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BPTF inhibition antagonizes colorectal cancer progression by transcriptionally inactivating Cdc25A

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ABSTRACT

As the largest subunit of the nuclear remodeling factor complex, Bromodomain PHD Finger Transcription Factor (BPTF) has been reported to be involved in tumorigenesis and development in several cancers. However, to date, its functions and related molecular mechanisms in colorectal cancer (CRC) are still poorly defined and deserve to be revealed. In this study, we uncovered that, under the expression regulation of c-Myc, BPTF promoted CRC progression by targeting Cdc25A. BPTF was found to be highly expressed in CRC and promoted the proliferation and metastasis of CRC cells through BPTF specific siRNAs, shRNAs or inhibitors. Based on RNA-seq, combined with DNA-pulldown, ChIP and luciferase reporter assay, we proved that, by binding to −178/+107 region within Cdc25A promoter, BPTF transcriptionally activated Cdc25A, thus accelerating the cell cycle process of CRC cells. Meanwhile, BPTF itself was found to be transcriptionally regulated by c-Myc. Moreover, BPTF knockdown or inactivation was verified to sensitize CRC cells to chemotherapeutics, 5-Fluorouracil (5FU) and Oxaliplatin (Oxa), c-Myc inhibitor and cell cycle inhibitor not just at the cellular level in vitro, but in subcutaneous xenografts or AOM/DSS-induced in situ models of CRC in mice, while Cdc25A overexpression partially reversed BPTF silencing-caused tumor growth inhibition. Clinically, BPTF, c-Myc and Cdc25A were highly expressed in CRC tissues simultaneously, the expression of any two of the three was positively correlated, and their expressions were highly relevant to tumor differentiation, TNM staging and poor prognosis of CRC patients. Thus, our study indicated that the targeted inhibition of BPTF alone, or together with chemotherapy and/or cell cycle-targeted therapy, might act as a promising new strategy for CRC treatment, while c-Myc/BPTF/Cdc25A signaling axis is expected to be developed as an associated set of candidate biomarkers for CRC diagnosis and prognosis prediction.

1. Introduction

As one of the most common malignant tumors globally, colorectal cancer (CRC) ranks the second and the third in morbidity and mortality rates [1,2]. Moreover, these two rates of CRC are increasing year by year, especially in younger individuals with the age of below 50 [3]. Thus, further revealing the pathogenesis and key factors involved in CRC occurrence and development is becoming more critical. Although surgery remains the preferred and the primary treatment form for CRC, its treatment varies according to the disease staging. Medication-assisted therapy, including the combined or sole use of chemotherapeutics, as well as targeted agents, is becoming increasingly essential for the

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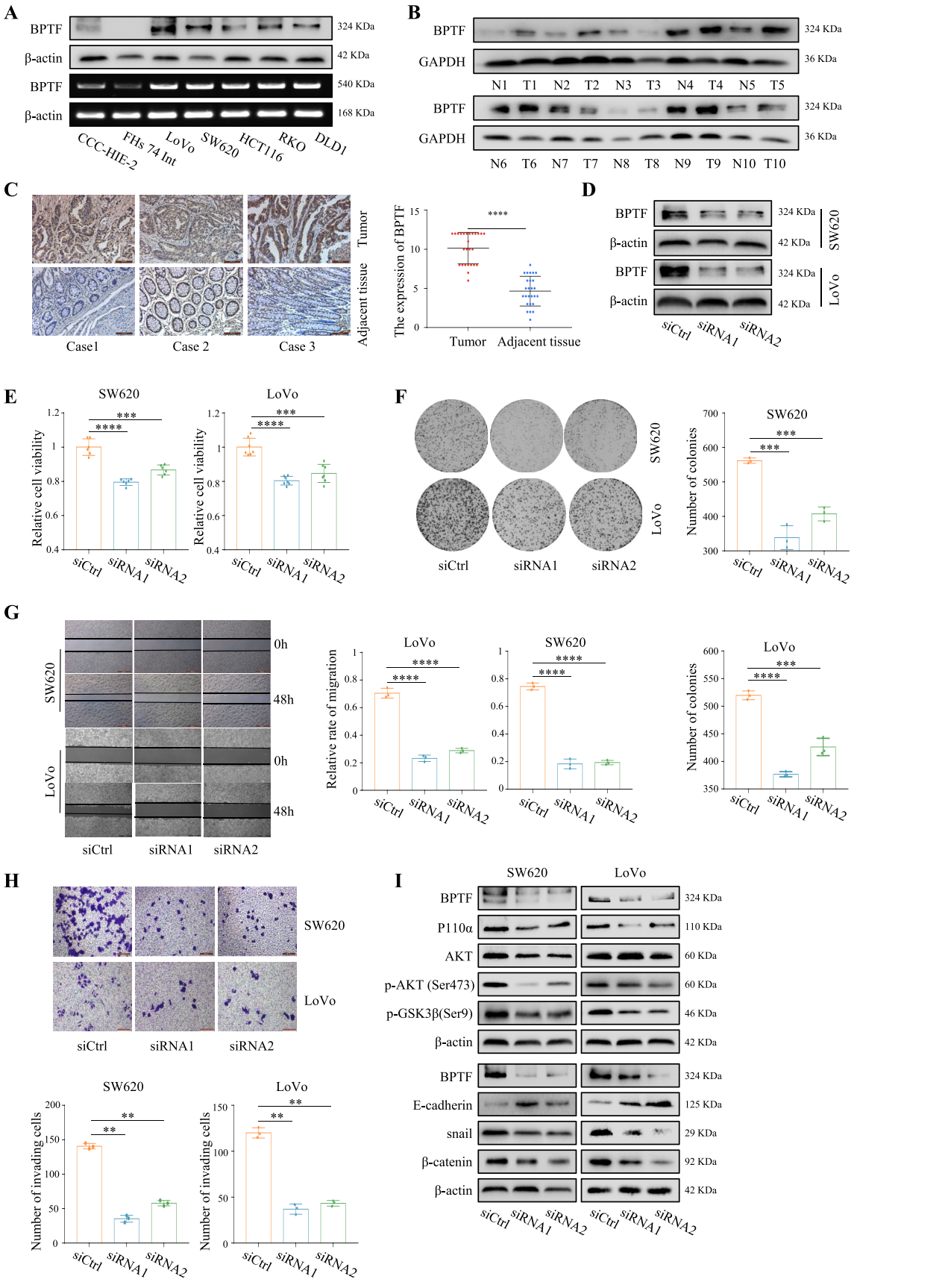

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Fig. 1. BPTF was highly expressed in CRC and promoted the proliferation and metastasis of CRC cells. (A) BPTF expression was detected in human embryonic intestinal mucosal tissue-derived cell line CCC-HIE-2, human small intestinal epithelial cell line FHs 74 Int and different CRC cell lines (LoVo, SW620, HCT116, RKO, DLD1) by Western Blot. (B–C) BPTF expression in tumor tissues (T) or adjacent normal tissues (N) from CRC patients was determined through Western Blot (B) or IHC (C). Representative pictures were shown. Score of IHC staining was displayed by scatter plot. Scale bars, 100 μm. (D–I) BPTF was knocked down by its specific siRNAs in CRC cell lines (LoVo, SW620). 48h after transfection, (D) BPTF expression, (E) cell viability, (F) colony formation, (G) cell migration, (H) cell invasion, (I) the expressions of proliferation-related PI3K/AKT pathway and EMT-related proteins were respectively analyzed. Representative images were shown. Scale bars in (G), 500 μm. Scale bars in (H), 200 μm. The data represented the mean ± SD of three independent experiments, and the level of significance was indicated by ***P < 0.001, **P < 0.01, *P < 0.05. siCtrl, control siRNA.

treatment of advanced and recurrent CRC [4–6]. However, due to individual differences in genetic background, susceptibility to drug resistance and unavoidable toxic and side effects, the therapeutic duration and efficacy of these drugs are greatly limited. There is an urgent need to find more potential candidate therapeutic targets and to further develop the targeting or combined treatment strategies to improve the prognosis of CRC patients.

Bromodomain PHD Finger Transcription Factor (BPTF), containing a DNA-binding domain, two zinc finger structures, and a bromine domain, is the largest subunit of chromatin remodeling complex [7]. It can act on chromatin remodeling process to regulate DNA accessibility, and can also act as transcriptional factor to regulate gene expression [8,9]. BFTF was initially known for affecting brain development, but in recent studies its role in promoting cancer progression has gradually attracted attention [10]. Firstly, BPTF acted as a transcription factor to regulate the expression of downstream genes to promote cancer development. It was reported to promote stemness and metastasis of hepatocarcinoma by transcriptional activating hTERT, and to accelerate lung cancer progression by synergizing with p50 to co-regulate the expression of COX-2 [11,12]. Next, BPTF functions as the downstream target of some critical tumor factors to be involved in cancer progression. MITF promoted the survival of melanoma cells by targeting BPTF, and m6A modification mediated by METTL14 reduces the stability of BPTF mRNA, leading to BPTF down-regulation and subsequent epigenetic change and metabolic reprogramming in clear renal cell carcinoma [13,14]. Furthermore, MicroRNA-3666 and MiR-1269 affected the metastasis and proliferation of lung cancer and liver cancer cells respectively by targeting BPTF [15,16]. And then, BPTF interacts with other proteins to co-participate in tumor progression. BPTF play an oncogenic role by affecting the Myc pathway in gliomas. It could also be recruited by H2A.Z to co-regulate the transcriptions of cell cycle proteins and accelerate cell division in bladder cancer. LncRNA NMR induced the recruitment of BPTF into specific chromatin regions in ESCC (esophageal squamous cell carcinoma), thereby activating ERK1/2 expression and promoting ECSS metastasis [17–19]. Last but not least, the latest studies have shown that circ-BPTF can adsorb miR-31–5p and further weaken the inhibition on RAB27A expression to promote the progression of bladder cancer [20]. Nevertheless, the precise functions and the responsible molecular mechanism of BPTF in CRC progression are still poorly understood and deserve to be further explored, although one study mentioned that BPTF expression in colon cancer tissues was correlated with EMT-associated proteins [21].

What's more, with the deepening of research on transcriptional regulatory mechanism of c-Myc, the critical role of BPTF involved in c-Myc activity has been gradually reported and clarified. On the one hand, BPTF was required for the transcriptional regulation of target genes mediated by c-Myc [22,23]. Its deficiency delayed B-cell lymphoma progression driven by c-Myc [24]. On the other hand, NURF complex containing BPTF regulated the expression of c-Myc [25]. Given the existence of E-box sequence, which could be specifically recognized and bound by c-Myc, within BPTF gene promoter, most likely, BPTF is transcriptionally regulated by c-Myc. This possibility will be further explored in our study to help us better understand the oncogenic function and mechanisms of c-Myc, and meantime to enrich our cognition for the expression regulation of BPTF in cancer, especially in CRC.

Cyclin-dependent kinases (CDKs) and their cyclin partners are the core regulatory factors related to cell cycle. At the different phases of a cell cycle, specific cyclin/CDK heterodimer is generated, during which CDK is activated to phosphorylate the corresponding substrate, thus ensuring the smooth progress of the cell cycle [26,27]. The abnormal expression or activation of positive regulators and functional suppression of negative regulators during cell cycle progression often initiates cancer. Therefore, targeting CDKs and the associated partners has become a considerable anti-cancer therapeutic strategy [28,29]. Although pan-CDK inhibitors have stalled in the clinical trials due to their toxicity within non-cancer cells, inhibitors targeting CDK4/6, such as Palbociclib, Ribociclib, and Abemaciclib, have been approved for the treatment of estrogen receptor-positive metastatic breast cancer [29–31]. Thus, based on cell cycle progress, the identification of new anti-cancer targets and the combined application of targeting therapy based on these targets and cell cycle blockade seem to be great significance in improving the efficacy of cell cycle blockers, reducing their dosage and toxicity, and delaying the development of their resistance.

By acting as an upstream regulator of CDKs, Cdc25A can remove inhibitory phosphorylation on CDKs, and contribute to the formation of cyclin-CDK complexes, thus promoting the transformation of cell cycle [32,33]. It was reported to be overexpressed in many different cancers to promote tumor progression [32,34], due to the deregulations at transcriptional and posttranscriptional level. Cell cycle regulation by c-Myc is also reported to be partially achieved by targeting Cdc25A [35]. Although it is known that p53 mutation or the destruction of E2F–Rb complex confers the loss of transcriptional inhibition for Cdc25A [36–38], while the ubiquitin hydrolase USP 17L2 is involved in the post-transcriptional regulation of Cdc25A [39], it is still of great significance to further clarify the expression regulatory mechanisms of Cdc25A, especially at the transcriptional level, in order to search for new cascade targets of Cdc25A in anti-cancer therapy.

Here, we set out to determine the function and the underlying molecular mechanisms of BPTF in CRC development. BPTF was silenced or inactivated to analyze its influence on CRC cell proliferation and metastasis. The related upstream and downstream regulatory mechanisms of BPTF were inspired through RNA-seq, DNA-pulldown, Chromatin Immunoprecipitation (ChIP) and luciferase reporter assay and BPTF was found to regulate the cell cycle process of CRC cells by transcriptionally targeting Cdc25A under the expression control of c-Myc. The synergy of BPTF knockdown or inhibition with cell-cycle specific chemotherapeutics, cell cycle inhibitor, or c-Myc inhibitor in anti-CRC efficacy in different CRC cell lines and tumor models was evaluated next, and the clinical significance of c-Myc/BPTF/Cdc25A signaling axis was further explored based on a cohort of CRC cases. Our study aimed to uncover the critical role of BPTF in CRC progression and related upstream and downstream regulatory mechanisms, thus providing potential candidate target and cascade targets and combinatorial therapeutic strategies based on them for CRC treatment.

2. Results

2.1. BPTF was highly expressed in human CRC and promoted the proliferation and metastasis of CRC cells

We firstly examined the expression of BPTF in CRC cells and tissues. Compared with normal colorectal cells, the expressions of BPTF at mRNA and protein level were both up-regulated in CRC cell lines (Fig. 1A). Consistently, although there are some exceptions, on the

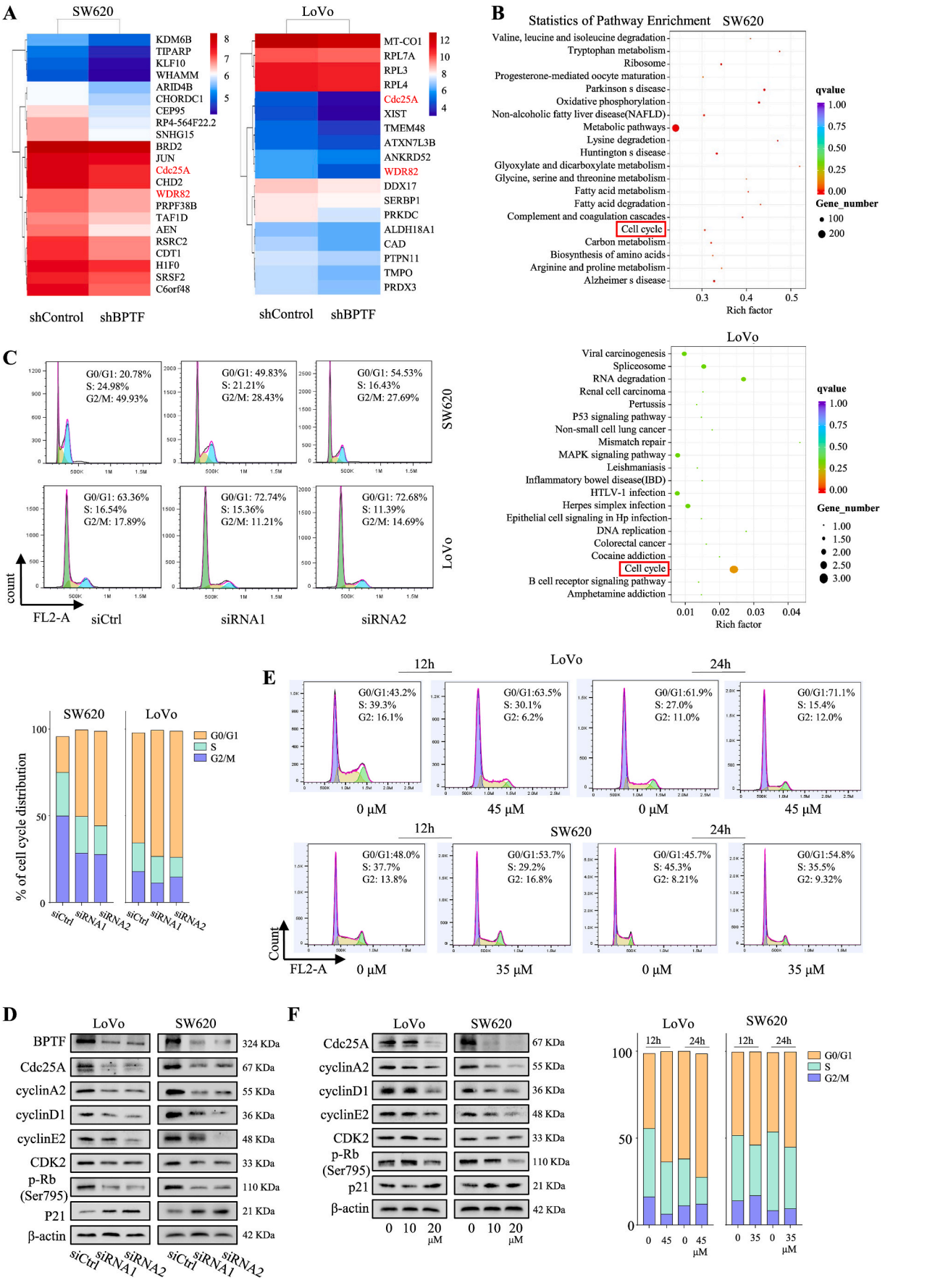

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Fig. 2. BPTF knockdown or inactivity caused G0/G1 phase arrest in CRC cells. (A) RNA-seq was performed in LoVo and SW620 cells with BPTF knockdown or not, and heat maps displayed the gene sets significantly down-regulated with BPTF silencing. (B) KEGG enrichment analysis showing the enrichment of differential genes caused by BPTF silencing in signaling pathways in SW620 or LoVo cells. (C–D) SW620 and LoVo cells transfected with BPTF specific siRNAs for 48 h were subjected to FACS cell cycle analysis using PI staining and cell cycle distribution was quantified (C). Meantime, cell cycle-related proteins were detected by Western Blot (D). (E) CRC cells treated with BPTF inhibitor (DC-BPi-07, 45 μM in LoVo and 35 μM in SW620) for 12 h or 24 h were subjected to FACS cell cycle analysis andthe cell cycle distribution was quantified. (F) Cell cycle-related proteins were detected by Western Blot in CRC cells treated with DC-BPi-07 for 48h at the indicated concentration. The data represented the mean ± SD of three independent experiments, and the level of significance was indicated by ***P < 0.001, **P < 0.01, *P < 0.05.

whole, western blot and IHC staining from CRC tissues and adjacent normal tissues indicated its high expression at protein level in most CRC tissues (Fig. 1B, C, Figs. S1A and B). The analysis based on Oncomine datasets also proved the high expression of BPTF at mRNA level in CRC tissue samples compared to normal tissues (Fig. S1C). Furthermore, the differential expression of BPTF was also observed between CRC proximal and distal metastasis tissues or between metachronous and synchronous liver metastasis tissues based on the analysis from GEO datasets (Figs. S1D and E). These results demonstrated that BPTF was highly expressed in CRC, and also suggested its potential tumor-promoting effect. We next investigated the possible role of BPTF in driving CRC. BPTF-specific siRNAs were used to knock down its expression (Fig. 1D) and cell viability and colony formation assay demonstrated that BPTF silencing significantly suppressed the proliferation of CRC cells (Fig. 1E and F). Meanwhile, cell migration and invasion assay indicated the reduced metastasis capacity of CRC cells upon BPTF silencing (Fig. 1G and H). Moreover, BPTF knockdown inactivated proliferation-related PI3K/AKT signaling pathway, which was accompanied by the reduced expression of p110α, p-AKT (Ser473) and p-GSK3β (Ser9) (Fig. 1I). Consistent with the previous report that BPTF was positively correlated with EMT pathway proteins [21], we also found that E-cadherin was up-regulated while snail and β-catenin were down-regulated upon knockdown of BPTF in CRC cells (Fig. 1I).

The specific small molecule inhibitor targeting BPTF bromodomain was also used to evaluate the function of BPTF in CRC. The significant proliferation suppression was seen in different CRC cells, but not in normal colorectal cells, with BPTF inhibitor DC-BPi-07 treatment in a dose-dependent way (Fig. S2A). Accordingly, the IC50 value of DC-BPi-07 in normal colorectal cells was much higher than that in CRC cells (Fig. S2B), suggesting the anti-proliferative function of BPTF inhibitor DC-BPi-07 in CRC cells and its weaker side effect to normal colorectal cells. Similarly, DC-BPi-07 treatment led to colony formation and metastasis inhibition in CRC cells (Figs. S2C–E). In addition, another BPTF inhibitor DC-BPi-11 treatment also reduced the proliferative ability of CRC cells by MTT experiment with a relatively low IC50, but also caused growth inhibition in normal colorectal cells to some extent (Figs. S3A and B). All these results preliminarily indicated that BPTF accelerated CRC progression, being manifested in the promotion of cancer cell proliferation and metastasis.

2.2. BPTF knockdown or activity inhibition caused G0/G1 phase cell-cycle arrest in CRC cells

To further clarify the possible molecular mechanism of BPTF in driving CRC, we performed RNA-seq in CRC cells with BPTF knockdown or not. Among the gene sets significantly down-regulated by BPTF knockdown, only WDR82 and Cdc25A appeared in common in both cell lines (Fig. 2A). Pathway enrichment analysis for differential genes indicated that BPTF knockdown affected cell cycle pathways in CRC cells (Fig. 2B). Combined with the functional enrichment analysis showing that BPTF knockdown mainly affected intracellular phosphatase activities and protein dephosphorylation (Fig. S4A) and the known function of Cdc25A as phosphatase necessary to maintain cell cycle progression [33,40], we speculated that BPTF might influence cell cycle progression in CRC by targeting Cdc25A. FACS analysis verified that BPTF knockdown or inactivity indeed caused G0/G1 phase arrest in CRC cells (Fig. 2C, E, Fig. S4B). In addition, some key proteins involved in cell cycle process, such as Cdc25A, cyclinA2, cyclinD1, cyclinE2, CDK2, p-Rb (ser785) and P21, were also changed in expression upon BPTF silencing or inactivity (Fig. 2D, F). All of these results indicated that BPTF drove CRC most possibly by promoting cell cycle progression of cancer cells.

2.3. BPTF promoted CRC survival by up-regulating Cdc25A expression

To further determine whether BPTF affects the cell cycle progression by targeting Cdc25A, we first analyzed the regulation of BPTF on Cdc25A expression. In line with RNA-seq results, BPTF knockdown decreased Cdc25A expression at both mRNA and protein levels, and its inactivity caused the same trend (Fig. 3A, Fig. S5A). We next analyzed Cdc25A expression in the same CRC cell lines and tissue samples as those used in Fig. 1. Cdc25A was highly expressed in CRC cells and tissues (Figs. S5B–F), and a positive correlation between BPTF and Cdc25A expression was found based on IHC staining of CRC tissue and the corresponding normal tissue samples (Fig. 3B). Moreover, such positive expression correlation was further confirmed in human CRC from microarray dataset analysis (R2: Genomics Analysis and Visualization Platform) (Fig. 3C). What's more, in CRC cell lines incubated for 0–24h in complete medium after starvation for 48h to synchronize cell cycles to G0/G1 phase, the expressions of BPTF and Cdc25A were all increased with the release of cell cycle arrest, and the expression trend between them was positively correlated to some extent, especially in LoVo (Fig. S5G), confirming again the regulation of BPTF on Cdc25A in CRC cells.

Given the expression regulation of BPTF on Cdc25A proved above, in other words, since Cdc25A has been proved to be the downstream gene of BPTF, most likely, BPTF plays its tumor-promoting function in CRC development by targeting Cdc25A. To confirm this, we knocked down BPTF and overexpressed Cdc25A simultaneously in CRC cells and determined the changes of cell proliferation and cell cycle progress. In comparison with the control group, BPTF knockdown suppressed cell viability and colony formation, caused GO/G1 phase arrest and the corresponding expression changes of cell cycle-associated proteins in vitro, while Cdc25A overexpression partially reversed such influences (Fig. 3D–G). Subcutaneous xenograft formation of CRC cells with stable BPTF knockdown and/or Cdc25A overexpression similarly showed that Cdc25A overexpression partially alleviated tumor growth inhibition caused by BPTF knockdown (Fig. 3H–J, Fig. S5H). Western Blot and IHC staining of the formed xenografts further proved that cell cycle arrest mediated by BPTF knockdown was rescued upon Cdc25A overexpression (Fig. 3K and L). Altogether, these results indicated that BPTF promoted CRC development by positively regulating Cdc25A expression.

2.4. BPTF transcriptionally regulated Cdc25A in CRC cells

Given that BPTF is a transcriptional factor that activates transcription by either directly binding or indirectly binding through H3K4me3 at the promoter region of target genes [8,41,42], we therefore hypothesized the transcriptional regulation of Cdc25A by BPTF in CRC. The binding peaks of BPTF and H3K4me3 within the same promoter segment of Cdc25A gene in CRC cells were found from the ChIP-seq database (Cistrome Date Browser) (Fig. 4A). Based on this, we designed a biotinylated DNA probe containing this binding sequence in the Cdc25A promoter region (−463-+392) for DNA-pulldown experiment

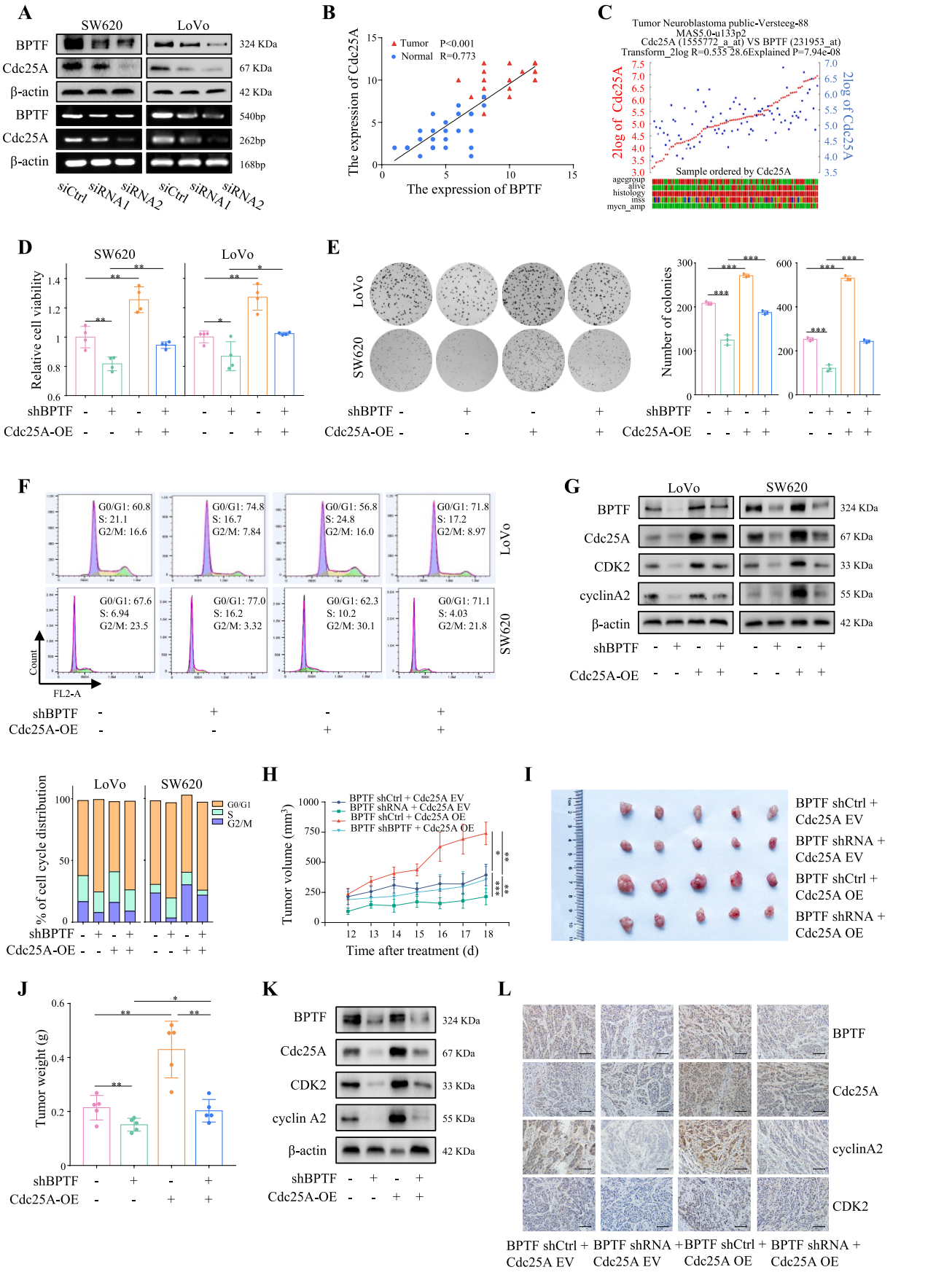

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Fig. 3. BPTF regulated cell cycle progress in CRC cells by targeting Cdc25A. (A) Protein and mRNA expression of BPTF and Cdc25A by Western Blot or RT-PCR in LoVo and SW620 cells transfected with BPTF specific siRNAs for 48h. (B) Linear regression shows a positive correlation between BPTF and Cdc25A expression based on IHC staining scores of cancer and adjacent normal tissues from 26 cases of CRC patients. (C) The expression correlation between BPTF and Cdc25A was explored through the analysis of microarray data from human CRC based on R2: Genomics Analysis and Visualization Platform. (D–G) LoVo and SW620 cells were co-transfected with shBPTF and Cdc25A overexpression plasmids for 48h, and then (D) cell viability assay was performed; (E) colony formation assay (10 days after seeding) was performed and representative images and the number of the formed colonies were shown and quantificated; (F) cell cycle analysis was performed by FACS and cell cycle distribution was quantified; (G) cell cycle-related proteins were detected by Western Blot. (H–L) LoVo cells with stable knockdown of BPTF and overexpression of Cdc25A simultaneously were injected subcutaneously into balb/c nude mice (n = 5 for each group) for 18 days, and (H) Tumor volumes were measured at different time points after injection; (I) Tumor images at the end of the experiment were shown; (J) the averaged tumor weight at the end of the experiment were displayed; (K–L) The expression of BPTF, Cdc25A, cyclin A2, CDK2 in tumor tissues were tested by Western Blot (K) or IHC staining (L), and representative images were shown. Scale bars in (L), 200 μm. The data represented the mean ± SD of three independent experiments, and the level of significance was indicated by ***P < 0.001, **P < 0.01, *P < 0.05.

(Fig. S6A). Both H3K4me3 and BPTF could be pulled down by Cdc25A promoter probe in different CRC cell lines, and the amount of BPTF pulled down was decreased upon BPTF silencing or inactivity, while the amount of H3K4me3 pulled down was inconsistent with that of BPTF (Fig. 4B, C, Fig. S6B), suggesting that the binding of BPTF to the Cdc25A promoter region is not, at least not entirely, dependent on H3K4me3. We further verified the binding of BPTF to Cdc25A promoter by ChIP. The promoter region of Cdc25A (−463-+392) was equally divided into three segments and only the segment ranging from −178-+107 could be amplified by PCR from the finally eluted DNA pellets in different colon cancer cells (Fig. 4D). Additionally, when BPTF was knocked down or inhibited, its binding at this segment was reduced accordingly (Fig. 4E, Fig. S6C), preliminarily demonstrating the specific binding of BPTF to Cdc25A promoter region (−178-+107). By employing dual-luciferase reporter assay, in which luciferase expression was driven by three truncated Cdc25A promoter regions, we found BPTF knockdown reduced −463-+392, −178-+392, but not +108-+392 fragment-driven luciferase expression (Fig. 4F), confirming again the precise binding of BPTF at Cdc25A promoter fragment −178-+108. BPTF activity inhibition caused the similar effect in −463-+392 fragment-driven luciferase expression (Fig. S6D). These results collectively indicated that BPTF transcriptionally regulated Cdc25A in CRC cells by anchoring at its promoter region.

2.5. BPTF itself was transcriptionally regulated by c-Myc in CRC cells

Considering the previous reports that BPTF was required for the transcriptional activity of c-Myc [22,23], and meantime BPTF could transcriptionally regulate c-Myc [25], we aimed to reassess the relationship between these two proteins in CRC cells. Based on the RNA-seq data mentioned above, we found that the differential genes caused by BPTF knockdown were also enriched at the c-Myc target genes (Fig. S7A). Interestingly, we noticed an E-box motif (cacgtg) and its variant sequence (cacctg) specifically bound by c-Myc exist in BPTF promoter region, and ChIP-seq database (Cistrome Data Browser) showed the binding peaks of c-Myc at BPTF promoter (Fig. S7B). Combined with the prediction of the top 20 transcriptional factors that possibly regulate BPTF transcription also based on Cistrome, in which c-Myc appeared as a main one (Fig. 4G), these results implied the possible transcriptional regulation of BPTF by c-Myc. Thus, we designed biotinylated DNA probe corresponding to -841–142 sequence of BPTF promoter region for pulldown assay and a pair of primers (−461–142), by which the amplified products contain E-box motif and its variant, for ChIP assay to verify the binding of c-Myc to BPTF promoter, and the results verified our conjecture (Fig. 4H and I). Furthermore, BPTF expressions at protein and mRNA level were both found to be decreased or increased with c-Myc knockdown or overexpression (Fig. 4J). By analyzing c-Myc expression in the same CRC cell lines and tissue samples as those used in Fig. 1, we found c-Myc was highly expressed in CRC cells and tissues compared to the normal colorectal cells and tissues (Fig. 4K, L, Fig. S7C), and its expression was positively correlated with BPTF expression based on IHC staining of tissue samples (Fig. 4M). Similarly, c-Myc expression was increased upon the release of cell cycle arrest, and its expression trend was positively correlated with BPTF, especially in SW620 (Fig. S5G).

Considering the positive transcriptional regulation of BPTF by c-Myc in CRC cells proved above, we therefore further determined the critical role of BPTF in the oncogenic function of c-Myc. Cell viability experiments in HCT-116 and RKO cells with BPTF knockdown and c-Myc overexpression demonstrated that BPTF knockdown partially reversed the proliferation promotion mediated by c-Myc overexpression (Fig. 4N). These data collectively indicated the expression regulation of BPTF by c-Myc in CRC cells and the key role of BPTF in c-Myc's tumor-promoting function.

2.6. BPTF knockdown or inactivity sensitized CRC cells to chemotherapeutics, c-Myc inhibitor and cell cycle inhibitor treatment

Given that chemotherapeutics usually lead to apoptosis or cellular senescence by inducing DNA damage in cells, activating the intra-S-phase checkpoint to inhibit DNA synthesis and causing cell cycle arrest [43,44], and c-Myc plays a vital role in maintaining cell cycle process [45,46], and combined with our study results mentioned above that BPTF promoted cell cycle process by targeting Cdc25A in CRC (Figs. 2–4), and meanwhile, BPTF was transcriptionally regulated by c-Myc in CRC (Fig. 4), we hypothesized knockdown or inactivity of BPTF might enhance anti-cancer effect of c-Myc inhibitor, cell cycle inhibitor, or common chemotherapeutics in CRC by synergistically targeting cell cycle progression. LoVo and SW620 cells with stable knockdown or inactivity of BPTF were used to verify these assumptions. BPTF silencing was observed to enhance the cytotoxic effect of 5-FU, Oxa, c-Myc inhibitor 10,058-F4 and cell cycle inhibitor roscovitine on colon cancer cells (Fig. 5A–C). Additionally, BPTF knockdown was also found to effectively improve the blockade of cell cycle process and the expression change of cell cycle-related proteins induced by chemotherapeutics in CRC cells (Figs. S8A–C). Similarly, BPTF inhibitor (LoVo: 20 μM; SW620: 15 μM) sensitized colon cancer cells to cell cycle inhibitor or c-Myc inhibitor treatment, to a certain extent (Fig. 5D and E), while colony formation assay further confirmed this effect with a relatively low-dose of BPTF inhibitor but a long-term incubation period (LoVo and SW620: 7.5 μM, 10 days treatment) (Figs. S8D and E). Furthermore, in the established subcutaneous xenografts model of the colon cancer cell LoVo with stable knockdown of BPTF or not in mice, compared to the control group, BPTF knockdown itself not only suppressed tumor growth, but also acted synergistically with oxaliplatin or c-Myc inhibitor (10, 058-F4), leading to more significant tumor inhibition (Fig. 5F–I). More notably, no significant difference in body weight changes of mice among different groups was observed during the administration (Fig. S8F). These results showed that BPTF knockdown or inactivity sensitized CRC cells to chemotherapeutics, c-Myc inhibitor or cell cycle inhibitor most possibly by functioning in a common pathway at cell cycle arrest.

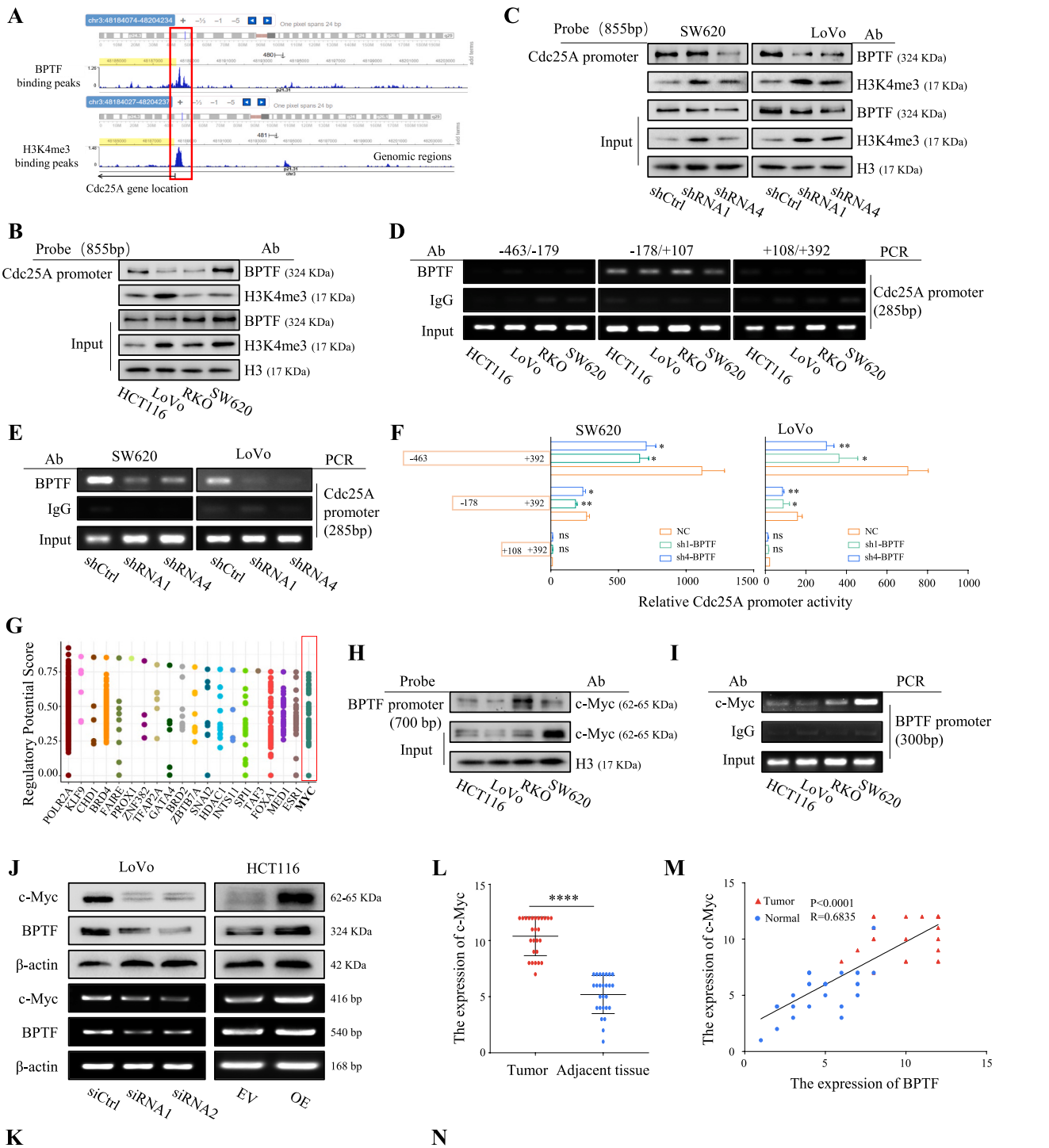

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Fig. 4. BPTF transcriptionally regulated Cdc25A and itself was transcriptionally regulated by c-Myc in CRC cells. (A) The binding peaks of BPTF and H3K4me3 at the same region of the Cdc25A promoter in CRC cells based on data from the ChIP-seq database: Cistrome Date Browser. (B–C) Pulldown assay using Cdc25A promoter probe was performed in different CRC cell lines (B), or in LoVo and SW620 cells with stable knockdown of BPTF or not (C), and BPTF and H3K4me3 proteins were detected in the pulled down complexes finally. (D) ChIP assay was performed using BPTF antibody or IgG in different CRC cell lines, and different segment of Cdc25A promoter region were detected finally. (E) ChIP assay was performed using BPTF antibody or IgG in LoVo and SW620 cells with stable knockdown of BPTF or not. The segment of Cdc25A promoter region corresponding to −178/+107 was detected. (F) Relative activities of different Cdc25A promoter fragments were detected in LoVo and SW620 cells stably transfected with shBPTF or shCtrl using dual-luciferase reporter assays. (G) Top 20 binding proteins in BPTF promoter predicted by the database: Cistrome Date Browser. (H) Pulldown assay using BPTF promoter probe was performed in different CRC cell lines, and c-Myc protein was detected in the pulled down complexes finally. (I) ChIP assay was performed using c-Myc antibody or IgG in different CRC cells and the segment of BPTF promoter region containing c-Myc-binding site was detected. (J) Protein or mRNA expression of BPTF and c-Myc in LoVo and HCT116 cells transfected with c-Myc specific siRNAs or overexpression plasmids for 48 h were detected by Western Blot or RT-PCR. (K–L) C-Myc expression in tumor tissues or adjacent normal tissues of CRC patients was determined through IHC (K). Representative pictures were displayed and scatter plot showed averaged expression level of c-Myc according to IHC score (L). (M) The positive correlation between BPTF and c-Myc protein expression based on IHC staining scores from cancer and adjacent normal tissues of 26 CRC patients. (N) Cell viability assay was performed in CRC cells co-transfected with c-Myc overexpression plasmids and BPTF specific siRNAs for 48h. The data represented the mean ± SD of three independent experiments, and the level of significance was indicated by ***P < 0.001, **P < 0.01, *P < 0.05.

2.7. BPTF activity inhibition delayed tumor progression and improved the anti-tumor effect of cell cycle inhibitor in AOM/DSS-induced mouse model of CRC

To better evaluate the therapeutic potential of BPTF blockade in CRC treatment, we established mouse model of colitis-associated colorectal tumorigenesis through AOM/DSS induction to better simulate occurrence and development of human CRC (Fig. S9A) [47,48]. Mice in AOM/DSS-induced group showed slow body weight gain and the increased levels of p-ERK in colon tissues after 14 days of induction, a key indicator of inflammatory responses, as compared to the normal mice without induction (Figs. S9B and C). Moreover, the distinct colorectal neoplastic tissues with concomitant colorectal shortening and splenomegaly were observed in mice with AOM/DSS induction for 107 days, and HE staining of colorectal tissues in these mice showed clear oncological features (Fig. 6A and B). Western Blot and IHC staining showed that Ki67, BPTF, c-Myc and Cdc25A were highly expressed in tissues of the formed tumors compared to normal colorectal tissues (Fig. 6C and D), especially in highly proliferating intestinal epithelial lining distributing in the crypts of intestinal villus (Fig. S9D), and a positive correlation between BPTF and Cdc25A or c-Myc expression was shown (Fig. 6E), proving the expression regulation of BPTF on Cdc25A and c-Myc on BPTF in CRC from another perspective.

Based on the established mouse model of CRC through AOM/DSS induction, we further evaluated the influence of BPTF inhibitor on tumor progression. Ten days after the fourth round of DSS treatment, the induced mice were divided into different groups with BPTF inhibitor or cell cycle inhibitor treatment individually or in combination. After 24 days treatment, BPTF inhibitor or cell cycle inhibitor application alone delayed tumor development to some extent, while the combined application of cell cycle inhibitor and BPTF inhibitor (whether DC-BPi-07 or DC-BPi-11) nearly detered tumor formation and relieved splenomegaly, compared with the solvent group or monotherapy groups (Fig. 6F–H; Fig. S10A). In agreement, Ki67, a key proliferative marker, showed the decreased expression in the formed tumors after single treatment, especially upon BPTF inhibitor administration. What's more, the combined administration further down-regulated its expression, compared to the cell cycle inhibitor treatment alone (Fig. 6I). No significant changes in body weight of mice in different groups during the administration were seen although DC-BPi-07 single administration caused slight body weight increase while the combined inhibitor treatment resulted in slight body weight decrease (Fig. S10B). Moreover, survival rate of mice was only 60% in the group with DC-BPi-07 monotherapy, 80% in the group with combined inhibitor treatment, but 100% in the other groups, suggesting a better tolerance in vivo for DC-BPi-11 than that for DC-BPi-07 (Fig. S10C). In line with this, the down-regulated expression of p-ERK/ERK indicated that DC-BPi-11 was more effective than DC-BPi-07 in relieving inflammatory reactions, and the combined administration caused better relief (Fig. S10D). Finally, we explored the effect of the combined treatment on cell cycle progress. Compared with the single administration, the expressions of c-Myc, CDK2 and Cdc25A in tumor tissues were significantly down-regulated upon co-administration. However, compared with the control group, the expressions of c-Myc, CDK2 and Cdc25A in the single-administration group were significantly increased, which was consistent with the trend in LoVo cells exposed to serum starvation to achieve cell cycle synchronization (Figs. S10E–G), suggesting the involvement of a multi-channel and more complex feedback regulatory mechanism. Altogether, these results indicated that BPTF inactivity delayed tumor progression and improved the anti-tumor effect of cell cycle inhibitor in AOM/DSS-induced mouse model of CRC by co-targeting cell cycle progress.

2.8. The expression of BPTF was positively correlated with Cdc25A or c-Myc in CRC tissues and their high expression predicted poor prognosis of patients with CRC

Furthermore, we performed IHC staining to analyze the expressions of BPTF, c-Myc and Cdc25A in cancer and the corresponding adjacent normal tissues based on tissue microarray containing 84 colon cancer patients. The related pathological information and expression levels of BPTF, c-Myc and Cdc25A according to the statistical analysis of the staining score were listed in Table S1. High expression of BPTF, c-Myc and Cdc25A in CRC tissues, compared to adjacent normal tissues, was demonstrated again (Fig. 7A and B). More than that, the positive expression correlation between BPTF and Cdc25A or c-Myc was found (Fig. 7C, D, Figs. S11A–D). Kaplan-Meier analysis showed that the expression of c-Myc and BPTF was significantly correlated with T stage, N stage and TNM stage respectively in patients with CRC, while the expression of Cdc25A was significantly correlated with N-stage and TNM-stage of CRC patients (Table S2). Importantly, the high expression of BPTF, Cdc25A or c-Myc was closely correlated with the poor prognosis of CRC patients (Fig. 7E, Figs. S11E and F), and their simultaneous high expression was significantly correlated with differentiation, T stage, N stage, TNM stage and poor prognosis of CRC patients (Fig. 7F and G). CRC tissues and the adjacent normal tissues could even be distinguished by the expression levels of c-Myc, BPTF, and Cdc25A among the 84 patients tested (Fig. S11G), and consistent conclusions were reached based on the analysis from database GEPIA (Fig. S11H).

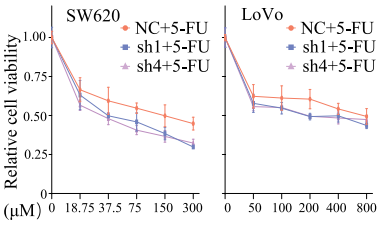
3. Discussion

So far, the reports on the involvement of BPTF in CRC have been limited only to its simple clinical significance, that is, the expression of BPTF is related to the poor prognosis of patients [21]. Thus, to our knowledge, we might for the first time demonstrate the significant tumor-promoting function of BPTF in CRC and the responsible molecular mechanisms. BPTF was found to be highly expressed in human CRC to promote the proliferation and metastasis of CRC cells, and its knockdown or inhibition caused cell cycle arrest. RNA-seq analysis suggested Cdc25A functioning as the critical downstream target of BPTF

A

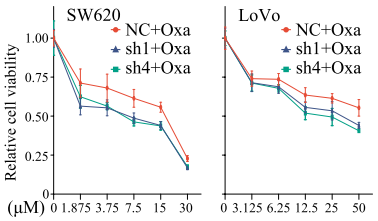
IC50 of 5-FU (μM)

	NC	sh1	sh4
SW620	146.5	49.57	32.91
LoVo	961.15	251.12	292.43



IC50 of Oxa (μM)

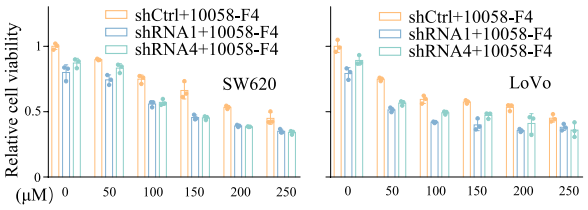
	NC	sh1	sh4
SW620	11.46	4.87	5.49
LoVo	97.17	29.33	21.61



B

IC50 of 10058-F4 (μM)

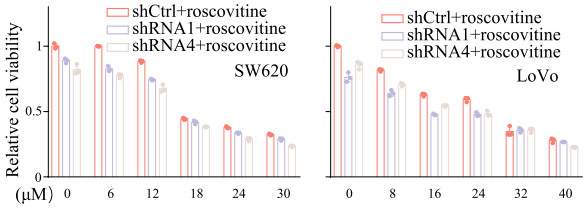
	NC	sh1	sh4
SW620	221.693	137.206	141.429
LoVo	209.174	49.694	94.6869



C

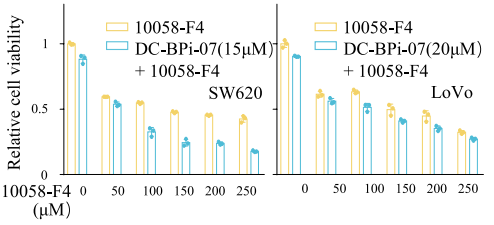
IC50 of roscovitine (μM)

	NC	sh1	sh4
SW620	19.7992	17.7156	15.9738
LoVo	23.8842	17.2286	19.6146



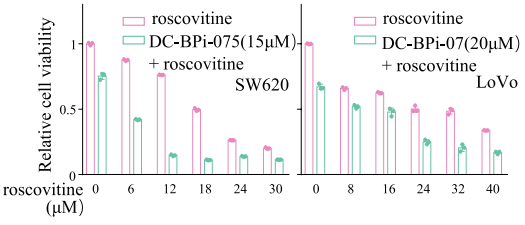
D

Cells	DC-BPi-07 (μM)	IC50 of 10058-F4 (μM)		Folds
		10058-F4	10058-F4+DC-BPi-07	
SW620	15	130.94	54.5593	2.40
LoVo	20	137.59	84.0102	1.64



E

Cells	DC-BPi-07 (μM)	IC50 of roscovitine (μM)		Folds
		roscovitine	roscovitine+DC-BPi-07	
SW620	15	17.6398	3.9469	4.47
LoVo	20	23.7098	11.2422	2.11



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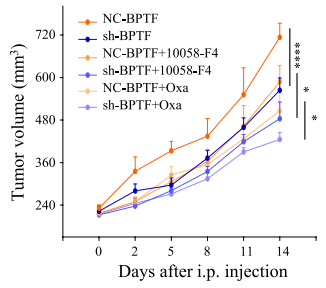

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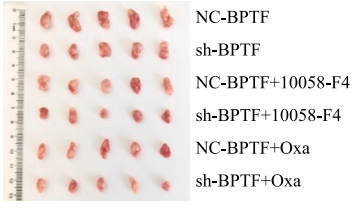

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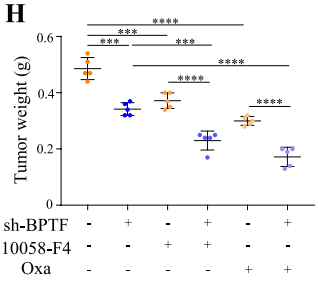

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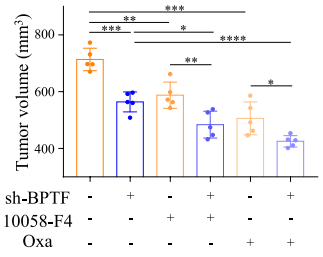

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Fig. 5. BPTF knockdown or inactivity improved the anti-tumor effect of chemotherapeutic, c-Myc inhibitor and cell cycle inhibitor in CRC. (A–C) LoVo and SW620 cells transfected with shBPTF or shCtrl were treated with varying concentrations of compound for 48 h and dose-response curves of cell viability and IC50 values for the corresponding drug were shown. Chemotherapeutic: 5-FU and Oxaliplatin; c-Myc inhibitor: 10,058-F4; cell cycle inhibitor: roscovitine. (D–E) LoVo and SW620 cells exposed to specific concentration of BPTF inhibitor (DC-BPi-07) were co-treated with varying concentrations of cell cycle or c-Myc inhibitor and dose-response curves of cell viability and IC50 values for the corresponding drug were shown (DC-BPi-07 concentration: LoVo: 20 μM; SW620: 15 μM). (F–I) LoVo cells with stable knockdown of BPTF or not were injected subcutaneously into balb/c nude mice. Then alternating doses of oxaliplatin at 3 mg/kg, once every two days, 7 cycles, or c-Myc inhibitor (10,058-F4) at 20 mg/kg, once a day for 14 days, were injected intraperitoneally into mice. 14 days after administration, (F) Tumor volumes were measured during the administration. (G) Tumor images at the end of the experiment were displayed. (H) The averaged tumor weight at the end of the experiment was shown. (I) The averaged tumor volume at the end of the experiment was shown. The data represented the mean ± SD of three independent experiments, and the level of significance was indicated by ***P < 0.001, **P < 0.01, *P < 0.05.

in CRC and our subsequent study proved this probability. The knockdown or inactivity of BPTF caused down-regulation of Cdc25A expression, while Cdc25A overexpression partially reversed the decreased proliferative capacity, cell cycle progression and subcutaneous xenograft growth of CRC cells mediated by BPTF silencing. In regard to the common role of BPTF as a transcriptional factor [9,49], we explored and verified the transcriptional regulation of BPTF on Cdc25A by anchoring at Cdc25A promoter region (−178-+107). Meanwhile, the upstream regulator of BPTF, c-Myc, was deduced and approved further in our study. BPTF knockdown or inactivity remarkably sensitized CRC cells to chemotherapeutics, c-Myc inhibitor or cell cycle inhibitor in vitro and in vivo. Finally and more significantly, the clinical significance of c-Myc/BPTF/Cdc25A signaling axis was explored and revealed. These three proteins were simultaneously highly expressed in CRC tissues and their expressions were highly relevant to tumor differentiation, TNM staging and poor prognosis of CRC patients. Hence, this study mentioned us that targeting BPTF itself, or the combination of BPTF inactivity with c-Myc inhibition or cell cycle inhibitor or cell cycle specific chemotherapeutics, can serve as a reinforcement to enhance the original therapy in CRC treatment.

It has been proved that BPTF directly or indirectly binds to the promoter region of target genes through H3K4me3 [8,42,50]. Combined with the data from ChIP-seq database showing the co-anchoring of BPTF and H3K4me3 to the same segment within the Cdc25A promoter in CRC cells, we reasonably deduced the involvement of H3K4me3 in the transcriptional regulation of Cdc25A mediated by BPTF. Although DNA pull-down results verified the binding of H3K4me3 at Cdc25A promoter region similarly bound by BPTF, this binding was not affected by BPTF level, indicating the binding of H3K4me3 to the Cdc25A promoter was BPTF independent. In other words, H3K4me3 might not be proposed as the major synergizer of BPTF in transcriptionally regulating Cdc25A. However, most likely, some other transcriptional factors or transcriptional coactivators or epigenetic events might be involved in the transcriptional regulation of BPTF on Cdc25A. As to what these factors or events are and how they influence the anchoring of BPTF at Cdc25A promoter and subsequent transcription and even CRC progression, all these queries remain to be further explored.

The abnormal high expression of BPTF in CRC prompted us to analyze its upstream regulatory mechanisms. Regarding the existence of E-box sequence within BPTF promoter and the binding peak of c-Myc at BPTF promoter region based on ChIP-seq database, we boldly hypothesized the transcriptional regulation of c-Myc on BPTF in CRC cells and conducted experimental verification on this prediction. As what we expected, the expression of BPTF was positively regulated by c-Myc level. Moreover, pulldown and ChIP analysis further confirmed the binding of c-Myc at BPTF promoter containing E-box sequence. Here, we firstly reported that c-Myc transcriptionally regulated BPTF in CRC cells. Combined with the previous reports that BPTF is required for the transcriptional activity of c-Myc, and BPTF could transcriptionally regulate c-Myc [22–25], our findings further clarified the relationship between BPTF and c-Myc and their contribution to tumorigenesis and development by acting as a positive feedback loop, implying co-targeting these two proteins simultaneously might be a considerable therapeutic strategy for c-Myc-driven tumors. In our study, BPTF knockdown or inactivity indeed sensitized CRC cells to c-Myc inhibitor, suggesting again the great potential of co-targeting BPTF and c-Myc to better eliminate cancer cells.

Given that BPTF promoted CRC development by affecting the cell cycle process, we therefore further evaluated the synergy of targeted suppression of BPTF with cell cycle-associated anti-tumor agents in CRC treatment. In line with the report from Tyutyunyk-Massey et al. [51], where genetic and pharmacologic inhibition of BPTF enhanced the effectiveness of TOP2-targeted cancer chemotherapeutic drugs, including doxorubicin and etoposide, in triple-negative breast cancer models, we also found that BPTF silencing or inactivity combined with cell cycle-specific chemotherapeutics, 5-Fu and oxaliplatin, or cell cycle inhibitor, roscovitine, showed varying degrees of synergy in antagonizing CRC cell growth in vitro. Of note, the strongest synergy was observed upon the combination of BPTF inhibitor with cell cycle inhibitor. Accordingly, in agreement with the in vitro evaluation, the synergistic anti-cancer effect was displayed upon BPTF silencing with co-treatment of the chemotherapeutic, oxaliplatin or the c-Myc inhibitor, 10,058-F4, in the xenograft of human CRC cells from nude mouse. More significantly, compared to oxaliplatin or 10,058-F4 administration alone, the combination treatment did not cause obvious toxic and side effects, being reflected by the result that no significant difference in body weight changes among different groups was seen during the administration. In regard to the synergy of BPTF inactivity with cell cycle inhibitor in vivo, the AOM/DSS in situ CRC model in mice that can simulate the progression of human CRC was employed. Encouragingly, compared with the single blocker treatment, BPTF inhibitor, whether DC-BPi-07 or DC-BPi-11, in combination with relatively low doses of cell cycle inhibitor, roscovitine, delayed CRC progression in situ as evidenced by the down-regulated number and volume of the formed tumors. Here, it cannot be ignored that BPTF inhibitors, especially DC-BPi-07, have shown some degree of neurotoxicity. The mice receiving BPTF inhibitor alone or its combination with roscovitine showed obvious hyperactivity and even died in very few individuals, suggesting the necessity of restructuring BPTF inhibitors to improve their efficacy and reduce their toxicity, especially neurotoxicity, for better clinical transformation. Moreover, it is notable that although, in general, cell cycle-related proteins, BPTF, c-Myc, and Cdc25A were all down-regulated upon co-administration of BPTF inhibitor and cell cycle inhibitor compared with the monotherapy group, they were up-regulated after single inhibitor treatment compared with the control group, implying the existence of intrinsic feedback regulatory mechanisms in response to cell cycle blockade caused by CDKs inactivity, which might partially contribute to the production of cell cycle inhibitor resistance [52–54]. Thus, our findings illustrated from another perspective the benefit of such combination therapy for delaying drug resistance development and maximizing drug utilization in cancer treatment.

Clinically, we analyzed the relationship between BPTF, c-Myc and Cdc25A expression and clinicopathological traits of CRC using IHC staining based on tissue microarray containing cancer and paracancerous tissues. Consistent with the analysis based on CRC tissue samples in situ from AOM/DSS-induced mice, the results also showed that the expression of BPTF was positively correlated with that of c-Myc or Cdc25A in human cancer and adjacent normal tissues. Furthermore, the simultaneous high expression of BPTF, c-Myc and Cdc25A could

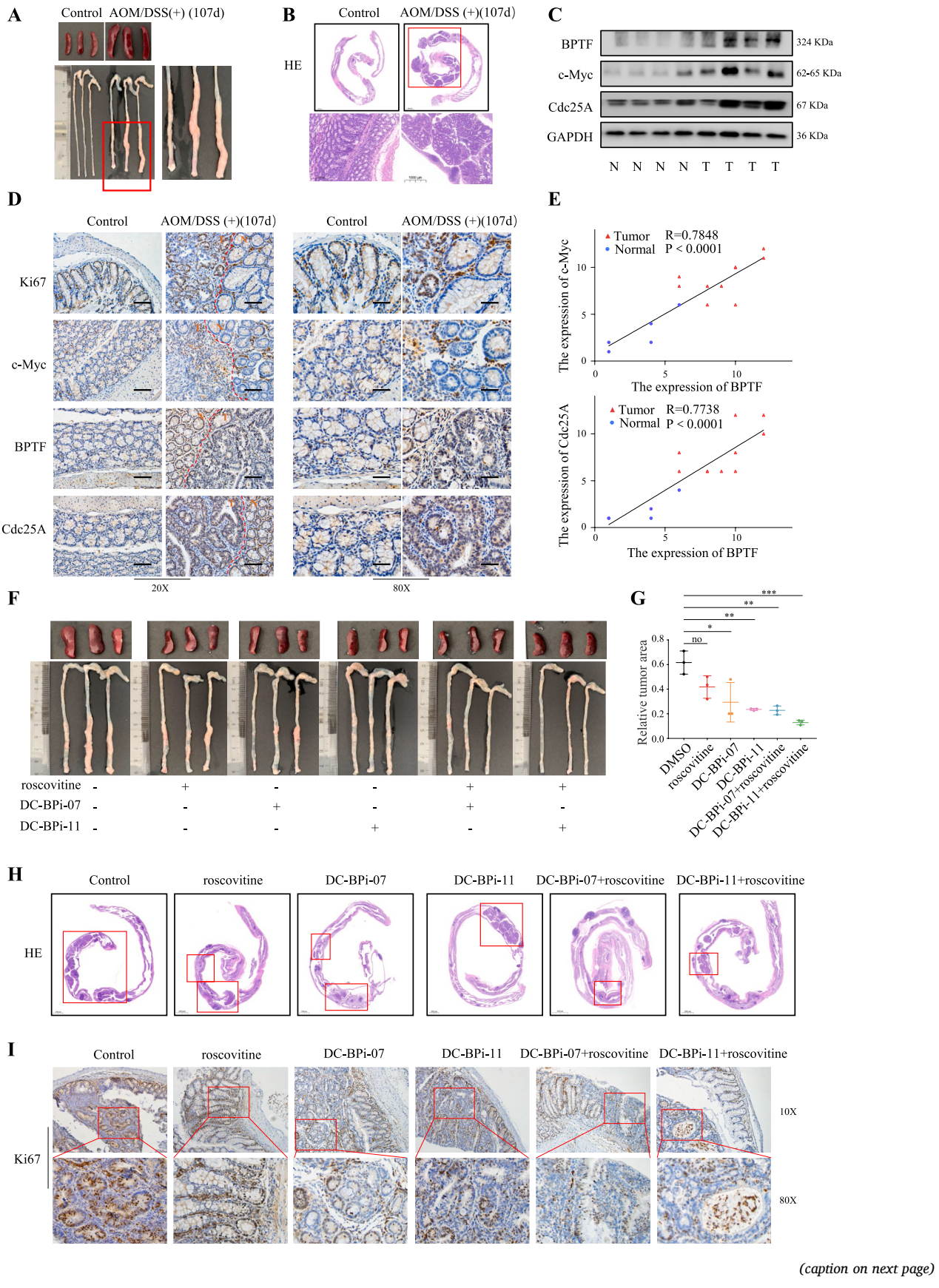

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Fig. 6. BPTF inactivity delayed tumor progression and improved the anti-tumor effect of cell cycle inhibitor in AOM/DSS-induced mouse model of CRC. (A) The representative photographs of colon, rectum and spleen in mice at the end of AOM/DSS treatment. (B) The representative photographs of HE staining for colon and rectum tissues in mice at the end of AOM/DSS treatment. Scale bars in up, 2000 μm; in lower left, 200 μm; in lower right, 1000 μm. (C) Western Blot assay for BPTF, Cdc25A and c-Myc expression in normal tissues and tumor tissues of colon and rectum in mice at the end of AOM/DSS treatment. (D) The representative photographs of IHC staining for Ki67, BPTF, Cdc25A and c-Myc in normal tissues and tumor tissues of colon and rectum in mice at the end of AOM/DSS treatment. Scale bars in left, 100 μm; in right, 25 μm. (E) Linear regression showing a positive correlation between BPTF and Cdc25A or BPTF and c-Myc expression based on IHC staining scores for cancer and adjacent normal tissues in AOM/DSS-induced mouse model. (F–I) In AOM/DSS-induced mouse model, alternating doses of cell cycle inhibitor (roscovitine) at 40 mg/kg, once every two days, 12 cycles; BPTF inhibitor (DC-BPi-07) at 50 mg/kg, once every two days, 12 cycles; BPTF inhibitor (DC-BPi-11) at 30 mg/kg, once every two days, 12 cycles, and the combination of roscovitine with DC-BPi-07 or DC-BPi-11 based on the doses mentioned above, was administered respectively. At the end of compound administration, (F) the representative photographs of colon, rectum and spleen in mice were shown (G) The quantification for the area of the formed tumor was shown. (H) The representative photographs of HE staining for colon and rectum tissues in mice were shown. Scale bars, 2000 μm. (I) The representative photographs of Ki67 staining for colon and rectum tissues in mice were shown. Scale bars in up, 200 μm; in down, 25 μm. The data represented the mean ± SD of three independent experiments, and the level of significance was indicated by ***P < 0.001, **P < 0.01, *P < 0.05.

effectively distinguish cancer from adjacent tissues, and was significantly related to TNM stage, differentiation and poor prognosis of CRC patients. Considering the early studies demonstrating that BPTF expression is associated with EMT transformation and poor prognosis in CRC patients, our results further suggested that the simultaneous high expression of BPTF, c-Myc and Cdc25A might be considered as a group of cascade biomarkers to predict the poor prognosis of CRC patients.

Summarily, BPTF knockdown or inactivity not only antagonized CRC progression, but also significantly sensitized CRC cells to cell cycle-specific chemotherapeutics, c-Myc inhibitor and cell cycle inhibitor in vitro and in vivo, suggesting that combined inhibition of c-Myc/BPTF/Cdc25A signaling axis is more effective in eliminating tumor cells than single targeted therapy or chemotherapy. In addition, DC-BPi-07 or DC-BPi-11, was identified as potent BPTF inhibitor combined with chemotherapeutics or targeted agents to increase their anti-CRC efficacy, although the structure of these two inhibitors remained to be further improved. More evaluations will be needed to further determine the efficacy and tolerability of this combination-based therapeutic strategy. However, our current study at least provided a high probability to develop c-Myc/BPTF/Cdc25A signaling axis as the promising therapeutic targets and prognosis biomarkers in CRC treatment.

4. Conclusion

Our study identified BPTF as a crucial target to cure CRC by targeting Cdc25A to accelerate cell cycle progression under the regulation of c-Myc. By binding to −178/+107 region of the Cdc25A promoter, BPTF promoted the transcription of Cdc25A in CRC cells. Meanwhile, BPTF itself was transcriptionally regulated by c-Myc, which might be partially responsible for the overexpression of BPTF in CRC. BPTF knockdown or activity blockade suppressed CRC progression and enhanced the sensitivity of CRC cells to chemotherapeutics, c-Myc inhibitor and cell cycle inhibitor in vitro and in vivo, while Cdc25A overexpression partially reversed the tumor growth inhibition caused by BPTF silencing. The simultaneous high expressions of c-Myc, BPTF and Cdc25A were highly relevant to tumor differentiation and TNM staging, and predicted poor prognosis of patients with CRC. The whole study collectively indicated that the targeted inhibition of BPTF may become a promising new strategy for adjuvant treatment or multi-target combination treatment of CRC, while BPTF, together with its upstream and downstream key regulatory factors c-Myc and Cdc25A, are expected to be an associated set of biomarkers for the diagnosis and prognosis prediction of CRC.

5. Materials and methods

5.1. Reagents

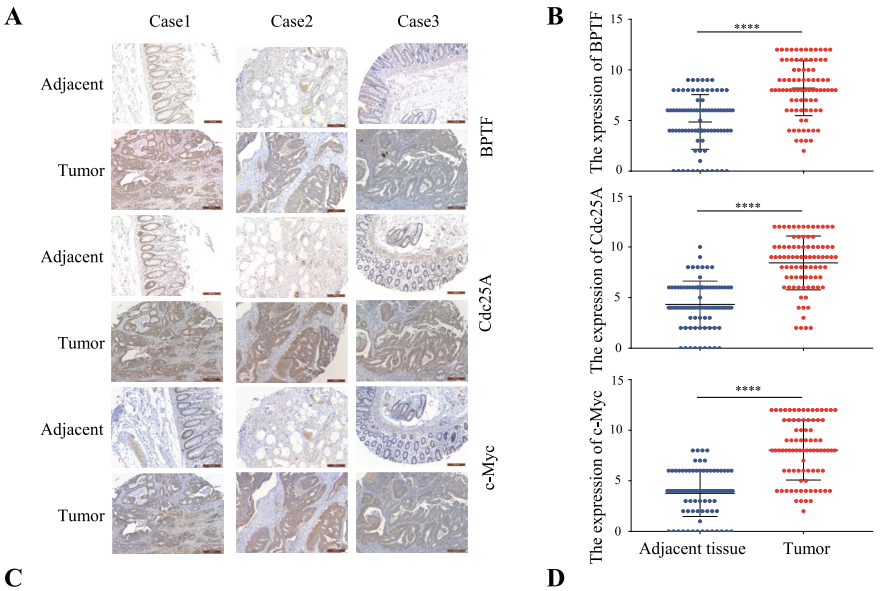
5-Fluorouracil (5-FU, #51-21-8) was purchased from Sigma-Aldrich. 10,058-F4 (#S715301) and Oxaliplatin (Oxa, #S122412) were purchased from Selleck Chemicals. roscovitine (#17817) was obtained from MedChem Express. BPTF inhibitors [55] DC-BPi-07 and DC-BPi-11 were kindly provided by Dr. Cheng Luo (Shanghai Institute of Materia Medica, Shanghai, China).

5.2. ChIP assay

Cells with different treatments were fixed with 1% formaldehyde for 10 min at RT, and the crosslink was ended by adding 10% 1.25 M glycine into the medium for 5 min at RT. The cells were washed with cold PBS for three times and then scraped and harvested in PBSI buffer. The cell pellets were resuspended with IP buffer (SDS buffer: Triton Dilution buffer 2:1) and sonicated on ice to shear the DNA into 200–500 bp fragments. The cell lysate with equal amounts of total proteins was subjected to incubation with the antibodies against BPTF or c-Myc or nonspecific IgG and protein A/G agarose beads for immunoprecipitation. The immunoprecipitated DNA was finally used as templates to amplify the Cdc25A or BPTF promoter fragment via PCR. The sequences of primers used in ChIP were listed in Table S3.

5.3. Animal studies

All animal maintenance and operational procedures were carried in accordance with the animal study protocol approved by Animal Care and Ethics Committee of Dalian Medical University. Male mice (Balb/c nude mice or Balb/c mice) aged 4–6 weeks were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd. For xenograft growth analysis, on the one hand, LoVo cells stably expressing BPTF shRNAs or control shRNAs were subcutaneously injected into left flank of Balb/c nude mice. Two weeks later, tumor-bearing mice with or without knockdown of BPTF were randomly divided into three groups: intraperitoneal injection of solvent, oxaliplatin (3 mg/kg, once every two days) or c-myc inhibitor 10,058-F4 (20 mg/kg, once a day). Tumor volume was measured during administration and calculated as V= (width^2 X length)/2. Two weeks after drug administration, the mice were sacrificed and the xenografts were isolated for western blot and IHC analysis. On the other hand, LoVo cells stably expressing BPTF shRNAs and/or Cdc25A overexpression plasmids were injected into left flank of Balb/c nude mice. Tumor volume was similarly measured and calculated as described above. 18 days later, the mice were sacrificed and the xenografts were isolated for western blot and IHC analysis. For azoxymethane (AOM) and dextran sulfate sodium (DSS)-induced colitis-associated tumorigenesis and development analysis, the operation was carried out according to the reports described previously [47,48]. Briefly, after intraperitoneal injection of AOM, DSS was added into drinking water for four cycles with six days for one cycle and an interval of 14 days between dosing. After the fourth rounds of DSS treatment, the induced mice were randomly divided into six groups: intraperitoneal injection of solvent, roscovitine, DC-BPi-07, DC-BPi-11, roscovitine in combination with DC-BPi-07 or roscovitine in combination with DC-BPi-11. At 14 days and 107 days after intraperitoneal injection of AOM, the mice were sacrificed and the colorectal parts were taken out respectively for further study.

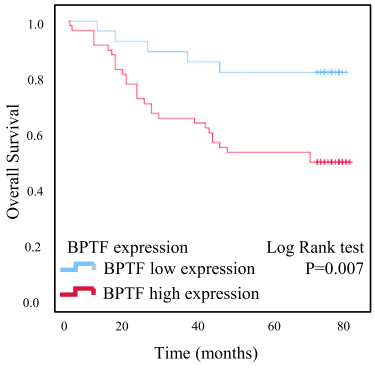


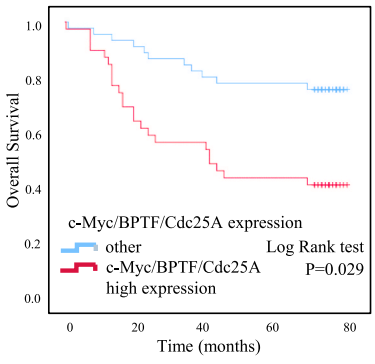
C

r=0.400		Cdc25A		P
		High	Low	
BPTF	High	46	11	<0.001
	Low	11	16	

D

r=0.358		c-Myc		P
		High	Low	
BPTF	High	44	13	0.001
	Low	11	16	





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Characteristics	c-Myc^{High}/BPTF^{High}/Cdc25A^{High}(%)	Other	P-value
Over all	39 (46.4)	45	
Sex			0.650
male	21 (48.8)	22	
female	18 (43.9)	23	
Age			0.287
≤65y	18 (40.9)	26	
>65y	21 (52.5)	19	
Differentiation			**0.001**
well	1 (33.3)	2	
moderate	26 (38.2)	42	
poor	12 (92.3)	1	
T states			**<0.001**
T1	0 (0)	1	
T2	1 (11.1)	8	
T3	18 (39.1)	28	
T4	20 (71.4)	8	
N states			**<0.001**
N0	13 (25)	39	
N1	20 (83.3)	4	
N2	6 (75)	2	
M states			0.243
M0	36 (45)	44	
M1	3 (75)	1	
TNM Stage			**<0.001**
I	1 (11.1)	8	
II	12 (28.6)	30	
III	23 (79.3)	6	
IV	3 (75)	1	

Fig. 7. The expression of BPTF was positively correlated with Cdc25A or c-Myc in CRC tissues and its high expression predicted poor prognosis in patients with CRC. (A) The representative photographs of IHC staining for BPTF, Cdc25A and c-Myc based on tissue microarray containing 84 cases of CRC patients. Scale bars, 200 μm. (B) Scatter plot showing the relative expression of BPTF, Cdc25A and c-Myc in tumor tissues and adjacent normal tissues according to the IHC score. (C) The expression correlation between BPTF and c-Myc according to the IHC score. (D) The expression correlation between BPTF and Cdc25A according to the IHC score. (E) The relation between overall survival and BPTF expression was analyzed by Kaplan–Meier analysis. (F) The relationship analysis between BPTF/c-Myc/Cdc25A expression and the clinicopathological parameters in colon cancer patients. (G) The relation between overall survival and BPTF/c-Myc/Cdc25A expression was analyzed by Kaplan–Meier analysis. The data represented the mean ± SD of three independent experiments, and the level of significance was indicated by ***P < 0.001, **P < 0.01, *P < 0.05.

5.4. Statistical analysis

The GraphPad Prism 7.0 software or R language was mainly used for chart generation in this paper. Statistical analysis was mainly performed using GraphPad Prism 7.0 or IBM SPSS Statistics 24 (SPSS, Inc., Chicago, IL, USA) software. The *t*-test or two-way ANOVA was used to analyze the differences between groups, and Spearman's rank correlation test was used for correlation analysis. $P < 0.05$ indicated significant difference.

Additional materials and methods are provided in supplemental information.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Author contributions

This work was carried out in collaboration between all authors. PG, WD, CL and WG defined the research theme, designed the experimental approach, and revised the manuscript critically. PG, SZ, SH, WY, XL, GX, ZS, XZ, HL, CH, XW, LR, ZG, HG, KL and GZ carried out the experiments. PG, SH, WD, and WG analyzed the data and interpreted the results. PG and WG wrote the manuscript. All authors have read and approved the final manuscript.

Ethical approval

This study was approved by the Biomedical Ethics Committee of Dalian Medical University (Liaoning, China) with the approval No. ChiCTR1900028751 and AEE18043.

Declaration of competing interest

All authors have no conflict of interests.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2022.102418.

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