Biochemical and Biophysical Research Communications 457 (2015) 681–687

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Inhibition of STAT3/cyclinD1 pathway promotes chemotherapeutic sensitivity of colorectal caner

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ARTICLE INFO

Article history:
Received 6 January 2015
Available online 21 January 2015

Keywords:
STAT3
CyclinD1
Colorectal cancer
Chemotherapy

ABSTRACT

Background: Chemotherapeutic resistance indicated the poor prognosis of colorectal cancer.
Objective: Our study aimed to investigate the role of STAT3/cyclinD1 pathway in the chemotherapeutic resistance of colorectal cancer.
Methods: We firstly measured the expression of cyclinD1 in the colorectal cancer tissues using immunohistochemistry in tissue microarray. Then cell viability and apoptosis were investigated in the HT-29 cell lines dealing with recombinant lentivirus and shRNA to increase or decrease cyclinD1 expression. Furthermore, luciferase and ChIP assays were applied to investigate whether STAT3 regulated cyclinD1 expression by binding to its promoter. Finally, we determined whether inhibition of STAT3 could decrease cyclinD1 and increase the chemotherapy sensitivity.
Results: CyclinD1 expression was significantly increased in the cancer cells and high level of cyclinD1 indicated the poor prognosis. Inhibition of cyclinD1 decreased the cell viability assessed by MTT and increased rate of apoptosis when exposed to 5-FU treatment while overexpression of cyclinD1 showed the reverse effect. ChIP assay showed that STAT3 directly bind to cyclinD1 promoter. Subclone of full promoter of cyclinD1 into pGL4 increased the luciferase activity while delete or mutation of any of STAT3 binding sites resulted in reductions of luciferase activity. Inhibition of STAT3 decreased cyclinD1 expression to decrease the cell viability and increase rate of apoptosis when exposed to 5-FU treatment.
Conclusions: Inhibition of STAT3/cyclinD1 pathway increased the sensitivity of colorectal cancer cell to chemotherapy.

1. Introduction

Colorectal cancer is the third leading cancer and the fourth most frequent cancer-related death worldwide [1,2]. Moreover, the incidence of colorectal cancer has rapidly increased in Asian population like Chinese people [3]. Significant progress has been made in the last decades [4] and the principal strategy is the surgical removal of the involved segment of colon tissue with free surgical margins [5]. In patients with metastatic lesions or high stage disease, adjuvant chemotherapy is applied to ablate the cancer cells to gain a free surgical margin [6]. It could extend the survival time after surgery and improve the life quality [7]. However, some patients remain refractory to chemotherapy [8] which indicates the poor prognosis [9]. Development of new drug target is necessary for the successful treatment of these patients.

Dysfunction of cell cycle plays an essential role in the development of colorectal cancer and chemotherapy resistance. The deregulation is mainly attributed to overexpression of cyclins and loss of expression of cyclin-dependent kinases (CDKs) inhibitors in caner cells [10]. As a major component of cyclins, cyclinD1 coupling with CDK4/6 serves as a key regulator of progression from the G1 to S phase of the cell cycle [11]. CyclinD1 is responsible for apoptosis resistance following treatment with cicplatin in the colorectal caner [12]. Restore of cyclinD1 would increase the chemotherapy sensitivity [13]. However, why cyclinD1 was deregulated in the colorectal cancer was not completely clear.

We did the bioinformatics analysis and found that there were two binding sites of signaling transduction and activation of transcription 3 (STAT3) in the promoter of cyclinD1. In our previous

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http://dx.doi.org/10.1016/j.bbrc.2015.01.048
0006-291X/

research, STAT3 was increased in the HT-29 cell line, one colorectal cancer cell line, and regulated the cell growth and cell cycle [14]. Given the role of cyclinD1 in the cell cycle, the finding that STAT3 regulated the cell growth and the bioinformatics analysis, it may be proposed that STAT3 regulate the expression of cyclinD1 by targeting its binding sites in the promoter of cyclinD1 to control the cell growth. In the present study, we aimed to investigate whether STAT3 could bind to the cyclinD1 promoter and inhibition of STAT3/cyclinD1 pathway could increase the sensitivity of colorectal cancer cell to chemotherapy.

2. Materials and methods

2.1. Patient profile

The whole procedure was performed according to the REMARK guideline [15]. From January, 2000 to August, 2008, patients with colorectal cancer who underwent curative resection were retrospectively screened. Patients with rectosigmoid cancer were also included. The research was conducted in compliance with current Good Clinical Practice standards and in accordance with the principles set forth under the Declaration of Helsinki (1989). The research was approved by the Ethics Committee of Suzhou Hospital affiliated to Nanjing Medical University (SZNMU 20001012). An informed written consent was obtained from each patient. The pathological stage was confirmed based on the International Union Against Cancer (UICC) classification system, 7th edition. The degree of lymphovascular and vascular invasion was classified as negative, mild or severe by two independent pathologists. Tissues from 50 patients were enrolled to make the microarray. The patients were followed up at 3, 6, 12 and every 12 months thereafter by an experienced nurse on clinic or by telephone interview.

2.2. Tissue microarray

Tissue microarray (TMA) was prepared from each tumor and its para-carcinoma tissue. The representative area was carefully selected based on a hematoxylin and eosin (HE)-stained section. A TMA was composed of small 1.0 mm cores of tissue obtained from paraffin blocks. For tissue stamping, the potential areas were marked on HE-stained sections and then marked directly on the corresponding formalin-fixed, paraffin-embedded tissue blocks. Paraffin-embedded material was cut into 5 μm sections and placed onto glass slides, followed by staining with HE or immunohistochemistry (IHC) by anti-cyclinD1 antibody (1:200, Cell signaling Tech, USA). The slices were then detected under microscope and graded semi-quantitatively by two blinded pathologists. The staining intensity was scored as follows: 0, no staining of cancer cells; 1, weak staining; and 2, strong staining while the percentage of stained tumor cells was classified on a scale of 3 grades: 0, no positive cells; 1, <50% positive cancer cells; and 2, >50% positive cancer cells.

To elucidate whether cyclinD1 and STAT3 coexpressed in cells, immunofluorescence was used. After rinsing in PBS (pH 7.4), the sections were incubated at 37 °C for 2 h with the primary antibody in PBS used at the following dilutions: anti-cyclinD1 (1/150, abcam), anti-STAT3 (1/150, Cell Signaling Tech). After 3 washes with PBS, antibody visualization was achieved by the incubation at room temperature for 60 min with Alexa Fluor 488-conjugated donkey anti-mouse (1/200) and Alexa Fluor 594-conjugated donkey anti-rabbit (Invitrogen, USA). Negative controls were prepared by omitting the primary antibodies. The sections were then cover slipped with a fluorescent mounting medium and viewed under microscope (Olympus X71, Japan).

2.3. Cell culture

HEK293 cells, HT-29 cells and HT-29-shSTAT3 cells (stably expressing shSTAT3 to knock down STAT3 [14]) were grown and maintained in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (FBS, Gibco), penicillin 100U/ml and streptomycin 100 μg/ml.

2.4. Construction of lentivirus

Human STAT3 and cyclinD1 gene fragments were subcloned into pcDNA3. ShRNA was designed to down-regulate the expression of cyclinD1. Three different non-overlapped sequences were designed (shRNA1, 5′-CACC*GCATGTTCGTGGCCTCTAAGA*CGAAT*CTTAGAGGCCACGAACATGC*-3'; shRNA2, 5′-CACC*GCTGTGCATCTACACCGACAA*CGAA*TTGTCGGTGTAGATGCACAGC*-3'; shRNA3, 5′-CACC*GCACGATTTCATTGAACACTT*CGAA*AAGTGTTCAATGAAATCGTGC*-3′). The shRNA1 targeting cyclinD1 was selectively subcloned into lentiviral vector pLVX-IRWS-ZsGreen1 due to its highest efficiency. And pcDNA3-STAT3, pcDNA3-cylinD1 or pcDNA3 was also subsequently cloned into pLVX-IRWS-ZsGreen1. Lentivirus was produced and the titration of purified virus was determined according to our previous report [14]. The virus was stored at −80 °C until use.

2.5. Realtime PCR

Total RNA was isolated from cells using the RNeasy Mini Kit (Qiagen, German) following the manufacturer's recommendation. Reverse transcript-reaction was carried out using the Firststrand cDNA synthesis kit (Promega, US) following the manufacturer's recommendations. The amplification and data acquisition were run on a realtime PCR system (Agilent MX3000P, USA) using SYBR green PCR Master Mix (Qiagen). The conditions were pre-denaturation at 95 °C for 15 min, followed by 40 cycles at 95 °C for 15 s, 60 °C for 1 min and 72 °C for 1 min followed by a dissociation stage at 95 °C for 15 s and 55 °C for 1 min. All samples were analyzed in triplicates in three independent experiments. Reactions without cDNA were used as no template control and no RT controls were also set up to rule out genomic DNA contamination. Relative quantification of mRNA expression was determined using the comparative Ct method.

2.6. Western blot

Cells were homogenized in RIPA buffer (Cell-Signaling Tech.). Protein concentrations were determined using a BCA kit (Pierce, USA). Equal amounts of protein were separated by 10% gel, and then transferred to 0.45 μm PVDF membranes (AmerSham, USA). The membranes were blocked in 5% bovine serum albumin (BSA) in Tris-buffered saline with Tween 20 buffer (TBST) for 2 h, and then incubated overnight at 4 °C with the following primary antibodies: anti-STAT3 (1:1000, Cell-signaling Tech.), anti-cyclinD1 (1:1000) and anti-β-actin (1:1000, Cell-signaling Tech.), which served as a loading control. Then the membranes were washed 3 times with TBST and incubated with horseradish peroxidaseconjugated secondary antibody (goat anti-rabbit IgG, 1:5000 or goat anti-mouse IgG, 1:5000, Cell-signaling Tech.) for 1 h under room temperature. Blots were developed using a chemiluminescence kit (Pierce) and exposed to X-ray film. The bands on the film were scanned and analyzed with Quality One (Bio-Rad).

2.7. Luciferase activity

A series of reporter constructs with the same 3′ terminus but different 5′ terminus, were constructed by PCR. All PCR products were subcloned into the plasmid pGL4-Minimal (Promega, USA). The mutant site was introduced into the plasmid by overlap extension PCR. First, two DNA fragments with an overlap containing the mutant site were amplified by PCR. Then the full length promoter, containing the mutant binding site, was obtained by mixing the two DNA fragments produced from the first-step PCR and then using them as the template in the second PCR reaction with the outermost primers. The subsequent PCR products were subcloned into the plasmids. All constructs were confirmed by DNA sequencing. Cells were plated into the 24-well plates one day before. For cylinD1 promoter assay, the cells were co-transfected with 1 μg of pcSTAT3 or pcDNA, 20 ng of a luciferase reporter plasmid and 10 ng of the pRL–CMV Renilla luciferase reporter plasmid (Promega). Twenty-four hours after transfection, the cells were harvested and determined the luciferase activity. Firefly luciferase activity was normalized to Renilla luciferase activity as reported previously [16].

2.8. ChIP

The ChIP assays were performed by the Magna ChIP A/G kit (Millipore, USA). The HT-29 cells were cross-linked with 1% formaldehyde for 10 min and quenched by glycine. The cross-linked cells were collected in cold PBS. The cells were sonicated for six 15 s pulses with 50 s rest in between pulses to reduce the total DNA size from 200 to 1000 bp. The sheared chromatins and magnetic beads were immunoprecipitated with anti-STAT3 or normal mouse IgG overnight at 4 °C on a rotator. Magnetic bead-antibody-chromatin complexes were washed once with low salt, high salt, LiCl buffers and TE buffer. The chromatin complexes were eluted and incubated at 62 °C for 2 h. The DNA samples were recovered by spin columns. For ChIP samples, real-time PCR was carried out using SYBR method as we described above.

2.9. MTT assay

Cells seeded on 96-well microplates at 4×10^3 cells per well were incubated with the anthocyanins at the indicated concentrations for 48 h. Following incubation with the anthocyanins, the medium was removed, and the cells were then incubated with 100 μl MTT solution (2 mg/ml MTT) for 4 h. The samples were then solubilized in dimethyl sulfoxide and the purple formazan dye, converted from MTT by viable cells, was quantified by absorbance at 560 nm.

2.10. Flow cytometry

The procedure of flow cytometry was according to our previous report [14]. In brief, 1×10^6 cells per sample were washed twice with ice-cold PBS. A total of 100 μL of cell suspension was incubated with 5 μL of annexin V-FITC (AV) and 10 μL of propidium iodide (PI) (BD, USA) for 15 min at room temperature. Then 400 μL of binding buffer was added to each sample and filtered by a 300-mesh nylon net to remove the cell debris before flow cytometry on the EPICS XL system (Beckman Coulter, USA). EXPO32 ADC analysis software was applied to analyze the data.

2.11. Statistics

Parameter data were expressed as mean ± standard deviation and were analyzed with unpaired *t*-test and analysis of variance (ANOVA) followed by a post-hoc *t*-test. Differences between proportions were assessed by the χ^2 test. The survival analysis was used Kaplan–Meier method. All the analyses were conducted using SPSS13.0 software. Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Overexpression of cyclinD1 associated with poor prognosis in colorectal cancer

We extracted cancer and paracarcinoma tissues from 50 patients with colon cancer to make a TMA. The patient information is listed in Table 1. The level of cyclinD1 was significantly increased in the cancer tissues compared with their corresponding paracarcinoma tissues (5.52 ± 0.42 vs. 1.06 ± 0.15, $P < 0.05$, n = 50, Fig. 1A and B). Then we investigated the relationship between cyclinD1 expression and patient survival. We defined 0-3 staining score as low expression while >3 as high expression. As shown in Fig. 1C, the survival rate was significantly decreased in the patients with high expression of cyclinD1 compared with that with low expression of cyclinD1 during the follow up ($P < 0.05$). This indicated that overexpression of cyclinD1 was a high risk of poor outcome of colorectal cancer patients.

3.2. Overexpression of cyclinD1 protecting cells against chemotherapy

We constructed the recombinant lentivirus, lenti-cyclinD1, to overexpress cyclinD1 in the cells. The realtime PCR (Fig. 2A) and western (Fig. 2C) blotting confirmed the high expressions of RNA and protein in the HT-29 cells after transducing of lenti-cyclinD1. Then MTT assay showed that overexpression of cyclinD1 increased the cell viability compared with sham group when exposed to 5-FU (74.1 ± 5.9% vs. 36.3 ± 4.8%, $P < 0.05$, Fig. 2B). Also the overexpression of cyclinD1 resulted in the reduction of Annexin V-PI positive cells in the HT-29 cells treated by 5-FU (76.2 ± 10.3 vs. 24.5 ± 3.2, $P < 0.05$, Fig. 2D).

Table 1
Characteristics of patients.

Characteristics	Number of case	Expression of cyclinD1		*P*
		Low	High	
Age	50	19	23	>0.05
<60	23	8	15	
>60	27	11	16	
Gender				>0.05
Male	22	9	13	
Female	28	10	18	
Tumor size (cm)				>0.05
<4	25	11	14	
>4	25	8	17	
Invasion depth				>0.05
T1, T2	26	10	16	
T3, T4	24	9	15	
Tumor stage				>0.05
I, II	27	10	17	
III, IV	23	9	14	
Metastasis				>0.05
Absent	25	12	13	
Present	25	7	18	

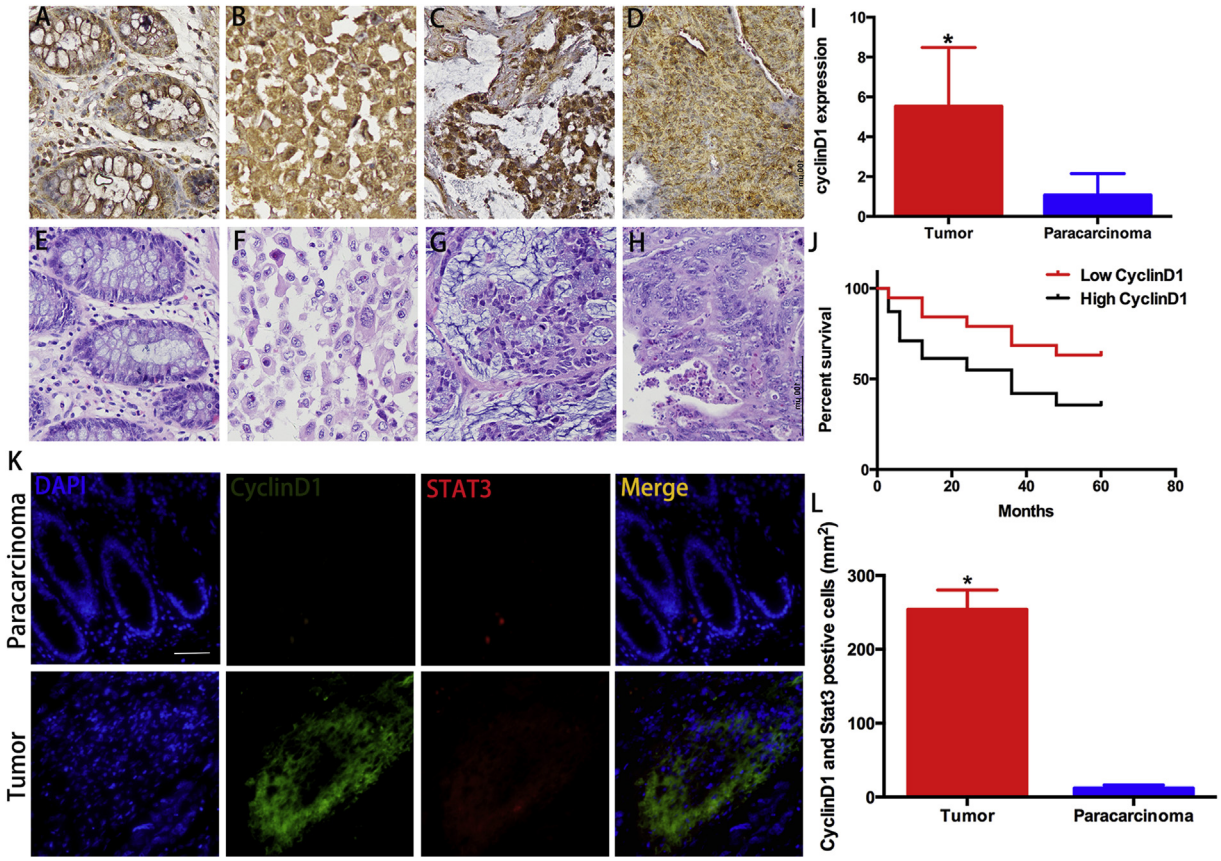

Fig. 1. Overexpression of cyclinD1 in colorectal cancer tissues. (A–E) Paracarcinoma tissue. (B–F) Cancer tissues. (C–G) High expression of cyclinD1 in cancer cells. (D–H) Low expression of cyclinD1 in cancer cells. (I) Overexpression of cyclinD1 in the cancer cells. (J) High grade of IHC score of cyclinD1 indicated the poor prognosis during the follow up. (K) Overexpression of cyclinD1 and STAT3 in colorectal cancer tissues. CyclinD1 and STAT3 were overexpressed in colorectal cancer tissues compared with paracarcinoma tissues. And overexpressed cyclinD1 and STAT3 occurred in the same cells. (L) The summary of double-positive cells. Scar bar: 50 μm. $P < 0.05$ as significant difference.

3.3. Inhibition of cyclinD1 increasing sensitivity of colon cancer to chemotherapy

The lentivirus, ShcyclinD1, was constructed to knock down the expression of cyclinD1. The realtime PCR and western blot confirmed the significant reductions of RNA (Fig. 2A) and protein (Fig. 2C) levels of cyclinD1 by ShcyclinD1. The cell viability of HT-29 cells was significantly decreased by ShcyclinD1 compared with the cells transduced with lenti-cyclinD1 when exposed to 5-FU (Fig. 2C, $P < 0.05$). However, there was no significant difference of cell viability between ShcyclinD1 and the normal HT-29 cells when exposed to 5-FU (Fig. 2C, $P > 0.05$). Also inhibition of cyclinD1 increased Annexin V-PI positive cells compared with overexpression of cyclinD1 in the HT-29 cells treated by 5-FU ($P < 0.05$, Fig. 2D).

3.4. STAT3 binding to the promoter of cyclinD1

Since there was overexpression of cyclinD1 in colorectal cancer, we investigated the mechanism of upregulation of cyclinD1. The bioinformatics analysis suggested two potential target sites of STAT3 in the cyclinD1 promoter. Hence, we determined whether STAT3 could bind to cyclinD1 promoter by ChIP real-time assay. The result demonstrated that STAT3 binding cyclinD1 was significantly increased when compared to IgG binding cycliD1 (Fig. 4F, $P < 0.05$). It indicated that STAT3 directly bind to the promoter of cylinD1. Then we investigate the expressions of STAT3 in cancer cells. We found that STAT3 overexpressed in cancer tissues compared with its paracarcinoma area. More importantly, STAT3 co-expressed with cyclinD1 in the same cells (Fig. 1K).

3.5. STAT3 regulating the expression of cyclinD1

We constructed Lenti-STAT3 and ShSTAT3 to increase and decrease expression of STAT3 which were confirmed by realtime PCR (Fig. 3B) and western blot (Fig. 3A and C). When the expression of STAT3 was upregulated by Lenti-STAT3, the expression of cyclinD1 was significantly increased (Fig. 3A and C). When the expression of STAT3 was inhibited, the cyclinD1 was also significantly decreased (Fig. 3A and C).

Different length and mutation of cyclinD1 promoter (Fig. 4A) were subcloned into pcGL4 and co-transfected with pcSTAT3 and pRL–CMV Renilla into HT-29 cells. Luciferase activity was measured 24 h after transfection. STAT3 increased the luciferase activity compared with pcDNA (Fig. 4B, $P < 0.05$). Deletion or mutation of the STAT3 binding site would reverse the effect of pcSTAT3 (Fig. 4B). To observe the direct effect STAT3 on cyclinD1, we subcloned different length of cyclinD1 promoter into pcDNA-GFP. When the binding site at -481 was deleted, the expression of GFP was decreased (Fig. 4).

3.6. STAT3 regulating the chemotherapy sensitivity by regulating cyclinD1

Since STAT3 regulated the expression of cyclinD1 by targeting its binding site in the promoter, we determined that whether STAT3 could regulate the sensitivity of cancer cell to 5-FU treatment. As shown in Fig. 3, the ShSTAT3 cell line with low expression of STAT3 had low cell viability compared with the HT-29 cells (Fig. 3, $P < 0.05$) while the cell viability was significantly increased by

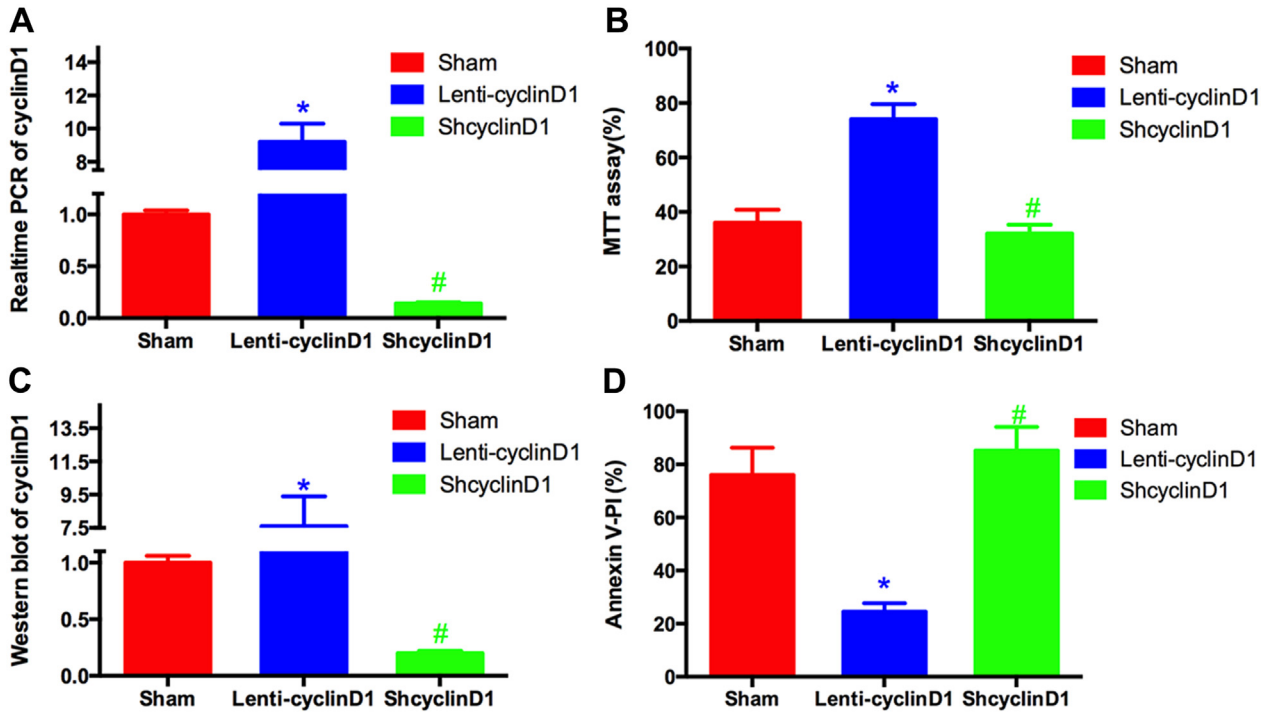

Fig. 2. Inhibition of cyclinD1 increasing sensitivity to chemotherapy. (A) The cells transduced with lenti-cyclinD1 had high level of cyclinD1 RNA assessed by Realtime PCR while shRNA could decrease it. (B) Inhibition of cyclinD1 had less cell viability while lenti-cyclinD1 could reverse the trend. (C) Western blot showed the cyclinD1 expression. (D) There was high ratio of Annexin V positive cells with low expression compared with high expression of cylinD1. * Compared with sham group; # compared with lenti-cyclinD1 group. $P < 0.05$ as significant difference.

Lenti-STAT3 (Fig. 3, $P < 0.05$). The Annexin V-PI positive cells showed the similar trend (Fig. 3, $P < 0.05$).

4. Discussion

In the present study, our data suggested that high level of cyclinD1 in the colorectal cancer patients indicated the poor prognosis. The expression of cyclinD1 was regulated by STAT3 via binding to its promoter. Inhibition of STAT3/cyclinD1 pathway improved the sensitivity of cancer cells to chemotherapy in the colorectal cancer. Our findings provided novel evidences of STAT3/cyclinD1 pathway as the potential new drug target for colorectal cancer.

Dysfunction of cyclinD1 results in the disturbance of cell cycle which is tightly connected with cancer development and resistance of chemotherapy [17]. We found that high level of cyclinD1 in the colorectal cancer cells. Our data was in consistent with previous researches which also demonstrated that high level of cylcinD1 in colorectal patients [18]. Moreover, overexpression of cyclinD1 was correlated with the poor prognosis of patients. Then we determined why high level of cyclinD1 lead to prognosis. It was found that cells transduced with Lenti-cyclinD1 showed resistance to 5-FU treatment in vitro. When the expression of cyclinD1 was decreased by shRNA, the cells showed sensitivity to 5-FU treatment. In Kisslov et al. study, it was found that inhibition of cyclinD1 increased the death of cancer cell when exposed to chemotherapy [18]. Takahashi

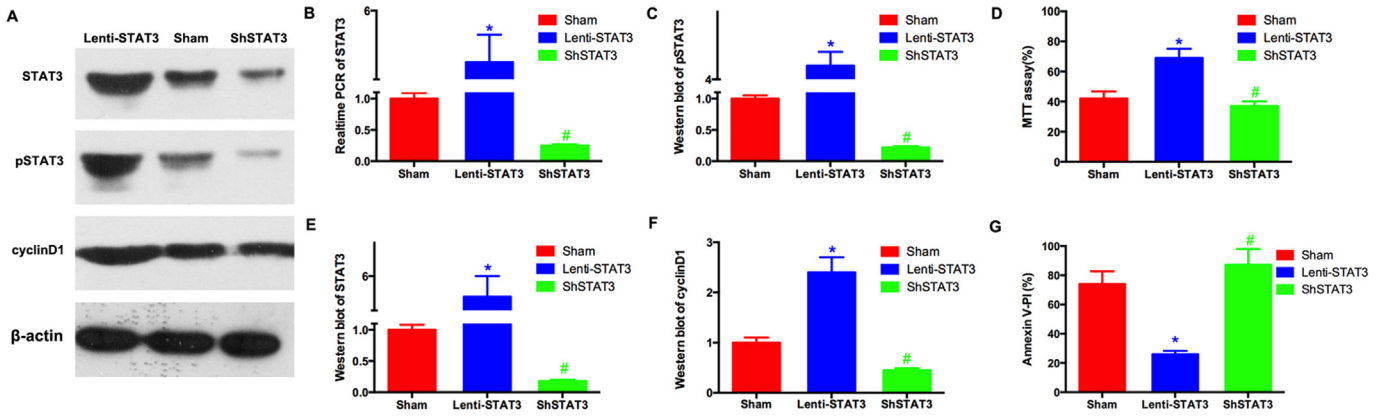

Fig. 3. Inhibition of STAT3/cyclinD1 pathway increasing sensitivity to chemotherapy. (A) Western blot of STAT3, pSTAT3 and cyclinD1 in the cells transduced with lenti-STAT3 and ShSTAT3 stable cell line. (B) Relative expression of STAT3 mRNA in the cells transduced with lenti-STAT3 or in the ShSTAT3 stable cell line. (C) Relative expression of pSTAT3. (D) MTT assay demonstrated that inhibition of STAT3 resulted in reduction of cell viability. (E) Relative expression of STAT3. (F) Relative expression of cyclinD1. (G) There was high ratio of Annexin V positive cells in ShSTAT3 stable cell line. * Compared with sham group; # compared with lenti-STAT3 group. $P < 0.05$ as significant difference.

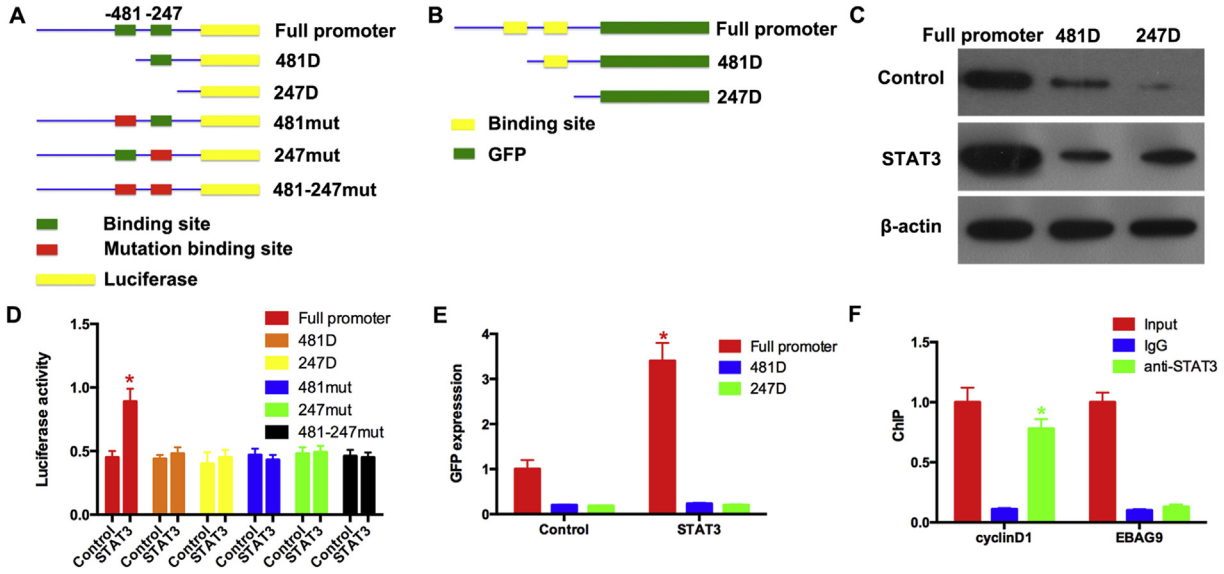

Fig. 4. STAT3 binding to the cyclinD1 promoter. (A) Different length and mutation sites of cyclinD1 promoter were subcloned into pGL4 vector. (B) Different lengths of cyclinD1 promoter were subcloned into pGFP vector with a full GFP fragment. (C) Mutation of binding sites in the cyclinD1 promoter inhibited the expression of GFP. (D) Luciferase activity showed that deletion or mutated binding sites reduced the luciferase activity in the cells cotransfected with STAT3. (E) Relative expression of GFP. (F) ChIP assay showed that STAT3 directly bind to the cyclinD1 promoter. * Compared with sham group; #compared with lenti-STAT3 group. $P < 0.05$ as significant difference.

et al. also found that the tumor volume was reduced by inhibition of cyclinD1 in mouse models [19]. Given these findings, we suggested that overexpression of cyclinD1 contributed to the poor prognosis of colorectal cancer.

Then we further determined the underlying mechanism of cyclinD1 dysregulation in colorectal cancer. The bioinformatics analysis showed that there two potential binding sites of STAT3 in the cyclinD1 promoter. ChIP assay confirmed that STAT3 directly bind to cyclinD1 promoter. This binding of STAT3 to the cylinD1 promoter enhanced the activity of promoter which was proved by luciferase assay. We subcloned different lengths of cyclinD1 promoter into pGL4 vector, cotransfected with pcSTAT3 into cells and measured the luciferase activity. Full length of cyclinD1 with two binding sites increased the luciferase activity while deletion of the first binding site (-481) resulted in significant reduction of luciferase activity. Mutation of any of the two binding sites also resulted in significant reduction of luciferase activity. These indicated that both the sites had function in the regulation of cyclinD1 expression. To further prove this, we also used the different length of cyclinD1 promoters to induce the GFP expression. The data also confirmed the result of luciferase activity. Furthermore, we constructed lenti-STAT3 and ShSTAT3 stable cell line to increase or decrease the expression of STAT3. In the HT-29 cells transduced with lenti-STAT3, cyclinD1 expression was also increased while cyclinD1 expression was inhibited in the ShSTAT3 stable cell line. STAT3 is a key transcription factor for many genes and our previous research also suggested overexpression of STAT3 in the colorectal cancer cell which was related to cell cycle [14]. Our data was similar with previous researches. In nasopharyngeal carcinoma, latent membrane protein 1 promoted STAT3 binding to the promoter region of cyclinD1, in turn, enhancing the promoter activity of cyclinD1. Furthermore, both transcriptional activity and mRNA levels of cyclinD1 were decreased by interference of STAT3 activity [20]. Samsonov et al. also identified that STAT3 regulated the cyclinD1 via binding to its promoter [21].

Considering the regulation of cyclinD1 by STAT3, inhibition of STAT3/cyclinD1 pathway may provide new insight into colorectal cancer therapy. In ShSTAT3 stable cell line with low expression of STAT3, the cell increased sensitivity to the 5-FU treatment. However, when ShSTAT3 was transduced with lenti-STAT3 to restore STAT3 expression, the sensitivity was decreased. Our data was supported by other researches. In DU145 cell line, Scoparone, a natural compound isolated from *Artemisia capillaris*, suppressed the transcription of cyclinD1 by inhibition of STAT3 [22].

In conclusion, our data indicated that STAT3/cyclinD1 pathway contributed to colorectal cancer and inhibition of STAT3/cyclinD1 increased the sensitivity of colon cancer to chemotherapy.

Conflict of interest

None.

Acknowledgments

This work was financially supported by the Suzhou Science &Technology Administration (SYS201362).

Transparency document

The transparency document associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrc.2015.01.048.

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