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HAS-CIRCpedia-5280 sponges miR-4712-5p inhibited colon cancer autophagyinduced by human beta-defensin-1

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Abstract

Background Among all malignancies, colorectal cancer ranks third in incidence rate and second in mortality rate. Human beta-defensin-1 (hBD-1) has broad-spectrum antimicrobial properties, and it plays an important role in the tumor microenvironment. Circular ribonucleic acids (circRNAs) regulate the proliferation and progression of colorectal cancer cells via cancer-related signaling pathways.

Methods Cell proliferation was assessed using the Cell Counting Kit-8 assay to determine the optimal hBD-1 concentration. Intracellular autophagic vesicles were visualized via monodansylcadaverine staining. In addition, the levels of AKT and mammalian target of rapamycin (mTOR)-associated signaling proteins were analyzed via Western blot analysis. CircRNA microarrays and quantitative real-time polymerase chain reaction were used to identify differentially expressed circRNAs in colon cancer cell lines. The functional role of HAS-CIRCpedia-5280 in vitro was demonstrated by overexpressing HAS-CIRCpedia-5280 and inhibiting miR-4712-5p. HAS-CIRCpedia-5280 could be a sponge of miR-4712-5p, mimicking the effect induced by HAS-CIRCpedia-5280 overexpression in colon cancer cells.

Results hBD-1 inhibited the proliferation of colon cancer cells and increased the number of intracellular autophagic vesicles. In addition, hBD-1 inhibited the AKT/mTOR signaling pathway, thereby enhancing cellular autophagy. Further, the interaction of HAS-CIRCpedia-5280 and miR-4712-5p was investigated. hBD-1 upregulated the expression level of HAS-CIRCpedia-5280 and downregulated the expression level of miR-4712-5p in colon cancer cells. Subsequently, the overexpression of HAS-CIRCpedia-5280 or the inhibition of miR-4712-5p activated the AKT/mTOR signaling pathway, leading to cellular autophagy inhibition. Conversely, the mimicry of miR-4712-p counteracted the effect of HAS-CIRCpedia 5280 overexpression in colon cancer cells by inhibiting the activation of the AKT/mTOR signaling pathway and, thereby, enhancing cellular autophagy.

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Conclusion hBD-1 can have an inhibitory effect against cell proliferation in colon cancer SW-620/HCT-116 cells via the HAS-CIRCpedia-5280/miR-4712-5p-mediated activation of autophagy.

Keywords Colon cancer, Human β-defensin-1, Circular RNAs, miRNA sponge, Autophagy

Introduction

Colorectal cancer (CRC) is the third most common cancer and a leading cause of cancer-related mortality worldwide, accounting for approximately 10% of all cancer diagnoses and 9.4% of tumor-related deaths [1]. CRC symptoms are often subtle, and CRC is challenging to predict due the lack of early diagnostic markers [2, 3]. Therefore, patients are commonly diagnosed at the late stage, leading to a higher mortality rate and posing a major health threat. Moreover, there is an urgent need for more effective treatment methods that suppress tumor progression and improve the prognosis and survival of late-stage CRC.

Defensins are endogenous defense peptides with well-defined broad-spectrum antimicrobial properties against different pathogens including bacteria, fungi, viruses, and parasites [4]. They also have other immune-related functions, thereby playing a relevant role in activating the adaptive immune system and generating anti-tumor immunity, which then contribute to the success of immunotherapy [5, 6]. Human beta-defensin-1 (hBD-1) promotes apoptosis and inhibits the migration and invasion of cancer cells in different cancers. Further, other studies have proposed that defensins are a tumor suppressor [7]. Biomarker analysis with various degrees of success in cancer diagnosis has been performed. Defensins are biomarkers for assessing disease activity and monitoring treatment response [8].The current study aimed to further investigate the impact of hBD-1 on cell proliferation and migration, anti-tumor mechanisms, and potential biomarkers in colon cancer.

Circular ribonucleic acids (circRNAs) are a type of non-coding RNAs that are generated during RNA splicing and that lack a 5′-terminal cap and a 3′-terminal poly-A tail, as they are formed by covalent bonding between the head and tail [9]. Due to the absence of a 3′end, circRNAs circRNAs are less susceptible to digestion by nucleic acid ectonucleases, which make them more stable than their linear mRNA counterparts linear mRNA counterparts [10]. These circRNAs may have significant regulatory effects on multiple genes including sponging microRNA (miRNAs) [11], interacting with RNA-binding proteins, regulating transcription and splicing, and translating protein [12]. Additionally, circRNAs exhibit unique properties such as abundance, stability, and tissue-specifc expression [13]. Current research has focused on the potential use of circulating tumor mRNA, microRNA, and cir culating cytokeratin as markers [14]. The expression of ci rcular RNAs was significantly downregulated in the colon cell line [15], i ndicating its potential as a biomarker for cancer detection and monitoring.This study aimed to investigate the role of hBD-1 in regulating autophagy in colon cancer cells via HAS-CIRCpedia-5280/miR-4712 5p. Results revealed that hBD-1 treatment significantly downregulated the HAS-CIRCpedia-5280 expression in colon cancer cells. HAS-CIRCpedia-5280, which is derived from the post-splicing (from exon 3 to exon 5) of LRP5 mRNA located on chromosome 11:6835764968365702 with a length of 537 nucleotides (nt), may play a ole in tumorigenesis by competitively binding to miR-4712-5p and affecting autophagy in colon cancer cells.

Methods

Cell culture

The human colon cancer cell line SW-620 was cultured in high-glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin. The human colon cancer cell line HCT-116 was cultured in DMEM with 10% fetal bovine serum and 1% penicillin–streptomycin. All cells were maintained at 37°C in a humidified atmosphere with 5% CO2. All cells were purchased from ORiCells Biotechnology, (Shanghai, China).

Cell proliferation assay

SW-620 and HCT-116 cells at a density of 7000 cells/well in the logarithmic growth phase were seeded into 96-well plates and diluted to 100 ìl/well. After cell adhesion to the plate, each well was treated with hBD-1 at various concentrations (35, 40, 45, 50, 55, 60, and 65 ng/mL). Subsequently, each well received an additional 10 ìL of the Cell Counting Kit-8 (CCK-8) reagent and was then incubated at 37°C for another hour. The absorbance was measured at a wavelength of 450 nm using an enzyme marker. Cell proliferation was assessed at different time points (12, 24, and 48 h). All experiments were conducted in triplicate. Additional file 1: Table 1 shows the RNA oligonucleotides sequences.

Cell migration

Approximately 2 × 10^5 cells/well were inoculated into a 6-well plate and incubated for 24 h. The monolayer was scraped using a sterile pipette tip (10 ìL). The cells were washed three times with phosphate-buffered saline and then incubated in serum-free DMEM. At 0 and 48 h, the scratch status of each well was captured under a

microscope. The ImageJ software was then utilized to analyze the total wound area to evaluate cell migration capability.

Cell transfection

SW-620 and HCT-116 cells were transfected with various constructs including the HAS-CIRCpedia-5280 overexpression plasmid, miR-4712-5p inhibitor, HAS-CIRCpedia-5280 overexpression plasmid combined with miR-4712-5p mimic, as well as the pcDNA3.1 vector NC, inhibitor-NC, HAS-CIRCpedia-5280 overexpression plasmid and mimic-NC.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from cell lines using TRIzol reagent. Subsequently, 500 ng of RNA was reverse transcribed to cDNA with a reverse transcription kit and then subjected to quantitative real-time polymerase chain reaction (qRT-PCR) using the SYBR green quantification kit. Three parallel wells were created for each sample. The expression levels of HAS-CIRCpedia-5280 and miR- 4712-5p were then examined. The circRNA levels were normalized to the GAPDH. Meanwhile, the miRNA levels were normalized to the U6 level. Additional file 1: Table 2 depicts the PCR primer sequences. The fold change in RNA expression was calculated using the 2-Δ Ct method.

Western blot analysis

Cellular protein was extracted using the radioimmunoprecipitation assay (RIPA) buffer. After bicinchoninic acid quantification, 20 μg of total protein was separated by SDS-PAGE and transferred to the NC membrane. The primary antibody was incubated overnight at 4 °C. The following day, the membrane was rinsed and the secondary antibody was incubated at room temperature for 1 h. Images were captured using the ECL chemiluminescence gel imaging system.

CircRNA sequencing and bioinformatics analysis

The transcriptome sequencing project was conducted using the Illumina sequencing platform. Differentially expressed circRNAs were identified using DESeq and screened for those with a logFC of > 1 and P value of < 0.05. Volcano plots were generated using the R language ggplot 2 software package. Clustering analysis of differentially expressed circRNAs was performed using the pheatmap package. Enrichment analysis of the biological pathways associated with differentially expressed circRNA target genes was carried out based on data from the KEGG database. (https://www.kegg.jp/).

Monodansylcadaverine staining

SW-620 and HCT-116 cells were transfected with or without hBD-1 at a density of 1000 cells/well in 96-wel plates. The plates were washed once with monodansylcadaverine (MDC) wash solution dissolved in double-distilled water (1:10).Subsequently, the cells were incubated for 30–45 min and washed three times with MDC wash solution. Thereafter, the cells were placed in 100 ìl of MDC wash solution, and three fields of view were captured to observe the cell images.

RNase R digestion

RNA from SW-620 and HCT-116 cells was isolated using TRIzol reagent. In total of 2 μg of RNA was incubated with 6 U/μL RNase R for 15 min at 37 °C to validate the characterization of the circRNA.

Radioimmunoprecipitation

The radioimmunoprecipitation assay was conducted to validate the direct interaction of hsa-circpedia-5280 and Ago2 as well as miR-4712-5p and Ago2 using the RNA Immunoprecipitation Kit. RNA was isolated and reverse transcribed into cDNA for subsequent qRT-PCR analysis.

Statistical analysis

Statistical analysis was conducted using the Statistical Package for the Social Sciences software version 26.0, and the data were presented as mean ± standard deviation. The independent samples t-test was used to compare two groups, and one-way analysis of variance was used to analyze multiple groups. A two-sided P value of less than 0.05 was considered statistically significant.

Results

HBD-1 suppressed the proliferation of colon cancer cells and induced autophagy

Defensins play a role in various cell death pathways, including apoptosis, pyroptosis, and necrosis [16]. However, the specific mechanism by which hBD-1 is involved in the progression of autophagy in colon cancer should be explored. The CCK-8 assay revealed that hBD-1 decreased the survival rate of colon cancer cells. The half maximal inhibitory concentration (IC50) values of SW-620 and HCT-116 in the hBD-1 group were measured at 12, 24, and 48 h. Subsequently, a concentration of 50 ng/mL was selected for further experiments (Fig. 1A and B). In addition, cell migration was reduced by hBD-1 via wound healing assays (Fig. 1C). MDC staining showed that hBD-1 increased the number of intracellular autophagic vesicles (Fig. 1D). Further, Western blotting analysis revealed that treatment with hBD-1 at a concentration of 50 ng/mL for 48 hours inhibited the phosphorylation of AKT/mTOR proteins and increased the ratio of autophagic protein LC3II/I and, the expression of

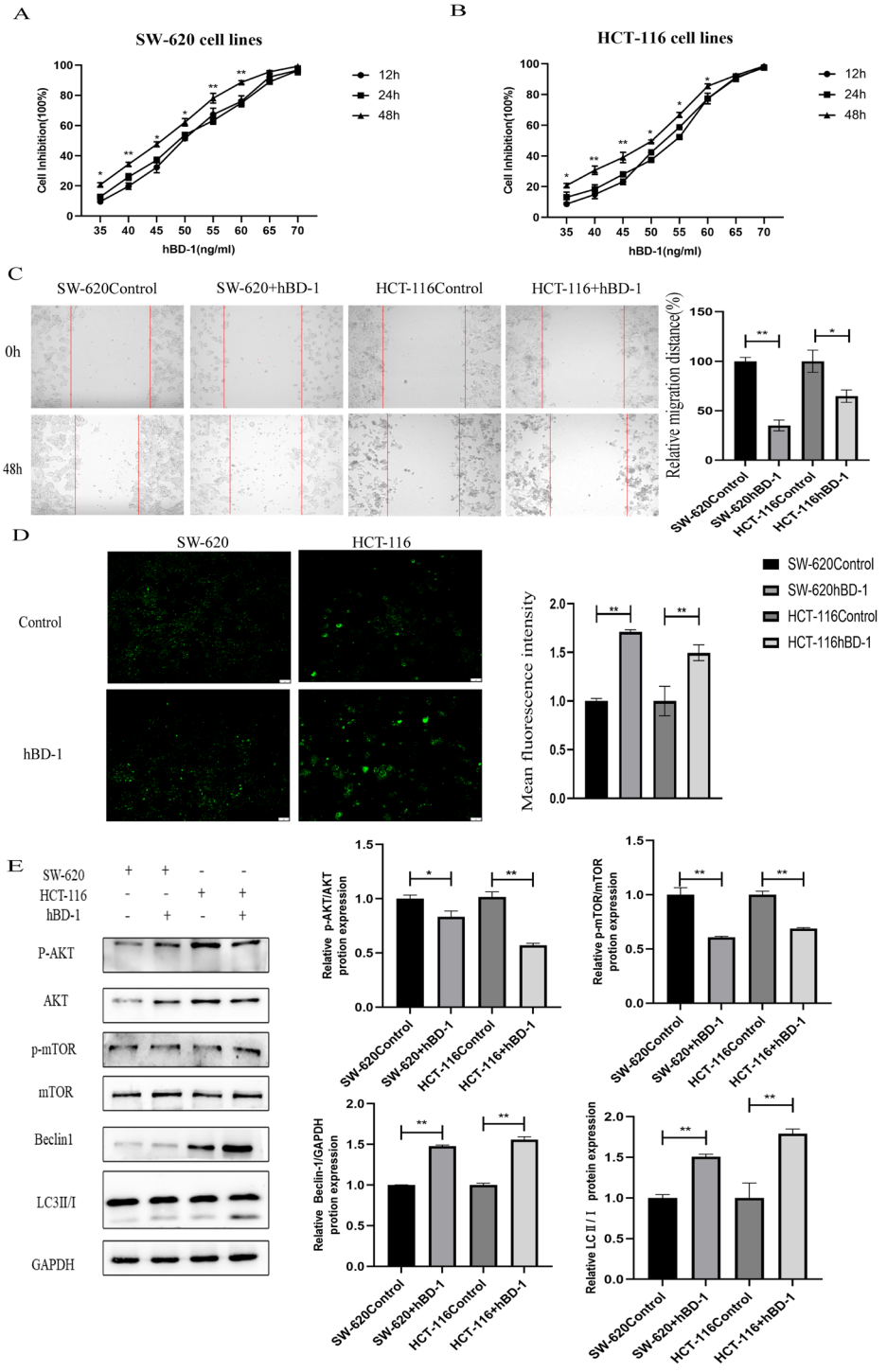

Fig. 1 HBD-1 inhibited the proliferation of colon cancer cells and promoted autophagy. (**A**, **B**): CCK-8 was used to detect the cell proliferation curve. (**C**): The c ell migration ability was detected using the scratch assay. (**D**): MDC staining was utilized to assess the presence of autophagic vesicles in the cells. (**E**): Western blotting analysis was performed to detect the AKT/mTOR signaling pathway-related proteins, LC3II/I ratio, and Beclin-1 expression. The representative Western blotting bands and the quantification of protein expression are shown. Statistical significance was denoted as the control group. hBD-1: Human beta-defensin-1,*p<0.05, **p<0.01

beclin-1 (Fig. 1E). Collectively, these results revealed that hBD-1 inhibits the proliferation and migration of colon cancer cells, thereby attenuating cancer progression and promoting autophagy in cancer cells.

The expression of HAS-CIRCpedia-5280 in colon cancer cells was downregulated by hBD-1

To elucidate the role of hBD-1 in colon cancer cells, RNA sequencing was performed on SW-620 cells. Results showed differential circrnas in SW-620 cells treated with hBD-1. In particular, the expressions of 8 and 20 circRNAs were upregulated and downregulated in hBD-1 treated SW-620 cells. The volcano plot showed the distribution of circRNAs (Fig. 2A), while an overall hierarchical cluster plot based on the RPKM values was generated for all differentially expressed circRNAs (Fig. 2B). In addition, the KEGG pathway enrichment analysis identified the top 20 most significantly enriched pathways (Fig. 2C) by sorting P-values for representation purposes. The top 20 most significantly enriched GO terms were selected for mapping based on the ordering of P-value. Moreover, the GO pathways were categorized according to biological process (BP), cellular component (CC) and molecu-lar function (MF) (Additional file 1: Figures 1 and 2). Three differentially expressed mRNAs and four differentially expressed circRNAs associated with the autophagy pathway were enriched based on the KEGG pathway analysis with a $|\log FC|$ of > 1 and a P value of <0.05 as the screening criteria (Additional file 1: Table 3). The expression of three differentially expressed circRNAs was quantitatively analyzed in two colon cancer cell lines (SW-620, HCT-116). Based on the qRTPCR, the expression level of has-circpedia-5280 was downregulated in all cancer cell lines (Fig. 2D). According to RNA sequencing, low-density lipoprotein receptor-related protein 5 (LRP5), which promotes bone gene differentiation of bone marrow mesenchymalstem cells, was the host gene of has-circpedia-5280 [17] LRP5 is a key regulator of the AKT/mTOR pathway [18], with an unclear role in colon cancer. To validate the loop structure of has-circpedia-5280, LRP5 was used as a control for linear RNA. After the RNase R treatment, the expression level of has-circpedia-5280 slightly decreased. Meanwhile, the expression level of LRP5 significantly decreased (Fig. 2E and F). Then, SW-620 and HCT-116 cell cDNA and genomic DNA (gDNA) were amplified with polymeric primers or divergent primers. Results showed that has-circpedia-5280 could only be observed in cDNAs amplified with different primers (Fig. 2G). Based on these data, the HAS-CIRCpedia-5280 expression is downregulated by hBD-1 to achieve a stable ring structure in colon cancer cells.

HAS-CIRCpedia-5280 functions as an mir-4712-5p sponge

To investigate the potential involvement of has-circpedia-5280 in the development of colon cancer at the post-transcriptional level, the LRP5 expression in colon cancer cells was examined. According to the qRT-PCR results, there was no significant difference in the expression level of LRP5 in colon cancer cells with or without hBD-1 treatment (Fig. 3A). The potential target microRNAs that may bind to the has-circpedia-5280 sequence were predicted using data from the Miranda and miRDB databases (Fig. 3B). The expression levels of these microRNAs were subsequently detected via qRT-PCR (Fig. 3C). Notably, miRNA-4712 -5p promotes the proliferation of vulvar squamous cell carcinoma cells by regulating the AKT signalling pathway [19]. Therefore, miRNA-4712-5p was used in for further studies. Radioimmunoprecipitation experiments revealed that has-circpedia-5280 and miR-4712 -5p directly bind to Ago2 (Fig. 3D). Based on these results, has-circpedia-5280 may function as a miR-4712-5p sponge.

HAS-CIRCpedia-5280 inhibits colon cancer cell proliferation and migration and promotes autophagy via hBD-1

Overexpressed has-circpedia-5280 was transfected into SW-620 and HCT-116 cells. The has-circpedia-5280 expression was upregulated, whereas the miR-4712-5p expression was downregulatedbased on qRT-PCR analysis (Fig. 4A).In addition, the proliferation and migration of cells were examined using CCK-8 and wound healing assays. The overexpression of circCUL2 increased cell proliferation and migration (Fig. 4B and C). MDC staining revealed a reduced number of intracellular autophagic vesicles in cells with upregulated has-circpedia-5280 expression (Fig. 4D). Further, Western blot analysis revealed that has-circpedia-5280 increased the levels of phosphorylated AKT/mTOR proteins and decreased the levels of Beclin -1 and LC3II/I expression (Fig. 4E). However, theoverexpression of hascircpedia-5280 treated with hBD-1 had the opposite effect. That is, it inhibited cell proliferation and migration and promote autophagy (Fig. 4B-E). Based on these results,has-circpedia-5280 can promote colon cancer cell proliferation and migration and inhibit autophagy, when the overexpression of has-circpedia-5280 exposed to hBD-1 reversed these phenomena.

Mir-4712-5p inhibits colon cancer cell progression via hBD-1

The effect of miR-4712-5p inhibitor on miR-4712-5p expression and the expression level of miR-4712-5p were down-regulated in SW-620 and HCT-116 cells via qRT-PCR (Fig. 5A). miR-4712-5p inhibitor enhances the proliferation and migration of colon cancer cells and reduces

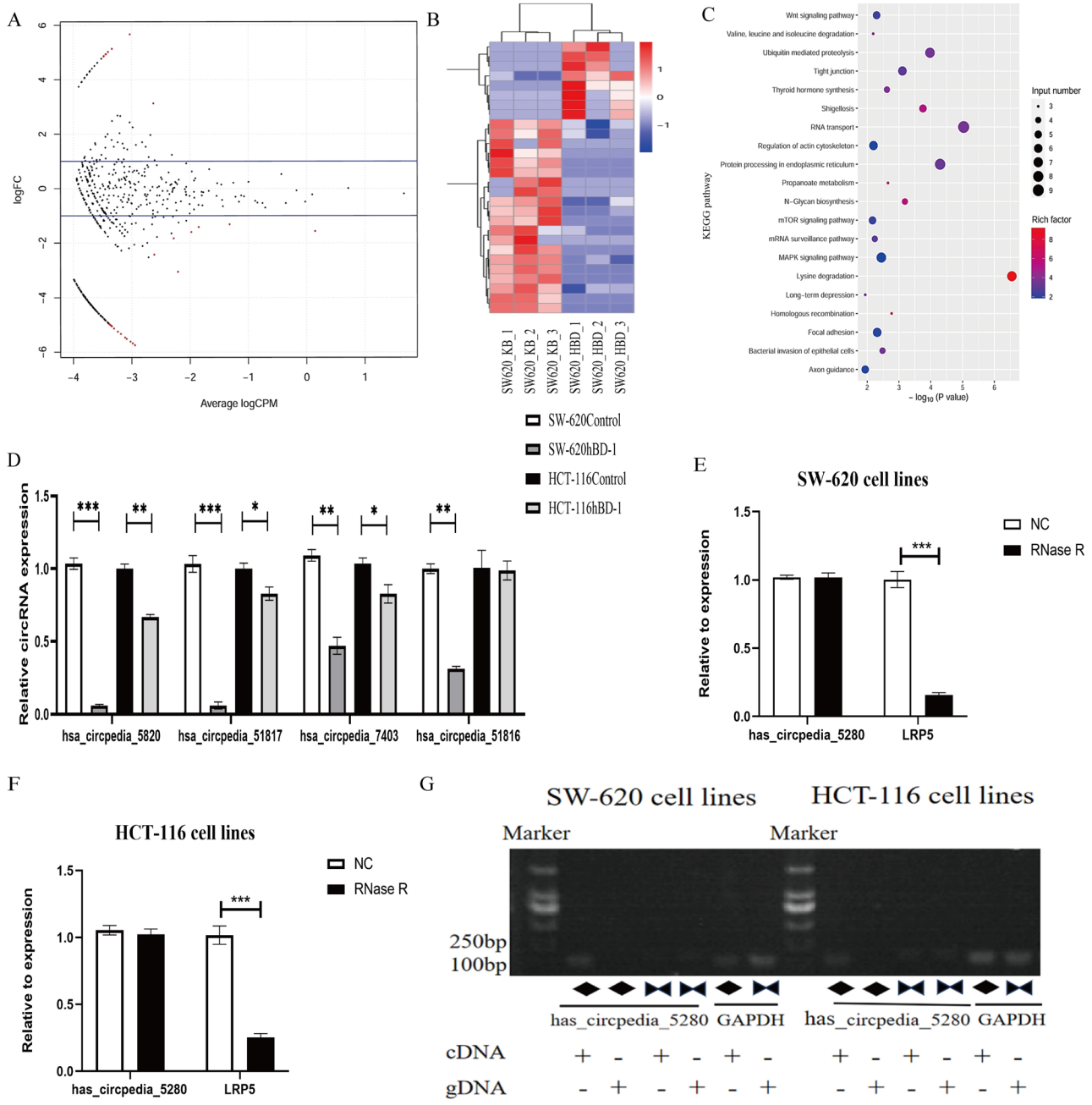

Fig. 2 The expression level of HAS-CIRCpedia-5280 was significantly down-regulated in colon cancer cells after the action of hBD-1. (**A**): Volcano plot of differentially expressed circRNAs. (**B**): Cluster analysis of differentially expressed circRNAs. (**C**): The KEGG pathway enrichment analysis. **D**): Detecti on of circRNAs expression vis qRT-PCR. (**E**-**G**): The RNaseR resistance test and PCR amplification validated the circular structure of has-circpedia-5280 with polymeric and divergent primers. Versus control group. hBD-1: Human beta-defensin-1, *p<0.05, **p<0.01

the number of intracellular autophagic vesicles (Fig. 5B-E).The inhibition of miR-4712-5p increased the phosphorylation levels of AKT/mTOR proteins and decreased the expression levels of Beclin-1 and LC3II/I Western blot analysis showed that autophagy was inhibited (Fig. 5E). Results showed that the overexpression of has-circpedia-5280 and the inhibition of miR-4712-5p had similar trends, which were subsequently reversed by hBD-1. Hence circCUL2 may exert its function via interaction with miR-142-3p.

Mir-4712-5p mimics mitigated the effects of overexpressed HAS-CIRCpedia-5280 in colon cancer cells via HBD-1

To further validate whether has-circpedia-5280 exerts its function via interaction with miR-4712-5p, the miR-4712-5p mimic and has-circpedia-5280 expression

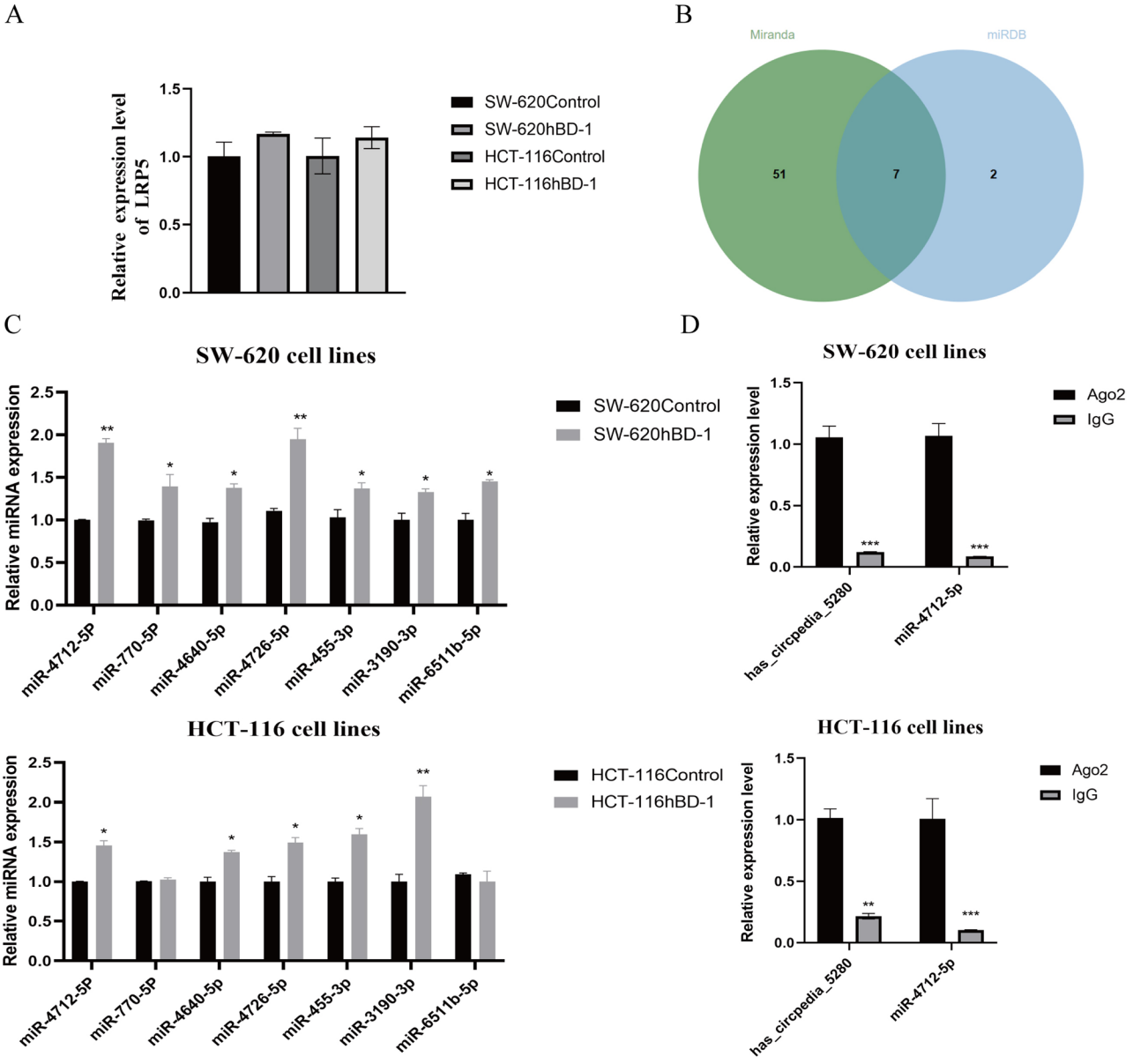

Fig. 3 HAS-CIRCpedia-52,802 functions as an miR-4712-5p sponge. (**A**): The qRT-PCR was used to detect LRP5 expression at the RNA level. (**B**): Potential target miRNAs that bind to has-circpedia-5280 were predicted using data from the Miranda and miRDB databases. (**C**): The qRT-PCR was used to detect microRNA expression. (**D**): The RIP experiment revealed the combined presence of both has-circpedia-5280 and miR-4712-5p with Ago2. Versus. control group. hBD-1: Human beta-defensin-1, *p<0.05, **p<0.01

plasmid were co-transfected into CRC cells. The qRT-PCR validated the downregulation of has-circpedia-5280 expression and the upregulation of miR-4712-5p expression in SW-620 and HCT116 cells overexpressing has-circpedia-5280 plasmids and miR 4712 5p mimics (Fig. 6A). The CCK8 assay revealed the inhibition of colon cancer cell proliferation (Fig. 6B). Meanwhile, the scratch assay showed the inhibitory effect against the migration ability of cells (Fig. 6C). The MDC staining revealed an increased number of intracellular autophagic vesicles (Fig. 6D). The western blot analysis further showed that miR-4712-5p inhibition decreased the phosphorylated expression levels of AKT/mTOR proteins and increased the Beclin-1 and LC3II/I expression levels (Fig. 6E). Interestingly, exposure to hBD-1 significantly inhibited the proliferation and migration of CRC cells while promoting autophagy. Therefore, has-circpedia-5280 suppresses CRC cell growth and metastasis via hBD-1 by sponging miR-4712-5p.

Discussion

Approximately 41% of CRCs develop in the proximal colon, 22% in the distal colon, and 28% in the rectum [20]. The tumors are classified as rectal and colorectal

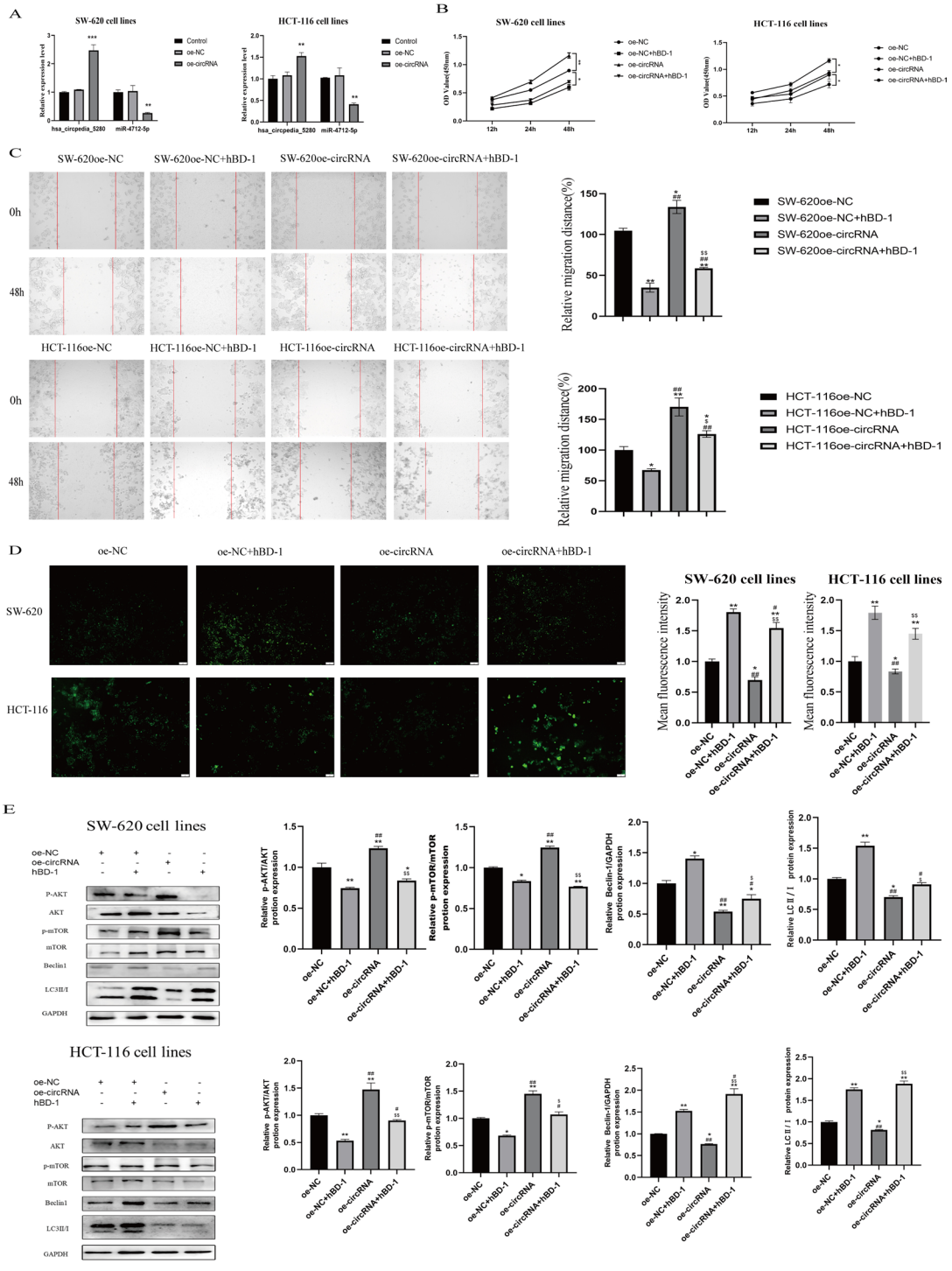

Fig. 4 HAS-CIRCpedia-5280 inhibits colon cancer cell proliferation and migration and promotes autophagy via hBD-1. (**A**): RT-qPCR detected has-circpedia-5280 and miR-4712-5p expression. (**B**): CCK-8 cell proliferation curve. (**C**): The cell migration ability was detected using the scratch assay. (**D**): MDC staining for intracellular autophagic vesicles. (**E**): Western blotting analysis for detecting the AKT/mTOR signaling pathway related proteins, LC3II/I ratio, and Beclin-1 expres-sion, the representative Western blotting bands, and the quantification of protein expression. Versus. oe-NC, *p<0.05, **p<0.01; Versus. oe-NC+hBD-1, #p<0.05, ##p<0.01; Versus. oe-circRNA, $p<0.05, $$p<0.01

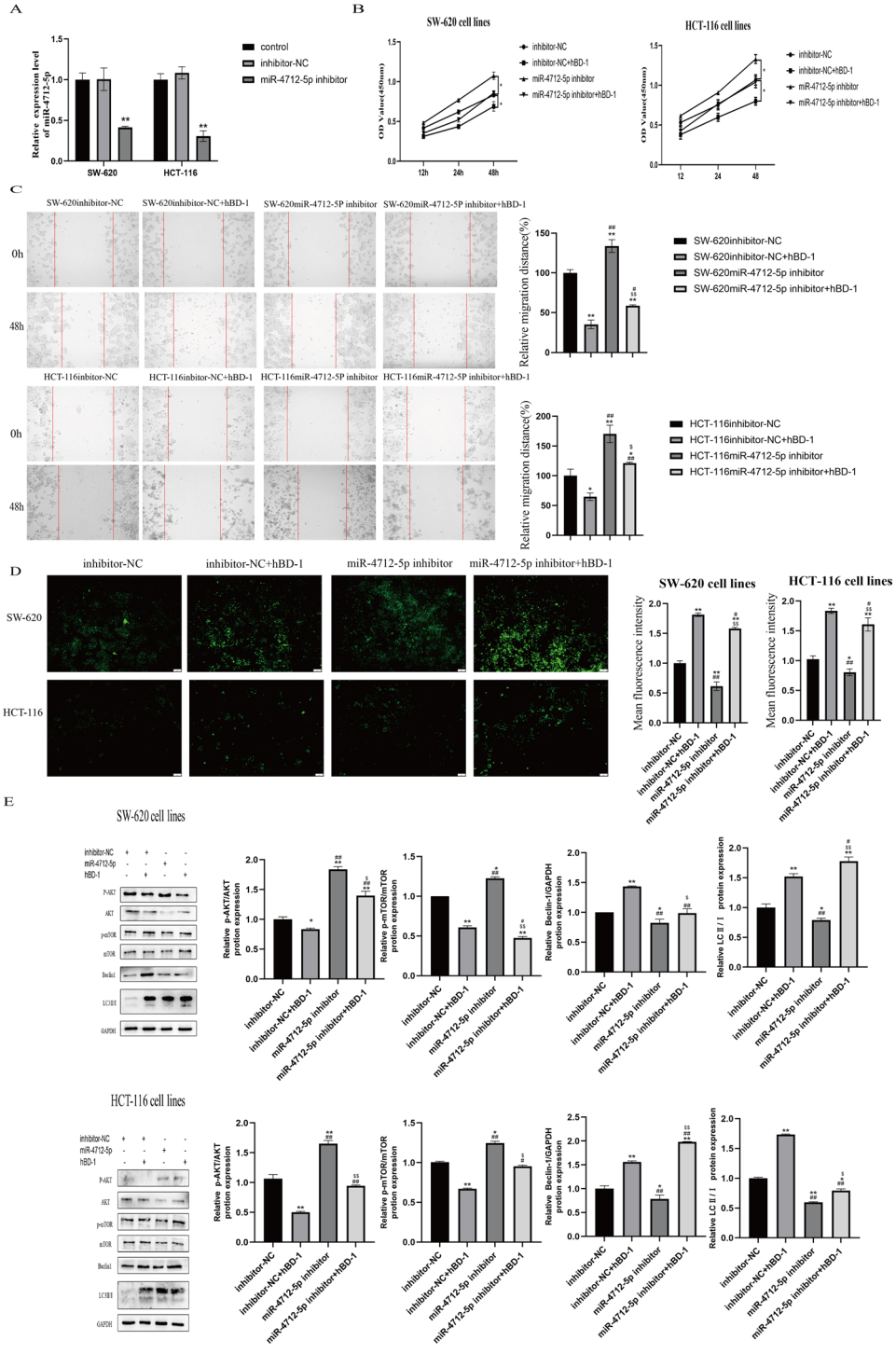

Fig. 5 miR-4712-5p inhibits colon cancer cell progression via hBD-1 (**A**): The qRT-PCR detected the miR-4712-5p expression at the RNA level. (**B**): CCK-8 cell proliferation curve; (**C**): The cell migration ability was using the scratch assay. (**D**): MDC staining for intracellular autophagic vesicles; (**E**): Western blotting for detecting the AKT/mTOR signaling pathway related proteins, LC3II/I ratio, and Beclin-1 expression, the representative Western blotting bands, and the quantification of protein expression. Versus. inhibitor-NC, *p< 0.05, **p< 0.01. Versus. inhibitor-NC+hBD-1, #p < 0.05, ##p< 0.01. Versus. miR-4712-5p inhibitor, $p<0.05, $$p<0.01

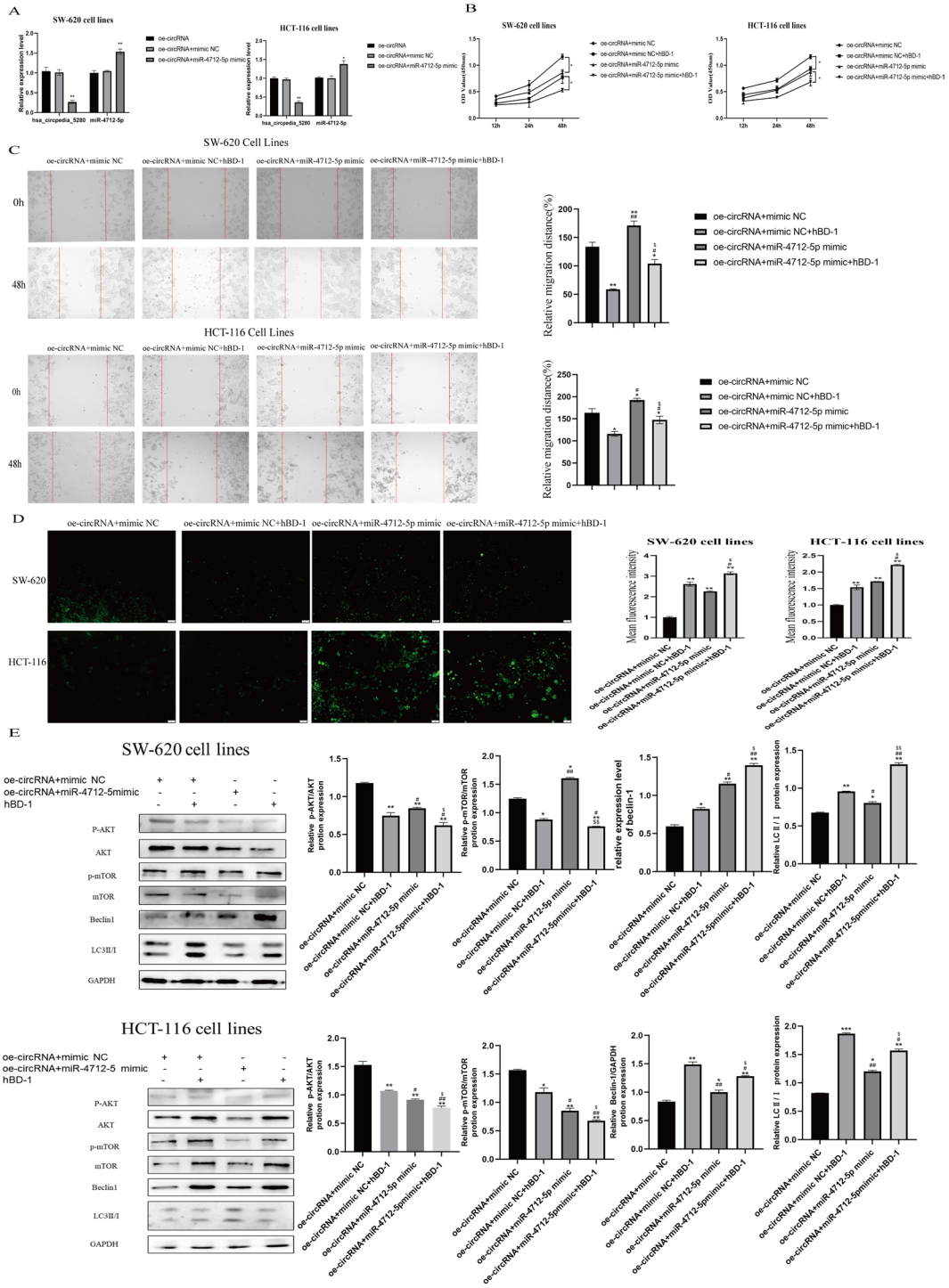

Fig. 6 shows demonstrates the ability of hBD-1 to mitigate the impact of HAS-CIRCpedia-5280 overexpression in colon cancer cells via miR-4712-5p mimics. (A): The qRT-PCR detected has-circpedia-5280 and miR-4712-5p expression. (B): CCK-8 cell proliferation curve. (C): The cell migration ability was using the scratch assay. (D): MDC staining for intracellular autophagic vesicles. (E): Western blotting for detecting the AKT/mTOR signaling pathway related proteins, LC3II/I ratio, and Beclin-1 expression, the representative Western blotting bands, and the quantification of protein expression. Versus. oe-circRNA+mimic NC, *p<0.05, **p<0.01. Versus. oe-circRNA+mimic NC+hBD-1, #p<0.05, ##p<0.01. Versus. oecircRNA+miR-4712-5p mimic, $p<0.05, $$p<0.01

cancers based on their sites of origin [21]. CRC treatment commonly involves the multimodal combinations of surgery, radiotherapy, and chemotherapy, which are individualized according to disease localization and progression. However, these treatments have significant side effects, are cytotoxic to normal cells, and have high rates of recurrence and metastasis.Further, they lack specificity. Therefore, there is an essential need for more effective therapies in patients with CRC [22–24]. hBD-1 can be a tumor suppressor. Previous studies have revealed its negative correlation with invasive potential in oral squamous cell carcinoma (OSCC) [25], prostate cancer [26], renal cancer [27], and triple-negative breast cancer (TNBC) [28]. This study confirmed that hBD-1 inhibited the viability and proliferative capacity of colon cancer cells.

Programmed cell death, which encompasses apotosis, necrotic apoptosis, cellular pyroptosis, iron death, PAN apoptosis, and autophagy, plays an important role in regulating the immunosuppressive tumor microenvironment and determining the clinical outcome of cancer therapeuticse [29]. Autophagy is a mechanism for suppressing tumors because genetic defects in various autophagy regulators lead to increased cancer incidence. Meanwhile, several oncogenes inhibit autophagy, and tumor suppressors increase autophagy [30].

Numerous studies have revealed that enhancing autophagy can prevent cancer progression in precancerous lesions. Conversely, in advanced-stage cancers, the enhancement of autophagy and inhibition of autophagy can be targets of therapeutic strategies. Interventions that stimulate and inhibit autophagy have been proposed as potential cancer therapies [31, 32]. The inactivation of the AKT-mTOR signaling pathway led to an increase in autophagosomes and LC3-dependent autophagy in atrophic endometrium. Hence, the AKT/ mTOR signaling pathway promotes anabolism and inhibits the induction of autophagy [33, 34]. In this study, hBD-1 promoted the protein expression of Beclin-1 and LC3II/I while decreasing the phosphorylation levels of AKT/mTOR proteins in colon cancer SW620 and HCT-116 cells. Therefore, hBD-1 promotes autophagy in colon cancer cells.

circRNAs are a class of RNA molecules characterized by a ring-like structure, formed via typical spliceosome-mediated or elongated splicing between an upstream splice acceptor and a downstream splice donor. Based on a recent study, circRNAs are widely distributed in eukaryotes [35]. The circRNA expression is dynamically regulated in different cancers, and it plays a role in cancer progression via various mechanisms, functioning as either tumor suppressors or oncogenes [36]. In this study, bioinformatics analysis identified the differential gene for hBD-1 acting on SW-620 in the mTOR signaling pathway. Results showed that the has-circpedia-5280 expression was downregulated in colon cancer SW-620 and HCT-116 cell lines, indicating its potential involvement in regulating colon cancer progression by hBD-1. Further, has-circpedia-5280 overexpression promoted colon cancer cell proliferation and inhibited autophagy. Circular RNAs typically comprise multiple exons, and they are enriched for functional miRNA bindingsites [37]. They can act as miRNA sponges to bind to miRNAs and affect tumorigenesis and metastasis in various cancer types [38–41]. In this study, miR-4712-5p was considered a candidate target miRNA for has-circpedia-5280 via database screening. Results showed that the miR-4712-5p expression was significantly upregulated in colon cancer cells, and decreased miR-4712-5p expression promoted cell proliferation while inhibiting autophagy. The co-transfection of has-circpedia-5280 overexpression plasmid and miR-4712-5p mimics was important in colon cancer. Interestingly, exposure to hBD-1 reversed the effects of has-circpedia-5280 overexpressing and miR-4712-5p inhibitor co-transfection by inhibiting AKT/mTOR phosphorylation expression while promoting the relative protein expression of Beclin-1 and LC3II. Similarly, co-transfection with hBD-1 exposed to hascircpedia-5280 overexpression plasmid and miR-4712-5p mimics promoted colon cancer autophagy while inhibiting cell proliferation. hBD-1 inhibits AKT/mTOR phosphorylation and promotes autophagy in colon cancer cells via the interaction between has-circpedia-5280 and miR-4712-5p.

Conclusions

In conclusion, hBD-1 inhibits the proliferation of colon cancer cells and promotes cell autophagy. hBD-1 induces autophagy in colon cancer cells by suppressing AKT/mTOR phosphorylation via has-circpedia-5280/miR-4712-5p. Further, has-circpedia-5280/miR-4712-5p can be a diagnostic and prognostic biomarker. Hence, it can be an important mechanism and therapeutic target for diagnosing and treating colon cancer.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-024-05860-x.

Supplementary Material 1

Author contributions

Shixiang An and Pengwei Zhao—wrote this article. Pengwei Zhao—designed, organized and reviewed this article. Shixiang An, Wenhong Yang, Jiaxian Cui, Lu Qiao—collected the data. Huiling Yu,Jingkun Lu, Yunpeng Tian, Mingyu Zhang—project administration. Shixiang An, Jiaxian Cui, Pengwei Zhao—acquired the fund. Xiumei Wang, Lili Bao, Pengwei Zhao—Scholarly Guidance. All authors have read and agreed to the published version of the manuscript.

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Data availability
All data generated or analyzes during this study are included in this published article and supplied materials. The datasets provided in this study can be found in the online repository below: https://www.ncbi.nlm.nih.gov/bioproject/ PRJNA1121975.We thank Bullet Edits Limited for the linguistic editing and proofreading of the manuscript.

Declarations

Ethics approval and consent to participate
The test was agreed by Ethics Committee of Inner Mongolia Medical University.

Conflict of interest
The authors declare no conflict of interest.

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