

Long Non-Coding RNA Myosin Light Chain Kinase Antisense I Plays an Oncogenic Role in Gallbladder Carcinoma by Promoting Chemoresistance and Proliferation

Yongliang Li^{1,*}

Mi Tian^{2,*}

Dongqing Zhang¹

Yifei Zhuang¹

Zhimin Li¹

Shenqi Xie¹

Keyu Sun¹

¹Department of Emergency, Minhang Hospital, Fudan University, Shanghai, 200040, People's Republic of China;

²Department of Intensive Care Unit, Huashan Hospital, Fudan University, Shanghai, 200040, People's Republic of China

*These authors contributed equally to this work

Background: Long non-coding RNAs (lncRNAs) have been reported to play critical roles in human tumours, including gallbladder carcinoma (GBC). However, their biological functions and molecular mechanisms in tumorigenesis and progression remain largely unknown.

Methods: Quantitative polymerase chain reaction (qPCR) was used to verify the expression of lncRNA myosin light chain kinase antisense RNA 1 (MYLK-AS1) in 120 pairs of GBC tissues and paired adjacent non-tumour tissues, as well as in six different GBC cell lines (NOZ, EH-GB1, OCUG-1, GBC-SD, SGC-996 and QBC-939). Cell counting kit 8 was applied to explore cell proliferation and drug sensitivity assays. The target miRNAs (miR) of MYLK-AS1 and downstream target genes were predicted using Starbase 3.0 software and confirmed by double luciferase reporting test. The expression of proteins was assessed using Western blot assay.

Results: Here, we demonstrated that MYLK-AS1 was significantly upregulated and correlated with a poor prognosis and poor clinical characteristics in GBC. Furthermore, the forced expression of MYLK-AS1 significantly promoted GBC cell proliferation and resistance to gemcitabine in vitro. Mechanistically, MYLK-AS1 functioned as an efficient miR-217 sponge, thereby releasing the inhibition of enhancer of zeste 2 polycomb repressive complex 2 (EZH2) subunit expression. MYLK-AS1 promoted GBC cell proliferation and resistance to gemcitabine by upregulating EZH2 expression, and EZH2 was confirmed as a direct target of miR-217.

Discussion: Our results confirmed that the chemoresistant driver MYLK-AS1 might be a promising candidate as a therapeutic target for the treatment of advanced GBC.

Keywords: chemoresistance, GBC, lncRNA, proliferation

Introduction

Gallbladder carcinoma (GBC) has a poor prognosis due to local recurrence, metastasis, and resistance to targeted therapies.¹ Gemcitabine is widely used as the first-line treatment in locally advanced and metastatic GBC.² However, patient prognosis is usually limited by natural and acquired chemotherapy resistance. Thus, it is critical to identify potential chemotherapy resistance molecular mechanisms to restore GBC cell sensitivity to gemcitabine.

Long non-coding RNAs (lncRNAs) are a class of non-coding RNAs characterized as transcripts of more than 200 nucleotides that do not have protein encoding

Correspondence: Keyu Sun
Department of Emergency, Minhang Hospital, Fudan University, Shanghai, 200040, People's Republic of China
Tel +86 18930536861
Email hoceima@126.com

functions.³ Recently, an increasing number of studies have reported that the dysregulation of lncRNAs participates in regulating the tumorigenesis, progression, and chemoresistance of human tumours.⁴ For example, lncRNA small Cajal body-specific RNA 2 (SCARNA2) promotes chemotherapy resistance by competitively binding to miR-342-3p to facilitate epidermal growth factor receptor (EGFR) and B-cell lymphoma 2 (BCL2) expression in colorectal cancer cells.⁵ Recently, several studies have reported that lncRNA myosin light chain kinase antisense RNA 1 (MYLK-AS1) expression is increased in several malignant tumours and functions as an oncogene in several types of cancer, including gastric cancer, neuroblastoma, and hepatocellular carcinoma.⁶⁻⁸ However, the clinical relevance or biological functions of MYLK-AS1 in GBC are currently unknown.

The forced expression of enhancer of zeste homologue 2 (EZH2) has been observed in numerous tumours, including GBC.⁹⁻¹² EZH2 plays a critical role in the physiological and pathological processes of cells, including cancer cell proliferation, invasion, metastasis, angiogenesis, and chemotherapy resistance.¹³ In this study, we found that the expression of MYLK-AS1 was upregulated in GBC tissues compared with adjacent non-tumour tissues. The forced expression of MYLK-AS1 predicted a worse prognosis in patients with GBC. MYLK-AS1 promoted proliferation and conferred resistance to gemcitabine by upregulating the expression of EZH2 by sponging miR-217 in GBC cells. Thus, our results revealed a novel role for the MYLK-AS1/miR-217/EZH2 axis in regulating the proliferation and chemotherapy resistance of GBC cells.

Materials and Methods

Cell Culture and Transfection

Human GBC cell lines (NOZ, EH-GB1, OCUG-1, GBC-SD, SGC-996 and QBC-939), an immortalized human non-tumorigenic biliary epithelial cell line (H69) and the HEK-293T cell line were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and cultured in optimal culture medium containing 10% foetal bovine serum (FBS, Gibco, USA). All of the cells were cultured in a humidified incubator containing 5% CO₂ at 37 °C. The microRNA (miRNA) mimics, miRNA inhibitor, and negative control utilized for transfection were purchased from GenePharma (Shanghai, China). MYLK-AS1 overexpression and short hairpin RNA adenoviruses were purchased from Genomeditech (Shanghai, China),

and the transfection procedure was performed in accordance with the manufacturer's protocol. The EZH2 plasmid was purchased from GenePharma (Shanghai, China) and transfected via Lipofectamine 2000 Reagent (Invitrogen) in accordance with the manufacturer's protocol.

Human Tissue Samples

GBC tissues and adjacent non-tumour tissues were obtained from patients who were diagnosed and underwent surgery in Minhang Hospital Affiliated to Fudan University and stored in liquid nitrogen and formalin. This study was approved by the Joint Ethics Committee of Minhang Hospital Affiliated to Fudan University, and written informed consent was obtained from all the patients. Experiments were conducted in accordance with the Declaration of Helsinki.

RNA Isolation and Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA was extracted from tissues and cells with TRIzol reagent (Invitrogen) according to the manufacturer's protocol. To detect MYLK-AS1 and EZH2 mRNA expression, the PrimeScript RT Reagent Kit (TaKaRa) and SYBR Premix Ex Taq II Kit (TaKaRa) were used according to the manufacturer's protocol. GAPDH was used as an internal control. To detect miR-217 expression, the Mir-X™ miRNA First Strand Synthesis Kit and Mir-X™ miRNA RT-qPCR SYBR® Kit were used according to the manufacturer's protocol. U6 was used as an internal control. The primers used, synthesized by BGI Co. Ltd., are listed as follows: MYLK-AS1-F: CCTAGAATCCAGTGTCTGCC; MYLK-AS1-R: CTTCGACGACGAGGCATTCG; EZH2-F: ATC CTGACTTCTGTGAGCTC; EZH2-R: TCATCTCCCAT ATAAGGAAT-G; GAPDH-F: GTACGTCGTGGAGTCC ACTG; and GAPDH-R: CTTCCACGATACC-AAAG TTG. The relative expression was analysed using the 2^{-ΔΔCt} method.

Proliferation and Drug Sensitivity Assays

Cell proliferation and drug sensitivity were carried out using the Cell Counting Kit-8 (CCK-8) (Beyotime Biotechnology) according to a previously published reference.⁵ For the proliferation assay, 500 tumour cells were cultured in 96-well plates for 24, 48, 72, 96, and

120 h. Then, the CCK-8 solution (Dojindo) was added to the cells and cultured in a humidified incubator containing 5% CO₂ at 37 °C. After 2 h, the optical density (OD) was measured at 450 nm. For the drug sensitivity assay, 2000 tumour cells were cultured in 96-well plates for 24 h. Then, the cultured medium containing different concentrations of the chemotherapy agent was added to the cells, which were then cultured in a humidified incubator containing 5% CO₂ at 37 °C. After 2 h, the optical density (OD) was measured at 450 nm, and the IC₅₀ was calculated.

Prediction of MYLK-AS1 and EZH2 mRNA Binding to miR-217 and Luciferase Reporter Assays

StarBase V3.0 was used to predict that MiR-217 could bind to MYLK-AS1 and EZH2 mRNA. Luciferase reporter assays were performed as described in

a previously published reference.¹⁴ HEK-293T cells were seeded in 96-well plates. After 24 h, cells were cotransfected with the miR-217 mimics/negative control and the corresponding luciferase reporter vectors (wt/mu MYLK-AS1 and EZH2), after which a dual luciferase reporter assay system (Promega, Madison, WI, USA) was used.

Western Blotting Analysis

Samples were rifted in RIPA lysis buffer plus PMSF with low temperature, and BCA assay kit (Beyotime Biotechnology) examined total protein concentration. Protein samples (20 µg) were separated via sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride (PVDF) membranes and then were incubated with prepared antibodies. Finally, this membrane was visualized by enhanced chemiluminescence (ThermoFisher). Antibodies against GAPDH and EZH2 were purchased from Abcam.

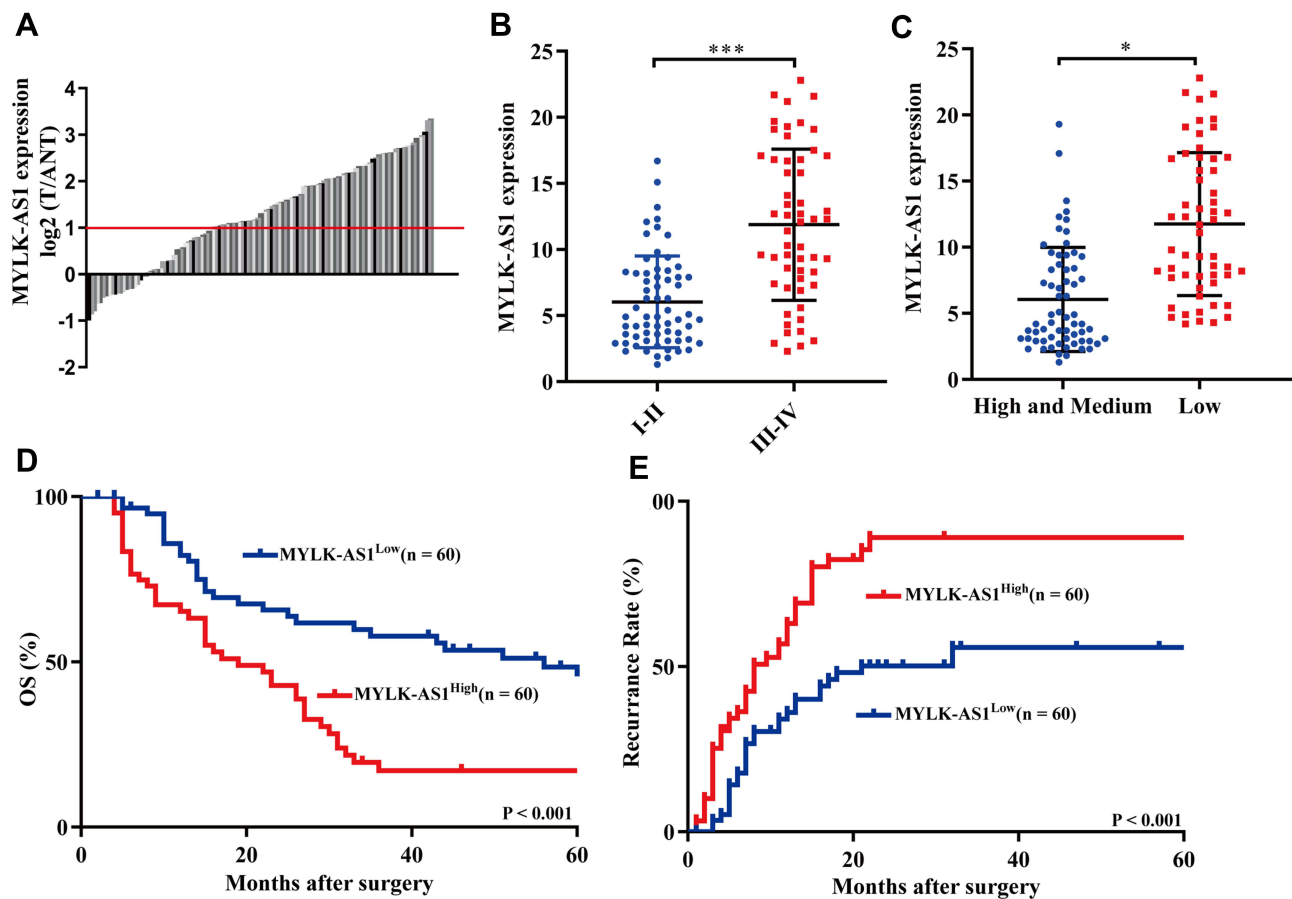


Figure 1 Overexpression of MYLK-AS1 predicts a poor prognosis in GBC patients. **(A)** The expression levels of MYLK-AS1 in GBC tissues and corresponding adjacent non-cancerous tissues were detected by RT-qPCR. **(B and C)** A total of 120 GBC patients were divided into well or moderate differentiation and low differentiation/I-II stage and III-IV stage groups. The diagram shows MYLK-AS1 expression in each group. *P < 0.05; ***P < 0.001. **(D and E)** Kaplan-Meier analysis of overall survival and PFS in GBC patients with low and high MYLK-AS1 levels.

Construction of Tissue Microarrays (TMAs) and Immunohistochemistry (IHC) Staining

TMA and IHC staining analyses of EZH2 were performed as previously described.⁵ In brief, TMA was prepared using classical methods, and EZH2 expression was detected using an immunoperoxidase method. According to the intensity and total area of the staining, EZH2 expression levels were classified as a reference.

Statistical Analysis

All statistical analyses were performed using SPSS 18.0 software (Chicago, IL, USA) and GraphPad Prism 7 as a reference.¹⁵ $P < 0.05$ was considered statistically significant. These experiments were carried out independently at least three times.

Results

MYLK-AS1 Overexpression Indicated the Poor Prognosis of GBC

To investigate the possible biological functions of MYLK-AS1 in the progression of GBC, we first detected the expression of MYLK-AS1 in 120 pairs of GBC tissues and paired adjacent non-tumour tissues. As shown by RT-qPCR results, MYLK-AS1 expression was higher in GBC tumour tissues than in paired adjacent non-tumour tissues (77/120) (Figure 1A). Next, we investigated the relationship between MYLK-AS1 expression and the clinicopathological characteristics of 120 GBC patients, as listed in Table 1. The results demonstrated that GBC patients with MYLK-AS1^{high} had an advanced TNM stage ($P = 0.001$) (Figure 1B) and low differentiation ($P = 0.006$) (Figure 1C). We then analysed the prognostic implications of MYLK-AS1 expression. Importantly, we found that patients with a high level of MYLK-AS1 expression had a significantly worse prognosis than those with MYLK-AS1^{low} expression (Figure 1D and E). Multivariate analysis identified that forced MYLK-AS1 expression was an independent prognostic predictor for GBC patients (Tables 2 and 3). These results indicated that MYLK-AS1 likely participates in the progression of GBC.

MYLK-AS1 Promoted Proliferation and Chemotherapy Resistance

Next, we explored the biological functions of MYLK-AS1 in GBC. Because GBC-SD and NOZ cells have a relatively high expression of MYLK-AS1, whereas EH-GB1 and

Table 1 The Correlation Between Clinicopathological Characteristics and MYLK-AS1 Expression

Clinicopathological Characteristics	MYLK-AS1 Expression		P
	Low	High	
Age			
≤60	23	28	0.767
>60	33	36	
Sex			
Male	42	47	0.845
Female	14	17	
Alcohol consumption			
Ever and current	31	42	0.250
Never	25	22	
Smoking status			
Ever and current	23	32	0.327
Never	33	32	
Tumor size			
<3 cm	30	31	0.574
≥3 cm	26	33	
Differentiation status			
Well or Moderate	37	26	0.005
Poor	19	38	
TNM stage			
I-II	39	26	0.001
III-IV	17	38	

SGC996 cells have a relatively low expression of MYLK-AS1 (Figure 2A), we silenced the expression of endogenous MYLK-AS1 in GBC-SD and NOZ cells using shRNA and overexpressed MYLK-AS1 in EH-GB1 and SGC996 cells using an expression vector containing full-length human MYLK-AS1. RT-qPCR analysis was used to detect the transfection efficiency (Figure 2B and C). The CCK-8 assay demonstrated that the knockdown of MYLK-AS1 inhibited the proliferation of GBC-SD and NOZ cells and increased the chemotherapy sensitivity of GBC-SD and NOZ cell to gemcitabine (Figure 2D and E). Conversely, the upregulation of MYLK-AS1 promoted the proliferation of EH-GB1 and SGC996 cells and induced EH-GB1 and SGC996 cell resistance to gemcitabine (Figure 2F and G).

MiR-217 Was a Direct Target of MYLK-AS1

Recently, accumulating evidence confirmed that lncRNAs mainly function as competing endogenous

Table 2 Univariate and Multivariate Analyses of Factors Associated with Overall Survival

Clinicopathological Characteristics	OS			
	Univariate	Multivariate		
	P	HR	95% CI	P value
Sex (female vs male)	0.317			NA
Age (years) (≤ 60 vs > 60)	0.822			NA
Alcohol consumption (Ever and current vs never)	0.671			NA
Smoking status (Ever and current vs never)	0.073			NA
Tumor size (diameter, cm) (> 3 vs ≤ 3)	0.064			NA
Differentiation status (Well or Moderate vs Poor)	0.024			NS
TNM (III/IV vs I/II)	0.016			NS
MYLK-AS1 expression (high vs low)	0.002	1.322	1.107–2.092	0.017

Abbreviations: NA, not adopted; NS, not significant; 95% CI, 95% confidence interval; HR, hazard ratio; Cox proportional hazards regression model.

Table 3 Univariate and Multivariate Analyses of Factors Associated with Cumulative Recurrence

Clinicopathological Characteristics	Cumulative Recurrence			
	Univariate	Multivariate		
	P	HR	95% CI	P value
Sex (female vs male)	0.146			NA
Age (years) (≤ 60 vs > 60)	0.276			NA
Alcohol consumption (Ever and current vs never)	0.733			NA
Smoking status (Ever and current vs never)	0.463			NA
Tumor size (diameter, cm) (> 3 vs ≤ 3)	0.084			NA
Differentiation status (Well or Moderate vs Poor)	0.031			NA
TNM (III/IV vs I/II)	0.007	1.034	0.811–1.972	0.042
MYLK-AS1 expression (high vs low)	0.004	1.472	1.085–2.723	0.025

Abbreviations: NA, not adopted; NS, not significant; 95% CI, 95% confidence interval; HR, hazard ratio; Cox proportional hazards regression model.

RNAs (ceRNAs) by sponging miRNAs. Thus, StarBase v3.0 was used to analyse the potential miRNAs that bound to MYLK-AS1, and the results showed that 28 miRNAs might bind to MYLK-AS1, including miR-217, a tumour suppressor miRNA. Therefore, we predicted miR-217 to be a potential target of MYLK-AS1 via StarBase V3.0 (Figure 3A). To further verify whether MYLK-AS1 could bind to miR-217 and act as a ceRNA, we constructed luciferase reporter vectors of MYLK-AS1 containing wild-type (wt) or mutated (mu) miR-217 binding sites. Luciferase reporter assays revealed that cotransfection of miR-217 with wt MYLK-AS1 significantly inhibited luciferase activity compared with cotransfection of miR-217 with mu MYLK-AS1 (Figure 3B). Furthermore, in MYLK-AS1-overexpressing GBC cells, miR-217 expression was repressed, while miR-217 expression was upregulated in MYLK-AS1 knockdown GBC cells (Figure 3C and

D). These results indicated that MYLK-AS1 sponges miR-217 in GBC cells.

Inhibition of miR-217 Restrained the Proliferation and Chemotherapy Resistance of GBC Cells

To further confirm the interaction of miR-217 with MYLK-AS1, we assessed its influence on the proliferation and chemotherapy resistance of GBC cells. A miR-217-specific inhibitor was synthesized and used to downregulate the expression of miR-217 in GBC-SD-MYLK-AS1 shRNA and NOZ-MYLK-AS1 shRNA cells (Figure 4A). Cell proliferation assays showed that downregulated miR-217 had inhibitory effects on cell proliferation capabilities in GBC-SD-MYLK-AS1 shRNA and NOZ-MYLK-AS1 shRNA cells (Figure 4B and C). In addition, a drug sensitivity assay

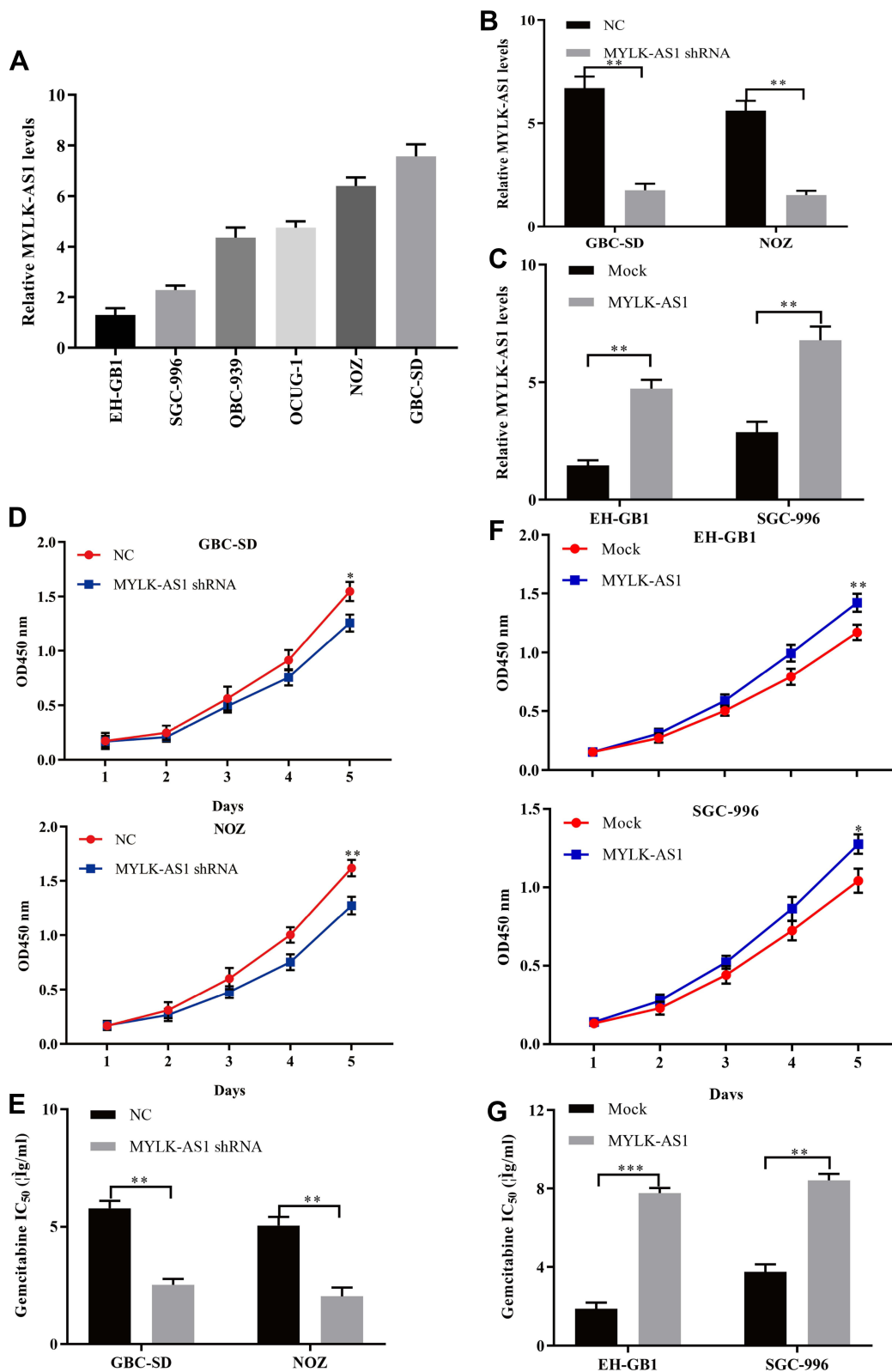


Figure 2 Forced MYLK-AS1 expression promotes GBC cell resistance to gemcitabine in vitro. (A) MYLK-AS1 expression in six GBC cell lines was examined using RT-qPCR analysis. (B and C) MYLK-AS1 expression in GBC cells was modified by shRNA interference and cDNA transfection. (D–G) Cell proliferation and drug sensitivity were measured using CCK-8 assays. The data are represented as the mean ± SD, n = 3. *P < 0.05; **P < 0.01; ***P < 0.001.

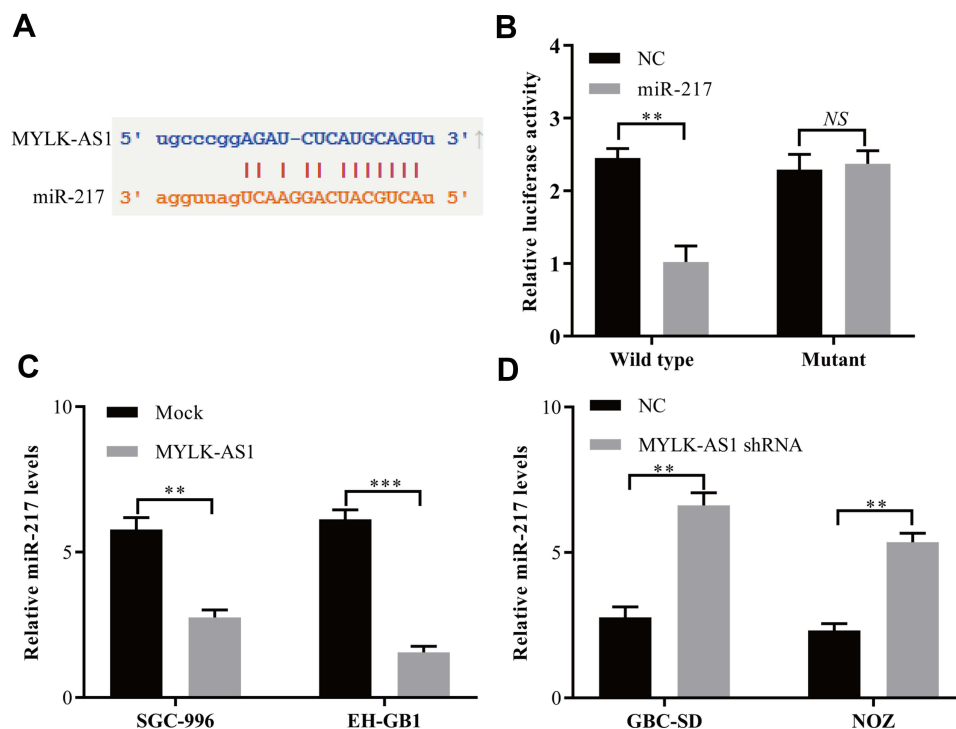


Figure 3 MYLK-AS1 functions as a ceRNA for miR-217 in GBC cells. **(A)** Target sequences in MYLK-AS1 predicted to bind to miR-217. **(B)** Wild-type or mutant MYLK-AS1 was transfected into HEK-293T cells with synthetic miR-217 or negative control. Luciferase activity was detected 48 h after transfection. **(C and D)** miR-217 expression after upregulation or knockdown of MYLK-AS1 expression was detected in GBC cells by using RT-qPCR. The data are represented as the mean \pm SD, $n = 3$. ** $P < 0.01$; *** $P < 0.001$.

demonstrated that inhibition of miR-217 significantly decreased the sensitivity of GBC-SD-MYLK-AS1 shRNA and NOZ-MYLK-AS1 shRNA cells to gemcitabine (Figure 4D). Taken together, our results suggested that MYLK-AS1 can promote the proliferation and chemotherapy resistance of GBC cells by targeting miR-217.

EZH2 Was Confirmed as a Direct Target Gene of miR-217

To further investigate the role of MYLK-AS1 in GBC, we predicted miR-217 targets using StarBase v3.0. As shown in Figure 5A, the 3'-UTR of EZH2 mRNA contains a putative binding site of miR-217. A luciferase reporter assay was also used to validate the direct interaction between EZH2 and miR-217 (Figure 5B). Furthermore, the mRNA levels of EZH2 in EH-GB1 and SGC996 cells were reduced after transfection with miR-217 mimics (Figure 5C). Conversely, the mRNA levels of EZH2 in GBC-SD and NOZ cells were increased after transfection with the inhibitor (Figure 5D).

MYLK-AS1 Regulated the miR-217/EZH2-Mediated Signalling Pathway in GBC

The upregulation of EZH2 is closely related to tumour progression, including GBC.^{16–19} Here, we posited that MYLK-AS1 may promote GBC cell resistance to chemotherapy agents by regulating the miR-217/EZH2-mediated signalling pathway. To test our hypothesis, we examined the expression of miR-217 and EZH2 in tissues from 120 GBC tissue samples. The results showed that there was a negative correlation between miR-217 and MYLK-AS1 in GBC tissues (Figure 6A). Furthermore, positive correlations between MYLK-AS1 and EZH2 were observed in GBC tissues (Figure 6B). To further investigate the results that MYLK-AS1 promoted GBC cell proliferation and chemotherapy resistance through regulation of the miR-217/EZH2 axis, we investigated the expression of MYLK-AS1 on EZH2 via Western blotting and RT-qPCR. The results showed that forced MYLK-AS1 expression led to increased protein and mRNA expression levels of EZH2, while decreased MYLK-AS1 downregulated EZH2 expression in GBC

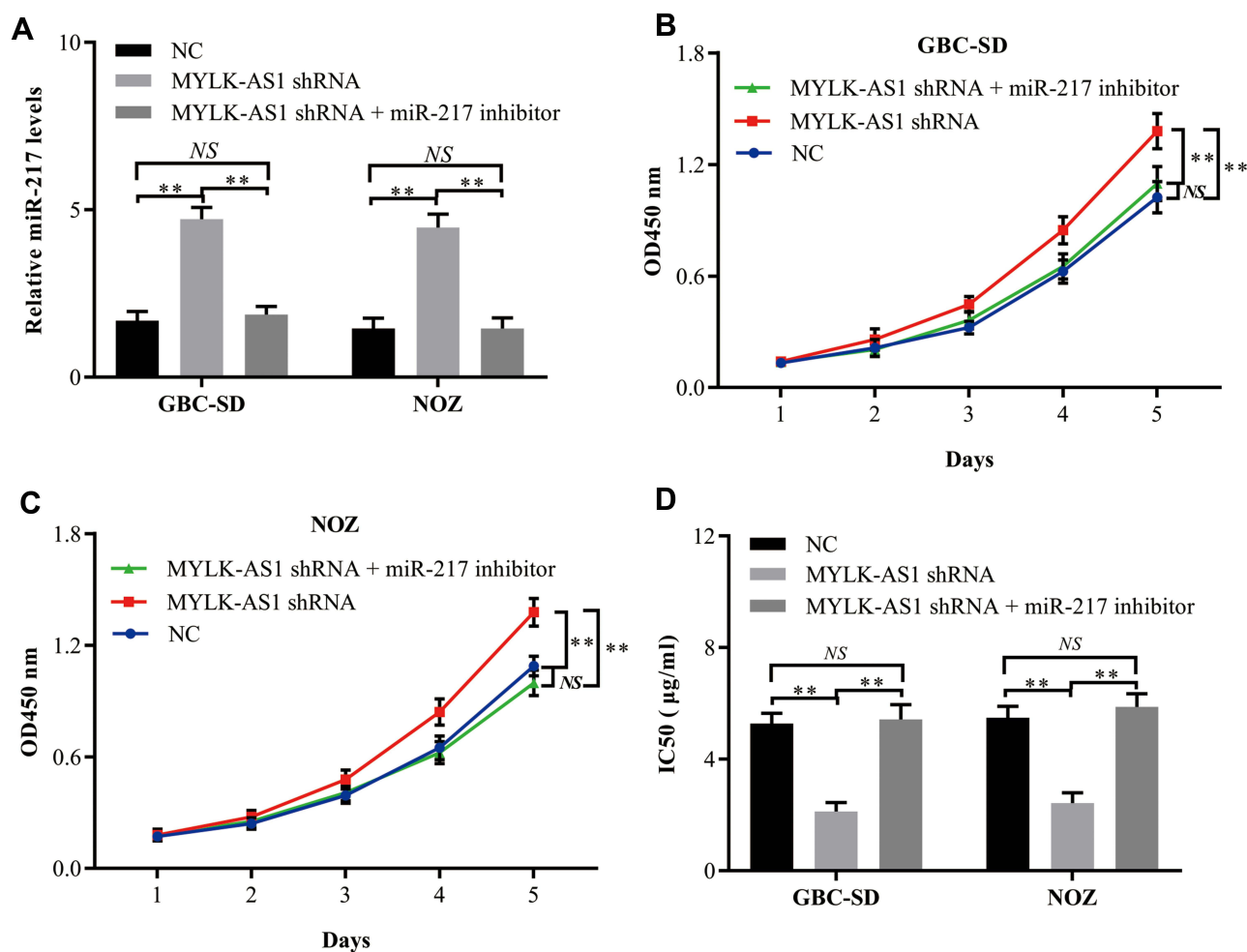


Figure 4 Silencing of miR-217 impairs the cell proliferation and chemoresistance inhibition of GBC cells with decreased MYLK-AS1 expression. **(A)** miR-217 expression in GBC cells was modified by shRNA interference. **(B and C)** Cell proliferation in GBC cells with reduced expression of miR-217 was assessed by a CCK-8 assay. **(D)** Drug sensitivity in GBC cells with reduced expression of miR-217 was evaluated via a CCK-8 assay. The data are represented as the mean \pm SD, $n = 3$. $**P < 0.01$; NS, not significant.

cells (Figure 6C and D). To further verify our hypothesis, we established EZH2-overexpressing EH-GB1 and SGC996 cells (EH-GB1-E and SGC996-E cells) through transfection with the EZH2 plasmid (Figure 6E). Proliferation and drug sensitivity assays showed that the increased EZH2 expression promoted the proliferation and chemoresistance of EH-GB1 and SGC996 cells (Figure 6F–H).

Discussion

In this study, we reported that MYLK-AS1 upregulated the expression of EZH2 by acting as a sponge for miR-217, as shown in molecular and functional assays, and thus promoted the proliferation and chemoresistance of GBC. This study is the first to report that the expression of EZH2 in GBC is regulated by MYLK-AS1 and the

first to show the molecular mechanism of MYLK-AS1 in GBC.

Recently, several studies reported that miR-217 functions as a tumour suppressor in multiple cancers by targeting oncogenes.^{20–24} For example, overexpression of miR-217 can suppress colony formation and the cell invasion capacity by targeting Rho-associated protein kinase 1 (ROCK1) in cervical cancer cells.²⁵ In colorectal cancer cell lines, upregulation of miR-217-5p expression induces cancer cell apoptosis by inhibiting PRKCI, BAG3, ITGAV and MAPK1.²⁴ Importantly, inhibiting miR-217 expression enhances chemoresistance in several types of cancer.^{26–28} However, the role of miR-217 in drug resistance in GBC remains unknown. To explore the underlying molecular mechanisms of MYLK-AS1, we validated miR-217 as a direct target of MYLK-AS1 in GBC cells. Dual-luciferase reporter and RIP

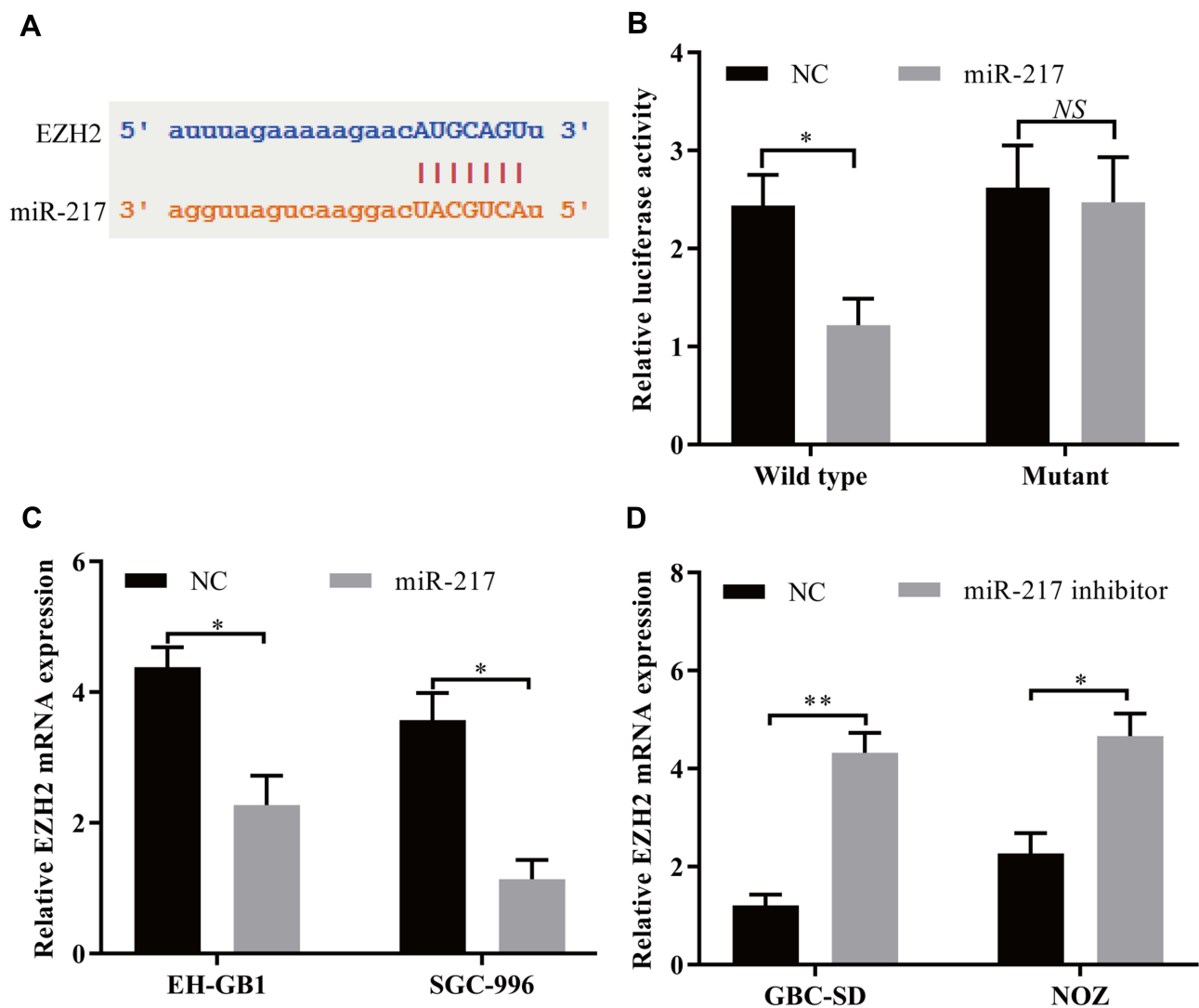


Figure 5 EZH2 mRNA acts as a target for miR-217 in GBC cells. **(A)** Target sequences in EZH2 mRNA predicted to bind to miR-217. **(B)** Wild-type or mutant EZH2 3'-UTR was transfected into HEK-293T cells with synthetic miR-217 or negative control. Luciferase activity was detected 48 h after transfection. **(C and D)** EZH2 mRNA expression after upregulation or knockdown of miR-217 expression was detected in GBC cells by using RT-qPCR. The data are represented as the mean \pm SD, $n = 3$. * $P < 0.05$; ** $P < 0.01$.

assays demonstrated the direct interaction between MYLK-AS1 and miR-217.

EZH2 is an important component of polycomb-repressive complex 2 (PRC2) and participates in suppressing gene expression by catalysing histone 3 lysine 27 trimethylation.²⁹ Recently, EZH2 has been reported to be an oncogene involved in the progression of various cancers, including GBC. Here, our results showed that aberrant MYLK-AS1/miR-217 may be associated with the regulation of EZH2, thus regulating chemotherapy resistance in GBC. Interestingly, EZH2 was predicted and validated as a target of miR-217 using StarBase 3.0 and luciferase reporter assays.

The expression of EZH2 in GBC cells treated with MYLK-AS1 was dramatically increased, and the knockdown of MYLK-AS1 significantly decreased the level of EZH2, revealing the regulatory role of MYLK-AS1 on EZH2 expression. Importantly, the dysregulation of EZH2 has been reported to be involved in chemotherapy resistance. For example, inhibition of EZH2 by inhibitor or miRNA mimics can reverse docetaxel resistance by suppressing cancer stem cells in prostate cancer.³⁰ In breast cancer, EZH2 confers tamoxifen resistance by inhibiting the expression of the oestrogen receptor α (ER α) cofactor GREB1.³¹ Overall, our results and previous studies suggest

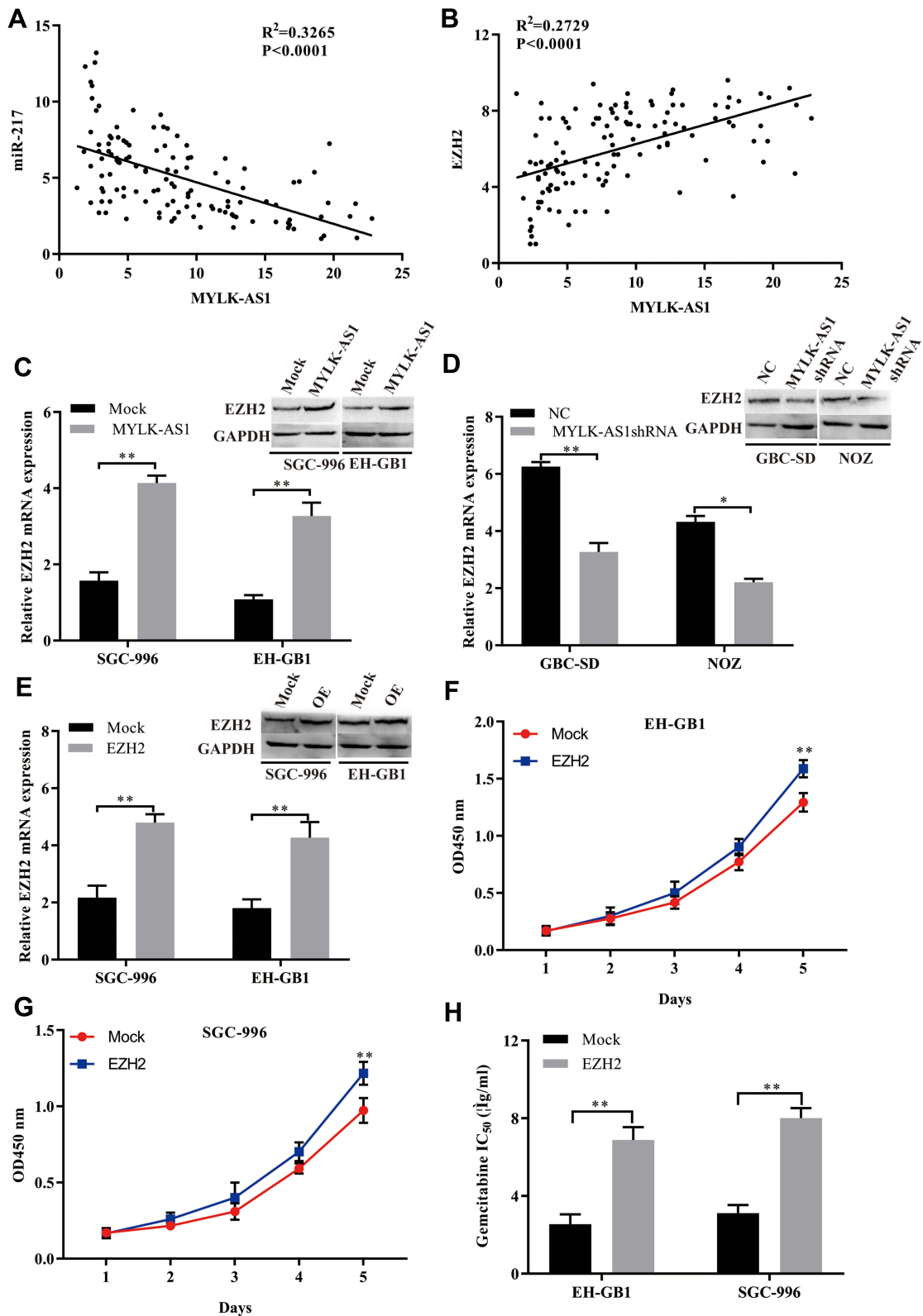


Figure 6 The relationship of MYLK-AS1, miR-217, and EZH2 mRNA in GBC tissues. **(A)** A negative correlation between MYLK-AS1 and miR-217 was observed in GBC tissues. **(B)** A positive correlation between MYLK-AS1 and EZH2 was observed in GBC tissues. **(C)** The levels of EZH2 were measured by Western blotting and RT-qPCR in GBC cells transfected with MYLK-AS1 and the MOCK control. **(D)** The levels of EZH2 were measured by Western blotting and RT-qPCR in GBC cells transfected with MYLK-AS1 shRNA or the negative control. **(E)** EZH2 expression in GBC cells was modified by plasmid transfection and detected by Western blotting and RT-qPCR. OE: overexpression of EZH2. **(F–H)** Cell proliferation and drug sensitivity were measured using CCK-8 assays. The data are represented as the mean \pm SD, $n = 3$. * $P < 0.05$; ** $P < 0.01$.

a new strategy to overcome chemotherapy resistance in GBC by targeting EZH2.

Taken together, we confirmed that MYLK-AS1 upregulated EZH2 expression through sponge-like binding to miR-217, thus affecting the chemotherapy sensitivity of GBC. Furthermore, forced MYLK-AS1 expression correlated with a poor prognosis and worse clinical characteristics in GBC. In conclusion, our results revealed a concrete molecular mechanism for MYLK-AS1 in GBC, which may serve as a promising biomarker and therapeutic target for GBC.

Data Sharing Statement

The datasets used during the present study are available from the corresponding author upon reasonable request.

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Disclosure

The authors state that they have no competing interests.

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