Cancer Research

Transcription Factor YY1 Promotes Cell Proliferation by Directly Activating the Pentose Phosphate Pathway

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Abstract

Tumor cells alter their metabolism to meet their demand for macromolecules and support a high rate of proliferation as well as cope with oxidative stress. The transcription factor yin yang 1 (YY1) is upregulated in various types of tumors and is crucial for tumor cell proliferation and metastasis. However, its role in tumor cell metabolic reprogramming is poorly understood. Here, we show that YY1 alters tumor cell metabolism by activating glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme in the pentose phosphate pathway. By stimulating the pentose phosphate pathway, YY1 enhanced production of nucleotides and DNA synthesis, decreased intracellular reactive oxygen species levels, and promoted antioxidant defense by supplying increased reducing power in the form of NADPH. Importantly, YY1-mediated regulation of the pentose phosphate pathway in tumor cells occurred not through p53, but rather through direct activation of G6PD transcription by YY1. Regulation of pentose phosphate pathway activity through G6PD was strongly related to YY1-induced proliferation of tumor cells and tumorigenesis. Together, our results describe a novel role for YY1 in regulating G6PD in a p53-independent manner, which links its function in tumorigenesis to metabolic reprogramming in tumor cells.

Significance: This study reveals a novel role for YY1 in regulating G6PD and activating the pentose phosphate pathway, linking its function in tumorigenesis to metabolic reprogramming. *Cancer Res; 78(16); 4549–62. ©2018 AACR.*

Introduction

Metabolic reprogramming is an important hallmark of tumor cells and a key feature of the malignant phenotype (1). As first discovered by Otto Warburg, compared with normal cells, tumor cells consume glucose at a significantly high rate and prefer to metabolize it through glycolysis even in the presence of an adequate oxygen supply (2–4). Through this metabolic alteration, known as the Warburg effect, more than 90% of glucose is converted to lactate (4), which conditions the tumor cell environment to favor their invasion (5). Metabolic reprogramming not only upregulates tumor cell adaptation potential to a fluctuating oxygen tension (6), but also allows production of glycolytic intermediates, which could in turn fuel the synthesis of macromolecules required for the formation of new cells (7). Metabolic reprogramming also enhances the pentose phosphate pathway (PPP), which produces ribose-5-phosphate for *de novo* nucleotide biosynthesis (5). The PPP also generates nicotinamide adenine dinucleotide phosphate (NADPH), which is crucial for supporting cell proliferation via the reductive biosynthesis of macromolecules such as lipids. Moreover, NADPH acts as a reactive oxygen species (ROS) scavenger and thus is critical for the antioxidant defense of the cells (5, 8). However, despite the crucial role of the PPP in tumorigenesis, its regulatory mechanism is not fully understood.

Yin yang 1 (YY1) is the first identified member of the Yin Yang (YY) family, a GLI-Krüppel zinc finger protein family that contains four highly conserved C2H2 zinc finger domains (9, 10). YY1 is a multifunctional transcription factor responsible for activating or inhibiting transcription of target genes depending on the context and the recruitment of specific cofactors (11). YY1 has been estimated to regulate about 7% of mammalian genes (12). Despite its function as a transcriptional regulator, recent studies have indicated that YY1 could regulate gene expression even through other mechanisms, for example, by stabilizing hypoxia-inducible factor 1 alpha (HIF1α; ref. 13). Not surprisingly, YY1 is implicated in various biological and physiological processes, including DNA replication, cell proliferation, embryonic development, differentiation, and disease pathways such as dystrophic muscle disease (9, 13–15). Meanwhile, YY1 overexpression could be observed in various cancers, including colon carcinoma, breast carcinoma, pancreatic cancer, and bladder cancer (16–19). We and other groups have also reported that YY1 plays crucial roles in tumorigenesis, promoting tumor cell proliferation and tumor angiogenesis in both a p53-dependent and p53-independent manner (11, 13, 18–20).

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In spite of its documented roles in tumorigenesis, whether YY1 is involved in the regulation of tumor cell metabolic reprogramming, and whether this regulation could have a determinant effect on tumor cell proliferation are still unclear. Here, we report that YY1 activates the expression of glucose-6-phosphate dehydrogenase (G6PD), the key enzyme of the PPP. By stimulating the PPP, YY1 alters tumor cell metabolism, enhances nucleotide production and DNA synthesis, and promotes antioxidant defense by increasing NADPH. All of these adaptations are crucial for tumor cell proliferation. Concomitantly, *G6PD* overexpression rescues the suppression of cell proliferation and tumorigenesis induced by *YY1* silencing. Importantly, we show that YY1 regulates G6PD through direct binding to the *G6PD* promoter, even in the absence of the tumor suppressor p53. Together, our findings not only unravel a pivotal role of YY1 in promoting tumor cell proliferation by inducing a metabolic shift, especially toward the PPP, but also provide new insights regarding the complex role of YY1 in driving tumorigenesis. Furthermore, our results strongly suggest that YY1 might be a potential target for cancer therapy regardless of the p53 status of the patient.

Materials and Methods

Cell lines and cell culture

HeLa, U2OS, and HepG2 cells were maintained in Dulbecco's modified Eagle's medium (Gibco, Life Technologies). HCT116^{WT} and HCT116^{p53null} cells were kindly provided by Dr. Bert Vogelstein at John Hopkins University School of Medicine, Baltimore, MD (21) and maintained in McCoy's 5A medium (Gibco). All cell lines were cultured with 10% fetal bovine serum (Biological Industries) and 1% penicillin–streptomycin. All cell lines were verified using short-tandem repeat profiling method at Genetic Testing Biotechnology (for HCT116^{p53null} cell) and at Cell Bank, Type Culture Collection, Chinese Academy of Science (for other cell lines), and tested for *Mycoplasma* contamination using Mycoplasma Detection Kit-Quick Test (Biotool) routinely every 6 months. Cells were transfected with indicated vectors using Lipofectamine 2000 (Invitrogen Life Technologies) according to the manufacturer's protocol.

For gene-silencing experiments, cells were seeded in 6-well plates, and transfected with 2 μg of indicated shRNA expression vectors. Twenty-four hours after transfection, puromycin selection was performed to eliminate untransfected cells. For establishing *YY1*-silenced HCT116^{p53null} cells (HCT116^{p53null}/shYY1 cells) and *YY1*-silenced, *G6PD*-overexpressed HCT116^{p53null} cells (HCT116^{p53null}/shYY1/G6PD cells), cells were seeded in 10-cm well plates, and transfected with 12 μg of shCon or shYY1 and 6 μg of pcEF9-Puro or pG6PD-Puro vectors. Stable cell lines were established by performing puromycin selection. For overexpression experiments, cells were seeded in 6-well plates and transfected with 2 μg of pcYY1. For rescue experiments, cells were transfected with 1 μg of shYY1 and 1 μg of pcG6PD vectors and subjected to puromycin selection to eliminate untransfected cells.

Clinical human colon carcinoma specimens

Human colon carcinoma fresh specimens were obtained from patients undergoing surgery at Chongqing University Cancer Hospital (Chongqing, China). Patients did not receive chemotherapy, radiotherapy, or other adjuvant therapies prior to the surgery. The specimens were snap frozen in liquid nitrogen. Prior patients' written informed consents were obtained. The studies were approved by the Institutional Research Ethics Committee of Chongqing University Cancer Hospital, and conducted in accordance with the Declaration of Helsinki.

Animal experiment

For the *in vivo* tumor study, BALB/c-nu/nu mice (male; body weight, 18–22 g; 6 weeks old) were purchased from the Third Military Medical University (Chongqing, China; permit number SYXK-PLA-20120031). Animal studies were approved by the Laboratory Animal Welfare and Ethics Committee of the Third Military Medical University and carried out in the Third Military Medical University. All animal experiments conformed to the guidelines of the Animal Care and Use Committee of the Third Military Medical University. All efforts to minimize suffering were made. To generate an experimental subcutaneous tumor model, BALB/c-nu/nu mice were randomly divided into three groups ($n = 6$), and each group was injected subcutaneously with 5×10^6 HCT116^{p53null}/Con, HCT116^{p53null}/shYY1, or HCT116^{p53null}/shYY1/G6PD stable cells. Tumor size (V) was evaluated by a caliper every 2 days with reference to the following equation: $V = a \times b^2/2$ (13), where a and b are the major and minor axes of the tumor, respectively. The investigator was blinded to the group allocation and during the assessment.

Plasmids and constructs

The shRNA control vector (shCon), shRNA expression vectors targeting YY1, YY2, and shp53, as well as YY1 overexpression vector were constructed as described previously (13, 22). The target sequences specific for YY1 are shYY1-1 (5′-GCAAGAAGAGTTACCTCAG-3′) and shYY1-2 (5′- GTCCAGAATACTTATAATT-3′), while those for YY2 are shYY2-1 (5′-GCATCAACATCAACATCAA-3′) and shYY2-2 (5′-ACATCAACATCAACCCAGA-3′), and those for p53 are shp53 (5′- GCAAGAAGGGAGACAAGAT-3′). For G6PD overexpression vector (pcG6PD), the coding region of human G6PD was amplified using the Takara Ex Taq Kit (Takara Bio) from human cDNA obtained by reverse-transcribing total RNA extracted from HCT116^{WT} cells using the PrimeScript RT Reagent Kit with gDNA Eraser (Takara Bio). The amplicon was inserted into the *Hind*III and *Eco*RI sites of pcDNA3.1(+; Invitrogen Life Technologies). For pG6PD-Puro vector, the amplicon was inserted into the pcEF9-Puro vector bringing puromycin resistance gene.

For G6PD luciferase reporter vectors, we cloned the −419 to +1,428 (for G6PD-Luc) or the +58 to +1,428 (for G6PD-del-Luc) of the G6PD promoter region into the *Eco*RV and *Hind*III sites of the pGL4.13 vector (Promega). Human genome DNA extracted from HCT116^{WT} cells using the TIANamp Genomic DNA Kit (Tiangen Biotech) was used as template for amplifying the promoter regions. G6PD-luciferase vector with mutated YY1 binding site (G6PD^{mut}-Luc) was constructed based on the site-specific mutagenesis method (17).

Western blotting and quantitative PCR analysis

Detailed methods for performing Western blotting and quantitative PCR analysis are described in the Supplementary Materials and Methods. The sequences of the primers and antibodies used are shown in Supplementary Tables S1 and S2, respectively. Images of uncropped blots are shown in Supplementary Fig. S8A to S8F.

Glucose consumption, lactate production, G6PD and P6GD enzyme activities, and the intracellular NADPH level

Cells were transfected with indicated shRNA expression vectors or overexpression vectors as described above. For glucose consumption and lactate production, the culture medium was then changed, and the cells were incubated for an additional 24 hours. Glucose levels in the culture medium were measured using the Glucose Colorimetric Assay Kit (BioVision). Lactate levels were determined using a Lactate Assay kit (KeyGen Biotech). The values were normalized with total protein amount determined using the BCA Protein Assay Kit (Beyotime). G6PD enzyme activity and the intracellular NADPH level were determined using G6PD assay kit (Yuanye) or Amplite Colorimetric NADP/NADPH Ratio Assay Kit (AAT Bioquest), respectively, after the cells being lysed.

For measuring the activities of G6PD and 6PGD in clinical tumor tissues and xenografted tissues, the tissues were homogenized, then the enzyme activities were measured using the G6PD assay and 6PGD assay kits (Solarbio), and the relative level to the control was calculated.

Intracellular ROS level

Cells were transfected with indicated expression vectors as described above, and then the same number of the cells was reseeded to 24-well plates. The intracellular ROS level was stained using CellROX Green Reagent (Life Technologies). Images were taken with DMI6000B (Leica) and analyzed using ImageJ software. The results were expressed as the mean fluorescence intensity per cell.

Liquid chromatography–mass spectrometry

For profiling PPP metabolites 6-phosphogluconate and ribose-5-phosphate, cells transfected with indicated shRNA expression vectors as described above were washed twice with cold PBS, and then the intracellular metabolites were extracted by sonication in ice-cold 80% methanol. Supernatants were collected with centrifugation, rotary evaporated, and dissolved in 95% acetonitrile. Liquid chromatography–mass spectrometry (LC-MS) analysis was carried out on Agilent 1290 Infinity II UPLC tandem Agilent Triple Quard (QQQ) MS equipped with electrospray source operating in the negative-ion multiple-reaction monitoring mode. The source parameters were as follows: gas temperature 300°C, gas flow 8 L/minute, nebulizer 35 psi, sheath gas temperature 350°C, sheath gas flow 11 L/minute, capillary 3500 V, and nozzle voltage 1500 V. Chromatographic separation was performed on Acquity UPLC BEH HILIC (2.1 × 100 mm, 1.7 μm) column. Mobile phase A used consists of water, while mobile phase B consists of 95% acetonitrile and 5% water. The quantitative multiple-reactions monitoring transition of each particular metabolite were m/z 275 > 80 for 6-phosphogluconate, and m/z 229 > 183 for ribose-5-phosphate, respectively. Data were acquired and processed using MassHunter Workstation Acquisition (Version B.08.00). Peak areas of individual metabolites were normalized against the total protein amount. The relative levels of each metabolite to those of the control are calculated.

Statistical analysis

All values of the experimental results were presented as mean ± SEM of three independent experiments. Statistical analysis was performed using the Student *t* test. For clinical samples and xenograft experiments, statistical analysis was performed using one-way ANOVA. A value of $P < 0.05$ was considered statistically significant.

Results

YY1 regulates glucose metabolism in tumor cells

To meet their highly proliferative growth, tumor cells alter their metabolism. We and others have shown that YY1 is crucial for tumor cell proliferation (11, 13, 17, 19, 20); however, it remains unclear whether this occurs via metabolic reprogramming. Therefore, we first analyzed whether YY1 expression levels affected glucose metabolism by measuring glucose consumption and lactate production in *YY1*-silenced tumor cells. To this end, we constructed two small-hairpin (shRNA) vectors against YY1 (Supplementary Fig. S1A and S1B) and a *YY1* overexpression vector (Supplementary Fig. S1C). Notably, our shRNA expression vectors against YY1 and the *YY1* overexpression vector did not affect the expression level of YY2, another member of the YY family (23, 24). Our results showed that knocking down *YY1* significantly reduced glucose consumption by $HCT116^{WT}$ colon carcinoma cells, whereas overexpressing *YY1* robustly enhanced it (Fig. 1A), indicating that YY1 positively regulates glucose metabolism in tumor cells. Moreover, *YY1* silencing grossly suppressed lactate production (Fig. 1B, left), whereas *YY1* overexpression conspicuously increased it (Fig. 1B, right). Thus, our results clearly demonstrated that YY1 alters glucose metabolism in tumor cells. To elucidate the underlying molecular mechanism, we screened for factors involved in glucose metabolism that could be potentially affected by YY1. As shown in Fig. 1C, *YY1* silencing clearly reduced the expression of several factors involved in glucose metabolism, such as glucose transporter 1 (GLUT1), G6PD, 6-phosphofructokinase 2 (PFK2), TP53-induced glycolysis regulatory phosphatase (TIGAR), phosphoglycerate kinase 1 (PGK1), pyruvate kinase M1/2 (PKM2), and lactate dehydrogenase A (LDHA). Among them, the expression level of G6PD was the most affected, decreasing below 50% in *YY1*-silenced cells.

To further confirm the role of YY1 in regulating G6PD, we assessed G6PD mRNA expression levels using two shYY1 vectors and found that both of them significantly suppressed G6PD mRNA (Fig. 2A, left); in contrast, *YY1* overexpression grossly upregulated G6PD mRNA (Fig. 2A, right). Furthermore, *YY1* silencing suppressed the G6PD protein level (Fig. 2B, top), whereas *YY1* overexpression upregulated it (Fig. 2B, bottom). Concomitantly, *YY1* silencing significantly repressed G6PD enzyme activity (Fig. 2C, left), while *YY1* overexpression induced it (Fig. 2C, right). To confirm that this regulation was specific to YY1, we silenced *YY2* using two different shRNA expression vectors and found that the level of G6PD was not affected (Supplementary Fig. S1D). Next, to assess whether this regulation was common to other tumor cells, we silenced YY1 expression in human hepatocarcinoma HepG2 cells, human cervical carcinoma HeLa cells, and human bone osteosarcoma epithelial U2OS cells (Supplementary Fig. S1E–S1G). We found that G6PD expression was conspicuously suppressed in these cells when *YY1* was silenced (Fig. 2D).

Next, we examined the expression levels of YY1 and G6PD in human clinical colon carcinoma tissues. Compared with adjacent normal tissue, mRNA expression of both YY1 and G6PD was robustly upregulated in tumor tissue (Fig. 2E, left and right, respectively). Furthermore, as shown by Western blotting and immunohistochemistry, YY1 and G6PD protein expression was

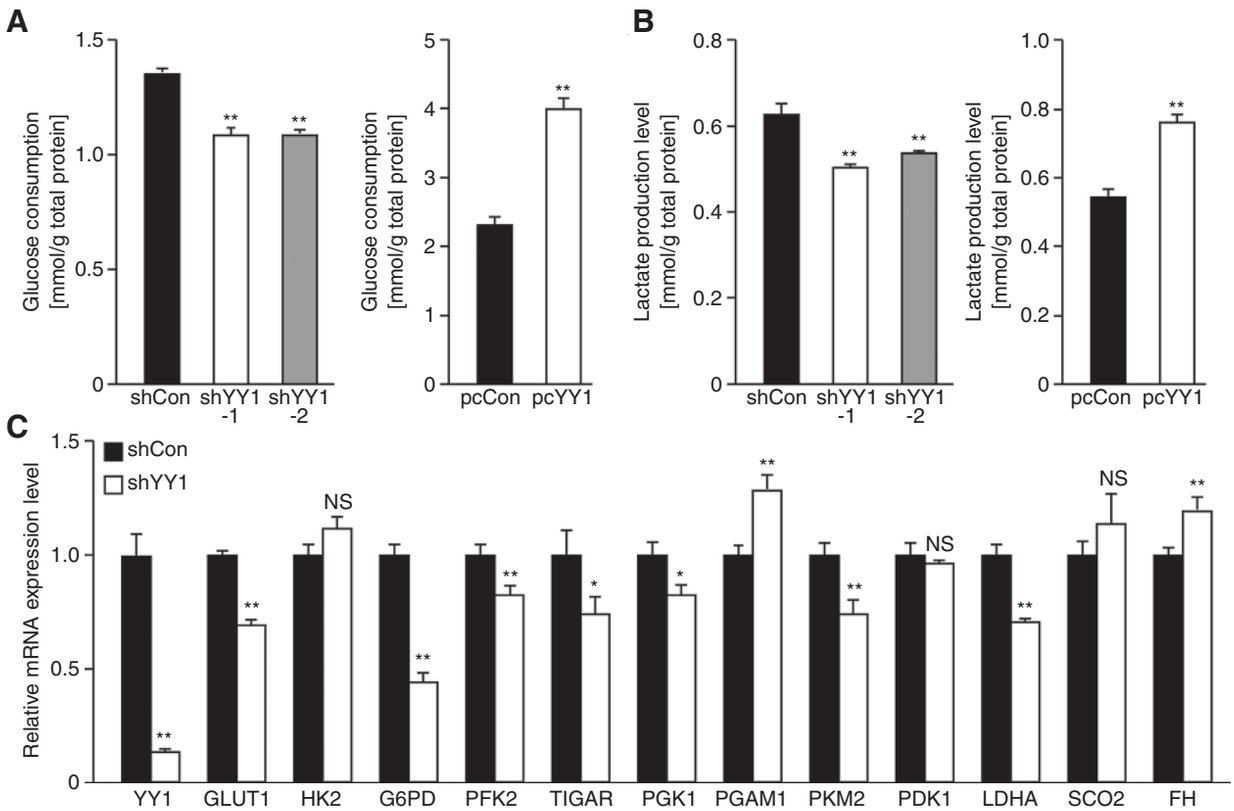

Figure 1.

YY1 regulates tumor cells' glucose metabolism. **A,** Glucose consumption level in *YY1*-silenced (left) and *YY1*-overexpressed (right) HCT116^{WT} cells. Total protein was used for normalization. **B,** Lactate production level in *YY1*-silenced (left) and *YY1*-overexpressed (right) HCT116^{WT} cells. Total protein was used for normalization. **C,** The mRNA expression levels of glucose metabolism-related genes in *YY1*-silenced HCT116^{WT} cells were analyzed using qPCR. Cells transfected with shCon or pcCon were used as control, and the results are shown as relative to control. β-Actin was used for qPCR normalization. Quantitative data were expressed as mean ± SEM of three independent experiments. *, $P < 0.05$; **, $P < 0.01$; NS, not significant; pcCon, pcDNA3.1(+).

significantly higher in tumor tissues compared with normal ones (Fig. 2F and G). In line with these results, we also detected a significant increase in G6PD enzyme activity in tumor tissue (Fig. 2H). Together, these results clearly suggest that YY1 is a positive regulator of G6PD expression, and that this regulatory mechanism is common to various tumor cell types.

YY1/G6PD regulation is crucial for the PPP

G6PD is known as the pacesetter of the PPP, and its activity determines the first committing step of this pathway (8). Aberrant G6PD expression and regulation had been found in tumor cells (25, 26); however, it remains unclear whether YY1 is involved in PPP regulation. To unravel the role of YY1 in PPP, we first examined the effect of YY1 expression on the intracellular NADPH level. As shown in Fig. 3A, the expression of YY1 correlated positively with the intracellular NADPH level. Concomitantly, *YY1* silencing robustly increased the amount of intracellular ROS (Fig. 3B). *YY1* silencing also reduced cell survival upon exposure to oxidative stress, whereas *YY1* overexpression upregulated it (Supplementary Fig. S2A). Furthermore, we measured the amounts of 6-phosphogluconate and ribose-5-phosphate, two typical metabolites of the PPP, using LC-MS (Supplementary Fig. S2B and S2C), and found that their amounts were also conspicuously lower as a result of *YY1* silencing (Fig. 3C). Ribose-5-phosphate is an intermediate in nucleotide production, which is crucial for *de novo* DNA synthesis. Not surprisingly, as demonstrated by the 5-ethynyl-2′-deoxyuridine (EdU) incorporation assay, *YY1* silencing suppressed *de novo* DNA synthesis (Fig. 3D). Concomitantly, *YY1* silencing grossly reduced the total number of HCT116^{WT} cells and the number of HCT116^{WT} colonies (Fig. 3E and F). Together, these results reveal that YY1 is critical for regulating the PPP and, consequently, the proliferation and colony formation potentials of tumor cells.

To investigate whether G6PD was involved in YY1-mediated PPP regulation, we constructed a *G6PD* overexpression vector (Supplementary Fig. S3A), which we used together with shYY1 vector to perform rescue experiment (Supplementary Fig. S3B). We observed that G6PD activity and cellular NADPH level, both of which were significantly suppressed by *YY1* silencing, were significantly restored when *G6PD* was overexpressed (Fig. 4A and B, respectively). NADPH is a key factor in suppressing intracellular ROS and enhancing lipid synthesis. Consistent with the trend shown by NADPH, *YY1* silencing robustly increased intracellular ROS, while suppressing cell viability; both of these were restored by *G6PD* overexpression (Fig. 4C; Supplementary Fig. S3C).

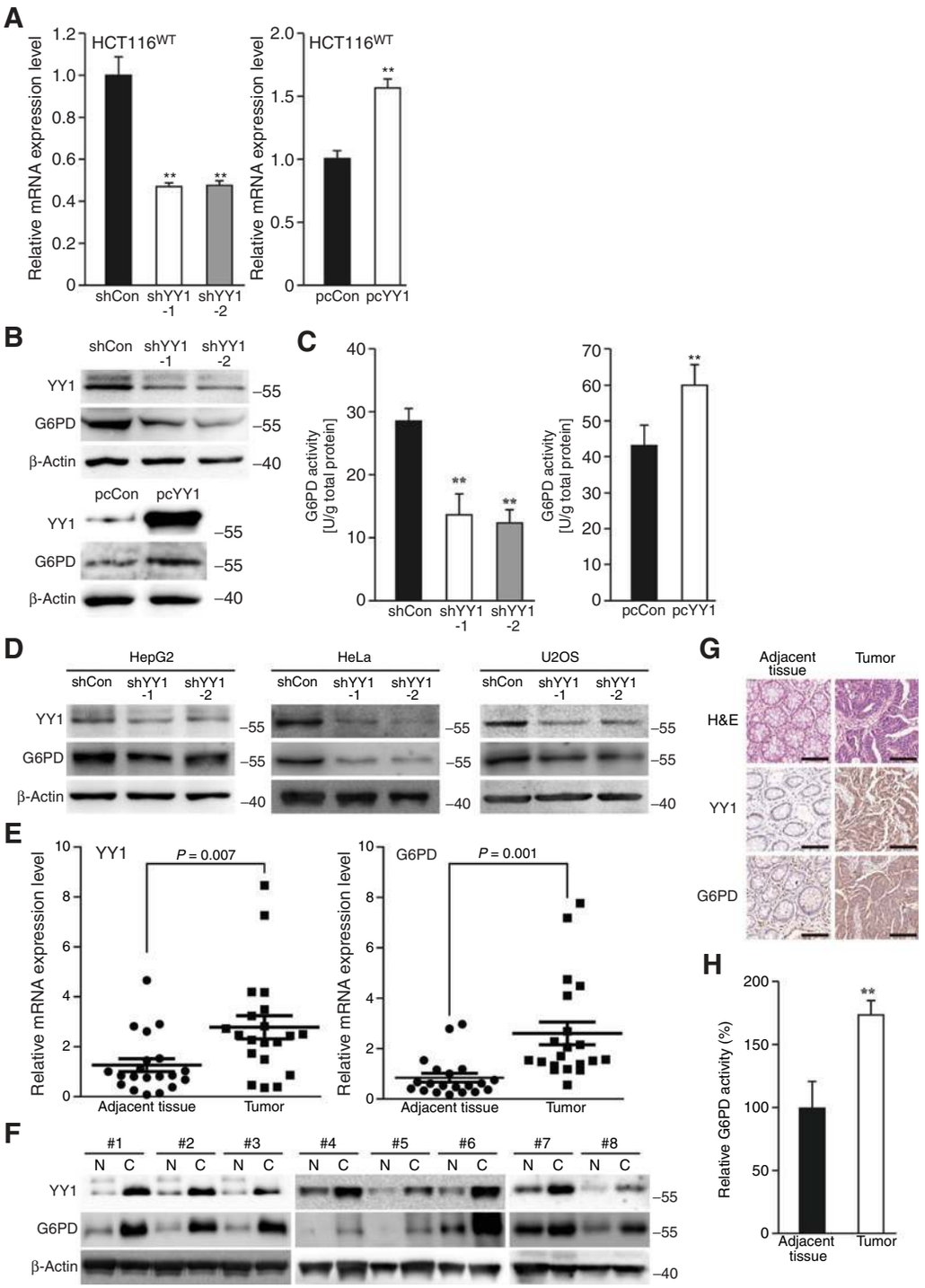

Figure 2.

YY1 positively regulates G6PD expression. **A,** The mRNA expression level of G6PD in *YY1*-silenced (left) and *YY1*-overexpressed (right) HCT116^{WT} cells was analyzed using qPCR. The results are shown as relative to control. **B,** The protein expression level of G6PD in *YY1*-silenced (top) and *YY1*-overexpressed (bottom) HCT116^{WT} cells was analyzed using Western blotting. **C,** G6PD activity in *YY1*-silenced (left) and *YY1*-overexpressed (right) HCT116^{WT} cells. Total protein was used for normalization. **D,** The protein expression level of G6PD in *YY1*-silenced HepG2 (left), HeLa (middle), and U2OS (right) cells was analyzed using Western blotting. **E** and **F,** The mRNA (**E**; $n = 20$) and protein (**F**) expression levels of YY1 and G6PD in clinical human colon carcinoma and corresponding adjacent tissues were analyzed using qPCR and Western blotting, respectively. **G,** The results of immunohistochemistry staining against YY1 and G6PD in clinical human colon carcinoma and corresponding adjacent tissues (scale bars, 100 μm). **H,** G6PD enzyme activity in clinical human colon carcinoma and corresponding adjacent tissue.

Total protein was used for normalization, and the results are shown as relative to control. Cells transfected with shCon or pcCon were used as control. β-Actin was used for qPCR normalization and Western blotting loading control. Quantitative data are expressed as mean ± SEM of three independent experiments.

**, $P < 0.01$. pcCon, pcDNA3.1(+).

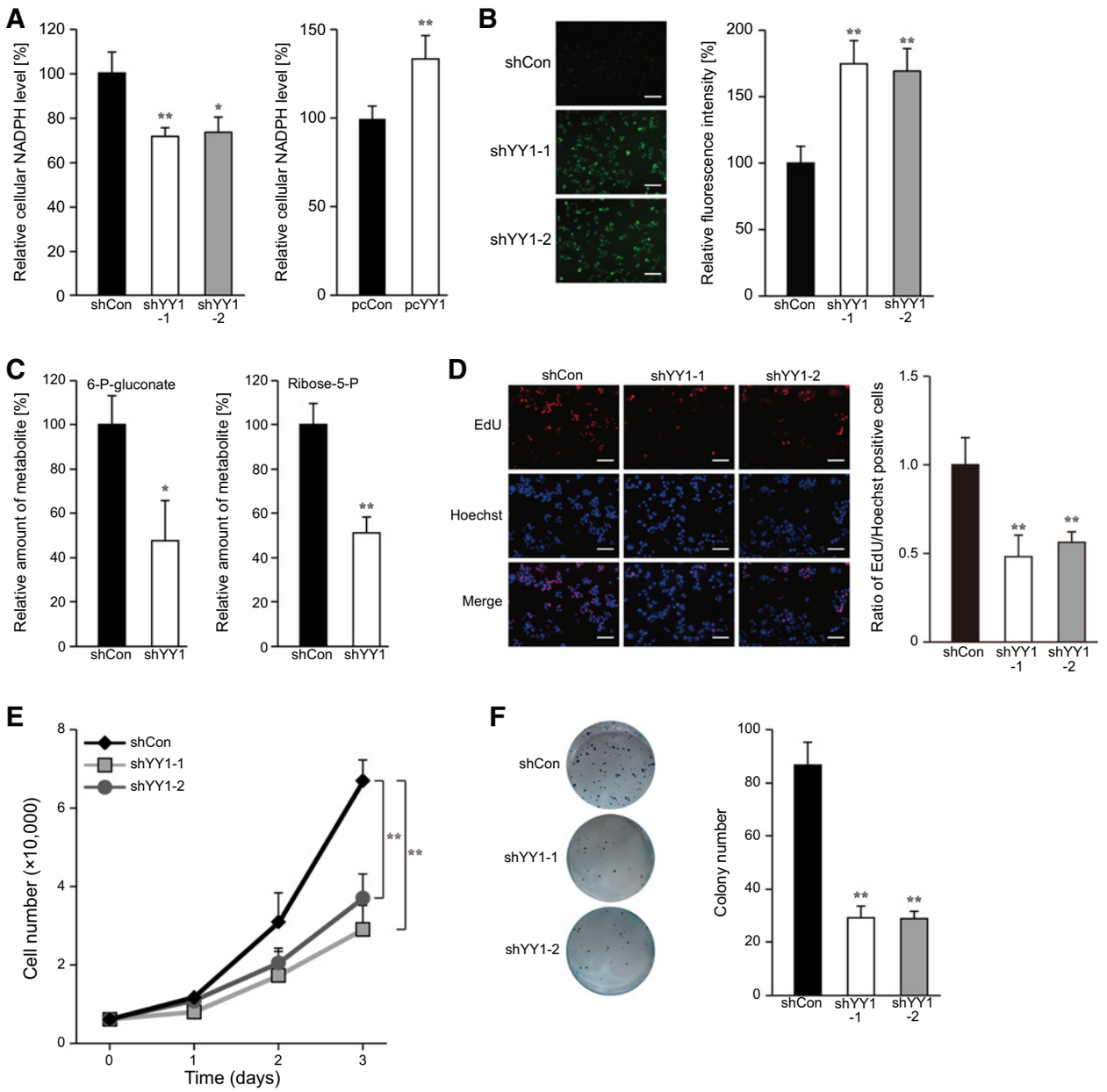

Figure 3.

YY1 affects the PPP. **A,** Intracellular NADPH level in *YY1*-silenced (left) and *YY1*-overexpressed (right) HCT116^{WT} cells. Total protein was used for normalization, and the results are shown as relative to control. **B,** Intracellular ROS level in *YY1*-silenced HCT116^{WT} cells. Representative images (left) and relative fluorescence intensity (right) were shown. **C,** The amount of 6-phosphogluconate (6-P-gluconate) and ribose-5-phosphate (ribose-5-P) as analyzed using LC-MS. Total protein was used for normalization, and the results are shown as relative to control. **D,** The ratio of *de novo* DNA synthesis determined using EdU incorporation assay. Representative images (left) and the quantitative results (right) are shown. Hoechst was used to stain the nuclei. The ratio of *de novo* DNA synthesis is shown as relative to control. **E,** Total cell number of *YY1*-silenced HCT116^{WT} cells was examined at indicated time points. **F,** Colony formation potentials of *YY1*-silenced HCT116^{WT} cells. Cells transfected with shCon or pcCon were used as control. Quantitative data are expressed as mean ± SEM of three independent experiments. *, $P < 0.05$; **, $P < 0.01$. pcCon, pcDNA3.1(+). Scale bars, 200 μm.

Similarly, lipid accumulation was also significantly restored in *YY1*-silenced cells overexpressing *G6PD* (Fig. 4D). Furthermore, *G6PD* overexpression eliminated the suppressive effect of *YY1* silencing on PPP intermediates (6-phosphogluconate and ribose-5-phosphate) and *de novo* DNA synthesis (Fig. 4E and F). Taken together, these results strongly suggest that YY1 regulation of the PPP occurs by controlling G6PD expression.

Finally, we examined whether YY1 regulation of G6PD could be linked to its role in tumor cell proliferation. *YY1* silencing was reported to reduce the proliferation rate of tumor

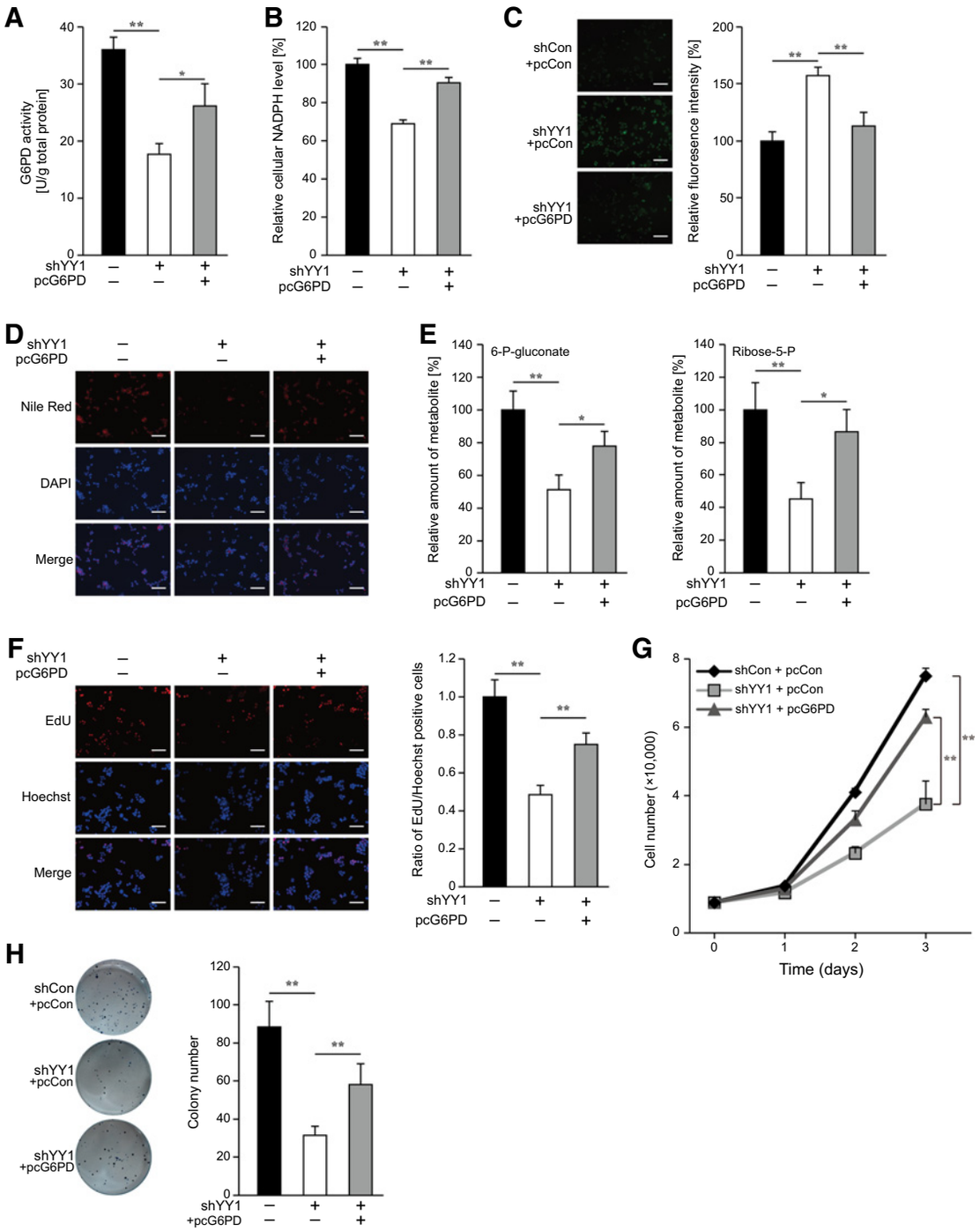

Figure 4.

G6PD restores the effect of *YY1* silencing on tumor cell proliferation by enhancing PPP. **A,** G6PD activity in *YY1*-silenced, *G6PD*-overexpressed HCT116^{WT} cells. Total protein was used for normalization. **B,** Intracellular NADPH level in *YY1*-silenced, *G6PD*-overexpressed HCT116^{WT} cells. Total protein was used for normalization, and the results are shown as relative to control. **C,** Intracellular ROS level in *YY1*-silenced, *G6PD*-overexpressed HCT116^{WT} cells. Representative images (left) and relative fluorescence intensity (right) are shown. **D,** Lipid accumulation in *YY1*-silenced, *G6PD*-overexpressed HCT116^{WT} cells was stained using Nile Red. Representative images are shown. **E,** The amounts of 6-phosphogluconate (6-P-gluconate) and ribose-5-phosphate (ribose-5-P) in *YY1*-silenced, *G6PD*-overexpressed HCT116^{WT} cells as analyzed using LC-MS. Total protein was used for normalization, and the results are shown as relative to control. **F,** The ratio of *de novo* DNA synthesis determined using EdU incorporation assay. Representative images (left) and the quantitative results (right) are shown. Hoechst was used to stain the nuclei. The ratio of *de novo* DNA synthesis is shown as relative to control. **G,** Total cell number of *YY1*-silenced, *G6PD*-overexpressed HCT116^{WT} cells was examined at indicated time points. **H,** Colony formation potentials of *YY1*-silenced, *G6PD*-overexpressed HCT116^{WT} cells. Cells transfected with shCon and pcCon were used as control. Quantitative data are expressed as mean ± SEM of three independent experiments. *, $P < 0.05$; **, $P < 0.01$. pcCon, pcDNA3.1(+). Scale bars, 200 μm.

cells (13, 17). However, whether the effect of YY1 on tumor cell proliferation could be linked to its regulation of the PPP, and particularly G6PD expression, remains unknown. As shown in Fig. 4G and H, *G6PD* overexpression robustly restored both the proliferation and colony formation potentials previously suppressed by *YY1* silencing. Together, these results strongly indicate that YY1 exerts its critical effect on tumor cell survival, proliferation, and colonization by regulating *G6PD* expression and enhancing the PPP.

YY1 regulates G6PD transcriptional activity by direct binding to the G6PD promoter region

Given that YY1 is a transcriptional factor, we used the UCSC Genome Browser (http://genome.ucsc.edu/, ref. 27) to search for a potential YY1 binding site within the G6PD promoter. Given that a YY1-binding site (ATGG, ref. 28) was predicted at position −205 to −202 of the G6PD promoter region, we constructed two luciferase reporter assay vectors with or without the predicted binding site (G6PD-Luc and G6PD-del-Luc, Fig. 5A, respectively). Luciferase reporter assay results clearly showed that knocking down *YY1* could grossly suppress the activity of G6PD-Luc; however, this suppressive effect could not be observed when G6PD-del-Luc was used (Fig. 5B, left). Similarly, although *YY1* overexpression could conspicuously promote the luciferase reporter activity of G6PD-Luc, it failed to have any significant effect on G6PD-del-Luc (Fig. 5B, right). These results indicated that the −419 to +57 region was critical for YY1 to regulate G6PD transcriptional activity. Next, we designed a set of primers for a chromatin immunoprecipitation (ChIP) assay, with which to confirm the binding of YY1 to the predicted promoter region (Fig. 5C, top). As shown in Fig. 5C (bottom), ChIP assay results clearly showed that YY1 could bind to the −312 to −110 region of the G6PD promoter. Finally, to assess whether the predicted binding site was functional, we constructed a G6PD luciferase reporter vector with four point mutations in the YY1 core binding site (G6PD^{mut}-Luc): the ATGG sequence from the wild-type G6PD promoter was mutated into TCTT (Fig. 5D). We found that while *YY1* silencing could robustly suppress the luciferase activity of the wild-type G6PD reporter vector (G6PD-Luc), this effect was diminished when G6PD^{mut}-Luc was used (Fig. 5E, left). Similarly, while *YY1* overexpression greatly induced G6PD-Luc activity, it failed to affect the luciferase activity of G6PD^{mut}-Luc (Fig. 5E, right). Together, these results strongly indicate that YY1 could directly bind to the G6PD promoter, and that such binding is critical for its transcriptional regulation.

p53 is known as a critical inhibitor of G6PD enzyme activity (8), whereas YY1 is known as a negative regulator of p53 (20). Our results indicated that YY1 regulated G6PD directly at its transcriptional level, most plausibly by binding to its promoter region. These facts suggested the possibility that YY1 might regulate G6PD expression in a p53-independent way. To verify this hypothesis, we first investigated the level of G6PD in HCT116^{WT} cells, in which both *YY1* and *p53* were transiently silenced. Our results showed that *YY1* silencing suppressed G6PD expression in *p53*-silenced cells (Supplementary Fig. S4A). Consequently, *YY1* silencing reduced G6PD activity and glucose consumption previously induced by *p53* silencing (Supplementary Fig. S4B and S4C, respectively), indicating the presence of a p53-independent way for YY1 to regulate G6PD. To further confirm this pathway, we investigated the effect of *YY1* silencing in HCT116^{p53null} cells. We first confirmed the knockdown efficacy of our shYY1 vectors in HCT116^{p53null} cells (Supplementary Fig. S5A) and measured G6PD expression in *YY1*-silenced or *YY1*-overexpressing HCT116^{p53null} cells. Our results clearly showed that in HCT116^{p53null} cells, *YY1* silencing suppressed G6PD mRNA and protein expression, whereas *YY1* overexpression induced it (Fig. 5F and G). Furthermore, manipulation of YY1 levels affected G6PD activity even in the absence of p53 (Fig. 5H). The fact that YY1 could directly regulate G6PD transcription and alter tumor metabolism even in the absence of p53 was further confirmed by the suppression of glucose consumption and lactate production in p53-null colon cancer cells upon *YY1* silencing, and their induction upon *YY1* overexpression (Supplementary Fig. S5B and S5C). These results suggest that transcriptional regulation of G6PD by YY1 could occur in a p53-independent manner, further confirming the direct action of YY1 in regulating G6PD activity.

G6PD-mediated YY1 regulation of the PPP occurs in a p53-independent manner

To further verify the functionality of the p53-independent YY1/G6PD axis on tumor cell PPP, we first analyzed the effect of *YY1* expression on the intracellular NADPH level in HCT116^{p53null} cells. In the absence of p53, *YY1* silencing significantly suppressed the level of intracellular NADPH (Fig. 6A, left); in contrast, *YY1* overexpression grossly upregulated it (Supplementary Fig. S6A). Moreover, the effect of *YY1* silencing on intracellular NADPH could be diminished by overexpressing *G6PD* (Fig. 6A, right). Concomitantly, the intracellular ROS level in HCT116^{p53null} cells was robustly increased by *YY1* silencing (Fig. 6B), but was restored by *G6PD* overexpression (Fig. 6C). YY1-dependent activation of G6PD and the PPP also regulate the antioxidant defenses of HCT116^{p53null} cells. Indeed, cell viability upon exposure to oxidative stress correlated positively with YY1 expression (Supplementary Fig. S6B), whereas G6PD overexpression restored the antioxidant defense suppressed by *YY1* silencing (Supplementary Fig. S6C). Intracellular lipid accumulation showed a similar trend: *G6PD* overexpression prevented the inhibition of lipid accumulation caused by *YY1* silencing (Fig. 6D).

Next, we examined the role of the YY1/G6PD pathway on *de novo* DNA synthesis in p53-null tumor cells. We found that while *YY1* silencing significantly suppressed the number of EdU-positive HCT116^{p53null} cells (Supplementary Fig. S6D), overexpression of *G6PD* restored it (Fig. 6E). Furthermore, *YY1* silencing suppressed the proliferation and colony formation potentials of HCT116^{p53null} cells (Supplementary Fig. S6E and S6F), whereas G6PD blocked these effects (Fig. 6F and G).

Given that the PPP could produce ribose-5-phosphate and NADPH, and thus is crucial for the biosynthesis of nucleotides and for suppressing intracellular ROS, we examined the effect of adding an ROS scavenger and/or a mixture of four deoxyribonucleosides and four ribonucleosides on the proliferation potential of *YY1*-silenced HCT116^{p53null} cells as described previously (25, 26). As shown in Fig. 6H, addition of both ROS scavenger and nucleosides mixture significantly reverted the effect of *YY1* silencing on the proliferation of HCT116^{p53null} cells. Thus, increased intracellular ROS level and impaired nucleotide biosynthesis contribute to the disruption of tumor cell proliferation caused by *YY1* silencing. Together, our results demonstrate, for the first time, that YY1 could activate the PPP in tumor cells in a p53-independent manner by directly regulating G6PD.

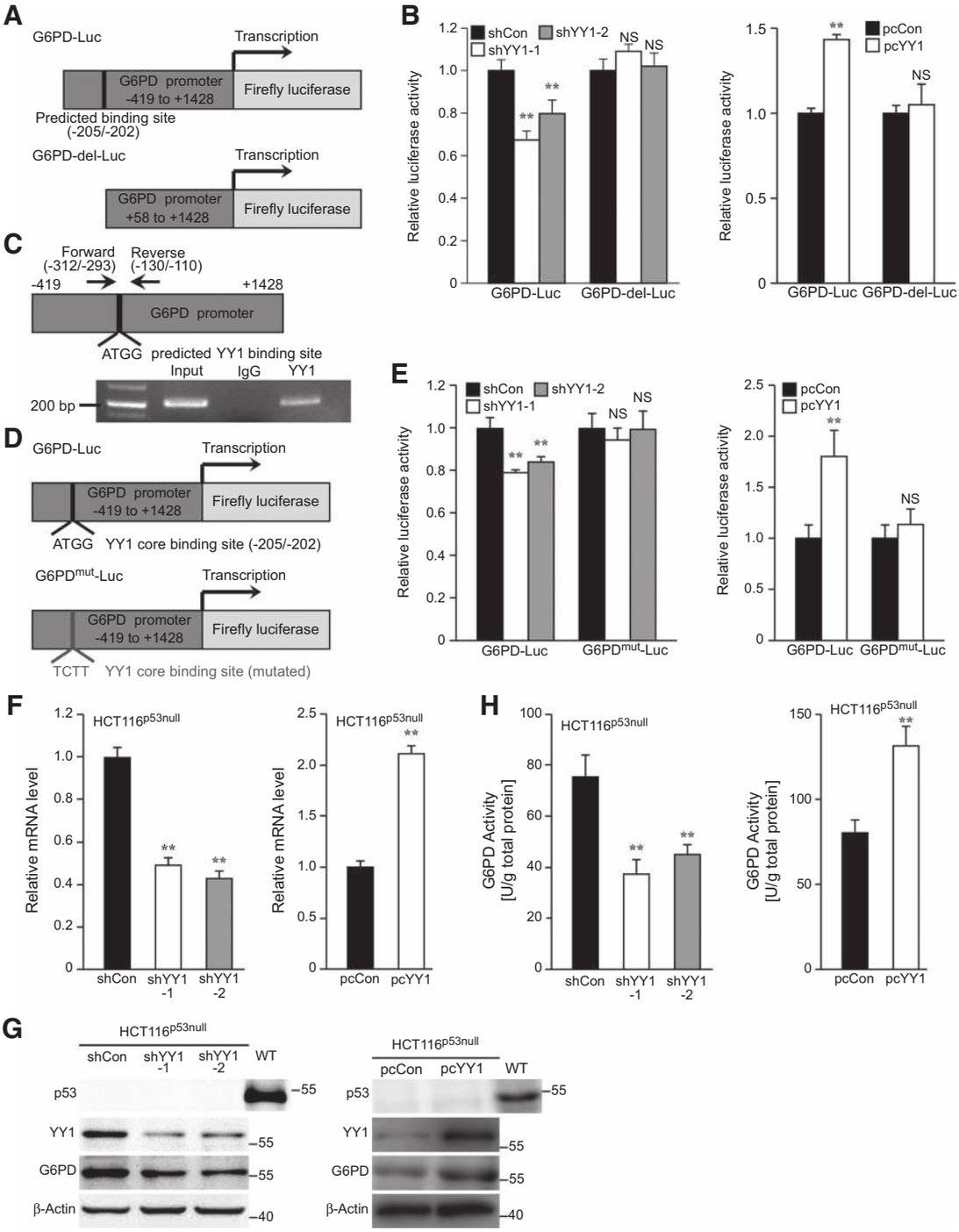

Figure 5.

YY1 directly binds G6PD promoter and regulates its transcriptional activity. **A** and **B,** The activities of G6PD-luciferase reporter vectors with or without predicted YY1 binding site (G6PD-Luc and G6PD-del-Luc, respectively). Schematic diagram (**A**) and relative luciferase activities to control (**B**) are shown. **C,** Binding of YY1 to the promoter region of G6PD was examined using ChIP assay with anti-YY1 antibody, followed by PCR in HCT116^{WT} cells. The predicted YY1 binding site on the promoter region of G6PD and the location of primer set used for PCR are shown. **D** and **E,** The activity of YY1 binding site mutated G6PD-luciferase reporter vector (G6PD^{mut}-Luc). Schematic diagram (**D**) and luciferase activities of G6PD-Luc and G6PD^{mut}-Luc in *YY1*-silenced (left) and *YY1*-overexpressed (right) HCT116^{WT} cells (**E**) are shown. Luciferase activities are shown as relative to control. Mutated base pairs are indicated in gray. **F** and **G,** The mRNA (**F**) and protein (**G**) expression levels of G6PD in *YY1*-silenced (left) and *YY1*-overexpressed (right) HCT116^{p53null} cells were analyzed using qPCR and Western blotting, respectively. β-Actin was used for qPCR normalization and Western blotting loading control. The mRNA expression levels are shown as relative to control. **H,** G6PD activity in *YY1*-silenced (left) and *YY1*-overexpressed (right) HCT116^{p53null} cells. Total protein was used for normalization. Cells transfected with shCon or pcCon were used as control. Quantitative data are expressed as mean ± SEM of three independent experiments. **, $P < 0.01$; NS, not significant; pcCon, pcDNA3.1(+).

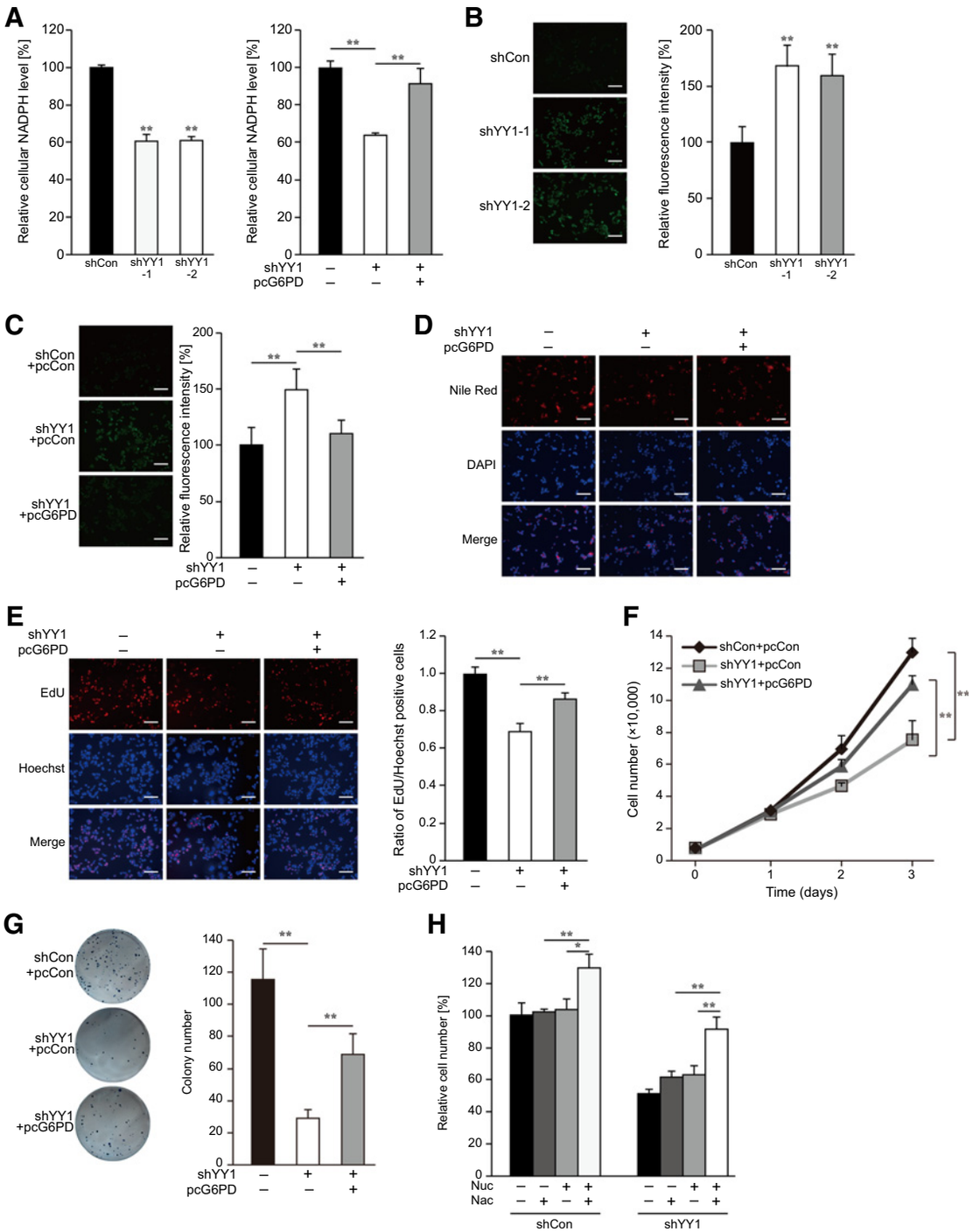

Figure 6.

YY1 regulates PPP p53 independently. **A,** Intracellular NADPH level in *YY1*-silenced (left) and *YY1*-silenced, *G6PD*-overexpressed (right) HCT116^{p53null} cells was determined. Total protein was used for normalization, and the results are shown as relative to control. **B** and **C,** The level of ROS in *YY1*-silenced (**B**) and *YY1*-silenced, *G6PD*-overexpressed (**C**) HCT116^{p53null} cells. Representative images (left) and relative fluorescence intensity (right) are shown. **D,** Lipid accumulation in *YY1*-silenced, *G6PD*-overexpressed HCT116^{p53null} cells was stained using Nile Red. Representative images are shown. **E,** The ratio of *de novo* DNA synthesis determined using EdU incorporation assay in *YY1*-silenced, *G6PD*-overexpressed HCT116^{p53null} cells. Representative images (left) and the quantitative results (right) are shown. Hoechst was used to stain the nuclei. The ratio of *de novo* DNA synthesis is shown as relative to control. **F,** Total cell number of *YY1*-silenced, *G6PD*-overexpressed HCT116^{p53null} cells was examined at indicated time points. **G,** Colony formation potentials of *YY1*-silenced, *G6PD*-overexpressed HCT116^{p53null} cells. **H,** Relative cell number of *YY1*-silenced HCT116^{p53null} cells cultured in the presence of nucleosides mixture (Nuc, final concentration, 200 μmol/L each) and/or ROS scavenger (N-acetyl-l-cysteine, Nac, final concentration, 2 mmol/L) as measured at the third day after the addition of nucleosides mixture and/or ROS scavenger. Cells transfected with shCon or shCon and pcCon were used as control. Quantitative data are expressed as mean ± SEM of three independent experiments. *, $P < 0.05$; **, $P < 0.01$. pcCon, pcDNA3.1(+). Scale bars, 200 μm.

The YY1/G6PD axis alters tumor growth in a p53-independent manner

Finally, we examined the pathological function of the YY1/G6PD pathway in tumorigenesis *in vivo* by transplanting HCT116^{p53null}/Con, HCT116^{p53null}/shYY1, and HCT116^{p53null}/shYY1/G6PD stable cell lines subcutaneously into BALB/c-nu/nu mice. As shown in Fig. 7A and B, while *YY1* silencing significantly decreased the tumorigenesis potential of HCT116^{p53null} cells, *G6PD* overexpression restored it. Furthermore, Western blotting revealed that expression of G6PD was negatively regulated in *YY1*-silenced cells (Fig. 7C), and immunohistochemistry of tissue sections from xenografted tumors further confirmed the above results (Fig. 7D). Moreover, a significant decrease in the enzyme activities of G6PD and 6-

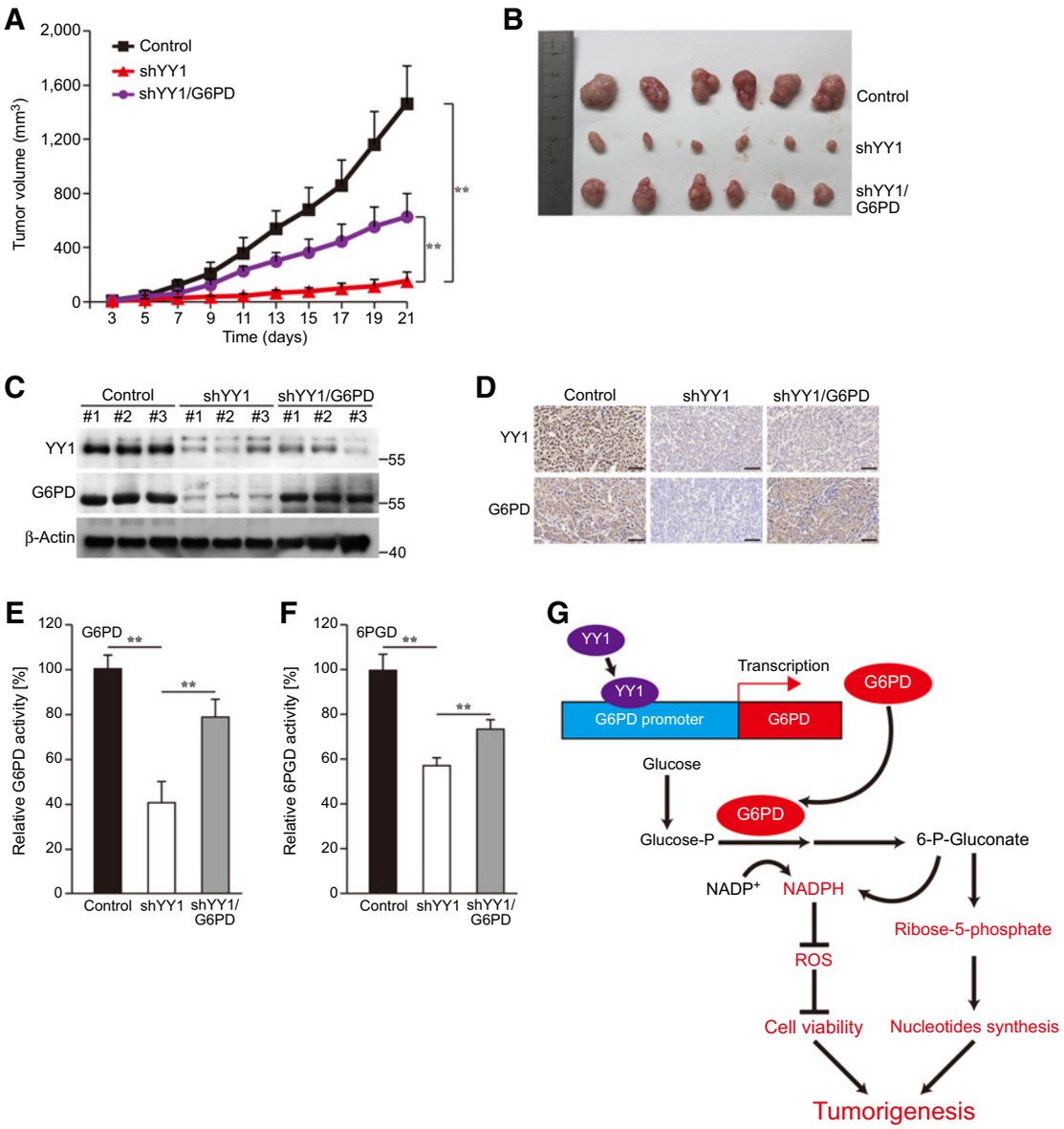

Figure 7.

G6PD is involved in YY1-mediated tumorigenesis. **A** and **B,** Tumorigenesis potentials of HCT116^{p53null}/Con (Control), HCT116^{p53null}/shYY1 (shYY1), and HCT116^{p53null}/shYY1/pcG6PD (shYY1/G6PD) stable cell lines were examined *in vivo* by subcutaneous injection into Balb/c-nu/nu mice ($n = 6$). Volume of the tumor generated was measured at indicated time points (**A**), and the representative images are shown (**B**). **C,** The protein expression levels of YY1 and G6PD in the tumor formed were examined using Western blotting. β-Actin was used as loading control. **D,** Immunohistochemistry staining images against YY1 and G6PD in tissue sections of xenografted tumors in Balb/c-nu/nu mice injected with indicated cell lines. Scale bars, 50 μm. **E** and **F,** G6PD (**E**) and 6PGD (**F**) enzyme activities in tissues of xenografted tumors in Balb/c-nu/nu mice injected with indicated cell lines. Total protein was used for normalization, and the results are shown as relative to control. **G,** Schematic diagram showing the mechanism of YY1 regulation on the tumorigenesis via G6PD-induced PPP. Quantitative data are expressed as mean ± SEM of three independent experiments. **, $P < 0.01$.

phosphogluconate dehydrogenase (6PGD), which had been reported to quantitatively correspond to G6PD activity (29), was observed in the tumors produced from HCT116^{p53null}/shYY1 cells; however, the activities of both enzymes were restored in xenografted tumors originating from HCT116^{p53null}/shYY1/G6PD cells (Fig. 7E and F).

Together, these findings reveal a novel role of YY1 in regulating the PPP in tumor cells. Through the direct activation of G6PD, YY1 induces tumor cell metabolic reprogramming in a p53-independent manner; this function is a crucial feature explaining the regulatory role of YY1 in tumorigenesis (Fig. 7G).

Discussion

Metabolic alteration is a hallmark of tumor cells as it confers them important growth advantages. Discovered first by Otto Warburg (2), it is now clear that metabolic reprogramming facilitates tumor cell growth not only through the generation of ATP, but also by providing tumor cells with nucleotide and lipid precursors for macromolecular synthesis, as well as NADPH (4). The latter acts as a reducing equivalent for synthesizing macromolecules such as lipids, and as a crucial factor for tumor cell antioxidant defense (8, 25, 26, 30, 31). Our findings show that YY1 positively regulates glucose consumption and lactate production by tumor cells, thus linking YY1 to tumor cell metabolic reprogramming.

We demonstrate that YY1 enhances the PPP, a highly conserved metabolic pathway (32). The PPP, which is activated in various tumor cells (33), generates ribose-5-phosphate for *de novo* synthesis of the DNA and RNA phosphoribosylamine backbone (3) and is the main source of intracellular NADPH (34, 35). Excessive oxidative stress is hazardous as it could damage cellular components including DNA, proteins, and lipids (3, 36, 37), eventually initiating cell death signaling pathways, while NADPH provides the reducing power for both the glutathione and thioredoxin systems, which are crucial for the clearance of ROS produced during rapid cell proliferation and for repairing ROS-induced damage (35). Hence, the PPP links glucose catabolism to the biosynthesis of macromolecules essential for supporting the rapid proliferation of tumor cells and also contributes to tumor growth and survival under oxidative stress condition, which arises due to rapid proliferation of tumor cells.

Indeed, our results show that an increase in intracellular oxidative stress and a decrease in nucleotides biosynthesis contribute to the impaired tumor cell proliferation caused by *YY1* silencing. Interestingly, restoration of either oxidative stress or the intracellular amount of nucleosides failed to significantly affect tumor cell proliferation, indicating that these two factors are of equal importance. Thus, our results strongly suggest that YY1 enhances the PPP and subsequently supports tumor cell viability and proliferation by acting as a guardian against excessive oxidative stress and by providing precursor for producing nucleic acids.

YY1 itself is a transcriptional regulator that could act positively or negatively, depending on its target genes (11). Aberrant YY1 expression has been confirmed in various cancers, including colon carcinoma, pancreatic cancer, bladder cancer, and breast cancer (13, 16–19). YY1 has been found to regulate various protumorigenic pathways by, for example, facilitating the interaction between MDM2 proto-oncogene and p53 to increase p53 ubiquitination (20), blocking p300-mediated p53 transcription and stabilization (38), stabilizing HIF1α (13), and inhibiting a tumor suppressor microRNA (18). Through these pathways, YY1 stimulates tumor cell proliferation, angiogenesis, and metastasis. Our present findings demonstrated a new role for YY1 in tumorigenesis, i.e., altering tumor cell metabolism by enhancing the PPP. Given that metabolic reprogramming is a key enabler of tumor cell proliferation, survival, and metastasis, the regulatory effect of YY1 on the PPP indicates that YY1 is critical for key functions of tumor cells.

As a transcription factor, YY1 operates on a wide range of targets involved in various biological pathways. Indeed, our results indicate that YY1 might affect, even if to a low extent, the expression of other glucose metabolism genes, including glycolysis-related factors *GLUT1*, *PFK2*, *PKM2*, and *LDHA* (Fig. 1C), as well as PPP-related factors *6PGD* and *RPE* (Supplementary Fig. S7). Our results show that *YY1*-silencing robustly suppresses the amount of 6-phosphogluconate, a PPP intermediate produced in the upstream of 6PGD and RPE enzymatic reactions, and is the substrate of 6PGD; and that excessive ROS and impaired nucleotides biosynthesis critically influenced *YY1* regulation of tumor cell growth, indicating that YY1 is critical for regulating PPP in tumor cells. Furthermore, given that G6PD restores cellular 6-phosphogluconate, ribose-5-phosphate, and NADPH levels, as well as tumor cell proliferation and colony formation potentials altered by *YY1* manipulation, our results highlight the importance of the YY1/G6PD pathway. Hence, while *YY1* might also affect other relevant metabolic pathways such as glycolysis, as well as ROS metabolism, our findings strongly suggest a novel, critical role of YY1 in regulating the PPP in tumor cells by acting on its pacesetter enzyme G6PD.

We show that regulation of the PPP by YY1 occurred regardless of p53 status, as it could bind directly and activate the G6PD promoter in p53-null cells. G6PD is the limiting enzyme that catalyzes the first committing step of the PPP, i.e., the oxidation of glucose-6-phosphate, and concomitantly reduces $NADP^+$ to NADPH (34, 39). Deregulated G6PD expression could be observed in various tumors, such as colon cancer, glioblastoma, and breast cancer (40–42) and is related to metabolic alteration of tumor cells. YY1 has been known as an inhibitor of the p53 pathway (20, 38), and a recent study has shown that p53 is involved in suppressing the PPP through inactivation of G6PD (8); however, our findings clearly indicate that YY1 regulates G6PD even in the absence of p53. Thus, regulation of G6PD by YY1 could occur in both p53-dependent and p53-independent manners. Several current anticancer drugs inhibit tumor cell growth by activating p53 (43, 44) and p53 mutation and down-regulation have been reported in many tumor patients (45). Therefore, together with our previous results describing the p53-independent function of YY1 in stabilizing HIF1α and inducing tumor angiogenesis (13), our present findings further emphasize the potential of targeting YY1 for cancer therapy. The benefit of such approach would entail a consistent clinical outcome regardless of p53 status.

In summary, we report a previously unknown YY1-dependent modulation of the PPP, which promotes tumor cell growth via enhanced biosynthesis of macromolecules required for tumor cell proliferation and protection from hazardous oxidative stress. These findings provide new mechanistic insights into the function of YY1 in tumorigenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S. Wu, V. Kasim
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S. Wu, H. Wang, Y. Li, Y. Xie, C. Huang
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Wu, H. Wang, Y. Li, Y. Xie, C. Huang, V. Kasim
Writing, review, and/or revision of the manuscript: S. Wu, H. Wang, Y. Li, V. Kasim
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S. Wu, H. Zhao, M. Miyagishi, V. Kasim
Study supervision: S. Wu, V. Kasim

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References

1. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? Nat Rev Cancer 2004;4:891–9.
2. Warburg O. On the origin of cancer cells. Science 1956;123:309–14.
3. DeBerardinis RJ, Chandel NS. Fundamentals of cancer metabolism. Sci Adv 2016;2:e1600200.
4. DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. Cell Metab 2008;7:11–20.
5. Kroemer G, Pouyssegur J. Tumor cell metabolism: cancer's Achilles' heel. Cancer Cell 2008;13:472–82.
6. Pouyssegur J, Dayan F, Mazure NM. Hypoxia signalling in cancer and approaches to enforce tumour regression. Nature 2006;441:437–43.
7. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646–74.
8. Jiang P, Du W, Wang X, Mancuso A, Gao X, Wu M, et al. p53 regulates biosynthesis through direct inactivation of glucose-6-phosphate dehydrogenase. Nat Cell Biol 2011;13:310–6.
9. Shi Y, Seto E, Chang LS, Shenk T. Transcriptional repression by YY1, a human GLI-Kruppel-related protein, and relief of repression by adenovirus E1A protein. Cell 1991;67:377–88.
10. Seto E, Shi Y, Shenk T. YY1 is an initiator sequence-binding protein that directs and activates transcription in vitro. Nature 1991;354:241–5.
11. Gordon S, Akopyan G, Garban H, Bonavida B. Transcription factor YY1: structure, function, and therapeutic implications in cancer biology. Oncogene 2006;25:1125–42.
12. Atchison M, Basu A, Zaprazna K, Papasani M. Mechanisms of Yin Yang 1 in oncogenesis: the importance of indirect effects. Crit Rev Oncog 2011;16:143–61.
13. Wu S, Kasim V, Kano MR, Tanaka S, Ohba S, Miura Y, et al. Transcription factor YY1 contributes to tumor growth by stabilizing hypoxia factor HIF-1alpha in a p53-independent manner. Cancer Res 2013;73:1787–99.
14. Donohoe ME, Zhang X, McGinnis L, Biggers J, Li E, Shi Y. Targeted disruption of mouse Yin Yang 1 transcription factor results in peri-implantation lethality. Mol Cell Biol 1999;19:7237–44.
15. Atchison ML. Function of YY1 in long-distance DNA interactions. Front Immunol 2014;5:45.
16. Chinnappan D, Xiao D, Ratnasari A, Andry C, King TC, Weber HC. Transcription factor YY1 expression in human gastrointestinal cancer cells. Int J Oncol 2009;34:1417–23.
17. Kasim V, Xie YD, Wang HM, Huang C, Yan XS, Nian WQ, et al. Transcription factor Yin Yang 2 is a novel regulator of the p53/p21 axis. Oncotarget 2017;8:54694–707.
18. Yuan P, He XH, Rong YF, Cao J, Li Y, Hu YP, et al. KRAS/NF-kappaB/YY1/miR-489 signaling axis controls pancreatic cancer metastasis. Cancer Res 2017;77:100–11.
19. Agarwal N, Dancik GM, Goodspeed A, Costello JC, Owens C, Duex JE, et al. GON4L drives cancer growth through a YY1-androgen receptor-CD24 axis. Cancer Res 2016;76:5175–85.
20. Sui G, Affar el B, Shi Y, Brignone C, Wall NR, Yin P, et al. Yin Yang 1 is a negative regulator of p53. Cell 2004;117:859–72.
21. Bunz F, Dutriaux A, Lengauer C, Waldman T, Zhou S, Brown JP, et al. Requirement for p53 and p21 to sustain G2 arrest after DNA damage. Science 1998;282:1497–501.
22. Miyagishi M, Taira K. Strategies for generation of an siRNA expression library directed against the human genome. Oligonucleotides 2003;13:325–33.
23. Nguyen N, Zhang X, Olashaw N, Seto E. Molecular cloning and functional characterization of the transcription factor YY2. J Biol Chem 2004;279:25927–34.
24. Klar M. Yin Yang 2: the great unknown within the Yin Yang 1 regulatory network. Crit Rev Oncog 2011;16:239–43.
25. Du W, Jiang P, Mancuso A, Stonestrom A, Brewer MD, Minn AJ, et al. TAp73 enhances the pentose phosphate pathway and supports cell proliferation. Nat Cell Biol 2013;15:991–1000.
26. Ma X, Wang L, Huang, Li Y, Yang D, Li T, et al. Polo-like kinase 1 coordinates biosynthesis during cell cycle progression by directly activating pentose phosphate pathway. Nat Commun 2017;8:1506.
27. Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, et al. The human genome browser at UCSC. Genome Res 2002;12:996–1006.
28. Klar M, Stellamanns E, Ak P, Gluch A, Bode J. Dominant genomic structures: detection and potential signal functions in the interferon-beta domain. Gene 2005;364:79–89.
29. Bijlsma R. Polymorphism at the G6pd and 6Pgd loci in Drosophila melanogaster. IV. Genetic factors modifying enzyme activity. Biochem Genet 1980;18:699–715.
30. Gillies RJ, Gatenby RA. Adaptive landscapes and emergent phenotypes: why do cancers have high glycolysis? J Bioenerg Biomembr 2007;39:251–7.
31. Jiang P, Du W, Wu M. Regulation of the pentose phosphate pathway in cancer. Protein Cell 2014;5:592–602.
32. Keller MA, Zylstra A, Castro C, Turchyn AV, Griffin JL, Ralser M. Conditional iron and pH-dependent activity of a non-enzymatic glycolysis and pentose phosphate pathway. Sci Adv 2016;2:e1501235.
33. Patra KC, Hay N. The pentose phosphate pathway and cancer. Trends Biochem Sci 2014;39:347–54.
34. Rao X, Duan X, Mao W, Li X, Li Z, Li Q, et al. O-GlcNAcylation of G6PD promotes the pentose phosphate pathway and tumor growth. Nat Commun 2015;6:8468.
35. Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. Nat Rev Cancer 2011;11:85–95.
36. Sena LA, Chandel NS. Physiological roles of mitochondrial reactive oxygen species. Mol Cell 2012;48:158–67.
37. Weinberg F, Hamanaka R, Wheaton WW, Weinberg S, Joseph J, Lopez M, et al. Mitochondrial metabolism and ROS generation are essential for Kras-mediated tumorigenicity. Proc Natl Acad Sci U S A 2010;107:8788–93.

38. Gronroos E, Terentiev AA, Punga T, Ericsson J. YY1 inhibits the activation of the p53 tumor suppressor in response to genotoxic stress. Proc Natl Acad Sci U S A 2004;101:12165–70.
39. Wang YP, Zhou LS, Zhao YZ, Wang SW, Chen LL, Liu LX, et al. Regulation of G6PD acetylation by SIRT2 and KAT9 modulates NADPH homeostasis and cell survival during oxidative stress. EMBO J 2014; 33:1304–20.
40. Ju HQ, Lu YX, Wu QN, Liu J, Zeng ZL, Mo HY, et al. Disrupting G6PD-mediated Redox homeostasis enhances chemosensitivity in colorectal cancer. Oncogene 2017;36:6282–92.
41. Wang Z, Liang S, Lian X, Liu L, Zhao S, Xuan Q, et al. Identification of proteins responsible for adriamycin resistance in breast cancer cells using proteomics analysis. Sci Rep 2015;5:9301.
42. Ahmad F, Dixit D, Sharma V, Kumar A, Joshi SD, Sarkar C, et al. Nrf2-driven TERT regulates pentose phosphate pathway in glioblastoma. Cell Death Dis 2016;7:e2213.
43. Piazza GA, Rahm AK, Finn TS, Fryer BH, Li H, Stoumen AL, et al. Apoptosis primarily accounts for the growth-inhibitory properties of sulindac metabolites and involves a mechanism that is independent of cyclooxygenase inhibition, cell cycle arrest, and p53 induction. Cancer Res 1997;57: 2452–9.
44. Bible KC, Bible RH Jr, Kottke TJ, Svingen PA, Xu K, Pang YP, et al. Flavopiridol binds to duplex DNA. Cancer Res 2000;60:2419–28.
45. Huang C, Wu S, Ji H, Yan X, Xie Y, Murai S, et al. Identification of XBP1-u as a novel regulator of the MDM2/p53 axis using an shRNA library. Sci Adv 2017;3:e1701383.