TET1-Mediated Tumor Inhibition and Dox Resistance in Colon Cancer by Blocking WNT / β-Catenin Pathway

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Research Article

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Abstract

Background

As DNA demethylation protein, Ten-eleven translocation 1 (TET1) has been widely reported that is related to tumorigenesis and tumor metastasis. This study is to investigate the role and regulation mechanism of TET1 in colon cancer.

Methods

The TET1 and Catenin beta-1 (CTNNB1) expression level in colon cancer samples and cancer cell lines HCT116/SW480 were observed to discover the relationship between these two genes. Knockdown and overexpression of TET1 through shRNA and CRISPR technology were used to elucidate the effect of TET1 on WNT/β-catenin pathway. The 5-hmC/5-mC level were explored by bisulfate sequencing (BSP) and Chromatin immunoprecipitation (ChIP) to further explain the regulation mechanism. Combined with the reverse assay and transwell invasion assay, the cell migration and invasion ability were tested. Finally, the role of TET1 on DOX resistance was analyzed.

Results

TET1 downregulated in colon cancer and showed an opposite expression trend with WNT pathway associated gene CTNNB1. TET1 bound to CTNNB1 promotor and catalyzed demethylation to activate transcription of CTNNB1, inhibiting WNT/β-catenin signaling pathways. Colon cancer cells proliferation was promoted by TET1 downregulation, which was further verified as shTET1 could upregulate the tumor invasion. The DOX addition could rescue the cell migration, compared with normal expression of TET1. Meanwhile, TET1 down-regulation was related to DOX resistances.

Conclusion

TET1 played as a DNA hydroxymethylation activates inhibitors of the WNT/β-catenin signaling pathway in colon tumor and TET1 down-regulation contributed to DOX-resistance, which might provide reference to targeting therapy in clinical practice.

1. Introduction

Colon cancer originated from the epithelial cells, ranging from benign and noncancerous to malignant(1). Despite the survival rate of colorectal cancer patients undergoing surgical and chemotherapeutic treatment has improved, the prognosis remains poor due to recurrence and metastasis(2, 3). However, the primary prevention of colorectal cancer including early diagnosis and elimination in early-stage carcinoma could reach 5-year event-free (EFS) above 95%, even get completely recover(4).

For the detection and treatment of metastatic colorectal cancer, it is necessary to further discover the mechanism that causes colorectal cancer to become invasive and migratory. Several researches show

that epigenetic alterations in colorectal cancer, especially aberrations in DNA methylation is widespread (5-9). Cause and intervention methods of DNA methylation aberrations are essential to develop effective strategies and explore novel targets of inhibiting metastasis.

Loss of ten-eleven translocation 1 (TET1) has been observed in a wide range of human solid tumors, including breast cancer, colorectal cancer, ovarian cancer and so on(10-13). Low levels of TET1 are associated with tumor aggressiveness, recurrence, and adverse prognosis(14). TET1 mediates a cross-talk between DNA methylation and histone modifications to orchestrate transcriptional silencing(15). TET1 catalyzes DNA demethylation by first converting 5-methylcytosine (5mC) to 5hmC and then producing demethylated cytosine(16). In glioblastoma cells, TET1 could mediate 5hmC production, acting as a recruitment signal to methylate H4R3 and activating the transcription of genes involved in glioblastoma genesis(16-18). From the perspective of cell phenotype, however, the role of TET1-mediated regulation in colon cancer and their mechanism of interaction is still unclear.

In this study, we tested the hypothesis that WNT1 and associated proteins enhanced upon TET1 knockdown in colon cancer cells and that this increase would lead to cell migration. Collectively, our results suggest that TET1-mediated change as a key switch to regulate colorectal cancer invasion.

2. Material And Methods

2.1 Clinical Tissue Samples

A total of five tumor tissue specimens without treatment with chemotherapy were collected from colon cancer patients in Putuo Hospital, Shanghai University of Traditional Chinese Medicine. The whole investigation was approved by the Ethics Committee of Shanghai University of Traditional Chinese Medicine affiliated Putuo Hospital and written consents were provided by all five patients. The clinical diagnosis was confirmed by two experienced pathologists without discrepancy.

2.2 Cell Culture

The human colon cancer cell lines of HCT116 and SW480 were acquired from the American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured in RPMI-1640 containing 10% FBS (fetal bovine serum) and 1% penicillin-streptomycin. Tumor cells are cultured in a 5% (v/v) CO_2 humidified incubator at 37°C.

2.3 Knockdown and overexpression

TET1 short hairpin RNA (shRNA, synthetized by Gene Pharma, CN) were utilized to transfect into HCT116 cells and SW480 cells for TET1 knockdown. Following please find the sequences of shRNA targeting TET1 (shTET1) 5'- TCAGAAGATTTAGAATTGATTTCAAGAGAATCAATTCTAAATCTTCTGTTTTTTC-3' or 5'-TTGTGCCTCTGGAGGTTATAATTCAAGAGATTATAACCTCCAGAGGCACAA-3'. CTNNB1 (shCTNNB1) containing the sequence

5-$GG\top\ \forall T\forall GGCTGCAG\top A\top TC\forall GAGAT\forall CTGCAG\mathbb{C}\top A\top\ \forall\mathbb{C}$ - 3 was designed and cloned into the pSicoR-GFP vector (Addgene, Cambridge, MA).

For TET1 overexpression, the gRNA for TET1 was designed at following website (http://sam.genome-engineering.org/database/). The TET1 overexpressing gRNA was designed to target the sequence 5'-AGGGGGTCGAGAGGGAGTCG-3'. Then, insert the gRNA oligo into lenti gRNA (MS2)-puro plasmid, by the process of Oligo annealing, digestion with BsmBI, ligation, transformation and plasmid extraction, we obtain the TET1 plasmid and transfect the plasmid in cells. 72h after transfection, the lentivirus for TET1-overexpression was collected and filtered through 0.45 μm filters. After infection with the virus for 24h, targeted cancer cells were replaced with fresh media for further analysis.

2.4 RNA Extraction and Detection of RT-PCR

Colonic cells were planted in 6-well plates. Total RNA was extracted from both human tissue samples and cultured cells with the TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Additionally, the cells with downregulated TET1 or TET1-activated were treated DOX with a concentration of 1μg /ml incubating for 24 h. Generally, High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) and SYBR Green master mix (Roche, Mannheim, Germany) were utilized to reverse transcribe mRNA into cDNA and were adopted to assay the relative expression of WNT1, SFRP1, MyC, TET1, CTNNB1, respectively. GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was used as control. The amplification was set as follows: 95°C for 10 min, 40 cycles of 95°C for 15 s, and 60°C for 1 min. Primer sequences were shown in Table 1.

Table 1
Primer sequences of RT-qPCR

Gene name	Primer sequence (5′-3′)
WNT1	F: CGATGGTGGGGTATTGTGAAC
	R: CCGGATTTTGGCGTATCAGAC
SFRP1	F: ACGTGGGCTACAAGAAGATGG
	R: CAGCGACACGGGTAGATGG
MyC	F: GGCTCCTGGCAAAAGGTCA
	R: CTGCGTAGTTGTGCTGATGT
TET1	F: CAGAACCTAAACCACCCGTG
	R: TGCTTCGTAGCGCCATTGTAA
CTNNB1	F: AAAGCGGCTGTTAGTCACTGG
	R: CGAGTCATTGCATACTGTCCAT
GAPDH	F: GGAGCGAGATCCCTCCAAAAT
	R: GGCTGTTGTCATACTTCTCATGG

2.5 Western Blot

Cultured cancer cells (5×10^6 cells/mL) were collected and lysed in cold RIPA buffer plus phosphatase and protein inhibitors, placed on ice for 30 min and collected at 12,000 g for 20 min at 4°C to remove cell debris. The protein concentration was detected by Bradford assay. 40 μg total protein was loaded, separated on 10% SDS-PAGE and electro-transferred onto 0.45μm PVDF membranes (Millipore, Billerica, MA). After blocking with 5% BSA (bovine serum albumin) in TBST solution for 2 h, the membrane was incubated a primary antibody against beta-catenin, Cyclin D1, cMyC, CTNNB1 and GAPDH (Cell Signaling Technology, CST) diluted at 1:1000 overnight at 4°C. After incubating with HRP-conjugated secondary antibody (Sigma-Aldrich, Dorset, UK) at a 1:5000 dilution was added to the membrane and incubated for 60 min at room temperature. The membrane was visualized by chemiluminescence assay with an ECL kit and the relative expression was quantified with Image J (National Institute of Health, Bethesda, MD).

2.6 Trans-well invasion assay

Cultured cancer cells were infected with shTET1 and with TET1-activated, treated with or without DOX, respectively. An equivalent of 1×10^5 cancer cells in 150 μL serum-free RMPI medium were added to upper chambers of 8μm-24 well Transwell culture dishes, with 750 μL of 10%FBS-containing medium in lower chambers. After 48 hours incubation at 37°C, cells were rinsed with calcium free PBS and fixed with methanol, then stained with crystal violet. These tumor cells which passed through the membranes were

evaluated in five randomly selected fields, then dissolved in methanol and quantified by microplate reader OD 405nm

2.7 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

The cells supernatants were added with 5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich) and incubated at 37°C for 4 hours. 100 μL dimethyl sulfoxide was then added. The optical density (OD) was measured at both 492 nm and 630 nm wavelength; Cell viability was calculated according to the following formula: cell viability = [OD value of treatment group–OD value of background] / [OD value of control group–OD value of background] × 100%.

2.8 Chromatin immunoprecipitation (ChIP)

ChIP was performed in HCT116 cells. Cells cultured in 10 cm plates were cross-linked with formaldehyde and processed following the ChIP kit protocol (EMD Millipore, Burlington, MA, USA). The target DNA fragments were detected by PCR. The primers used to detect target DNA fragments are as follows: CTNNB1-Forward 5'-AAACAGAGAAGCCTGGCCG-3', CTNNB1-Reverse 5'-TTCGGACTGGGGCAAAACAA-3'.

2.9 Bisulfate sequencing

Genomic DNA was bisulfate-treated using the EpiTect Bisulfite Kit Qiagen following the manufacturer's protocol. Bisulfate-treated DNA was amplified by PCR with the following primers: CTNNB1 Forward: 5'-GAGTTGGCGCTGTTAACCATGTT-3' and Reverse: 5'-CCGAGACACACACACAAAGTC-3'. PCR products were then cloned into the T-Easy Vector (Quanshijin Biotechnology, Beijing, China). For each treated group, 10 clones were randomly selected for sequencing, and the results were analyzed by QUMA, an online CpG methylation analysis tool (http://quma.cdb.riken.jp/).

2.10 Statistical Analysis

Statistical analysis was performed using Prism 6 software. All experiments were carried out at least three times, and the results are presented as the mean ± standard deviation. The sample sizes were determined by power analyses, based on variation shown in our previous experiments and predicted effect sizes considered to be biological significant. No data were excluded from any analyses and all replicates were true biological replicates. Statistical significance between two samples and among multiple samples was assessed by using the one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Survival curves were plotted using the Kaplan–Meier method and compared using the log-rank test. Correlations were determined by Pearson's correlation. P-values < 0.05 were considered statistically significant.

3. Results

3.1 Expression of TET1 in colon cancer is reduced

To uncover the role of TET1 in the progression of colon cancer, we analyzed the expression of TET1 in colon tumor tissues and normal tissues according to the data from TCGA databases. The analysis results showed that TET1 level was lower in tumor (Figure 1A). Additionally, we extracted RNA in five colon cancer patients' tissue and one normal tissue. The level of TET1 was analyzed with PCR (Figure 1B) and real-time quantitative reverse transcriptase PCR (RT-qPCR) (Figure 1C). The results showed that the relative TET1 level was significantly reduced in three of five primary colon cancer samples when compared with normal tissues. In two of these patients, the relative TET1 content was reduced to approximately 20%, and in the other three patients by about a half.

3.2 TET1 regulates the activity of the WNT/β-catenin pathway

The relative CTNNB1 expression was further assayed in the colon cancer tissues by PCR (Figure 1D) and RT-PCR(Figure 1E), which showed a opposite expression trend compared with the expression of TET1. Moreover, the expression of CTNNB1 in colon tumor and normal tissues according to the TCGA data was explored and the result showed that CTNNB1 level was higher in normal tissues (Figure 1F). GO enrichment analyses showed that WNT/β-catenin signaling pathway was the most enriched pathway which was related to CTNNB1 (Figure 1G).

In order to explore the relationship between TET1 and WNT signaling pathway in depth, we first transiently knockdown TET1 of HCT116 and SW480 by using shRNA. WB analyses demonstrated effective silencing of TET1 expression (Figure 2A). Additionally, the relative expression of WNT1, SFRP1, MyC and CTNNB1 in HCT116 was detected with PCR and RT-qPCR. shTET1 induced an upregulation of the WNT1, MyC and CTNNB1 indicating increasing WNT/β-catenin signaling in cancer cells (Figure 2B, C). Expression of β-catenin, Cyclin D1 and cMyC were also promoted by TET1 knockdown both in HCT116 and SW480 cells (Figure 2D, E). Then, TET1 was specially overexpressed by CRISPR-SAM method to further verify whether TET1 could regulate WNT/β-catenin pathway. According to the WB analyses results, TET1 overexpression system was successfully established (Figure 2F). As we expected, protein levels of β-catenin, c-MyC, Cyclin D1 and CTNNB1 were downregulated when TET1 overexpression detected by WB analyses (Figure 2G, H). These results suggested that TET1 regulates the activity of the WNT/β-catenin pathway.

3.3 TET1-dependent demethylation induces transcriptional activation of CTNNB1

To further explore the relationship between TET1 and CTNNB1, CTNNB1 mRNA expression in TET1 OV and KD HCT116 cells was detected. Results showed that CTNNB1 expression was promoted in TET1 KD cells but was decreased in TET1 OV cells (Figure 3A). The classical molecular mechanism underlying the upregulation of target genes by TET1 consists of TET1 binding to the promoter of the target gene, catalyzing 5-mC hydroxylation to 5-hmC in CpG islands, inducing demethylation, and ultimately activating gene transcription. In accordance with this mechanism, we first performed ChIP analysis and found TET1 directly bound the CTNNB1 promoter (Figure 3B). To confirm the influence of the TET family in hydroxymethylation, we also determined the 5-hmC and 5-mC content in paired tissues. RT-PCR analysis indicated the level of 5-hmC was downregulated but the 5-mc was promoted in patience's tissue

(Figure 3C). Then, we analyzed 5-hmC and 5-mC enrichment in CTNNB1 via Bisulfate sequencing PCR (BSP), which revealed that 5-hmC levels were significantly increased by TET1 overexpression, and showed the opposite trend by TET1 knockdown (Figure 3D). Together, these results demonstrate that TET1 directly binds to CTNNB1, catalyzing demethylation via conversion of 5-mC to 5-hmC, ultimately inhibiting the WNT/β-catenin signaling pathway.

3.4 TET1 inhibits carcinoma cell migration and proliferation by regulating WNT/β-catenin pathway

To detect the effect of TET1 on tumor invasion, cell transwell migration assay was performed. It found that the knockdown of TET1 increased cell migration, whereas the overexpression of TET1 reduced cell invasion (Figure 4A). Moreover, TET1 knockdown significantly increased colon cancer growth, while TET1 overexpression inhibited tumor cell proliferation (the data of HCT116 was shown in Figure 4B, the data of SW480 was not shown). Additionally, we knocked down CTNNB1 by shRNA transfection in HCT116 cell (Figure 4C) and found that the inhibition effect of TET1 on HCT116 proliferation was offset when CTNNB1 was knocked down (Figure 4D). Several reports have shown that optimal doxycycline (Dox) concentration to maintain TET1 gene expression(19). Therefore, we tested the effect of DOX on HCT116 proliferation. Figure 4E showed that adding DOX can rescue the knocking down efficiency. These results suggested that TET1 inhibits colon cancer cell migration and proliferation by regulating WNT/β-catenin pathway.

3.5 TET1 down-regulation is related to DOX resistances in the colon cancer cells HCT116

To further investigated whether TET1 down-regulated was associated with DOX resistances, we first established DOX-resistances HCT116 cells. By incubating HCT116 cells with chronic increasing concentration of DOX, DOX-resistant cell line HCT116/DDP was established with its IC50 raised to 20.82 μg/mL, which has been elevated to 6 times the original. Incubation of DOX decreased the cell viability in a concentration-dependent manner (Figure 5A). In the exposure of 50 μg/mL DOX, the viability of HCT116-DOX resistances was 24.2% while that of the parental counterparts was 7% (Figure 5A). As our expected, TET1 expression was significantly decreased in DOX-resistance cells (Figure 5B).

In the experiment to explore the effects of TET1 on drug-resistant HCT116 migration, it was found that 10ug/ml DOX inhibited almost all HCT116 migration, but had little effect on the migration of HCT116-DOX resistance. At the same time, when TET1 was over-expressed, the resistance of HCT116-DOX resistance cells could be effectively reversed, resulting in a significant metastasis of the drug-resistant cells under the effect of 10ug/ml DOX (Figure 5C, D). Finally, in the cell vitality assay, results showed that TET knockdown could effectively inhibit the growth inhibition caused by DOX in HCT116 (Figure 5E), while TET1 overexpression could also reverse the resistance of drug-resistant cells (Figure 5F). Therefore, we verified that TET1 down-regulation was related to DOX resistances in the colon cancer cells.

4. Discussion

Cytosine hydroxymethylation was recently reported as an important epigenetic modification of DNA in mammalian cells. Similar to methylation, hydroxymethylation replaces the C5-position in cytosine with a hydrogen atom by a hydroxymethyl group(20). Cytosine hydroxymethylation is also involved in gene regulation and cell development(21, 22). Abnormal DNA hydroxymethylation can lead to cellular dysfunction and affect tumor progression and hematopoiesis function (23, 24). The TET family proteins, which are related to leukemia and various solid cancers, have become a hotspot in cancer research. Among the three proteins of TET family, TET2 is a widely recognized tumor suppressor gene, and TET2 mutations are closely related to the occurrence and development of leukemia(25-27). TET2 knockout embryonic stem cells become tumorigenic. TET3 is expressed at low levels in tissues and has rarely been studied. In acute myeloid leukemia, TET1 was first identified as a translocation partner that fuses with hybrid-lineage leukemia gene (28). Compared with healthy normal epithelia cells, significantly downregulation of TET1 can be observed in colon, prostate, breast, and liver tumors, which contributes to enhanced cell proliferation and growth capacity. However, whether TET1 is an oncogene or an anticancer gene is unclear, and there have been conflicting reports about its role in tumorigenesis(29-32). As for now, TET1 is downregulated with the absence of 5-hydromethylation in various human cancers (33). Downregulation of TET1 has been proved related to breast cancer invasion and metastasis through HMGA2/TET1/HOXA9 axis, as well as having an inhibitor effect to EMT in pancreatic tumor [34]. In recent research, TET1-mediated 5-hmC has been demonstrated to play an important role in epigenetic regulation and gene expression(34). Neri F *et.al* reports that TET1 is downregulated in initial stage of colon cancer, however, the biding of DKK promoter inhibitor and TET1 could maintain hypomethylated in a normal condition, which means TET1 downregulated lead to constitutive activation of WNT pathway(35, 36).

In our work, we found that patients with colon cancer always exhibit downregulated TET1. Using shRNA and CRISPR-SAM technology, we constructed the TET1 knockdown and TET1 overexpression colonic cancer cells to explore the relationship between TET1 and WNT/β-catenin pathway. We found that TET1 KD could induce WNT/β-catenin pathway associated gene WNT, MyC, CTNNB1 dys-activation and high invasion, whereas TET1 overexpression could reverse these effects. The results implicit that TET1 plays a onco-suppressor role mediated by inhibiting WNT/β-catenin pathway.

In addition, the TET family proteins has been found in the mammalian genomes to oxidase 5mC to 5hmC, suggesting that DNA methylation in mammalian cells may be reversible(38-40). Following the classical molecular mechanism of TET1-mediated transcriptional activation, we demonstrated that TET1 bound directly to the CTNNB1 promoter, catalyzed 5-mC hydroxylation to 5-hmC in the promoter CpG islands, induced demethylation, initiated CTNNB1 transcriptional activation. Moreover, since optimal Dox concentration can maintain TET1 gene expression, we tested the effect of DOX on HCT116 proliferation, as well as the relationship between TET1 expression level and DOX resistance. The results suggested that TET1 down-regulation was related to DOX resistances in the colon cancer cells.

Studies have shown that overexpression of TET1 can down-regulate the proliferation of cultured colon cancer cells and cells inoculated in nude mice through demethylation of the CTNNB1 promoter. Changes

in TET1 expression could disrupt the DNA methylation and demethylation balance, which was associated with colon cancer(41). More researches are needed to explain the detailed mechanism of TET1 and its relationship to colon cancer before considering it as a therapeutic option.

Colorectal cancer is the third most common malignancy and one of the deadliest cancers. Most patients are diagnosed as advanced stage when cancer cells have formed and metastasis to other vital organs. (37). Thus, inhibition of tumor metastasis may improve survival of colorectal cancer patients.

Declarations

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Conflicts of interest

The authors declare that they have no competing interests.

Author contributions

Lu Shiyu and Feng Mingli were responsible for designing and doing experiments. Tian Jiyun, Wu Chenquand Jiang Yuanyewere responsible for manuscript draft. Cao Qinand Lin Hui were responsible for project design and manuscript edit.

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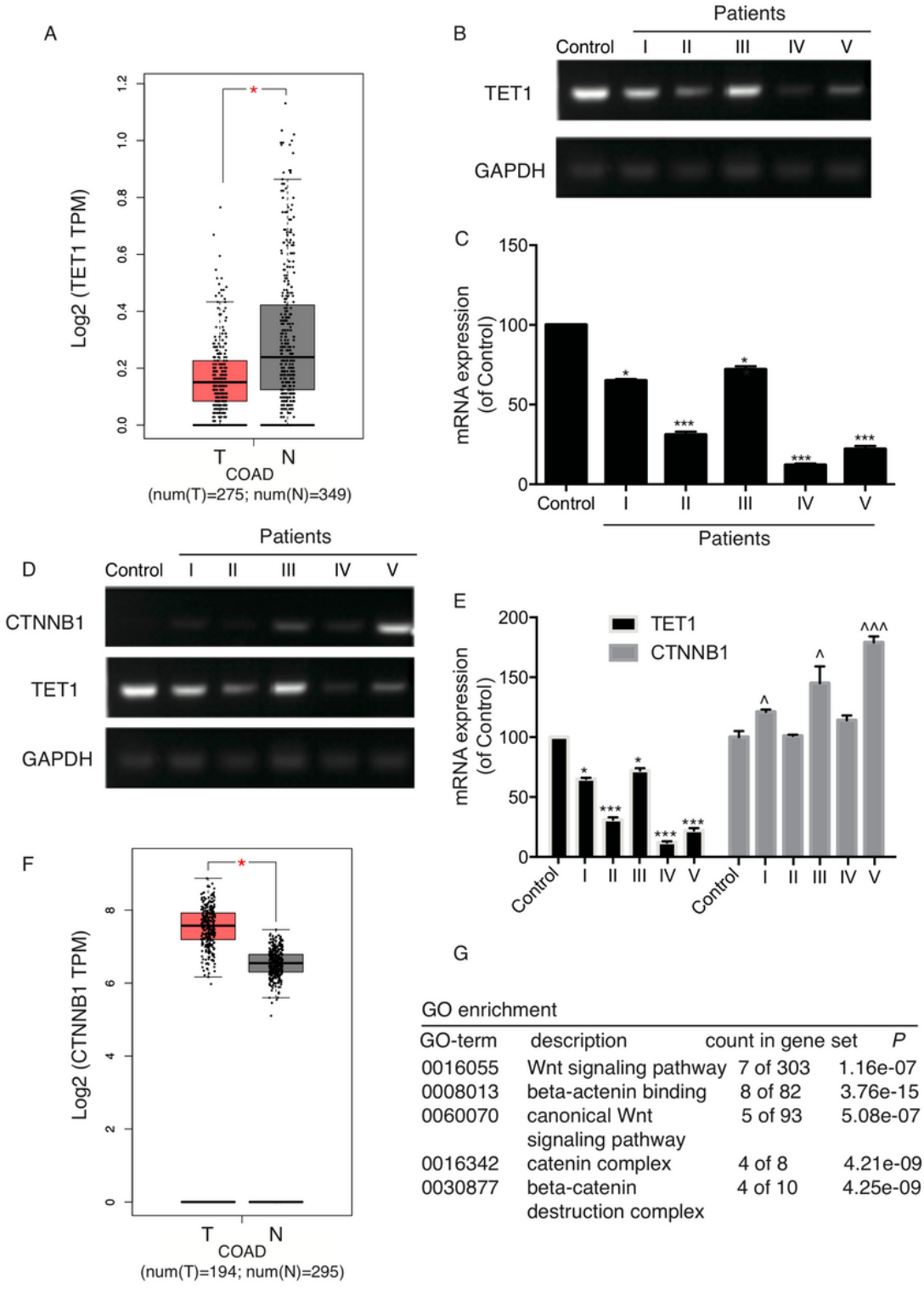
Figures



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GO enrichment

GO-term	description	count in gene set	*P*
0016055	Wnt signaling pathway	7 of 303	1.16e-07
0008013	beta-actenin binding	8 of 82	3.76e-15
0060070	canonical Wnt signaling pathway	5 of 93	5.08e-07
0016342	catenin complex	4 of 8	4.21e-09
0030877	beta-catenin destruction complex	4 of 10	4.25e-09

Figure 1

Expression of TET1 and CTNNB1 in colon cancer. (A) TET1 mRNA expression in colon patience and normal person based on TCGA database. (B, C) The relative TET1 mRNA expression detected by PCR (B) and RT-PCR (C) in human samples of colon tumors, relevant adjacent healthy tissues. (D, E) TET1 and CTNNB1 relative mRNA level in colon cancer patients was detected by PCR (D) and RT-qPCR (E). (F) Analyses of CTNNB1 expression in tumor and normal tissue based on TCGA database (G) Enriched pathways with indicated p-values related to CTNNB1 using Gene Ontology (GO) enrichment analysis. (*P < 0.05; **P < 0.01; ***P < 0.001(of TET1 control). ^P < 0.05; ^^P < 0.01; ^^^P < 0.001(of CTNNB1 control).)

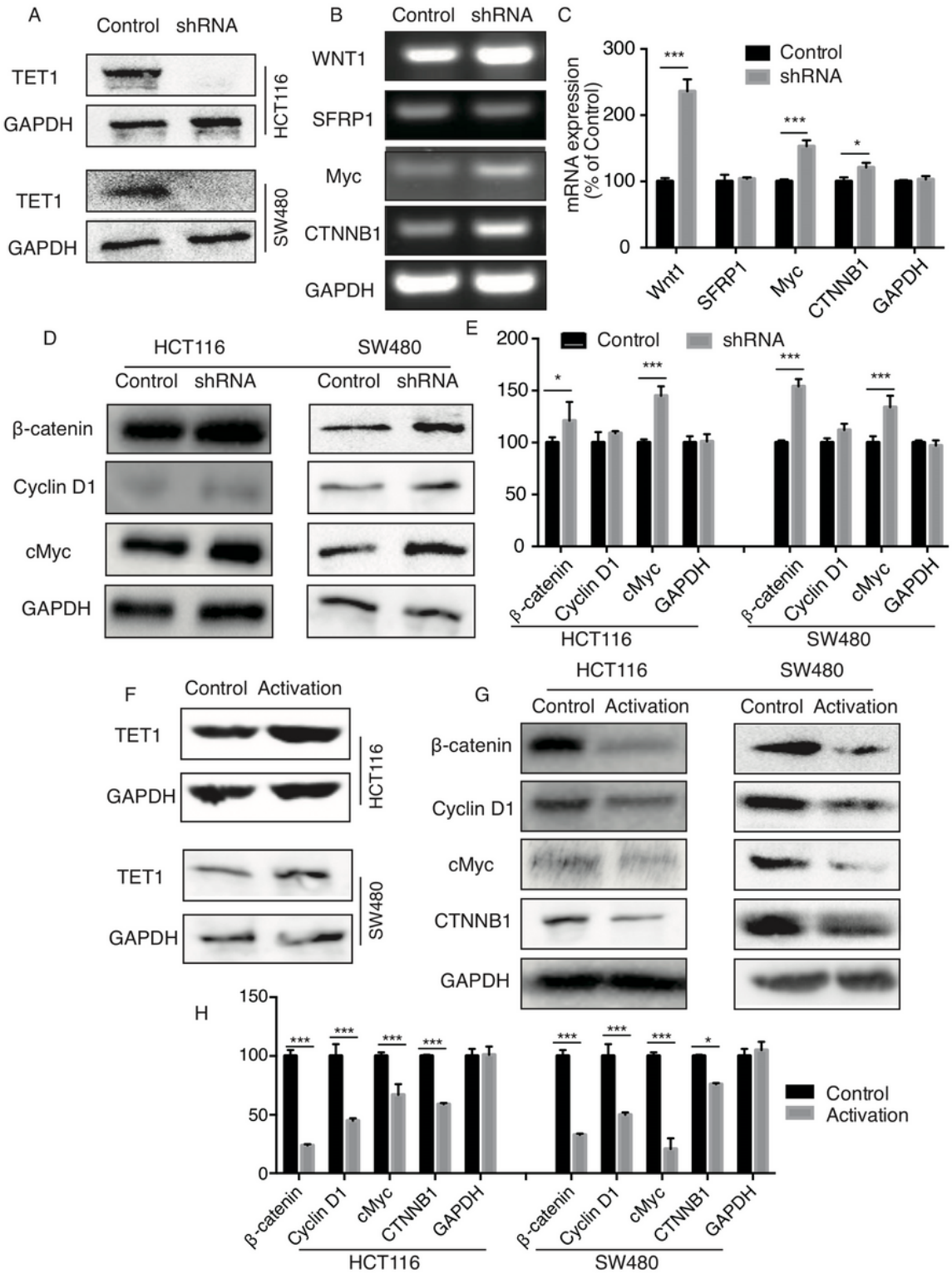

Figure 2

TET1 regulates the activity of the WNT/β-catenin pathway (A) Western blot analysis of the TET1 expression in the HCT116 and SW480 cells transfected with shRNA. (B, C) The WNT/β-catenin pathway associated gene expression of WNT1, SFRP1, CTNNB1 and MyC were detected by PCR (B) and RT-PCR (C) in HCT116 control cell and HCT116-shTET1 cell. (D) Protein expression of β-catenin, Cyclin D1 and cMyC were detected in HCT116 and SW480 cells. Results were shown in (E). (F) Western blot analysis of

the TET1 expression in the HCT116 and SW480 cells after TET1 overexpression. (G) Protein expression of β-catenin, Cyclin D1, cMyC and CTNNB1 were detected in HCT116 and SW480 cells. Data were shown in (H). (*P < 0.05; **P < 0.01; ***P < 0.001.)

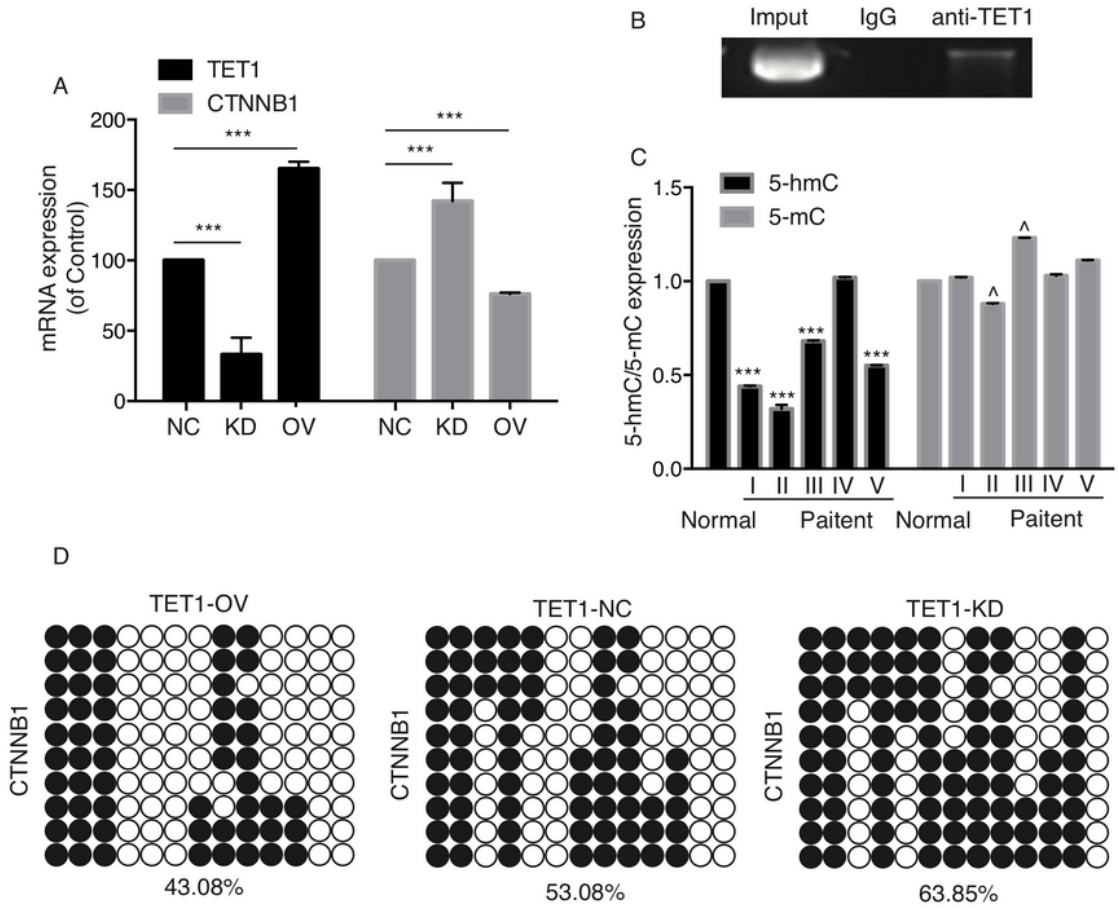

Figure 3

TET1-dependent demethylation induces expression of CTNNB1 (A) Analyses of CTNNB1 expression in normal, TET1 KD and TET1 OV HCT116 cells. (B) ChIP analysis of TET1 binding to the CTNNB1. (C) 5-

hmC and 5-mC levels in patience's tissues and normal tissue were detected by RT-PCR. (D) BSP indicated the changes in methylation levels in CTNNB1. (*P < 0.05; **P < 0.01; ***P < 0.001(of 5-hmc control). ^P < 0.05; ^^P < 0.01; ^^^P < 0.001(of 5-mc control).)

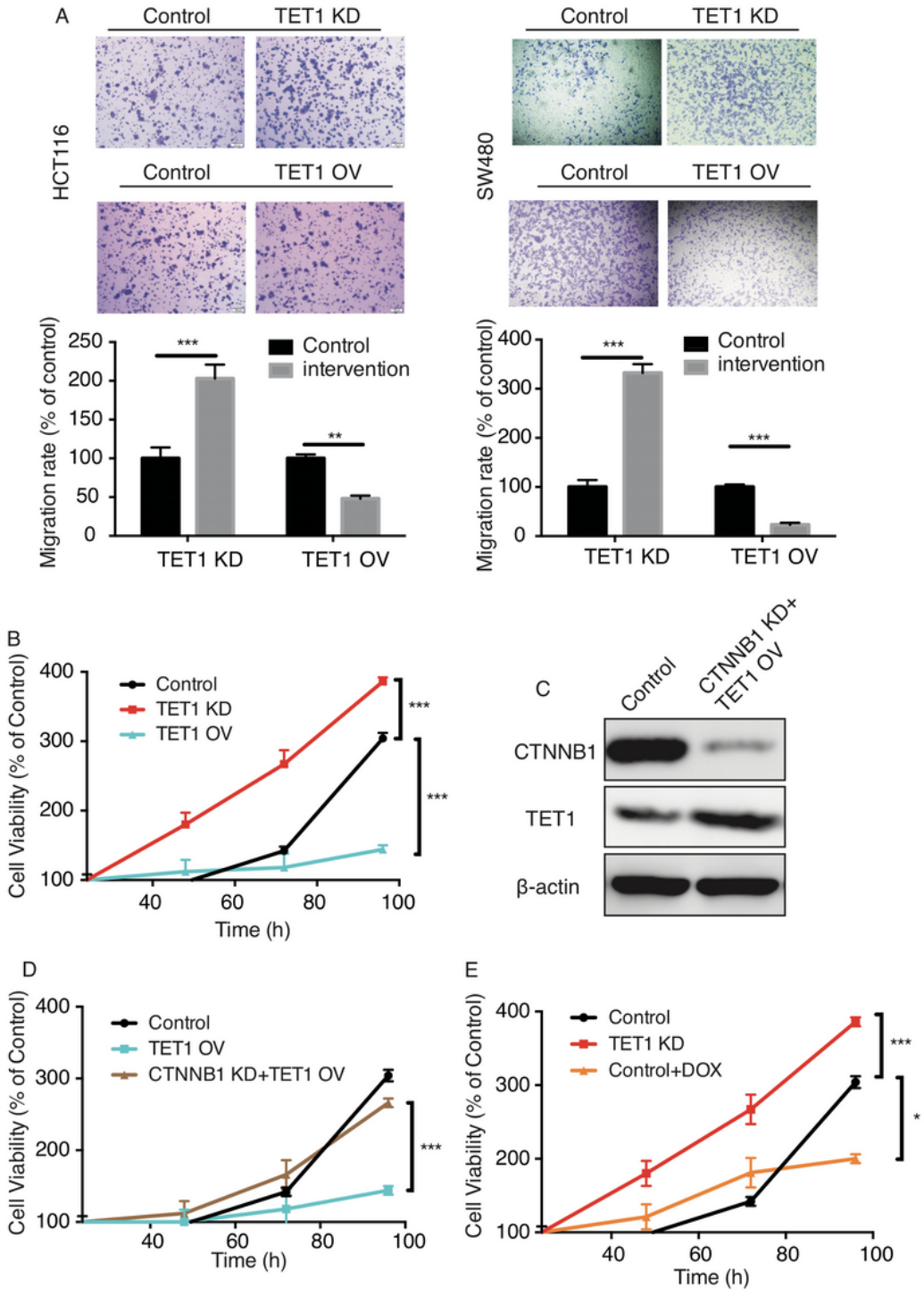

Figure 4

TET1 inhibits HCT116 carcinoma cell migration and proliferation by regulating WNT/β-catenin pathway. (A) Trans-well invasion assay of HCT116 and SW480 cells in the condition of TET1 Knockdown or TET1

overexpression. Representative figures from three independent experiments were shown. Cell invasion statistics graph of Trans-well invasion assay showed below. (B) Effect of TET1 on HCT116 proliferation was detected by MTT assay. (C) Western blot analysis of the TET1 and CTNNB1 expression in the HCT116 cells after transfected with CTNNB1 shRNA or TET1 overexpression. (D) Effect of CTNNB1 on TET1 overexpression HCT116 cells. Cell proliferation was detected by MTT assay. (E) Effect of DOX on HCT116 cells proliferation was detected by MTT assay. (*P < 0.05; **P < 0.01; ***P < 0.001.)

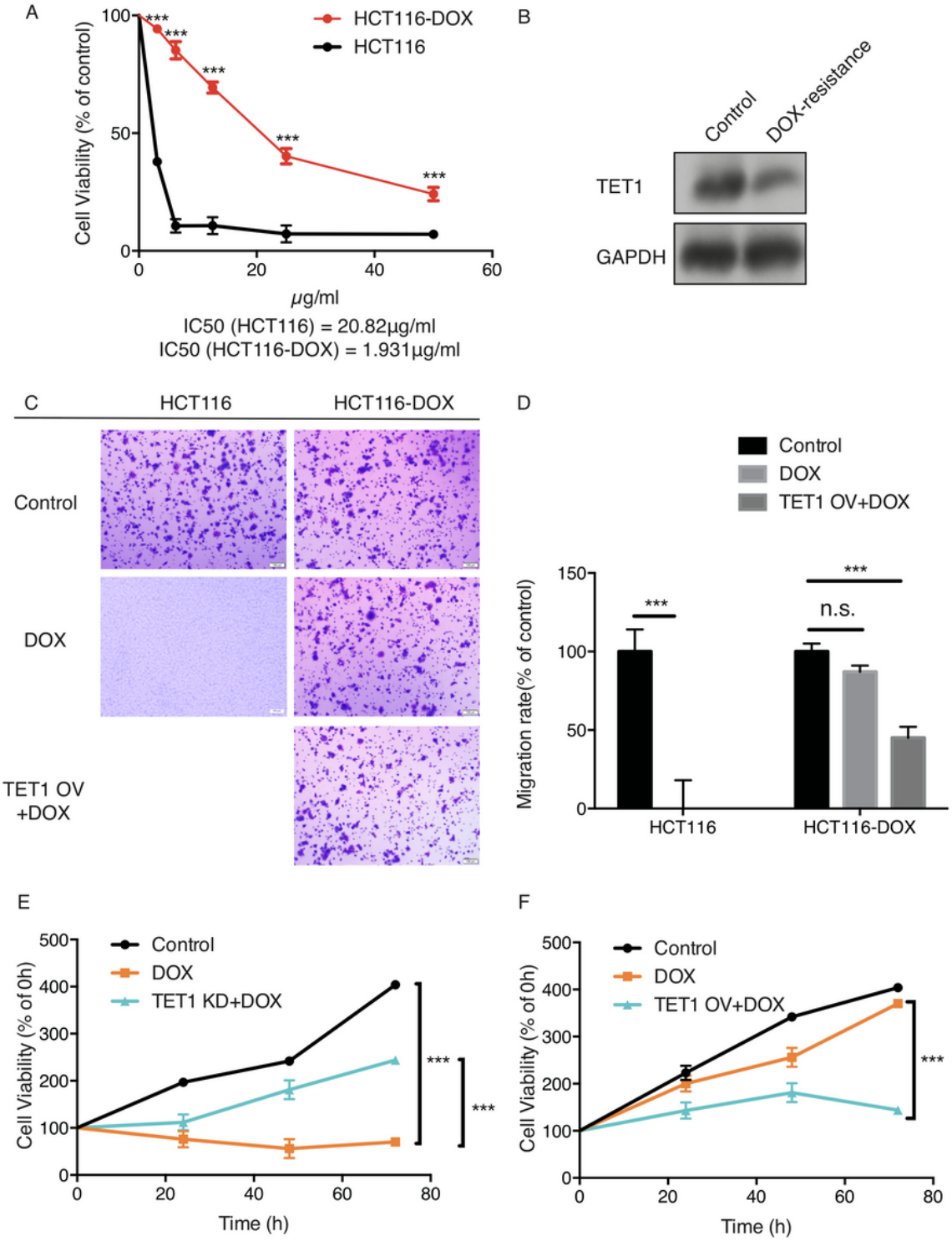

Figure 5

TET1 down-regulation is related to DOX resistances in the colon cancer cells HCT116 (A) Establishment of DOX-resistant cell line. HCT-116-DOX, by chronic exposing HCT116 cells with increasing concentrations of DOX. HCT116 and HCT116-DOX cells were incubated with serial concentrations of DOX for 48h. Cell viability(%) was measured and normalized with the corresponding untreated cells. (B) Western blot analysis of the TET1 expression in the HCT116-DOX cells. (C) Trans-well invasion assay of HCT116 cells or HCT116-DOX cells in the condition of TET1 overexpression with or without DOX treated. Representative figures from three independent experiments were shown. Migration rate of Trans-well assay was shown in (D). (E) Effect of DOX on HCT116 cells proliferation was detected by MTT assay. (F) Effect of DOX on HCT116-DOX cells proliferation was detected by MTT assay. (*P < 0.05; **P < 0.01; ***P < 0.001.)