Protein Phosphatase 2A Inhibits Gastric Cancer Cell Glycolysis by Reducing MYC Signaling

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Abstract

Background: The Warburg effect is closely associated malignant phenotypes and poor prognosis in cancer patients. PP2A is a highly conserved eukaryotic serine/threonine protein phosphatase that functions as a tumor suppressor in a variety of human cancers. However, the relationship between PP2A and the Warburg effect has yet to be fully understood.

Methods: The expression profile of two endogenous inhibitors of PP2A, SET and CIP2A, are detected by real-time qPCR. Loss-of-function and gain-of-function are performed to demonstrate the roles of PP2A in gastric cancer cell proliferation and glycolysis. Cell biological, molecular, and biochemical approaches are used to uncover the underlying mechanism.

Results: In this study, we find that SET and CIP2A are overexpressed in gastric cancer and associates a decreased PP2A activity. Pharmacological activation of PP2A with FTY-720 and DT-061 significantly reduces gastric cancer cell proliferation and glycolytic ability. Importantly, inhibition of PP2A activity by genetic silencing of PPP2R5A induces a growth advantage, which can be largely compromised by addition of the glycolysis inhibitor 2-Deoxy-D-glucose, suggesting a glycolysis-dependent effect of PP2A in gastric cancer. Mechanistically, the well known transcription factor and glycolysis regulator c-Myc is discovered as the functional mediator of PP2A in regulating cell glycolysis. Ectopic expression of a phosphorylation-mutant c-Myc resistant to PP2A (MycT58A) restores the inhibitory effect of FTY-720 and DT-061 on the lactate production and glucose uptake. Furthermore, there is a close association between SET and CIP2A expression and c-Myc gene signatures in gastric cancer samples.

Conclusions: This study provides strong evidence of the involvement of PP2A in the Warburg effect and indicates that it could be a novel antitumor strategy to target tumor metabolism in gastric cancer.

1. Introduction

Gastric cancer is one of the most common malignant tumors in the world. The incidence and mortality of gastric cancer in East Asia and China remain high [1, 2]. Due to the low frequency of regular gastroscopy, insidious early symptoms, poor results of radiotherapy and chemotherapy, the mortality rate of gastric cancer has remained high, the clinical outcome of patients with advanced and metastatic gastric cancer is still not optimistic [3]. Therefore, in-depth studying of the molecular mechanism involved in gastric cancer occurrence and development and developing effective diagnosis and treatment methods are urgently needed.

Due to the hypoxia and low pH microenvironment, the glucose metabolism of tumor cells is different from that of normal cells, which is manifested by high glycolysis but not oxidative phosphorylation and known as the Warburg effect [4, 5]. Abnormal energy metabolism especially the Warburg effect is an emerged as a key feature of most tumor cells [6]. Increased Warburg effect is associated many oncogenic phenotypes in cancers, such as rapid cell proliferation, drug resistance, and stemness [7]. Therapies targeting the Warburg effect have a profound inhibitory effect on tumor progression. In gastric cancer,

glycolytic proficiency negatively affects survival outcomes of metastatic gastric cancer patients treated with paclitaxel-ramucirumab systemic therapy [8]. Notably, several reports have documented the regulators of the Warburg effect in gastric cancer, such as MACC1 [9], FBP1 [10], WTAP [11], and MUC16 [12]. However, the molecular mechanisms underlying the Warburg effect in gastric cancer remain unclear.

Protein Phosphatase 2A (PP2A) is a highly conserved eukaryotic serine/threonine protein phosphatase with functions that counter-balance kinase-mediated phosphorylation [13]. PP2A is a heterotrimeric enzyme comprised of a scaffolding subunit (A), regulatory subunit (B) and a catalytic subunit (C) [14]. PP2A is frequently inactivated in human cancers and is considered as a tumor suppressor. Therefore, strategies of improving PP2A activity have been regarded as a promising therapeutic intervention. PP2A activity is tightly regulated by several endogenous inhibitors especially SET and cancerous inhibitor of PP2A (CIP2A), which have been shown to be overexpressed in a variety of human cancers [15]. SET is first identified as inhibitor of PP2A through isolation from bovine kidney and is upregulated in many cancers such as leukemia and breast cancer [16]. CIP2A is an oncoprotein that prevents PP2A-mediated dephosphorylation of c-Myc at Ser 62 and contributes to the stabilization of c-Myc [15]. PP2A has been shown to regulate many cellular processes in gastric cancer including, but not limited to, growth, stemness, and apoptosis [17, 18, 19, 20]. However, the link between PP2A and the Warburg effect remains largely unknown.

In this study, we show that highly expressed SET and CIP2A lead to reduced PP2A activity in gastric cancer. Pharmacological activation of PP2A with FTY-720 or DT-061 decreases the colony formation ability and glycolytic capacity of gastric cancer cells. PP2A activation decreases phosphorylated c-Myc levels and c-Myc transcriptional activity. Taken together, our study suggests that PP2A plays an important role in the Warburg effect by modulating c-Myc signaling.

2. Materials And Methods

2.1. Gene expression analysis

For analysis of SET and CIP2A expression in gastric cancer samples, data from The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) were used and analyzed by the GEPIA database [21]. TCGA RNA-seq data of gastric cancer samples (n = 408) was used for correlation analysis and the correlation coefficient was determined by Spearman method.

2.2. Cell culture and reagents

The eight human gastric cancer cell lines: AGS, BGC-823, HGC-27, MGC-803, MKN-28, MKN-45, N87 and SGC-7901 were acquired from ATCC or the Institute of Biochemistry and Cell Biology, Chinese Academy of Science (Shanghai, China). These cancer cells were routinely cultured in RPMI-1640 medium or DMEM, supplemented with 10% (v/v) of fetal bovine serum (FBS, Gibco, Grand Island, NY, USA) and 1% (v/v) streptomycin-penicillin (Sigma-Aldrich, Shanghai, China). All cells were maintained at 37°C in a 5% CO_2

incubator. FTY-720 (SET inhibitor, S5002), DT-061 (PP2A activator, S8774), and 2-Deoxy-D-glucose (2-DG, S4701) was obtained from Selleck (Shanghai, China).

2.3. Cell transfection

Two specific siRNAs against PPP2R5A were used to knockdown PPP2R5A in gastric cancer cells. MycT58A was cloned into pcDNA3.1 for ectopic expression. For siRNA experiment, the siRNAs of PPP2R5A were obtained from Genepharma Biotechnology (Shanghai, China); the antisense sequences were: PPP2R5A-#1: TTAGTTGAAACATACTCAACCA; PPP2R5A-#2: TTTAATTATATTATACTGATGA. Scramble non-target siRNAs were used as negative controls. Cell transfection was performed with 15 μmol siRNAs by Lipofectamine RNAiMAX reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Western blotting was used to determine knockdown efficiency.

2.4. Detection of PP2A activity

The PP2A activity in response to FTY-720, DT061, and PPP2R5A knockdown was detected by PP2A Immunoprecipitation Phosphatase Assay Kit (Millipore, USA) in according to the manufacturer's protocol. Briefly, gastric cancer cells with indicated treatment were lysated by NP-40 lysis buffer supplemented with protease inhibitor cocktail. Then, cell lysates was mixed with PP2A antibody and protein A slurry. After washing with TBS and assay buffer for three times, phosphopeptide was added and allowed to incubation for 10 min at room temperature. Finally, Malachite green solution was added, followed by absorbance detection at 650 nm.

2.5. Real-time quantitative PCR

Total RNA was extracted from gastric cancer cell lines with RNAiso Plus reagent (Takara, Japan) according to the manufacturers' instruction and then reverse transcribed with a PrimeScript RT-PCR kit (Takara, Japan). cDNA was amplified by PCR with 10 μl reaction system using SYBR-Green PCR Master Mix in a Fast Real-time PCR 7500 System (Applied Biosystems, USA). All real-time qPCR reactions were completed in triplicate. The primer sequences used in this study were shown as follows: HK2 forward, 5'-TTGACCAGGAGATTGACATGGG-3', HK2 reverse, 5'-CAACCGCATCAGGACCTCA-3'; PFKL forward, 5'-GCTGGGCGGCACTATCATT-3', PFKL reverse, 5'-TCAGGTGCGAGTAGGTCCG-3'; LDHA forward, 5'-ATGGCAACTCTAAAGGATCAGC-3', LDHA reverse, 5'-CCAACCCCAACAACTGTAATCT-3'. GAPDH forward, 5'-CTGGGCTACACTGAGCACC-3', GAPDH reverse, 5'- AAGTGGTCGTTGAGGGCAATG-3'. GAPDH was served as an internal control.

2.6. Western blotting

Gastric cancer cells with indicated treatment were rinsed with cold PBS before treated with RIPA lysis buffer (50 mM Tris, 0.15 M NaCl, 1 mM EGTA, 1% NP40, 0.25% SDS, pH 7.4) containing protease and phosphatase inhibitors. Cell pellets were incubated for 30 min on ice to homogenize fully and then total proteins in the supernatant of cell lysates were collected by centrifuging at 4°C at 12,000 rpm for 10 min. Total protein concentrations were measured by the BCA protein assay kit (Pierce, USA) before experiment. Then, standard western blotting assays (SDS-PAGE) were used to analyze protein expression. The

antibodies used for western blotting analysis in this study included: anti-PPP2R5A (Abcam, ab89621, 1:2,000 dilution), anti-c-Myc antibodies (Cell Signaling Technology, #5605, 1:1,000 dilution), anti-p-c-Myc (S62) antibodies (Cell Signaling Technology, #13748,1:1,000 dilution), anti-β-actin antibody (Sigma, A2228, 1:5,000 dilution). After incubation with corresponding species-specific secondary HRP-conjugated antibodies, the target protein bands were visualized by an ECL imaging system.

2.7. Glucose uptake and lactate production

Glucose and lactate levels in the culture medium were detected using Glucose Assay kit (Biovision, Milpitas, CA, USA) and Lactate Assay kit (Biovision, Milpitas, CA, USA), respectively. In brief, gastric cancer cells were seeded at 5 x 10^5 cells in 6-cm cell culture dishes and allowed to grow for 24 h with indicated treatment. After 24 h, the culture medium collected and centrifuged at 2,000 rpm for 5 min to obtain the supernatant without cell debris. Finally, glucose and lactate assays were performed according to the manufacturer's instructions, and colorimetric density was assessed using a Multifunctional microplate reader.

2.8. Plate colony formation assay

Gastric cancer cells with were resuspended and plated in 6-well plates at a density of 500 cells per well and allowed to grow for 10–14 days with indicated treatment. Then the cells were fixed with 4% paraformaldehyde for 15 min at room temperature and stained with 0.5% crystal violet for 15 min. The experiment was performed in triplicate.

2.9. Measurement of c-Myc transcriptional activity

The c-Myc transcription factor assay Kit (Abcam, ab207200) was used to quantify c-Myc activation in nuclear extracts from gastric cancer cells. In brief, HGC-27 and MGC-803 cells were subjected to treatment with 5 μM FTY-720 or 10 μM DT-061 for 24h, followed by detection of c-Myc transcriptional activity in according to the manufacturer's instructions.

2.10. Statistical analysis

All data were represented as mean ± standard deviation (SD). GraphPad 6.0 (GraphPad Software Inc., San Diego, CA) was used for statistical analysis. The correlation of gene expression was evaluated by Spearman's correlation. P-values were calculated by two-tailed unpaired Student's t-test or one-way ANOVA. A value of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Increased SET and CIP2A expression are associated with reduced PP2A activity in gastric cancer

To begin investigating the role of PP2A in gastric cancer, we first determined the expression of SET and CIP2A, two known oncogenic inhibitors of PP2A. Consistent with previous reports, data obtained from

TCGA and GTEx cohorts showed that SET and CIP2A expression levels were high in gastric cancer tissues compared with normal samples (Fig. 1A). By real-time qPCR analysis, we found that SET and CIP2A mRNA expression were significantly increased in 87.5% (7/8) and 75% (6/8) of the gastric cancer cell lines, respectively (Fig. 1B). By measuring the PP2A activity, we found that 75% (6/8) of the gastric cancer cell lines had an increased PP2A activity compared with the nonmalignant GES1 cells (Fig. 1C). Of note, SET or CIP2A expression was closely associated with a reduced PP2A activity in gastric cancer cell lines (Fig. 1D). Therefore, decreased PP2A activity in gastric cancer appears to be induced by aberrant SET and CIP2A expression.

3.2. FTY-720 and DT-061 suppress gastric cancer proliferation in a dose-dependent manner

To better understand the role of PP2A in gastric cancer, we used a known SET inhibitor FTY-720 that activates PP2A and a small-molecule activator of PP2A DT-061 to treat HGC-27 and MGC-803 cells [22]. As shown in Fig. 2A-B, FTY-720 and DT-061 treatment led to a significant increase in PP2A activity in gastric cancer cells. Plate colony formation assay showed that FTY-720 and DT-061 suppressed the colony formation ability of HGC-27 and MGC-803 cells in a dose-dependent manner (Fig. 2C-D), suggesting the tumor-suppressive role of PP2A in gastric cancer.

3.3. PP2A activation inhibits the Warburg effect in gastric cancer

Occasionally, we found that cell culture medium was acidified much slower after FTY-720 and DT-061 treatment for 24 h. Therefore, we speculated a potential role of PP2A in regulating lactate production, a classical character of the Warburg effect. To test this hypothesis, we measured the lactate levels in the culture medium of HGC-27 and MGC-803 cells after treatment with different concentrations of FTY-720 and DT-061. After normalization with total cell protein, we observed that the lactate level was significantly reduced by FTY-720 in HGC-27 and MGC-803 cells in a dose-dependent manner (Fig. 3A). Likewise, similar effects were also induced by DT-061 (Fig. 3B). To further confirm the impact of PP2A on the Warburg effect, we tested the glucose uptake upon FTY-720 and DT-061 treatment in gastric cancer cells. As a result, both FTY-720 and DT-061 resulted in a marked reduction of glucose uptake in HGC-27 and MGC-803 cells (Fig. 3C-D). Moreover, using real-time qPCR, we found that glycolytic genes including HK2, PFKL and LDHA were also significantly downregulated by FTY-720 or DT-061 treatment (Fig. 3E). Consistently, correlation analysis revealed that there was a high correlation between SET and CIP2A expression and glycolytic genes (HK2, PFKL and LDHA) in gastric cancer samples (Fig. 3F).

3.4. Growth advantage induced by PP2A inhibition is glycolysis-dependent in gastric cancer

Because that PP2A can inhibit gastric cancer proliferation and reduce the glycolytic ability, we next tested whether PP2A affect tumor growth via modulation tumor glycolysis. To address this hypothesis, we first genetically silenced PPP2R5A, a regulatory B subunit of the major PP2A protein complex [23], in two gastric cancer cell lines, MKN28 and SGC-7901. The knockdown efficiency was shown in Fig. 4A. PPP2R5A knockdown led to significant decrease in PP2A activity (Fig. 4B). Interestingly, lactate production (Fig. 4C) and glucose uptake (Fig. 4D) of MKN28 and SGC-7901 were remarkably upregulated by PPP2R5A knockdown. Moreover, plate colony formation assay showed that PPP2R5A knockdown boosted cell proliferation of MKN28 and SGC-7901 cells, which can be largely abrogated by addition of the known glycolysis inhibitor 2-DG (Fig. 4E). Collectively, these data above suggest that PP2A couples cell glycolysis to tumor cell proliferation in gastric cancer.

3.5. PP2A modulates c-Myc expression to suppress gastric cancer glycolysis

PP2A complex dephosphorylates serine 62 (S62) of c-Myc, rendering c-Myc as a substrate for FBXW7-mediated ubiquitination and subsequent degradation by the 26S proteasome. Importantly, c-Myc is a known transcriptional factor of the Warburg effect [24]. Therefore, we reasoned that PP2A targets c-Myc to modulate gastric cancer cell glycolysis. By western blotting, we found that PP2A activation by FTY-720 or DT-061 decreased the S62-phosphorylated c-Myc and total c-Myc levels in HGC-27 and MGC-803 cells (Fig. 5A). As the second line of evidence, c-Myc transcriptional activity was also suppressed by FTY-720 and DT-061 treatment (Fig. 5B). To determine whether aberrant expression of c-Myc can restore the suppressive effect induced by PP2A activation, we ectopic introduced MycT58A, a phosphorylation-mutant resistant to PP2A in HGC-27 and MGC-803 cells. As shown in Fig. 5C, the inhibitory effect of FTY-720 and DT-061 treatment on the lactate production and glucose uptake was largely restored by MycT58A. Furthermore, correlation analysis showed that SET or CIP2A expression was positively associated with c-Myc gene signature in gastric cancer samples (Fig. 5D). Collectively, c-Myc is a critical mediator for PP2A-induced suppressive effects on cell proliferation and glycolysis in gastric cancer (Fig. 5E).

4. Discussion

PP2A plays diverse roles in human cancers [22, 25, 26]. In this study, we investigated (i) the expression pattern of two cellular inhibitors of PP2A, SET and CIP2A, in gastric cancer, (ii) the effect of PP2A activation on tumorigenic potential and the Warburg effect, (iii) whether increased growth advantage induced by PP2A inhibition is glycolysis-dependent, (iv) the underlying molecular mechanism by which PP2A regulates the Warburg effect. Our results suggest that PP2A is profoundly implicated in the Warburg effect and be exploited as potential targets for gastric cancer therapy.

It is well documented that increased expression of PP2A-inhibitory proteins such as SET and CIP2A contributes to decreased PP2A activity in cancers [27]. SET protein levels are highly expressed in various human tumors, including chronic myeloid leukemia, pancreatic cancer, and colorectal cancer; in gastric

cancer, SET is reported to maintain cancer cell stemness by suppressing PP2A activity and stabilizing E2F1 protein [28]. Moreover, CIP2A is overexpressed in gastric cancer and predicts a poor prognosis; CIP2A knockdown is shown to negatively affect clonogenicity and senescence of tumor cells [29, 30, 31]. In this report, we confirmed that SET and CIP2A expression was highly expressed in gastric cancer samples and their dysregulation led to reduced PP2A activity.

SET can be targeted by FTY-720 (Fingolimod), a sphingosine analogue, is an FDA approved immunosuppressant used for the treatment of multiple sclerosis [13]. FTY720 is able to disrupt the interaction between SET and PP2A, thus resulting in elevated PP2A activity. FTY720 has been reported to increase the expression of Cip1/p21, p27, and BH3-only proteins to induce gastric cancer cell apoptosis and has an additive effect in killing cancer cells when in combination with Cisplatin [32]. Consistently, we revealed that FTY720 suppressed gastric cancer cell proliferation in a dose-dependent manner. Moreover, activation of PP2A with DT-061, a phenothiazine derivative, induced similar tumor-suppressive effects in gastric cancer cells. Apart from previous reports regarding the roles of PP2A in gastric cancer, we for the first time identified a novel link between PP2A and the Warburg effect. FTY720 and DT-061 treatment significantly boosted the Warburg effect of gastric cancer cells as evidenced by increased lactate release, glucose uptake, and expression of glycolytic genes. Moreover, PP2A inactivation by knockdown of PPP2R5A facilitated cell growth and blocking glycolysis with 2-DG largely abrogated this effect. Therefore, our findings further broaden the roles of PP2A in gastric cancer and suggest a glycolysis-dependent effect of PP2A.

Prior works have highlighted c-Myc as critical substrate of PP2A complex in cancers [25, 33, 34, 35]. For instance, PP2A activation by a novel SET antagonist OP449 decreases S62 phosphorylation of c-Myc and reduces c-Myc activity and expression of downstream target genes in breast cancer [36]. In MYC-driven cancer, DT-061 treatment inhibited c-Myc expression via proteasome-mediated degradation, resulting in tumor growth inhibition [37]. Given the important role of c-Myc in regulating the Warburg effect, we also tested c-Myc changes in the presence of PP2A activation. Expectedly, S62 phosphorylation of c-Myc was reduced by FTY720 or DT-061 treatment in gastric cancer cells and ectopic expression of c-MycT58A was competent to restore the inhibitory effect of PP2A on the Warburg effect. However, future work comprehensively illustrating the full spectrum of substrates of PP2A and whether these substrates are involved in the regulation of the Warburg effect may be very important.

Conclusion

In conclusion, our results reveal that dysregulation of PP2A-inhibitory proteins leads to reduced PP2A activity. We propose that PP2A regulates c-Myc activity to transcriptionally repress the expression of glycolytic genes and the Warburg effect. Given the important role of the Warburg effect in facilitating tumor growth, our data further supports the pursuit of PP2A as a target for cancer therapy in gastric cancer.

Abbreviations

PP2A: Protein phosphatase 2A; CIP2A: cancerous inhibitor of PP2A; TCGA: The Cancer Genome Atlas; GTEx: Genotype-Tissue Expression; 2-DG: 2-deoxy-D-glucose.

Declarations

Ethical Approval and Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All data needed to evaluate the conclusions in the paper are present in the paper. Additional data related to this paper may be requested from the authors.

Competing interests

The authors declare that they have no competing interests.

Finding

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Author contributions

WZ and ZC carried out in vitro cell experiments, manuscript preparation and statistical analysis; WZ, ZC, RZ, XL, and YH carried out in vitro experiments; WZ, ZC, RZ, XL, and YH conceived, designed, supervised, analyzed and interpreted the study and provided critical review.

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References

1. Allemani C, Matsuda T, Di Carlo V, Harewood R, Matz M, Niksic M, Bonaventure A, Valkov M, Johnson CJ, Esteve J, Ogunbiyi OJ, Azevedo ESG, Chen WQ, Eser S, Engholm G, Stiller CA, Monnereau A, Woods RR, Visser O, Lim GH, Aitken J, Weir HK, Coleman MP, Group CW. Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): analysis of individual records for 37 513 025 patients

diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. Lancet. 2018;391:1023–75.

2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J Clin (2021).
3. Yoshida N, Doyama H, Yano T, Horimatsu T, Uedo N, Yamamoto Y, Kakushima N, Kanzaki H, Hori S, Yao K, Oda I, Katada C, Yokoi C, Ohata K, Yoshimura K, Ishikawa H, Muto M. Early gastric cancer detection in high-risk patients: a multicentre randomised controlled trial on the effect of second-generation narrow band imaging. Gut. 2021;70:67–75.
4. Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. Nat Rev Cancer. 2011;11:85–95.
5. Doherty JR, Cleveland JL. Targeting lactate metabolism for cancer therapeutics. J Clin Invest. 2013;123:3685–92.
6. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646–74.
7. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? Nat Rev Cancer. 2004;4:891–9.
8. Ruzzo A, Graziano F, Bagaloni I, Di Bartolomeo M, Prisciandaro M, Aprile G, Ongaro E, Vincenzi B, Perrone G, Santini D, Fornaro L, Vivaldi C, Tomasello G, Loupakis F, Lonardi S, Fassan M, Valmasoni M, Sarti D, Lorenzini P, Catalano V, Bisonni R, Del Prete M, Collina G, Magnani M. Glycolytic competence in gastric adenocarcinomas negatively impacts survival outcomes of patients treated with salvage paclitaxel-ramucirumab. Gastric Cancer. 2020;23:1064–74.
9. Lin L, Huang H, Liao W, Ma H, Liu J, Wang L, Huang N, Liao Y, Liao W. MACC1 supports human gastric cancer growth under metabolic stress by enhancing the Warburg effect. Oncogene. 2015;34:2700–10.
10. Liu X, Wang X, Zhang J, Lam EK, Shin VY, Cheng AS, Yu J, Chan FK, Sung JJ, Jin HC. Warburg effect revisited: an epigenetic link between glycolysis and gastric carcinogenesis. Oncogene. 2010;29:442–50.
11. Yu H, Zhao K, Zeng H, Li Z, Chen K, Zhang Z, Li E, Wu Z. N(6)-methyladenosine (m(6)A) methyltransferase WTAP accelerates the Warburg effect of gastric cancer through regulating HK2 stability. Biomed Pharmacother. 2021;133:111075.
12. Zhao H, Zhang L. MUC16 mutation predicts a favorable clinical outcome and correlates decreased Warburg effect in gastric cancer. Biochem Biophys Res Commun. 2018;506:780–6.
13. O'Connor CM, Perl A, Leonard D, Sangodkar J, Narla G. Therapeutic targeting of PP2A. Int J Biochem Cell Biol. 2018;96:182–93.
14. Shi Y. Serine/threonine phosphatases: mechanism through structure. Cell. 2009;139:468–84.
15. Soofiyani SR, Hejazi MS, Baradaran B. The role of CIP2A in cancer: A review and update. Biomed Pharmacother. 2017;96:626–33.
16. Kauko O, Westermarck J. Non-genomic mechanisms of protein phosphatase 2A (PP2A) regulation in cancer. Int J Biochem Cell Biol. 2018;96:157–64.

17. Goguet-Rubio P, Amin P, Awal S, Vigneron S, Charrasse S, Mechali F, Labbe JC, Lorca T, Castro A. PP2A-B55 Holoenzyme Regulation and Cancer, Biomolecules 10 (2020).
18. Bao Y, Oguz G, Lee WC, Lee PL, Ghosh K, Li J, Wang P, Lobie PE, Ehmsen S, Ditzel HJ, Wong A, Tan EY, Lee SC, Yu Q. EZH2-mediated PP2A inactivation confers resistance to HER2-targeted breast cancer therapy. Nat Commun. 2020;11:5878.
19. Coles GL, Cristea S, Webber JT, Levin RS, Moss SM, He A, Sangodkar J, Hwang YC, Arand J, Drainas AP, Mooney NA, Demeter J, Spradlin JN, Mauch B, Le V, Shue YT, Ko JH, Lee MC, Kong C, Nomura DK, Ohlmeyer M, Swaney DL, Krogan NJ, Jackson PK, Narla G, Gordan JD, Shokat KM. J. Sage, Unbiased Proteomic Profiling Uncovers a Targetable GNAS/PKA/PP2A Axis in Small Cell Lung Cancer Stem Cells. Cancer Cell. 2020;38:129–43 e127.
20. Uddin MH, Pimentel JM, Chatterjee M, Allen JE, Zhuang Z, Wu GS. Targeting PP2A inhibits the growth of triple-negative breast cancer cells. Cell Cycle. 2020;19:592–600.
21. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 2017;45:W98–102.
22. Mazhar S, Taylor SE, Sangodkar J, Narla G. Targeting PP2A in cancer: Combination therapies. Biochim Biophys Acta Mol Cell Res. 2019;1866:51–63.
23. Liu Z, Yoshimi A, Wang J, Cho H, Chun-Wei Lee S, Ki M, Bitner L, Chu T, Shah H, Liu B, Mato AR, Ruvolo P, Fabbri G, Pasqualucci L, Abdel-Wahab O, Rabadan R. Mutations in the RNA Splicing Factor SF3B1 Promote Tumorigenesis through MYC Stabilization. Cancer Discov. 2020;10:806–21.
24. Dang CV. MYC on the path to cancer. Cell. 2012;149:22–35.
25. Allen-Petersen BL, Risom T, Feng Z, Wang Z, Jenny ZP, Thoma MC, Pelz KR, Morton JP, Sansom OJ, Lopez CD, Sheppard B, Christensen DJ, Ohlmeyer M, Narla G, Sears RC. Activation of PP2A and Inhibition of mTOR Synergistically Reduce MYC Signaling and Decrease Tumor Growth in Pancreatic Ductal Adenocarcinoma. Cancer Res. 2019;79:209–19.
26. Sangodkar J, Perl A, Tohme R, Kiselar J, Kastrinsky DB, Zaware N, Izadmehr S, Mazhar S, Wiredja DD, O'Connor CM, Hoon D, Dhawan NS, Schlatzer D, Yao S, Leonard D, Borczuk AC, Gokulrangan G, Wang L, Svenson E, Farrington CC, Yuan E, Avelar RA, Stachnik A, Smith B, Gidwani V, Giannini HM, McQuaid D, McClinch K, Wang Z, Levine AC, Sears RC, Chen EY, Duan Q, Datt M, Haider S, Ma'ayan A, DiFeo A, Sharma N, Galsky MD, Brautigan DL, Ioannou YA, Xu W, Chance MR, Ohlmeyer M, Narla G. Activation of tumor suppressor protein PP2A inhibits KRAS-driven tumor growth. J Clin Invest. 2017;127:2081–90.
27. Sangodkar J, Farrington CC, McClinch K, Galsky MD, Kastrinsky DB, Narla G. All roads lead to PP2A: exploiting the therapeutic potential of this phosphatase. FEBS J. 2016;283:1004–24.
28. Enjoji S, Yabe R, Tsuji S, Yoshimura K, Kawasaki H, Sakurai M, Sakai Y, Takenouchi H, Yoshino S, Hazama S, Nagano H, Oshima H, Oshima M, Vitek MP, Matsuura T, Hippo Y, Usui T, Ohama T, Sato K. Stemness Is Enhanced in Gastric Cancer by a SET/PP2A/E2F1 Axis. Mol Cancer Res. 2018;16:554–63.

29. Wang J, Okkeri J, Pavic K, Wang Z, Kauko O, Halonen T, Sarek G, Ojala PM, Rao Z, Xu W, Westermarck J. Oncoprotein CIP2A is stabilized via interaction with tumor suppressor PP2A/B56. EMBO Rep. 2017;18:437–50.
30. Khanna A, Bockelman C, Hemmes A, Junttila MR, Wiksten JP, Lundin M, Junnila S, Murphy DJ, Evan GI, Haglund C, Westermarck J, Ristimaki A. MYC-dependent regulation and prognostic role of CIP2A in gastric cancer. J Natl Cancer Inst. 2009;101:793–805.
31. Li W, Ge Z, Liu C, Liu Z, Bjorkholm M, Jia J, Xu D. CIP2A is overexpressed in gastric cancer and its depletion leads to impaired clonogenicity, senescence, or differentiation of tumor cells. Clin Cancer Res. 2008;14:3722–8.
32. Zheng T, Meng X, Wang J, Chen X, Yin D, Liang Y, Song X, Pan S, Jiang H, Liu L. PTEN- and p53-mediated apoptosis and cell cycle arrest by FTY720 in gastric cancer cells and nude mice. J Cell Biochem. 2010;111:218–28.
33. Scarpa M, Singh P, Bailey CM, Lee JK, Kapoor S, Lapidus RG, Niyongere S, Sangodkar J, Wang Y, Perrotti D, Narla G, Baer MR. PP2A-activating Drugs Enhance FLT3 Inhibitor Efficacy through AKT Inhibition-Dependent GSK-3beta-Mediated c-Myc and Pim-1 Proteasomal Degradation, Mol Cancer Ther (2021).
34. Zhang L, Zhou H, Li X, Vartuli RL, Rowse M, Xing Y, Rudra P, Ghosh D, Zhao R, Ford HL. Eya3 partners with PP2A to induce c-Myc stabilization and tumor progression. Nat Commun. 2018;9:1047.
35. Tan J, Lee PL, Li Z, Jiang X, Lim YC, Hooi SC, Yu Q. B55beta-associated PP2A complex controls PDK1-directed myc signaling and modulates rapamycin sensitivity in colorectal cancer. Cancer Cell. 2010;18:459–71.
36. Janghorban M, Farrell AS, Allen-Petersen BL, Pelz C, Daniel CJ, Oddo J, Langer EM, Christensen DJ, Sears RC. Targeting c-MYC by antagonizing PP2A inhibitors in breast cancer. Proc Natl Acad Sci U S A. 2014;111:9157–62.
37. Farrington CC, Yuan E, Mazhar S, Izadmehr S, Hurst L, Allen-Petersen BL, Janghorban M, Chung E, Wolczanski G, Galsky M, Sears R, Sangodkar J, Narla G. Protein phosphatase 2A activation as a therapeutic strategy for managing MYC-driven cancers. J Biol Chem. 2020;295:757–70.

Figures

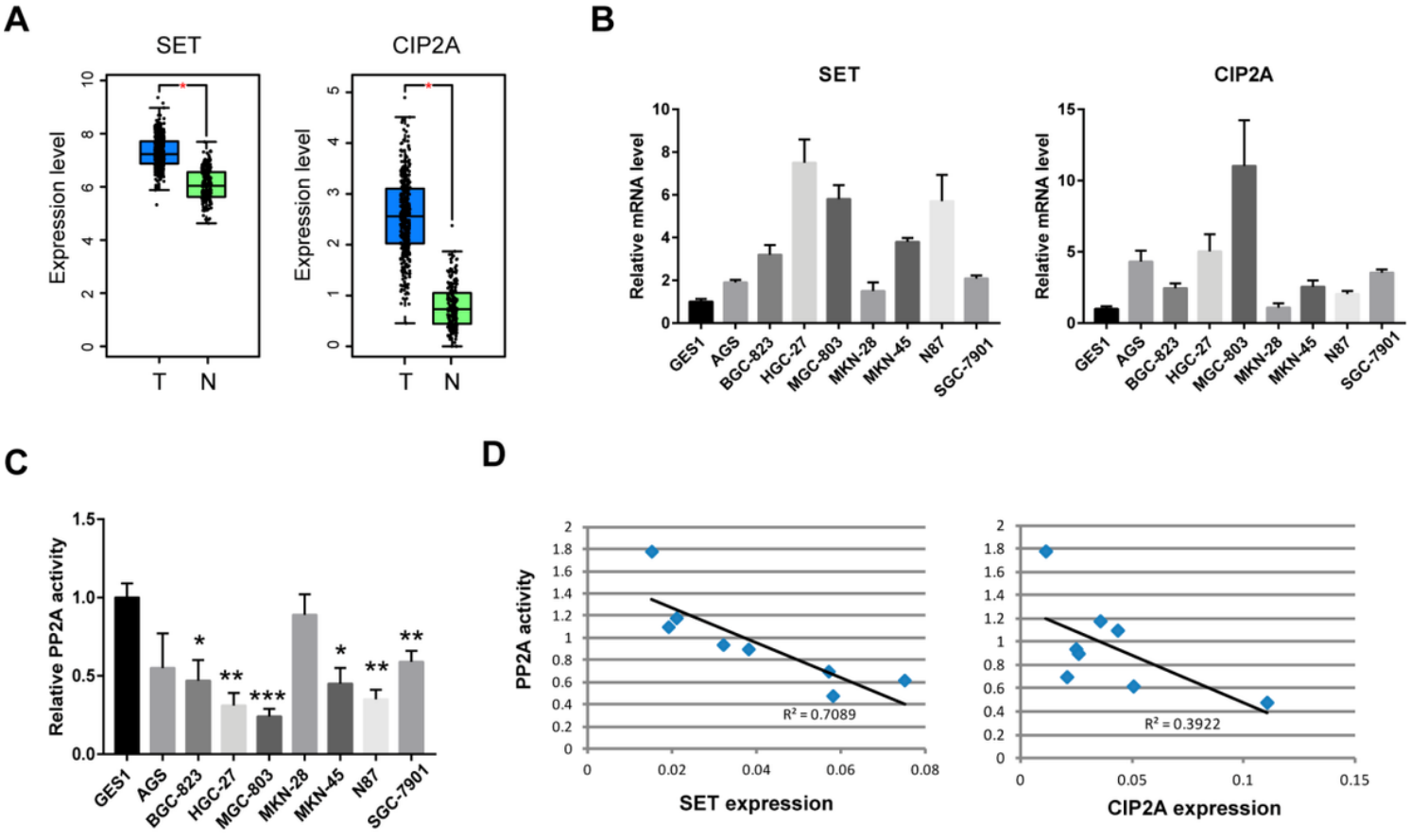

Figure 1

Increased SET and CIP2A expression are associated with reduced PP2A activity in gastric cancer. (A) Data from TCGA and GTEx cohort showed the expression levels of SET and CIP2A in gastric cancer tissues (n = 408) and normal tissues (n = 211). (B) Real-time qPCR analysis of SET and CIP2A expression in gastric cancer cell lines; GES1 cell line was set as a normal control. (C) Measurement of PP2A activity in 8 gastric cancer cell lines and GES1 cell line. (D) Correlation analysis of SET and CIP2A expression and PP2A activity in gastric cancer cell lines. *p < 0.05; **p < 0.01; ***p < 0.001

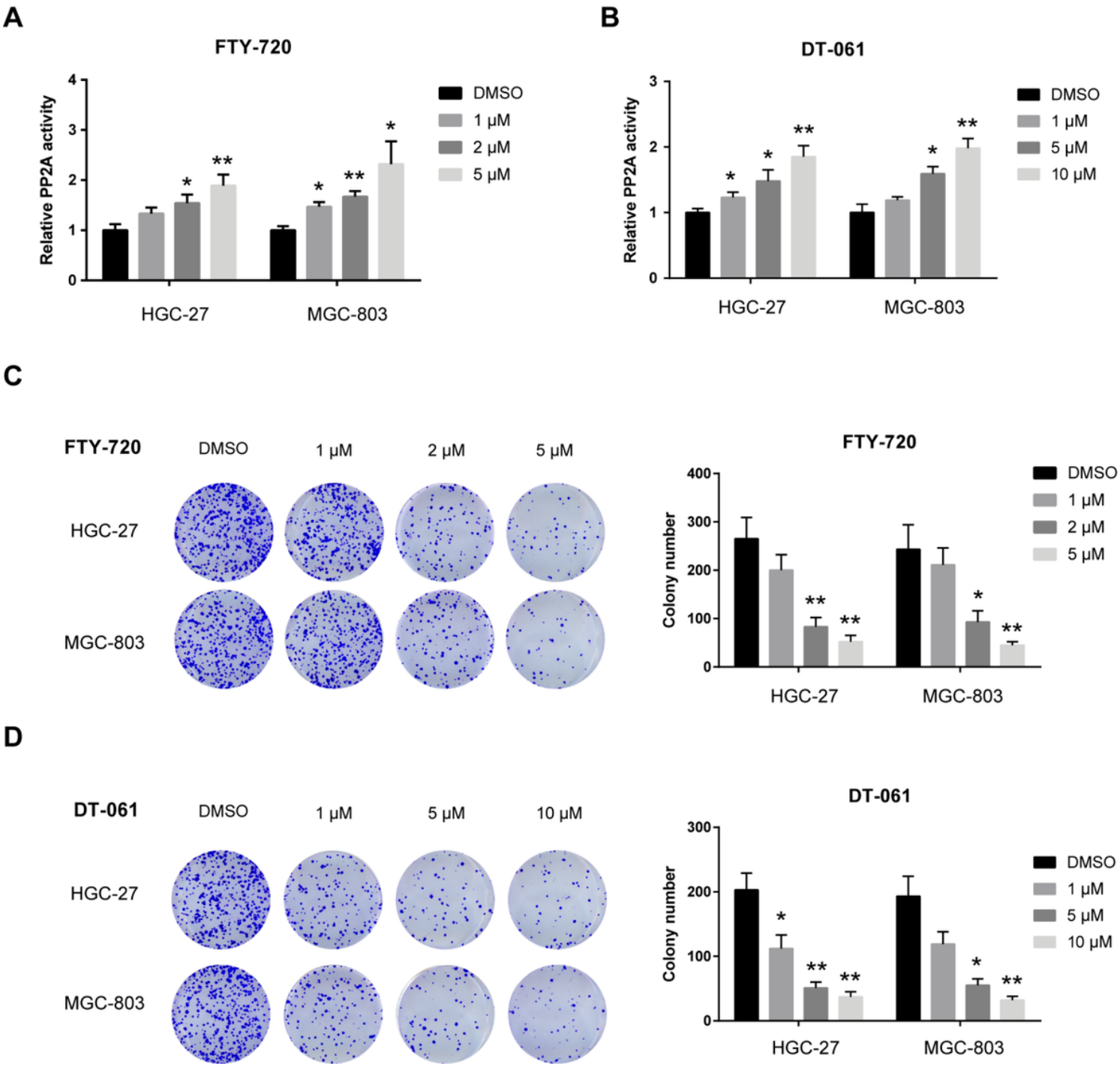

Figure 2

FTY-720 and DT-061 suppress gastric cancer proliferation in a dose-dependent manner. (A) Measurement of PP2A activity upon treatment with different concentrations of FTY-720 (1 μM, 2 μM and 5 μM) in HGC-27 and MGC-803 cells. (B) Measurement of PP2A activity upon treatment with different concentrations of DT-061 (1 μM, 5 μM and 10 μM) in HGC-27 and MGC-803 cells. (C) The effect of FTY-720 (1 μM, 2 μM and 5 μM) on the proliferation of HGC-27 and MGC-803 cells was analyzed by plate colony formation assay. (D) The effect of DT-061 (1 μM, 5 μM and 10 μM) on the proliferation of HGC-27 and MGC-803 cells was analyzed by plate colony formation assay. *$p < 0.05$; **$p < 0.01$.

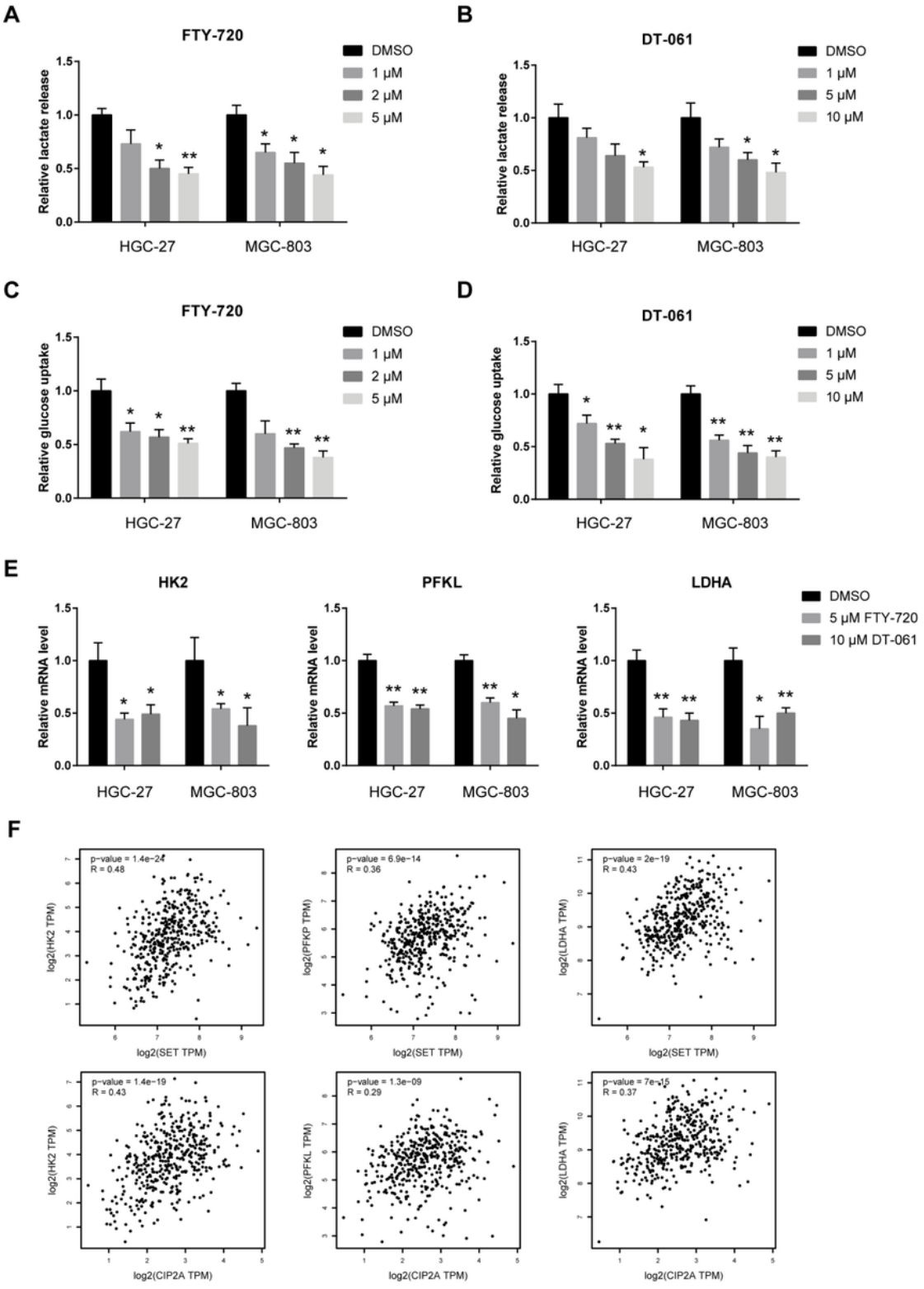

Figure 3

PP2A activation inhibits the Warburg effect in gastric cancer. (A) The effect of FTY-720 (1 μM, 2 μM and 5 μM) on the lactate production of HGC-27 and MGC-803 cells. (B) The effect of DT-061 (1 μM, 5 μM and 10 μM) on the lactate production of HGC-27 and MGC-803 cells. (C) The effect of FTY-720 (1 μM, 2 μM and 5 μM) on the glucose uptake of HGC-27 and MGC-803 cells. (D) The effect of DT-061 (1 μM, 5 μM and 10 μM) on the glucose uptake of HGC-27 and MGC-803 cells. (E) Real-time qPCR analysis of

glycolytic genes (HK2, PFKL and LDHA) in HGC-27 and MGC-803 cells in the presence or absence of treatment with 5 μM FTY-720 or 10 μM DT-061. (F) Correlation analysis of SET and CIP2A expression and glycolytic genes (HK2, PFKL and LDHA) in gastric cancer; data were acquired from TCGA cohort. *p < 0.05; **p < 0.01.

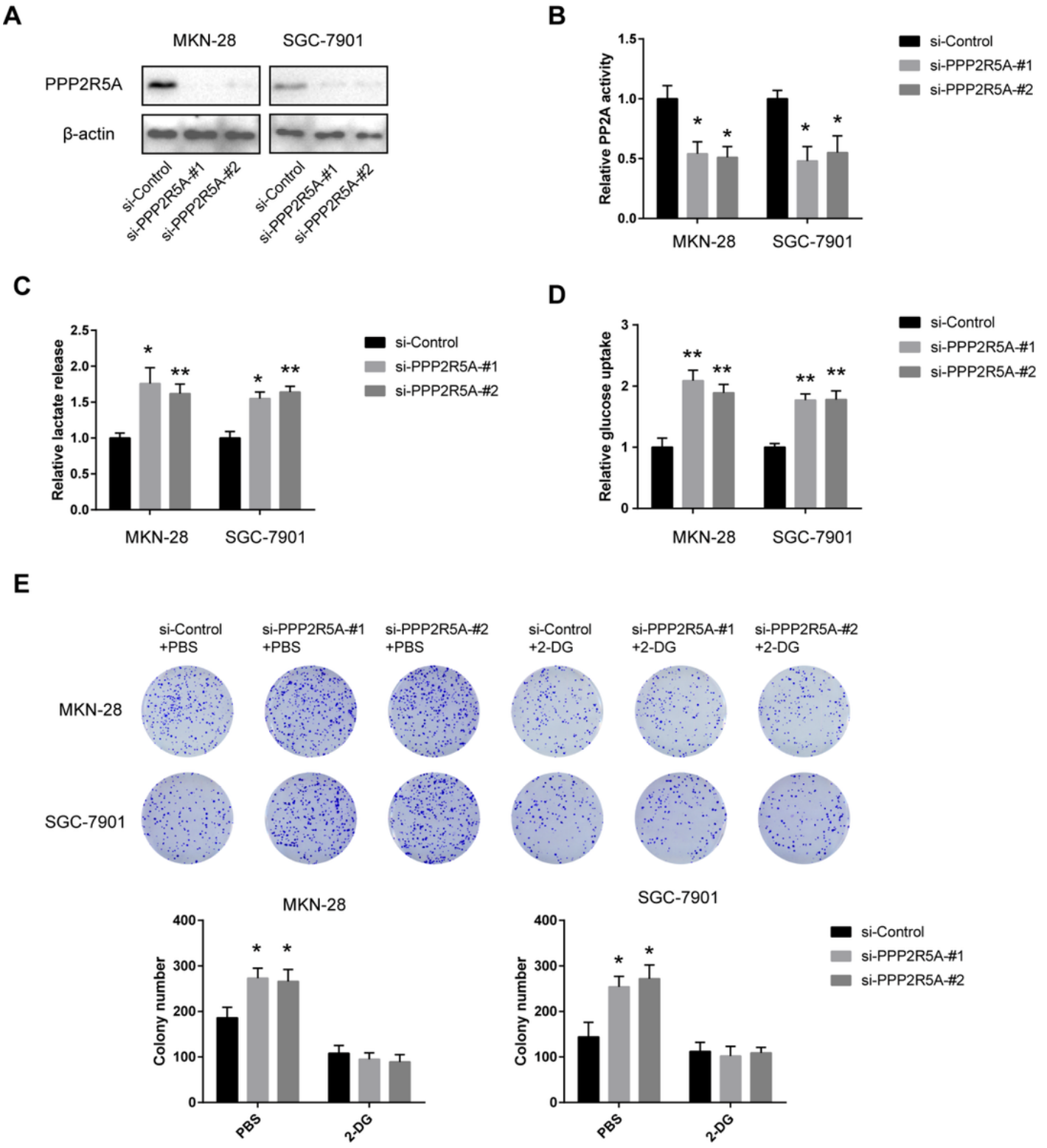

Figure 4

Growth advantage induced by PP2A inhibition is glycolysis-dependent in gastric cancer. (A) The knockdown efficiency of PPP2R5A in MKN28 and SGC-7901 cells was verified by western blotting. (B) Measurement of PP2A activity in si-Control and si-PPP2R5A MKN28 and SGC-7901 cells. (C) The effect of PPP2R5A knockdown on the glucose uptake of MKN28 and SGC-7901 cells. (D) The effect of PPP2R5A knockdown on the lactate production of MKN28 and SGC-7901 cells. (E) The effect of PPP2R5A knockdown on the proliferation of MKN28 and SGC-7901 cells in the presence or absence of 2-DG was analyzed by plate colony formation assay. *p < 0.05; **p < 0.01.

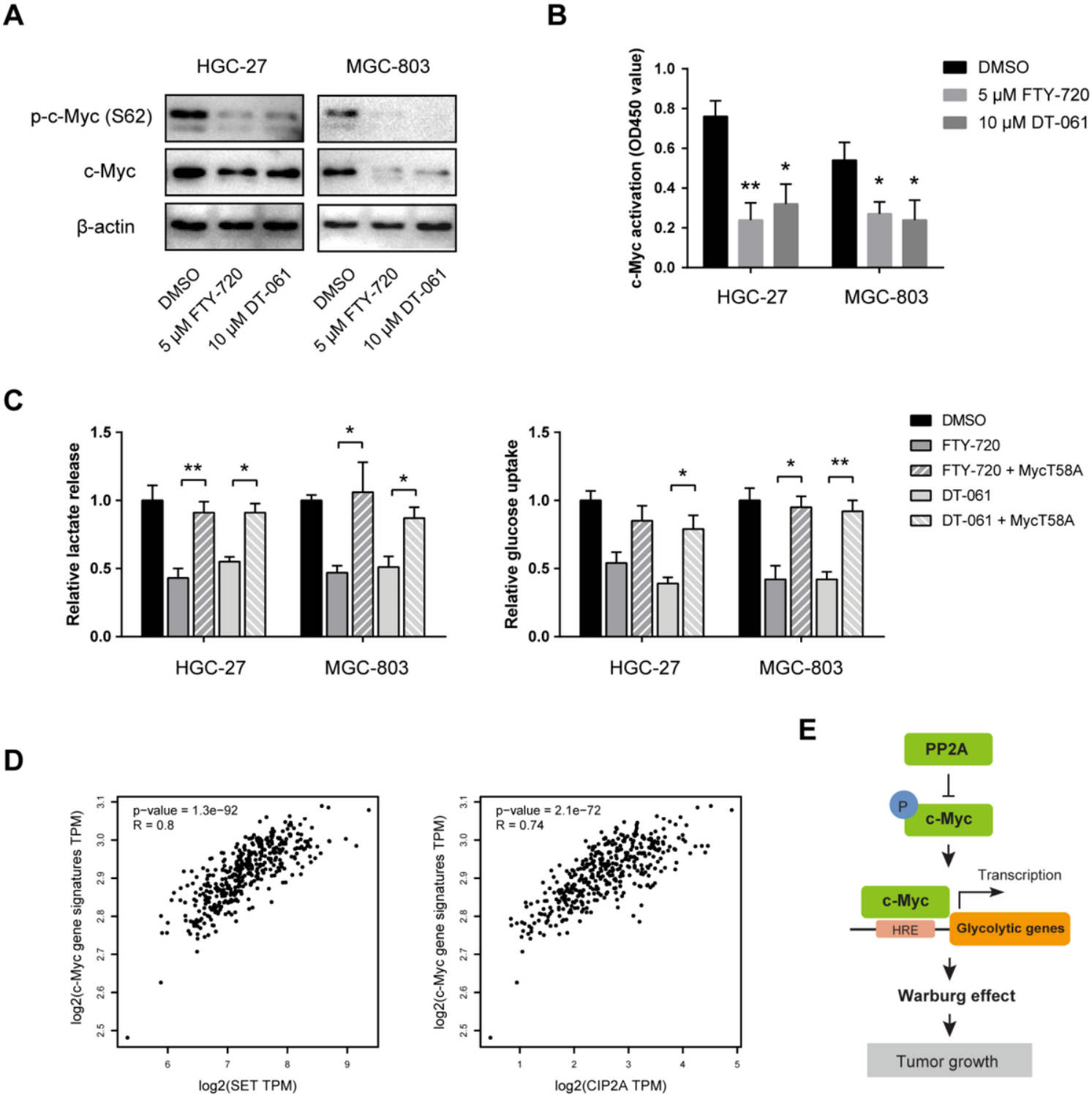

Figure 5

PP2A modulates c-Myc expression to suppress gastric cancer glycolysis. (A) Western blotting analysis of c-Myc and p-c-Myc (S62) levels in HGC-27 and MGC-803 cells in the presence or absence of treatment with 5 μM FTY-720 or 10 μM DT-061. (B) Measurement the effect of 5 μM FTY-720 or 10 μM DT-061 treatment on c-Myc transcriptional activity in HGC-27 and MGC-803 cells. (C) The effect of 5 μM FTY-720 or 10 μM DT-061 treatment on lactate production or glucose uptake in HGC-27 and MGC-803 cells transfected with or without MycT58A. (D) Correlation analysis of SET and CIP2A expression and c-Myc gene signatures in gastric cancer; data were acquired from TCGA cohort. (E) Model illustrating the mechanism by which PP2A regulates c-Myc to inhibit the Warburg effect and cell proliferation in gastric cancer. *$p < 0.05$; **$p < 0.01$.