TDG interacts with DNMT3A to inhibit the migration and invasion of human colon cancer cells

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Research

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Abstract

Background

Colorectal cancer (CRC) is one of the most common malignant tumors with high recurrence and mortality. Thymine DNA glycosylase (TDG) is one of the key molecules involved in base excision repair pathway. Recently, more and more attentions have been paid to the role of TDG on tumor development. However, the specific functions of TDG in CRC remain unclear.

Methods

The biological functions of TDG and DNA methyltransferase 3 alpha (DNMT3A) in CRC were evaluated using migration and invasion assay. Tumor metastasis assay was performed in nude mice to detect the role of TDG *in vivo*. The interaction of TDG with DNMT3A was determined by co-immunoprecipitation (Co-IP). Chromatin immunoprecipitation analysis (ChIP) was applied to predict the DNA binding site of DNMT3A. We also performed methylation-specific PCR (MSP) to detect the changes in TIMP2 methylation levels.

Results

We found that TDG could inhibit the migration and invasion of human colon cancer cells *in vitro* and *in vivo*. TDG promoted the ubiquitination and degradation of DNMT3A by binding with it. Interference with siDNMT3A also inhibited the migration and invasion of human colon cancer cells. Further ChIP, MSP, and rescue experiments data confirmed that TDG accelerated the degradation of DNMT3A, and then significantly regulated the transcription and expression of TIMP2, thereby affecting the migration and invasion of human colon cancer cells.

Conclusion

Our findings reveal that TDG inhibit the migration and invasion of human colon cancer cells through DNMT3A-TIMP2 axis which may be potential therapeutic strategies in the development and treatment of CRC.

Background

Colorectal cancer (CRC) is one of the most common malignant tumors, which seriously threaten human life and health[1]. The development of CRC is a complex multi-step process, which is induced by various environmental and genetic factors, such as gene mutations and epigenetic modifications. The current treatment for CRC patients is still based on surgery, supplemented by radiotherapy and chemotherapy[2].

However, the 5-year survival of CRC patients is relatively low, and the prognosis is not ideal. The main reason for it is the high metastasis rate and the high recurrence rate of CRC[3].

Thymine DNA Glycosylase (TDG) is one of the key molecules in the base excision repair (BER) pathway that can remove thymine from G•T mispairing and restore G•C base pairs following a 5-methylcytosine deamination[4]. With the deepening of research, other functions of TDG have been emerged, especially those involved in epigenetic modifications, such as DNA demethylation[5, 6]. TDG can recognize various derivatives of 5-methylcytosine and remove them to achieve demethylation. TDG can also interact with DNA methyltransferase 3 alpha (DNMT3A) to inhibit its methylation activity[7]. As a kind of methyltransferase, DNMT3A performs its catalytic function to directly methylate the CpG island of the target gene promoter and change its methylation level to achieve transcriptional silencing effect[8, 9]. Cells with DNA repair defects have increased genomic instability and are more likely to acquire mutations that bring about cell transformation and cancer, such as Lynch syndrome tumors and *MUTYH*-associated polyposis syndrome (MAP) [10]. However, current researches on TDG mainly focus on the gene mutation caused by its abnormality, and the research on its role and mechanism in carcinogenesis or cancer development is still very limited, which needs to be explored in specific cancer.

In the present study, we firstly analyzed the significance of TDG in CRC using publicly available CRC gene expression RNA sequencing (RNAseq) datasets from The Cancer Genome Atlas (TCGA). TDG is closely related to the metastasis of CRC. Then the *in-vitro* and *in-vivo* experiments were applied to examine the role of TDG in CRC. And the interaction of TDG-DNMT3A and its function on the role of TDG in CRC were explored in depth further. Our results strongly demonstrate the epigenetic regulatory role of TDG in inhibiting the metastasis of CRC, and would enrich the regulatory mechanism and therapeutic strategies of TDG in CRC.

Materials And Methods

Bioinformatics analysis

The TCGA database with designed web tool (<https://xenabrowser.net/>) was used to investigate the relative expression of TDG and the correlation with CRC stage and prognosis. We used UCSC Genome Browser (<http://genome.ucsc.edu/>) to download 2000bp DNA Psequence upstream of TIMP2 transcription initiation region as TIMP2 promotor, then Primer 5.0 software was applied to design PCR primers for ChIP. we also put it into MethPrimer 2.0(<http://www.urogene.org/methprimer2/>) to predict the methylation sites of the TIMP2 promoter and design PCR primers for MSP.

Clinical patient samples

Colorectal cancer samples were collected from patients of the Second Affiliated Hospital, Xi'an Jiaotong University. All were validated by pathologic examination. The study was approved by the Institute Research Ethics Committee at Cancer, Xi'an Jiaotong University, and all patients provided written

informed consent. Tissue samples were immediately frozen in liquid nitrogen until subsequent protein extraction.

Cell lines and cell culture

Human colon cancer cells (HCT116, RKO, SW480 and SW620) and normal colonic epithelial cell line, NCM460 were purchased from the American Type Culture Collection (ATCC). Cells were cultured in RPMI-1640 medium supplemented with 10% (v/v) fetal bovine serum (Gibco BRL, New York, USA) at 37°C, 95% air and 5% CO₂ in a humidified atmosphere.

Quantitative real-time PCR

Total RNA was extracted using TRIzol reagent (Invitrogen Life Technologies, USA). cDNA was generated using the StarScript II First-strand cDNA Synthesis Mix Kit (GenStar, Beijing, China). Quantitative real-time PCR (qRT-PCR) was performed using FTC-3000TM System (Funglyn Biotech Inc., Canada). Relative gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method.

Migration and invasion assay

Transfected HCT116 and SW480 cells were resuspended in 200 µl serum-free RPMI-1640 medium and plated in the upper chamber of the transwell coated with or without matrigel. The lower chamber was filled with RPMI-1640 complete medium. After incubation for 24 h and 36 h respectively, the cells on the bottom surface were fixed with 4% polyformaldehyde and stained with 0.1% crystal violet. Images were captured using an inverted microscope. Add 500 µl of glacial acetic acid, and shake for 30 minutes to dissolve the crystal violet. Transferred the solution to a microplate 100 µl per well, and measured the absorbance at 570nm using Spectrophotometer (FLUOstar OPTIMA, BMG, Germany).

Western blot

Cells were lysed using RIPA buffer (Pioneer, Shanghai, China) containing PMSF (Sigma, Germany). BCA protein assay kit (GenStar, Beijing, China) was used to measure protein concentrations. Primary antibodies (1:1000) (Proteintech, Wuhan, China) were dropped on membranes at 4°C overnight. Then all membranes were incubated with secondary antibody (Abways Technology, China) for 1 h at room temperature. Protein expression was normalized to GAPDH levels in each sample.

Chromatin immunoprecipitation analysis (CHIP)

CHIP is to detect DNA fragments bound to specific proteins through co-precipitation with chromatin and PCR technology. DNA and protein are cross-linked and ultrasonically broken into small fragments. The DNA fragments are captured with DNMT3A antibody and purified for PCR using 3 pairs of ChIP specific TIMP2 primers (Table S1). The products are separated by agarose gel electrophoresis. Gel imaging system is used to obtain images.

Methylation-specific PCR (MSP) analysis

After DNA is treated with sodium bisulfite, all unmethylated cytosines are deaminated into uracil, while methylated cytosines remain unchanged. Based on this base change, primers for methylated and unmethylated sequences are then designed and amplified by polymerase chain reaction (PCR). Finally, the methylation status of DNA sequences complementary to the primers was determined by agarose gel electrophoresis analysis. EpiTect Bisulfite Kit (QIAGEN, Germany) is used for sulfite conversion. All operations are carried out according to the operating instructions. Four pairs of Methylated and unmethylated TIMP2 primers are listed in Table S1.

***In vivo* Tumor Metastasis Assay**

The 4-6-week-old male nude mice were purchased and raised in a pathogen-free SPF room in the Animal Center of Xi'an Jiaotong University. All tumorigenic experiments were conducted under the guidance of the Animal Health and Utilization Committee of Xi'an Jiaotong University. HCT116 Lv-ctrl and HCT116 Lv-TDG stable transfected cell suspension 100 μ l (5×10^6 cells) were injected into mice respectively through tail vein. Then mice were raised for 3 weeks and intraperitoneally injected 100 μ l D-fluorescein sodium salt solution (15 mg/ml), Lung metastasis was monitored by the IVIS bioluminescence imaging system.

Statistical analysis

Data are performed as the mean $\pm$ SD in at least three independent experiments. Student's *t*-test was used to analyze the differences between groups. $P < 0.05$ was statistically significant. All statistical analyses were performed using IBM SPSS statistics 23 software.

Results

TDG is down-regulated in mCRC samples and CRC cells.

To explore the role of TDG in CRC, we analyzed the relationship between TDG expression and TNM and stage in CRC patient samples using publicly available CRC gene expression RNAseq data from TCGA database. The relationship between its expression and the overall survival of CRC patients was also analyzed. TDG expression was not associated with tumor topography (T), lymphatic metastasis (N), and stages in patients, but had a significantly correlation with distant metastasis (M). In patients of metastatic CRC (M1), TDG expression decreased significantly (Fig. 1a). In addition, low TDG expression was associated with poor overall survival in CRC patients (Fig. 1b). We further examined TDG expression in clinical CRC patient tissues and human colon cancer cells. Compared with the CRC tissue without metastasis, TDG protein was remarkably down regulated in CRC tissue with tumor metastasis (Fig. 1c). Lower TDG mRNA (Fig. 1d) and protein expression (Fig. 1e) were also detected in human CRC cells compared to those of normal human colonic epithelial cell NCM460. These results indicate that TDG is down regulated in mCRC (metastasis CRC) samples and human CRC cells and is associated with CRC metastasis and prognosis of CRC patients.

TDG inhibits CRC metastasis in vitro and in vivo.

We constructed a TDG overexpression vector with Flag-tag to explore its role in CRC. The control vector and it were transfected into two kinds of human CRC cells, HCT116 and SW480, respectively. qRT-PCR and Western blotting were applied to verify the overexpression of TDG. The results showed that compared with the control (Ctrl.), the mRNA level (Fig. 2a) and protein level (Fig. 2b) of TDG in TDG overexpression group (TDG) were significantly increased in both two kinds of cells. Transwell assay was used to detect the effect of TDG on the migration and invasion of CRC cells (Fig. 2c). The results showed that the number of HCT116 and SW480 cells passed through the chamber with or without Matrigel had a significant reduction in the TDG group compared with the control, indicating the declining of cell migration and invasion induced by TDG. Then, we detected the molecular changes related with tumor migration and invasion. The results of qRT-PCR showed that there was no significant difference of the mRNA levels of MMP2, MMP9, and TIMP1 between the TDG group and the Ctrl. group, however, the mRNA level of TIMP2 increased significantly in the TDG group compared with the control (Fig. 2d). Data of western blotting also showed that the protein level of TIMP2 increased significantly in the TDG group, with no changes of the TIMP1 protein level and decreases of the MMP2 and MMP9 protein in the TDG group compared with the control (Fig. 2e). These results indicate that TDG could significantly inhibit the migration and invasion ability of CRC cells.

We then established a mice metastasis model to explore the effect of TDG on the metastasis of CRC cells *in vivo*. Lentiviruses of TDG-Flag and the control were constructed and infected into HCT116 respectively to make the stable overexpression cells, HCT116-Lv-TDG, and the control cells, HCT116-Lv-Ctrl. They were injected into the tail of nude mice, and 3 weeks later, the metastasis imaging was obtained using small animal live imaging system. There were metastases sites in the lung of nude mice in the HCT116-Lv-Ctrl group, but no metastases in the HCT116-Lv-TDG group (Fig. 2f), that is, the overexpression of TDG could inhibit the metastases of human colon cancer cells *in vivo*. This data is consistent with the results of TDG inhibiting the migration and invasion of colon cancer cells.

TDG could bind to DNMT3A and promote the ubiquitination and degradation of DNMT3A

Previous researches reported that TDG played an important role in the epigenetic regulation of gene demethylation by interacting with DNMT3A. So, we focused our research on the interaction of TDG and DNMT3A in human CRC cells. We transfected the TDG overexpression vector and the control vector into HCT116 and SW480 cells, respectively, and examined the changes of DNMT3A using qRT-PCR and Western blotting. The results showed that there was no significant difference in the mRNA level of DNMT3A between two groups (Fig. 3a), however, the protein level of DNMT3A in TDG overexpression group decreased significantly compared with that of the Ctrl. group (Fig. 3b). Co-IP assay confirmed that TDG could bind to DNMT3A in HCT116 cell (Fig. 3c and d). So, there is an interaction of TDG and DNMT3A, and TDG could downregulate the expression of DNMT3A post-translationally in human CRC cells.

To further explore the mechanism of TDG regulating the expression of DNMT3A, we added 1 μ M of imine cyclohexanone (CHX), a protein synthesis inhibitor, into HCT116 cells of the TDG group, extracted protein lysates after 0h, 1h, 2h and 3h, and performed Western blotting. The results showed that CHX addition made the TDG-induced DNMT3A protein declining significantly faster than the control (Fig. 3e). Moreover, 1 μ M of MG132, a proteasome inhibitor, was applied into HCT116 cells of the Ctrl. group and the TDG group. After 24 hours, protein lysate was extracted and subjected to Western blotting. The results showed that MG132 inhibited the TDG-induced downregulation of DNMT3A protein (Fig. 3f). These data suggested that TDG might be involved in the proteasomal degradation of DNMT3A protein. We then performed Co-IP experiment to capture the DNMT3A protein and its interactors, and detect the binding amount of ubiquitin (Fig. 3g). The ubiquitination of DNMT3A protein in the TDG group increased significantly compared with that of the Ctrl. Group. So, we propose that TDG binds to DNMT3A and promotes its ubiquitination and degradation.

Knockdown of DNMT3A suppress the migration and invasion of CRC cells.

To verify the role of DNMT3A in the migration and invasion of CRC cells, we designed and purchased DNMT3A interference fragments, and transfected them into HCT116 and SW480, respectively. The results of qRT-PCR (Fig. 4a) and western blotting (Fig. 4b) verified the knockdown of both DNMT3A mRNA and DNMT3A protein in cells of the siDNMT3A group (siDNMT3A-1, siDNMT3A-2). Transwell assay was then used to detect changes in the migration and invasion of these two cell lines (Fig. 4c). The results showed that compared with that of the control group (NC), the migration and invasion ability of HCT116 and SW480 of the siDNMT3A group was reduced. Therefore, knockdown of DNMT3A could suppress the migration and invasion ability of human CRC cells.

DNMT3A binds to the TIMP2 promoter to regulate its methylation level.

Previous studies have reported that TIMP2 was hypermethylated in CRC, which may contribute to the high metastasis of CRC. We used online databases UALCAN (<http://ualcan.path.uab.edu/>) and found that the promoter region of TIMP2 was hypermethylated in CRC samples (Fig. 5a). Next, we treated HCT116 cells with different concentrations of 5-aza-2'-deoxycytidine (5-Aza) for 72 hours, and detect the changes of TIMP2 mRNA. As the concentration of 5-Aza increased, the mRNA level of TIMP2 increased significantly, which suggested that the TIMP2 mRNA level could be regulated by methylation in human CRC cells (Fig. 5b).

DNMT3A belongs to the family of methyltransferases, which can regulate gene expression by methylating CpG islands in the promoter region of target genes. To explore the regulatory effect of DNMT3A on TIMP2 expression, we transfected DNMT3A interference fragment and the control into HCT116 and SW480 respectively, and examined the changes of TIMP2 expression by qRT-PCR and Western blotting. The results showed that the mRNA level and protein level (Fig. 5c) of TIMP2 in the siDNMT3A group (siDNMT3A-1, siDNMT3A-2) were significantly higher than those in the control group (NC). Therefore, we assumed that DNMT3A may regulate the transcription level of TIMP2 by methylating the CpG island region in the TIMP2 promoter.

Using Online software (<http://www.urogene.org/methprimer2/>), we analyzed the 2000bp DNA sequence upstream of TIMP2, found 3 potential CpG islands, and designed 3 pairs of Chip primers for these region (Fig. 5d and Table s1). Then they were utilized to perform PCR amplification on the DNA fragments that was precipitated by DNMT3A antibody or IgG from HCT116 lysate. The PCR products were identified by agarose gel electrophoresis. Among them, the product amplified using primer pair 2 was significantly higher in the DNMT3A group than in the IgG group (Fig. 5d). This data indicates that DNMT3A could bind with the primer pair 2 region of TIMP2 promoter. Next, we used MSP experiment to directly verify the methylation effect of DNMT3A on the TIMP2 promoter. Methylated primers (TIMP2-M1 and TIMP2-M2) and non-methylated primers (TIMP2-U1 and TIMP2-U2) were designed for potential CpG islands in the TIMP2 promoter region. The imaging results showed that consistent with the result after 5-Aza treatment, the siDNMT3A group had a significant reduction in the products amplified using the methylated primers, while the products amplified using non-methylated primers significantly increased compared to those of the NC group, (Fig. 5e). These data show that DNMT3A could directly bind to the TIMP2 promoter region to change its methylation status, thereby regulating the transcription of TIMP2 in CRC cells.

TDG-DNMT3A-TIMP2 affects colon cancer cell migration and invasion

Since TDG could bind to DNMT3A and promote its degradation, and DNMT3A could bind to the TIMP2 promoter to regulate its methylation and TIMP2 transcription, Does TDG also change the methylation of TIMP2 promoter, and inhibit CRC metastasis through these TDG-DNMT3A-TIMP2 pathway? We firstly used MSP experiment to examine the TDG-influenced methylation of TIMP2 promoter. The imaging results showed that compared with the Ctrl. group, the products amplified using the methylated primers in the TDG overexpression group were significantly reduced, while the products amplified using the non-methylated primers were significantly increased (Fig. 6a). Thus, TDG could induce the hypomethylation of the TIMP2 promoter, which in turn increase the transcription and expression of TIMP2 (Fig. 2d and e).

Next, to verify that TDG inhibits the migration and invasion of colon cancer cells through DNMT3A-TIMP2, we performed rescue experiment. TDG overexpression plasmid and DNMT3A overexpression plasmid were co-transfected into HCT116 and SW480 separately or not. Data of transwell assay showed that overexpression of DNMT3A could partially reverse the decreased migration and invasion ability of colon cancer cells induced by TDG overexpression (Fig. 6b). Similarly, overexpression of DNMT3A also partially reversed the increase in TIMP2 mRNA and protein levels in CRC cells induced by TDG overexpression (Fig. 6c). Therefore, TDG promotes the ubiquitin and degradation of DNMT3A by binding with it, decreases the methylation level of TIMP2 promoter and increases the transcription and expression of TIMP2, and inhibits the migration and invasion ability of human CRC cells. Our finding will enrich the regulatory mechanism of TDG in tumors, and provide new ideas for the treatment of CRC.

Discussion

TDG is a mismatch-specific DNA glycosidase firstly found in Hela cells. It acts on G/T or G/U mismatches, repairs bases mismatched with G, and initiated the BER pathway[4]. Moreover, TDG can remove new cytosine derivatives produced by Ten-Eleven translocation enzymes during active DNA demethylation and participate in the genome demethylation[11, 12]. TDG also interacts with some transcription factors or transcription co-activators to regulate gene transcription[13, 14]. Considering its roles in DNA repair, DNA demethylation, and transcription regulation, TDG might contribute to carcinogenesis or cancer progression. In multiple myeloma, TDG expression is decreased, which leads to a deficient in the DNA repair activity against hydrogen peroxide-induced DNA damage[15]. In osteosarcomas that arose in *p53* heterozygous mice *tdg* expression levels are increased[16]. At present, the relationship between BER deficiency and cancer is limited to the autosomal-recessive MAP [10], and the researches of TDG in carcinogenesis or cancer development is limited, which needs to be explored in specific cancer.

In our study, we found that TDG expression was significantly related to the metastasis and prognosis of patients with CRC (Fig. 1a and b). Data of clinical CRC samples and human CRC cells also showed lower TDG expression in mCRC tissues and human CRC cells (Fig. 1c, d and e). This suggests that TDG plays an important role in the progression of CRC and is worthy of in-depth study. Data of *in-vitro* and *in-vivo* overexpression experiments demonstrated that TDG significantly inhibited the migration and invasion of human CRC cells, with the increases of TIMP2 mRNA and protein levels (Fig. 2). Therefore, TDG has a role of inhibiting metastasis in CRC. Yatsuoka et al detected low expression of TDG mRNA in pancreatic cancer tissues and pancreatic cancer cell lines[17]. TDG expression was also found lost due to a heterozygous missense mutation in patients with malignant rectal cancer. TDG inactivation might have contributed to the aggressive phenotype of the rectal cancer in patients[10]. Our findings also strongly suggest that TDG might inhibit metastasis in CRC and play a similar role as tumor suppressors.

DNMT3A regulates DNA methylation patterns during mammalian development, and the abnormal DNA methylation patterns induced by DNMT3A changes can cause many types of cancer[18, 19]. Fabbri reports that in lung cancer tissues, miR-29s directly targets the 3'UTR region of the DNMT3A gene and degrade the mRNA of DNMT3A[20]. In patients with CRC and acute leukemia, miR-143 negatively regulates the expression of DNMT3A[21, 22]. In our study, although TDG did not affect the mRNA level of DNMT3A, it significantly caused a decrease in the protein level of DNMT3A (Fig. 3a and b). DNMT3A has been reported bound with TDG to achieve genome demethylation. Li et al find that both the PWWP domain and catalytic domain of DNMT3A could interact with the N-terminus of TDG and affect their enzyme activities[7]. In acute myeloid leukemia, TDG interacts with DNMT3A, reducing its methylation ability[23]. TDG can bind to DNMT3A-H3 tail and play dominant roles in the modulation of DNMT3A activity[24]. Here using Co-IP and inhibitors, we also found TDG bound with DNMT3A and promoted its degradation to induce hypomethylation status of DNMT3A-targeting genes, such as TIMP2 (Fig. 3).

TIMPs inhibit the degradation of extracellular matrix, suppress angiogenesis, and play an important role in the occurrence, invasion and metastasis of tumor cells[25]. TIMP2 is reported dysregulated in more than 30 clinical studies about breast cancer[26], lung cancer[27], prostate cancer[28]. EZH2 can methylate

the promoter of TIMP2, thereby inhibiting TIMP2 expression and promoting the metastasis of ovarian cancer[29]. Here, we also find TIMP2 is regulated by methylation in human colon cancer cells (Fig. 5a and b). Using interference of DNMT3A, ChIP, and MSP experiments, we demonstrate DNMT3A can bind to the TIMP2 promoter to methylate, thereby inhibiting TIMP2 mRNA expression (Fig. 5c, d and e). TDG overexpression reduces the methylation level of TIMP2 promoter (Fig. 6A) and increases the expression of TIMP2 (Fig. 2d and e). DNMT3A overexpression partially rescues the TDG-decreased migration and invasion and TDG-increased TIMP2 expression in colon cancer cells (Fig. 6b, c and d). TDG itself has no demethyltransferase activity. Therefore, we propose that TDG can inhibit migration and invasion by reducing DNMT3A, decreasing TIMP2 promoter methylation, and increasing the expression of TIMP2. TIMP2 can hydrolyze MMP2 and MMP9 and make their protein level decreased [30, 31]. This is also consistent with our results (Fig. 2d and e).

Conclusion

Our study reveals that TDG interacts with DNMT3A to promote its ubiquitination degradation, induces hypomethylation state of TIMP2 promoter, increases TIMP2 expression, and significantly inhibits the migration and invasion ability of human colon cancer cells (Fig. 7). This epigenetic regulatory role of TDG in inhibiting the metastasis of CRC enriches the regulatory mechanism and therapeutic strategies of TDG in CRC.

Abbreviations

TDG: Thymine DNA glycosylase; DNMT3A: DNA methyltransferase 3 alpha; CRC: Colorectal cancer; Co-IP: Co-immunoprecipitation; CHIP: Chromatin immunoprecipitation analysis; MSP: Methylation-specific PCR; TIMP2: TIMP metalloproteinase inhibitor 2; TCGA: The Cancer Genome Atlas.

Declarations

Supplementary Information

The online version contains supplementary material available at XXX.

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Author's contributions

CH conceived and supervised the project. JYM, CAZ, KJT, WJX, HHZ, XTJ and WL performed the experiments. JYM, XFX, FW, BJD and WL performed statistical analysis of the data. CAZ, NH and CH provided critical comments. JYM and NH wrote and revised the manuscript.

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Availability of data and materials

All datasets supporting the findings of this study are available from the corresponding authors on reasonable request.

Ethics approval and consent to participate

All animal procedures were approved by Institute Research Ethics Committee at Cancer, Xi'an Jiaotong University.

Consent for publication

All authors have agreed to publish this manuscript

Competing Interests

The authors have declared that no competing interest exists.

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Figures

Figure 1

Bioinformatics analysis and TDG expression in CRC patient tissues and cells. a TDG expression in different pathologic classification of CRC patients. b Low TDG expression related to poor prognosis. c The expression of TDG was decreased in mCRC (metastasis CRC) compared with CRC. d and e The expression of TDG in NCM460 and CRC cells were detected by qRT-PCR and Western blotting. * $P<0.05$, ** $P<0.01$, *** $P<0.001$

Figure 2

TDG inhibited the migration, invasion and metastasis of colon cancer cells. a and b The overexpression efficiency of TDG was detected by qRT-PCR and Western blotting. c The transwell assay was performed for migration and invasion of colon cancer cells. d and e Migration and invasion related molecules were detected by qRT-PCR and Western blotting. f Construction of metastatic tumor model in nude mice.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Figure 3

TDG interacts with DNMT3A. a and b The expression of DNMT3A was detected by qRT-PCR and Western blotting after transfected with TDG plasmid. c and d Co-IP was applied to vivificate the combination of TDG and DNMT3A. e CHX promoted the degradation rate of DNMT3A. f MG132 inhibited the decrease of DNMT3A caused by TDG. G TDG increased the ubiquitination level of DNMT3A. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Figure 4

siDNMT3A suppressed the migration and invasion of colon cancer cells. a and b The inhibition efficiency of DNMT3A was detected by qRT-PCR and Western blotting. c The transwell assay was performed for migration and invasion of colon cancer cells. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Figure 5

DNMT3A could regulate the expression of TIMP2. a TIMP2 was hypermethylated in CRC analyzed from UCLCAN database. b 5-Aza promoted the expression of TIMP2 mRNA. c The expression of TIMP2 was detected by qRT-PCR and Western blotting after transfected with siDNMT3A-1/2. d ChIP was performed to detected the DNMT3A binding region of TIMP2 promotor. e MSP was performed to detected the methylation level of TIMP2 promotor. *P<0.05, **P<0.01, ***P<0.001

Figure 6

DNMT3A Partially reversed the effect of TDG in colon cancer cells. a MSP was performed to detect the methylation level of TIMP2 promoter. b The transwell assay was performed for migration and invasion of colon cancer cells (C+C:Ctrl.+Control, T+C: TDG+Control, C+D: Ctrl.+DNMT3A, T+D: TDG+DNMT3A). c and d The expression of TIMP2 was detected by qRT-PCR and Western blotting. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Figure 7

A model for TDG-mediated TIMP2 demethylation in colorectal cancer.

Supplementary Files

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