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Serotonin acts through YAP to promote cell proliferation: mechanism and implication in colorectal cancer progression

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Abstract

Serotonin, also known as 5-hydroxytryptamine (5-HT), is a key messenger that mediates several central and peripheral functions in the human body. Emerging evidence indicates that serotonin is critical in tumorigenesis, but its role in colorectal cancer remains elusive. Herein, we report that serotonin transporter (SERT) transports serotonin into colorectal cancer cells, enhancing Yes-associated protein (YAP) expression and promoting *in vitro* and *in vivo* colon cancer cell growth. Once within the cells, transglutaminase 2 (TG2) mediates RhoA serotonylated and activates RhoA-ROCK1/2 signalling to upregulate YAP expression in SW480 and SW1116 cells. Blocking SERT with citalopram reversed the serotonin-induced YAP expression and cell proliferation, inhibiting serotonin's effects on tumour formation in mice. Moreover, SERT expression was correlated with YAP in pathological human colorectal cancer samples and the levels of 5-HT were highly significant in the serum of patients with colorectal cancer. Together, our findings suggested that serotonin enters cells via SERT to activate RhoA/ROCK/YAP signalling to promote colon cancer carcinogenesis. Consequently, targeting serotonin-SERT-YAP axis may be a potential therapeutic strategy for colorectal cancer.

Keywords Serotonin, Serotonin transporter, YAP, Colorectal cancer, Serotonylation

Introduction

Recently, the biological and therapeutic implications of neural regulation in cancers have drawn considerable interest [1, 2]. For example, in some malignancies, such as pancreatic and prostate cancer, nerves have been shown to infiltrate the tumour microenvironment and actively stimulate cancer cell growth and dissemination

[3, 4]. This mechanism involves cancer cells inducing the outgrowth of nerves in the tumour microenvironment by releasing neurotrophic factors. Moreover, in return, nerves liberate neurotransmitters, such as adrenaline [5], acetylcholine (ACh) [6], and others, directly into the vicinity of cancer and stromal cells to activate cancer growth and dissemination. However, the overall significance of neural-associated tumorigenesis remains unclear, especially in colorectal cancers.

Serotonin, also termed 5-hydroxytryptamine (5-HT), is an omnipresent biogenic monoamine expressed in the central nervous system and gastrointestinal tract. Peripheral 5-HT is primarily synthesised by tryptophan hydroxylase-1 (Tph1) in the intestine enterochromaffin cells [7]. 5-HT participates in extensive physiological activities (e.g., platelet aggregation, vascular tone, and intestinal motility) by coupling with the 5-HT receptors (5-HTRs). Seven 5-HTR classes and 13 subtypes exist

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in mammals, reflecting the diversity and importance of serotonergic functions in the human body [8]. Along with 5-HTRs, SERT, a member of Na⁺-dependent transporter family, also biologically regulates the transportation and function of 5-HT [9]. SERT is responsible for the reuptake of 5-HT released into the presynaptic terminal, terminating the synaptic signal and ensuring replenishment of intracellular 5-HT stores during neurotransmission. SERT's physiological role in 5-HT signalling is substantiated by the drugs targeting SERT. This approach is used to clinically treat various diseases, such as depression, anxiety, obsessive–compulsive disorder, and neuropathic pain [10, 11].

Numerous studies have revealed that 5-HT plays an essential role in the tumorigenesis of many cancers [12]. 5-HT facilitates tumour growth by directly activating downstream mTOR targets in human hepatocellular cancer [13]. Furthermore, via 5-HT_{2BR}, 5-HT also induces the Warburg effect under metabolic stress, promoting cell growth in pancreatic cancer [14]. In some malignancies, particularly in prostate cancer [15], breast cancer [16], and cholangiocarcinoma [17], 5-HT functions as a self-secreting factor stimulating and maintaining cancer cell proliferation. Even though 5-HT is secreted from intestinal tract mucosa, its exact role in colon carcinogenesis has yet to be fully elucidated. The binding property to different 5-HTRs is a key step for its ability to induce signal transduction and malignant biological effects. Blocking 5-HTR could prevent cancer cell responses to 5-HT signalling, inhibiting tumour growth in various cancers [18]. As a unique transporter protein involved in 5-HT metabolism, SERT may also play a crucial role in tumour growth and progression. Drugs such as Selective Serotonin Reuptake Inhibitors (SSRIs) such as fluoxetine may inhibit SERT's functioning, thereby suppressing colon cancer cell proliferation [19]. Notably, several epidemiological studies have reported that administering SSRIS to patients with long-term clinical depression greatly reduced their risk of suffering colon cancer [20–22]. This outcome suggests that SERT might also participate in colon cancer tumorigenesis.

Previous studies have reported that the central component of Hippo pathway, YAP, is frequently overexpressed in many human tumours. These studies also confirmed that dysregulated YAP expression is essential for cancer initiation and progression [23]. In colon cancer, YAP overexpression is considered an independent predictor of prognosis and metastasis. Moreover, colon cancers appeared to proliferate when YAP was dysregulated [24]. However, the mechanism underlying abnormal YAP overexpression remains unknown. Hayakawa et al. reported that neurotransmitter ACh could activate YAP expression in human gastric

cancer cells via aberrant NGF/Trk and cholinergic signalling, promoting tumour growth [25]. Given the unique nature of neurotransmitters in coordinating crosstalk between nerves and cancer, we hypothesized that 5-HT might also act via activating YAP signalling to promote colon cancer carcinogenesis. Accordingly, we studied the interrelationship between neurotransmitter 5-HT signalling and dysregulated YAP pathway, as well as YAP pathway's implications in colorectal tumorigenesis.

Materials and methods

Cell culture, reagents, and animals

The Chinese Academy of Sciences' (Shanghai, China) Cell Biology Institute supplied the human colon cell lines SW480 and SW1116. Dulbecco's Modified Eagle's Medium (DMEM, high glucose, Gibco, Grand Island, NY, USA) was responsible for culturing all cells and supplied a 10% fetal bovine serum (FBS) (Gibco) in a humidified incubator at 37 °C and 5% CO₂, respectively. MP Biomedicals (Solon, OH, USA) and Lundbeck (Valby, Denmark) supplied the dextran sulfate sodium (DSS) and citalopram. MCE (Monmouth Junction, NJ, USA) supplied the study's chemical reagents, except for azoxymethane (AOM) and Sigma (St Louis, MO, USA) supplied the rabbit anti-serotonin antibody, and Cell Signalling Technology (Danvers, MA, USA) supplied all other antibodies. The DaSuo Laboratory Animal Co., LTD (Chengdu, China) supplied C57/BL mice.

Cell proliferation assay

SW480 and SW1116 cells were seeded in 96-well plates at 1 × 10³ cells/well and cultured for 12 h to evaluate the effect of exogenous 5-HT on cell proliferation. After 24 h of serum-free starvation, the cells were treated with a medium containing 5-HT at a range of concentrations for 48 h. Cell viability was monitored using Cell Counting Kit-8 (CCK8, Dojingdo, Kumamoto, Japan) according to the manufacturer's protocol. Briefly, 10 μL of CCK8 solution was added to each well, and the plates were incubated at 37 °C for 2 h. A microplate reader (Bio-Rad Laboratories, CA, USA) was used to detect the optical densities (ODs) of wells at 450 nm. All experiments were performed in triplicate.

RNA interference and real-time PCR

This study used the sequences of the small interfering RNAs (siRNAs), and primer pairs are listed in the Additional file 1: Table S1. All siRNAs were designed by GenePharma (Shanghai, China), and the primer pairs were obtained from TSINKE Biological Technology Co., LTD (Chengdu, China). Cells were transfected with siRNA using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The

knockdown efficiencies were verified by western blot or real-time PCR. To analyse YAP and its target gene in cells stimulated with 5-HT and the knockdown efficiencies of G α subunits, TRIzol (Invitrogen) was utilized according to the manufacturer's protocol. The procedure included extracting total RNA and reversing transcribed into cDNA using Reverse Transcriptase M-MLV (Takara, Dalian, China). The SYBR Premix ExTaq kit (Takara) was used to perform real-time PCR. Furthermore, the procedure was performed via iQ5 Real-Time PCR Detection System (Bio-Rad). Lastly, $2^{-\Delta\Delta CT}$ method was used to model the detected genes relative to that of GAPDH.

Western blot analysis

Cells were seeded in 6-well plates at 2×10^4 cells/well and starved for 24 h, and then pre-treated with drugs for 2 h before 5-HT stimulation. After 4 h the cells were harvested and lysed with RIPA lysis buffer containing protease and phosphatase inhibitors. Protein extracts were divided equally, separated by 12% SDS-PAGE, and then transferred to nitrocellulose membranes via Bio-Rad semi-dry transfer system. The membranes were blocked in 5% non-fat dried milk for 1 h and then sequentially incubated with the primary antibodies overnight at 4 °C and the DyLight 800 AffiniPure goat anti-rabbit IgG or DyLight 680 AffiniPure goat anti-mouse IgG secondary antibodies (EarthOx, CA, USA) for 2 h at room temperature. Lastly, ODYSSEY Infrared Imaging System (LI-ORBiosciences, Lincoln, NE, USA) visually modelled and quantified the immunoreactive bands.

Measurement of RhoA activity

About 2×10^4 cells were seeded and cultured as described above, after 4 h with 10 μ M 5-HT or 5-HT and citalopram (100 μ M) stimulation, cells were harvested and measured the activity of RhoA in SW480 and SW1116. A pulldown assay was used via Rhotekin's Rho binding domain to assess RhoA activity [26]. A Western blot test was conducted to analyze the precipitated GTP-bound RhoA and total RhoA.

Coimmunoprecipitation

SW480 and SW1116 cells were seeded at the concentration of 5×10^4 /mL in culture dish and then pre-treated with 10 μ M 5-HT alone or 10 μ M 5-HT combined with 100 μ M citalopram for 4 h, the cells were harvested in NETF buffer (100 mM NaCl, 2 mM EGTA, 50 mM Tris-Cl [pH 7.4], and 50 mM NaF) containing 1% Nonidet P-40, 2 mM orthovanadate, protease inhibitors, and a phosphatase inhibitor mixture (Life Technologies, Waltham, MA, USA). The 40 μ L of protein A or protein G Sepharose beads were

used to pre-clear the sample, and an anti-RhoA antibody was used to carry out immunoprecipitation reactions. A NETF buffer was first used with Nonidet P-40 (1% w/v) to wash. The protein A/G Sepharose-bound immune complexes. The procedure was carried out again without the detergent. The immunoprecipitated pellets were heated at 95 °C for 5 min in 70 μ L of the sample for SDS-PAGE and analysed for 5-HT via immunoblotting.

LC-MS/MS analysis

The concentrations of intracellular-stimulated 5-HT in SW480 and SW1116 cells were analysed according to a prior procedure [27]. Briefly, 5×10^6 cells in 10 mL of medium were plated in 60 cm² culture dishes. After incubation with 10 μ M 5-HT for 24 h. To remove exogenous 5-HT contaminates, cell suspensions were obtained and washed with pre-chilled PBS three times. The pellets were then extracted using four times the volume of acetonitrile, and the mixtures were centrifuged at 13,000 rpm for 15 min at 4 °C; the supernatants (100 μ L) were then subjected to analysis. Ultra-high performance liquid chromatography (UPLC) separation was performed on an ACQUITY UPLCTM BEH C18 column (Waters Corporation, Milford, MA, USA). The mobile phases comprised 0.1% formic acid (A) and methanol (B). In succession, isocratic elution was performed with 90% A and 10% B. MS/MS analysis employed the Waters Quattro Premier XE Mass Spectrometer (MS) coupled with an electrospray ionization (ESI) source; Masslynx V 4.1 software was used to control UPLC-MS/MS system. The mass spectrometer was operated in positive ion mode with a source temperature of 110 °C, a cone voltage of 20 V, and a capillary voltage of 2.8 kV. Lastly, selective multi-reaction monitoring in positive ionization mode was used to quantify intracellular 5-HT.

Immunofluorescence of RhoA and intracellular 5-HT

About 1×10^3 cells/well were seeded in 6-well plates which with a slide at the bottom, after treated with 10 μ M 5-HT alone or 10 μ M 5-HT combined with 100 μ M citalopram for 24 h, taken the slides out and then underwent the following procedure: first, they were fixed with 4% paraformaldehyde for 15 min; second, they were permeabilized with 1% TritonX-100 for 15 min; third, they were blocked with 1% BSA for 30 min; and lastly, they were incubated with primary RhoA (1:1000) and 5-HT (1:5000) antibodies overnight at 4 °C. After being washed three times with PBS, the slides were incubated with FITC- or Alexa Fluor 488-labelled secondary

antibodies for 20 min. Finally, the positive staining signals in the cytoplasm were observed by a fluorescence microscope.

In vivo experiments

Twenty-five adult female C57BL/6 mice were divided into five groups. Colon tumours in mice were induced by AOM and DSS, as previously described [28]. Briefly, 10 mg/kg AOM was intraperitoneally injected into mice, and their normal drinking water was replaced with a 2.5% (wt/vol) DSS solution in the next seven days. This procedure was then repeated every fourteen days for four cycles. Respectively, 10 mg/kg 5-HT was intraperitoneally injected (5-HT group), 20 mg/kg citalopram in normal saline was administered intragastrically (citalopram group), and then both 5-HT and citalopram were administered in combination (combined group). Following the procedure, the mice were sacrificed after 90 days, and their blood serum was collected to quantify 5-HT contents. The tumours were separated from each animal, and their sizes and volumes were measured. Immunohistochemistry (IHC) was performed on the tumours to detect YAP and SERT expression. Animals were bred and housed under pathogen-free conditions. All animal experiments were performed according to the guidelines of Zunyi Medical University's Institutional Animal Experimental Ethics Committee (approval no. 2019-2-215).

YAP and SERT expression in human CRC tissues

In colorectal cancer (CRC) cohort, 56 formalin-fixed, paraffin-embedded tissue samples were obtained from the Department of Pathology of the Third Affiliated Hospital of ZMU between August 2021 and October 2022. The clinical pathological parameters were included in the bank, including IHC staining for YAP and SERT, tumour size, and axillary lymph node metastasis status. The WHO classification criteria determined the histological type and clinical stage of tumours. Paraffin-embedded sections (normal and tumour tissues) were immunohistochemically stained for YAP and SERT expression. Briefly, slides were deparaffinized, blocked for nonspecific protein binding, and incubated with either YAP (1:200) or SERT (1:100) primary antibodies for 24 h at 4 °C overnight. These slides were then incubated with the biotinylated secondary antibody for 30 min at room temperature (68–72 °F). The 3,3-diaminobenzidine (DAB) chromogen performed a visualization for 2 min. Replacing the primary antibody with preimmune rabbit serum prepared the negative controls. Then, two independent pathologists scored the patients' clinical characteristics via YAP and SERT staining.

Measurement of serotonin

After patients and animals were given serotonin and tryptophan-free diet for 48 h, these anticoagulants-free serum samples were then collected and centrifuged at 1000 g at room temperature for 30 min. The supernatant was collected and also stored at –80 °C. The 5-HT level in serum was measured via a 5-HT ELISA Kit (Jiancheng, Nanjing, China). This procedure was conducted according to manufacturer's instructions.

Statistical analysis

GraphPad Prism software used a one-way analysis of variance (ANOVA) with Tukey's post-test and the student's t-test. The data were expressed as the mean \pm SD. *P* values <0.05 and <0.01 were considered statistically significant by the researchers.

Results

Serotonin acts by enhancing YAP expression to promote colon cancer cell proliferation

To verify the biological effects of serotonin on colon cancer cell proliferation, we performed an in vitro test on two colon cancer cell lines. As shown in Fig. 1A, 10 μ M serotonin treatment was administered to SW480 and SW1116, showing cell proliferation after starving the cells for 24 h. Moreover, these proliferative effects were apparent upon 100 μ M serotonin stimulation (*P*<0.05). After serotonin stimulation, an EdU proliferation procedure re-confirmed staining for positive cells (Fig. 1B). Western blot analysis showed that YAP expression, and its downstream proteins c-Myc, Cyr61 in SW480 and SW1116 cells, increased gradually after 4 h of serotonin treatment as the concentrations increased. This analysis confirmed YAP's expression in a time-dependent manner from 30 min to 4 h, peaking at 4 h under 10 μ M serotonin stimulation (Fig. 1C). Moreover, immunofluorescence staining showed that 10 μ M 5-HT stimulation for 4 h dramatically increased nuclear YAP expression in both SW480 and SW1116 cells (Additional file 2: Fig. S1).

Because YAP protein has a strong ability to proliferate, many cancers, including colon cancer [29], are frequently viewed as cancer oncopromoter. Given YAP's effect on cancers, we hypothesized that the enhancement of 5-HT on YAP might be the primary underlying cause of cell proliferation. Our experiment was conducted as follows: first, we used verteporfin to block YAP function. This drug has been proven to prohibit YAP activity from binding its main target transcriptional factor TEAD in the nucleus. Using 5-HT for cell proliferation caused a

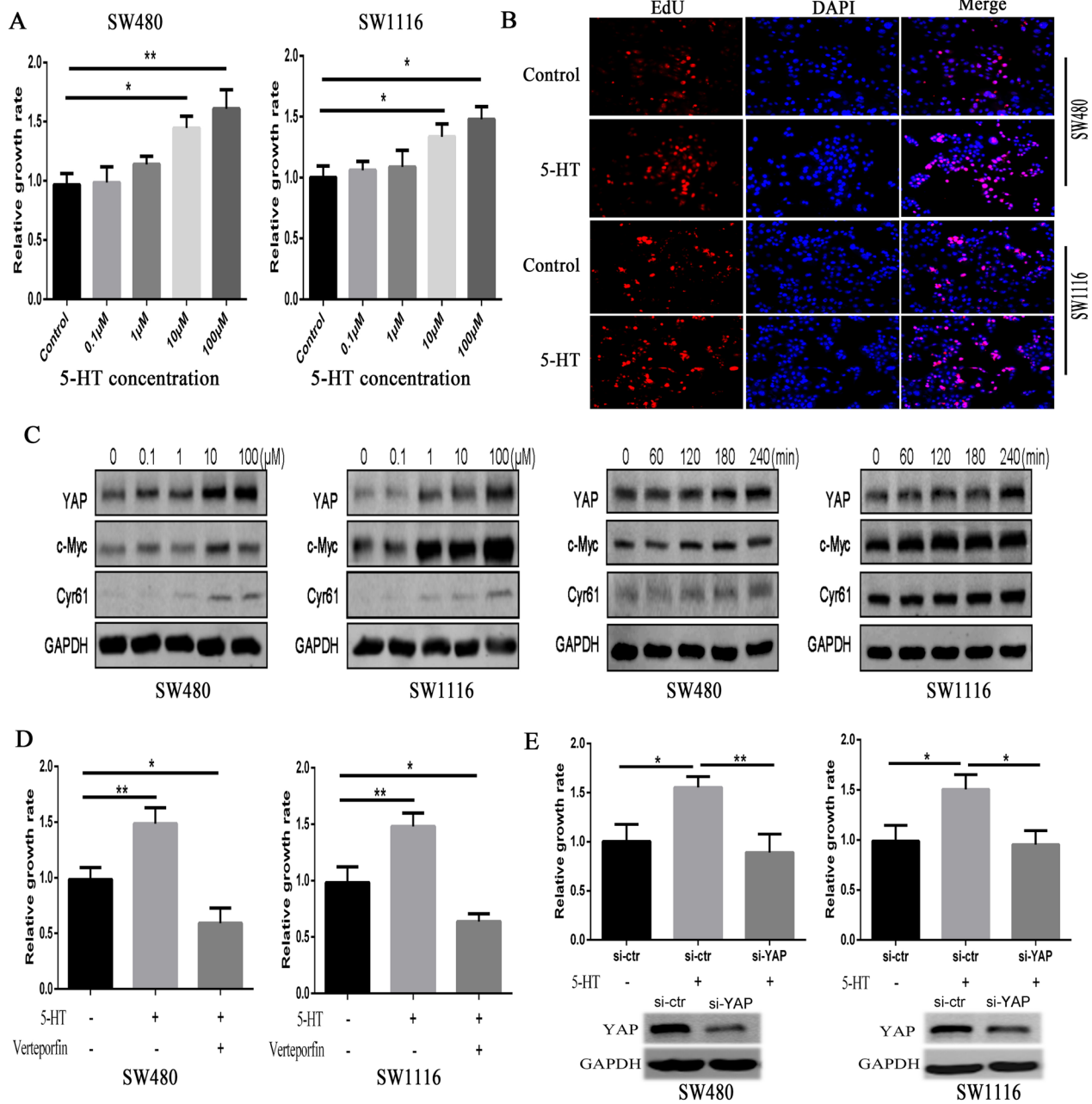


Fig. 1 5-HT proliferates colon cancer cells through enhancing YAP expression. **A** 5-HT incubated SW480 and SW1116 cells for 48 h at their indicated concentrations. Furthermore, the CCK-8 assay was used to evaluate the proliferation of cancer cells. *, $P < 0.05$; **, $P < 0.01$. **B** EdU staining (100 ×) determined the cell proliferation of SW480 and SW1116 cells after 10 μM 5-HT stimulation was determined. **C** 5-HT was used to treat the cells at 0.1 μM-100 μM concentrations for 4 h or with 10 μM 5-HT from 30 to 240 min; a western blot test was performed to determine changes in YAP's expression of its target proteins in SW480 and SW1116 cells. **D** Cells were pre-treated with 1 μM verteporfin 2 h before 10 μM 5-HT stimulation, and 5-HT was combined with a CCK-8 assay to induce proliferation abilities of SW480 and SW1116 cells. *, $P < 0.05$; **, $P < 0.01$. **E** After RNAs were transferred and YAP expression was knocked down in SW480 and SW1116 cells, 5-HT induced cell proliferation was used in combination with CCK-8 assay for this experiment. *, $P < 0.05$; **, $P < 0.01$

significant 10 μ M verteporfin inhibition (Fig. 1D). Additionally, YAP downregulation restrained colon cancer cell growth (Fig. 1E). Together, these results indicated that 5-HT acted by enhancing YAP expression to promote the proliferation of colon cancer cells in vitro.

SERT transports extracellular 5HT entry into colon cancer cells

Both 5-HTRs and SERT, located in the cell membrane (plasma membrane), are functional receptors responsible for transducing 5-HT signals. To determine which receptor primarily mediates 5-HT effects in colon cancer cells, we first pre-treated the colon cancer cells with a 5-HTR broad-spectrum inhibitor (asenapine) and two SSRIs (citalopram and fluvoxamine). This procedure was performed 2 h before adding 10 μ M 5-HT. 5-HT treatment combining citalopram and fluvoxamine appeared to reverse the growth of SW480 and SW1116 cells. In contrast, asenapine had nearly no effect on cell proliferation (Fig. 2A). At the protein level, western blot analysis showed that administering 5-HT dramatically inhibited YAP and Cyr61's expression levels when cells were pre-treated with 100 μ M citalopram or 100 μ M fluvoxamine. However, their expression was nearly unaltered by asenapine (Fig. 2B). To further exclude the role of 5-HTR in YAP expression, we also targeted the knockdown of four G α subunits (G α 12, G α 13, G α q, and G α 11) in colon cancer cells and subsequently stimulated the cells with 10 μ M 5-HT. As shown in Additional file 3: Fig. S2A, after downregulating the G α subunits via siRNA transfection, YAP expressions in SW480 and SW1116 cells were not remarkably altered by 10 μ M 5-HT stimulation. However, SERT's expression knockdown inhibited 5-HT-induced YAP expression (Fig. 2C). Moreover, when hSERT pcDNA3 vector was transferred and SERT was overexpressed in HeLa cells, YAP expression was remarkably increased upon 10 μ M 5-H stimulation (Additional file 3: Fig. S2B). Consequently, SERT (not 5-HTR) mainly activates YAP expression by transducing 5-HT signals and promoting cell proliferation in the colon cancer cells.

SERT has traditionally been viewed as a transporter of 5-HT into cells. Due to the results above expressing SERT as a primary mediated 5-HT-induced YAP expression and cell proliferation mechanism, we naturally suspected whether these promotional effects in SW480 and SW1116 cells were correlated with the transporting function of SERT. To verify this hypothesis, we treated SW480 and SW1116 cells with a 10 μ M 5-HT for 4 h. Then we tested the LC-MS/MS and the intracellular solutes to detect the cytoplasmic 5-HT contents. The prominent dissociative peak (Additional file 4: Fig. S3) appeared at 1.49 min in SW480 and SW1116 cells. This observation was similar to that of the standard one,

indicating that SERT was responsible for transporting extracellular 5-HT into the cytoplasm and that 5-HT stimulation resulted in intracellular 5-HT appearing in the cytoplasm of SW480 and SW1116 cells. These data indicated that intracellular 5-HT transported by SERT might mainly mediate YAP upregulation in CRC cells.

TG2 mediates intracellular 5-HT serotonylates and activates RhoA to promote YAP in colon cancer cells

Intracellular 5-HT is capable of covalently coupling with other proteins under the catalysation of transglutaminase 2 (TG2). In effect, this intracellular modification alters the molecular conformation of the covalented protein and activates it [30]. Proteins of the small G family are the main cellular substrates of TG2. RhoA as a core member of the small GTPase family, has been reported modifies by intracellular 5-HT, leading to it constitutive activity [31]. And the active RhoA has been viewed as an upstream regulator of YAP expression in Hippo pathway [32]. Next, we investigated whether TG2 could internalize 5-HT-stimulated cells with 5-HT as a substrate to regulate YAP expression. Cells were first transfected with TG2 siRNA (siTG2) or controlled with siRNA (siCtrl) for 48 h. Then, these cells were stimulated with 5-HT for 4 h. As displayed in Fig. 3A, 5-HT-induced YAP expression was significantly reversed in SW480 and SW1116 cells upon TG2 knockdown. Next, we used siRNA transfection 48 h before 5-HT stimulation to downregulate RhoA expression in SW480 and SW1116 cells. As a result, RhoA knockdown (Fig. 3B) remarkably reversed 5-HT-induced YAP expression. Next, GST pulldown assays were used to measure RhoA activity in colon cancer cells. Active RhoA expression (Fig. 3C) was dramatically increased in SW480 and SW1116 cells subjected to 10 μ M 5-HT treatment. Meanwhile, 100 μ M citalopram reversed 5-HT-induced active RhoA expression, suggesting that RhoA was responsible for 5-HT-induced YAP expression in colon cancer cells.

Subsequently, we performed immunoprecipitation experiments to investigate whether RhoA was modified by 5-HT in SW480 and SW1116 cells. The expression of serotonylated RhoA (Fig. 3D) was increased upon stimulation with 10 μ M 5-HT, where 100 μ M citalopram for 4 h before 5-HT stimulation dramatically reversed this effect. Immunofluorescence analysis also showed that 10 μ M 5-HT treatment co-expressed RhoA and intracellular 5-HT in SW480 and SW1116 cells, whereas citalopram remarkably weakened the expression of intracellular 5-HT (Additional file 5: Fig. S4). These results indicated that RhoA was indeed serotonylated and activated by intracellular 5-HT in SW480 and SW1116 cells.

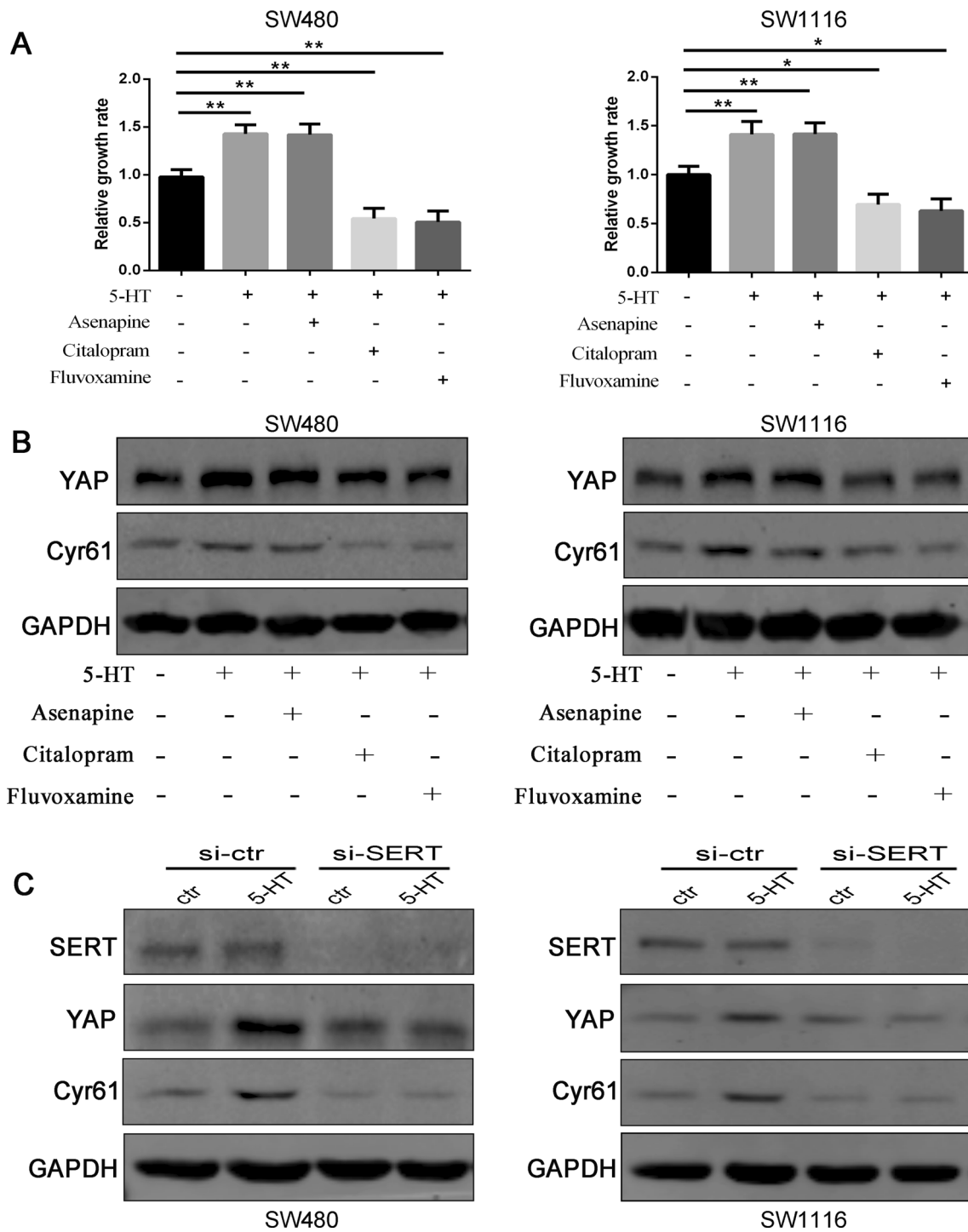


Fig. 2 SERT transports extracellular 5HT entry into colon cancer cells. **A** CCK-8 assay treated the cells with the broad spectrum 5-HTR inhibitor asenapine (100 μ M) and SERT antagonists citalopram (100 μ M) or fluvoxamine (100 μ M) for 2 h before 5-HT stimulation. In succession to this procedure, cell proliferation was detected by the CCK-8 assay. *, $P < 0.05$; **, $P < 0.01$. **B** Moreover, a western blot analysis of YAP and Cyr61 expression in SW480 and SW1116 cells were treated with 100 μ M asenapine or SSRIs citalopram, and fluvoxamine for 2 h before using 5-HT stimulation. **C** A western blot test was used to stimulate YAP and Cyr61 after knocking down SERT expression in SW480 and SW1116 cells

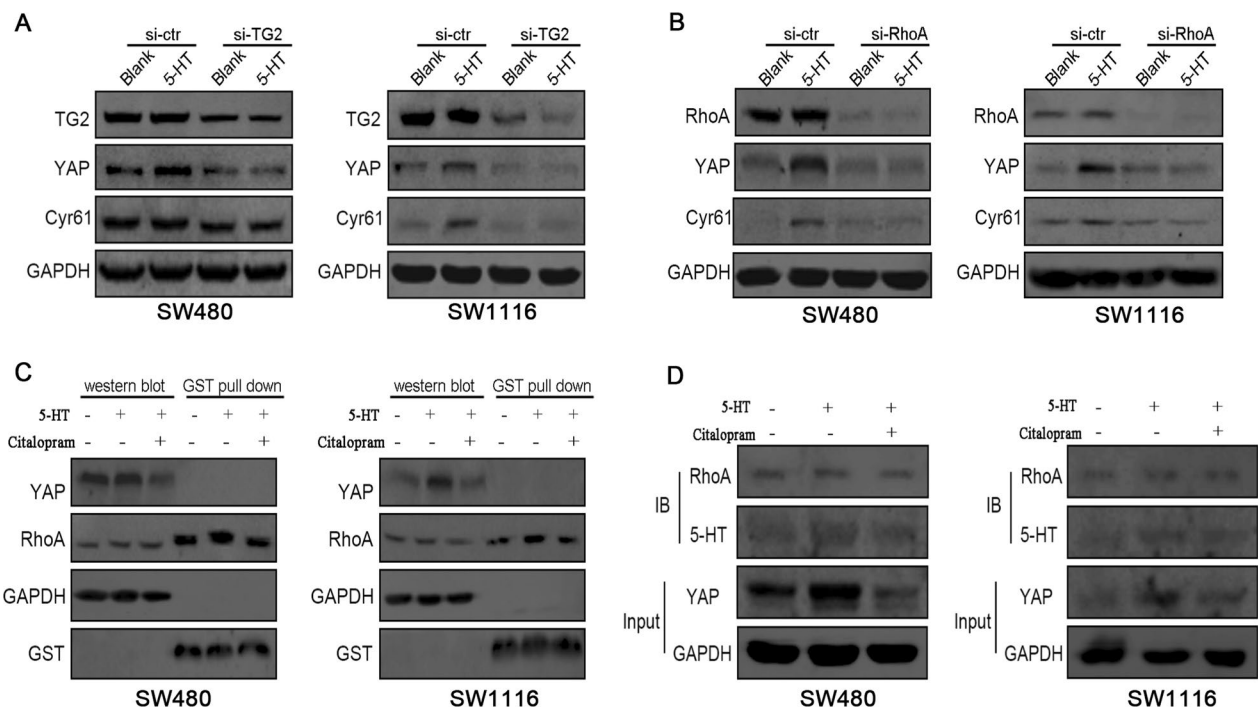


Fig. 3 TG2 mediates intracellular 5-HT seronylates and activates RhoA to promote YAP in colon cancer cells. **A** After siRNA transfection downregulated TG2 expression in SW480 and SW1116 cells, a western blot was used to analyse YAP and Cyr61 expression in cells treated before with 10 μ M 5-HT. **B** After downregulating RhoA expression in SW480 and SW1116 cells, YAP and Cyr61 expressions in cells were treated with 10 μ M 5-HT. For this procedure, a western blot test modelled these expressions. **C** GST pull-down analysis was used to detect the levels of total and active RhoA in cells stimulated with 5-HT (10 μ M) or pre-treated with 100 μ M citalopram before 5-HT stimulation. **D** Immunoprecipitation was performed to analyse the expression of RhoA, 5-HT in cells stimulated with 5-HT (10 μ M) or pre-treated with 100 μ M citalopram before 5-HT stimulation

ROCK1/2 mediates 5-HT-induced YAP expression in colon cancer cells

Lats1/2 and ROCK1/2 regulated YAP expression and phosphorylation [33, 34] via downstream effectors of RhoA. To investigate which molecules participated in 5-HT-induced signalling transduction in colon cancer cells, we first downregulated Lats1/2 expression in SW480 and SW1116 cells by introducing siRNA transfection 48 h before 5-HT stimulation. This procedure had nearly no effect on YAP expression (Fig. 4A,B). When cells were pre-treated with either 10 μ M Y27632 (a selective ATP-competitive inhibitor of ROCK1 and ROCK2.) or 10 μ M SLx-2119 (a selective inhibitor of ROCK2) for 2 h before 5-HT stimulation, the enhanced YAP expressions in SW480 and SW1116 cells were dramatically reversed (Fig. 4C). This outcome suggested that ROCK1/2 mainly mediated 5-HT-induced YAP expression in SW480 and SW1116 cells.

5-HT promotes primary colon carcinogenesis in mice

An inducible mouse model of colonic cancer was founded using AOM + DSS treatment to investigate the roles of exogenous 5-HT in the carcinogenesis of colon cancer. After 90 days of treatment with 10 mg/kg 5-HT

(Fig. 5A), the tumour number and volume in the mice's intestinal mucosa were 11.60 ± 1.52 and 14.35 ± 2.72 mm³, respectively. This outcome showed significantly higher numbers than those of the control group's mice (7.20 ± 0.84 and 9.86 ± 2.45 mm³, respectively). We have also examined citalopram's effect on the tumorigenesis of colon cancer. The results revealed that citalopram could reduce 5-HT-induced tumour promotion. The tumours in mice and combined groups treated with the citalopram were 4.40 ± 1.14 and 4.60 ± 1.82 , respectively. This outcome displayed a significantly lower number than that in the control group (Fig. 5B). The tumour volumes in the citalopram and combined groups were 4.17 ± 1.55 and 4.96 ± 1.83 mm³, respectively. This outcome presents a significantly lower number than that in the control group. There were no statistically significant differences in tumour number or volume between combined and citalopram groups ($P > 0.05$, Fig. 5C).

No structural morphology differences in each group's intestinal mucosa measurement of haematoxylin and eosin (H&E) staining were observed following chemical treatment (Additional file 6: Fig. S5A). YAP, the core effector of Hippo pathway, plays an essential role in CRC's carcinogenesis. Subsequently, we detected YAP

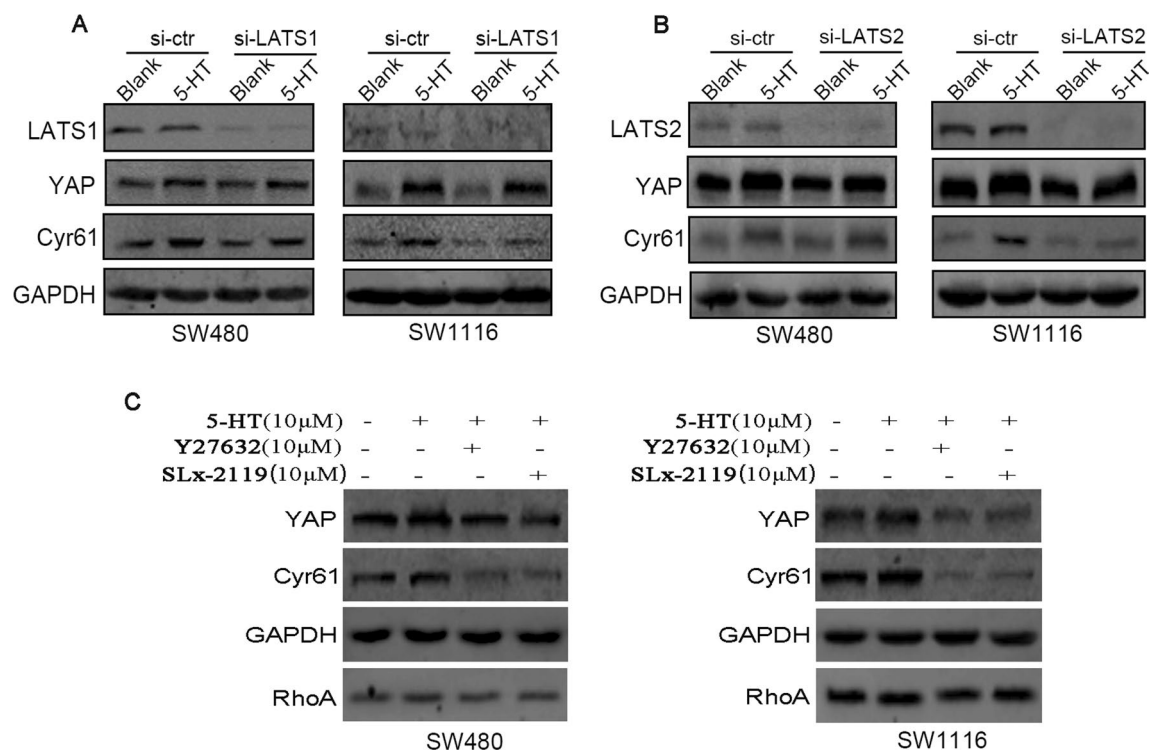


Fig. 4 ROCK1/2 but not Lats1/2 mediates 5-HT-induced YAP expression in colon cancer cells. **A** A western blot test was used to analyse YAP and Cyr61 expression in cells treated with 10 μ M 5-HT. This procedure was done after Lats1 expression was knocked down in SW480 and SW1116 cells. **B** After Lats2 expression was knocked down in SW480 and SW1116 cells, YAP and Cyr61 expressions in cells treated with 10 μ M 5-HT were analysed by western blot. **C** Cells were treated with ROCK1/2 antagonists Y27632 or SLx-2119 (10 μ M) for 2 h before 10 μ M 5-HT stimulation; a western blot was performed to detect the expression of YAP and Cyr61 in SW480 and SW1116 cells

expression in the tissues. The results indicated that the percentage of YAP-positive cells in intestinal tissues from 5-HT group was remarkably higher than that in the control group ($31.67 \pm 3.06\%$ vs. $22.33 \pm 3.41\%$, $P < 0.01$). The data in citalopram and combined groups were decreased to $13.68 \pm 3.08\%$ and $13.33 \pm 3.21\%$, respectively. YAP-positive cells were the lowest among the four groups, measured at $9.67 \pm 3.52\%$ (Fig. 5D and Additional file 6: Fig. S5B). Moreover, AOM plus DSS-induced serum 5-HT contents in tumour-bearing mice reached 243.90 ± 41.74 ng/mL. This result was remarkably higher than that in normal mice (100.65 ± 15.92 ng/mL, $P < 0.05$, Fig. 5E). Together, these data suggested that 5-HT promoted tumour growth in colon cancer and, in effect, enhanced YAP expression in colon mucosa. Concurrently, SSRI citalopram prevented colon carcinogenesis in mice.

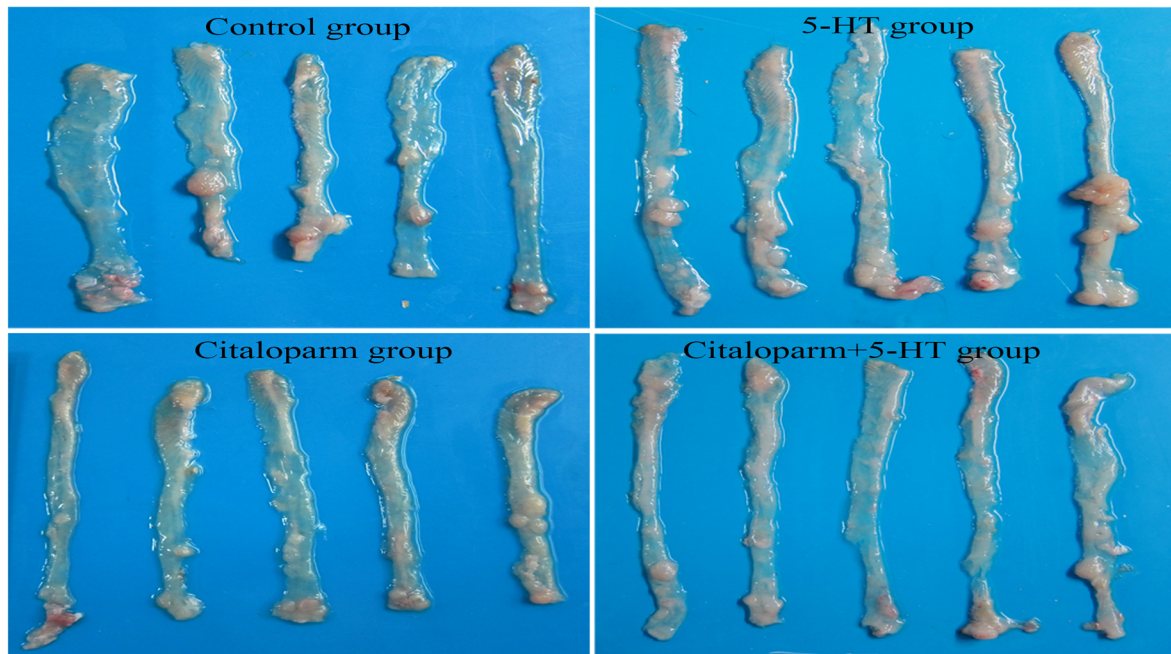
Clinical significance of YAP and SERT expression in human colon cancer tissues and high 5-HT content in the serum of CRC patients

To further determine YAP and SERT's clinical significance and prevalence in human CRC, we used IHC to assess the expression levels of these proteins in tumour tissue samples from our retrospective cohort of 56 CRC patients after

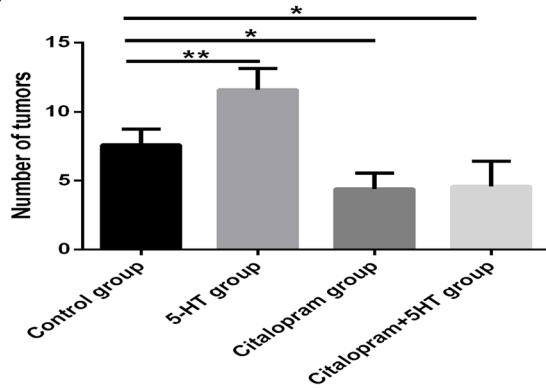
tumour resections (Fig. 6A). Among 56 patients, 43 (76.8%) samples were positive and 13 (23.2%) samples were negative for YAP expression, while 39 (69.6%) samples were positive and 17 (30.4%) samples were negative for SERT expression. YAP and SERT expressions were significantly associated with the lymph node status (both $P < 0.01$), tumour sizes (both $P < 0.01$), and TNM stage (both $P < 0.01$) in colon tumours. However, these expressions did not significantly correlate with gender, tumour location, metastasis, tumour status, or cell differentiation (Table 1). Moreover, YAP and SERT ($r = 0.354$, $P < 0.01$, Table 2) showed a significant positive correlation, indicating a potential correlation between these proteins in human CRC.

Furthermore, we used ELISA to detect 5-HT levels in the serum of patients with colorectal cancer. We found that 5-HT content in patients before surgery was (359.05 ± 38.90) ng/mL, while the probability decreased to (250.09 ± 29.32) ng/mL in the patients following their surgery ($P < 0.01$, Fig. 6B). Meanwhile, 5-HT levels in the normal population were (145.96 ± 5.46) ng/mL, whereas its levels in patients with multiple metastatic colorectal cancers were as high as (393.04 ± 28.69) ng/mL ($P < 0.01$, Fig. 6C). These results reveal that 5-HT is highly significant in the serum of patients with colorectal cancer.

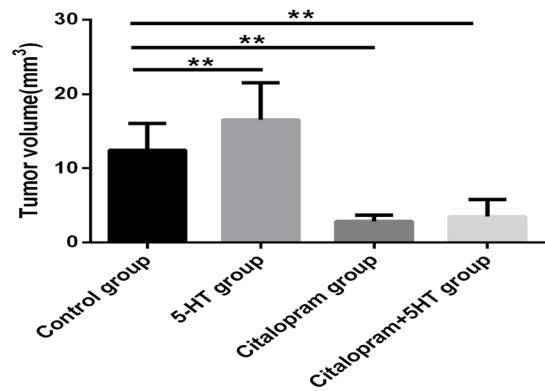
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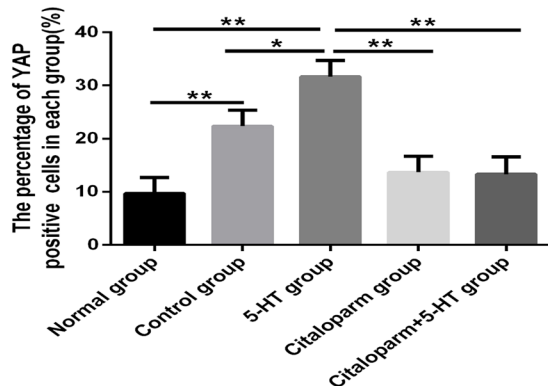
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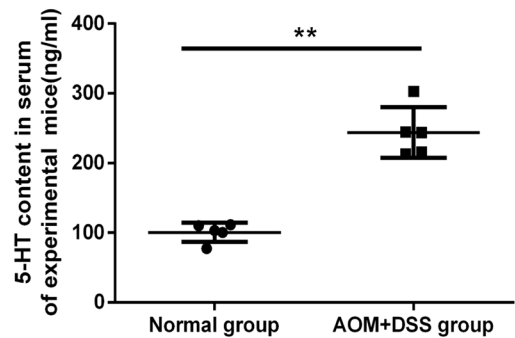


Fig. 5 5-HT promotes primary colon carcinogenesis in mice. **A** AOM combined with DSS in indicative groups was used to treat tumour images in the mice's intestinal tissues. **B** Tumour numbers in the indicated groups of mice. *, $P < 0.05$; **, $P < 0.01$. **C** Tumour volume in the indicated groups of mice. *, $P < 0.05$; **, $P < 0.01$. **D** Immunohistochemistry was performed to analyse YAP expression in colorectal carcinoma tissues of the indicated group of mice. *, $P < 0.05$; **, $P < 0.01$. **E** ELISA analysis detected the serum of the mice's indicated group's contents of 5-HT. *, $P < 0.05$; **, $P < 0.01$

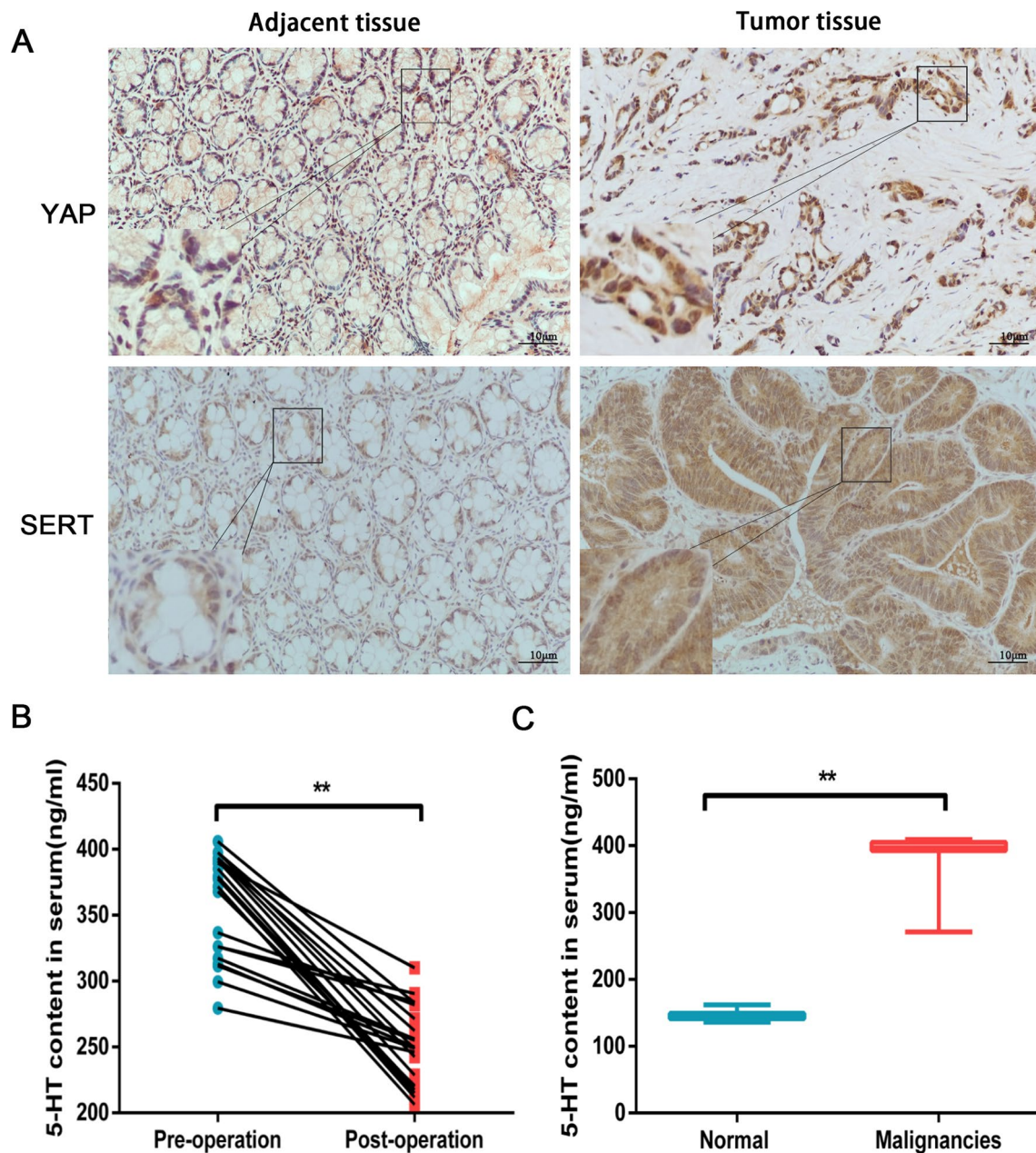


Fig. 6 YAP and SERT expression levels are elevated in human colorectal cancer. **A** Immunohistochemistry was used to detect YAP and SERT expression in a representative CRC sample and a matched adjacent normal intestinal tissue (original magnification, 200 ×). **B** ELISA analysis detected the 5-HT content in the serum of patients suffering from CRC before and after surgery. **, $P < 0.01$. **C** ELISA analysis detected 5-HT content in the patients' serum with multiple metastatic CRC and that of normal people. **, $P < 0.01$

Discussion

As an essential functional molecule in the gastrointestinal tract, 5-HT can regulate gastrointestinal homeostasis via direct innervations of gastrointestinal crypts. However, a consensus on the role of 5-HT in colon cancer development and progression has not been reached. Tutton et al. [35] reported injecting intraperitoneal 5-HT

into dimethylhydrazine (DMH)-treated rats significantly promoted colonic tumours, this effect did not influence crypt cell proliferation. Moreover, subsequent studies expand on the proliferative activity of 5-HT in colonic carcinoma [36]. However, 5-HT has been reported to inhibit tumour growth partially because of its restrictive effect on tumour blood flow and immunomodulation

Table 1 Clinical correlation of YAP and SERT expression in human colorectal cancer

Clinicopathologic features	Number of patients	YAP positive (%)	P value	SERT positive (%)	P Value
Age			0.665		0.890
≤ 60	24	21 (87.5)		19 (79.2)	
> 60	32	22 (68.8)		20 (62.5)	
Sex			0.680		0.747
Male	31	27 (87.1)		20 (64.5)	
Female	25	16 (64.0)		19 (76.0)	
Tumour status			0.010*		0.149
T1–2	23	19 (82.6)		18 (78.3)	
T3–4	33	24 (72.7)		21 (63.6)	
Nodal status			0.004**		0.002**
N0	15	7 (46.7)		5 (33.3)	
N1	17	14 (82.4)		13 (76.5)	
N2	24	22 (91.7)		21 (87.5)	
Metastasis			0.314		0.054
M0	50	40 (80.0)		36 (72.0)	
M1	6	3 (50.0)		3 (50.0)	
Tumour location			0.900		0.278
Right	20	16 (80.0)		15 (75.0)	
Left	23	17 (73.9)		16 (69.6)	
Rectum	13	10 (76.9)		8 (61.2)	
Tumour size (cm)			0.025*		0.042*
≤ 3.0	22	14 (63.6)		13 (59.1)	
> 3.0	34	29 (85.3)		26 (76.5)	
TNM stage			0.009**		0.002**
I	4	2 (50.0)		1 (25.0)	
II	11	5 (45.5)		3 (27.3)	
III	35	33 (94.3)		31 (88.6)	
IV	6	3 (50.0)		4 (66.7)	
Differentiation			0.326		0.454
Well	5	2 (40.0)		3 (60.0)	
Moderately	34	25 (73.5)		22 (64.7)	
Poorly	17	16 (94.1)		14 (82.4)	

* $P < 0.05$; ** $P < 0.01$

Table 2 Correlation between the positive staining of YAP and SERT in human colorectal cancer

	YAP	SERT
<i>Spearman's rho</i>		
YAP		
Correlation coefficient	1.000	0.354
Sig. (2-tailed) P value		0.008**
N	56	56
SERT		
Correlation coefficient	0.354	1.000
Sig. (2-tailed) P value	0.008**	
N	56	56

**Correlation is significant at the 0.01 level (2-tailed)

of tumour growth [37, 38]. Additionally, 5-HT participates in tumour angiogenesis [39] and gastrointestinal inflammation in mice [40]. Various 5-HTR antagonists and SERT inhibitors appear to suppress colonic tumour growth [41, 42]. These findings are sufficient and ambivalent and elucidate the mechanisms underlying 5-HT's role in colon cancer. Herein, our data demonstrated that 5-HT acted as a mitogenic factor to facilitate colon cell proliferation and tumour formation. To explore 5-HT as a neurotransmitter in colon cancer development, this process was performed via SERT-RhoA-ROCK1/2-YAP signalling pathway both in vitro and in vivo.

Being a hydrophilic compound, 5-HT cannot permeate cell membranes by diffusion, and it has long been

considered a classical extracellular hormone that transduces signals via GPCRs. In our study, SERT, but not 5-HTRs, mainly mediated the increased YAP expression and the proliferation of SW480 and SW1116 cells. This process was done by transporting 5-HT into the cytoplasm. Although we did not identify which 5-HTR subtypes were expressed in these two colon cancer cell lines, we verified that SERT transduced 5-HT signals using SERT siRNAs and SSRI inhibitor citalopram *in vitro* and in animals. Furthermore, SERT and YAP expression levels were correlated in pathological human CRC tissues. Both displayed clinicopathological features, suggesting that SERT was a key player in mediating 5-HT's effects on colon carcinogenesis. However, the researchers do not all agree on 5-HTR's effectiveness in controlling colon cancer. This disagreement might stem from the tremendous heterogeneity in receiving 5-HT signals despite the results in the cell lines mentioned. One or more 5-HTR subtypes presented are responsible for similar malignant biological characteristics in colon cancer, and the same 5-HTR subtype may underlie diverse malignant activities in a single cell line [43, 44]. Consequently, we proposed that 5-HTRs might exert other malignant effects on the tumour progression of SW480 and SW1116 cells, such as metastasis or angiogenesis.

Traditionally, SERT has been considered an intracellular transporter, promoting degradation of 5-HT into the cytoplasm. Nevertheless, various studies have recently shown that intracellular 5-HT initiates additional signaling pathways and induces several biological activities before its metabolic degradation [45]. Notably, 5-HT's effect in SERT-driven cells is its conjunction with glutamine residues of specific acceptor proteins, such as small GTPases of the Rho and Rab families, in a TG-mediated reaction, culminated in constitutive activation of the targets [31, 46]. This post-translational protein modification was termed 'serotonylation' [47]. In addition, serotonylation presents an important molecular event promoting disease progression in many physiological and pathological processes, such as platelet activation [47], insulin secretion [30], and formation of pulmonary arterial hypertension [31]. Histone serotonylation at the site of H3Q5 in neuronal cells exerts a permissive transcriptional activity and sensitives to cellular differentiation [48]. More than 40 intracellular proteins with 50 serotonylation sites at their glutamine residues have been identified in SW480 cells [49], indicating that modifying this post-translational protein significantly affects the development and progression of colorectal cancer.

During serotonylation, TG2 catalyses covalent isopeptide bond formation between the lysine residues' ϵ -amino groups and the glutamine residues' γ -carboxamide groups

on proteins [30, 46]. In our findings, TG2 regulated intracellular 5-HT to serotonylate and activate RhoA in SW480 and SW1116 cells. RhoA protein's Gln residues at positions 52, 63, and 136 can be transamidated under TG2 catalysis [50]. RhoA's Gln-63 transamidation is especially essential for the intrinsic and protein-stimulating of its GTPase activity. Moreover, transamidated 5-HT-induced RhoA's serotonylation is constitutively active [47]. Active RhoA acts as an upstream regulator to modulate YAP expression via various molecules. This process occurs in Lats-dependent and Lats-independent manners [33, 34]. Our results confirmed the correlation between 5-HT and YAP's transcriptional activity: 5-HT stimulation increased YAP's transcriptional activity and accelerated its nuclear localization in SW480 and SW1116 cells. The pharmacological inhibition of Rho-associated protein kinase (ROCK) proteins with Y27632 or SLx-2119 reversed 5-HT-induced YAP expression. This outcome confirmed RhoA/ROCK's critical effect on its signaling axis in YAP-mediated regulation of 5-HT in colon cancer cells. RhoA-ROCK signalling appears to control YAP expression to maintain intestinal homeostasis and stem cell regeneration in the small intestinal epithelium [51]. Hyperactivate RhoA/ROCK1/actomyosin signalling drives oncogenic TEAD/YAP transcription, a frequent mechanism activating RhoA function in human malignancies [52].

Notably, we found that citalopram may inhibit CRC cell proliferation *in vitro* and the tumorigenesis of colon cancer *in vivo*, as well as the content of 5-HT, is abnormally highly expressed in the serum of no matter tumour-bearing mice and patients with colorectal cancer. While SSRIs have been widely used as a first-line drug in clinics for patients suffering some mental disorders (e.g., depression), several epidemiological data confirmed that SSRI treatment might decrease colorectal cancer in depressed patients [20–22]. Despite a depression diagnosis, these results were not conclusively associated with cancer risk [53]. However, increasing evidence indicated that a bad prognosis may cause depression increasing the mortality rate in the cancer population overall [54]. In sum, we provided some new evidence on SSRI administration aiming to prevent and treat colorectal cancer, especially in patients with depression. However, further clinical investigations should be conducted to validate this novel strategy in CRC patients.

In summary, we reported that SERT was responsible for transporting serotonin into colon cancer cells which TG2-mediated serotonylation to activate RhoA. This effect enhances YAP expression and promotes the carcinogenesis of colon cancer, blocking SERT by potentially impeding 5-HT's intracellular signalling effect on cell growth (Fig. 7). These findings provided insight into

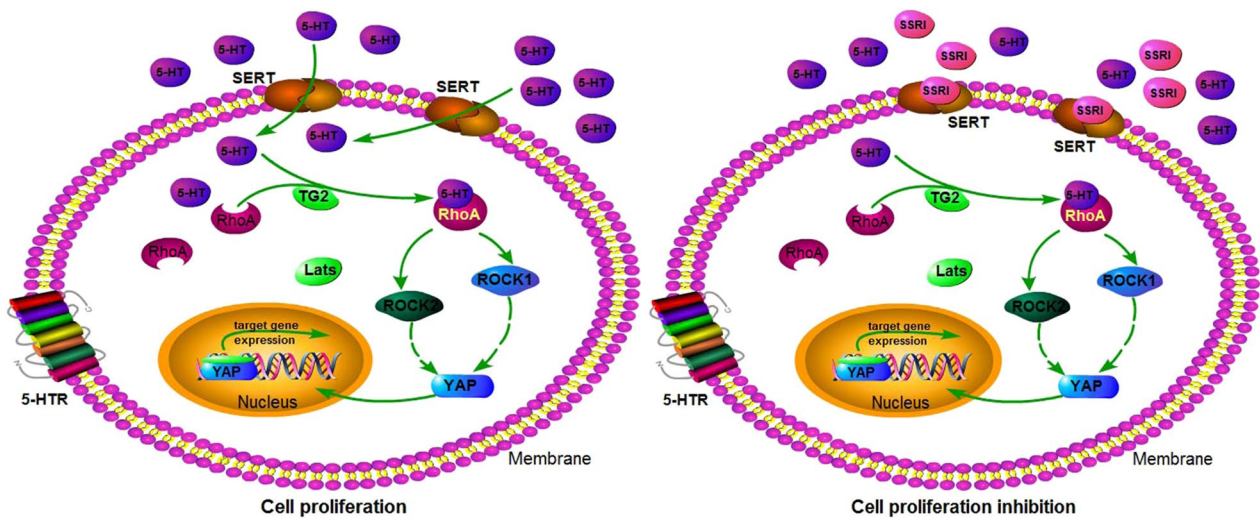


Fig. 7 Schematic representation of the proposed mechanism by which 5-HT modulates signalling pathways in colon cancer. **A** In colon cancer cells, extracellular 5-HT is transported into cells by SERT located in the cytomembrane and then transamidates and activates RhoA via seronylation regulated by TG2. Subsequently, ROCK1/2 is activated, leading to the expression of YAP and its target proteins. **B** When SERT function is blocked by specific antagonists (such as citalopram), 5-HT intracellular transportation is weakened to some degree, blocking the cellular signalling transduction and colon cancer cell proliferation induced by 5-HT. This strategy suggests that targeting 5-HT signalling may be feasible for treating colon cancer

serotonin’s role in promoting colorectal carcinogenesis and provided new strategies for colorectal cancer therapy.

Abbreviations

- 5-HT 5-Hydroxytryptamine
- 5-HTRs 5-HT receptors
- SERT Serotonin transporter
- SSRIs Serotonin reuptake inhibitors
- YAP Yes-associated protein
- LC–MS/MS Liquid chromatography–tandem mass chromatography
- TG2 Transglutaminase-2
- ROCK Rho-associated protein kinase
- Tph1 Tryptophan hydroxylase-1
- Cyr61 Cysteine-rich angiogenic inducer 61

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12964-023-01096-2>.

Additional file 1. Table S1. The sequences of primers for siRNA and qPCR.

Additional file 2. Figure S1. Subcellular localization of YAP in colon cancer cells induced by 5-HT. Subcellular localization of YAP expression in SW480 or SW1116 cells after 10 μM 5-HT stimulation for 4 h was shown by immunofluorescence (400×).

Additional file 3. Figure S2. GPCR has not affected YAP expression in colon cancer cells. **A** Subtypes of GPCR in SW480 and SW1116 cells were knocked down by transfecting small interfering RNAs; a western blot was used to analyse YAP and Cyr61 expression in cells treated with 10 μM 5-HT. **, *P* < 0.01. **B** Hela cells were stimulated with 10 μM 5-HT after transfection with hSERT pcDNA3 vector, and YAP expression was analysed by western blot.

Additional file 4. Figure S3. LC-MS/MS detected cytoplasmic 5-HT in colon cancer cells. Cytoplasmic 5-HT is observable in colon cancer cells

following 5-HT stimulation. Subsequently, LC–MS/MS identified a prominent dissociative peak at 1.49 min in both SW480 and SW1116 cell lysates after 10 μM 5-HT stimulation, similar to the standard.

Additional file 5. Figure S4. Colocalization of 5-HT and RhoA in colon cancer cells. Immunofluorescence was performed to show that the co-expression of 5-HT and RhoA in SW480 and SW1116 cells stimulated with 10 μM 5-HT in the absence and presence of citalopram (100 μM), added 2 h before 5-HT stimulation (400×).

Additional file 6. Figure S5. Morphological changes of intestinal mucosa structures in mice. **A** HE staining showed intestinal mucosa structures in experimental mice of indicated groups (200×). **B** YAP expression in colorectal carcinoma tissues of indicated group of mice was analysed by immunohistochemistry (200×).

Author contributions

HY designed and conceived this project. TQ, LY, and QD performed the experiments. TQ, LY participated in the analysis and interpretation of data, HY drafted the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data are included in the study and available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

All animal procedures were conducted in accordance with government published recommendations for the Care and Use of Laboratory Animals and were approved by the Animal Experimental Ethics Committee of Zunyi Medical University (approval no. 2019-2-215). The studies involving human participants were reviewed and approved by Institutional Review Board and the Ethics

Committee of the Third Affiliated Hospital of Zunyi Medical University. The patients/participants provided their written informed consent to participate in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Jobling P, Pundavela J, Oliveira SM, Roselli S, Walker MM, Hondermarck H. Nerve-cancer cell cross-talk: a novel promoter of tumor progression. *Cancer Res.* 2015;75:1777–81.
2. Faulkner S, Jobling P, March B, Jiang CC, Hondermarck H. Tumor neurobiology and the war of nerves in cancer. *Cancer Discov.* 2019;9:702–10.
3. Renz BW, Takahashi R, Tanaka T, Macchini M, Hayakawa Y, Dantes Z, Maurer HC, Chen X, Jiang Z, Westphalen CB, Ilmer M, Valenti G, Mohanta SK, Habenicht AJR, Middelhoff M, Chu T, Nagar K, Tailor Y, Casadei R, Di Marco M, Kleespies A, Friedman RA, Remotti H, Reichert M, Worthley DL, Neumann J, Werner J, Iuga AC, Olive KP, Wang TC. beta2 adrenergic-neurotrophin feedforward loop promotes pancreatic cancer. *Cancer Cell.* 2018;33:75–90.
4. Zahalka AH, Arnal-Estape A, Maryanovich M, Nakahara F, Cruz CD, Finley LWS, Frenette PS. Adrenergic nerves activate an angio-metabolic switch in prostate cancer. *Science.* 2017;358:321–6.
5. Kamiya A, Hayama Y, Kato S, Shimomura A, Shimomura T, Irie K, Kaneko R, Yanagawa Y, Kobayashi K, Ochiya T. Genetic manipulation of autonomic nerve fiber innervation and activity and its effect on breast cancer progression. *Nat Neurosci.* 2019;22:1289–305.
6. Yin QQ, Xu LH, Zhang M, Xu C. Muscarinic acetylcholine receptor M1 mediates prostate cancer cell migration and invasion through hedgehog signaling. *Asian J Androl.* 2018;20:608–14.
7. Gershon MD. 5-Hydroxytryptamine (serotonin) in the gastrointestinal tract. *Curr Opin Endocrinol Diabetes Obes.* 2013;20:14–21.
8. Liu N, Sun S, Wang P, Sun Y, Hu Q, Wang X. The mechanism of secretion and metabolism of gut-derived 5-hydroxytryptamine. *Int J Mol Sci.* 2021;22:7931.
9. Zhang YW, Turk BE, Rudnick G. Control of serotonin transporter phosphorylation by conformational state. *Proc Natl Acad Sci USA.* 2016;113:E2776–2783.
10. Bonilla-Jaime H, Sanchez-Salcedo JA, Estevez-Cabrera MM, Molina-Jimenez T, Cortes-Altamirano JL, Alfaro-Rodriguez A. Depression and pain: use of antidepressants. *Curr Neuropharmacol.* 2022;20:384–402.
11. Andrews PW, Bharwani A, Lee KR, Fox M, Thomson JA Jr. Is serotonin an upper or a downer? The evolution of the serotonergic system and its role in depression and the antidepressant response. *Neurosci Biobehav Rev.* 2015;51:164–88.
12. Balakrishna P, George S, Hatoum H, Mukherjee S. Serotonin pathway in cancer. *Int J Mol Sci.* 2021;22:1268.
13. Soll C, Jang J, Riener M, Moritz W, Wild P, Graf R, Clavien P. Serotonin promotes tumor growth in human hepatocellular cancer. *Hepatology.* 2010;51:1244–54.
14. Jiang SH, Li J, Dong FY, Yang JY, Liu DJ, Yang XM, Wang YH, Yang MW, Fu XL, Zhang XX, Li Q, Pang XF, Huo YM, Li J, Zhang JF, Lee HY, Lee SJ, Qin WX, Gu JR, Sun YW, Zhang ZG. Increased serotonin signaling contributes to the warburg effect in pancreatic tumor cells under metabolic stress and promotes growth of pancreatic tumors in mice. *Gastroenterology.* 2017;153:277–91.
15. Shinka T, Onodera D, Tanaka T, Shoji N, Miyazaki T, Moriuchi T, Fukumoto T. Serotonin synthesis and metabolism-related molecules in a human prostate cancer cell line. *Oncol Lett.* 2011;2:211–5.
16. Gautam J, Banskota S, Regmi SC, Ahn S, Jeon YH, Jeong H, Kim SJ, Nam TG, Jeong BS, Kim JA. Tryptophan hydroxylase 1 and 5-HT7 receptor preferentially expressed in triple-negative breast cancer promote cancer progression through autocrine serotonin signaling. *Mol Cancer.* 2016;15:75.
17. Alpini G, Invernizzi P, Gaudio E, Venter J, Kopriva S, Bernuzzi F, Onori P, Franchitto A, Coufal M, Frampton G, Alvaro D, Lee SP, Marziani M, Benedetti A, DeMorrow S. Serotonin metabolism is dysregulated in cholangiocarcinoma, which has implications for tumor growth. *Can Res.* 2008;68:9184–93.
18. Sarrouilhe D, Mesnil M. Serotonin and human cancer: a critical view. *Biochimie.* 2018;161:46–50.
19. Radin DP, Patel P. A current perspective on the oncopreventive and oncolytic properties of selective serotonin reuptake inhibitors. *Biomed Pharmacother.* 2017;87:636–9.
20. Xu W, Tamim H, Shapiro S, Stang MR, Collet JP. Use of antidepressants and risk of colorectal cancer: a nested case-control study. *Lancet Oncol.* 2006;7:301–8.
21. Coogan PF, Strom BL, Rosenberg L. Antidepressant use and colorectal cancer risk. *Pharmacoepidemiol Drug Saf.* 2009;18:1111–4.
22. Chubak J, Boudreau DM, Rulyak SJ, Mandelson MT. Colorectal cancer risk in relation to antidepressant medication use. *Int J Cancer.* 2011;128:227–32.
23. Zanconato F, Cordenonsi M, Piccolo S. YAP/TAZ at the roots of cancer. *Cancer Cell.* 2016;29:783–803.
24. Gregorieff A, Liu Y, Inanlou MR, Khomchuk Y, Wrana JL. Yap-dependent reprogramming of Lgr5(+) stem cells drives intestinal regeneration and cancer. *Nature.* 2015;526:715–8.
25. Hayakawa Y, Sakitani K, Konishi M, Asfaha S, Niikura R, Tomita H, Renz BW, Tailor Y, Macchini M, Middelhoff M, Jiang Z, Tanaka T, Dubeykovskaya ZA, Kim W, Chen X, Urbanska AM, Nagar K, Westphalen CB, Quante M, Lin C-S, Gershon MD, Hara A, Zhao C-M, Chen D, Worthley DL, Koike K, Wang TC. Nerve growth factor promotes gastric tumorigenesis through aberrant cholinergic signaling. *Cancer Cell.* 2016;31:21–34.
26. Ren XD, Kiouss WB, Schwartz MA. Regulation of the small GTP-binding protein Rho by cell adhesion and the cytoskeleton. *EMBO J.* 1999;18:578–85.
27. Zheng X, Kang A, Dai C, Liang Y, Xie T, Xie L, Peng Y, Wang G, Hao H. Quantitative analysis of neurochemical panel in rat brain and plasma by liquid chromatography-tandem mass spectrometry. *Anal Chem.* 2012;84:10044–51.
28. Neufert C, Becker C, Neurath MF. An inducible mouse model of colon carcinogenesis for the analysis of sporadic and inflammation-driven tumor progression. *Nat Protoc.* 2007;2:1998–2004.
29. Wang L, Shi S, Guo Z, Zhang X, Han S, Yang A, Wen W, Zhu Q. Overexpression of YAP and TAZ is an independent predictor of prognosis in colorectal cancer and related to the proliferation and metastasis of colon cancer cells. *PLoS ONE.* 2013;8:e65539.
30. Walther DJ, Stahlberg S, Vowinckel J. Novel roles for biogenic monoamines: from monoamines in transglutaminase-mediated post-translational protein modification to monoaminylation deregulation diseases. *FEBS J.* 2011;278:4740–55.
31. Guilluy C, Eddahibi S, Agard C, Guignabert C, Izikki M, Tu L, Savale L, Humbert M, Fadel E, Adnot S, Loirand G, Pacaud P. RhoA and Rho kinase activation in human pulmonary hypertension: role of 5-HT signaling. *Am J Respir Crit Care Med.* 2009;179:1151–8.
32. Yu FX, Zhao B, Panupinhu N, Jewell JL, Lian J, Wang LH, Zhao J, Yuan H, Tumaneng K, Li H, Fu XD, Mills GB, Guan KL. Regulation of the Hippo-YAP pathway by G-protein-coupled receptor signaling. *Cell.* 2012;150:780–91.
33. Zhang Y, Xia H, Ge X, Chen Q, Yuan D, Chen Q, Leng W, Chen L, Tang Q, Bi F. CD44 acts through RhoA to regulate YAP signaling. *Cell Signal.* 2014;26:2504–13.
34. Dai X, Liu H, Shen S, Guo X, Yan H, Ji X, Li L, Huang J, Feng XH, Zhao B. YAP activates the Hippo pathway in a negative feedback loop. *Cell Res.* 2015;25:1175–8.
35. Tutton PJ, Barkla DH. Neural control of colonic cell proliferation. *Cancer.* 1980;45:1172–7.
36. Tutton PJ, Barkla DH. Biogenic amines as regulators of the proliferative activity of normal and neoplastic intestinal epithelial cells (review). *Anti-cancer Res.* 1987;7:1–12.
37. Lubbe AS, Huhnt W. Microvessel diameters of human colon adenocarcinoma during acute treatment with serotonin. *Int J Microcirc Clin Exp.* 1994;14:218–25.

38. Jenkins DC, Wilkinson J, Lindon JC, Farrant RD, Carpenter TA, Stables JN, Topley P, Lindstead DJ, Holmes LS. Anti-tumor effects of 5-hydroxytryptamine (5-HT) are not entirely attributable to its effects on tumor vasculature. *Int J Radiat Biol.* 1991;60:243–8.
39. Peters MAM, Walenkamp AME, Kema IP, Meijer C, de Vries EGE, Oosting SF. Dopamine and serotonin regulate tumor behavior by affecting angiogenesis. *Drug Resist Updates.* 2014;17:96–104.
40. Ghia JE, Li N, Wang H, Collins M, Deng Y, El-Sharkawy RT, Côté F, Mallet J, Khan WJ. Serotonin has a key role in pathogenesis of experimental colitis. *Gastroenterology.* 2009;137:1649–60.
41. Nocito A, Dahm F, Jochum W, Jang JH, Georgiev P, Bader M, Graf R, Clavien PA. Serotonin regulates macrophage-mediated angiogenesis in a mouse model of colon cancer allografts. *Can Res.* 2008;68:5152–8.
42. Sarrouilhe D, Clarhaut J, Defamie N, Mesnil M. Serotonin and cancer: What is the link? *Curr Mol Med.* 2015;15:62–77.
43. Ataee R, Ajdary S, Zarrindast M, Rezayat M, Shokrgozar MA, Ataee A. Y25130 hydrochloride, a selective 5HT3 receptor antagonist has potent antimitogenic and apoptotic effect on HT29 colorectal cancer cell line. *Eur J Cancer Prev Off J Eur Cancer Prev Organ (ECP).* 2010;19:138–43.
44. Ataee R, Ajdary S, Zarrindast M, Rezayat M, Hayatbakhsh MR. Antimitogenic and apoptotic effects of 5-HT1B receptor antagonist on HT29 colorectal cancer cell line. *J Cancer Res Clin Oncol.* 2010;136:1461–9.
45. Jiang SH, Wang YH, Hu LP, Wang X, Li J, Zhang XL, Zhang ZG. The physiology, pathology and potential therapeutic application of serotonylation. *J Cell Sci.* 2021;134:jcs257337.
46. Hummerich R, Thumfart JO, Findeisen P, Bartsch D, Schloss P. Transglutaminase-mediated transamidation of serotonin, dopamine and noradrenaline to fibronectin: evidence for a general mechanism of monoamination. *FEBS Lett.* 2012;586:3421–8.
47. Walther DJ, Peter JU, Winter S, Holtje M, Paulmann N, Grohmann M, Vowinckel J, Alamo-Bethencourt V, Wilhelm CS, Ahnert-Hilger G, Bader M. Serotonylation of small GTPases is a signal transduction pathway that triggers platelet alpha-granule release. *Cell.* 2003;115:851–62.
48. Farrelly LA, Thompson RE, Zhao S, Lepack AE, Lyu Y, Bhanu NV, Zhang B, Loh YE, Ramakrishnan A, Vadodaria KC, Heard KJ, Erikson G, Nakadai T, Bastle RM, Lukasak BJ, Zebroski H 3rd, Alenina N, Bader M, Berton O, Roeder RG, Molina H, Gage FH, Shen L, Garcia BA, Li H, Muir TW, Maze I. Histone serotonylation is a permissive modification that enhances TFIIID binding to H3K4me3. *Nature.* 2019;567:535–9.
49. Lin JC, Chou CC, Tu Z, Yeh LF, Wu SC, Khoo KH, Lin CH. Characterization of protein serotonylation via bioorthogonal labeling and enrichment. *J Proteome Res.* 2014;13:3523–9.
50. Schmidt G, Selzer J, Lerm M, Aktories K. The Rho-deamidating cytotoxic necrotizing factor 1 from *Escherichia coli* possesses transglutaminase activity. Cysteine 866 and histidine 881 are essential for enzyme activity. *J Biol Chem.* 1998;273:13669–74.
51. Liu M, Zhang Z, Sampson L, Zhou X, Nalapareddy K, Feng Y, Akunuru S, Melendez J, Davis AK, Bi F, Geiger H, Xin M, Zheng Y. RHOA GTPase controls YAP-mediated EREG signaling in small intestinal stem cell maintenance. *Stem cell reports.* 2017;9:1961–75.
52. Esposito D, Pant I, Shen Y, Qiao RF, Yang X, Bai Y, Jin J, Poulidakos PI, Aaronson SA. ROCK1 mechano-signaling dependency of human malignancies driven by TEAD/YAP activation. *Nat Commun.* 2022;13:703.
53. Ahn HK, Bae JH, Ahn HY, Hwang IC. Risk of cancer among patients with depressive disorder: a meta-analysis and implications. *Psychooncology.* 2016;25:1393–9.
54. Orive M, Anton-Ladislao A, Lazaro S, Gonzalez N, Bare M, Fernandez de Larrea N, Redondo M, Bilbao A, Sarasqueta C, Aguirre U, Quintana JM, REDISSEC-CARESS/CCR Group. Anxiety, depression, health-related quality of life, and mortality among colorectal patients: 5-year follow-up. *Support Care Cancer.* 2022;30:7943–54.

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