ISSN 1948-5204 (online)

World Journal of *Gastrointestinal Oncology*

World J Gastrointest Oncol 2024 April 15; 16(4): 1091-1675

Published by Baishideng Publishing Group Inc

World Journal of Gastrointestinal Oncology

Contents

Monthly Volume 16 Number 4 April 15, 2024

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Contents

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Contents

ABOUT COVER

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WJGO mainly publishes articles reporting research results and findings obtained in the field of gastrointestinal oncology and covering a wide range of topics including liver cell adenoma, gastric neoplasms, appendiceal neoplasms, biliary tract neoplasms, hepatocellular carcinoma, pancreatic carcinoma, cecal neoplasms, colonic neoplasms, colorectal neoplasms, duodenal neoplasms, esophageal neoplasms, gallbladder neoplasms, *etc*.

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RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Xiang-Di Zhang*; **Production Department Director:** *Xiang Li*; **Cover Editor:** *Jia-Ru Fan*.

NAME OF JOURNAL
World Journal of Gastrointestinal Oncology

ISSN
ISSN 1948-5204 (online)

LAUNCH DATE
February 15, 2009

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Monjur Ahmed, Florin Burada

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/1948-5204/editorialboard.htm

PUBLICATION DATE
April 15, 2024

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World J Gastrointest Oncol 2024 April 15; 16(4): 1465-1478

DOI: 10.4251/wjgo.v16.i4.1465

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Basic Study

Human β-defensin-1 affects the mammalian target of rapamycin pathway and autophagy in colon cancer cells through long non-coding RNA TCONS_00014506

Yu-Xin Zhao, Yan Cui, Xin-Hong Li, Wen-Hong Yang, Shi-Xiang An, Jia-Xian Cui, Min-Yu Zhang, Jing-Kun Lu, Xuan Zhang, Xiu-Mei Wang, Li-Li Bao, Peng-Wei Zhao

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Bordonaro M, United States

Received: November 14, 2023
Peer-review started: November 14, 2023
First decision: January 5, 2024
Revised: January 15, 2024
Accepted: February 18, 2024
Article in press: February 18, 2024
Published online: April 15, 2024

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Abstract

BACKGROUND

Colorectal cancer has a low 5-year survival rate and high mortality. Human β-defensin-1 (hBD-1) may play an integral function in the innate immune system, contributing to the recognition and destruction of cancer cells. Long non-coding RNAs (lncRNAs) are involved in the process of cell differentiation and growth.

AIM

To investigate the effect of hBD-1 on the mammalian target of rapamycin (mTOR) pathway and autophagy in human colon cancer SW620 cells.

METHODS

CCK8 assay was utilized for the detection of cell proliferation and determination of the optimal drug concentration. Colony formation assay was employed to assess the effect of hBD-1 on SW620 cell proliferation. Bioinformatics was used to

screen potentially biologically significant lncRNAs related to the mTOR pathway. Additionally, p-mTOR (Ser2448), Beclin1, and LC3II/I expression levels in SW620 cells were assessed through Western blot analysis.

RESULTS

hBD-1 inhibited the proliferative ability of SW620 cells, as evidenced by the reduction in the colony formation capacity of SW620 cells upon exposure to hBD-1. hBD-1 decreased the expression of p-mTOR (Ser2448) protein and increased the expression of Beclin1 and LC3II/I protein. Furthermore, bioinformatics analysis identified seven lncRNAs (2 upregulated and 5 downregulated) related to the mTOR pathway. The lncRNA TCONS_00014506 was ultimately selected. Following the inhibition of the lncRNA TCONS_00014506, exposure to hBD-1 inhibited p-mTOR (Ser2448) and promoted Beclin1 and LC3II/I protein expression.

CONCLUSION

hBD-1 inhibits the mTOR pathway and promotes autophagy by upregulating the expression of the lncRNA TCONS_00014506 in SW620 cells.

Key Words: Colon cancer; Human β-defensin-1; LncRNA; Mammalian target of rapamycin; Autophagy

Core Tip: Colorectal cancer has a low 5-year survival rate and high mortality. Human β-defensin-1 (hBD-1) is likely to play an integral function in the innate immune system, contributing to the recognition and destruction of cancer cells. Hence, we explored the effect of hBD-1 on colon cancer SW620 cells, which could be good for the development of therapy for colorectal cancer.

Citation: Zhao YX, Cui Y, Li XH, Yang WH, An SX, Cui JX, Zhang MY, Lu JK, Zhang X, Wang XM, Bao LL, Zhao PW. Human β-defensin-1 affects the mammalian target of rapamycin pathway and autophagy in colon cancer cells through long non-coding RNA TCONS_00014506. *World J Gastrointest Oncol* 2024; 16(4): 1465-1478
URL: https://www.wjgnet.com/1948-5204/full/v16/i4/1465.htm
DOI: https://dx.doi.org/10.4251/wjgo.v16.i4.1465

INTRODUCTION

Colorectal cancer is a prevalent and highly aggressive malignancy of the gastrointestinal tract. Having the third highest incidence among all malignancies worldwide, colorectal cancer is considered one of the primary contributors to cancer-related fatalities globally[1]. According to the location of disease onset, colorectal cancer is divided into rectal cancer and colon cancer. Colon cancer often has no specific symptoms in the early stage, and changes in bowel habits, stool characteristics, abdominal pain, abdominal mass, intestinal obstruction, and systemic symptoms (anemia, weight loss, fatigue, low-grade fever, *etc*.) gradually appear in the late stage of disease progression[2,3]. A 5-year survival rate of only 11% was recorded in patients with colorectal cancer at TMN stage 4 or with distant metastases[4,5].

Human β-defensin-1 (hBD-1) is a small cationic host defense peptide produced by neutrophils and epithelial cells. It plays a vital role in innate immunity by effectively combating microbial pathogens or neutralizing bacterial toxins. Additionally, it contributes to adaptive immunity by acting as a chemical inducer and activator of immune cells and is a vital component of innate immune responses[6,7]. hBD-1 demonstrates varying expression patterns in diverse types of cancer. It is recognized as a tumor suppressor due to its ability to promote cancer cell apoptosis while simultaneously inhibiting the migration and invasion of cancer cells[4,8,9]. Hence, hBD-1 may play an integral function in the innate immune system, contributing to the recognition and destruction of cancer cells.

Autophagy is a highly conserved process of eukaryotic cell cycling[10]. In the context of cancer, autophagy is a general metabolic adaptation for cancer cell development, enabling the circulation of cellular components (CCs) under metabolic stress or during anticancer therapy. This allows cancer cells to survive in the challenging hypoxic and low-nutrient tumor microenvironments[11].

Despite the identification of numerous long non-coding RNAs (lncRNAs) through high-throughput RNA sequencing, a limited portion of them has undergone comprehensive functional characterization. The functional and biological relevance of most of these lncRNAs remains unelucidated. These already characterized lncRNAs are involved in the process of cell differentiation and growth. Moreover, these lncRNAs have been implicated in the onset and progression of numerous diseases, including cancer[12]. Research has confirmed that lncRNAs are vital in the onset and progression of colon cancer, and approximately 200 differentially expressed lncRNAs have been identified in colon tumors[13]. Hence, it is reasonable to speculate that lncRNAs may hold potential as biomarkers and potential targets for colon cancer therapy.

This study focused on investigating whether hBD-1 affects autophagy *via* lncRNAs in colon cancer SW620 cells and its impact on the expression of these lncRNAs within SW620 cells. This research will lay the groundwork for a more comprehensive examination of the role of hBD-1 in colon cancer. Furthermore, bioinformatics analysis of differentially expressed

lncRNAs was performed to offer novel insights that could aid in the diagnosis and treatment of colon cancer.

MATERIALS AND METHODS

Cells and main reagents

The human colon cancer cell line SW620 was obtained from Wuhan Procell Life Science & Technology Co., Ltd, and hBD-1 was purchased from Sino Biological, Inc. (China). The total RNA extraction kit was purchased from TIANGEN Biotech (Beijing) Co., Ltd. The Trizol kit was acquired from Takara Bio Inc. (Japan), and chloroform was purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd. EL Transfection Reagent was purchased from TransGen Biotech Co., Ltd (Beijing). Additionally, si-lncRNA (Suzhou Gemma Biotechnology Co., Ltd.), BCA kit (Shanghai Epizyme Biotech Co., Ltd.), and Western blot reagents (Beyotime Biotech. Inc., Shanghai) were used. The antibody was purchased from Cell Signaling Technology Inc. (United States).

Cell culture

SW620 cells were grown in high-glucose DMEM with 10% fetal bovine serum and 1% penicillin/streptomycin at 37 °C in an incubator with 5% carbon dioxide.

CCK-8 assay for detection of proliferation of colon cancer SW620 cells

SW620 cells (100 μL) in the logarithmic growth phase were inoculated into 96-well plates at a density of 7000 cells/well. After the cells had adhered to the plate, hBD-1 was added at final concentrations of 35, 40, 45, 50, 55, 60, 65, and 70 ng/mL, respectively. These cultures were placed at 37 °C with 5% CO_2 in an incubator for 12, 24, and 48 h. Afterward, 10 μL of CCK-8 reagent was added into each well and incubated at 37 °C for 1 h. A microplate reader was utilized for assessing the optical density (OD) value at the wavelength of 450 nm. Cell inhibition rate (%) was calculated as [(OD value of control group - OD value of hBD-1 group)/(OD value of control group - OD value of blank group)] × 100%. The three assessed groups were as follows: Control group: SW620 cells + medium + CCK8 solution; hBD-1 group: SW620 cells + each concentration of hBD-1 + medium + CCK8 solution; and blank group: Medium + CCK8 solution.

Colony formation assay

SW620 cells in the logarithmic growth phase were diluted with a complete culture medium after trypsin digestion and seeded in 6-well plates at a density of 700 cells/well. The cells were categorized as a control group (without any treatment) and a hBD-1 group (exposure to 40 ng/mL hBD-1 after cell attachment). Following this process, 0.1% crystal violet was utilized to stain the cells. After 2 wk of cultivation, cell fixation was carried out, and a camera was utilized to capture the images. The cell count was subsequently determined using Image J software.

Western blot analysis

RIPA buffer was used to extract total cellular protein. After bicinchoninic acid quantification, the supernatant was mixed with 5 × loading buffer at a 4:1 ratio and placed in boiling water for 10 min. Then, 20 μg of total protein was separated by SDS-PAGE and transferred to an NC membrane. After blocking with 5% nonfat dry milk for 1 h, the primary antibody was added and incubated at 4 °C for 12-18 h. After washing with TBST thrice (10 min each), the secondary antibody was added and incubated at room temperature for 1 h, followed by three washes with TBST (10 min each). ECL chemiluminescence was evenly dropped onto the NC film. After several seconds of reaction, photographs were captured with a gel imaging system, and gray values were examined using Image J software.

LncRNA sequencing and bioinformatics analysis

The transcriptome sequencing project was completed on the Illumina sequencing platform. For this, the Illumina PE library (approximately 300 bp) was constructed and sequencing was conducted in a paired-end (PE sequencing) manner. After unloading the sequencing data, a thorough quality control process was implemented. This involved assessing the base mass distribution, base balance analysis, and repeat sequence level. Trimmomatic software was used to clean the raw data, removing the joint and low-quality reads. Subsequently, a new round of quality control was conducted on the cleaned reads to obtain comprehensive data. The clean data were then aligned with reference genomes to obtain detailed transcriptomic information. RPKM (Reads per Kilobase per Million Reads) value was used to measure gene expression, and the differentially expressed genes were identified using $|\log FC| > 1$ and $P < 0.05$ as the criteria.

Differential expression analysis of lncRNAs

Differential expression analysis of lncRNAs was performed using DESeq to identify differentially expressed genes in terms of fold difference and significance level. The absolute value of $\log FC > 1$ and $P < 0.05$ were used as the screening criteria. Volcano plot of differentially expressed lncRNAs was generated using the R package ggplot2.

Cluster analysis (heatmap)

A two-way cluster analysis of the merged set and the samples of all differentially expressed genes was performed. The process involved the use of the Pheatmap package in R, with the aim of clustering based on two criteria: The expression levels of the same lncRNA in various samples and the expression patterns of diverse lncRNAs in the same sample.

Target gene prediction

The lncRNA target genes were predicted by cis- and co-expression (differential co-expression network). The intersection of prediction results was visualized in the form of interaction networks.

KEGG and GO pathway enrichment analysis of target genes of differentially expressed lncRNAs

Based on the KEGG database (https://www.kegg.jp/), enrichment analysis of the biological pathways, wherein the differentially expressed lncRNA target genes were located, was performed. The analysis predicted the signaling pathways in which these differentially expressed lncRNAs are involved. Through GO annotation (http://amigo.geneontology.org/amigo), gene function was annotated. The degree of enrichment was calculated according to several parameters such as rich factor, false discovery rate, and count. The top 20 most significant terms were selected after ranking for further analysis.

Transfection of si-lncRNA TCONS_00014506

Target genes of lncRNAs and differentially expressed mRNAs associated with the mTOR pathway were analyzed. Specifically, the study focused on assessing lncRNA-mRNA co-expression to select the pertinent lncRNAs associated with the mTOR pathway.

The negative control (si-NC) and the small interfering RNA (si-lncRNA) of lncRNA TCONS_00014506 were transfected into SW620 cells. Subsequent experiments were carried out after an appropriate incubation period. Si-NC was utilized to ensure that any observed changes in the experiments can be attributed specifically to the presence or absence of si-lncRNA TCONS_00014506 and not to other factors. This process was executed *via* the EL Transfection Reagent. The si-lncRNA sequence is shown in Supplementary Table 1.

Real-time quantitative PCR

Total cellular RNA was extracted and converted into cDNA 24 h after transfection. LncRNA expression levels were detected by RT-qPCR. The sequence of primers used is shown in Supplementary Table 2.

Statistical analysis

SPSS 26.0 was utilized for statistical analyses, and the experiment was repeated more than three times. The data acquired from the experiments are presented as the mean ± SD. To assess group differences, the study employed various methods. Comparative assessment between two groups was executed *via* independent sample *t*-test, whereas comparisons among multiple groups were executed through one-way analysis of variance (ANOVA). Furthermore, the LSD *t*-test was utilized for pairwise comparisons. Two-sided $P < 0.05$ was deemed statistically significant.

RESULTS

HBD-1 regulates viability, clonogenic potential, and autophagy in SW620 cells

HBD-1 exerted significant effects on SW620 colon cancer cells, inhibiting their viability and clonogenic potential and promoting autophagy. The survival rate of SW620 cells decreased with increasing concentrations of hBD-1, as demonstrated *via* the CCK-8 assay (Figure 1A). The half maximal inhibitory concentration values of hBD-1 at 12, 24, and 48 h were measured at 43.32, 43.97, and 42.18 ng/mL, respectively. For subsequent experiments, a concentration of 40 ng/mL and a duration of 48 h were selected ($P < 0.05$).

The number of colonies of colon cancer SW620 cells decreased in the 40 ng/mL hBD-1 group ($P < 0.05$, Figure 1B and C).

To explore the effect of hBD-1 on p-mTOR, Beclin1, and LC3II/I in SW620 cells, Western blot analysis was performed. In comparison with the control cells, SW620 cells treated with 40 ng/mL hBD-1 for 48 h exhibited a decrease in the expression level of p-mTOR ($P < 0.05$; Figure 1D). In addition, the expression of autophagy-related proteins Beclin1 and LC3II/I was increased ($P < 0.05$; Figure 1D-G).

Differential expression analysis of lncRNAs

A total of 1040 differentially expressed lncRNAs (447 upregulated and 593 downregulated) were observed in the hBD-1 group (Figure 2A). Volcano plot shows the distribution of lncRNAs (Figure 2B). The overall hierarchical clustering plot of all differentially expressed lncRNAs clustered by RPKM values was generated (Figure 2C). The relationship between differentially expressed lncRNAs and their targeted mRNAs is shown in Figure 2D.

KEGG pathway enrichment analysis

The top 20 KEGG pathways with the most significant enrichment were selected for presentation by rank order of *P* values (Figure 3A). Classification analysis of KEGG pathways was executed, and the resulting data are illustrated (Figure 3B). These pathways include cellular processes, genetic information processing, environmental information processing, metabolism, organismal systems, and human diseases.

GO enrichment analysis

The top 20 most significant GO terms were selected for plotting by rank order of *P*-values (Figure 3C). GO pathways were classified according to biological process (BP), CC, and molecular function (Figure 3D).

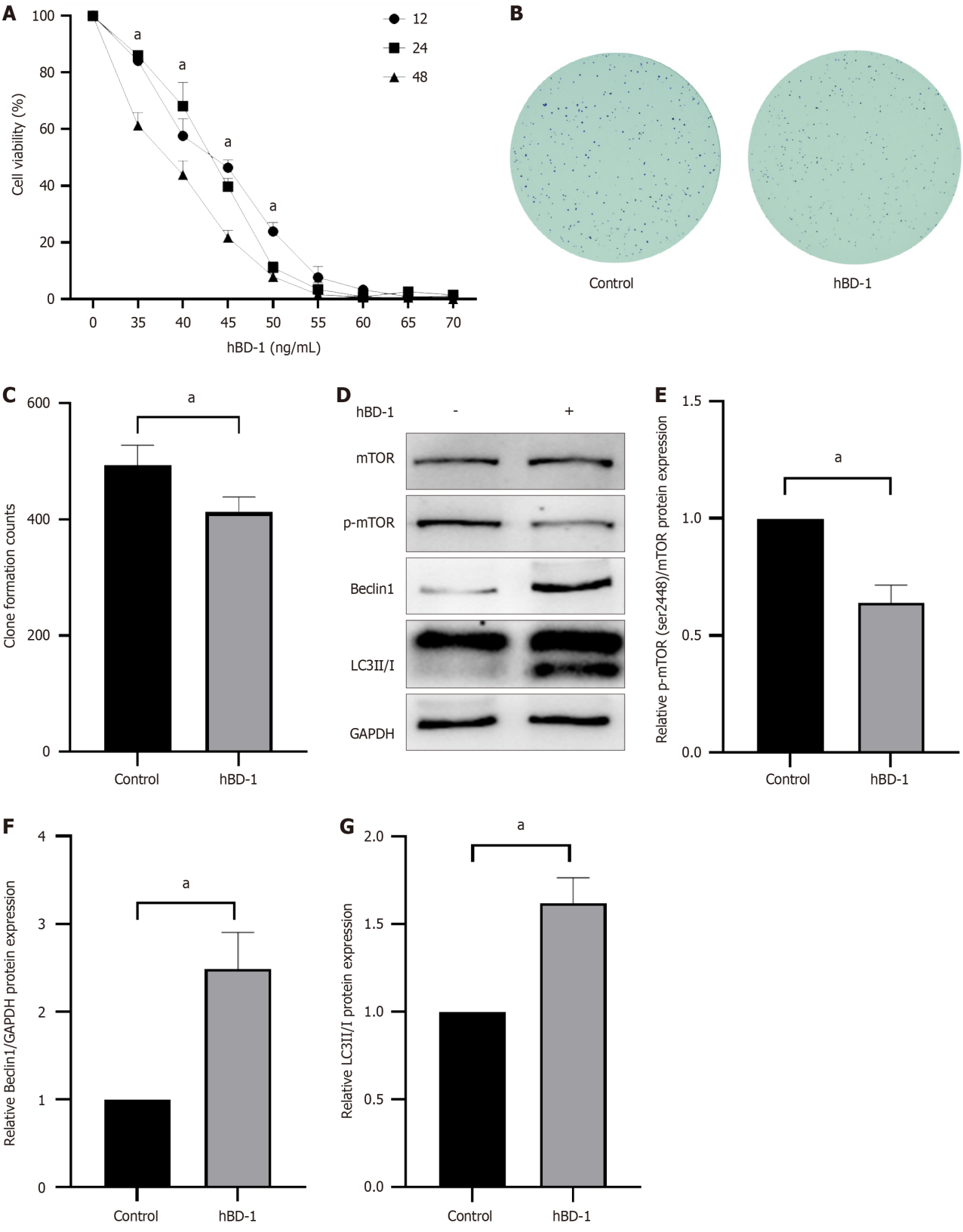


Figure 1 Human β-defensin-1 affects the viability, clonogenesis, and autophagy of SW620 cells. A: Human β-defensin-1 (hBD-1) suppresses cell viability; B: hBD-1 inhibits clonal formation; C: Clonal formation count; D: hBD-1 inhibits phosphorylation of mTOR and promotes expression of Beclin-1 and LC3II/I; E-G: Relative expression of p-mTOR, Beclin-1, and LC3II/I. $^{a}P < 0.05$ *vs* control group. hBD-1: Human β-defensin-1.

Analysis of differentially expressed lncRNAs

A total of 31 differentially expressed mRNAs were obtained by intersecting KEGG and GO enrichment terms, including 8 upregulated and 23 downregulated genes (Supplementary Table 3).

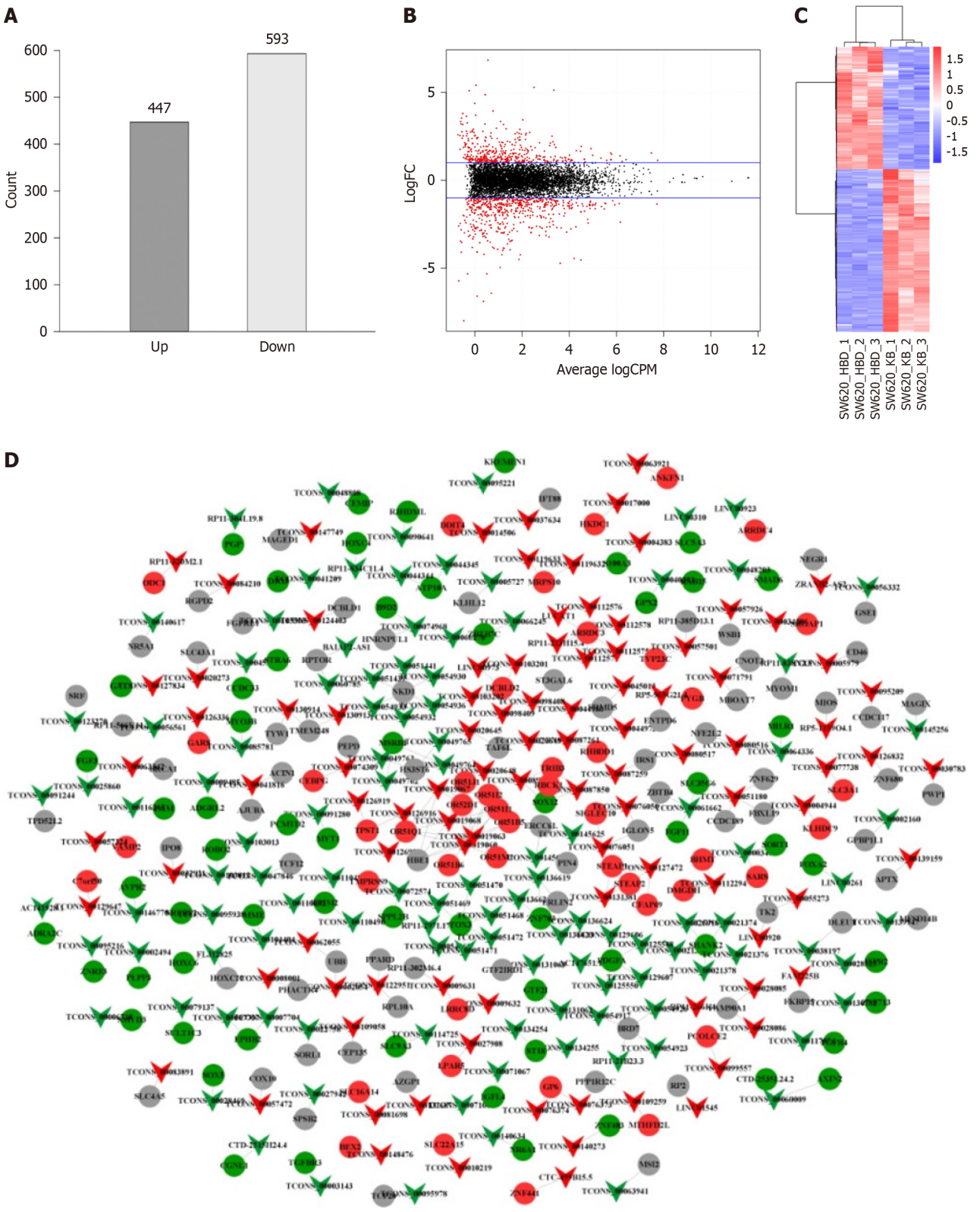
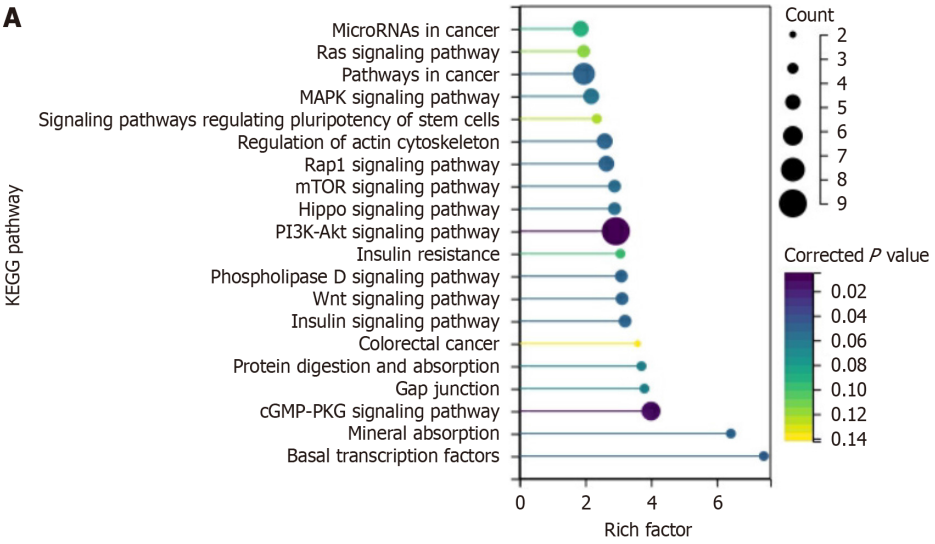


Figure 2 Differential expression analysis of long non-coding RNAs. A: Histogram of differentially expressed long non-coding RNAs (lncRNAs); B: Volcano plot of differentially expressed lncRNAs. The two blue lines in the figure signify the threshold for downregulating genes with differential expression on the upper panel (logFC > 1 indicates upregulation and logFC < -1 indicates downregulation), where black represents no differentially expressed lncRNAs and red represents differentially expressed lncRNAs; C: Cluster analysis of differentially expressed lncRNAs. The horizontal axis denotes lncRNAs from different samples, each listed as a sample, red denotes highly expressed lncRNAs, and blue denotes lncRNAs with decreased expression. The vertical axis corresponds to gene names, where each row denotes an individual gene. The color scale is used to indicate the abundance of gene expression, with red denoting significant upregulation and blue denoting significant downregulation; D: Relationship between differentially expressed lncRNAs and mRNAs targeted. Inverted triangles denote lncRNAs, circles denote mRNAs, red denotes upregulated expression, green denotes downregulated expression, and gray denotes no difference in expression.


A
KEGG pathway
MicroRNAs in cancer
Ras signaling pathway
Pathways in cancer
MAPK signaling pathway
Signaling pathways regulating pluripotency of stem cells
Regulation of actin cytoskeleton
Rap1 signaling pathway
mTOR signaling pathway
Hippo signaling pathway
PI3K-Akt signaling pathway
Insulin resistance
Phospholipase D signaling pathway
Wnt signaling pathway
Insulin signaling pathway
Colorectal cancer
Protein digestion and absorption
Gap junction
cGMP-PKG signaling pathway
Mineral absorption
Basal transcription factors
0
2
4
6
Rich factor
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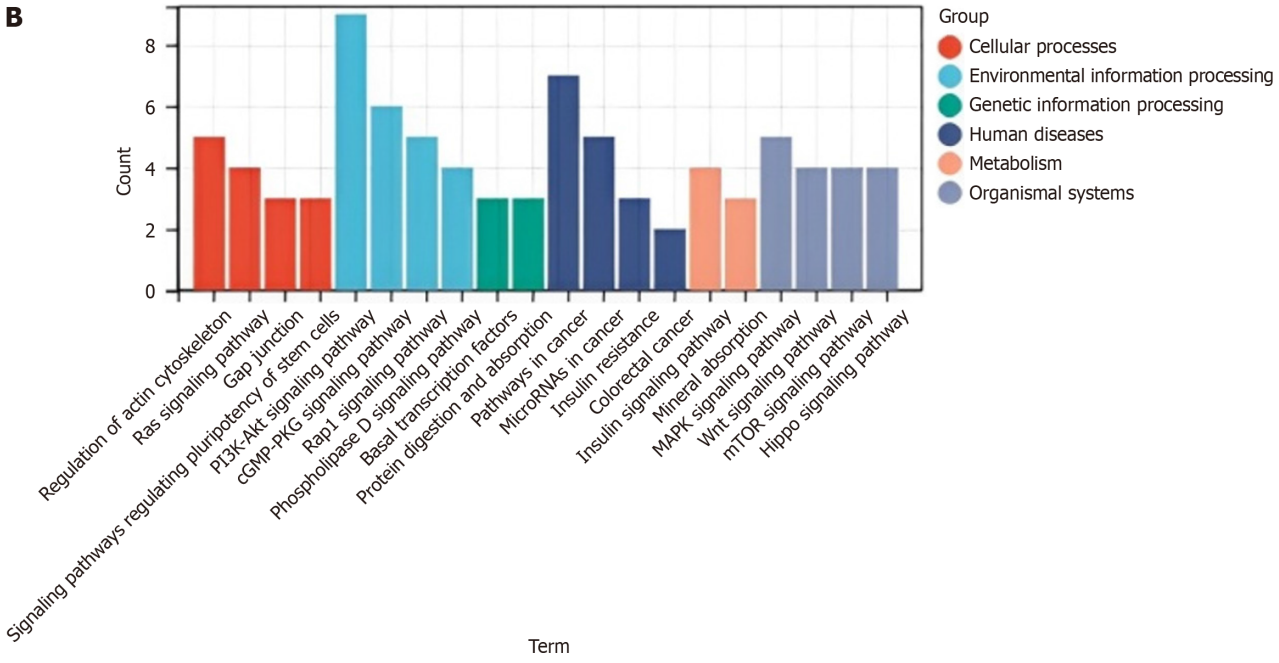
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cGMP-PKG signaling pathway
Rap1 signaling pathway
Phospholipase D signaling pathway
Basal transcription factors
Protein digestion and absorption
Pathways in cancer
MicroRNAs in cancer
Insulin resistance
Colorectal cancer
Insulin signaling pathway
Mineral absorption
MAPK signaling pathway
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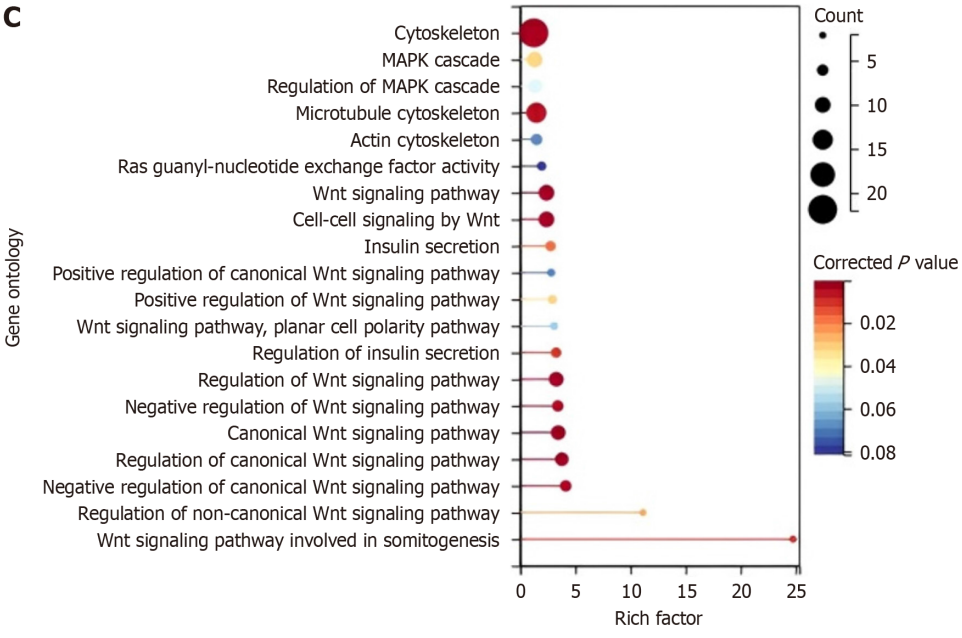
Genetic information processing
Human diseases
Metabolism
Organismal systems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MAPK cascade
Regulation of MAPK cascade
Microtubule cytoskeleton
Actin cytoskeleton
Ras guanyl-nucleotide exchange factor activity
Wnt signaling pathway
Cell-cell signaling by Wnt
Insulin secretion
Positive regulation of canonical Wnt signaling pathway
Positive regulation of Wnt signaling pathway
Wnt signaling pathway, planar cell polarity pathway
Regulation of insulin secretion
Regulation of Wnt signaling pathway
Negative regulation of Wnt signaling pathway
Canonical Wnt signaling pathway
Regulation of canonical Wnt signaling pathway
Negative regulation of canonical Wnt signaling pathway
Regulation of non-canonical Wnt signaling pathway
Wnt signaling pathway involved in somitogenesis
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25
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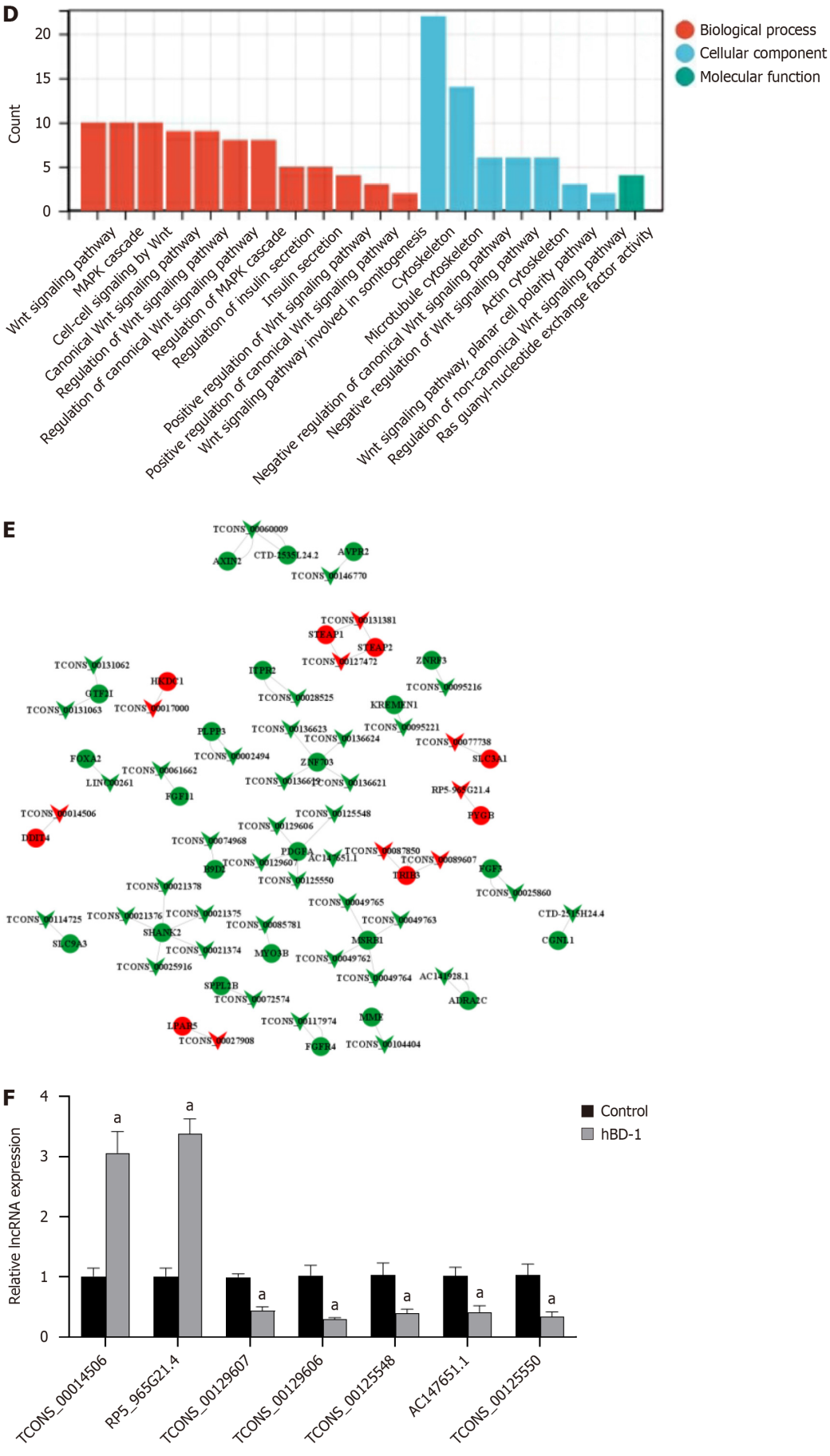

Figure 3 Bioinformatics analysis of differentially expressed long non-coding RNAs and selection of the target long non-coding RNA. A:

KEGG pathway enrichment analysis. The bubble size denotes the number of long non-coding RNAs (lncRNAs) target genes enriched in this pathway. Larger bubbles denote a higher number of target genes associated with the pathway. Darker color indicates more significant enrichment. The abscissa count indicates the number of genes in this pathway, and the ordinate signifies the KEGG pathway; B: GO pathway enrichment analysis. Bubble size signifies the number of lncRNA target genes enriched in this pathway, with a larger bubble indicating a larger number of target genes. Darker color indicates more significant enrichment. The abscissa count denotes the number of target genes, and the ordinate denotes a GO term; C: Co-expression analysis of differentially expressed lncRNAs and differentially expressed mRNAs. Inverted triangles indicate lncRNAs, circles indicate mRNAs, red color indicates upregulated expression, and green color indicates downregulated expression; D: KEGG pathways classification analysis. From left to right are cellular processes, environmental information processing, genetic information processing, and human diseases, metabolism, organismal systems; E: GO pathways classification analysis. Red parts indicate biological processes, blue parts indicate cellular components, and green parts indicate molecular function; F: LncRNA expression was detected *via* qRT-PCR. $^{a}P < 0.05$ *vs* control group. hBD-1: Human β-defensin-1.

Differentially expressed lncRNAs and mRNAs were analyzed for lncRNA-mRNA co-expression (Figure 3E). A total of 46 lncRNAs (9 upregulated and 37 downregulated) were obtained (Supplementary Table 4). These lncRNAs hold promise as therapeutic targets for diagnosing and treating colon cancer.

As per the KEGG pathway analysis, using $|\log FC| > 1$ and $P < 0.05$ as screening criteria, three differentially expressed mRNAs and seven differentially expressed lncRNAs associated with the autophagy pathway were enriched. These results were further confirmed by the lncRNA-mRNA co-expression analysis (Supplementary Table 5). qPCR data suggested an upregulation in the levels of TCONS_00014506 and RP5-965G21.4. Furthermore, the acquired data indicated that TCONS_00129607, TCONS_00129606, TCONS_00125548, AC147651.1, and TCONS_00125550 were downregulated (Figure 3F).

Identification of lncRNAs

LncRNA-mRNA co-expression analysis revealed that the target gene of the lncRNA TCONS_00014506 (TCONS_00014506) was DDIT4 (K08270: REDD1). DDIT4 has been confirmed to be a crucial regulator of the mTOR pathway[14]. Hence, the lncRNA TCONS_00014506 was selected as the subject of subsequent studies.

Inhibition of lncRNA TCONS00014506 by hBD-1 promotes autophagy and regulates p-mTOR expression in colon cancer cells

qPCR results showed a decrease in the expression of the lncRNA TCONS_00014506 in the si-lncRNA group in comparison with the control group (Figure 4A). Western blot analysis further revealed that the si-lncRNA group exhibited an increase in the expression level of p-mTOR, along with a decrease in Beclin1 and LC3II/I expression. Interestingly, following exposure to hBD-1, the level of p-mTOR decreased, along with an increase in Beclin1 and LC3 II/I expression ($P < 0.05$; Figure 4B-E).

hBD-1 promotes autophagy by inhibiting phosphorylation of mTOR in colon cancer cells

Western blot analysis revealed that MHY1485, an mTOR activator, increased the expression of p-mTOR while simultaneously reducing Beclin1 and LC3II/I expression in SW620 cells. Following exposure to hBD-1, the MHY1485-induced increase in p-mTOR was attenuated, along with an increase in Beclin1 and LC3 II/I expression ($P < 0.05$; Figure 5).

DISCUSSION

In China, the incidence of colon cancer has increased significantly in the past two decades. There is a tendency for colon cancer to be more common than rectal cancer[15]. The standard treatments for colorectal cancer include surgery, chemotherapy, radiotherapy, or a combination of several methods, but many patients relapse despite a series of treatments[16]. In this study, hBD-1 inhibited the viability and proliferation of SW620 cells.

hBD-1, as an antimicrobial peptide in humans, has garnered significant attention due to its involvement in cytotoxicity, cytolysis, and tumor immunity[7,8]. Notably, hBD-1 exhibits differential expression between normal tissue and tumor tissue, with varying levels along with tumor development. The significance of hBD-1 in tumorigenesis and progression is being increasingly acknowledged[17,18]. In this study, hBD-1 promoted the protein expression of Beclin1 and LC3II/I in SW620 cells, suggesting that hBD-1 promoted autophagy in SW620 cells. In cancer, autophagy is vital in the regulation of the tumor microenvironment. However, in later stages, autophagy can promote cancer cell proliferation and tumor growth, transitioning into a factor that supports cancer maintenance and contributes to treatment resistance[17-20]. Various chemotherapeutic and radiotherapeutic agents cause metabolic stress in cancer, which is accompanied by autophagy inhibition[21]. Regulation of autophagy could serve as an effective intervention strategy to prevent cancer development, limit tumor progression, and improve the efficacy of cancer treatment.

Recent research has unveiled the significant role of non-coding RNAs (ncRNAs) in various BPs. Dysregulation of ncRNAs has been linked to a range of diseases, including cancer. Although there is no evidence that lncRNAs encode peptides, an increasing number of studies have elucidated the crucial functions of lncRNAs in diverse BPs. These processes encompass proliferation, differentiation, neurogenesis, embryogenesis, stem cell pluripotency, tumorigenesis, and pathogenic infection[22,23]. It has been confirmed that lncRNAs affect autophagy in a variety of cancers[24-26]. In colon cancer, a multitude of lncRNAs have been demonstrated to be crucial players in cell proliferation, apoptosis, cell metastasis and invasion, cell cycle, drug resistance, and patient prognosis[27]. In the present research, it was observed

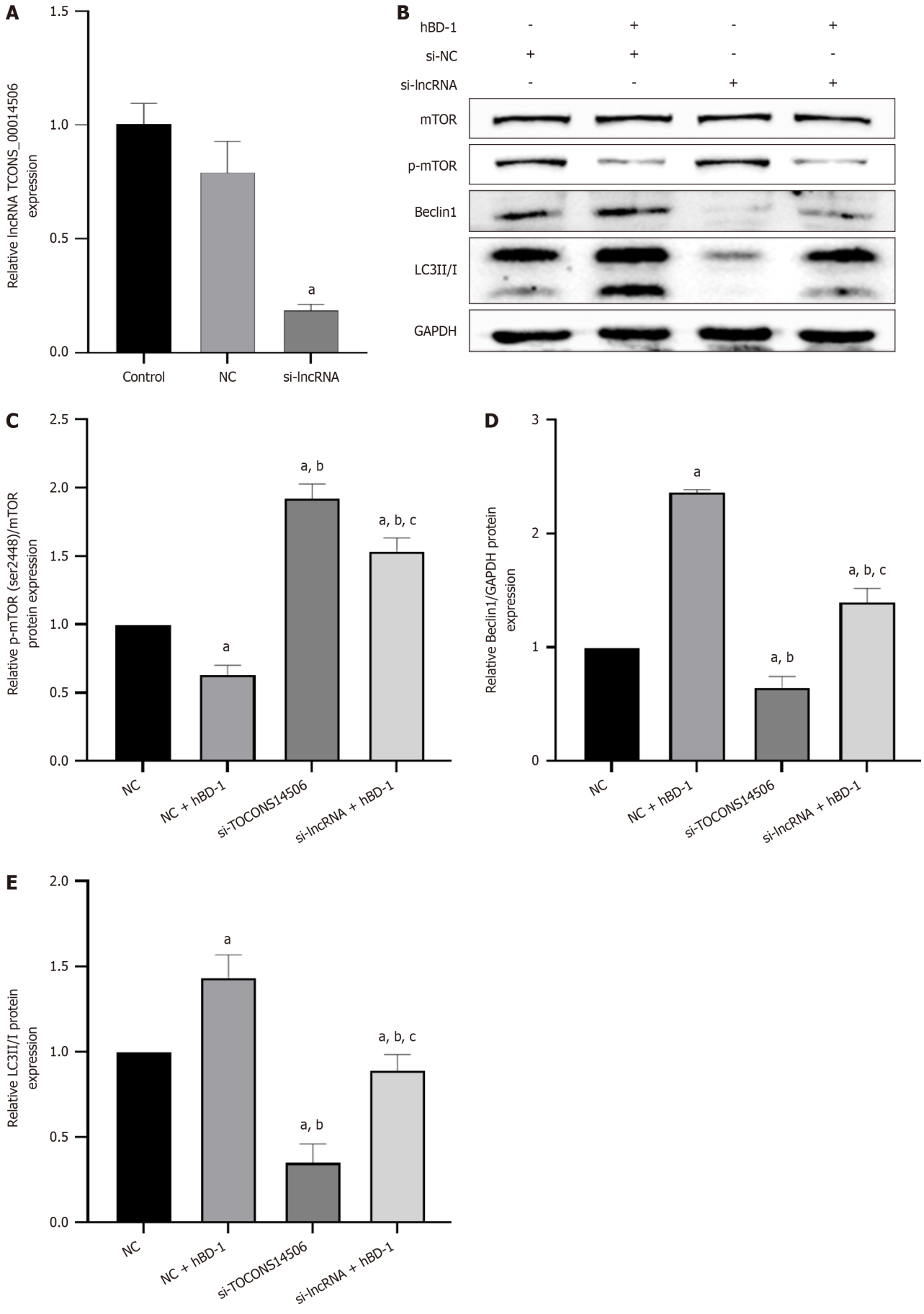

Figure 4 Human β-defensin-1 inhibits phosphorylation of mTOR and promotes autophagy in SW620 cells through long non-coding RNA

TCONS_14506. A: Long non-coding RNA (lncRNA) TCONS_00014506 expression at RNA level; B: Western blot analysis demonstrated the protein expression levels of p-mTOR (Ser2448), Beclin-1, and LC3II/I; C-E: Relative expression of p-mTOR, Beclin-1, and LC3II/I. $^{a}P < 0.05$ *vs* control group; $^{b}P < 0.05$ *vs* human β-defensin-1 group; $^{c}P < 0.05$ *vs* si-lncRNA group. $P < 0.05$ was considered statistically significant. hBD-1: Human β-defensin-1; lncRNA: Long non-coding RNA.

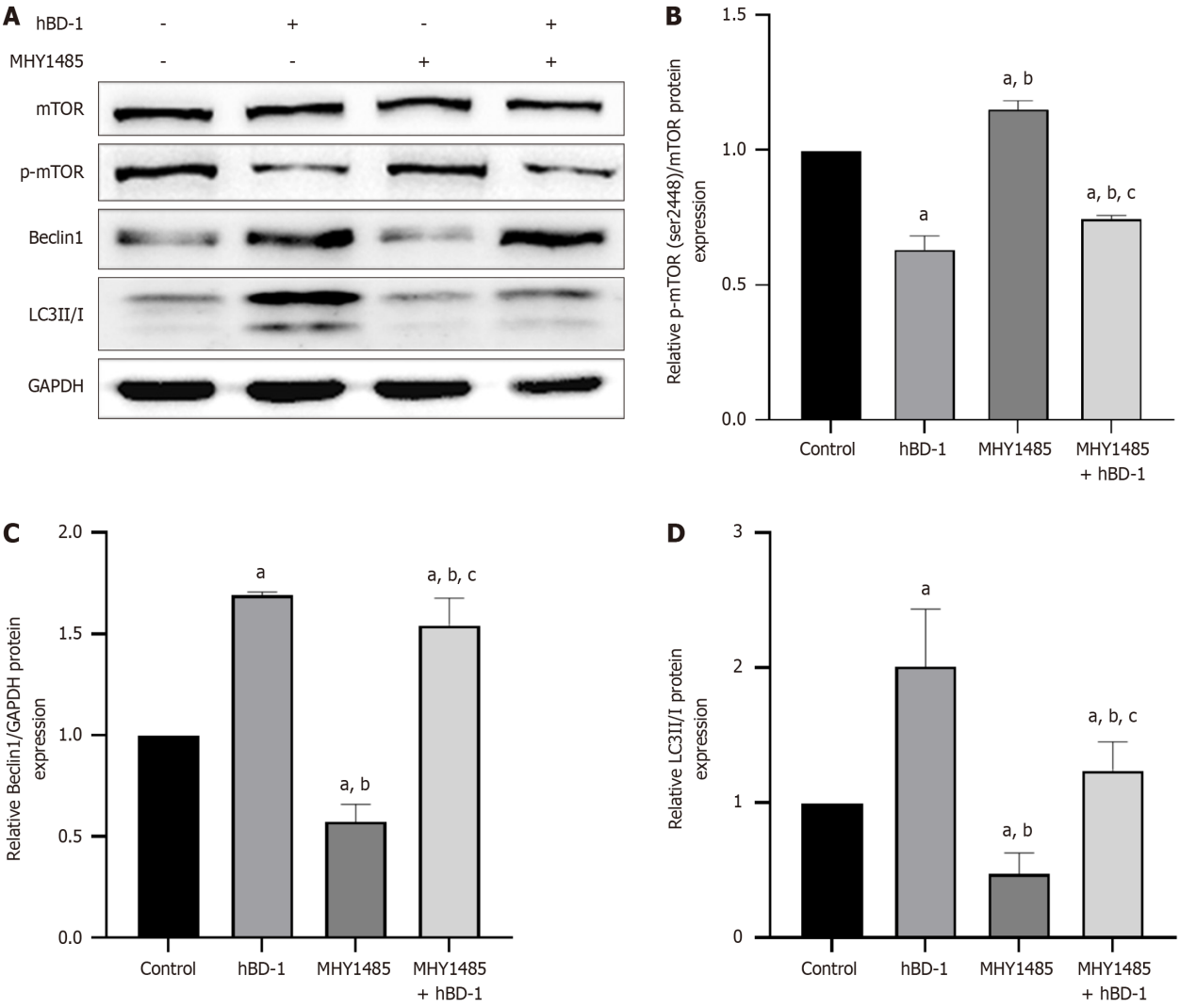

Figure 5 Human β-defensin-1 inhibits phosphorylated mTOR expression in SW620 cells. A: Western blot analysis demonstrated that the addition of MHY1485 (mTOR activator) increased p-mTOR (Ser2448) expression and reduced Beclin-1 and LC3II/I expression in SW620 cells; B-D: Relative expression of p-mTOR, Beclin-1, and LC3II/I. $^{a}P < 0.05$ *vs* control group; $^{b}P < 0.05$ *vs* human β-defensin-1 group; $^{c}P < 0.05$ *vs* MHY1485 group. $P < 0.05$ was considered statistically significant.

that hBD-1 stimulated the expression of the lncRNA TCONS_00014506 and concurrently inhibited p-mTOR expression. The resultant decrease in p-mTOR was accompanied by an increase in the relative protein expression of Beclin1 and LC3 II. Additionally, it was observed that inhibition of the lncRNA TCONS_00014506 resulted in increased p-mTOR expression, along with decreased Beclin1 and LC3 II protein expression. These effects were reversed upon exposure to hBD-1. It is inferred that hBD-1 inhibits mTOR phosphorylation and promotes autophagy in SW620 cells *via* the lncRNA TCONS_00014506.

CONCLUSION

In summary, this study verified that hBD-1 may induce autophagy in colon cancer SW620 cells by inhibiting phosphorylation of mTOR through the lncRNA TCONS00014506 at the cellular level. Additionally, associated lncRNAs hold potential as diagnostic and prognostic biomarkers, offering a novel theoretical foundation for diagnosing and treating colon cancer.

ARTICLE HIGHLIGHTS

Research background

Colorectal cancer has a low 5-year survival rate and high mortality. Human β-defensin-1 (hBD-1) may play an integral function in the innate immune system, contributing to the recognition and destruction of cancer cells. Long non-coding RNAs (lncRNAs) are involved in the process of cell differentiation and growth.

Research motivation

There is an urgent need for innovative treatment approaches for colon cancer. Our investigation into hBD-1 has revealed its effect on autophagy in colon cancer cells. During this exploration, we hypothesized that lncRNAs might play a crucial role in influencing autophagy. Consequently, our study delves into understanding the specific functions of lncRNAs in the context of hBD-1-mediated autophagy in colon cancer cells.

Research objectives

To investigate the effect of hBD-1 on the mTOR pathway and autophagy in human colon cancer SW620 cells.

Research methods

CCK8 assay was utilized for the detection of cell proliferation and the determination of the optimal drug concentration. Cell colony formation assay was employed to assess the effect of hBD-1 on SW620 cell proliferation. Bioinformatics was used to identify lncRNAs related to the mTOR pathway, aiming to identify those of potential biological significance. Additionally, p-mTOR (Ser2448), Beclin1, and LC3II/I expression levels in SW620 cells were assessed through Western blot analysis.

Research results

hBD-1 inhibited the proliferative ability of SW620 cells, as evidenced by the reduction in the colony formation capacity of SW620 cells upon exposure to hBD-1. hBD-1 decreased the expression of p-mTOR (Ser2448) protein and increased the expression of Beclin1 and LC3II/I protein. Furthermore, bioinformatics analysis identified seven lncRNAs (2 upregulated and 5 downregulated) related to the mTOR pathway. The lncRNA TCONS_00014506 was ultimately selected. Following the inhibition of the lncRNA TCONS_00014506, exposure to hBD-1 inhibited p-mTOR (Ser2448) and promoted Beclin1 and LC3II/I protein expression.

Research conclusions

HBD-1 inhibits the mTOR pathway and promotes autophagy by upregulating the expression of the lncRNA TCONS_00014506 in SW620 cells.

Research perspectives

We for the first time found that the lncRNA TCONS_00014506 can regulate mTOR during hBD-1-affected autophagy.

FOOTNOTES

Co-first authors: Yu-Xin Zhao and Yan Cui.

Co-corresponding authors: Li-Li Bao and Peng-Wei Zhao.

Author contributions: Zhao YX and Zhao PW wrote this article; Bao LL and Zhao PW designed, organized, and reviewed this article; Li XH, Yang WH, and An SX collected the data; all authors have read and agreed to the published version of the manuscript. Zhao YX and Cui Y contributed equally to this work as co-first authors. The reasons for designating Zhao YX and Cui Y as co-first authors are threefold. First, the research was performed as a collaborative effort, and the designation of co-first authorship accurately reflects the distribution of responsibilities and burdens associated with the time and effort required to complete the study and the resultant paper. Second, the overall research team encompassed authors with a variety of expertise and skills from different fields of the co-first authors best reflects this diversity. They also did many works on the test. Third, Zhao YX and Cui Y contributed efforts of equal substance throughout the research process. The choice of these researchers as co-first authors acknowledges and respects this equal contribution, while recognizing the spirit of teamwork and collaboration of this study. In summary, we believe that designating Zhao YX and Cui Y as co-first authors is fitting for our manuscript as it accurately reflects our team's collaborative spirit, equal contributions, and diversity. Bao LL and Zhao PW contributed equally to this work as co-corresponding authors. The reasons for designating Bao LL and Zhao PW as co-corresponding authors are threefold. First, the research was performed as a collaborative effort, and the designation of co-corresponding authorship accurately reflects the distribution of responsibilities and burdens associated with the time and effort required to complete the study and the resultant paper. This also ensures effective communication and management of post-submission matters, ultimately enhancing the paper's quality and reliability. Second, the overall research team encompassed authors with a variety of expertise and skills from different fields, and the designation of co-corresponding authors best reflects this diversity. This also promotes the most comprehensive and in-depth examination of the research topic, ultimately enriching readers' understanding by offering various expert perspectives. Third, Bao LL and Zhao PW contributed efforts of equal substance throughout the research process. The choice of these researchers as co-corresponding authors acknowledges and respects this equal contribution, while recognizing the spirit of teamwork and collaboration of this study. In summary, we believe that designating Bao LL and Zhao PW as co-corresponding authors is fitting for our manuscript as it accurately reflects our team's collaborative spirit, equal contributions, and diversity.

Supported by National Natural Science Foundation of China, No. 82360329; Inner Mongolia Medical University General Project, No. YKD2023MS047; Inner Mongolia Health Commission Science and Technology Plan Project, No. 202201275.

Institutional review board statement: The study was agreed by Ethics Committee of Inner Mongolia Medical University (Approval No. YKD202201151).

Informed consent statement: Our research was conducted using cell cloning, bioinformatics, and molecular biology techniques. No human samples were involved and informed consent is not required.

Conflict-of-interest statement: The authors declare no conflict of interest for this article.

Data sharing statement: All data generated or analyzes during this study are included in this published article and supplied materials.

Country/Territory of origin: China

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S-Editor: Lin C
L-Editor: Wang TQ
P-Editor: Zheng XM

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