GPD1L Suppresses Colon Adenocarcinoma Via Repressing TGFβ1/EMT

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Abstract

Colon adenocarcinoma is one of the most prevalent malignant tumors in human beings. Hence, the identification of valuable biomarkers and therapeutic targets is vital for improved treatment and patient outcomes. The role of glycerol-3-phosphate dehydrogenase 1-like (GPD1L) in several tumors has been achieved in recent years. However, the underlying mechanisms of GPD1L in colon adenocarcinoma remain elusive. In this study, we identified that GPD1L was associated with better prognosis in colon adenocarcinoma patients using gene expression omnibus (GEO) and the cancer genome atlas (TCGA) database. In addition, knockdown of GPD1L promoted the proliferation, migration and invasion and reversed by re-expression GPD1L in colon adenocarcinoma cells in vitro. According to gene set enrichment analysis (GSEA), GPD1L is closely correlated with transforming growth factor-β (TGFβ) signaling pathway in colon adenocarcinoma. Moreover, GPD1L downregulates epithelial mesenchymal transition (EMT) marker proteins via TGFβ1 due to Western blot analysis. These findings demonstrate that GPD1L inhibits the growth of colon adenocarcinoma cells by inhibiting EMT induced by TGFβ1. GPD1L may be a promising molecular target for the treatment of colon adenocarcinoma patients.

Introduction

Colon adenocarcinoma is one the most common malignant colon tumor with high incidence rate and increased mortality [1]. The number of colon adenocarcinoma new cases is much higher than that of other digestive system tumors. It is widely believed that colon adenocarcinoma is associated with lots of factors such as genetics, diet, inflammation, immunity, etc. However, treatment of colon adenocarcinoma remains a contentious issue. Therefore, investigation of the molecular mechanism is crucial to improve the outcome of colon adenocarcinoma patients.

The glycerol-3-phosphate dehydrogenase (GPD) protein is a dimer involved in the glycerol phosphate shuttle that transfers electrons from cytosolic nicotinamide adenine dinucleotide(NADH) to the mitochondrial transport chain and may play roles in energy production, osmoregulation, tumor growth, and apoptosis [2]. GPD1L has 84% homology with the GPD and its functions remain not fully explored. In 2007, GPD1L was identified that may affect trafficking of the cardiac Na+ channel to the cell surface. A GPD1L mutation decreases sodium voltage-gated channel alpha subunit 5 (SCN5A) surface membrane expression, reduces inward Na+ current, and causes Brugada syndrome [3]. Moreover, GPD1L may downregulate human cardiac sodium channels by altering the oxidized to reduced NADH [4]. Kelly found that GPD1L, targeting miR-210, can regulate hypoxia-inducible factor 1-alpha (HIF-1α) expression negatively by the suppression of prolyl hydroxylase (PHD) activity [5]. In recent years, GPD1L has been shown to play a vital role in several tumors. In head and neck squamous cell carcinoma, high GPD1L expression has a significantly better prognosis [6]. Meanwhile, low GPD1L and high HIF1α expression can predict lymph node metastasis in early-stage head and neck squamous cell carcinoma [7]. In pancreatic cancer, GPD1L is inhibited by a 3-microRNA signature, which promotes cell proliferation and clone formation, and inhibits apoptosis [8]. However, to date, investigations on the efficacy and function of GPD1L in colon adenocarcinoma are still poorly understood.

We thus performed the present study to explore the correlation between GPD1L and colon adenocarcinoma. GPD1L was significantly downregulated in colon adenocarcinoma tissues by comprehensive analysis using TCGA. GPD1L was proved to induce colon adenocarcinoma cell proliferation, migration and invasion by undergoing TGFβ1/ EMT signaling pathway. These findings suggest that GPD1L may be a promising tumor suppressor in colon adenocarcinoma.

Materials And Methods

Cell lines and reagents

Human colon adenocarcinoma cell lines HCT116 and COLO205 were acquired from China Center for Type Culture Collection (CCTCC, China), and cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) with 10% fetal bovine serum (FBS, Gibco, USA). All cells were grown and contained in a humidified incubator at 37 ℃ in 5% CO_2. Anti-p-Smad3 (ab52903) and anti-Snail (ab216347) were from Abcam (ab251867). Anti-TGFβ1 (sc-130348), anti–GPD1L (sc-517404), anti-E-cadherin (sc-8426), anti-Vimentin (sc-6260), anti-Slug (sc-166476), anti- anti-Smad3 (sc-7960), anti-GAPDH (sc-47724) were from Santa Cruz Biotechnology.

Plasmid construction and transfection

Lipofectamine 2000 reagent and Lipofectamine RNAiMAX were used for transfections of plasmids and siRNAs, respectively, according to the manufacturer's guidelines (Invitrogen). The sequence of GPD1L siRNAs were 5'- AAAUUUCUGAAGUUUCUUGDADC-3' and 5'- ACGUGACACGUUCGGAGAADTDT-3'.

Cell proliferation and colony formation assay

Cell Counting Kit-8 (CCK-8) assays were performed to check the proliferation rate of HCT116 and COLO205 cells according to the manufacturer's instructions. For colony formation assays, transfected cells were seeded in 6-well plates at a density of 1×10^3 cells per well. 2 weeks later, the colonies were fixed with 4% paraformaldehyde and stained with a crystal violet solution for 30 min. The number of colonies containing at least 50 cells was counted.

Wound healing assays

Cell migration was assessed by scratch assays. Transfected cells grown to 90% in 6-well plates were scratched by a 200 μl pipette tip to create a straight wound followed by washing twice with PBS. Cultured cells were grown for 24 h to allow wound closure. The relative wound healing rates were measured and compared to the width at 0 h.

Invasion assay

Cell invasion assays were performed with Matrigel Invasion Chambers following the producer's protocols (BD Bio- sciences). Transfected cells were seeded into the upper well. After 24 h, the invasive cells were

fixed with 4% paraformaldehyde and stained with 0.5% crystal violet dye solution for 30 min respectively. The number of invasive cells were counted in casually selected microscope visions and photographed.

Western blotting (WB)

The collected cells were lysed in radioimmunoprecipitation assay (RIPA) buffer and separated by 10% gel electrophoresis and electrophoretic transferred onto nitrocellulose PVDF membranes. The membrane was detected by Pierce ECL Western Blotting Substrate (Thermo Fisher Scientific, CA, USA) under ChemiDoc imaging system (Bio-Rad, USA).

Immunohistochemistry (IHC)

Tissues were deparaffinized and rehydrated with xylene and ethanol. Then, tissues were incubated for 5–10 min. After blocked with 10% goat serum for 10 min, tissues were incubated with primary antibody working solution and incubate at 4°C overnight. HRP labeled secondary antibody was added and incubated for 30 min, then horseradish phosphatase-labeled streptavidin was added and incubated for 30 min. DAB reagent stained tissues for 3 min, fully rinsed with tap water, counterstained, dehydrated, and mounted.

Statistical analysis

All in vitro experiments were performed in triplicate and repeated 3 times. GraphPad Prism software (GraphPad Software, USA) was used to evaluate the data shown as the mean ± SD. Estimation of overall survival was conducted using the Kaplan-Meier method, and differences between survival curves were examined with the log-rank test. Standard two-tailed student t-test was used to assess the group statistical analysis. The SPSS 22.0 statistical software package was used to perform the statistical analyses. p□0.05 was considered statistically significant.

Results

GPD1L plays a pivotal role in colon adenocarcinoma

To investigate the potentially involved genes in colon adenocarcinoma, we analyzed the RNA-Seq data of colon adenocarcinoma from TCGA and GEO databases (GSE151179 and GSE136630). In brief, we screened the upregulated and downregulated genes in three databases according to the differentially expressed genes between normal and tumor tissues. We identified 926 upregulated and 769 downregulated genes from the GEO database (GSE151179), 751 upregulated and 601 downregulated genes from the GEO database (GSE136630) and 7531 upregulated and 1541 downregulated genes from the TCGA database (Fig. 1a-c). Finally, 291 differentially crossed genes were obtained (Fig. 1d). Among these, GPD1L exhibited the best prognosis in colon adenocarcinoma based on the survival analysis in the GEPIA database (http://gepia.cancer-pku.cn/index.html), which was selected for further study (Fig. 1e). Further, the expression of GPD1L was significantly downregulated in colon adenocarcinoma tissues

compared with the adjacent non-tumor tissues according to TCGA database (Fig. 1f). Taken together, these data indicates that GPD1L may plays a pivotal role in colon adenocarcinoma.

GPD1L inhibits colon adenocarcinoma proliferation, migration and invasion in vitro.

Since database analysis indicates GPD1L expression has clinical significance in colon adenocarcinoma patients, we tested whether GPD1L mediates proliferation, migration and invasion in colon adenocarcinoma cells. HCT116 and COLO205 cells transfected with GPD1L siRNAs grew faster than those of control cells. These effects were reversed by GPD1L re-expression in the GPD1L knockdown cells in the above-mentioned cells. The trend of colony formation was similar to the proliferation (Fig. 2a, c). These findings indicate that GPD1L inhibits the growth of colon adenocarcinoma cells. Moreover, we performed wound-healing and transwell assays to verify the migration and invasion ability of GPD1L in the two cells. As expected, knockdown of GPD1L promoted the migration and invasion, while GPD1L re-expression reversed the effect of GPD1L knockdown (Fig. 2b, d-f). Collectively, GPD1L may play an important role in colon adenocarcinoma cell proliferation, migration and invasion in vitro.

GPD1L downregulates TGFβ1 in colon adenocarcinoma cells

To further study the molecular mechanism of GPD1L, we performed GSEA on the colon adenocarcinoma data from TCGA. Interestingly, among the signaling pathways closely related to GPD1L, the TGFβ1 signaling pathway was negatively correlated with GPD1L expression (Fig. 3a). According to the database of Starbase (https://starbase.sysu.edu.cn/) based on TCGA data, we observed a significant negative correlation between GPD1L and TGFβ1 (Fig. 3b).

Next, we investigated whether GPD1L affects the phenotype of colon adenocarcinoma through TGFβ1 in cell growth, migration and invasion. We used SD-208 as an inhibitor of TGFβ1. As expected, SD-208 reduced cell proliferation, migration and invasion. More importantly, SD-208 almost abolished the ability of GPD1L knockdown to increase cell proliferation, migration and invasion (Fig. 3c-f). In summary, GPD1L inhibits cell proliferation, migration and invasion via downregulating TGFβ1.

GPD1L regulates EMT via TGFβ1

One of the important roles of TGFβ1 in tumor is positively affecting EMT. In our study, overexpressed GPD1L in HCT116 and COLO205 cells caused a significant change in classical EMT markers, such as E-cadherin and Vimentin (Fig. 4a). Knockdown of GPD1L in both colon adenocarcinoma cells can promote the positive EMT marker proteins N-cadherin, Vimentin, Snail and Slug, and inhibit the negative marker proteins E-cadherin and Beta-catenin. These effects could be reversed by re-overexpression of GPD1L (Fig. 4b). GPD1L knockdown elevated the levels of TGFβ1, N-catenin, p-Smad and downregulated E-catenin. More importantly, SD-208 almost abolished the effect of GPD1L knockdown exhibiting a similar trend with Fig. 3 (Fig. 4c), Our results indicated that GPD1L regulates EMT through inhibiting the expression of TGFβ1.

Correlations between GPD1L and TGFβ1 in colon adenocarcinoma patients

IHC was utilized to detect the expression of GPD1L, TGFβ1 and ki67 in 36 human colon adenocarcinoma patients. Consistent with the findings in vitro, the expression level of GPD1L in colon adenocarcinoma tissues was negatively correlated with TGFβ1 and ki67 expression (Fig. 5a). Taken together, these findings suggest the important pathological role of the GPD1L/TGFβ1 axis in colon adenocarcinoma patients.

Discussion

In the present study, we identified that GPD1L acts as a tumor suppressor in colon adenocarcinoma. In detail, GPD1L is associated with better prognosis and closely correlated with TGFβ1 pathway by comprehensive data mining in various databases, including GEO and TCGA. We found that GPD1L significantly downregulated in colon adenocarcinoma tissue compared to the nontumor tissue. Further, GPD1L inhibits the proliferation, migration and invasion of colon adenocarcinoma cells in vitro. Mechanistically, though negative regulating the TGFβ1/EMT pathway, GPD1L eventually contribute to the colon adenocarcinoma formation. Overall, our data suggest that GPD1L may be a promising target for the treatment of colon adenocarcinoma.

In 2002, GPD1L [9] was reported to be expressed in cardiac tissue and colocalizes with the SCN5A-encoded cardiac sodium channel. There have been increasing studies on mediating mechanisms of GPD1L in cardiac related diseases [5, 10]. In recent years, GPD1L has been shown to be associated with tumor formation and metastasis[6-8, 11]. In triple-negative breast cancer, miR-210 confers growth-advantage and anti-apoptotic activity by targeting GPD1L. Meanwhile, miR-210 represses GPD1L, contributing to suppression of prolyl hydroxylase (PHD) activity, which leads to cancer metastasis [12, 13]. A key finding of this study is that the molecular mechanisms of GPD1L in colon adenocarcinoma is directly involve TGFβ1 pathway. To our knowledge, we first found that GPD1L is associated with colon adenocarcinoma regulating the TGFβ1/EMT pathway. TGFβ1 plays a key role in cancer progression [14-17]. EMT is also considered as an important step in tumor progression[18-20]. Indeed, TGFβ1/EMT axis is one of molecular mechanisms in many diseases[21]. In colorectal cancer, TGFβ1 is one of the dominating cytokines associated microenvironment[22]. The up/downstream key protein of TGF-β/Smad/EMT signaling pathway in colorectal cancer could be potential biomarkers [23-25], such as a disintegrin and metalloproteinase 8, Isthmin 1, Runt-related transcription factor 1, etc.

GPD1L, proved to be a potential regulator factor in our study, may be highlighted in colon adenocarcinoma. Therefore, exploring the more exact roles of GPD1L in colon adenocarcinoma oncogenesis and progression as well as the underlying mechanisms will provide powerful and direct evidence to prove GPD1L as a therapeutic target for colon adenocarcinoma.

Declarations

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by YL, ML, XY and HL. The first draft of the manuscript was written by YL and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflict of interest The authors declare that they have no conflicts of interest in this work.

Ethical approval This article does not include any studies with human participants or animals performed by any of the authors.

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Figures

Figure 1

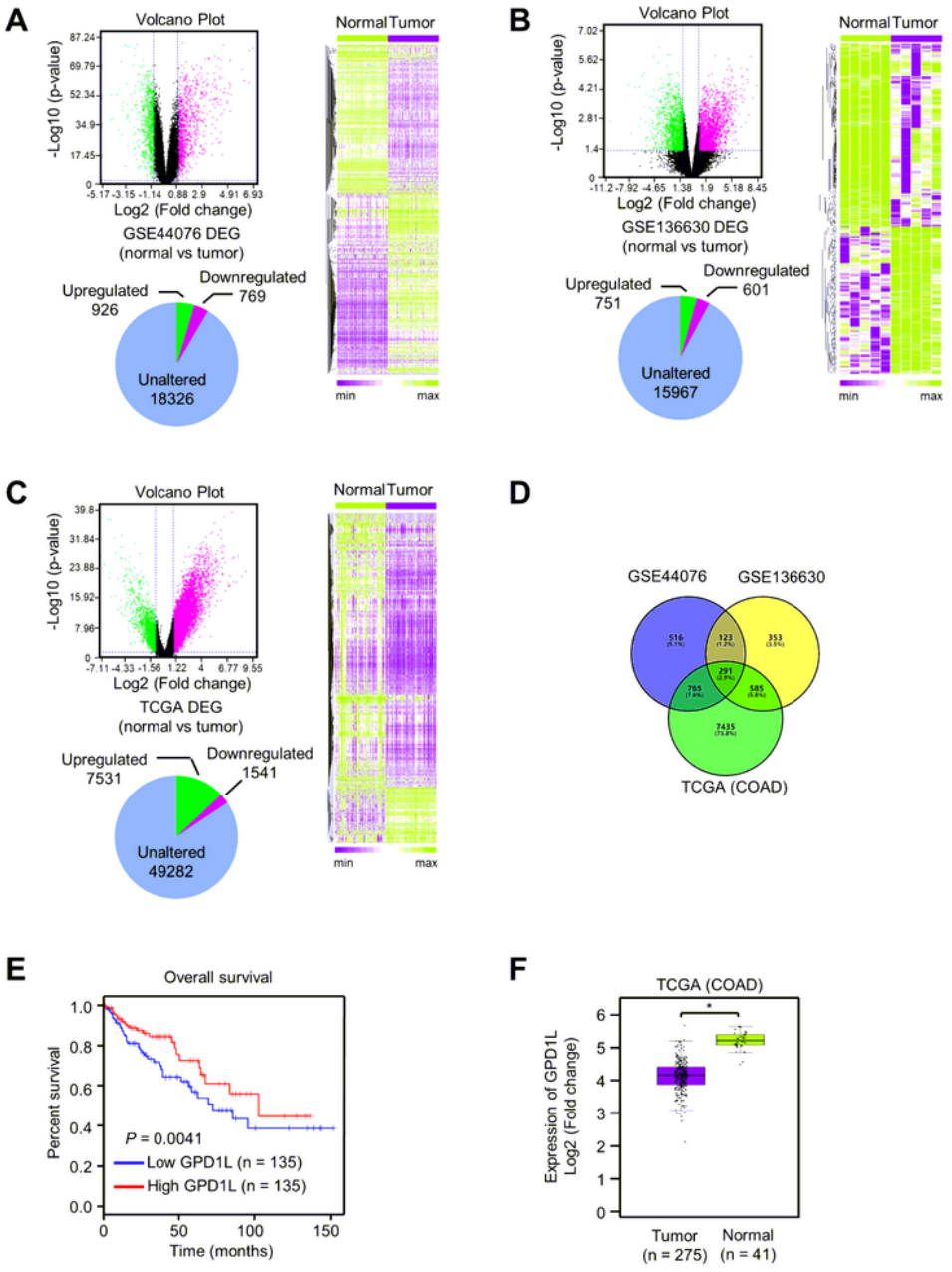

Figure 1

GPD1L plays a pivotal role in colon adenocarcinoma. a-c Volcano plots illustrating differentially expressed genes between the normal and tumor tissues in GSE44706, GSE136630, TCGA database, respectively. Green and red represent upregulated and downregulated genes. Values are presented as the log10 of tag counts. Pie chart revealed 926, 751 and 7531 upregulated genes and 769, 601 and 1541 downregulated genes, respectively. Heat maps represent the significant DEGs. d Venn analysis represents

the DEGs in each screening database, and the middle part represented the intersection of the results. e GPD1L is screened as a gene differentially expressed in normal and tumor and compared using one-way ANOVA. f Kaplan-Meier estimates of overall survival of colon adenocarcinoma patients from TCGA database (*P<0.05, **P<0.01).

Figure 2

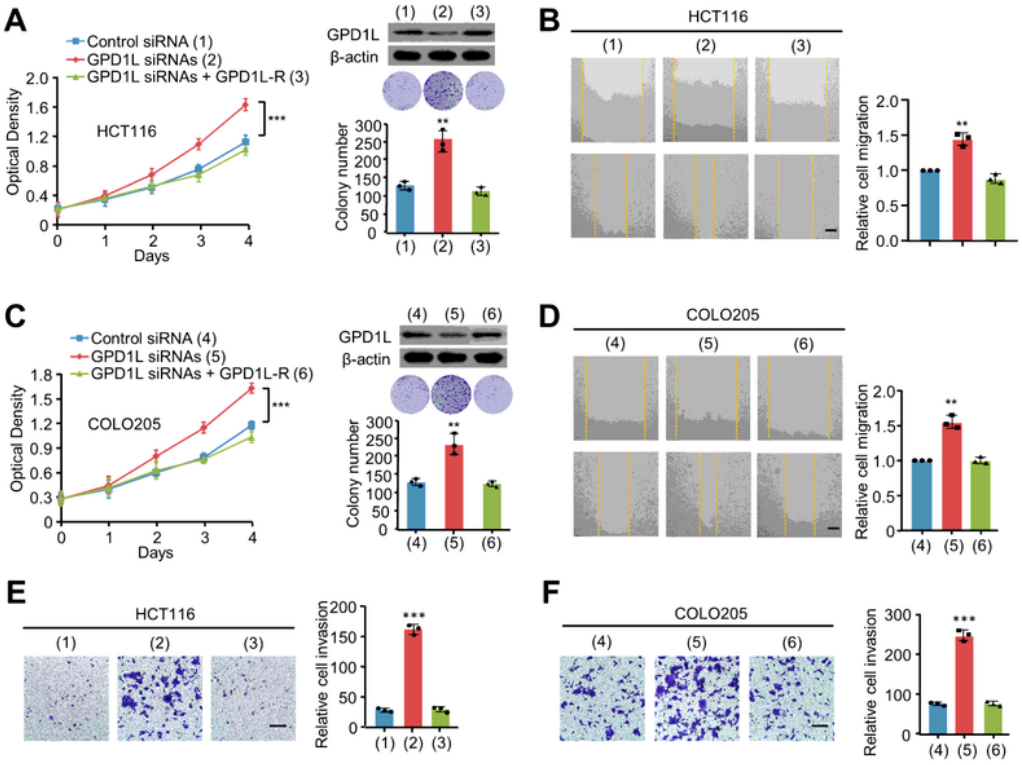

Figure 2

GPD1L inhibits colon adenocarcinoma proliferation, migration and invasion in vitro. a HCT116 cells were transfected with Control siRNA or GPD1L siRNAs or GPD1L siRNAs plus GPD1L and labeled as (1), (2) and (3), respectively. The immunoblot shows GPD1L expression. CCK-8 assay was performed to detect relative proliferation compared to day 0 of the indicated groups of cells. Illustrative colony formation images show colonies in plates. Histograms show colony number. b Wound healing/migration assay at 0 h and 24 h post wounding and percentage wound closure in groups (1), (2) and (3). c, d COLO205 cells were transfected as in a and labeled as (4), (5) and (6), respectively. CCK-8 assay, colony formation, wound healing/migration assay were used as in HCT116. e, f Invasion assay showing the number of invading cells through transwell chamber 24 h after stimulation with 5% FBS in HCT116 and COLO205. All data are represented as mean ± SD. Student's t-test was used for all statistical analyses (*P<0.05, **P<0.01, ***P<0.001).

Figure 3

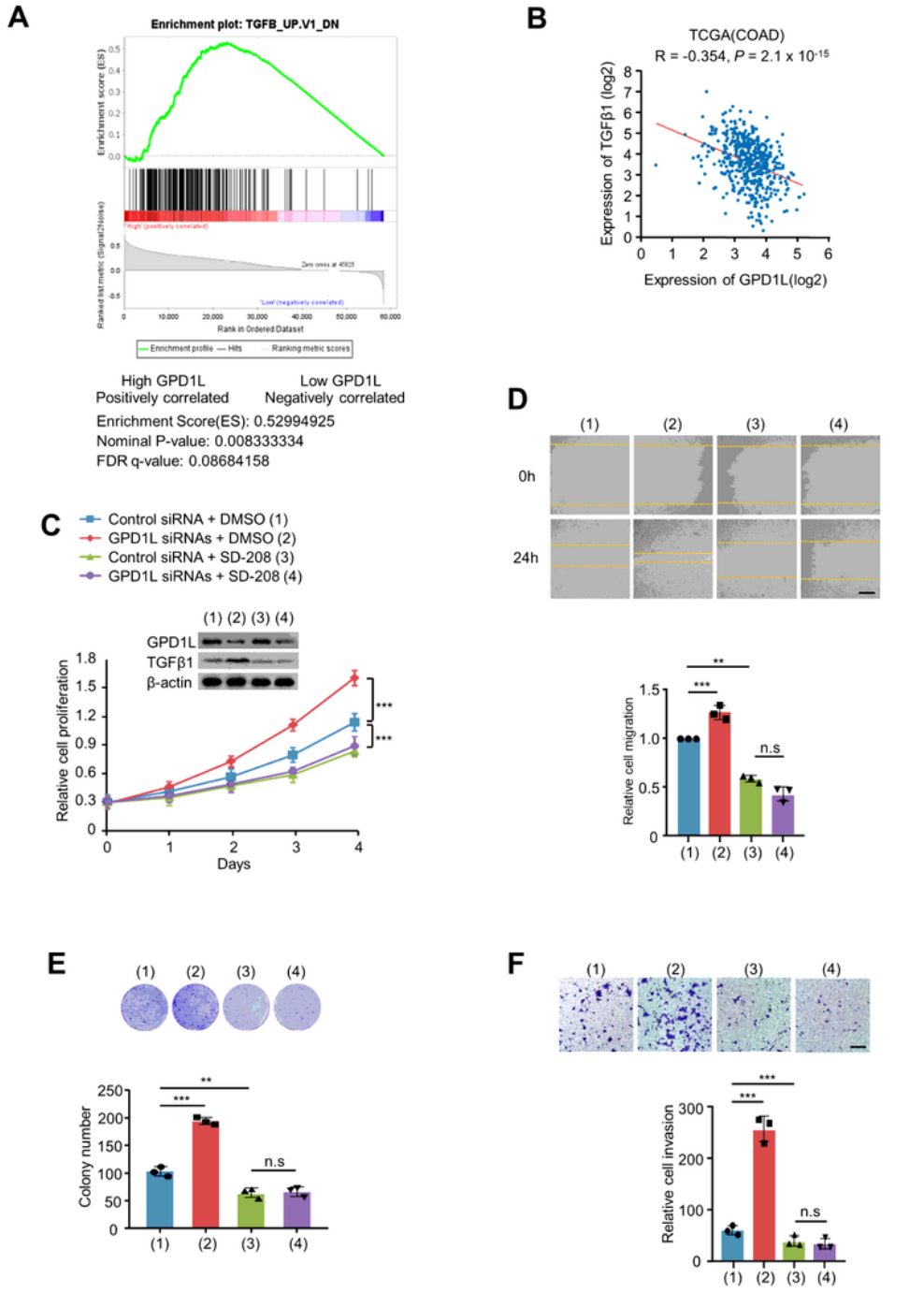

Figure 3

GPD1L downregulates TGFβ1 in colon adenocarcinoma cells. a GSEA plot showing that GPD1L expression is negatively correlated with TGFβ1 signaling in TCGA database. b GPD1L and TGFβ1 expression is negatively correlated based on the online analysis of Starbase. c HCT116 cells were transfected with Control siRNA or GPD1L siRNAs or Control siRNA plus SD-208 or GPD1L siRNAs plus SD-208. The immunoblot shows GPD1L and TGFβ1 expression. CCK-8 assay was performed to detect

relative proliferation compared to day 0 of the indicated groups of cells. d-f Illustrative images show colonies in plates, cell migration and invasion. Histograms show colony number, comparative cell migration and invasion. All data are represented as mean ± SD. Student's t-test was used for all statistical analyses (*P<0.05, **P<0.01, ***P<0.001).

Figure 4

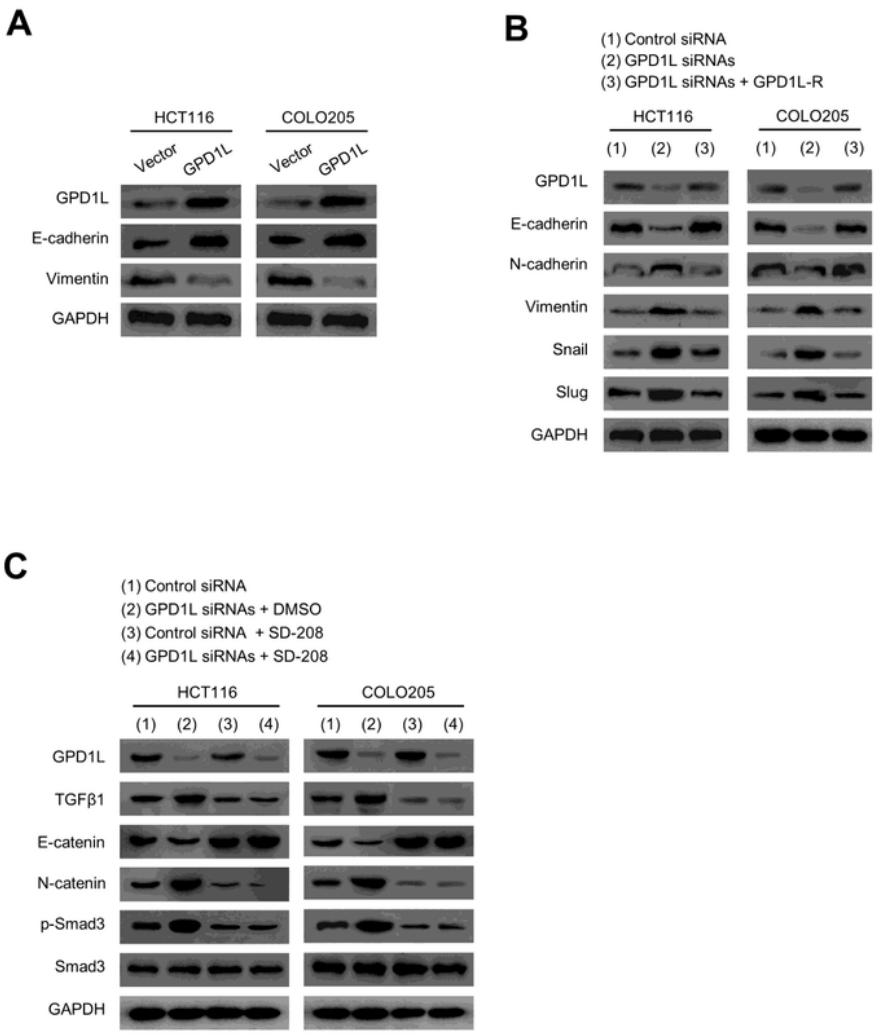

Figure 4

GPD1L regulates EMT via TGFβ1. a HCT116 and COLO205 cells were transfected with pcDNA3.0-Empty vector (EV) or pcDNA3.0-GPD1L (GPD1L). The Western-blot assay was performed to detect the proteins involved in the EMT. b HCT116 and COLO205 cells were transfected with Control siRNA or GPD1L siRNAs or GPD1L siRNAs plus GPD1L and labeled as (1), (2) and (3). The Western-blot assay was performed to detect the proteins involved in the EMT. c HCT116 and COLO205 cells were transfected with Control siRNA or GPD1L siRNAs or Control siRNA plus SD-208 or GPD1L siRNAs plus SD-208. The Western-blot assay was performed to detect the proteins involved in the EMT.

Figure 5

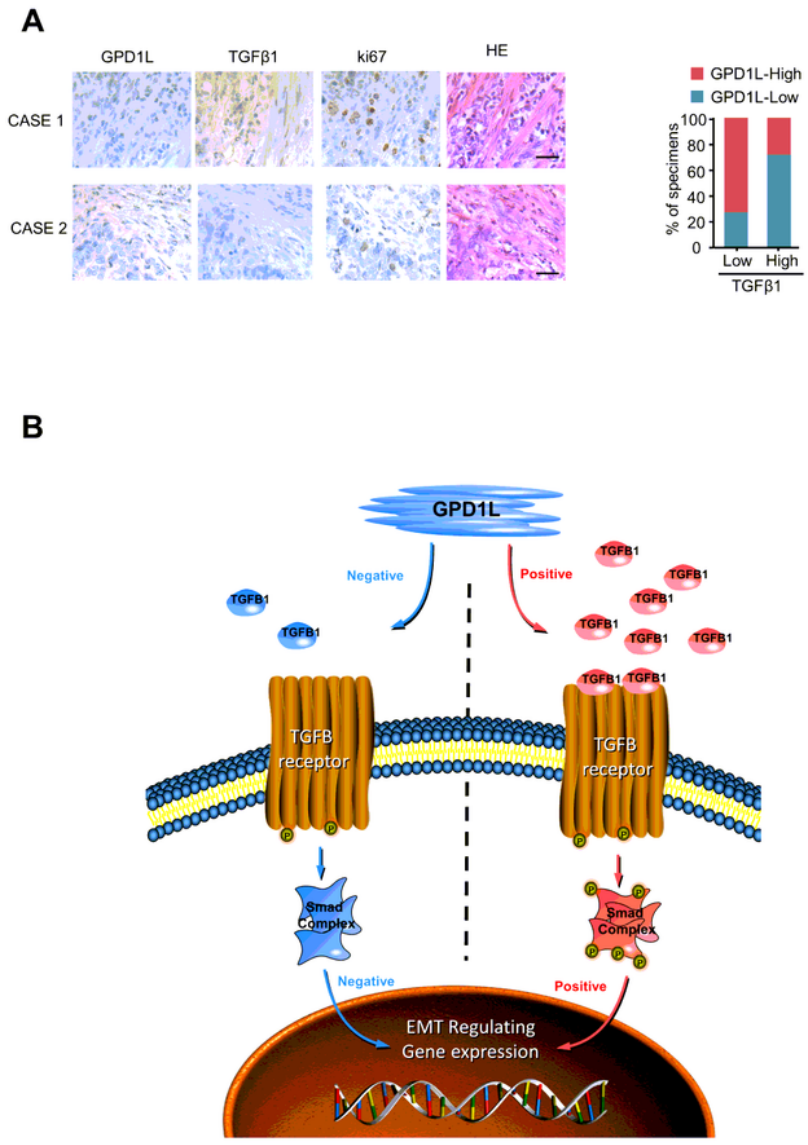

Figure 5

Correlations between GPD1L and TGFβ1 in colon adenocarcinoma patients. a Representative IHC of 36 colon adenocarcinoma patients. GPD1L and TGFβ1 were determined by IHC. b Proposed model for GPD1L inhibits EMT by negatively regulating TGFβ1, and inhibits the growth, migration and invasion of tumor cells, thus leading to colon adenocarcinoma.