastric cancer cells. BGC823 and MKN45 cells were co-transfected with CRYM-AS1-OE / Vector and CRYM overexpressing plasmid (CRYM-OE) / Vector control (Ctrl). (A) mRNA levels of CRYM were examined by RT-qPCR. (B) Protein levels of CRYM were detected by western blot. Actin was determined as control. (C) Glucose consumption, (D) Lactate production, (E) ATP production and (F and G) cell viabilities during a period of 72 hours (MTT assay) were examined. *, P<0.05; **, P<0.01; ***, P<0.001.