ARTIFICIAL CELLS, NANOMEDICINE, AND BIOTECHNOLOGY
2019, VOL. 47, NO. 1, 767–775
https://doi.org/10.1080/21691401.2019.1577880

RETRACTED ARTICLE

RETRACTED ARTICLE: Long non-coding XIAP-AS1 regulates cell proliferation, invasion and cell cycle in colon cancer

Xiaohong Lu, Yuanjie Yu and Shiyun Tan

Department of Gastroenterology, Renmin Hospital of Wuhan University, Hubei Key Laboratory of Digestive System Disease, Wuhan, Hubei, China

ABSTRACT

Colon cancer is one of the most commonly diagnosed and deadly cancers worldwide. Further understanding of the biological mechanisms is important for exploring the molecular biomarkers and therapeutic targets of this disease. Dysregulation of long non-coding RNAs (lncRNAs) has been reported to be associated with the development and progression of various cancers. XIAP-AS1 is a novel lncRNA, which can regulate apoptosis in gastric cancer cells. However, the role of XIAP-AS1 in colorectal cancer (CRC) remains unclear. In this study, we found that XIAP-AS1 expression was significantly increased in CRC tissues and its expression showed a positive correlation with TNM stage and cumulative survival rate of CRC. To investigate whether XIAP-AS1 regulates the progression of CRC, we knocked down its expression in several CRC cell lines. CCK-8 assays showed that XIAP-AS1 knockdown remarkably suppressed CRC cell growth and arrested the cell cycle at the G0/G1 phase (flow cytometric analysis). Furthermore, XIAP-AS1 knockdown also remarkably blocked cell invasion of colon cancer cells by regulating the expression of EMT markers, such as E-cadherin, ZO-1, vimentin, and N-cadherin. Importantly, we found that XIAP-AS1 knockdown significantly reduced STAT3 phosphorylation. Overall, this study suggests that lncRNA XIAP-AS1 might serve as a potential oncogene for colon cancer.

Introduction

Colon cancer is one of the third most common type of malignancy in the population, colon cancer affects millions of people worldwide [1]. The morbidity of colon cancer is rapidly increasing in eastern Asian countries including China [2]. About 50% of patients with colon cancer will present with metastasis either at the time of diagnosis or develop distant relapses after therapy [3]. Current primary treatments of colon cancer include surgery, chemotherapy, and radiotherapy [4]. Chemical drugs targeting epidermal growth factor receptor (EGFR) and KRAS have been reported to significantly prolong the survival of CRC patients [5]. A progressive accumulation of genetic changes during the development and progression of colon cancer results in tumour transformation from the normal colonic mucosa [6]. A deeper understanding of the intracellular and biological mechanisms in the pathogenesis of colon cancer is beneficial for exploring novel therapeutic approaches and agents.

Rapid development of genome sequencing results in the identification of non-coding RNA (ncRNA), which have similar properties to mRNA but are not translated into proteins [7]. However, these molecules have important biological functions. Long non-coding RNAs (lncRNAs) are a class of non-protein coding transcripts over 200 nucleotides (nt) in length [8]. LncRNAs have been reported to regulate gene expression at the epigenetic level, transcriptional level, and post-transcriptional level through interacting with other molecules such as proteins, RNAs, and DNAs [9]. LncRNAs have been shown to be intensively involved in the pathogenesis of human diseases. Notably, lncRNAs have been identified as an important regulator of cancer progression and metastasis and play key roles in the physiological and pathological processes of various types of cancer [10]. The physiological roles of lncRNAs in colon cancer development have attracted considerable attention [11]. LncRNAs such as H19, CCAT1, CCAT2, and TUG1 are involved in the initiation and progression of colon cancer and regulate the biological properties of cancer cells [3]. The protein X-linked inhibitor of apoptosis (XIAP) acts as an important regulator and one of the most promising targets for a variety of cancers [12]. XIAP-AS1 is a novel lncRNA complementary to the XIAP transcript. It has been reported that XIAP-AS1 regulates apoptosis in gastric cancer cells by interacting with Sp1 [13].

In this study, we analyzed the expression of lncRNA XIAP-AS1 in normal and CRC tissues by real-time PCR analysis. Our results show that lncRNA XIAP-AS1 expression was significantly increased in CRC tissues. Next, using CCK-8 and flow cytometric analysis assays, we found that lncRNA XIAP-AS1 promotes CRC cell growth by regulating the cell cycle as well.

CONTACT Xiaohong Lu km68wu26@sina.com Department of Gastroenterology, Renmin Hospital of Wuhan University, Hubei Key Laboratory of Digestive System Disease, No. 238, Jiefang Road, Wuhan, Hubei 430060, China

© 2019 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
as cell migration and invasion. These results suggest that IncRNA XIAP-AS1 might act as an oncogene during CRC progression and may also provide a potential target for the treatment of CRC.

Materials and methods

Patients and tissue samples

A total of 75 cancer tissue samples and corresponding adjacent normal tissues were obtained from stages I/II and III/IV (according to the seventh version of the American Joint Committee on Cancer staging system) patients who underwent surgery for colon cancer without preoperative chemotherapy or radiotherapy. This study was approved by the Medical Ethics Committee of Sun Yat-Sen University. Experiments were guided by the World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects. We confirmed that all methods were performed in accordance with the relevant guidelines and regulations of the Medical Ethics Committee of Sun Yat-Sen University. Tumour specimens and corresponding adjacent normal tissues were collected and stored in liquid nitrogen until use.

Cell culture, treatment, and transfection

The human colon cancer cell lines SW480, LOVO, and normal colonic mucosa epithelial cells (NCM460) were obtained from the American Type Culture Collection (ATCC, USA). Cells were routinely maintained in DMEM/F-12 (1:1) medium (HyClone, South Logan, UT) supplemented with 10% foetal bovine serum (FBS, USA) at 37 °C in humidified air containing 5% CO₂. For knockdown experiments, cells were transfected with siRNA targeting XIAP-AS1 and non-targeting siRNA (GenePharma, Shanghai, China) using Lipofectamine 2000 (Life Technologies, Eugene, OR) in accordance with the manufacturer’s instructions. Successful knockdown was confirmed by real-time PCR (RT-PCR). The sequence for si-XIAP-AS1 was 5’-GATCCAAAAGAGAGAGAGGGATTCGAGAATCCCTCTCTCTTTTG-3’ (sense) and 5’-AATTCAAAAAAAAGAGAGAGAGAGAGGATTCGAGAATCCCTCTCTCTCTTTTG-3’ (antisense). The negative control siRNA sequence was 5’-GATCTTTTCTCCCGAACGTGCACGTGCAGTGAAGCTTTTG-3’ (sense) and 5’-AATTCAAAAAAAATTCGAGAATCCCTCTCTCTCTTTTG-3’ (antisense).

Real-time polymerase chain reaction (PCR)

Total intracellular RNA was extracted from cells and tissues using Trizol reagent (Life Technologies, Eugene, OR) in accordance with the manufacturer’s instructions. Equal amounts of total RNA (2 μg) were subjected to TaqMan one-step reverse transcription PCR (Applied Biosystems, Foster City, CA), followed by real-time PCR on an ABI PRISM 7700 sequence detection system (Applied Biosystems, Foster City, CA) according to the manufacturer’s protocols. The expression levels of relative genes were normalized to the internal control glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using the 2-ΔΔCT method. The following primers were used in this study: XIAP-AS1: 5’-TACCCCTGGGAGACAGAATGAAAAGC-3’ (forward) and 5’-TGATGGCATG-GACTGTGGTC-3’ (reverse); human GAPDH: 5’-GGAGAAGGGCTGGGGCTCAT-3’ (forward) and 5’-ACCTGTTTTTCTAGCCCTTCTC-3’ (reverse).

Western blot analysis and antibodies

Protein lysates were prepared using cell lysis buffer (Cell Signaling, Boston, MA) supplemented with a protease inhibitor cocktail and phenylmethylsulphonyl fluoride (Roche, Basel, Switzerland). Protein concentrations were determined using a protein assay kit (Bio-Rad, Hercules, CA). Equal amounts of protein extract (20 μg) were separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), then transferred onto a polyvinylidene fluoride (PVDF) membrane (Sigma-Aldrich, St. Louis, MO). Membranes were sequentially probed with specific primary antibodies and secondary antibodies. Blots were visualized using the ECL chromogenic substrate (Thermo Fisher Scientific, Waltham, MA). After a blocking in 5% non-fat milk, the membrane was probed with primary anti-cyclin D1 (dilution 1:1000, Santa Cruz Biotechnology, Santa Cruz, CA), anti-cyclin E (dilution 1:2000, Santa Cruz Biotechnology, Santa Cruz, CA), anti-cyclin A (dilution 1:500, Santa Cruz Biotechnology, Santa Cruz, CA), c-Myc (dilution 1:2000, Santa Cruz Biotechnology, Santa Cruz, CA), anti-β-catenin (dilution 1:800, Santa Cruz Biotechnology, Santa Cruz, CA), anti-ZO-1 (dilution 1:1000, Santa Cruz Biotechnology, Santa Cruz, CA), anti-E-cadherin (dilution 1:1000, Santa Cruz Biotechnology, Santa Cruz, CA), anti-N-cadherin (dilution 1:1000, Santa Cruz Biotechnology, Santa Cruz, CA), anti-vimentin (dilution 1:1000, Santa Cruz Biotechnology, Santa Cruz, CA), and anti-actin (dilution 1:5000, Santa Cruz Biotechnology, Santa Cruz, CA) antibody. After washing, the membrane was incubated with horseradish peroxidase-conjugated (HRP) secondary antibody (1:2000, Bio-Rad, Hercules, CA). Blots were visualized using the ECL detection system (Thermo Fisher, Waltham, MA) and quantified by densitometry using Quantity One software (Bio-Rad, Hercules, CA).

Cell-cycle analysis

Cell cycle was evaluated by flow cytometric analysis. After the necessary transfection, cells were fixed with 70% ethanol and stored at 4 °C overnight. After washing three times with PBS, cells were incubated with 50 μg/ml PI with 100 μg/ml of RNase A in 0.5% Triton-X 100 in darkness at 37 °C for 30 min. Signals were analyzed using a FACS Calibur Cytometer (BD Biosciences, Franklin Lakes, NJ).

Cell viability assay

After the indicated transfection, the cell viability was determined using a cell counting kit-8 (CCK-8) assay. Briefly, cells were seeded into 96-well plates at the density of 5 × 10³ cells
per well and transfected with siRNA-XIAP-AS1 or siRNA-scramble. The CCK-8 reagent was added and incubated with the cells for 1 h. Absorbance measured at 450 nm was used to index cell proliferation.

Cell invasion assay
Matrigel with Transwell inserts (8.0 mm pore size with polyethylene terephthalate membrane) was used to determine cell invasion. Briefly, 10 mg of Matrigel (BD Biosciences, Franklin Lakes, NJ) was added to pre-coat filters. After the necessary transfection, cells (10^5) in serum-free medium were seeded into the upper chamber and incubated for 24 h. Cells that invaded to the lower side of the membrane were fixed with methanol and stained with 0.1% crystal violet.

Statistical analysis
Statistical analysis was carried out using SPSS (version 12.0) software (SPSS, Chicago, IL). Experimental data are expressed as means ± standard error (SE) using two-way analysis of variance (ANOVA). p values less than .05 were considered statistically significant.

Results
To investigate the role of IncRNA XIAP-AS1 in CRC progression, we first examined its expression in CRC. Samples were obtained from 75 tissue specimens and an equal number of adjacent normal tissues. Quantitative PCR showed that the level of XIAP-AS1 was significantly increased in primary CRC tumour tissues as compared to adjacent normal tissues (Figure 1(A)). This result suggests that IncRNA XIAP-AS1 may play a functional role in the progression of CRC. To address whether XIAP-AS1 expression is correlated with the clinicopathological development of colon cancer, its expression was determined in different stages of CRC. As expected, XIAP-AS1 expression was higher in TMN stage IV samples than in TMN stage I/II/III tissue samples (Figure 1(B)). In addition, we examined the effects of XIAP-AS1 on disease outcomes in colon cancer patients using Kaplan–Meier survival analysis. We found that the relapse-free survival rate was significantly lower in patients with high expression of XIAP-AS1 than in those with low XIAP-AS1 expression (Figure 1(C)).

To investigate how XIAP-AS1 regulates CRC progression, we first examined its expression in several CRC cell lines. Interestingly, we found that expression of XIAP-AS1 in the colon cancer cell line SW480 (Figure 2(A)) and LoVo cells (Figure 2(B)) was significantly higher than that in normal colonic mucosa epithelial cells (NCM460). The transcriptional factor p63 has been reported to regulate the expression of IncRNAs in colon cancer cells [14]. The expression of p63 was silenced by transfection with p63-siRNA in SW480 and LoVo cells. The results in Figure 2(C,D) indicate that knockdown of p63 increased the expression of XIAP-AS1 in both cell lines, whereas p63 overexpression produced the opposite result (Figure 2(E,F)). Furthermore, the effects of XIAP-AS1 on cell proliferation of colon cancer cells were assessed. Cell proliferation assay (CCK-8) results indicate that XIAP-AS1 knockdown significantly suppressed the growth of SW480 (Figure 3(A)) and LoVo cells (Figure 3(B)), whereas p63 overexpression produced the opposite result (Figure 3(C,D)). To investigate how XIAP-AS1 suppresses CRC cell growth, the effects of XIAP-AS1 on the CRC cell cycle were also examined. As shown in Figure 4(A,B), the cell cycles of both SW480 and LoVo cells, which were transfected with XIAP-AS1-siRNA, were arrested in the G0/G1 phase, whereas p63 overexpression produced the opposite result (Figure 4(C,D)). Additionally, we determined the effects of XIAP-AS1 on the expression of cell cycle–related proteins. As expected, XIAP-AS1 overexpression significantly reduced the expression of cyclin D1, cyclin E, and c-Myc in both SW480 (Figure 5(A)) and LoVo cells (Figure 5(B)). However, cyclin A levels remained consistent. As we know, β-catenin is a crucial signalling factor regulating oncogenesis. Cyclin D1 and c-Myc are important target genes of the Wnt/β-catenin pathway. Therefore, we measured the expression of β-catenin. As expected, the results in
Figure 5(C,D) indicate that silencing of XIAP-AS1 reduced the expression of β-catenin.

Cell invasion plays an important role in tumour progression and results in an unfavourable prognosis in colon cancer. Hence, we determined the effects of XIAP-AS1 on cell invasion in SW480 and LoVo cells. Interestingly, our results show that transfection with XIAP-AS1-siRNA significantly reduced the number of invasive SW480 cells (Figure 6(A)) and LoVo cells (Figure 6(B)), whereas p63 overexpression produced the opposite result (Figure 6(C,D)).

Epithelial–mesenchymal transition (EMT) plays a key role in tumour metastasis. Here, we investigated the role of XIAP-AS1 on the expression of EMT markers, such as ZO-1, E-cadherin, N-cadherin, and vimentin. Our results indicate that...
Figure 3. Knockdown of XIAP-AS1 inhibited cell proliferation. Cells were transfected with XIAP-AS1-siRNA or scrambled siRNA in human SW480 cells and LoVo cells. NS: non-specific siRNA. (A and C). Cell proliferation was determined by CCK-8 assay in XIAP-AS1 knockdown (A) or overexpressing (C) SW480 cells. (B and D) Cell proliferation was determined by CCK-8 assay in XIAP-AS1 knockdown (B) or overexpressing (D) LoVo cells (*p < .05 versus NS group).

Figure 4. Knockdown of XIAP-AS1 arrested human colon cancer cell lines in the G0/G1 phase. Cells were transfected with XIAP-AS1-siRNA or scrambled siRNA in human SW480 cells and LoVo cells. NS: non-specific scrambled-siRNA. (A and C) Cell cycle was determined by flow cytometry in XIAP-AS1 knockdown (A) or overexpressing (C) SW480 cells. (B and D) Cell cycle was determined by flow cytometry in XIAP-AS1 knockdown (B) or overexpressing (D) LoVo cells (*p < .05 versus NS group).
Figure 5. Effects of XIAP-AS1 on the expression of β-catenin and cell cycle proteins. Cells were transfected with XIAP-AS1 siRNA or scrambled siRNA in human SW480 cells and LoVo cells. NS, non-specific scrambled-siRNA. (A) Expression of cyclin D1, cyclin E, c-Myc, and cyclin A in SW480 cells. (B) Expression of cyclin D1, cyclin E, c-Myc, and cyclin A in LoVo cells. (C) Expression of β-catenin in SW480 cells. (D) Expression of β-catenin in LoVo cells (*p < .05 versus NS group).

Figure 6. Transfection with lncRNA XIAP-AS1 siRNA reduced human colon cancer SW480 cells and LoVo cells metastasis in vitro. Cells were transfected with XIAP-AS1 siRNA or scrambled siRNA in human SW480 cells and LoVo cells. NS: non-specific siRNA. (A and C) Cell invasion of XIAP-AS1 knockdown (A) or overexpressing (C) SW480 cells was determined by Matrigel assay. (B and D) Cell invasion of XIAP-AS1 knockdown (B) or overexpressing (D) LoVo cells was determined by Matrigel assay (*p < .05 versus NS group).
silencing of XIAP-AS1 significantly increased the expression of ZO-1 and E-cadherin, but decreased the expression of N-cadherin and vimentin in both SW480 (Figure 7A) and LoVo cells (Figure 7B). These results indicate that XIAP-AS1 might regulate colon cancer cell metastasis via EMT.

STAT3 has been considered as an important transcriptional factor which plays an essential role in gene transcription and cell fate determination. Here, we found that XIAP-AS1 knockdown reduced the phosphorylation of STAT3 in both SW480 (Figure 8A) and LoVo cells (Figure 8B), suggesting a possible molecular mechanism.

Discussion

Colon cancer is the third most diagnosed malignancy in the world, therefore, posing to a serious threat to human health. The incidence is increasing in young adults, especially in developing countries [15]. In past decades, efforts have been made to study the pathological mechanisms as a means to reduce the mortality of colon cancer. However, the prognosis of this disease is still poor due to late clinical diagnosis of the malignancy [16]. The accumulation of genetic alterations such as NRAS, KRAS, and BRAF has been associated with the malignancy [16]. The current study demonstrates that silencing of XIAP-AS1 causes G0/G1 phase cell-cycle arrest via downregulation of cyclin D1, cyclin E, and c-Myc levels, which appears to be the underlying mechanism in colon cancer cell growth inhibition. The expression of cell-cycle-related proteins is regulated by the Wnt-signalling pathway. Stabilization and subsequent nuclear translocation of β-catenin are critical steps in activating downstream signalling and expression of cell cycle proteins. β-Catenin activates the transcription of various genes involved in cell proliferation, migration, and metastasis [21]. Hyperactivation of Wnt/β-catenin signalling and increased expression of β-catenin have been considered as characteristic features of colon cancer development. Here, we found that XIAP-AS1 knockdown significantly suppressed CRC cell growth and arrested the cell cycle at the G0/G1 phase. Furthermore, cell-cycle-related genes, including cyclin D, cyclin A, and cyclin E, are also regulated by XIAP-AS1 silencing. Additionally, our findings indicate that XIAP-AS1 knockdown significantly reduced the expression level of β-catenin in colon cancer cells, implying that XIAP-AS1 promoted cell growth and invasion by potentiating the Wnt/β-catenin pathway.

As a critical mechanism of tumour metastasis [22], EMT has been shown to be involved in the aggressive tumour biology of a variety of cancers, including colon cancer [23]. During the process of EMT, cells lose their cellular epithelial features and gain mesenchymal properties with increased migration and invasion abilities, which can invade and migrate through the body [24]. Epithelial markers such as E-cadherin and ZO-1 and mesenchymal markers such as vimentin and N-cadherin have been widely used to index EMT [25]. Indeed, decreased levels of E-cadherin and ZO-1 in tumour cells have been associated with liver metastasis of colon cancer [26]. Meanwhile, increased expression of vimentin and N-cadherin has been found in colon cancer cells [27]. A recent study reported that another IncRNA ATB promotes the progression of colon cancer and predicts poor prognosis by
repressing the expression of E-cadherin [28]. In the current study, our results indicate that silencing of XIAP-AS1 decreased the expression of E-cadherin (E-cad) and ZO-1 but increased the expression of vimentin and N-cadherin, suggesting that XIAP-AS1 might have an important potential role in facilitating EMT colon carcinoma. Agents or therapeutic approaches targeting XIAP-AS1 might be beneficial for the blockage of EMT and metastasis of colon cancer.

The pathological mechanism of colon cancer is complicated and needs to be elucidated. A variety of risk factors have been linked with the pathogenesis of colon cancer, including genetics, ageing, obesity, and a personal history of inflammatory bowel disease [29]. The biological function of XIAP-AS1 in vivo is rarely reported. In an in vivo mouse xenograft gastric cancer model, tumour cell proliferation was inhibited by XIAP-AS1 knockdown in response to tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) administration [13]. It should be noted that a major limitation of this study is that all the findings here are based on in vitro cell culture model experiments. More future in vivo studies are necessary to confirm the physiological function of XIAP-AS1 on colon cancer.

In summary, our findings indicate that XIAP-AS1 promoted cell growth and invasion by facilitating the Wnt/β-catenin pathway and EMT. Based on these observations, we speculate that XIAP-AS1 plays an oncogenic role in colon cancer progression and serves as a potential target for cancer prevention and treatment.

**Disclosure statement**

None of the authors of this work need to disclose has any conflicts of interest.
colorectal adenomas and adenocarcinomas. Dis Colon Rectum. 2006;49:588–594.

[15] Kiliaraki V, Pallangyo CK, Greten FR, et al. Mesenchymal cells in colon cancer. Gastroenterology. 2017;152:964–979.

[16] Pattison AM, Merlino DJ, Blomain ES, et al. Guanylyl cyclase C signaling axis and colon cancer prevention. World J Gastroenterol. 2016;22:8070–8077.

[17] Cunningham D, Atkin W, Lenz HJ, et al. Colorectal cancer. Lancet. 2010;375:1030–1047.

[18] Zhai HY, Sui MH, Yu X, et al. Overexpression of long non-coding RNA TUG1 promotes colon cancer progression. Med Sci Monit. 2016;22:3281–3287.

[19] Wu WK, Wang XJ, Cheng AS, et al. Dysregulation and crosstalk of cellular signaling pathways in colon carcinogenesis. Crit Rev Oncol Hematol. 2013;86:251–277.

[20] Strey CW, Schamell L, Oppermann E, et al. Valproate inhibits colon cancer growth through cell cycle modification in vivo and in vitro. Exp Ther Med. 2011;2:301–307.

[21] Haase G, Gavert N, Brabletz T, et al. The Wnt target gene L1 in colon cancer invasion and metastasis. Cancers (Basel). 2016;8:48.

[22] Yue B, Qiu S, Zhao S, et al. lncRNA-ATB mediated E-cadherin repression promotes the progression of colon cancer and predicts poor prognosis. J Gastroenterol Hepatol. 2016;31:595–603.

[23] Carethers JM. Risk factors for colon location of cancer. Transl Gastroenterol Hepatol. 2018;3:76.