Downregulation of 5-hydroxytryptamine receptor 3A expression exerts an anticancer activity against cell growth in colorectal carcinoma cells *in vitro*

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Received October 25, 2016; Accepted March 7, 2018

DOI: 10.3892/ol.2018.9351

Abstract. 5-hydroxytryptamine receptor 3A (*HTR3A*) is an important member of the 5-HT family, which has been suggested to contribute to human tumor development. However, the functions of *HTR3A* in human cancer, particularly in colorectal carcinoma (CRC) have not been well-characterized. Reverse transcription quantitative polymerase was performed to detect endogenous *HTR3A* expression in 6 CRC cell lines. *HTR3A* was then knocked down via a lentivirus-mediated shRNA system to detect the effect of *HTR3A* silencing on cell proliferation and apoptosis by MTT, colony formation, flow cytometry and western blotting assays in CRC. *HTR3A* was expressed at different levels in the 6 CRC cell lines. In addition, *HTR3A* knockdown inhibited CRC cell proliferation and colony formation, resulting in cell cycle arrest and the promotion of cell apoptosis. Additionally, the expression levels of apoptosis-associated proteins including BAD and BAX were increased, while Bcl-2 expression was decreased following *HTR3A* knockdown. In summary, the data of the present study indicated that *HTR3A* serves an important role in colon carcinogenesis, but in-depth studies of the mechanisms underlying these data are required to demonstrate whether it may be used as a novel target for CRC therapy.

Introduction

Colorectal carcinoma (CRC) is the third most common cancer worldwide (1). Additionally, it is the fifth leading cause of death in China and is a major public health problem (2). The activation of oncogenes coupled with the inactivation of tumor suppressor genes may lead to the carcinogenesis of CRCs (3). In previous years, increasing numbers of oncogenes and tumor suppressor genes, including Adenomatous polyposis coli, Tumor protein 53 and KRAS proto-oncogene, GTPase have been clearly identified as key factors in tumor formation (4-7). However, the identification of these authenticated targeting molecules, which contribute to an improved understanding of the occurrence and development of CRCs, is insufficient for the development of a cure.

5-Hydroxytryptamine (5-HT, serotonin) was first identified as a vasoconstrictor from the blood (8). It was subsequently characterized as a neurotransmitter in the central nervous system (CNS) (9). It is primarily isolated in the gastrointestinal tract, platelets, and the CNS (10). 5-HT exerts its biological function through binding numerous cognate receptors, including the 5-HT1/5, 5-HT2, 5-HT3 and 5-HT4/6/7 subtypes (11). Generally, the serotonin receptor genes encode G-protein-coupled serotonin receptors, with the exception of the 5-HT3 subtype. The 5-HT3 subtype, which has 3 subunits including *5-HT3A* (also termed *HTR3A)*, *5-HT3B* (also termed *HTR3B)*, and *HTR3C*, may encode a subunit of the ligand-gated cation channel (12-15). The *HTR3A* subunit forms a functional channel as a homo-pentamer; however, the *HTR3B* subunit alone does not and the underlying cause has not yet been determined.

5-HT, as a mitogenic factor, has been suggested to contribute to certain malignancies including breast, prostate and bladder cancer (16-18). Tutton *et al* (19) demonstrated the effect of serotonin in dimethylhydrazine-induced adenocarcinoma of the colon: Intra-peritoneal injection of a small dose (10 mg/kg) of serotonin resulted in an increase in the tumor cell mitotic rate. Similarly, 5HT3 and 5HT4 agonists caused significant proliferation of the colorectal cancer HT29 cell line, while 5HT3 and 5HT4 antagonists inhibited the cell growth (20). A previous study also revealed that *HTR3A* expression was directly associated with tumor grade in follicular lymphoma, suggesting that *HTR3A* may participate in its carcinogenesis (21). However, the precise role of *HTR3A* in CRC has not been fully evaluated.

In the present study, to detect the functional role of *HTR3A* in CRC, *HTR3A* expression was silenced in human CRC HCT116 and SW1116 cell lines via construction of a short hairpin RNA (shRNA) lentiviral vector. Furthermore, the

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Key words: 5-hydroxytryptamine receptor 3A, short hairpin RNA, cell proliferation, apoptosis, colorectal carcinoma

effects of *HTR3A* silencing on the growth and apoptosis of CRC cells were determined by MTT, colony formation and flow cytometry assays.

Materials and methods

Analysis of Oncomine data. In order to determine the expression of *HTR3A* in human colorectal cancer, data mining using the Oncomine database (www.oncomine.org; date of access, 03/06/2016) was performed. The gene expression of *HTR3A* in cancer tissues was compared with normal colorectal tissues, collected from a number of datasets containing colon and colorectal data (Bittner, colon; Gaedcke, colorectal; Hong, colorectal; Jorissen, colorectal 2; Laiho, colon; Notterman, colon; Reid, colon; Sabates-Bellver, colon; Watanane, colon.) (22-25). An Oncomine outlier analysis was also performed in the colorectal cancer tissues.

Cell culture. The human embryonic kidney (HEK) 293T cell line and CRC DLD-1, HCT116, LoVo, RKO, SW1116 and SW620 cell lines were obtained from The Cell Bank of Type of Culture Collection of Chinese Academy of Science (Shanghai, China). SW1116 and 293T cells were cultured in Dulbecco's modified Eagle's medium (Hyclone; GE Healthcare Life Sciences, Logan, UT, USA) with 10% fetal bovine serum (FBS; Biowest, Nuaillé, France). DLD-1, HCT116, LoVo, RKO and SW620 cells were cultured in RPMI-1640 medium (Hyclone; GE Healthcare Life Sciences) with 10% FBS. All cells were incubated at 37˚C in 5% CO_2 humidified air.

Lentivirus plasmids and transfection. To knock down *HTR3A* expression in the colon cancer cell lines, a lentivirus-mediated shRNA vector was constructed. A total of 100 ng of oligonucleotides was mixed with 100 ng linearized pFH-L vector (Shanghai Hollylab Co., Ltd., Shanghai, China) to perform the ligation under the catalytic action of ligase for 2 h at 25˚C. The sequences of the oligonucleotides cloned into the pFH-L vector were as follows: Control shRNA (shCon), 5'-TTC TCCGAACGTGTCACGT-3'; human *HTR3A* gene shRNA #1 [shHTR3A(S1)], 5'-CTACAGCATCACCCTGGTTAT-3'; and #2 [shHTR3A(S2)], 5'-CAAATATCCCGTACGTGT ATA-3'. Lentiviruses were generated following the co-transfection of recombinant pFH-L vector with the pHelper plasmids including pVSVG-I and pCMV-ΔR8.92 (Shanghai Hollybio Co., Ltd., China) into 293T cells using Lipofectamine 2000® (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol for 48 h. Then, SW1116 (40,000 cells/well) and HCT116 (50,000 cells/well) cells were plated into 6-well plates overnight and transfected with different lentiviruses including shCon, shHTR3A(S1) and shHTR3A(S2) vectors with a multiplicity of infection (MOI) of 20 and 50, respectively. The infection efficiency was monitored through observation of the expression level of green fluorescent protein (GFP) under an Olympus CKX41 microscope (Olympus Corporation, Tokyo, Japan) with a magnification x100 after 96 h.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR) analysis. Total RNA was extracted from colon cancer cell lines using TRIzol® reagent (Life Technologies; Thermo Fisher Scientific, Inc.), and then cDNA was synthesized using 2.0 μg Total RNA and 1 μl Oligo dT1 (0.5 μg/μl, Shanghai Shenggong, China) by M-MLV Reverse Transcriptase and M-MLV Reverse Transcriptase Buffer (Promega Corporation, Madison, WI, USA) according to the manufacturer's protocol at 40˚C for 60 min. RT-qPCR was then performed using the Bio-Rad Connect Real-Time PCR platform (CFX96 Touch™, Bio-Rad Laboratories, Inc., Hercules, CA, USA). The solution contained 10 μl 2X SYBR premix ex Taq (Bio-Rad, USA), 150 ng cDNA and 0.8 μl forward primer and reverse primer (2.5 μM), respectively in a total volume of 20 μl. The primers used for amplification of human *HTR3A* gene were as follows: Forward, 5'-CATCTTCATTGTGCGGCTGGTG-3'; and reverse, 5'-AGTCATCAGTCTTGGTGGCTTGG-3'. As an internal standard, *β-actin* was amplified using the following primers: Forward, 5'-GTGGACATCCGCAAAGAC-3'; and reverse, 5'-AAAGGGTGTAACGCAACTA-3'. Absorbance value was read at the extension stage and the $2^{-\Delta\Delta Cq}$ method was used to quantify the results (26).

Western blot analysis. The lentivirus transfected cells were lysed using lysis buffer (100 mM Tris, 4% SDS, 10% glycerin, 200 mM NaCl, 2 mM EDTA) to extract total protein, and the protein concentration was measured using a bicinchoninic acid Protein Assay kit (P0012, Beyotime Institute of Biotechnology, Haimen, China). Protein samples (30 μg) were loaded onto 10% SDS-PAGE at 80 V for 2 h and transferred to polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA, USA). Then, the membranes were blocked for 1 h at 25˚C in 5% non-fat milk (in TBST buffer: 10 mM Tris, 100 mM NaCl, 0.1% Tween-20; pH=7.4) and incubated with primary antibodies as follows: Rabbit anti-HTR3A (1:400 dilution; cat. no., 10443-1-AP, ProteinTech Group, Inc., Chicago, IL, USA), rabbit anti-Bcl-2-associated death promoter (BAD; 1:1,000 dilution; cat. no., 10435-1-AP; ProteinTech Group, Inc.), rabbit anti-B-cell-2-associated X protein (BAX; 1:500 dilution; cat. no., 2774; Cell Signaling Technology, Inc., Danvers, MA, USA), rabbit anti-Bcl-2 (1:1,000 dilution; cat. no., 2876; Cell Signaling Technology, Inc.) and rabbit anti-GAPDH (1:100,000 dilution; cat. no., 10494-1-AP; ProteinTech Group, Inc.) at 4˚C for overnight. Following three wash steps with TBST (10 mM Tris, 100 mM NaCl, 0.1% Tween-20; pH=7.4), the membranes were incubated for 1 h with anti-rabbit horse-radish peroxidase-conjugated secondary antibodies (1:5,000 dilution; cat. no., SC-2054; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) at room temperature. The target bands were detected with Pierce™ ECL Western Blotting Substrate (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol.

MTT assay. The effect of *HTR3A* silencing on CRC cell proliferation was assessed by MTT assay. SW1116 (2,500 cells/well) and HCT116 (2,000 cells/well) cells infected with shCon and shHTR3A lentiviruses were seeded in 96-wells plates. At the indicated time points (1, 2, 3, 4 and 5 days), MTT (5 mg/ml, Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) was added to the plate and incubated for 4 h at 37˚C. Then, 100 μl acidic isopropanol (10% SDS, 5% isopropanol and 0.01 mol/l HCl) was added to dissolve the formazan. The absorbance at 595 nm was measured

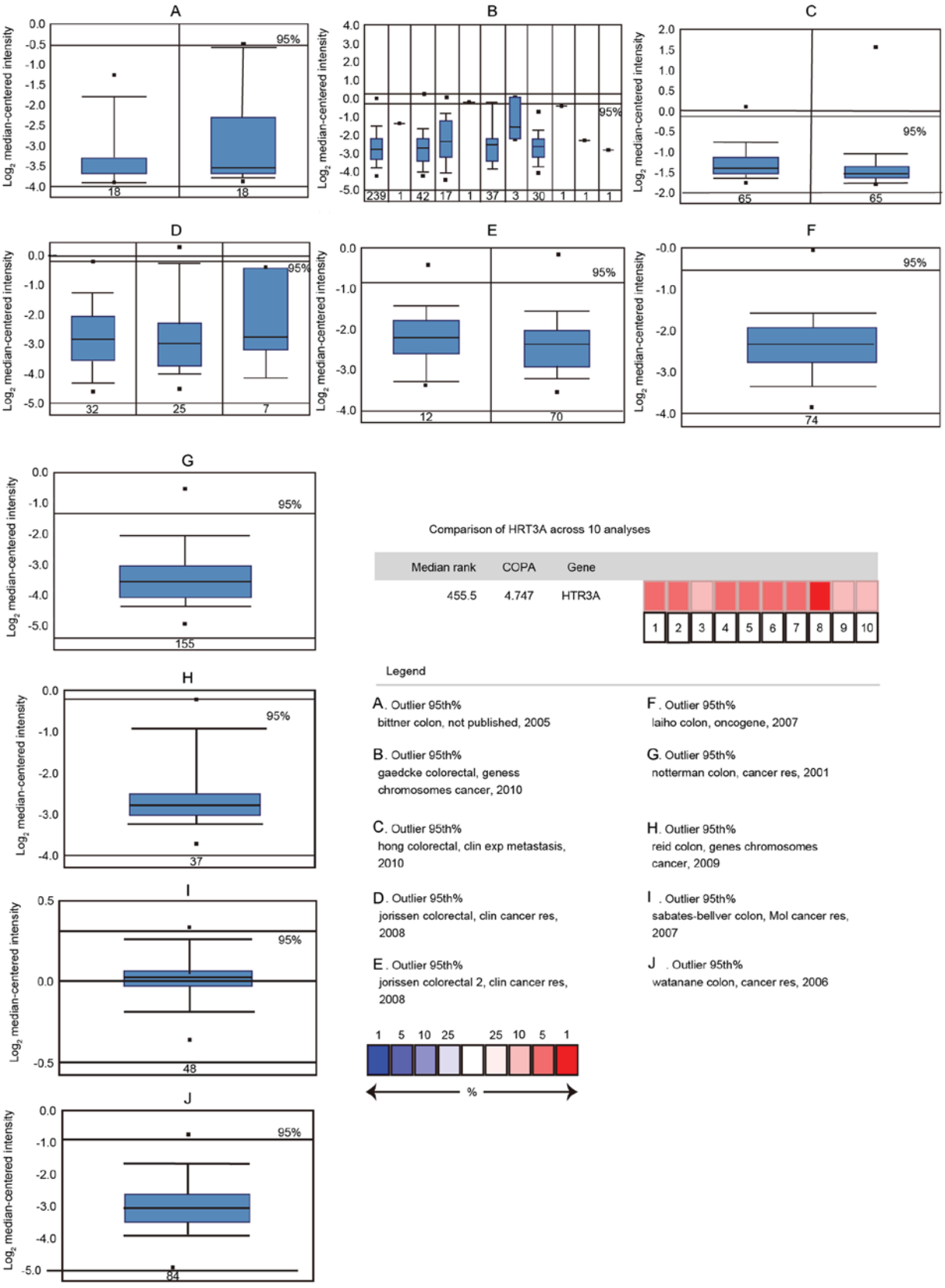

Figure 1. Expression of *HTR3A* in CRC cancer and normal tissue using the Oncomine database. (A) Outlier 95th% analysis in bittner colon. (B) Outlier 95th% analysis in laiho colon. (C) Outlier 95th% analysis in gaedcke colorectal. (D) Outlier 95th% analysis in jorissen colorectal. (E) Outlier 95th% analysis in jorissen colorectal 2. (F) Outlier 95th% analysis in laiho colon. (G) Outlier 95th% analysis in notterman colon. (H) outlier 95th% analysis in reid colon. (I) Outlier 95th% analysis in sabates-bellver colon. (J) Outlier 95th% analysis in watanane colon.

quantitatively using a microplate reader (Epoch™, BioTek Instruments, Inc.).

Colony formation assay. The effect of *HTR3A* silencing on CRC cell colony formation was measured using colony

formation assay. Equal numbers (200 cells/well) of SW1116 and HCT116 cells transfected with shCon or shHTR3A were inoculated in 6-wells plate and cultured continuously at 37˚C for 11 and 8 days, respectively. The cell medium (DMEM or RPMI-1640 as described above) was changed every 2 days. Then, the cell colonies were washed with PBS, fixed in 4% paraformaldehyde for 30 min at 4˚C, and stained with crystal violet (C0121; Beyotime Institute of Biotechnology) for 20 min at 25˚C. The single colonies (>50 cells) was counted and images were captured under a CH-2 light microscope at a magnification of x40 (Olympus Corporation, Tokyo, Japan).

Flow cytometric analysis. The effects of *HTR3A* silencing on CRC cell cycle distribution and apoptosis were examined using flow cytometric analysis. SW1116 (80,000 cells/well) and HCT116 (120,000 cells/well) cells infected with shCon or shHTR3A were seeded in 6 cm dishes and cultured for 5 days at 37˚C. For the cell cycle analysis, the cells were harvested with centrifugation at 5,000 g for 3 min at 25˚C, washed in cold PBS and fixed in 70% ethanol for overnight at 4˚C. Then, cells were stained with 500 μl propidium iodide solution containing RNase (C1052; Beyotime Institute of Biotechnology) for 30 min in darkness according to the manufacturer's protocol. For apoptosis analysis, the cells were also harvested, washed in PBS and then stained using the Annexin V-APC/7-AAD Apoptosis Detection kit (KGA1026, Nanjing KeyGen Biotech Co., Ltd., Nanjing, China) according to the manufacturer's protocol. Finally, the cells were measured using a flow cytometer (Gallios™, Beckman Coulter, Inc., Brea, CA, USA). Cell cycle data were analyzed using modifit software (version 5.0; Verity Software House, ME, USA) and apoptosis data were analyzed using FlowJo software (version 7.6; FlowJo LLC, Ashland, OR, USA).

Statistical analysis. Data are presented as mean ± standard deviation from three independent experiments. Statistical analysis was performed using GraphPad Prism software (version 5.0; GraphPad Software, Inc., La Jolla, CA, USA). An unpaired Student's t-test was used when comparing data between two groups. For multi-group analysis, one-way and two-way analysis of variance with Bonferroni post hoc test was used. P<0.05 was considered to indicate a statistically significant difference.

Results

HTR3A is efficiently silenced in CRC cell lines. *HTR3A* expression levels in colorectal cancer tissues were investigated using the publicly available Oncomine database (www.oncomine.org) in Sep. 2017. The results in Fig. 1 indicated that no significant changes in *HTR3A* expression were observed in the colorectal tumor samples compared with normal tissue. Considering that cancer heterogeneity, including cellular morphology, gene expression, metabolism, motility, proliferation and metastatic potential was a potential challenge in proto-oncogene screening, an Oncomine outlier analysis was performed in colorectal cancer samples for *HTR3A* expression, with the outlier set at the 95th percentile. As indicated in Fig. 1, the expression of *HTR3A* was demonstrated to be significantly increased in 10 databases from different studies.

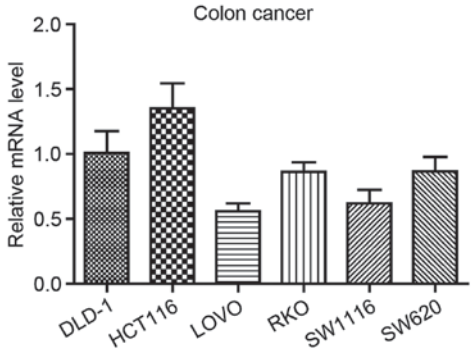

Figure 2. mRNA expression of endogenous 5-hydroxytryptamine receptor 3A was analyzed by reverse transcription polymerase chain reaction in CRC cell lines.

following this outlier analysis. These results demonstrated that HTR3A was overexpressed in certain colorectal cancer samples and may be a proto-oncogene in colorectal cancer.

To analyze the role of *HTR3A* in the occurrence and development of CRC *in vitro*, the endogenous expression of *HTR3A* was examined in the 6 CRC DLD-1, HCT116, LOVO, RKO, SW1116 and SW620 cell lines. As demonstrated in Fig. 2, it was identified that *HTR3A* was widely expressed in CRC cell lines, and the highest expression level was observed in HCT116 cells. Therefore, this cell line was selected for the subsequent analyses. In addition, to demonstrate the universality of the functional role of *HTR3A* in CRC cells, SW1116 was also randomly selected. They were used to perform the subsequent *in vitro* experiments through RNA interference. The infection efficiency, as measured using a lentivirus-mediated shRNA vector expressing GFP and observed using fluorescence microscope, was >80% in the HCT116 cells following infection with shHTR3A(S1) and shHTR3A(S2) groups (Fig. 3A), indicating that the off-target effect did not occur. As the off-target effect was excluded and it exhibited sufficient knockdown efficiency, only shHTR3A(S1) was used in SW1116 cells to silence *HTR3A* expression. Additional confirmation that *HTR3A* mRNA and protein levels were significantly decreased in HCT116 and SW1116 cells infected with shHTR3A was obtained by RT-qPCR and western blot analysis (P<0.01; Fig. 3B and C). Collectively, these results indicated that the lentivirus-mediated shRNA may silence *HTR3A* expression successfully in CRC cell lines *in vitro*.

HTR3A silencing inhibits CRC cell growth. The effect of *HTR3A* silencing on CRC cell growth was explored by MTT and colony formation assays. As indicated in Fig. 4A and B, the proliferative ability of the CRC cells was significantly decreased in HTR3A knockdown group compared with those in the shCon group (P<0.001). Concurrently, it was identified that the size and number of colonies formed in the shHTR3A (S1) or shHTR3A (S2) groups was decreased compared with those in shCon group in the HCT116 and SW1116 cells (Fig. 4C). The quantitative analysis results confirmed that the number of colonies was significantly decreased in the shHTR3A(S1) and shHTR3A (S2) groups in HCT116 cells (Fig. 4D; P<0.05), and shHTR3A (S1) group in the SW1116 group compared with shCon groups (Fig. 4D; P<0.05). Together, the data

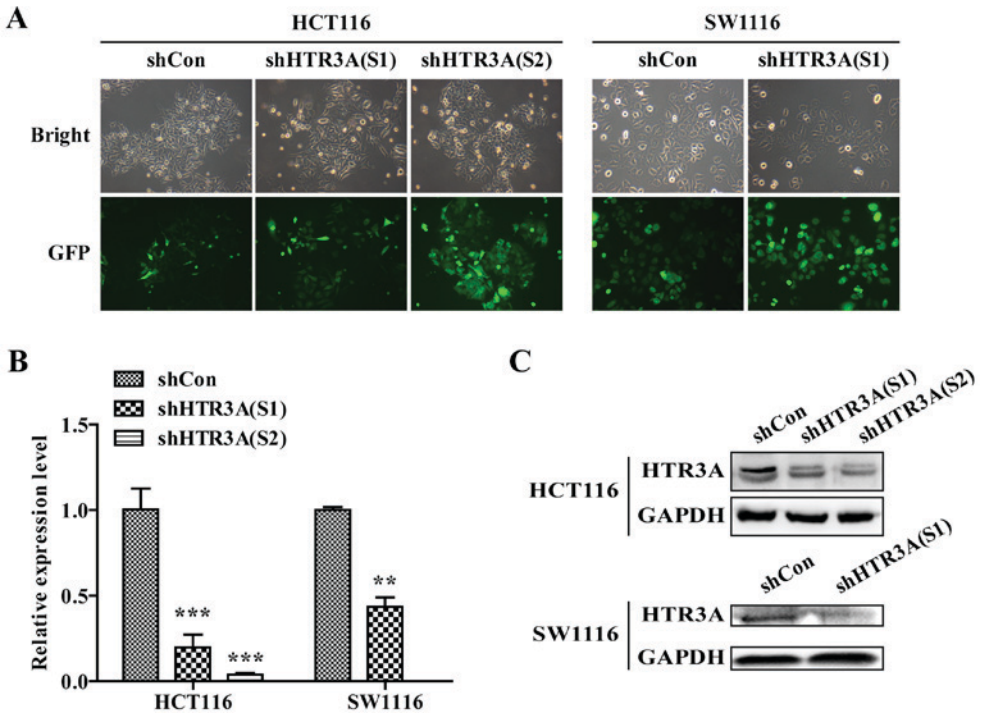

Figure 3. *HTR3A* is efficiently knocked down in CRC cell lines. (A) CRC HCT116 and SW1116 cell lines were infected with lentivirus expressing shCon, shHTR3A(S1) or shHTR3A(S2) lentiviruses for 96 h. Representative bright-field (top panel) and fluorescent-field (lower panel) microscopy images were captured using a fluorescent microscope (magnification, x100). Knockdown efficiency of *HTR3A* at (B) mRNA and (C) protein levels were examined by reverse transcription polymerase chain reaction and western blot analysis, respectively. GAPDH was used as an internal reference. **P<0.01 and ***P<0.001 vs. shCon. *HTR3A*, 5-hydroxytryptamine receptor 3A; CRC, colorectal cancer; sh, short hairpin; Con, control; GFP, green fluorescent protein.

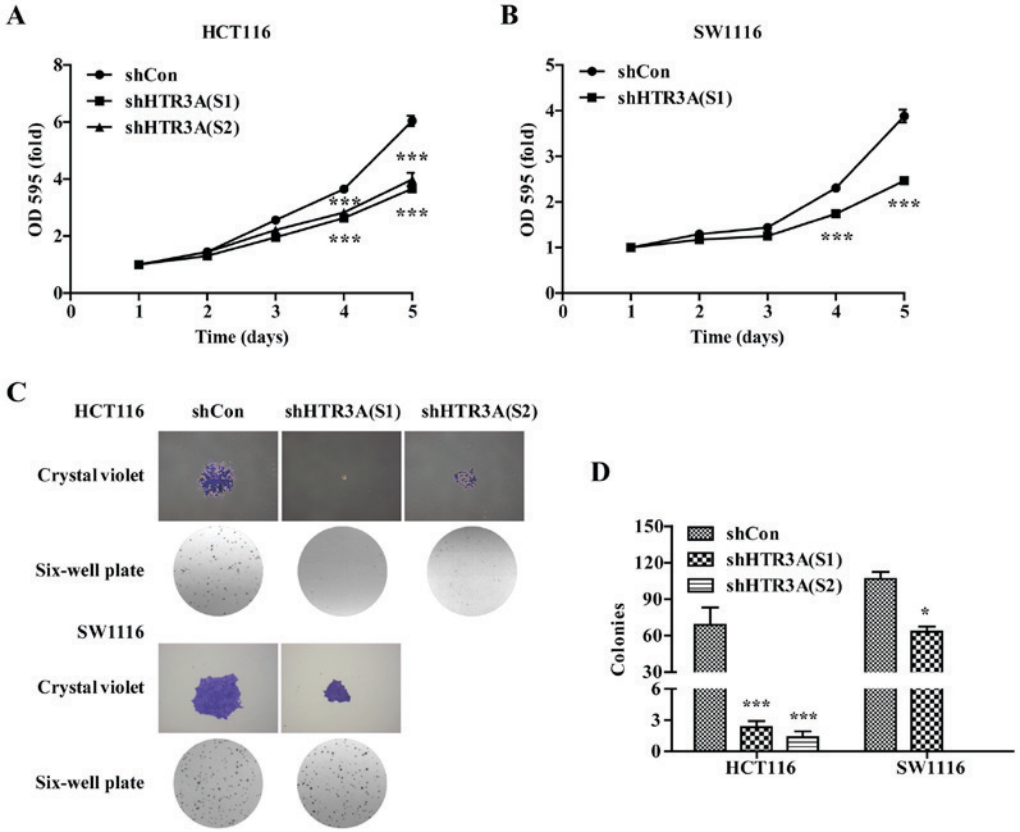

Figure 4. *HTR3A* silencing inhibits CRC cell growth. CRC (A) HCT116 or (B) SW1116 cell lines infected with shCon, shHTR3A(S1) or shHTR3A(S2) lentiviruses were seeded into 96-well plates. The cell proliferation curve was determined by MTT assay from days 1 to 5. (C) Representative images of colony formation assay in HCT116 and SW1116 cells infected with shCon, shHTR3A(S1) or shHTR3A(S2) lentiviruses. (D) Quantification of colony numbers in HCT116 and SW1116 cells. *P<0.05 and ***P<0.001 vs. shCon. *HTR3A*, 5-hydroxytryptamine receptor 3A; CRC, colorectal cancer; sh, short hairpin; Con, control; OD, optical density.

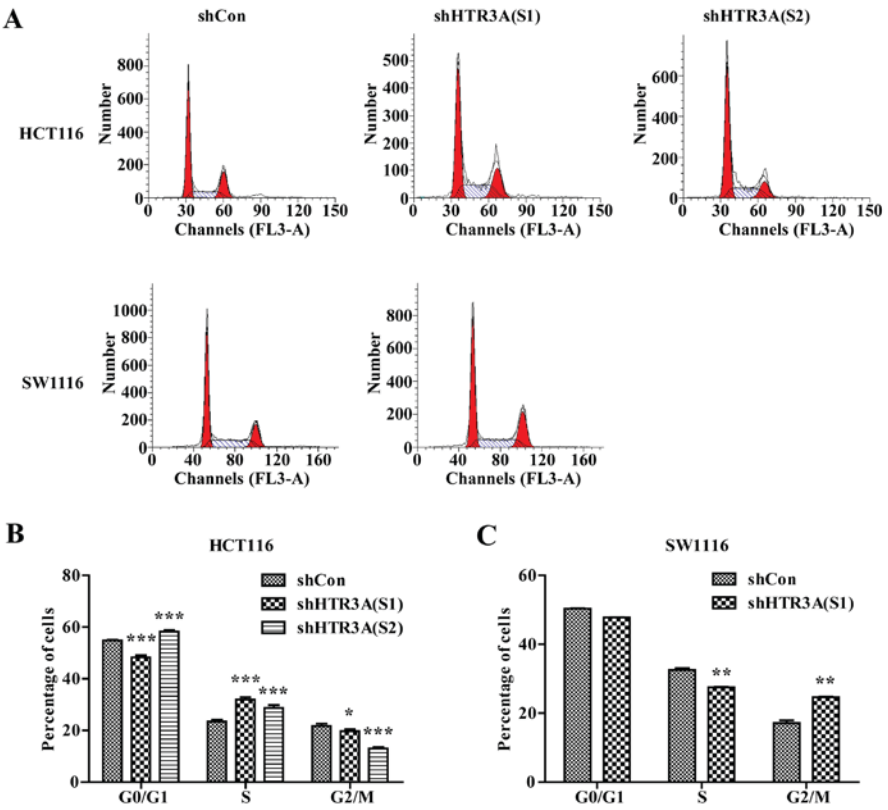

Figure 5. *HTR3A* depletion induces cell cycle arrest in CRC cells. (A) Representative cell cycle distribution data of HCT116 and SW1116 cells following *HTR3A* silencing by flow cytometry. Statistical analysis of the percentage of (B) HCT116 and (C) SW1116 cells in G_0/G_1, S and G_2/M phases. *P<0.05, **P<0.01 and ***P<0.001 vs. shCon. *HTR3A*, 5-hydroxytryptamine receptor 3A; sh, short hairpin; Con, control.

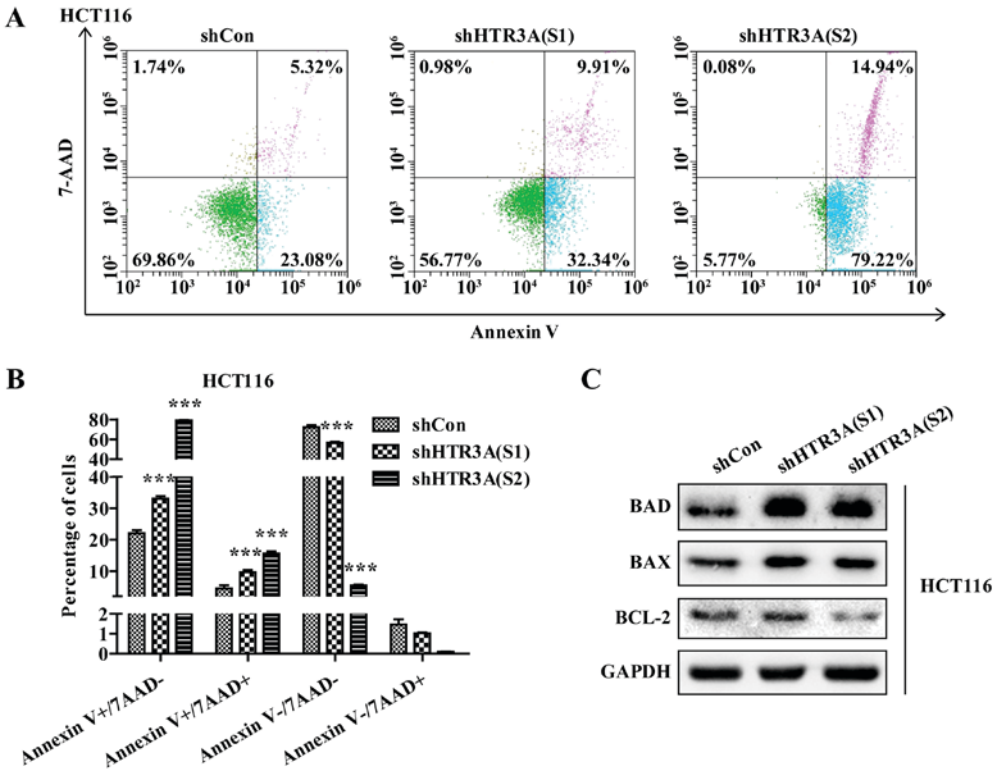

Figure 6. *HTR3A* depletion induces colorectal cancer cell apoptosis by regulating apoptosis-associated molecules. (A) Representative flow cytometry data of apoptosis in HCT116 cells following *HTR3A* silencing. (B) Statistical analysis of cell apoptosis. (C) Apoptosis-associated proteins including BAD, BAX and Bcl-2 were measured in HCT116 cells following *HTR3A* silencing by western blot analysis. GAPDH was used as an internal reference. ***P<0.001 vs. shCon. *HTR3A*, 5-hydroxytryptamine receptor 3A; sh, short hairpin; Con, control; BAD, BAX and Bcl-2, B-cell lymphoma 2; BAD, Bcl-2-associated death promotor; BAX, B-cell-1-associated X protein; early apoptosis, Annexin V+/7AAD-; late apoptosis, Annexin V+/7AAD+; viable cells, Annexin V-/7AAD-; necrotic cells, Annexin V-/7AAD+.

indicated that CRC cell growth was inhibited following *HTR3A* knockdown *in vitro*.

HTR3A silencing induces CRC cell cycle arrest. In light of the effect of *HTR3A* silencing on CRC cell growth, whether *HTR3A* depletion also affected the cell-cycle progression was investigated. Flow cytometric analysis indicated that *HTR3A* silencing significantly increased the percentage of cells in S phase in the shHTR3A(S1) and shHTR3A(S2) groups [shHTR3A(S1) group, 31.98±0.92 vs. 23.43±0.77%, P<0.01; shHTR3A(S2) group, 28.69±1.13 vs. 23.43±0.77%, P<0.001], accompanied with decreased G_2/M phase [shHTR3A(S1) group, 19.70±0.83% vs. 21.70±0.95%, P<0.05; shHTR3A(S2) group, 13.03±0.57% vs. 21.70±0.95%, P<0.001] compared with those in the shCon group in HCT116 cells (Fig. 5A and B). Additionally, in the SW1116 cells, an increase in the percentage of cells in S phase in the shHTR3A(S1) group (27.51±0.12 vs. 32.50±0.64%; P<0.01) was observed, but a decrease in G_2/M phase cells [24.68±0.10 vs. 17.14±0.77%; P<0.01 in shHTR3A(S1) group] in comparison with the shCon group was indicated (Fig. 5A and C). These results suggested that *HTR3A* knockdown may damage cell growth through arresting cell cycle in CRC cells.

Knockdown of HTR3A induces apoptosis by regulating apoptosis-associated molecules. Next, the effect of *HTR3A* silencing on apoptosis was examined to initially explore how *HTR3A* was involved in CRC cell growth. As expected, *HTR3A* knockdown led to an increased proportion of early apoptotic cells [Annexin V+/7AAD-shHTR3A(S1) group, 33.04±0.78%; shHTR3A(S2) group, 78.85±0.55%] and late apoptotic cells [Annexin V+/7AAD+ shHTR3A(S1) group, 9.65±0.72%; shHTR3A(S2) group, 15.64±0.66%] compared with the shCon group (Annexin V+/7AAD-, 22.05±0.96%, P<0.001; Annexin V+/7AAD+, 4.45±1.06%, P<0.001) in HCT116 cells (Fig. 6A and B). Accordingly, the expression of apoptosis-associated proteins including BAD, BAX, and Bcl-2 were also detected, and it was identified that the expression levels of BAD and BAX were upregulated while Bcl-2 was downregulated in *HTR3A*-knockdown cells (Fig. 6C). Taken together, these results demonstrated that *HTR3A* knockdown accelerated CRC cell apoptosis by the regulation of partial Bcl-2 family protein expression, including BAD, BAX and BCL-2 protein.

Discussion

HTR3A is considered to be involved in various biological processes, including heart arrhythmias, organismal energy homeostasis, including inhibited thermogenesis through Htr3 in BAT and increased lipogenesis, and interneuron migration (27-29). Additionally, it has been revealed that HTR3A is present in large B cell lymphomas and its agonists may promote growth of CRC cells, which implies that HTR3A may contribute to tumor development (30,31). In the present study, the potential role of HTR3A in CRC was first demonstrated by silencing HTR3A expression, and it was demonstrated that HTR3A regulated the growth of CRC cells *in vitro* by controlling partial Bcl-2 family protein expression, including BAD, BAX and BCL-2 protein.

The involvement of sustaining uncontrolled proliferation is one of most fundamental traits of cancer cells (30). In the present study, the effect of *HTR3A* on the proliferation of CRC cells *in vitro* was examined. Using MTT and colony formation assays, it was identified that *HTR3A* silencing was able to inhibit the growth in CRC HCT116 and SW1116 cells. The cell cycle controls the transition from quiescence to proliferation in cells (31,32). Notably, knockdown of *HTR3A* arrested the cell cycle at S phase in HCT116 cells, but at G_2/M phase in SW1116 cells.

It was suggested that 5-HT, as the ligand of *HTR3A*, may decrease apoptosis rate through 5-HT1B receptors and 5-HT transporters in pulmonary artery smooth muscle cells (33). Concurrently, 5-HT1B receptor antagonists exerted anti-mitogenic and apoptotic effects on the CRC HT29 cell line (34). Additionally, the *HTR3A* antagonist tropisetron was able to mediate apoptosis in the breast cancer MCF-7 cell line (34,35). An additional *HTR3A* antagonist, Y25130 hydrochloride, was also demonstrated to induce apoptosis in the HT29 cell line (36). These results indicate that the inhibition of 5HT3A-induced apoptosis may be a widespread phenomenon in cancer cells. Consistent with the previous results, the present study identified that *HTR3A* depletion accelerated cell apoptosis in CRC cells HCT116 and altered the expression of apoptosis-associated proteins including BAD, BAX and Bcl-2. Cell apoptosis is important for maintaining homeostasis, and impaired apoptosis is widely considered to be a pivotal process in oncogenesis. It has been established that the Bcl-2 family proteins mediate cell apoptosis primarily through the involvement of apoptosis-associated signaling pathways (37). Among these proteins, BAD and BAX are pro-apoptotic regulators (38); however, Bcl-2 is primarily a regulator against apoptosis (39). However, the exact mechanisms of *HTR3A* in CRC require additional investigation.

HTR3A has been demonstrated to induce emesis during chemotherapy and radiotherapy administered during cancer treatment (40). It was revealed that *HTR3A* antagonists exhibited anti-emetic effects during chemotherapy and radiotherapy (40). At present, *HTR3A* antagonists remain the primary drugs used in clinical settings to treat emesis. Considering the fact that dysregulation of *HTR3A* in CRC may induce apoptosis, it is hypothesized that *HTR3A* antagonists may enhance the efficiency in CRC treatment. Additional studies are required to demonstrate this hypothesis and determine whether the combined use of *HTR3A* antagonists and medicine for CRC is an improved choice compared with current strategies for CRC treatment.

In summary, the data of the present study demonstrated that the dysregulation of *HTR3A* primarily participated in the proliferation, cell cycle progression and apoptosis process of CRC cells. Additional in-depth studies of the mechanism underlying these observations are required to demonstrate whether *HTR3A* may be used as a novel target for CRC therapy.

Acknowledgements

Not applicable.

Funding

The present study was supported by Program for Clinical Research and Innovation of Renji Hospital, School of Medicine, Shanghai Jiao Tong University (grant no. PYZY16-007).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JC performed the vector construction experiments and drafted the manuscript. JT participated in the research design, reviewed the literature and examined the data. ZW participated in the qPCR and western blot experiments. JL participated in the cellular function experiments. CZ participated in the data analysis and figure formatting.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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