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Aberrant expression of LncRNA CASC2 mediated the cell viability, apoptosis and autophagy of colon cancer cells by sponging miR-19a via NF-κB signaling pathway

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Abstract

Abnormal and rapid proliferation of colon cancer cells is a severe problem that can be regulated by non-coding RNAs. Thus, our study focused on effects of lncRNA CASC2 and miR-19a on colon cancer cells. Expressions of lncRNA CASC2, miR-19a, Bcl-2, Bax and NF-κB/p65 were examined by RT-qPCR. Cell viabilities were detected by CCK-8. A luciferase report assay was used for measuring binding conditions between lncRNA CASC2 and miR-19a. Western blotting was used to evaluate expression of LC3-I, LC3-II and p62 related to autophagy. Expression of lncRNA CASC2 lower in cancer cell lines and the overexpression reduced the cell viability of HT29 and SW480. Furthermore, Bcl-2 was suppressed by overexpressed lncRNA CASC2, while Bax was upregulated. LC3-Ⅰ and p62 were both inhibited, but LC3-Ⅱ was promoted. MiR-19a was predicted to bind lncRNA CASC2 and expressed higher in cancer cell lines. Overexpressed miR-19a reduced expression of lncRNA CASC2 and increased cell viability. This was repressed by upregulated lncRNA CASC2. Bcl-2 and Bax expression and proteins implicated in autophagy that are regulated by lncRNA CASC2 upregulation were reversed by miR-19a overexpression. NF-κB was upregulated in colon cancer cell lines, while inhibition of NF-κB reversed functions of lncRNA CASC2 and magnified roles of miR-19a. Our findings showed that lncRNA CASC2 inhibited cell viability in colon cancer cell lines and miR-19a reversed its functions through the NF-κB signalling pathway, suggesting that these could be factors in treating colon cancer in the future.

KEYWORDS

apoptosis, autophagy, cell viability, colon cancer, lncRNA CASC2, NF-κB

1 | INTRODUCTION

Colon cancer, ranking the third most frequent form of cancer in terns of in mortality, is one of the most malignant forms of cancers worldwide.[1] In China, morbidity from colon cancer is behind gastric cancer and oesophagus cancer in human digestive system. With the improvement of living standard, changes in way of life, environment and nutrition habits, morbidity of colon cancer is increasing year by year, having risen from the sixth to the third most frequent cause over a 10 year period.[2] Though developments have been achieved in surgery and other treatments, even some new molecular therapeutics have been introduced, nonetheless there continues to be a high death rate

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in colon cancer, resulting from continual recurrences and or transition into more aggressive forms.[3,4]

Long non-coding RNA is a kind of RNA which lacks protein-coding features[5] These RNAs are over 200 nucleotides in length.[6] LncRNAs could inhibit expressions of imminent genes through disturbing combinations between transcript factors and promoters, induction of protein modification and promoting the reconstruction of chromosomes,[7] which could affect expressions of genes through binding target genes. LncRNA Cancer Susceptibility Candidate 2 (CASC2) has been reported to be downregulated in many kinds of cancers, such as lung cancer, melanoma, and gastric cancer.[8-12] LncRNA CASC2 was also found to be a tumour suppressor in colorectal cancer (CRC) by sponging miR-18a; this has been proven in tumour tissues of CRC patients and 5 CRC cell lines.[13] LncRNA CASC2 has been found to be significantly inhibited in colon cancer tissues and cells and its overexpression induced apoptosis and autophagy through suppressing TRIM16.[14] Nevertheless, new ways of lncRNA CASC2-mediating progression of colon cancer are needed for providing more methods in cancer treatment. Therefore, this study would focus on mechanisms and functions of lncRNA CASC2 in colon cancer cells in order to find a new biomarker in treating this cancer.

MicroRNAs are endogenous small RNAs playing important roles by targeting 3'UTR of mRNAs.[15] In detection of the miR-17-92 cluster in cancers, miR-19a has been determined as a key factor that mediates progression and cell proliferation of cancers.[16,17] Roles for miR-19a have also been examined in the study of Yanqing Liu et al, showing that miR-19a facilitated CRC cell proliferation and metastasis and promoted tumour volume in xenograft mice model through binding TIA1.[18] Besides that, miR-19a has been reported to enhance resistance to DDP in gastric cancer through binding lncRNA CASC2,[19] indicating that miR-19a has correlation with lncRNA CASC2 in gastric cancer. Based on these detections, we implied that the correlation between miR-19a and lncRNA CASC2 also operated in colon cancer. Therefore, miR-19a was chosen to figure out its functions with lncRNA CASC2.

Autophagy is a highly controlled process, and the dysregulation of autophagy has correlation with diseases.[20] In colon cancer, autophagy can accelerate tumour growth, which can also induce cell apoptosis.[21-23] Moreover, lncRNA CASC2 has been reported to induce autophagy and repress TRM16, resulting in inhibition of colon cancer cells.[14] In contrast, overexpression of miR-19a suppressed autophagy in the in vitro hepatocytes injury model.[24] Therefore, we wondered that whether lncRNA CASC2 and miR-19a regulated progression of autophagy in colon cancer cell models, resulting in changed cell viability and apoptosis of colon cancer cells.

In colon cancer studies, NF-κB signalling pathway was reported to be a tumour promotion pathway that contributed to cell metastasis and epithelial-mesenchyme transition.[11,12,25] Moreover, lncRNA CASC2 repressed hepatoma carcinoma cell viability via the NF-κB signalling pathway.[26] In colitis-associated colon cancer, TNF-α induced upregulation of miR-19a and the latter one activated NF-κB signalling pathway by targeting TNF-α.[18] Based on these studies, we have deduced that lncRNA CASC2 and miR-19a might have close correlation during progression of colon cancer cells. Meanwhile, the NF-κB signalling pathway might also a role as a key factor in this progression. Therefore, we determined to analyse their functions in this study.

2 | METHODS

2.1 | Cell culture

HT29, SW620 and SW480 are human colon cancer cell lines, and all those three cell lines are epithelial and adherent. The HT29 cell line was acquired from a 44-year-old woman with colorectal adenocarcinoma. SW480 was from a 55-year-old man who has Dukes' type B, colorectal adenocarcinoma and SW620 is from a 51-year-old man with Dukes' type C, colorectal adenocarcinoma. These three colon cancer cell lines were all bought from ATCC. NCM460, a human normal colonic epithelial cell line, was purchased from BLUEFBIO. Culture media were preheated to 37℃ first, and then, cancer cells were transferred into RPMI-1640 medium (Thermo Fisher) contained 10% foetal bovine serum (FBS) from Thermo Fisher, and NCM460 cells were incubated in DMEM medium supplemented with 10% FBS (Gibco). After that, cells were stored and cultured in hygro-saturated incubator at 37℃, 5%CO_2. Cells in log phase were selected for the following experiments, and other cells were frozen by liquid nitrogen.

2.2 | RT-qPCR

Total RNAs were extracted from cells according to manufactures' instructions of Trizol reagent (Thermo Fisher). Next, 3 μg of total RNAs was reverse transcribed to cDNAs using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) and cDNAs were stored at −20℃. Thereafter, PowerUp™ SYBR™ Green Master Mix (Applied Biosystems) and 7500 Fast Food Safety Real-Time PCR System (Applied Biosystems) were used for amplification. Primers were designed and sequences about primers of lncRNA CASC2 were shown as below: forward, 5′-GCTGATCAGAGCACATTGGA-3′, reverse, 5′-ATAAA GGTGGCCACAACTGC-3′.[13] Sequences about primers of miR-19a: sense, 5′-GGGGGGGTGTGCGCTCTCT-3′, antisense, 5′-GTGCGTGTCGTGGAGTCG-3′.[9,10] Primer's

sequences of Bcl-2 were shown: forward, 5′-CGCCAAC ATTCTCTCCACAG-3′, reverse, 5′-CTGGGCCAGAGCTA CATCTT-3′. Primer's sequences of Bax were displayed as below: sense 5′-GGCCTGAGTCCAGCTCTTTA-3′, antisense, 5′-GTCCTGGAGACAGGGACATC-3′ and sequences of β-actin primers: sense, 5′-CACCATGTACCCAGGCATTG-3′, antisense, 5′-CCTGCTTGCTGATCCACATC-3′. Predenaturation was at 95℃, 3 minutes, and denaturation was at 95℃, 10 seconds. Annealing was at 60℃ and extension for 30 seconds, 40 cycles. RNA expressions were qualified by $2^{-\Delta\Delta Ct}$ methods with β-actin as the negative control.

2.3 | Transfection

Cells were plated into 24-well plate and incubated overnight at 4℃. Cells were resuspended until 50% confluences. 1 μg of RNAs was added into serum-free RPMI-1640 medium to get RNA dilution. To confirm the role of miR-19a, inhibitor of miR-19a and negative control was purchased form GenePharma. For overexpression of lncRNA CASC2, pcDNA3.1 was used for upregulating RNAs and lncRNA CASC2 was inserted into vectors. Lipofectamine™ 3000 Transfection Reagent (Thermo Fisher) was applied for transfection. Mimics of miR-19a were compounded and provided by GenePharma. Later, miR-19a mimics were mixed with Lipofectamine™ 3000 Transfection Reagent (Thermo Fisher) and the mixture was added into cells in serum-free RPMI-1640 medium and incubated at 37℃, 5%CO_2 for 24 hours. RNA expressions were quantified by RT-qPCR.

2.4 | CCK-8

A 100 μL of suspension of cells was planted into 96-well plate and adjusted numbers of cells to 1×10^5 cells per well. Thereafter, plate was cultured in saturated humidity incubator for 24 hours at 37℃, 5%CO_2. Next, 10 μL CCK-8 (Beyotime) was mixed into each well and cultured for 2 hours. Optical density (OD) values of cells was evaluated at 450 nm wavelength through microplate reader (Thermo Fisher).

2.5 | Luciferase report assay

Cells were digested with 0.25% trypsin and seeded into a 35-mm plate. Cells then were cultured in saturated humidity incubator at 37℃, 5%CO_2 overnight. After confluences reached 70%, Thermo Scientific pCMV-Cypridina Luc Vectors (Thermo Fisher) was used for the cells to be inserted by wild type of lncRNA CASC2 and mutant type of lncRNA CASC2. Thereafter, lncRNA CASC2-wt/mut were co-transfected into cells with NC mimics and miR-19a mimics by Lipofectamine™ 3000 (Thermo Fisher). Cells were incubated for 24 hours. Next, cells to be assayed were removed from the medium and rinsed twice with PBS. Afterwards, cells were split with Passive Lysis Buffer (PLB) (Promega). Fluoroskan™ FL Fluoroskan and Luminoskan microplate readers (Thermo Scientific) were used to detect activities after lysing.

2.6 | Western blotting

Total proteins were acquired from cells using RIPA lysis buffer (Beyotime) on ice. Supernatants were collect for quantification of proteins using a BCA protein assay kit (Beyotime). After qualification, proteins were adjusted to the same densities and 50 μg of proteins was transferred into PVDF membranes by SDS-PAGE separately. Then membranes were blocked with QuickBlock™ Blocking Buffer (Beyotime) and cultured with primary antibodies at 4℃ overnight. Primary antibodies were shown as below: anti-LC3-Ⅰ (1:1000; ab128025), anti-LC3-Ⅱ (1:1000) and anti-β-actin (1:2000; ab8226) as internal reference. After incubation, membranes were rinsed by QuickBlock™ Blocking Buffer three times and then membranes were cultured with goat anti-Rabbit IgG (1:800; ab150077) marked with HRP or goat anti-Mouse IgG (1:800; ab150117) marked with HRP at room temperature for 2 hours. Grey values then were detected, and β-actin was the standard.

2.7 | Statistical analysis

Data were displayed as mean ± SD and analysed by SPSS 19.0 (IBM). Experiments were repeated three times. Analysis between two was using t-test and one-way ANOVA was for examining groups over two. $P < .05$ was considered to have statistical meaning.

3 | RESULTS

3.1 | LncRNA CASC2 was downregulated in colon cancer cell lines and inhibited cell viability but promoted apoptosis and autophagy

Expression of lncRNA CASC2 was evaluated in NCM460, HT29, SW620 and SW480. Compared to the NCM460 cell line, expression in HT29, SW620 and SW480 cell lines were significantly decreased (Figure 1A). Expression of upregulated lncRNA CASC2 was detected in HT29 and SW480 cell lines, which indicated that overexpressed lncRNA CASC2 could promote expression of lncRNA CASC2 in RNA level Figure 1B.

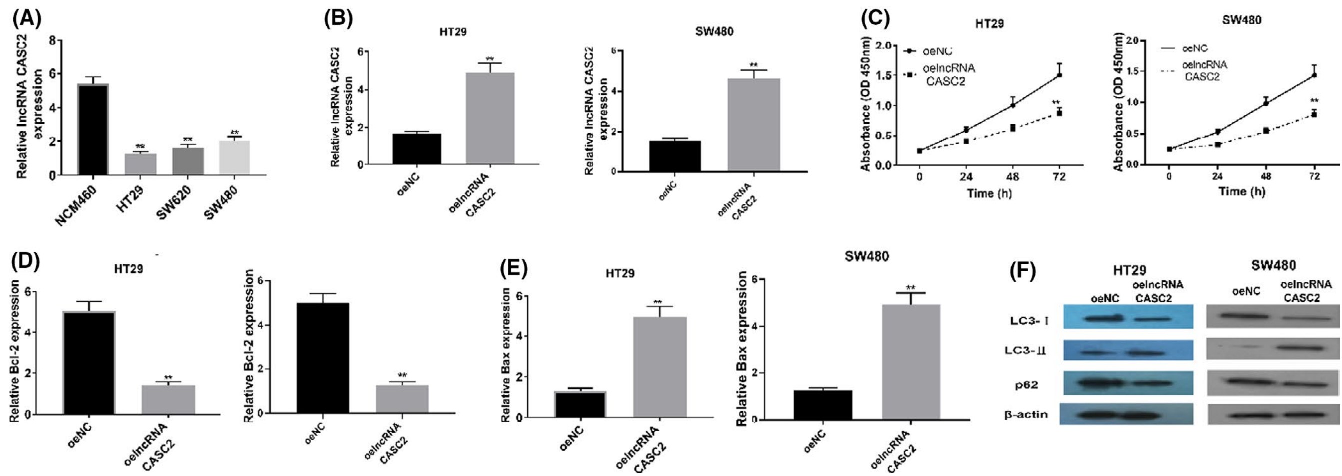

FIGURE 1 Downregulated lncRNA CASC2 inhibitory cell viability and promoted apoptosis and autophagy A, RNA expression of lncRNA CASC2 in NCM460, HT29, SW480 and SW620 were checked by RT-qPCR, $^{**}P < .05$ compared to NCM460 group. B, lncRNA CASC2 RNA expressions in HT29 and SW480 cells were detected after overexpressed transfection was evaluated using RT-qPCR, $P < .05$ compared with oeNC group. C, CCK-8 was applied to validate cell viabilities of HT29 and SW480 cells, $^{**}P < .05$ compared with oeNC group. D, E, RNA expressions of Bcl-2 and Bax in HT29 and SW480 cells were assessed by RT-qPCR, $P < .05$ in comparison with oeNC group. F, Protein expressions of LC3-I, LC3-II and p62 in HT29 and SW480 cells were measured by Western blot, $P < .05$ compared to oeNC group

After evaluating expression of lncRNA CASC2, the cell viabilities of HT29 and SW480 cell line with upregulated lncRNA CASC2 were examined. Cell viabilities were significantly reduced after lncRNA CASC2 overexpressed in HT29 and SW480 Figure 1C. Factors related to apoptosis were evaluated as well. Compared to negative control group in HT29 as well as SW480 cells, expressions of Bcl-2 were obviously inhibited in upregulated lncRNA CASC2 groups, while expressions of Bax were significantly increased (Figure 1D,E). Meanwhile, expressions of proteins implicated in autophagy were also examined. Expressions of LC3-Ⅰ and p62 were both significantly lower in oelncRNA CASC2 compared to control groups, but expression of LC3-Ⅱ was promoted Figure 1F.

3.2 | MiR-19a was the direct target gene of lncRNA CASC2

Previous experiments determined that lncRNA CASC2 could inhibit cell viability in colon cancer cells. Therefore, connections at the RNA level needed to be documented. Through miRcode (http://www.mircode.org/), miR-19a was predicted to have binding sites with lncRNA CASC2 (Figure 2A). Then, the binding condition was detected to confirm the correlation. The luciferase report assay was applied for detecting binding situation between lncRNA CASC2 and miR-19a, revealing that the luciferase activity was significantly lower in wild type of lncRNA CASC2 with miR-19a mimics group compared to other groups in both HT29 and SW480 cells Figure 2B. Hence, it was miR-19a that could bind wild type of lncRNA CASC2 in colon cancer cells. Expressions of miR-19a in RNA level were evaluated in normal cell line and tumour cell lines, which showed that miR-19a was expressed at much higher levels in tumour cell lines Figure 2C. Then, levels of miR-19a were detected in HT29 cell line and SW480 cell line after suppression of miR-19a, indicating that knockdown of miR-19a remarkably reduced level of miR-19a RNA expression (Figure 2D). Cell viabilities were also tested, which demonstrated that miR-19a inhibitor could decrease cell viabilities of both HT29 and SW480 cells Figure 2E.

3.3 | Overexpressed miR-19a repressed inhibitory effects on colon cancer cell lines caused by upregulated lncRNA CASC2

Expressions of lncRNA CASC2, miR-19a and cell viabilities with lncRNA CASC2 overexpression or miR-19a suppression were evaluated individually. Correlation between lncRNA CASC2 and miR-19a was calculated later. In HT29 cells and SW480 cells, expression of lncRNA CASC2 was significantly increased because of lncRNA CASC2 overexpression, but its expression was reversed because of upregulation of miR-19a (Figure 3A). The promoted cell viabilities of HT29 cells and SW480 cells after lncRNA CASC2 overexpression were enhanced by miR-19a upregulation (Figure 3B). Meanwhile, RNAs associated with apoptosis were measured, which demonstrated that oelncRNA CASC2 inhibited expression of Bcl-2 and promoted expression of Bax, while overexpressed miR-19a could resume functions of lncRNA CASC2 resulting in high

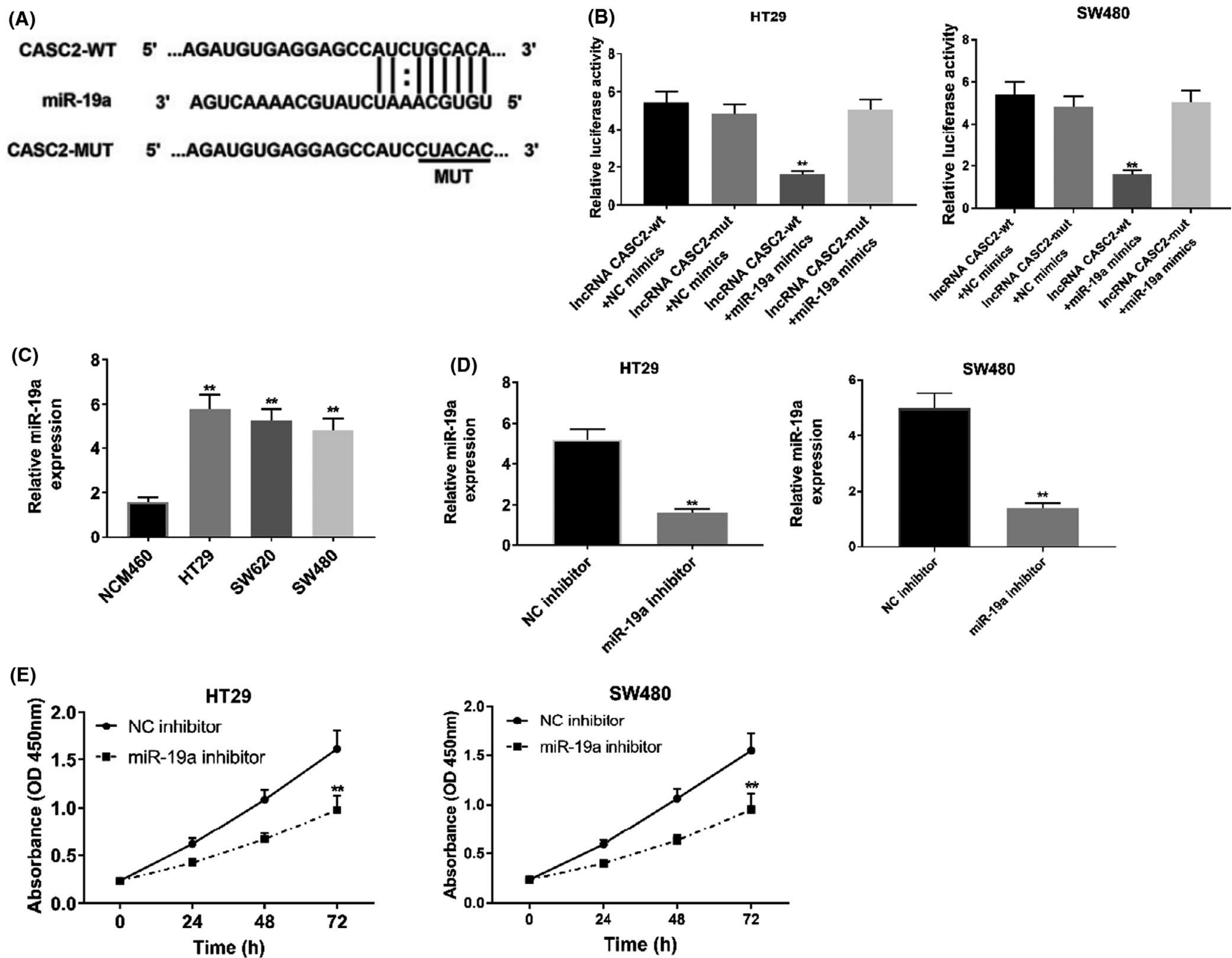

FIGURE 2 MiR-19a was the direct target gene of lncRNA CASC2 A, Putative binding site was provided by miRcode (http://www.mircode.org/). B, Binding conditions between CASC2 and miR-19a were detected in HT29 and SW480 cells using dual luciferase report assay, $^{**}P < .05$. C, RNA expressions of miR-19a in NCM460, HT29, SW62 and SW480 were assessed using RT-qPCR, $^{**}P < .05$ compared to NCM460 group. D, RNA levels of miR-19a in HT29 and SW480 cells after inhibition were measured by RT-qPCR, $^{**}P < .05$ compared with NC inhibitor group. E, Cell viabilities of HT29 cells and SW480 cells were measured after knockdown of miR-19a, $^{**}P < .05$ compared with NC inhibitor group

level of Bacl-2 and low level of Bax (Figure 3C,D). Proteins included in autophagy were evaluated as well. MiR-19a mimics could reduce inhibition of lncRNA CASC2 in LC3-Ⅰ as well as p62 and upregulation of LC3-Ⅱ by over expressed lncRNA CASC2 was restored by upregulated miR-19a (Figure 3E).

3.4 | MiR-19a regulated functions of lncRNA CASC2 through NF-κB signalling pathway

Expressions of NF-κB/p65 were validated at RNA level. In a normal cell line, the RNA level of NF-κB/p65 was significantly decreased compared to its expression in cancer cell lines (Figure 4A). After treatment by QNZ, a type of NF-κB inhibitor, NF-κB/p65 expression was evaluated in HT29 and SW480 cells, which indicated that QNZ inhibited the level of NF-κB/p65 in HT29 and SW480 cells (Figure 4B). Previous experiments indicated that miR-19a could resume functions of lncRNA CASC2 in colon cancer cell lines. Therefore, functions of the NF-κB signalling pathway were detected. Downregulated NF-κB/p65 suppressed cell viabilities in oelncRNA CASC2 with miR-19a overexpression group (Figure 4C). Compared to oelncRNA CASC2 and miR-19a mimics group, level of Bcl-2 was reduced but expression of Bax was increased after NF-κB was inhibited (Figure 4D,E). Expressions of LC3-Ⅰ and p62 were decreased in inhibited NF-κB with overexpressed lncRNA CASC2 and overexpressed miR-19a, while LC3-Ⅱ expression was promoted (Figure 4F).

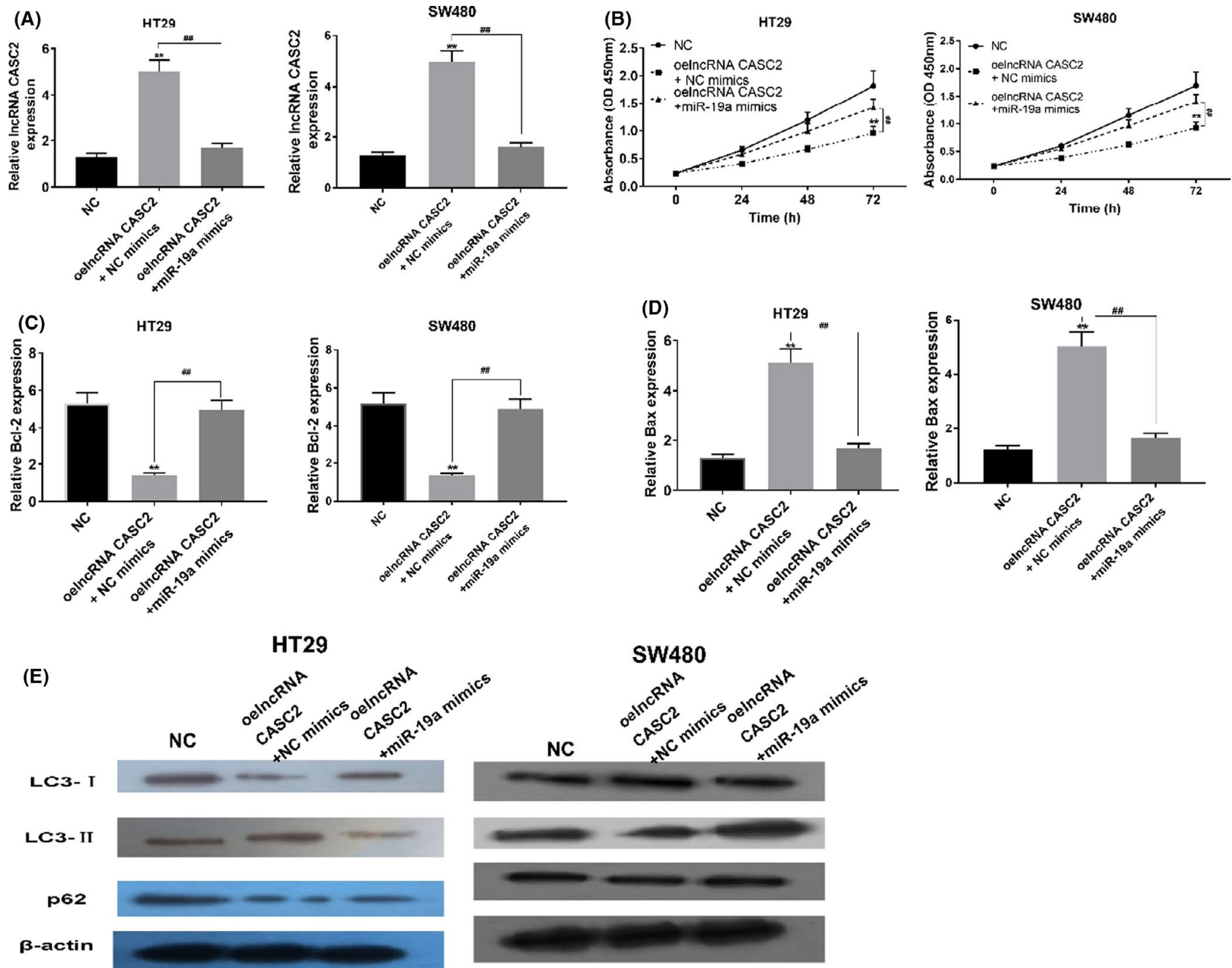

FIGURE 3 Overexpressed miR-19a repressed inhibited effects on colon cancer cell lines caused by upregulated lncRNA CASC2 A, lncRNA CASC2 RAN expressions were detected by RT-qPCR in HT29 and SW480 cells after lncRNA CASC2 and miR-19a upregulation, $^{**}P < .05$ compared with NC group and $^{\#\#}P < .05$ compared with oelncRNA CASC2 with NC mimics group. B, Cell viabilities in HT29 and SW480 cells were evaluated using CCK-8 after overexpression of lncRNA CASC2 and miR-19a mimics, $^{**}P < .05$ compared with NC group and $^{\#\#}P < .05$ compared with oelncRNA CASC2 with NC mimics group. C,D, Bcl-2 and Bax RNA expressions were detected by RT-qPCR in HT29 and SW480 cells after lncRNA CASC2 and miR-19a overexpressed transfection, $^{**}P < .05$ compared with NC group and $\#\#P < .05$ compared with oelncRNA CASC2 with NC mimics group. E, Western blot was applied to measure protein expressions of LC3-I, LC3-II and p62 in HT29 and SW480 cells after lncRNA CASC2 and miR-19a upregulation, $^{**}P < .05$ compared with NC group and $^{\#\#}P < .05$ compared with oelncRNA CASC2 with NC mimics group

4 | DISCUSSION

Cancer is a major killer causing of human death causing serious medical and social problems.[27] The occurrence of cancer is related to multigenes and steps including instabilities of microsatellites, restricted apoptosis functions and signalling pathway anomalies and so on.[28] People over 50 have increasing probability of colon cancer.[29] in addition, high fat, high calories and alcohol also contribute to occurrences of colon cancer. Researchers have shown that genes have connections with colon cancer as well, morbidity of which is 2 or 3 times higher in first-degree relatives than ordinary people. About 35% of colon cancers have been linked to genetic predisposition.[30]

Previous studies have found many kinds of lncRNAs were expressed abnormally in colon cancer. CCAT1 was discovered in tissues of colon cancer, expressed at apparently higher levels than either in normal colon tissues or in blood samples. In about 40% of patients CCAT 1 expression was upregulated, which indicated that high expressions of CCAT1 has a connection with occurrence of colon cancer.[31] Other studies showed that transcripts of CRNDE were upregulated in colon cancer tissues.[32] CRNDE could regulate metabolism of cells through insulin or insulin-like growth factors to

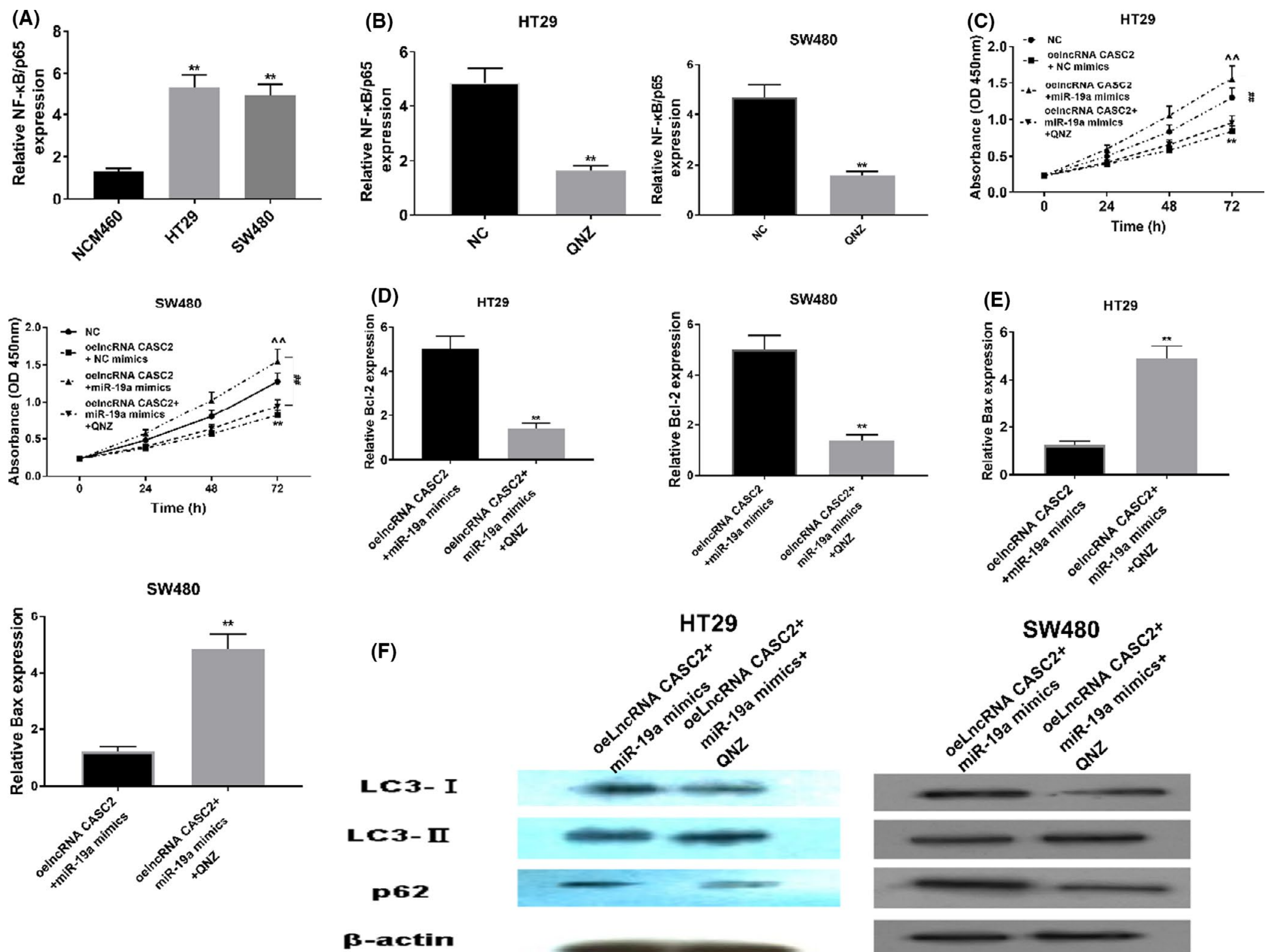

FIGURE 4 MiR-19a regulated functions of lncRNA CASC2 through NF-κB signalling pathway A, RNA expression of NF-κB/p65 in NCM460, HT29 and SW480 cells were measured through RT-qPCR, $P < .05$ compared with NCM 460 group. B, NF-κB/p65 RNA levels were evaluated in HT29 and SW480 cells after QNZ treatment (a NF-κB signalling pathway inhibitor), $^{**}P < .05$ compared with NC group. C, Cell viabilities of HT29 and SW480 cells were examined by CCK-8 after lncRNA CASC2 or miR-19a upregulation and QNZ treatment, $^{**}P < .05$ compared with NC group, $^{\wedge\wedge}P < .05$ compared with oelncRNA CASC2 with NC mimics group and $^{\#\#}P < .05$ compared with oelncRNA CASC2 with miR-19a mimics group. D,E, Bcl-2 and Bax RNA expressions were detected after QNZ treatment in HT29 cells and SW480 cells transfected with oelncRNA CASC2 and miR-19a mimics, $^{**}P < .05$ compared with oelncRNA CASC2 and miR-19a mimics group. F, Protein expressions of LC3-I, LC3-II and p62 in HT29 and SW480 cells were measured by western blot after lncRNA CASC2 and miR-19a upregulation and QNZ treatment, $^{**}P < .05$ compared with oelncRNA CASC2 and miR-19a mimics group

promote its expressions in colon cancer.[33] LncRNA CASC2 has been reported as a newly discovered tumour suppressor in oesophageal squamous cancer, pancreatic cancer and ovarian cancer.[34-36] However, studies of lncRNA CASC2 were rare in colon cancer. Thus, this study would focus on connections between lncRNA CASC2 and colon cancer. First, expression of lncRNA CASC2 were evaluated, which demonstrated that expression of lncRNA CASC2 were lower in tumour cell lines. Cell viabilities also showed negative functions of lncRNA CASC2. RNAs related to apoptosis were detected as well, which showed that lncRNA CASC2 could inhibit expression of Bcl-2 but promote expression of Bax. Expression of proteins involved in autophagy displayed that lncRNA CASC2 suppressed expressions LC3-Ⅰ and p62 but upregulated expressions of LC3-Ⅱ. Therefore, lncRNA CASC2 could suppress cell viability of colon cancer cells and promote apoptosis and autophagy of cell lines.

A serious of studies indicated that correlations between lncRNAs and miRNAs could affect neoplasia and tumour progression.[37] LncRNAs transcribed from ultraconserved region (UCR) in leukaemia were indicated to have negative correlation with 13 kinds of miRNAs. Among those miRNAs, five of them could bind lncRNA in UCR and miR-155 was one of those five which was proved that overexpression of miR-155 could reduce expressions of target lncRNA in MEGO1 leukaemia cell line. Meanwhile, expressions of miRNAs were controlled by lncRNAs.[38] In this study, binding site was predicted in lncRNA CASC2 and miR-19a by Starbase v2.0.

Thus, whether there was a connection between miR-19a and lncRNA CASC2 needed to be analysed. According to luciferase report assay, miR-19a was proven to bind wild-type lncRNA CASC2. Afterwards, miR-19a expressions were evaluated, which showed that miR-19a expressed higher in colon cancer cell lines. Level of miR-19a was decreased after miR-19a suppression. About cell viabilities, inhibited miR-19a suppressed cell viabilities in HT29 and SW480 cells. As lncRNA CASC2 and miR-19a had different functions to colon cancer cells, correlations between them needed to be figure out. In colon cancer cell lines, overexpressed lncRNA CASC2 could increase expression of lncRNA CASC2, but the function was reversed by upregulated miR-19a as well as cell viabilities. In regard of RNAs in apoptosis, expression of Bcl-2 was inhibited, but Bax was promoted with lncRNA CASC2 overexpression. However, overexpressed miR-19a reversed functions of lncRNA CASC2 by improving level of Bcl-2 and suppressing Bax. Besides that, increased LC3-II by lncRNA CASC2 was restored by miR-19a and low expressions of LC3-I and p62 were upregulated. Therefore, miR-19a had negative effects on lncRNA CASC2 and it could restore functions of lncRNA CASC2 in colon cancer cells.

NF-κB was discovered in 1986, which is related to immunization, inflammations and transcriptions of genes in proliferation.[39] In cancers, many carcinogens were proved to promote proliferation, anti-apoptosis through activating NF-κB. Thus, NF-κB suppression becomes a new target in cancer treatment.[40] Previous studies discovered that many factors could induce activation of NF-κB. TNF-α and IL-1β had positive effects for activation of NF-κB, which could cause Unrestricted proliferation of cancer cells.[41] Therefore, NF-κB signalling pathway was examined. Expressions of NF-κB/p65 RNA level were evaluated in cell lines, which showed that NF-κB/p65 expressed higher in colon cancer cells. Because of high expression, QNZ, a kind of inhibitor, was used to suppress NF-κB/p65, resulting in decreased NF-κB/p65 expression. After NF-κB was inhibited, cell viabilities were suppressed, which is even lower than the cell viability in oelncRNA CASC2 and miR-19a mimics group. Results of RT-qPCR also proven that suppressed NF-κB repressed expression of Bcl-2, which was upregulated by overexpressed miR-19a, and increased Bax expression, which was repressed by upregulated miR-19a. As for autophagy, expressions of LC3-I and p62 were reduced by inhibited NF-κB. Expression of LC3-II was inhibited with mimics of miR-19a, but the result was restored by inhibited NF-κB. Thus, NF-κB was the signalling pathway in colon cancer cells, which could regulate cell process by correlation between lncRNA CASC2 and miR-19a.

5 | CONCLUSION

LncRNA CASC2 was detected to express lower and repressed cell viability in colon cancer cells by sponging miR-19a and suppressing NF-κB/p65 in NF-κB signalling pathway, which suggested that lncRNA CASC2 could be a potential factor in treating colon cancer at clinical stage. However, further studies in vivo and clinical stage are requested for a better understanding.

CONFLICT OF INTEREST

There is no conflict of interest.

ETHICAL APPROVAL

Not applicable.

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