



TRANSCRIPTION FACTOR E2F7 ACTIVATES PKMYT1 TO PARTIALLY SUPPRESS ADRIAMYCIN SENSITIVITY IN GASTRIC CANCER THROUGH THE MAPK SIGNALING PATHWAY

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ABSTRACT

Background: Adriamycin (ADM) resistance remains an obstacle to gastric cancer chemotherapy treatment. **Objective:** The objective of this study was to study the role and mechanism of transcription factor E2F7 in sensitivity to ADM chemotherapeutic agents in gastric cancer. **Methods:** Cell viability and cell sensitivity were assessed by CCK-8 and IC₅₀ values of ADM were calculated. The impact of ADM on cellular proliferative capacity was assessed through colony formation assay. The binding relationship between E2F7 and PKMYT1 was then verified by dual luciferase assay and chromatin immunoprecipitation assay. ERK1/ERK2 and p-ERK1/p-ERK2 protein expression levels were detected by western blot. **Results:** In both gastric cancer tissue and ADM-resistant cells, a conspicuous upregulation of E2F7 and PKMYT1 was observed. Upregulated PKMYT1 was notably enriched in the MAPK signaling pathway. Enhanced levels of E2F7 were shown to not only drive gastric cancer cell proliferation but also engender a reduction in the sensitivity of these cells to ADM. Furthermore, PKMYT1 emerged as a downstream target of E2F7. Activation of E2F7 culminated in the transcriptional upregulation of PKMYT1, and silencing E2F7 reversed the inhibitory impact of PKMYT1 overexpression on ADM sensitivity in gastric cancer cells. **Conclusion:** E2F7/PKMYT1 axis might promote the proliferation and partially inhibit ADM sensitivity of gastric cancer cells by activating the MAPK pathway. (REV INVEST CLIN. 2024;76(1):6-17)

Keywords: E2F7. PKMYT1. MAPK pathway. Gastric cancer. Adriamycin.

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Received for publication: 11-08-2023

Approved for publication: 09-12-2023

DOI: 10.24875/RIC.23000180

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INTRODUCTION

Gastric cancer (GC) is a common gastrointestinal cancer¹. Despite significant advances in diagnostic techniques and treatments for GC in recent years, the 5-year survival rate for GC patients remains low, which is approximately 20-30%². Since GC is typically asymptomatic in the early stage, most patients are diagnosed at an advanced stage, missing the opportunity for radical resection. Chemotherapy is considered the major GC treatment³. First used against advanced GC in 1980⁴, Adriamycin (ADM), an anthracycline-based antineoplastic chemotherapy agent has since been approved by the US FDA as an effective agent for GC treatment⁵. ADM functions by inhibiting the macromolecule biosynthesis using DNA insertions of its glycosyl groups and cyclohexane to disrupt topoisomerase II and eventually lead to DNA damage (cell death)⁶. However, ADM resistance severely limits chemotherapy efficacy in GC patients. Therefore, the mechanism of chemoresistance is currently a major concern in GC research. An in-depth explanation of the resistance mechanisms of ADM in GC is essential for therapeutic efficacy improvement.

E2F7 transcription factor belongs to the E2F/DP family winged-helix DNA-binding domain⁷. Through two separate DNA-binding domains that distinguish E2F7 from others, E2F7 binds to promoter sites of target genes in a DP protein-independent manner⁸. In addition, the presence of upregulated E2F7 has been proven in a variety of cancers and so does its involvement in cell cycle, cell proliferation, angiogenesis, and DNA damage⁹. For example, Liang et al.¹⁰ found that SNHG6 promotes migration, proliferation, epithelial mesenchymal transition (EMT), and invasion of lung adenocarcinoma cells through the miR-26a-5p/E2F7 axis. According to Yang et al.⁹, E2F7 promotes EZH2 transcription and activates the PTEN/AKT/mTOR pathway to promote cell proliferation, cell metastasis, and tumorigenesis in glioblastoma. Furthermore, that, E2F7 is also involved in regulating drug resistance development in tumor cells. For example, in ER-positive breast cancer, the miR-26a/E2F7 feedback loop promotes tamoxifen resistance¹¹. Hazar-Rethinam et al.¹² demonstrated that, in head and neck squamous cell carcinoma, E2F7 promotes ADM resistance by regulating Sphk1 to activate AKT pathway. Based on the previous

studies, we suspected that E2F7 was associated with ADM sensitivity in GC and intended to conduct relevant investigation.

Located on human chromosome 16p13.3, protein kinase membrane-associated tyrosine/threonine 1 (PKMYT19) is an important gene encoding the Wee kinase family¹³. PKMYT1 functions to inhibit Cdk1 phosphorylation during cell cycle transitions¹⁴, with aberrant PKMYT1 expression implicated in promoting progression of various cancers^{13,15,16}. One study has reported that PKMYT1 interacts with MCRS1 to affect GC cell proliferation, migration, invasion and EMT¹⁷. However, whether PKMYT1 affects that the sensitivity of GC cells to ADM is unclear.

In this study, we demonstrated the interaction relationship between E2F7 and PKMYT1 and their effect on GC progression. Our results indicated that E2F7 and PKMYT1 were highly expressed in GC, and E2F7 high expression promoted GC cell proliferation and suppressed ADM sensitivity in GC cells. E2F7 could target and regulate PKMYT1 expression and inhibit ADM sensitivity through the MAPK signaling pathway. At present, the mechanism of E2F7 affecting ADM sensitivity in GC cells has not been reported. This study revealed the key role of E2F7 in ADM sensitivity of GC, providing new directions and approaches for improving ADM sensitivity in GC patients.

MATERIALS AND METHODS

Bioinformatics

Expression data of mRNAs in GC were downloaded from TCGA database (<https://portal.gdc.cancer.gov/>) (normal: 32, tumor: 375). Then, differentially expressed mRNAs were obtained through differential expression analysis using edgeR package with $|logFC| > 2$ and $padj < 0.05$ as the thresholds. Downstream target genes of transcription factors were forecasted by hTFTtarget (<http://bioinfo.life.hust.edu.cn/hTFTtarget#!/tf>) and GRNdb (<http://www.grndb.com/>) and then overlapped with differential genes to obtain differentially expressed target genes. JASPAR (<http://jaspar.genereg.net/>) was used to analyze the association between transcription factors and target genes and their motif sites. Gene set enrichment analysis (GSEA) of enriched pathways for target genes was conducted.

Table 1. The qRT-PCR primers sequences

Gene	Forward primer	Reverse primer
E2F7	5'-GTCAGCCCTCACTAACCTAAG-3'	5'-TGCAGTGGATGCTCTGG-3'
PKMYT1	5'-CATGGCTCCTACGGAGAGGT-3'	5'-ACATGGAACGCTTACCGCAT-3'
GAPDH	5'-CACCCACTCCTCACCTTG-3'	5'-CCACCACCCCTGTTGCTGTAG-3'

qRT-PCR: quantitative reverse transcription-polymerase chain reaction.

Cell culture and *in vitro* ADM-resistant model

GC cell lines (SNU-1, MKN-45, and NCI-N87), gastric epithelial cell line, and 293T cell line were purchased from BeNa Culture Collection (BNCC, China). These cell lines were kept in RPMI 1640 medium (Gibco, USA) with 10% fetal bovine serum (FBS) (Gibco, USA) and 1% penicillin-streptomycin (Solarbio, China). Incubation was conducted in a humidified incubator at 37°C with 5% CO₂¹⁸.

ADM-resistant cell lines MKN-45/ADM and SNU-1/ADM were constructed through a progressive ADM concentration induction method. MKN-45 and SNU-1 cells in logarithmic phase were subjected to treatment with 0.5 μM ADM (MedChemExpress, USA). After 48 h, the culture medium was replaced with ADM-free medium. Cells were re-treated with the drug, and when the cells were resistant to the current concentration, ADM concentration was increased. The procedures above were repeated. Cellular resistance was continuously assessed throughout the cultivation period until resistance levels increased approximately fivefold. Cells were consistently cultured under high ADM concentrations to stabilize resistance and were used for subsequent investigations¹⁹.

Cell transfection

GC cells were placed at a density of 1 × 10⁶ cells/well in six-well plates and cultured until 70% confluent. The oe-NC (negative control)/oe-E2F7, sh-NC (negative control)/sh-E2F7, and oe-PKMYT1 (Ribobio, China) were transfected into GC cells or 293T cells utilizing Lipofectamine 2000 (Invitrogen, USA). Subsequent experiments were carried out after transfection for 24 h.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was extracted by TRIzol reagent (Invitrogen, USA). Subsequently, 500 ng of total RNA were reversely transcribed into cDNA using the FastQuant RT kit (Tiangen, China). qRT-PCR was performed using AceQ qPCR SYBR Green Master Mix (Vazyme, China) on an Applied Biosystems 7300 instrument (Thermo Fisher, USA), and gene expression was computed utilizing 2^{-ΔΔCt} method. β-actin was used as an internal reference control. Primers used for qRT-PCR are shown in table 1.

Cell viability

Viability of GC cells was detected using CCK-8 kit (EZB, USA). Cells were placed into 96-well plates with a seeding density of 2000 cells per well and incubated for 0, 24, 48, and 72 h. After PBS rinses and 10 μL CCK-8 solution addition, cells were incubated at 37°C for 1.5 h. Cell viability was assayed at 450 nm using a microplate reader (Thermo Fisher, USA). Each group had 3 replicates, and the mean value was calculated.

To analyze ADM resistance, transfected cells were kept with different concentrations (0, 0.5, 1, 2, 4, and 8 μM) of ADM for 36 h. After adding 10 μL CCK-8 reagent, cells were kept at 37°C for 2 h. Absorbance values were measured at 450 nm and IC₅₀ values were calculated. Each group had three replicates, and the mean value was calculated.

Western blot (WB)

Cells from each group were collected after trypsin digestion. RIPA lysis buffer (Beyotime, China) was mixed with the sample, and the mixture was lysed on ice for 30 min, followed by centrifugation at 12,000 rpm and

4°C for 10 min. Protein concentrations in the supernatant were quantified employing a bicinchoninic acid protein assay kit (Beyotime, China). Protein lysates from each sample were isolated by 10% SDS-PAGE and then transferred to PVDF membranes. Membranes were blocked with 5% skim milk for 1 h at 37°C and then incubated overnight at 4°C with primary antibodies rabbit anti-human E2F7 (1:1000, #DF2444, Affinity, USA), PKMYT1 (1:1000, ab307146, Abcam, UK), ERK1/2 (1:1000, #AF0155, Affinity, USA), p-ERK1/2 (1:1000, #AF1015, Affinity, USA), JNK (1:1000, ab307802, Abcam, UK), p-JNK (1:1000, #AF3318, Affinity, USA), p38 (1:1000, ab178867, Abcam, UK), p-p38 (1:1000, #AF4001, Affinity, USA), and β-actin (1:1000, ab8227, Abcam, UK). After 1 h of incubation with secondary antibody goat anti-rabbit IgG (1:2000, ab6721, Abcam, UK) at room temperature, development of protein bands in skim milk was performed using ECL hypersensitive chemiluminescence reagent on a chemiluminescence imager (Clinx, China)¹⁸.

***In vitro* colony formation analysis**

Cells were cultured in six-well plates (approximately 1000 cells per well) that were supplemented with RPMI-1640 medium (plus FBS). After 10 days, cells were stained with 0.1% crystal violet solution (Solarbio, China) after fixation with 100% methanol for 15 min at room temperature. After using PBS for development, a camera (Nikon, Japan) was used to take photos.

Luciferase reporter assay

The PKMYT1 wild-type (PKMYT1-wt) and PKMYT1 mutant (PKMYT1-mut) reporter vectors (Promega, USA) containing E2F1 binding site were constructed. PKMYT1-wt or PKMYT1-mut plasmids and oe-E2F7/oe-NC were cotransfected into 293T cells using Lipofectamine 2000 (Invitrogen, USA) according to the manual. After 48 h of cultivation, luciferase activity was detected using the dual-luciferase reporter system (Promega, USA)¹⁸.

Chromatin immunoprecipitation (ChIP) assay

The experiment was conducted according to the protocol described in the study of Sun et al.²⁰. A Pierce Agarose ChIP Kit (Thermo Fisher, USA) was used for

ChIP assay following the manufacturer's guidance. Chromatin was mechanically sheared using sonication after MKN-45 and SNU-1 cells were collected and cross-linked by formaldehyde and then subjected to immunoprecipitation with the appropriate antibodies. Immunoprecipitated and total chromatin was then reverse cross-linked and recovered using column purification. The amount of DNA was further assessed by qRT-PCR, using the PKMYT1-specific ChIP primers and SYBR Select Master Mix (Applied Biosystems, USA). Antibodies used included anti-E2F7 (Abcam, UK) and anti-IgG (Abcam, UK). ChIP primers used were as follows: forward primer 5'-ATG-GACCCAAACACTACGCCA-3', reverse primer 5'-GCTCGCCAAAATTCCAA-3.'

Data analysis

Statistical analysis was performed using Prism 8.0 software (GraphPad, USA). Results are presented as mean ± standard deviation (SD). t-test was used for two groups, and the one-way analysis of variance (ANOVA) test was used to determine the significance between more than two groups. All experiments were performed with at least three independent biological replicates. p < 0.05 was considered significant.

RESULTS

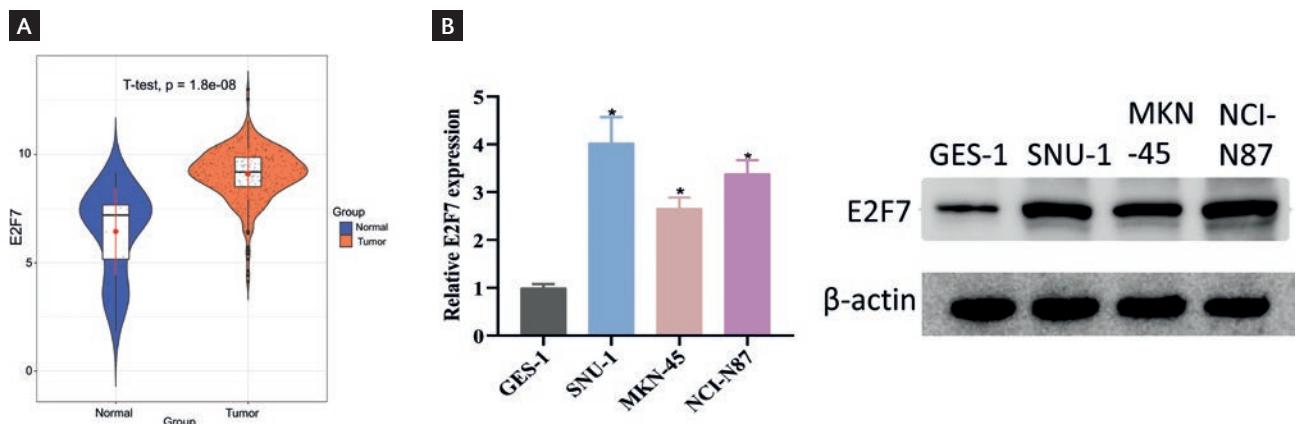
E2F7 was significantly highly expressed in GC

With upregulation of E2F7 found across a variety of cancers, E2F7 is considered an important driver of tumor growth⁹. After investigating E2F7 in GC tissues using bioinformatics, the results found substantially higher E2F7 expression in GC tissue (Fig. 1A). Further, *in vitro* cell experiments confirmed that, compared to gastric epithelial cells, GC cells presented a higher E2F7 expression (Fig. 1B). Taken together, substantially high E2F7 expression in GC was confirmed.

Downregulation of E2F7 enhanced the sensitivity of gastric cancer cells to Adriamycin

According to the previous research, E2F7 can promote ADM resistance in head and neck squamous cell carcinoma cells²¹. Therefore, we conjectured

Figure 1. E2F7 is significantly highly expressed in GC. **A:** E2F7 expression in GC tissues, with blue representing normal and orange representing tumor (normal: 32, tumor: 375). **B:** detect the mRNA and protein expression of E2F7 in GC cells by qRT-PCR and WB. * $p < 0.05$.



that high E2F7 expression in GC could suppress GC sensitivity to ADM. To confirm the hypothesis, we established ADM-resistant GC cell lines *in vitro*, MKN-45/ADM, and SNU-1/ADM (Fig. 2A). qRT-PCR and WB analyses revealed a substantial elevation in the expression of E2F7 within the MKN-45/ADM and SNU-1/ADM cell lines compared with MKN-45 and SNU-1 cells (Fig. 2B). We generated cell lines with low E2F7 expression (sh-E2F7) as well as negative controls (sh-NC) using the two established ADM-resistant cell lines. After detecting transfection efficiency through qRT-PCR and WB analyses, it was observed that E2F7 expression was markedly reduced in both MKN-45 and SNU-1 cells on E2F7 silencing (Fig. 2C). As per the outcomes of the CCK-8 assay, a notable attenuation in viability was observed in both MKN-45/ADM and SNU-1/ADM cells after E2F7 silencing (Fig. 2D). Correspondingly, the heightened sensitivity of these cells to ADM was illustrated in figure 2E. We also examined ADM resistance in non-resistant MKN-45 and SNU-1 cells, and found that silencing E2F7 increased the susceptibility of non-resistant strains of MKN-45 and SNU-1 cells to ADM (Fig. 2F). Notably, the colony formation assay revealed a more pronounced inhibitory effect of ADM on the sh-E2F7 transfected drug-resistant cell lines (Fig. 2G). Similarly, the inhibitory effect of ADM on cells was more pronounced in non-resistant cell lines transfected with sh-E2F7 (Fig. 2H). All these results led us to conclude that high E2F7 expression in GC inhibited the sensitivity of cancer cells to ADM partially.

PKMYT1 is the target gene downstream of E2F7

To further investigate the molecular mechanism by which E2F7 inhibits ADM sensitivity partially, the hTFtarget and GRNdb databases were searched for the downstream target of E2F7. After overlapping predicted results with differentially upregulated mRNAs, 18 candidate genes were obtained (Fig. 3A and Supplementary Table 1). A Pearson correlation analysis of E2F7 expression with candidate target genes revealed a significant positive correlation between E2F7 and PKMYT1 (Fig. 3B). Therefore, PKMYT1 was screened as the gene of interest. According to bioinformatics analysis, PKMYT1 expression was higher in GC tissues than in adjacent normal tissues (Fig. 3C). Through validation at the cellular level, PKMYT1 expression was found to be higher in GC cells than in gastric epithelial cells. Among GC cells, the most substantial upregulation was found in SNU-1 cells (Fig. 3D). Motif sites in E2F7 with the PKMYT1 promoter region were identified by bioinformatics analysis (Fig. 3E). After using dual luciferase assay and ChIP assay for validation, we found overexpressed E2F7 led to enhanced luciferase activity of PKMYT1-WT (Fig. 3F). ChIP results demonstrated a clear enrichment of PKMYT1 by the E2F7 antibody (Fig. 3G). Subsequently, successful oe-E2F7 was achieved in both MKN-45 and SNU-1 cells, with the qRT-PCR and WB analyses illustrating a substantial elevation in the expression level of PKMYT1 (Fig. 3H). These observations led us to conclude that highly expressed PKMYT1 was a downstream target gene of the E2F7 transcription factor.

Figure 2. Downregulation of E2F7 enhances the sensitivity of GC cells to ADM-E2F7. **A:** CCK-8 was used to detect the IC_{50} of MKN-45, MKN-45/ADM, SNU-1 and SNU-1/ADM cell lines to ADM; **B:** qRT-PCR and WB were used to detect the expression of E2F7 in MKN-45, MKN-45/ADM, SNU-1 and SNU-1/ADM cell lines; **C:** qRT-PCR and WB were used to detect the expression of E2F7-silenced MKN-45/ADM and SNU-1/ADM cell lines was assessed using the CCK-8 assay; **D:** viability of E2F7-silenced MKN-45/ADM, MKN-45, and SNU-1 cell lines; **E** and **F:** CCK-8 assay was employed to determine the IC_{50} of ADM in E2F7-silenced MKN-45/ADM, SNU-1/ADM, MKN-45, and SNU-1 cell lines; **G** and **H:** Colony formation assays were conducted to assess the impact of ADM on E2F7-silenced MKN-45/ADM, SNU-1/ADM, MKN-45, and SNU-1 cell lines.

*p < 0.05.

#p < 0.05.

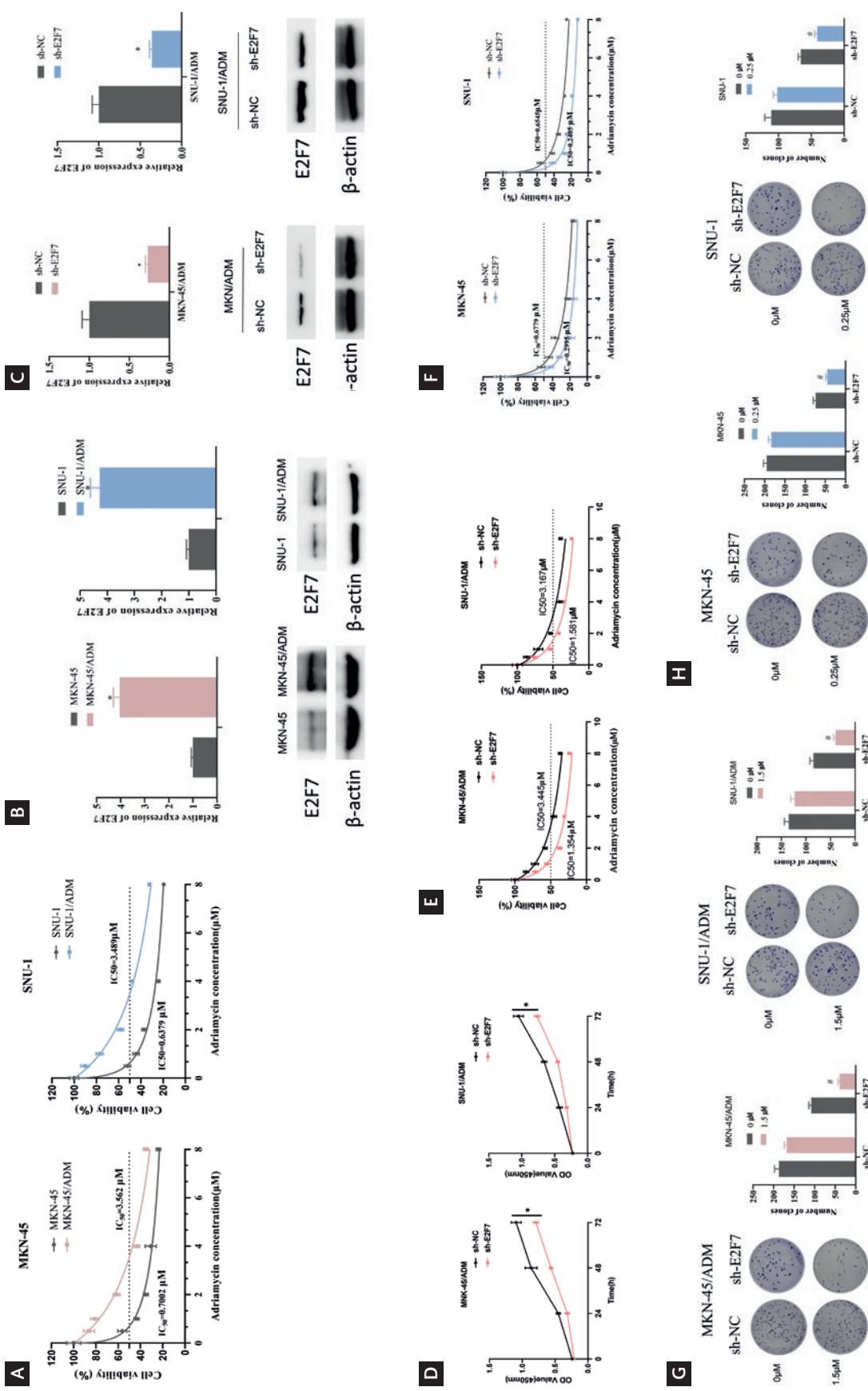
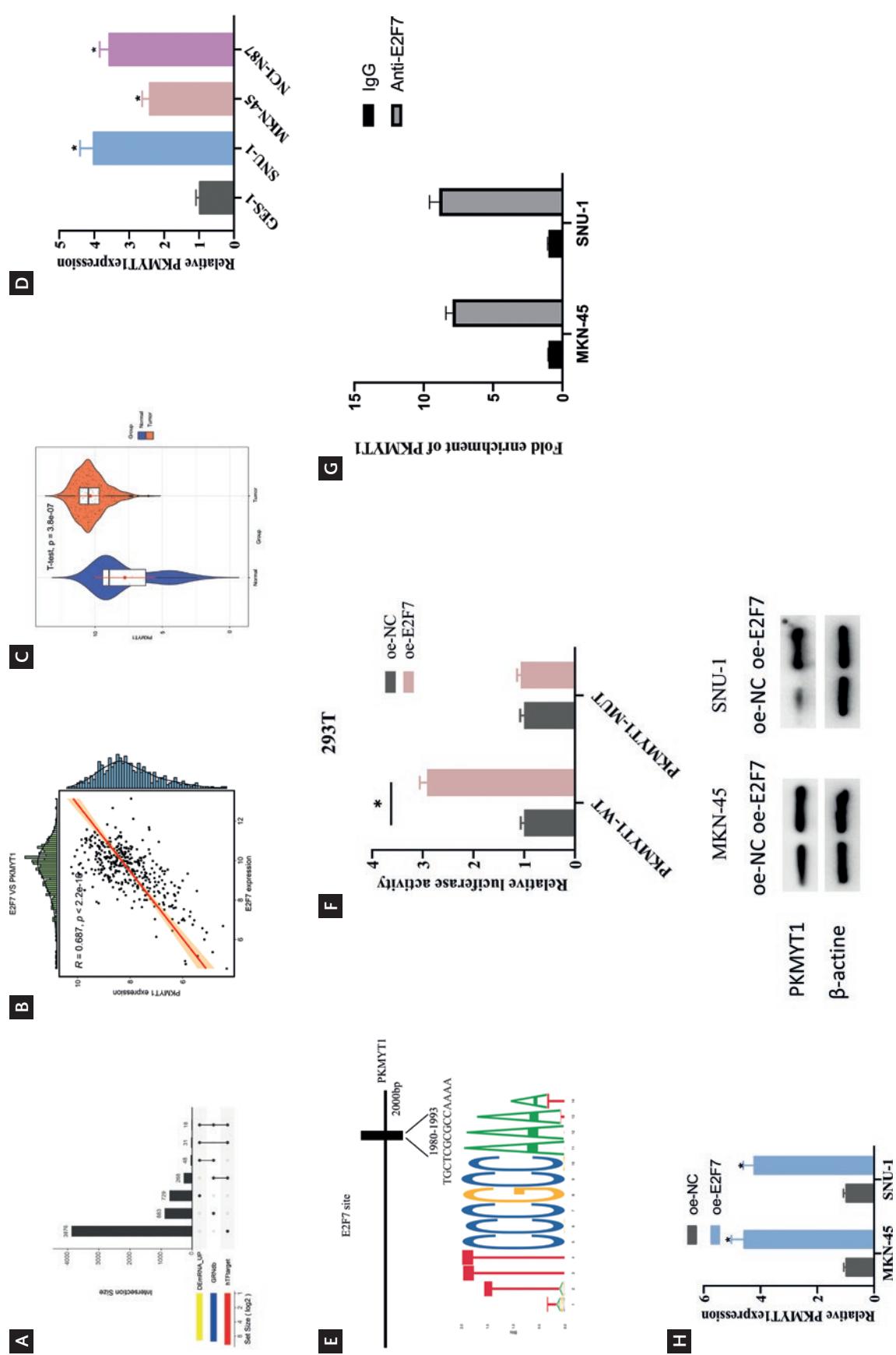


Figure 3. PKMYT1 is the target gene downstream of E2F7 and differentially expressed genes. A: upset plot of predicted target genes downstream of E2F7. B: Pearson correlation analysis of E2F7 and PKMYT1. C and D: PKMYT1 expression in GC tissues and cells; E: binding sequence of E2F7 and target gene PKMYT1; F and G: binding relationship between E2F7 and PKMYT1 detected by dual luciferase assay and ChIP assay. H: qRT-PCR and WB analysis. *p < 0.05.



E2F7 activated PKMYT1 to suppress gastric cancer sensitivity to Adriamycin

To mine the molecular mechanism of E2F7/PKMYT1 axis affecting GC cell sensitivity to ADM, the following groups sh-NC+oe-NC, sh-NC+oe-PKMYT1, and sh-E2F7+oe-PKMYT1 were constructed based on MKN-45/ADM and SNU-1/ADM cells. After assessing PKMYT1 expression in each group with qRT-PCR and WB, we found substantial increases in PKMYT1 expression after overexpression of PKMYT1, yet the increases could be reversed by silencing E2F7 (Fig. 4A). CCK-8 assay results found significantly enhanced vitality of cells – after overexpression of PKMYT1, and this effect was reversed after further silencing of E2F7 (Fig. 4B). By determining IC_{50} values for each treatment group, we observed that the elevation of PKMYT1 expression hindered the sensitivity of GC cells to ADM, whereas the attenuation of E2F7 offset this effect (Fig. 4C). Colony formation assay indicated a reduction in the inhibitory effect of ADM on the clonogenic potential of cells overexpressing PKMYT1. However, simultaneous overexpression of PKMYT1 along with E2F7 silencing restored the suppressive impact of ADM on the clonogenic potential of GC cells (Fig. 4D).

E2F7 activates PKMYT1 to suppress gastric cancer sensitivity to Adriamycin through the MAPK signaling pathway

The samples were grouped according to the median PKMYT1 expression in tumor samples, and KEGG pathway enrichment analysis was performed using GSEA software. The results revealed that the PKMYT1 low expression group was significantly enriched on KEGG_MAPK_SIGNALING_PATHWAY (Fig. 5A, FDR = 0.014, Supplementary Table 2). We postulated that PKMYT1 affected the sensitivity of GC cells to ADM through the MAPK signaling pathway. To address this, we established PKMYT1 overexpressing cell lines (oe-PKMYT1) based on MKN-45/ADM and SNU-1/ADM cells (Fig. 5B). CCK-8 assay results revealed that sensitivity to ADM was diminished in oe-PKMYT1 group; however, the addition of the MAPK signaling pathway inhibitor AZD6244 restored ADM sensitivity in the oe-PKMYT1 cells (Fig. 5C). WB analysis, further, demonstrated that overexpression of PKMYT1 led to an increase in the expression of

ERK1/2, p-ERK1/2, p-JNK, and p-p38 proteins, which was reversed by AZD6244 (Fig. 5D). In summary, E2F7 activated PKMYT1, thereby suppressing the sensitivity of GC cells to ADM partially through the MAPK pathway.

DISCUSSION

The resistance of GC to ADM poses a formidable obstacle to the effective treatment of this disease. Many previous studies have reported the molecular mechanism that impacts the sensitivity of ADM chemotherapeutic agents in GC. For example, Shang et al.²² confirmed that silencing lncRNA UCA1 could inhibit malignant proliferation and improve the chemosensitivity to ADM. Zhu et al.²³ found that the knockdown of MDR1 leads to increases in the sensitivity of drug-resistant GC cells to ADM. A comprehensive understanding of the mechanisms underlying ADM resistance in GC remains pivotal for advancing therapeutic strategies in GC treatment. We found that by silencing upregulated E2F7 in GC, the sensitivity to ADM was improved in GC. According to the research on head and neck squamous cell carcinoma cells conducted by Hazar-Rethinam et al.¹², E2F7 activates AKT by directly increasing the transcriptional activity of the Sphk1/S1P axis, which, in turns, led to developed ADM resistance. In conclusion, driven by relevant experiment results, the critical role of E2F7 in ADM chemosensitivity was confirmed. In other words, silencing E2F7 might be a novel strategy for slowing ADM resistance in GC cells.

Besides, we identified that there was a downstream regulatory molecule PKMYT1 in E2F7. More and more studies have shown that PKMYT1 plays a role as a promoting factor in the progression of cancer^{24,25}. It is worth noting that Zhang et al.¹³ indicated elevated PKMYT1 expression in GC, and PKMYT1 is discovered to promote proliferation and inhibit apoptosis in GC through activating MAPK pathway, which echoed our findings. In addition, one study has reported that upregulation of PKMYT1 expression can promote resistance to gemcitabine in gallbladder cancer²⁶. However, in our investigation, we uncovered the regulatory influence of the transcription factor E2F7 on PKMYT1, whereby PKMYT1 overexpression was observed to suppress the sensitivity of MKN-45 and SNU-1 cells to ADM.

Figure 4. E2F7 activates PKMYT1 to inhibit the sensitivity of GC to ADM. A: expression of PKMYT1 was assessed in various transfected groups of MKN-45/ADM and SNU-1/ADM cell lines utilizing qRT-PCR and WB analyses; B: cell viability of different transfected groups in MKN-45/ADM and SNU-1/ADM cell lines was evaluated using the CCK-8 assay; C: IC_{50} values of ADM for different transfected groups in MKN-45/ADM and SNU-1/ADM cell lines were determined utilizing CCK-8 assay; D: colony formation assays were employed to evaluate the inhibitory effect of ADM on clonogenic potential of different transfected groups in MKN-45/ADM and SNU-1/ADM cell lines. Group: sh-NC+ oe-NC, sh-NC+oe+PKMYT1, and sh+E2F7+oe+PKMYT1. $* p < 0.05$.

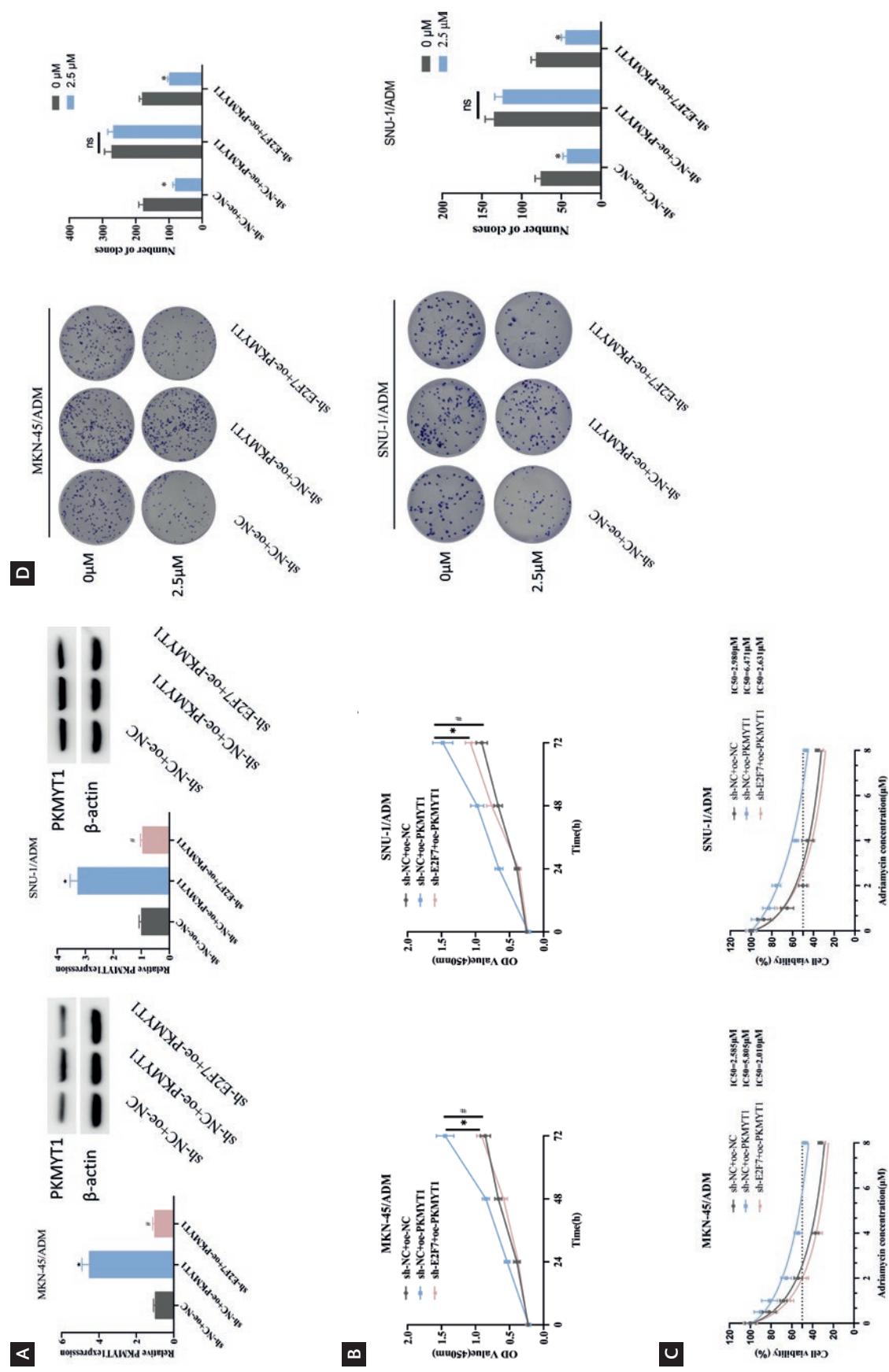
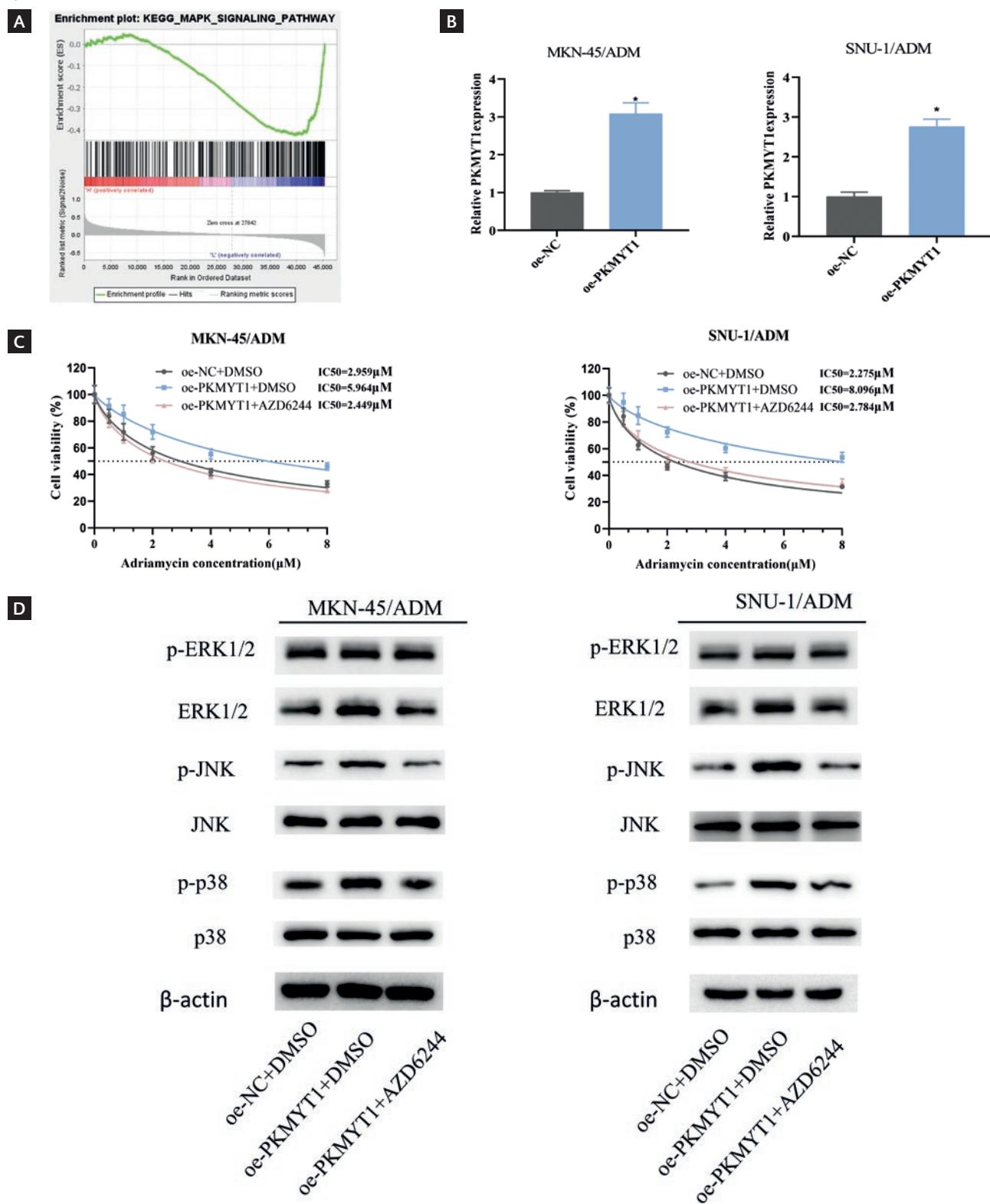


Figure 5. E2F7 activates PKMYT1 to inhibit the sensitivity of GC to ADM through the MAPK signaling pathway. **A:** PKMYT1 enrichment in the MAPK signaling pathway detected by bioinformatics; **B:** qRT-PCR was conducted to validate the transfection efficiency of PKMYT1 overexpression; **C:** IC₅₀ values of various treatment groups were assessed utilizing CCK-8 assay; **D:** protein expression levels of ERK1/2, p-ERK1/2, JNK, p-JNK, p38, and p-p38 detected by WB. Group: oe-NC+DMSO, oe-PKMYT1+AZD6244.

*p < 0.05.



As reported, E2F7 functions as an oncogenic factor participating in tumor cell progression and resistance. For instance, E2F7 is upregulated in gallbladder carcinoma that facilitates EMT and metastasis of cancer cells²⁷. E2F7 was found to inhibit the sensitivity of head and neck squamous cells to ADM by activating the PI3K/AKT pathway through upregulation of Rac-GAP1 15 by Hazar-Rethinam et al.²¹. Within this investigation, high expression of E2F7 in GC was observed, which activated the MAPK pathway through PKMYT1, thereby impeding the sensitivity of GC cells to ADM. The prognostic value of E2F7 in GC was analyzed by bioinformatics, and, intriguingly, was found that increased E2F7 expression was significantly associated with good overall survival in GC patients^{28,29}. We speculate that this may be related to whether GC patients receive chemotherapy or not. During the process of ADM treatment in GC patients, the impact of E2F7 on ADM resistance in GC patients may outweigh the benefits of its overexpression, which requires further investigation. It has been shown that GC cell-derived extracellular vesicles increase E2F7 expression and activate the MAPK/ERK signaling to promote peritoneal metastasis in GC patients by delivering SNHG2³⁰, suggesting that E2F7 may be present as a pro-cancer factor in GC. In conclusion, this study elucidated a novel molecular regulatory network impacting GC progression, suggesting the E2F7/PKMYT1/MAPK pathway as a feasible novel target for counteracting chemoresistance in GC.

Our results demonstrated the role of E2F7 in GC progression and its underlying regulatory mechanisms. Targeting PKMYT1 by E2F7 promotes GC cell malignant behavior and suppresses GC sensitivity to ADM partially. The role of E2F7 in GC progression and chemosensitivity may be completed through the activation of MAPK signaling pathways. The E2F7/PKMYT1/MAPK axis may be a target for GC therapy. Although this study provides new insights and directions, there are still shortcomings in this paper. In this study, only *in vitro* cell experiments were performed and no further validation was performed through animal experiments. In addition, whether E2F7 can influence GC development through other signaling pathways has not yet been investigated, which is also the direction of our future studies.

In conclusion, this study found that E2F7 targets PKMYT1 to regulate PKMYT1, which promotes GC

cell proliferation and partially inhibits ADM sensitivity through activation of the MAPK pathway, providing a promising therapeutic target for the treatment of GC.

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