

Epigenetic Mechanisms Underlying Organic Solute Transporter β Repression in Colorectal Cancer[§]

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ABSTRACT

Colorectal cancer (CRC) is known to be the third most common cancer disease and the fourth-leading cause of cancer-related deaths worldwide. Bile acid, especially deoxycholic acid and lithocholic acid, were revealed to play an important role during carcinogenesis of CRC. In this study, we found organic solute transporter β (OST β), an important subunit of a bile acid export transporter OST α -OST β , was noticeably downregulated in CRC. The decline of OST β expression in CRC was determined by Western blot and real-time polymerase chain reaction (RT-PCR), whereas chromatin immunoprecipitation (ChIP) was used to evaluate the histone acetylation state at the OST β promoter region in vivo and in vitro. CRC cell lines HT29 and HCT15 were treated with trichostatin A (TSA) for the subsequent determination, including RT-PCR, small interfering RNA (siRNA) knockdown, ChIP, and dual-luciferase reporter gene assay, to find out which histone acetyltransferases and deacetylases exactly participated in regulation. We demonstrated that after TSA treatment, OST β expression increased noticeably because of upregulated H3K27Ac state at OST β

promoter region. We found that stimulating the expression of p300 with CTB (Cholera Toxin B subunit, an activator of p300) and inhibiting p300 expression with C646 (an inhibitor of p300) or siRNA designed for p300 could control OST β expression through modulating H3K27Ac state at OST β promoter region. Therefore, downregulated expression of p300 in CRC may cause low expression of OST β in CRC via epigenetic regulation. Generally, we revealed a novel epigenetic mechanism underlying OST β repression in CRC, hoping this mechanism would help us to understand and inhibit carcinogenesis of CRC.

SIGNIFICANCE STATEMENT

Organic solute transporter β (OST β) expression is lower in colon cancer tissues compared with adjacent normal tissues. We revealed the epigenetic mechanisms of it and proved that p300 controls OST β expression through modulating H3K27Ac state at OST β promoter region and hence causes low expression of OST β in colorectal cancer.

Introduction

Colorectal cancer (CRC) is known to be the third most common cancer disease and the fourth-leading cause of cancer-related deaths worldwide (Favoriti et al., 2016). CRC rate is geographically relevant; North America, Oceania, and Europe have higher incidence of CRC, whereas Asia, Africa, and South America show the lowest CRC incidence rates. Such phenomenon may be connected to risk factors, such as obesity and physical inactivity (Center et al., 2009). Although surgery intervention and neoadjuvant and adjuvant chemotherapy were the

most common and efficient methods in CRC treatment (Brenner et al., 2014), early diagnosis and useful therapeutic targets in CRC are becoming more and more important to assist therapy.

During carcinogenesis and development of CRC, bile acids seem to play a vital role. Bile acids are physiologic products synthesized from cholesterol in liver and functioned as generating bile flow as well as promoting intestinal absorption and transportation of lipids, nutrients, and vitamins (Chiang, 2009). Narisawa et al. reveals lithocholic acid (LCA) and deoxycholic acid (DCA) promote CRC in rats after MNNG (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine) treatment (Narisawa et al., 1974). Bernstein et al. further confirms the effect of DCA in a mouse experiment; they fed 18 mice a 0.2% DCA-containing diet, and after 8–10 months, 10 of them had CRC (Bernstein et al., 2011). Recently, more studies have found the secondary bile acids LCA and DCA are the main factors involved in carcinogenesis and development of CRC (Plotnikoff, 2014; Farhana et al., 2016).

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ABBREVIATIONS: CRC, colorectal cancer; CTB, cholera toxin B subunit; DAC, decitabine; DCA, deoxycholic acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HAT, histone acetyltransferase; HDAC, histone deacetylase; LCA, lithocholic acid; OST, organic solute transporter; PPIB, peptidylprolyl isomerase B; qRT-PCR, quantitative real-time polymerase chain reaction; siRNA, small interfering RNA; TSA, trichostatin A.

Organic solute transporter (OST) α and OST β form a heteromeric transporter known as OST- α/β , which participates in enterohepatic circulation of bile acids (Ballatori et al., 2013). The two subunits of the transporter are expressed together in human small intestine, kidney, liver, colon, testis, and ovary, with a high expression in ileum, as shown when searched in the Variability of Drug Transporter Database (Yin et al., 2020). They localize at basolateral plasma membrane of epithelial cells responsible for bile acids efflux (Seward et al., 2003; Ballatori et al., 2005). Although maintenance of the transport activity of this transporter complex requires the normal interaction between OST α and OST β subunits, OST β was reported to function as an accessory factor to promote the egress of OST α as well as stabilize the heterodimerization complex, traffic the complex to plasma membrane, and maintain the bile acid transport activity (Christian et al., 2012). Therefore, it is reasonable to speculate that abnormal OST β expression can influence transport capability of OST α -OST β transporter, and the OST β is the main object in our research.

Epigenetic alterations have attracted more attention in recent years and are considered to play a key role during CRC development (Okugawa et al., 2015). Epigenetics refers to the study of heritable alterations in gene expression without undergoing changes in DNA sequence, including DNA methylation, histone modification, noncoding RNA expression, and nucleosome remodeling (Bird, 2002; Liu et al., 2016). Among those, histone modifications include acetylation, methylation, phosphorylation, ubiquitylation, ADP-ribosylation, and deamination (Minucci and Pelicci, 2006), controlled by the involvement of some key enzymes such as histone acetyltransferases (HATs) and deacetylases (HDACs). Actually, histone acetylation is an indicator of active gene transcription because the hyperacetylation state of histones relaxes the chromatin structure with increasing transcriptional activity, whereas hypoacetylation state of histones condenses the chromatin structure with decreasing transcriptional activity (Codd et al., 2009). In other words, the two different enzymes HATs and HDACs can cause gene activation and silencing on the chromosome level, respectively, as well as control specific gene expression *in vivo* (Wang et al., 2009).

In this study, we found that OST β expression was distinctly repressed in CRC tissues. To clarify which factors were involved, we determined regulatory mechanisms via epigenetic control that underlie this phenomenon.

Materials and Methods

Tissues and Cell Culture. A total of 93 paired tissue samples from CRC patients in this study were collected from the Specimen Bank of Zhejiang Cancer Hospital (Hangzhou, China), with the approval by the Institutional Review Board of Zhejiang Cancer Hospital. Detailed patient information is provided in Supplemental Table 1. All the experiments of patient tissues were in line with the Helsinki Declaration. Those samples were well-kept under -80°C without specific classification. The reference gene (PPIB, peptidylprolyl isomerase B) values were first detected, and paired normal and cancer tissues that contained similar PPIB values were chosen for the subsequent analysis. Colon cancer cell lines HCT15 were gifted from the Institute of Modern Chinese Medicine, College of Pharmaceutical Sciences, Zhejiang University; HT29 cells were purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Both cell lines were cultured in RPMI 1640 medium (Gibco) containing 10% FBS, 100 U/ml penicillin, and 100 mg/ml streptomycin in a humidified atmosphere at 37°C with 5% CO_2 , under the guidance of instructions

from American Type Culture Collection. During the whole process of the experiment, cell growth situation and cell culture medium were checked every time before the cell operations, and nonexistence of mycoplasma contamination has been spotted.

Cell Drug Treatments. Decitabine (DAC), cholera toxin B subunit (CTB), and C646 were purchased from Sigma-Aldrich, and TSA was purchased from TCI. HCT15 cells were previously seeded in a six-well plate with 10%–20% cell density and cultured with medium containing different doses of DAC for 72 hours, with refreshing culture medium every day. For CTB treatment, cells were precultured in 40%–50% confluence, and after the overnight culture, cells were treated with medium containing indicated doses of CTB for another 48 hours. The medium was refreshed every day. For TSA and C646 treatment, HCT15 was precultured to 30% confluence and HT29 was precultured to 60% confluence in a six-well plate, and then cells were subsequently cultured with medium containing indicated doses of TSA and C646 for 24 hours.

RNA Extraction and Real-Time Quantitative Polymerase Chain Reaction Analysis. Total RNA was isolated from tissues using an RNA mini-prep kit (Tiangen, Beijing, China), and total RNA from cells was isolated using Multisource total RNA mini-prep kit (Axygen, Suzhou, China). For quantitative real-time polymerase chain reaction (qRT-PCR), RNA was reverse-transcribed to cDNA using PrimeScript RT Master Mix (Takara, Tokyo, Japan) following the manufacturer's instructions. qRT-PCR was performed by SYBR Premix EX Taq (Takara), and the sequences of primers are listed in Supplemental Table 2. RT-PCR assays were performed in Applied Biosystem Step-One Plus system with the following procedure: denaturation at 95°C for 30 seconds, followed by 40 cycles at 95°C for 5 seconds and then at 60°C for 30 seconds. As PPIB was stable and highly expressed in patients' tissues and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was suitable for cell analysis, results were quantified by normalization to GAPDH in cell lines and PPIB in patient tissues. $2^{-\Delta\Delta\text{Ct}}$ was used to calculate relative gene expression.

Western Blot and Antibodies. Tissues and cells proteins were lysed using radioimmunoprecipitation assay lysis buffer [50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, sodium orthovanadate, sodium fluoride, EDTA, leupeptin; Beyotime, China]. The protein lysis was separated by centrifugation at 20,000g under 4°C for 10 minutes, and protein concentration was quantified by BCA protein assay kit (Beyotime). Protein lysates were heated at 100°C for 10 minutes for denaturation with loading buffer (Takara). Protein lysates were isolated by 90 V, 30 minutes in 5% stacking gel, and 120 V, 70 minutes in 15% separating gel. Proteins were then transferred to $0.45\ \mu\text{m}$ polyvinylidene difluoride membranes (Millipore, MA), blocked using 5% skim milk at room temperature for 2 hours, and then incubated with anti-SLC51A antibody (1:250 dilution, PAB20435; Abnova) and anti-GAPDH antibody (1:4000 dilution, KC-5G4; Kangchen), respectively. The immune complexes were detected and visualized with EZ-ECL (4A Biotech, China) according to the manufacturer's protocol.

Chromatin Immunoprecipitation. Tissues and cells were cross-linked using 1.1% formaldehyde (Thermo Forma); tissues and cells were incubated for 30 and 15 minutes, respectively, at room temperature, and the crosslinking state was quenched with 0.125 M glycine. To shear the chromatin, tissues and cells were treated with 10 U MNase ml^{-1} (Thermo Fisher Scientific) for 10 minutes at 37°C , followed by tissue sonication for 30 minutes (cells for 15 minutes) at 4°C . Sheared chromatin was incubated with 1.5 μg antibody at 4°C overnight, and then the mixture was incubated with Protein G beads (Millipore) for 4–6 hours at 4°C . The beads were then washed off, and DNA was collected for subsequent RT-PCR. The enrichment was indicated as percent of input (Lee et al., 2006). Primers used in chromatin immunoprecipitation (ChIP)-qPCR were listed in Supplemental Table 2. The antibodies used for the ChIP were as follows: anti-H3 (Ab1791; Abcam, Cambridge, MA), anti-H3Ac (06-599; Millipore), anti-H3K4me3 (ab8580; Abcam), anti-H3K4me2 (07-030; Millipore), anti-H3K9Ac (ab4441; Abcam), anti-H3K18Ac (ab1191; Abcam), anti-H3K27Ac (ab4729; Abcam), anti-H3K27me3 (07-449; Millipore), anti-H4Ac (41384; Abcam),

anti-H3K9me3 (Active motif; 39161), and normal rabbit IgG (sc-2027; Santa Cruz Biotechnology) as control. Every value of reference genes in samples were assayed, and the results of patient numbers 13, 24, and 34 were confidential and representative, owing to the good normalization of negative reference genes. These three representative results were presented.

Small Interfering RNA-Mediated Gene Knockdown. HCT116 and HT29 cells were transfected using small interfering RNA (siRNA) for HDACs and p300, which were synthesized by GenePharma (Shanghai). Transfect reagent lipofectamine 3000 (Life Technologies) was previously incubated with suitable amounts of siRNAs for 5 minutes at room temperature, and then the mixture was added into precultured cells in 60%–80% confluence for transient transfection. Negative control siRNA was also provided by GenePharma and added to cells as the negative control group. For HCT15, final concentration of siRNAs targeted at HDACs were 30 nM; for HT29, the final concentrations of siRNAs were 40 nM for HDACs and 100 nM for si-p300. After 48 hours, total RNA was extracted from cells for later analysis. The sequences of siRNAs were listed in Supplemental Table 3.

Luciferase Assay. *OSTβ* promoter fragments were amplified with primers listed in Supplemental Table 2 and cloned into pGL3-basic (Promega) for the construction of reporter plasmid. The luciferase assay was performed in HEK293 cell line. HEK293 cells were precultured to 70%–90% confluence in a 24-well plate and then transfected with *OSTβ* promoter reporter plasmid. Cells were respectively treated with 50 μM CTB and si-p300 for 48 hours to compare relative light units 1 and 2, which indicated the firefly luciferase activity from promoter constructs normalized to the corresponding *Renilla* luciferase.

Statistical Analysis. Results were expressed as means ± S.D. Paired *t* test was used for the paired cancer and adjacent normal tissues obtained from patients. A two-tailed *t* test was used for the statistical comparison between two experiment groups.

Results

***OSTα* and *OSTβ* Are Repressed in CRC.** Data gained from the database Oncomine (Compendia Bioscience, Ann Arbor, MI) showed that *OSTα* and *OSTβ* mRNA expression were distinctly downregulated in CRC tissues (Fig. 1a and b).

We analyzed 93 pairs of colon cancer tissues and the *OSTα* and *OSTβ* expression levels of pair-matched adjacent normal cancer tissues by using qRT-PCR. We found that *OSTα* and *OSTβ* mRNA expression was obviously declined in CRC tissues compared with that in normal tissues, which was accordant with the findings from Oncomine (Fig. 2a and b). As shown in Fig. 2e, protein levels of *OSTβ* expression also confirmed this discovery. To conclude, regardless of gender, age, location, TNM stage (T: tumor, N: regional lymph node, M: metastasis), and cancer subtype (Supplemental Figs. 1 and 2), *OSTα* and *OSTβ* transporter expression was repressed in CRC.

Reduced H3K27Ac at *OSTβ* Promoter Region Leads to *OSTβ* Repression in CRC. Next, considering that epigenetics plays key roles in gene expression regulation, we attempted to confirm the epigenetic mechanism accounting for *OSTα* and *OSTβ* repression in CRC. DAC, a demethylating reagent that blocks the cellular DNA methyltransferases process (Nie et al., 2014), and TSA, an HDAC inhibitor that elevates the level of histone acetylation and promotes the transcription process, were used to treat CRC cell lines HT29 and HCT15 to see whether they would reduce *OSTα* and *OSTβ* expression (Codd et al., 2009).

After DAC treatment, neither *OSTα* nor *OSTβ* mRNA expression were elevated (Supplemental Fig. 3b; Supplemental Fig. 4a), so we speculated that the DNA methylation state was not the main regulator of *OSTα-OSTβ* repression in CRC. However, after TSA treatment, we found *OSTβ* transcription was positively related to TSA dosage in HT29 and HCT115 (Fig. 3a and b), and the same results were discovered in protein levels (Supplemental Fig. 3c). But for *OSTα*, expression of mRNA was slightly influenced after TSA treatment (Supplemental Fig. 3a), which was also parallel with ChIP-qPCR results

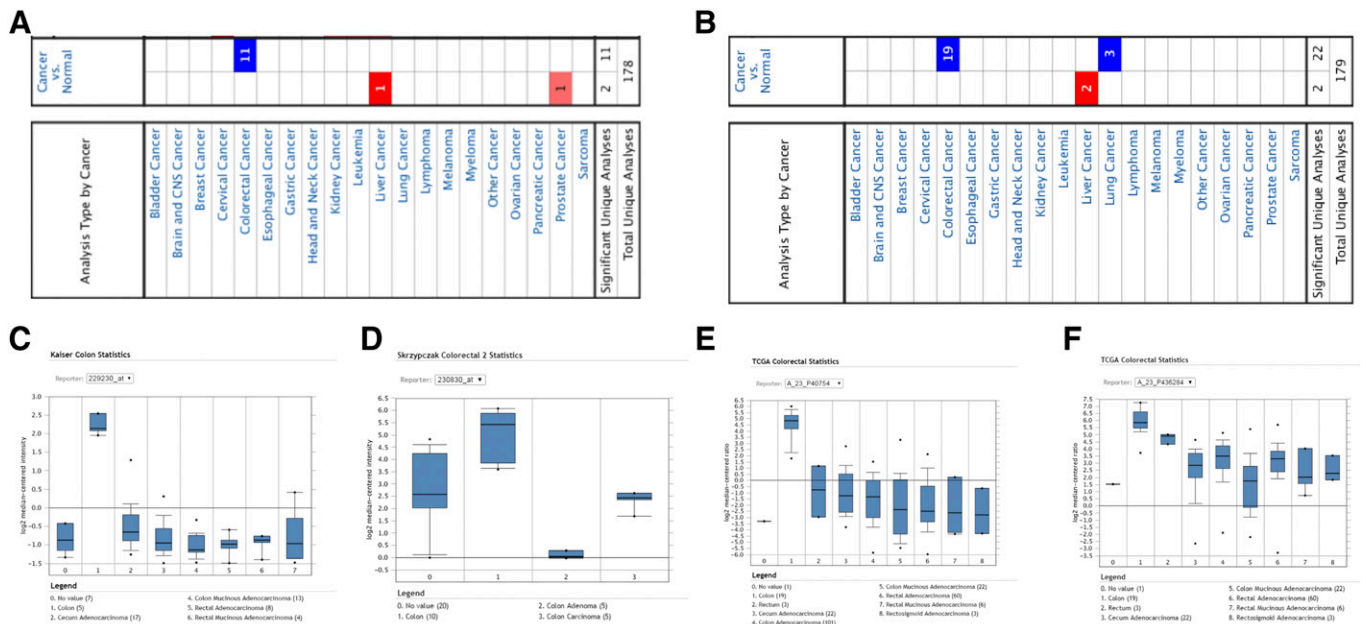


Fig. 1. (A and B) Summary of *OSTα* and *OSTβ* expression in various human cancers compared with normal tissues in Oncomine. Blue: downregulated in cancer; red: upregulated in cancer. Cell color is determined by the best gene rank percentile for the analyses within the cell. (C and D) *OSTβ* expression in Kaiser Colon Statistics and Skrzypczak Colorectal 2 Statistics. (E and F) *OSTα* and *OSTβ* expression in TCGA Colorectal statistics.

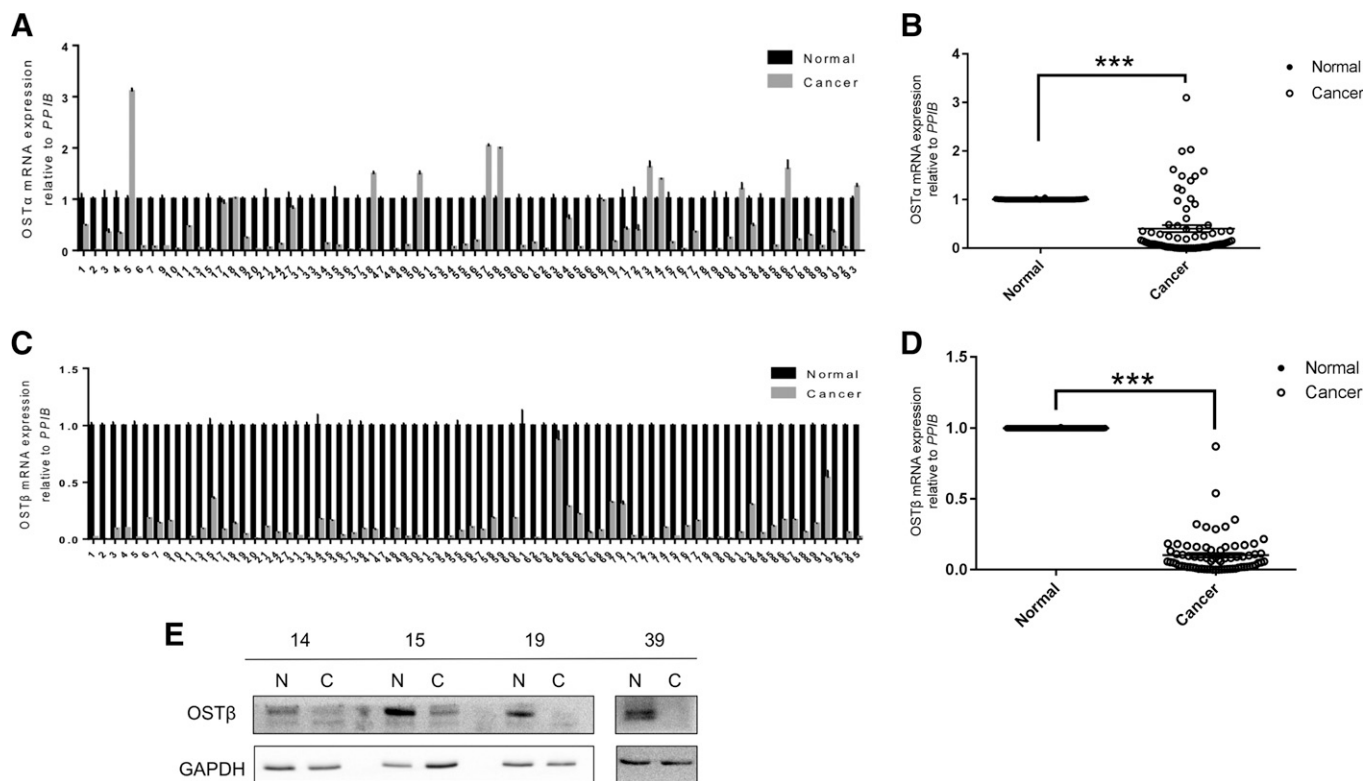


Fig. 2. *OSTβ* is repressed in CRC. (A) RT-PCR analysis of *OSTα* transcription in matched CRC tissues and adjacent normal tissues normalized to reference gene *PPIB*. The x-axis represents number of tissues, mean \pm S.D., $n = 70$ (B) Individual data points of *OSTα* expression are shown, with means and S.D. indicated by horizontal lines; $n = 70$. Paired t tests were used. $***P < 0.001$. (C) RT-PCR analysis of *OSTβ* transcription in matched CRC tissues and adjacent normal tissues normalized to reference gene *PPIB*. (D) Individual data points are shown, with means and S.D. indicated by horizontal lines; $n = 70$. Paired t tests were used. $***P < 0.001$. (E) Western blot of *OSTβ* and GAPDH in matched CRC tissues and adjacent normal tissues (14N, 14C; 15N, 15C; 19N, 19C; 39N, 39C). C, cancer tissues; N, adjacent normal tissues.

(Supplemental Fig. 3c). Collectively, DNA methylation and histone acetylation may not be responsible for *OSTα* repression in CRC.

To verify whether histone acetylation repressed *OSTβ* expression in CRC, we performed ChIP-qPCR experiments. HT29 and HCT15 cells were cultured in medium with 1 μ M TSA for 24 hours. Then, in both cells, we found the H3K27Ac level at *OSTβ* promoter region was upregulated whereas H4Ac was downregulated after TSA treatment (Fig. 3d and e). As we know, TSA was intended to inhibit HDAC and promote the histone acetylation. The result indicated that TSA could upregulate the expression of H3K27Ac as well as the mRNA expression of *OSTβ*, whereas H4Ac state was not consistent with this tendency. In that case, we speculated that H3K27Ac played more important roles compared with H4Ac. To test this hypothesis, we also did ChIP-qPCR experiments with CRC tissues, and the results were in accordance with cells (Fig. 3f and g). We found that CRC tissues presented consistently a lower H3K27Ac state at *OSTβ* promoter region than adjacent normal tissues. However, the H4Ac state at *OSTβ* promoter region varied without rules. This data may provide more evidence that indicates that H3K27Ac plays a dominant role in modulating *OSTβ* expression in CRC.

We designed two different pairs of primers for all ChIP-qPCR analysis mentioned above to cover the whole 2000 bp of the the *OSTβ* promoter region from an overall perspective rather than only focus on a specific range in the promoter. Both primers presented similar results, which indicated that all parts of the promoter region were intended to be acetylated.

The RT-PCR results for both pairs of primers are shown in Fig. 3 and Supplemental Fig. 4. Taken together, these results revealed that lower H3K27Ac state at *OSTβ* promoter region leads to *OSTβ* repression in CRC.

Downregulation of p300 in CRC Causes *OSTβ* Repression in CRC. Previous reports have proved that histone acetylation state is influenced by enzymes, such as HATs and HDACs, and TSA was reported to inhibit Zinc-dependent class I and class II histone deacetylases, including HDAC1-10 (Seto and Yoshida, 2014). Therefore, ChIP evaluation was performed in CRC tissues to find out which specific HAT or HDAC was responsible for *OSTβ* repression during CRC development.

We found that HDAC2 and HDAC7 were upregulated in CRC tissues compared with adjacent normal tissues, and HDAC9 was downregulated (Supplemental Fig. 5). It seemed HDAC2 and HDAC7 might be the main factors to influence *OSTβ* expression; however, further siRNA knockdown assay did not support this speculation. Expression of *OSTβ* was not accordingly elevated after cells were transfected with siRNAs targeted at corresponding HDACs (Supplemental Figs. 6 and 7). Hence, it can be concluded that HDACs might not be the dominant factors responsible for *OSTβ* repression in CRC.

Then, we began research on HATs to further test which HATs participated in modulating *OSTβ* expression. Unlike HDACs, HATs modify specific sites on histones. Combining the previous research in our laboratory on HATs (Ye et al., 2018), we found p300 modified H3K27Ac and H3K18Ac in vivo (Holmqvist and Mannervik, 2013). Interestingly, mRNA expression of *p300* was

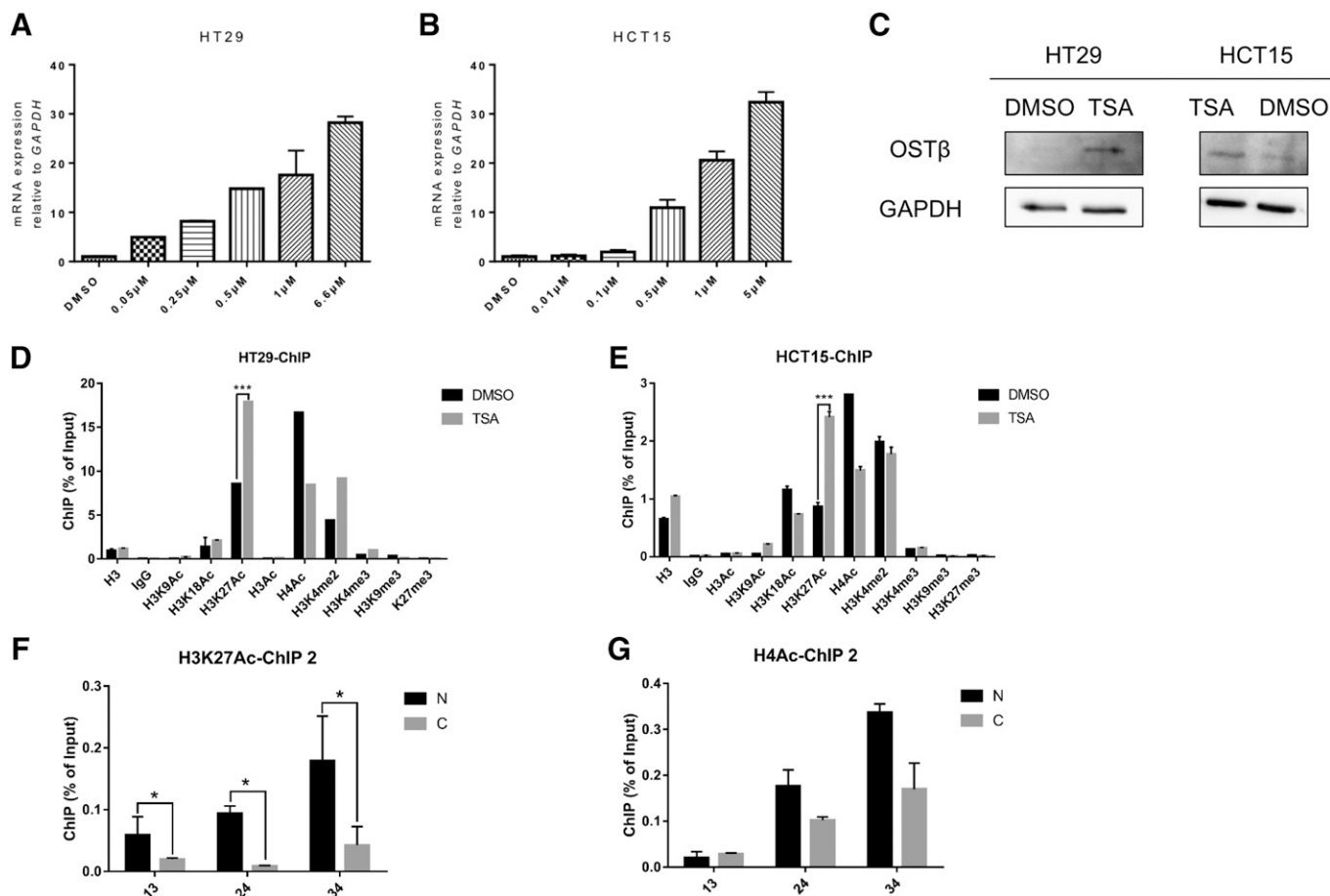


Fig. 3. Reduced H3K27Ac at *OSTβ* promoter region leads to *OSTβ* repression in CRC. (A and B) The expression of *OSTβ* normalized to *GAPDH* in HT29 and HCT15 after treated with TSA in concentration gradient for 24 hours; mean \pm S.D. (C) Western blot of *OSTβ* and *GAPDH* in colorectal cancer cell lines HT29 and HCT15 after treatment with 1 μ M TSA for 24 hours. (D and E) 1 μ M TSA treatment in CRC cell lines HCT15 and HT29 reorganized histone modification profile at *OSTβ* promoter region. (F and G) ChIP-qPCR analysis of H3K27Ac and H4Ac regions in *OSTβ* promoter between human CRC tumors and paired adjacent normal tissues. C, cancer tissue; N, adjacent normal tissue. All data above presented as mean \pm S.D., two-tailed unpaired *t* test. * $P < 0.05$; *** $P < 0.001$.

demonstrated to be downregulated in CRC tissues as predicted (Fig. 4a). In vitro experiments were all performed in CRC cell line HT29, and because *OSTβ* expression in HCT15 was relatively low, it was difficult to detect its expression accurately. We then treated HT29 cells with p300 activator CTB (Mantelingu et al., 2007; Furdas et al., 2012; Dastjerdi et al., 2013) and inhibitor C646 (Bowers et al., 2010; Gaddis et al., 2015). We found that when p300 was activated, *OSTβ* expression was elevated as well (Fig. 4b); when p300 was inhibited, *OSTβ* expression also decreased dramatically (Fig. 4c). Moreover, to further confirm the association between p300 and *OSTβ* expression, we also designed siRNA for *p300* to observe variation on *OSTβ* expression after *p300* is degraded. As predicted, after p300 knockdown, *OSTβ* expression was also downregulated (Fig. 4d).

We had already proved H3K27Ac state-controlled *OSTβ* expression in CRC, so we wanted to verify if p300 focused on regulating H3K27Ac at *OSTβ* promoter region to control its expression. ChIP-qPCR results were performed to support this speculation. Results show that after being treated with p300 activator, H3K27Ac state was enhanced at *OSTβ* promoter region; conversely, inhibiting p300 decreased H3K27Ac state (Fig. 5a–d). Results of luciferase assay also showed similar outcomes, which were that CTB promoted *OSTβ* promoter

activity and si-*p300* downregulated its activity (Fig. 5e and f). In conclusion, p300 regulates H3K27Ac level at *OSTβ* promoter region to control its expression in CRC.

Discussion

The results of recent studies have confirmed that bile acid accumulation in vivo could promote the tumorigenesis of colorectal cancer and trigger the colonic mucosa with undesirable outcomes, such as DNA damage, oxidative/nitrosative stress, and apoptosis (Ajouz et al., 2014; Gadaleta et al., 2017). Primary bile acid was synthesized from cholesterol catabolism in liver and absorbed in distal small intestine, where they are transformed into secondary bile acids by bacteria in the intestine. Bile acids are efficiently reabsorbed via the portal venous circulation in the ileum and continue to go through cycles between the liver and intestine (Ballatori et al., 2009).

It can be seen that the accumulation of bile acid was closely associated with various bile acid transporters. Raufman et al. (2015) found that mice deficient in one specific bile acid transporter (ASBT, ileal apical sodium-dependent bile acid transporter) were more likely to induce colon tumor formation and formed larger colon adenocarcinomas after treatment because of a lack of bile acid uptake in the distal small

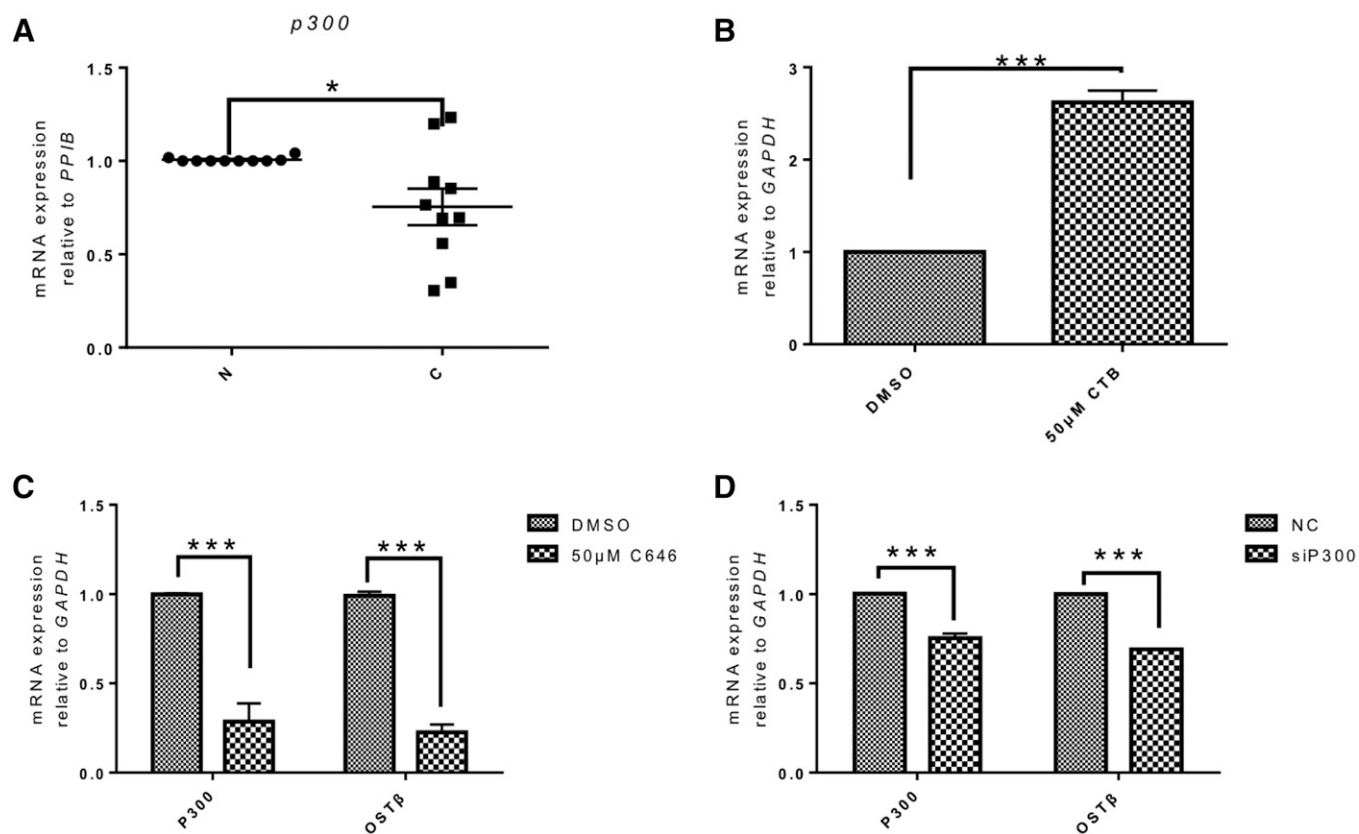


Fig. 4. P300 regulates *OSTβ* expression. (A) RT-PCR analysis of *p300* transcription in matched CRC tissues and adjacent normal tissues normalized to reference gene *PPIB* ($n = 10$, two-tailed paired t test). Lines represent mean with S.D. C cancer tissue; N, adjacent normal tissue. (B) The mRNA expression of *OSTβ* normalized to *GAPDH* in HT29 after treatment with 50 μ M CTB for 48 hours. (C) The mRNA expression of *OSTβ* normalized to *GAPDH* in HT29 after treatment with 50 μ M C646 for 24 hours. (D) The mRNA expression of *OSTβ* normalized to *GAPDH* in HT29 after transfected with siRNA for *p300* for 48 hours. NC, cells transfected with negative control siRNA; si-*p300*, cells transfected with siRNA-*p300*. All data above presented as mean \pm S.D., two-tailed unpaired t test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

intestine. We found the expression of efflux bile acid transporters *OSTα-OSTβ* was noticeably reduced in colorectal cancer tissues compared with control, and this result was demonstrated by Oncomine Cancer Transcriptome database and Western blot RT-PCR assay. *OSTα-OSTβ* complex is a bile acid transporter first identified in 2001 (Wang et al., 2001). So far, there have been no studies that have clarified the direct interaction between *OSTα-β* transporters and colon rectal cancer development. Except for the common list substrates of *OSTα-OSTβ* transporters, nonbile acids such as estrone-3-sulfate, digoxin, prostaglandin E2, and dehydroepiandrosterone-3-sulfate were also the substrates for *OSTα-β* (Ballatori et al., 2009; Dawson et al., 2009; Klaassen and Aleksunes, 2010). In this study, we mainly focused on the *OSTα-β* transporters localized in the intestine, and the dominant substrates of *OSTα-β* in the intestine are bile acids. As for *OSTα-β* transporters localized in other organs such as brain or adrenal gland, they could modulate steroid homeostasis, contributing to neurosteroid action (Fang et al., 2010), and those localized in kidney or liver are responsible for conjugated steroids traffic (Ballatori et al., 2008). The function of intestinal *OSTα-β* transporters in modulating bile acid reabsorption and its relationship with colon cancer carcinogenesis was the main focus in this article. Their regulation mechanism was rarely uncovered, especially in the process of tumorigenesis.

We continue to investigate the potential epigenetics mechanism of these two subunits of the transporter in colorectal

cancer. Actually, studies have proven that epigenetics alterations play a vital role in regulating colorectal cancer development and at the same time are considered as biomarkers for CRC therapeutic applications (Okugawa et al., 2015). For example, in our laboratory's previous study, TSA induced the expression of concentrative nucleoside transporter 2 through reducing the level of deacetylation in the promoter region of concentrative nucleoside transporter 2 gene as well as reversed drug resistance in colorectal cancer therapy (Ye et al., 2018). DNA methylation inhibitor decitabine and histone deacetylase inhibitor vorinostat could noticeably evaluate the expression of human organic cation transporter OCT2 (Organic cation transporter 2) in renal cell carcinoma (Zhu et al., 2019), and the abnormal expression of OCT2 caused resistance to anticarcinogen-like oxaliplatin in renal cell carcinoma, bringing difficulties to cancer therapy (Chen et al., 2019). Furthermore, silencing of other family members of histone acetyltransferases, such as MOF (a member of the Moz-Ybf2/Sas3-Sas2-Tip60 family of HATs), causes genomic instability, suppressing of gene transcription, and promotion of different tumorigenesis, including colorectal cancer (Su et al., 2016). Generally, the histone acetylation level was positively correlated with transcriptional activity of genes. Our study found that not only DNA methylation but also histone acetylation levels of the *OSTβ* promoter region were evaluated after treated with DNA methylase (by DAC) and HDAC (by TSA), respectively, and we finally concluded

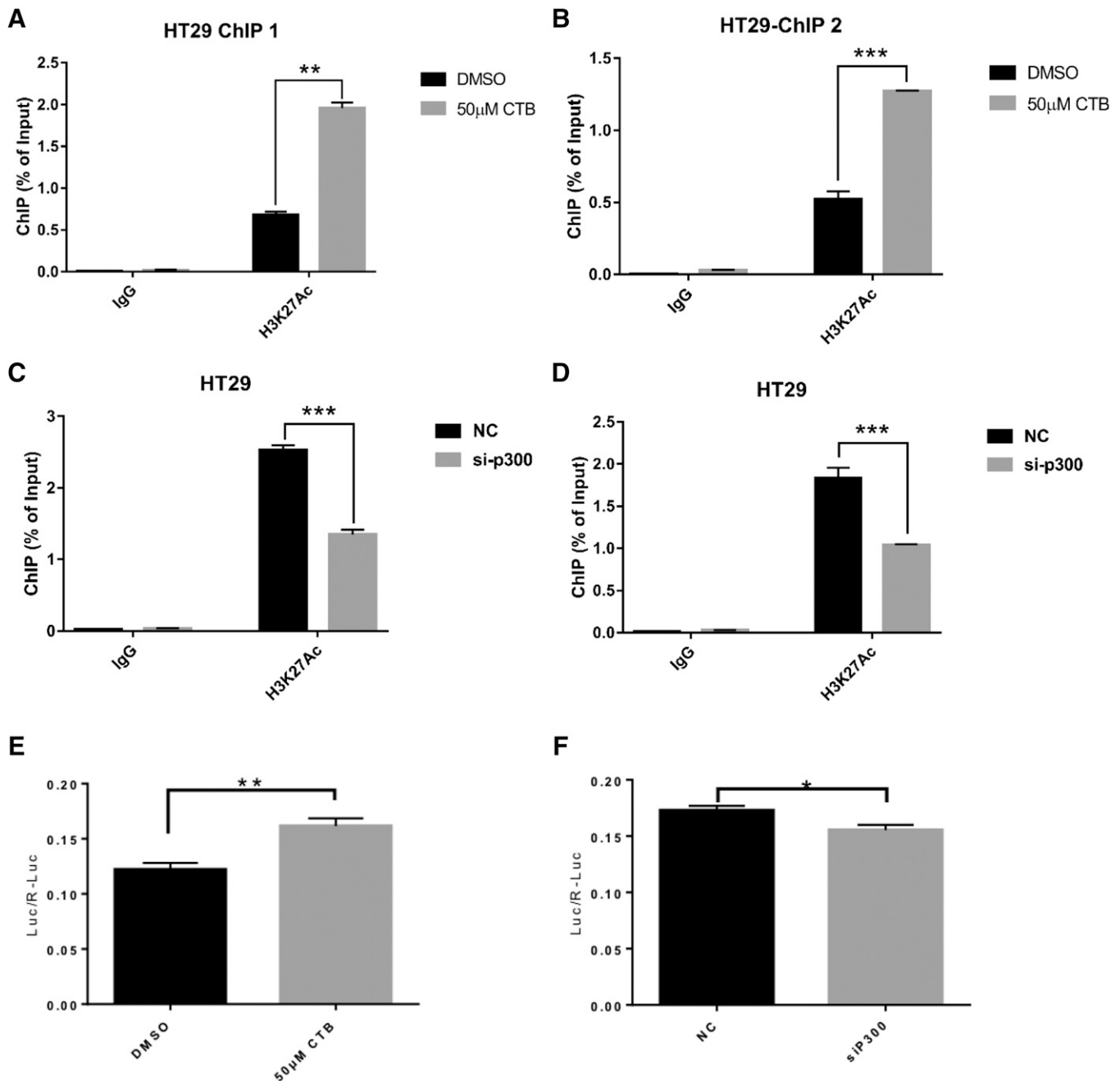


Fig. 5. P300 regulates H3K27Ac state in *OSTβ* promoter region. (A and B) 50 μM CTB treatment for 48 hours in CRC cell line HT29 upregulated H3K27Ac at *OSTβ* promoter region. (C and D) P300-siRNA treatment for 48 hours in CRC cell line HT29 downregulated H3K27Ac at *OSTβ* promoter region. NC, cells transfected with negative controls; si-p300, cells transfected with si-p300. (E and F) Dual-luciferase reporter gene assay in HEK293 cells. All data above presented as mean ± S.D., two-tailed unpaired *t* test. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

that histone acetylation was the dominant reason responsible for repressing *OSTβ* expression. By ChIP assay, we discovered that the downregulation of *OSTβ* transcription was mediated by acetylation level of H3K27Ac site. In colorectal cancer tissues, the acetylation level was also reduced compared with normal adjacent tissues.

Based on the understanding of the epigenetics regulation of *OSTβ* transcription, we continued to discover the potential factors within HATs and HDACs to regulate H3K27Ac state. From the previous study, p300 is a main regulator of modulating H3K27Ac state and a well-studied HAT (Raisner et al., 2018; Wong et al., 2018). Combined with the qRT-PCR, drug

treatment, siRNA knockdown, and luciferase assay, we could finally draw the conclusion that p300 regulates H3K27Ac at *OSTβ* promoter region to control its expression in CRC.

To our knowledge, our study first revealed the epigenetics regulation mechanism of *OSTβ* in colorectal cancer. This finding could be clinically meaningful because *OSTβ* dysregulation may be related to higher concentrations of DCA and LCA, which have already been proved to be carcinogenic factors, in the colorectal region. Taken together, our study indicated that the reduced expression of p300 caused H3K27 deacetylation in *OSTβ* promoter region and hence led to the downregulated expression of *OSTβ* in CRC to mediate the export of bile acid.

This result gives new insight into the epigenetic effects toward the main factors in colorectal cancer and suggests that in the future, cancer therapy could focus on targeting epigenetic transcriptional factors, such as p300, to treat cancer.

Authorship Contributions

Participated in research design: Zhou, C. Ye, Zeng, Yu.

Conducted experiments: Zhou, C. Ye, Lou, Chen, Lei, Guo.

Contributed new reagents or analytic tools: C. Ye, Liu, S. Ye.

Performed data analysis: Zhou, Lou, Zeng, Yu.

Wrote or contributed to the writing of the manuscript: Zhou, C. Ye, Yu.

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