

Cervical carcinoma high-expressed long non-coding RNA 1 may promote growth of colon adenocarcinoma through interleukin-17A

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Abstract. Cervical carcinoma high-expressed long non-coding RNA 1 (CCHE1) has been demonstrated to promote several different types of cancer; however, the involvement of CCHE1 in other types of cancer remains unknown. In the present study, the expression levels of CCHE1 and interleukin (IL)-17A were increased in the plasma of patients with metastatic and non-metastatic colon adenocarcinoma (MC and NMC, respectively) compared with the healthy controls. There was no significant difference in the plasma expression levels of CCHE1 and IL-17A in patients with MC compared with patients with NMC. The plasma expression levels of CCHE1 and IL-17A were positively associated with the primary tumor diameter. A significant correlation as demonstrated between the serum levels of CCHE1 and IL-17A in patients with colon adenocarcinoma, but not in the healthy controls. CCHE1 and IL-17A overexpression promoted colon adenocarcinoma cell proliferation. Transfection of small interfering RNA against IL-17A partially reversed the effects of CCHE1 overexpression on cancer cell proliferation. Upregulation of IL-17A was observed after CCHE1 overexpression, while IL-17A overexpression did not significantly change the expression level of CCHE1. Therefore, CCHE1 may promote growth of colon adenocarcinoma through interactions with IL-17A.

Introduction

Colorectal cancer is one of the most frequently diagnosed malignancies and one of the leading causes of cancer-related deaths worldwide (1,2). In the United States, colorectal cancer affects ~140,000 new cases and causes more than 50,000 deaths per year (3). In developing countries, such as China, changes in people's dietary habits in have lead to an increase in

the incidence rate of this disease (4,5). Although the survival rates of patients with colon cancer have improved significantly over the past several decades, successful and complete treatment is complicated by the high prevalence of distant tumor metastasis at the first diagnosis (6).

The role inflammation plays during the pathogenesis of colon cancer and inflammatory bowel disease is well established and is considered to be a significant risk factor for the development and progression of colon cancer (7). As a pro-inflammatory cytokine, interleukin (IL)-17A participates in tumor growth in different types of malignancies (8,9). Previous studies have shown that IL-17A achieves its functions through interactions with both proteins and non-coding RNAs, including miRNAs and long non-coding RNAs (lncRNAs) (10,11). However, the interactions between IL-17A and long non-coding RNAs have rarely been studied, to the best of our knowledge. Previous studies demonstrated that cervical carcinoma high-expressed long non-coding RNA 1 (CCHE1) promoted several different types of cancer (12-15); however its involvement in colon cancer has only been recently established (16). The present demonstrated that CCHE1 may have promoted the growth of colon adenocarcinoma through interactions with IL-17A and may be used a potential biomarker for early detection of colon adenocarcinoma.

Materials and methods

Human samples and cell lines. Blood was extracted from 62 patients with colon adenocarcinoma (patient group) and 36 healthy volunteers (control group) to extract the plasma. These participants were admitted to The Inner Mongolia People's Hospital (Inner Mongolia, China) between July 2015 and July 2018. The inclusion criteria were: i) Diagnosed by pathological examinations; and ii) patients and their families understood the experimental protocol and signed informed consent. The exclusion criteria were: i) Patients who were diagnosed with multiple diseases; ii) patients who failed to cooperate with researchers; and iii) patients who were treated within the 3 months prior to their admission at The Inner Mongolia People's Hospital. Distant tumor metastasis was observed in 30 cases. A total of 19 patients were in American Joint Committee on Cancer (AJCC) stage I and II, both of which are considered as the early stages of cancer (17). The patient group consisted of 33 males and 29 females, with an age range of 28-67 years, and a mean age of 47.5 ± 5.3 years.

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The control group consisted of 20 males and 18 females, with an age range of 27-68 years, and a mean age of 46.9 ± 4.9 years. The patient and the control groups had a similar age range and sex distributions. The present study was approved by The Ethics Committee of Inner Mongolia People's Hospital. All patients signed written form informed consent.

Hs 698.T and SNU-C1 human colon adenocarcinoma cell lines were purchased from The American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were cultured in RPMI-1640 medium (ATCC) containing 10% fetal bovine serum (ATCC) at 37°C with 5% CO₂ (95% humidity).

Transfection. IL-17A and CCHE1 expression vectors were purchased from GeneCopoeia, Inc. (Rockville, MD, USA). IL-17A small interfering (si)RNA (5'-CCUACGUUGUUU GCUACUU-3') and scrambled siRNA control (5'-UUCUCC GAACGUGUCACGUdTdT-3') were purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). Lipofectamine® 2000 reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used to transfect 50 nM vector and 15 nM siRNA, respectively. Untransfected cells were treated as the control and the cells transfected with scrambled siRNA control or an empty vector were treated as the negative control. The interval between transfection and subsequent experimentation was 24 h.

Reverse transcription-quantitative (RT-q)PCR. The TRIzol® Plus RNA Purification kit (Thermo Fisher Scientific, Inc.) was used to extract total RNA from plasma, Hs 698.T and SNU-C1 cells. Following reverse transcription using the RevertAid RT kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol, PCR reaction systems were prepared using qScript One-Step RT-qPCR kit (Quantabio, Beverly, MA, USA). Sequences of primers used in the PCR reactions were: CCHE1 forward, 5'-AAGGTCCCAGGATACTCGC-3', and reverse, 5'-GTGTCGTGGACTGGCAAAT-3'; β-actin forward, 5'-GACCTCTATGCCAACACAGT-3', and reverse, 5'-AGTACTTGCCTCAGGAGGA-3'; IL-17 forward, 5'-CTG GAGGATAAACTGTGAGAG-3, and reverse 5'-GCTGAA TGGCGACGGAGTTC-3'. IL-17 primer pairs were purchased from SinoBiological (Wayne, PA, USA) The thermocycling conditions used were: 95°C for 45 sec, followed by 40 cycles of 95°C for 14 sec and 58.5°C for 38 sec. All data were processed using the 2^{-ΔΔCq} method (18).

Cell proliferation assay. Proliferation assays were performed when CCHE1 and IL-17A overexpression rates were between 200-250% and the IL-17A knockdown rate was >50%. Cell suspensions were prepared with a cell density of 4×10^4 cells/ml. Cells were cultured at 37°C in a 5% CO₂ incubator, followed by addition of 1 μl Cell Counting Kit-8 solution (cat. no. ab228554; Abcam, Cambridge, UK) 4, 48, 72 and 96 h later. Subsequently, cells were cultured at 37°C in a 5% CO₂ incubator for an additional 4 h and a Fisherbrand™ accuSkan™ GO UV/Vis Microplate Spectrophotometer (Thermo Fisher Scientific, Inc.) was used to measure optical density values at 450 nm.

Western blotting. Western blotting was only performed if the CCHE1 and IL-17A overexpression rates were between 200-250% or the IL-17A knockdown rate was <50%. A Total

Protein Extraction kit (Merck KGaA, Darmstadt, Germany) was used to extract the total protein from cells. Protein samples were quantified using a bicinchoninic acid assay kit (Sangon, Shanghai, China). Following denaturing in boiling water for 5 min, electrophoresis was performed using a 10% SDS-PAGE gel (35 μg per lane). Following transfer to PVDF membranes and blocking in 5% non-fat milk for 2 h at room temperature, western blotting was performed using conventional methods; briefly, the membranes were incubated with the following primary antibodies at 37°C for 15 h: Rabbit anti-human IL-17A (1:2,000; cat. no. ab136668) and GAPDH (1:1,000; cat. no. ab8245) (both from Abcam, Cambridge, UK). A subsequent incubation of 15 h at 37°C was performed with the secondary, goat anti-rabbit Immunoglobulin G-horse radish peroxidase-conjugated antibody (1:1,000; cat. no. MBS435036; MyBioSource, Inc., San Diego, CA, USA). Signals were developed using enhanced chemiluminescence (Sigma-Aldrich; Merck KGaA) and were detected using a MYECL™ Imager (Thermo Fisher Scientific, Inc.). Densitometry analysis was performed using ImageJ version 1.46 software (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis. All the data are expressed as the mean ± standard deviation of three experimental repeats. Statistical analysis was performed using SPSS version 19.0 (IBM Corp., Armonk, NY, USA). Correlations between expression levels of CCHE1 and IL-17A were analyzed using Pearson's rank correlation coefficient. The diagnostic value of plasma CCHE1 expression levels for the detection of early stage disease was evaluated using a receiver operating characteristic curve. Comparisons between two groups were performed by a Student's t-test. Comparisons between multiple groups were performed by one-way analysis of variance followed by a post-hoc Tukey's test. P<0.05 was considered to indicate a statistically significant difference.

Results

Plasma CCHE1 and IL-17A levels are upregulated in patients with colon adenocarcinoma, but are not affected by the presence of distant tumor metastases. RT-qPCR results showed that, compared with healthy controls, plasma levels of CCHE1 (Fig. 1A) and IL-17A mRNA (Fig. 1B) were significantly increased in patients with metastatic colon adenocarcinoma (MC) and non-metastatic colon adenocarcinoma (NMC; all P<0.05). However, there were no significant differences in plasma levels of CCHE1 (Fig. 1A) and IL-17A mRNA (Fig. 1B) MC and NMC groups (both P>0.05).

Plasma levels of CCHE1 and IL-17A are positively associated with the primary tumor diameter. Based on the diameter of primary tumors, colon adenocarcinoma were divided into 0-2 cm group (n=22), 2-4 cm group (n=18) and >4 cm group (n=22). The plasma expression levels of CCHE1 (Fig. 2A) and IL-17A (Fig. 2B) were significantly increased, in each instance, as the primary tumor diameter increased (all P<0.05).

Plasma levels of CCHE1 and IL-17A are positively correlated in patients with colon adenocarcinoma, but not in healthy controls. Pearson correlation coefficient analysis showed that

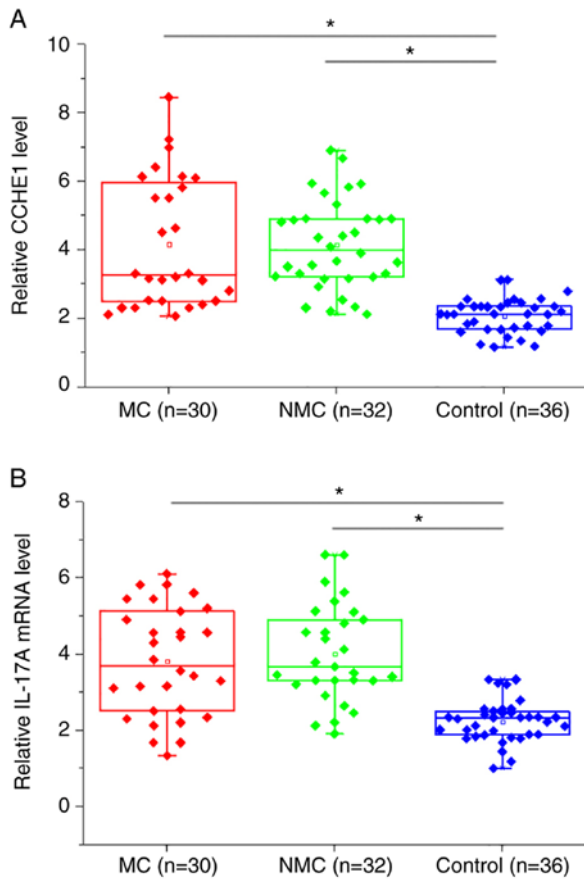


Figure 1. CCHE1 and IL-17A expression levels are upregulated in patients with colon adenocarcinoma, but are not affected by the presence of distant tumor metastases. Plasma levels of (A) CCHE1 and (B) IL-17A mRNA were significantly increased in patients with MC and NMC, while no significant differences in plasma levels of CCHE1 and IL-17A mRNA were found between MC and NMC groups. * $P < 0.05$. MC, metastatic colon adenocarcinoma; NMC, non-metastatic colon adenocarcinoma; CCHE1, cervical carcinoma high-expressed long non-coding RNA 1; IL-17A, interleukin-17A.

plasma levels of CCHE1 and IL-17A were positively correlated in patients with colon adenocarcinoma (Fig. 3A; $P < 0.0001$). In contrast, the correlation between plasma levels of lncRNA CCHE1 and IL-17A was not significant in healthy controls (Fig. 3B; $P = 0.1721$).

Downregulation of plasma CCHE1 distinguishes patients with early stage colon adenocarcinoma from healthy controls. The present study included 19 patients with colon adenocarcinoma in AJCC stages I and II, which are considered the early stages of cancer. ROC curve analysis was performed to evaluate the diagnostic value of plasma CCHE1 for early stage colon adenocarcinoma. As shown in Fig. 4, the area under the curve was 0.8745, with standard error of 0.05599 and 95% confidence interval of 0.7378-0.9573.

CCHE1 overexpression increases IL-17A expression in colon adenocarcinoma cell lines Hs 698.T and SNU-C1. Compared with the control group and negative control group, cells transfected with CCHE1 showed a significantly upregulated expression of CCHE1 in cells of both colon adenocarcinoma cell lines Hs 698.T and SNU-C1 (Fig. 5A, $P < 0.05$). Similarly, CCHE1 overexpression significantly increased the mRNA

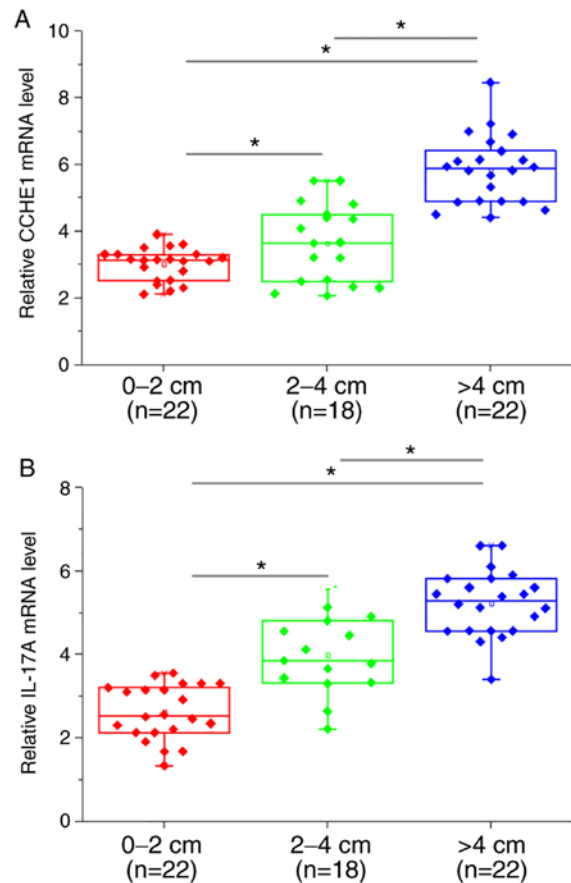


Figure 2. Plasma levels of CCHE1 and IL-17A increase concurrently with primary tumor diameter. Plasma levels of (A) CCHE1 and (B) IL-17A increased with an increase in the primary tumor diameter. * $P < 0.05$. CCHE1, cervical carcinoma high-expressed long non-coding RNA 1; IL-17A, interleukin-17A.

levels of IL-17A (Fig. 5A; $P < 0.05$). In contrast, whilst IL-17A overexpression did significantly increase the relative IL-17A mRNA levels in both cell lines (Fig. 5B; $P < 0.05$), there was no significant increase in the expression of CCHE1 mRNA levels following IL-17A overexpression in either cell line (Fig. 5B; $P > 0.05$).

CCHE1 overexpression promotes colon adenocarcinoma cell proliferation through IL-17A. siRNA-mediated knock-down of IL-17A significantly reduced relative IL-17A mRNA expression levels compared with the control and negative control groups in both cell lines (Fig. 6A; $P < 0.05$). Compared with the control group and the negative control group, proliferation was increased in cells overexpressing CCHE1 or IL-17A in both cell lines (Fig. 6B; $P < 0.05$). In addition, transfection with IL-17A siRNA significantly reduced cell proliferation and attenuated the increase in proliferation when co-transfected with CCHE1 in both cell lines (Fig. 6; $P < 0.05$ vs. CCHE1 alone).

Discussion

The functionality of CCHE1 as an oncogene has been characterized in cervical cancer (14), non-small lung cancer, gastric cancer and hepatocellular carcinoma (12,13,15), while its roles in other malignancies are unknown. The key finding of the

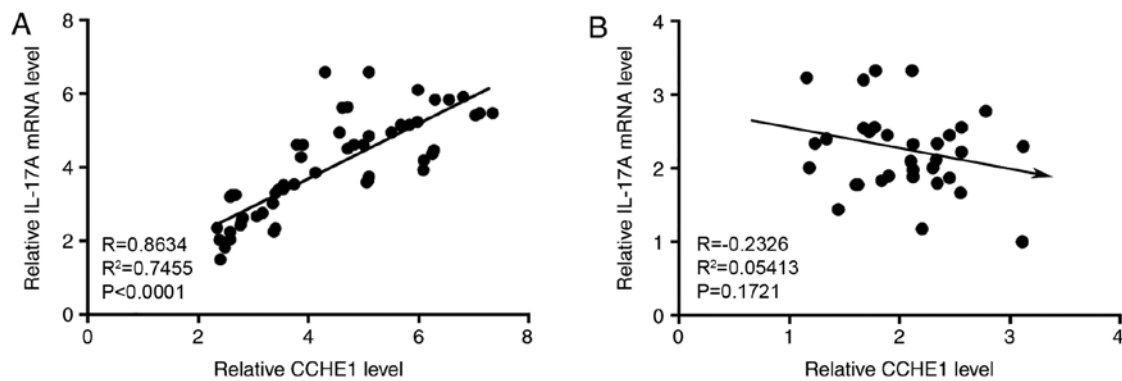


Figure 3. Plasma levels of CCHE1 and IL-17A are positively correlated in patients with colon adenocarcinoma, but not in healthy controls. Pearson's correlation coefficient analysis showed that plasma levels of CCHE1 and IL-17A were positively correlated in (A) patients with colon adenocarcinoma (B) but not in healthy controls. CCHE1, cervical carcinoma high-expressed long non-coding RNA 1; IL-17A, interleukin-17A.

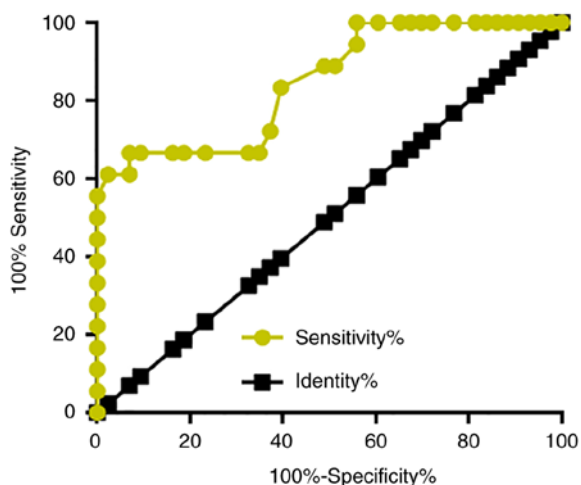


Figure 4. Downregulation of plasma cervical carcinoma high-expressed long non-coding RNA 1 distinguishes early stage patients with colon adenocarcinoma from healthy controls. Receiver operating characteristic curve analysis showed that the area under the curve was 0.8745, with standard error of 0.05599 and 95% confidence interval of 0.7378-0.9573.

present study was that CCHE1 was also an oncogenic lncRNA in colon adenocarcinoma, the major type of colon cancer accounting for more than 95% of all colon cancer cases. The CCHE1-induced oncogenicity in colon adenocarcinoma was mediated, at least in part, through interactions with IL-17A.

It has been demonstrated that the development of colon adenocarcinoma is accompanied by changes in expression levels of a large set of lncRNAs (19). The altered expression of those lncRNAs reflects the development, progression and prognosis of this disease (20). However, most of the differentially expressed lncRNAs were likely involved in the promoting a number of aspects of cancer development and progression (19,20). lncRNAs only involved in a specific aspect of cancer development, such as growth or metastasis are rare by comparison (19,20). CCHE1 as an oncogene is overexpressed in cervical cancer, non-small lung cancer, gastric cancer and hepatocellular carcinoma (12-15). Based on the results of the present study, CCHE1 may also be upregulated in colon cancer and the expression levels of CCHE1 were affected by tumor size, but not the number of metastases. *In vitro* cell proliferation experiments additionally demonstrated that CCHE1

overexpression promoted proliferation of colon adenocarcinoma cell lines. Therefore, CCHE1 may specifically participate in the growth, but not metastasis of colon adenocarcinoma. However, it has been reported that CCHE1 is involved in the metastasis of non-small lung cancer (12). Therefore, CCHE1 may serve different roles in different types of malignancies.

As a pro-inflammatory cytokine, IL-17A promotes tumor growth in different types of human malignancies (8,9). Our study also showed that IL-17A overexpression promoted, while siRNA-mediated silencing inhibited, proliferation of human colon adenocarcinoma cell lines. Therefore, anti-IL-17A agents, such as Secukinumab, may be used to treat human colon adenocarcinoma. However, further studies are required to test this hypothesis. The present study additionally demonstrated that CCHE1 is likely an upstream inhibitor of IL-17A in the regulation of colon adenocarcinoma cell proliferation. However, the upstream regulation may be through indirect mechanisms, as there was a lack of correlation between CCHE1 and IL-17A in the healthy patients. Future studies should investigate the role of CCHE1 and IL-17A function in *in vivo* models of colon adenocarcinoma.

The clinical relevance of CCHE1 as a potential biomarker was also demonstrated in the present study, effectively distinguishing patients with early stage colon adenocarcinoma from the healthy controls. Therefore, circulating CCHE1 may potentially be used to assist the early screening of colon adenocarcinoma. However, more clinical trials are needed to evaluate this possibility, particularly the diagnostic specificity.

It was previously reported that IL-17A interacts with the IL-6-Stat3 signaling pathway to promote tumor growth (21). Therefore, future studies should investigate the involvement of the IL-6-Stat3 signaling pathway, as a potential downstream effector of IL-17A in colon adenocarcinoma. However, IL-17A plays a complex role in tumorigenesis. IL-17A inhibits anti-tumor immunity by recruiting myeloid derived suppressor cells (22). In contrast, IL-17 knockout in mice increases the risk of metastatic lung melanoma (23), suggesting that IL-17A may stimulate cytotoxic T cells to produce the potent anti-tumor cytokine interferon- γ . The complex role of IL-17A in colon adenocarcinoma requires further study.

Although the functionality of CCHE1 in cancer biology has been extensively studied in different types of cancers (12-15), the interactions between lncRNA CCHE1 and chemotherapeutic

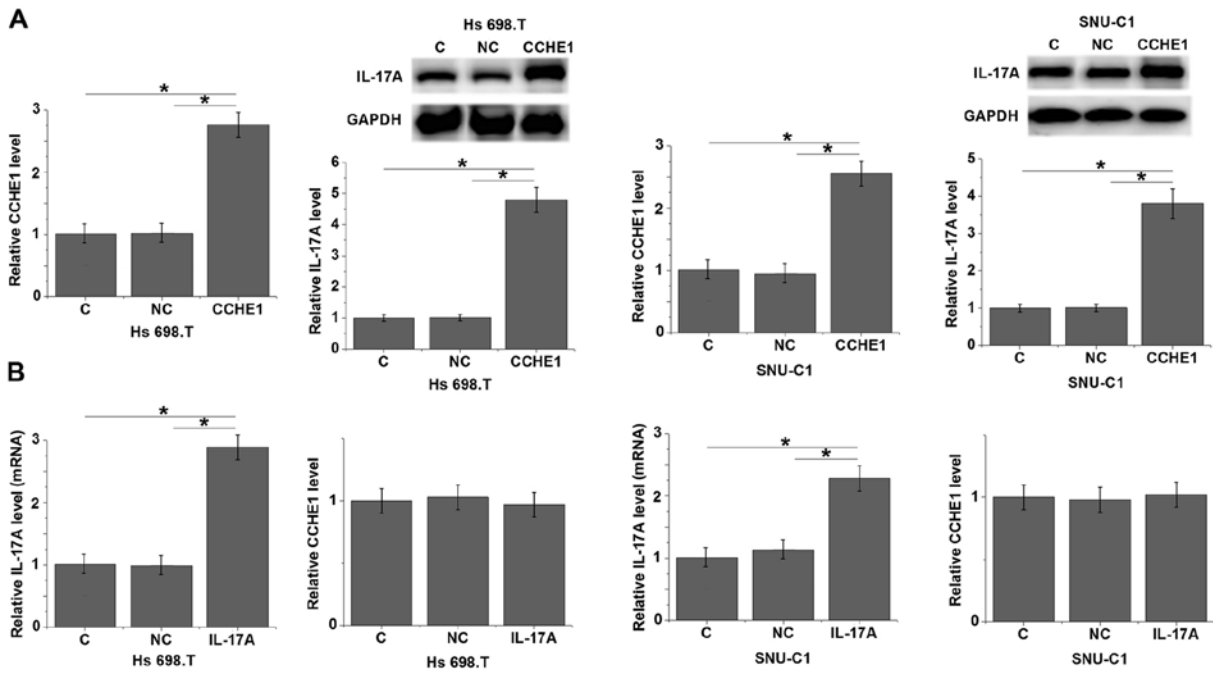


Figure 5. CCHE1 overexpression leads to an increase in IL-17A expression in the colon adenocarcinoma cell lines Hs 698.T and SNU-C1. (A) CCHE1 overexpression significantly upregulated expression of IL-17A in both Hs 698.T and SNU-C1 colon adenocarcinoma cell lines. (B) IL-17A overexpression did not significantly alter the expression of CCHE1 in Hs 698.T or SNU-C1 colon adenocarcinoma cell lines. *P<0.05. C, control; NC, negative control; CCHE1, cervical carcinoma high-expressed long non-coding RNA 1; IL-17A, interleukin-17A.

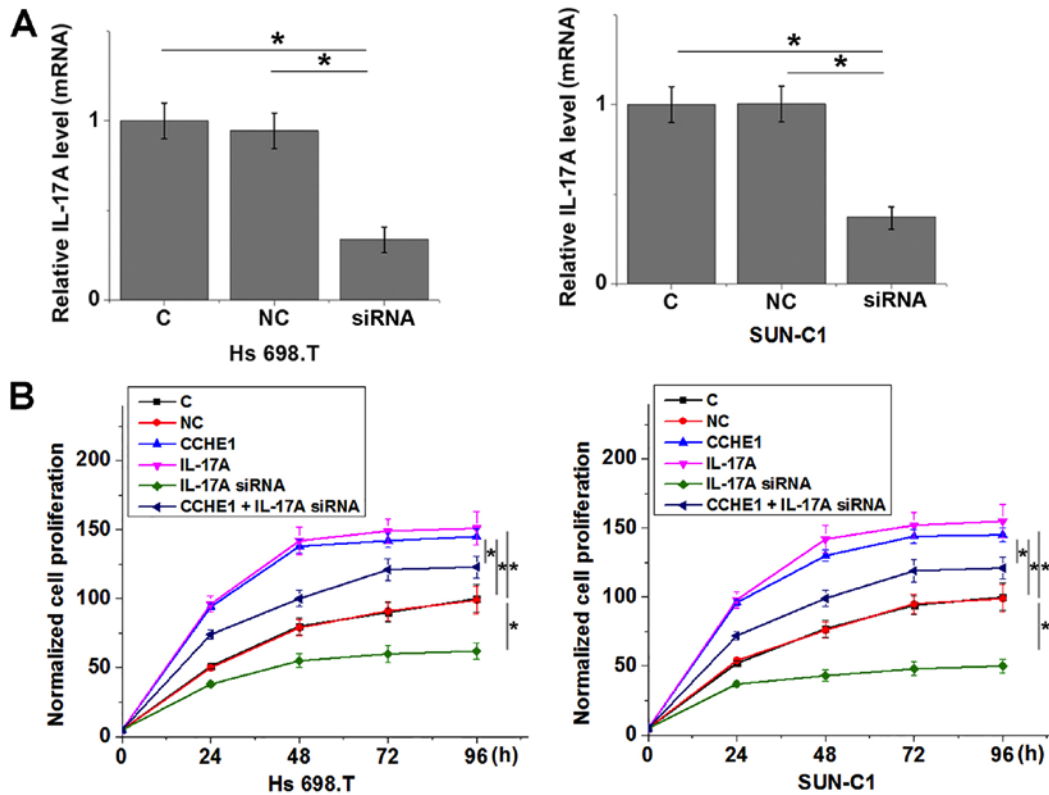


Figure 6. CCHE1 overexpression promotes colon adenocarcinoma cell proliferation through IL-17A. (A) CCHE1 and IL-17A overexpression were achieved. (B) CCHE1 and IL-17A overexpression significantly promoted proliferation of Hs 698.T or SNU-C1 colon adenocarcinoma cell lines. IL-17A small interfering RNA silencing inhibited cell proliferation and attenuated the enhancing effects of CCHE1 overexpression on cell proliferation. *P<0.05. CCHE1, cervical carcinoma high-expressed long non-coding RNA 1; IL-17A, interleukin-17A; siRNA, small interfering RNA; C, control; NC, negative control.

drugs is still unknown. Therefore, future studies are required to elucidate the role of CCHE1 in chemotherapy.

In conclusion, CCHE1 and IL-17A were both upregulated in colon adenocarcinoma. CCHE1 was involved in growth

possibly through indirect interactions with IL-17A, but may not be involved in the metastasis of colon adenocarcinoma.

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Availability of data and materials

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

Authors' contributions

JW performed the majority of the experiments, analyzed all data and was a major contributor in writing the manuscript. HL, CZ, LX and ZC all performed some of the experiments. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by The Ethics Committee of Inner Mongolia People's Hospital (Inner Mongolia, China). All patients signed written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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