INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignancies; approximately 1.4 million patients are diagnosed with CRC each year worldwide.\(^1\) According to the latest cancer statistics, in the USA and Europe, new cases of CRC and death both ranked third.\(^2\) The incidence of CRC in China is second only to esophageal cancer and gastric cancer, and the incidence and mortality are increasing year by year.\(^3,4\) Apart from familial genetic factors, the occurrence of CRC is also related to bad eating habits, obesity, smoking, and other factors.
but the exact pathogenesis is still unclear. Therefore, further study of the molecular mechanisms of CRC is essential to ease the burden of the disease.

Only approximately 1.5% of genes in the human genome can encode proteins, and most genes are transcribed into noncoding RNAs (ncRNA). Noncoding RNAs can be divided into short ncRNAs and long ncRNAs (lncRNA) according to their length (less than or greater than 200 nt). Long ncRNA is the most common and most versatile class. Long ncRNA lacks a significant ORF and is widely present in the nucleus and cytoplasm. It does not participate in protein coding. Recent studies have shown that IncRNA plays a critical role in carcinogenesis. For example, upregulation of SNHG6 regulates ZEB1 expression by competitively binding microRNA-101-3p and interacting with UPF1 in hepatocellular carcinoma. Our previous studies also reported that IncRNA SPRY4-IT1 increases the proliferation of human breast cancer cells by upregulating ZNF703 expression. However, the function of IncRNAs in human CRC remains largely unknown. In this study, we identified MAPKAPK5-AS1, a novel lncRNA previously mentioned in published reports, as being upregulated in CRC tissue and associated with tumorigenesis, larger tumor size, proliferation, and poor prognosis. In-depth study of IncRNA MAPKAPK5-AS1 not only enables us to further understand the pathogenesis of CRC, but also establish it as a new diagnostic marker and therapeutic target of CRC. To explore the role of IncRNA in the progression of CRC, we have previously analyzed the differential expression of IncRNAs in The Cancer Genome Atlas (TCGA). Therefore, it is necessary to further explore the causes of abnormal expression, to provide a theoretical basis and research direction for the early diagnosis and early treatment of CRC.

**2 MATERIALS AND METHODS**

**2.1 Clinical specimens**

From January 2015 to December 2018, 50 pairs of matched tumor tissues and corresponding normal tissues were collected from CRC patients undergoing surgical treatment in the Second Affiliated Hospital of Nanjing Medical University (Nanjing, China). None of the patients received any local or systemic treatment before surgery. The pathological stage, grade, and nodal status were appraised by an experienced pathologist. All experiments were approved by the research ethics committee of Nanjing Medical University. Written informed consent was obtained from all patients.

**2.2 Cell culture**

The CRC cell lines HCT116, SW480, HT-29, and DLD-1, and the normal epithelium of the intestinal mucosa HCoEpic were purchased from the Cell Culture Bank of the Chinese Academy of Sciences (Shanghai, China). Colorectal cancer cells HCT116, HT-29, SW480, and DLD-1 were cultured in DMEM containing 10% FBS; normal CRC epithelial cells HCoEpic were cultured in RPMI-1640 medium containing 10% FBS in an incubator at 37°C, with 5% CO2 and relative humidity of 90%. Medium was replaced every 2-3 days.

**2.3 RNA isolation and qPCR assays**

Total RNA was extracted from tissues or cultured cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s protocol. RNA quantity and quality were determined by NanoDrop2000c (Thermo Fisher Scientific, Waltham, MA, USA). For qRT-PCR, 1 μg RNA was reverse transcribed into cDNA using a reverse transcription kit (Takara, Dalian, China). Quantitative PCR assays were carried out on an ABI 7500 (Carlsbad, CA, USA). Data were normalized to GAPDH. The primer sequences were: MAPKAPK5-AS1, forward AAGCCCGAGTCTGATGCTAA, and reverse CTGCACACCTCTTCTGGTCA; p21, forward GAGACTCT, and reverse CTGCACACCTCTTCTGGTCA; p21, forward GAGACTCT, and reverse CTGCACACCTCTTCTGGTCA; and reverse GAPDH, forward AGCCACATCGCTCAGACAC, and reverse GCCCAATACGACCAATTC; and U6 forward ATGGAAACG ATACAGGAGATTT, and reverse GGAAGGCCTCAGCAGATT. The ChIP-qPCR primers for p21 were: forward, CAGGATTGCTCCTAATGATCG; and reverse, GGAATTCACTTCCACACAGCC. Each sample was analyzed in triplicate.

**2.4 Cell transfection**

Two individual MAPKAP5-AS1 (MAPKAP5-AS1 2# and 3#), si-EZH2, and scrambled negative control (NC) siRNAs were purchased from Invitrogen and transfected into cells using Lipofectamine 2000 (Invitrogen). Plasmid vectors (sh-MAPKAP5-AS1 and empty vector) for transfection were extracted by a DNA Midiprep kit (Qiagen, Hilden, Germany) and transfected into cells using FuGENE (Roche, Basel, Switzerland). The full-length
cDNA of MAPKAPK5-AS1 was synthesized by Realgene (Nanjing, China) and subcloned into the pCDNA3.1 (+) vector (Invitrogen) according to the manufacturer's instructions. Cells were harvested after 48 hours for qRT-PCR and western blot analyses. The sequences of the siRNAs/shRNAs were: si-MAPKAPK5-AS1 2#, GGUGACGUGGUGCGUGAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAU
2.13 | Western blot assay

SW480 cells were harvested, and proteins were extracted from the transfected cells and quantified using a 12% polyacrylamide gradient SDS gel as previously described. Quantification was undertaken using a density assay (Quantity One software; Bio-Rad, Hercules, CA, USA) using ECL chromogenic substrate. A GAPDH Ab (#2118; Cell Signaling Technology, Boston, MA, USA) was used as a control. Anti-p21 was purchased from Abcam.

2.14 | Nude mouse tumor formation experiment

To determine whether the level of MAPKAP5-AS1 expression could affect CRC tumorigenesis in vivo, sh-MAPKAP5-AS1 2#, or negative control-transfected DLD-1 cells were s.c. injected into male nude mice. Subcutaneous tumors are observed every 3 days. After the appearance of tumors, the tumor size of the mice in different groups was measured and recorded once every 2 days. After 16 days of tumor formation, the mice were killed. The tumors were removed, photographed, weighed, and measured. All the animal experiments in this study were undertaken in strict accordance with the guidelines and regulations for the Care and Use of Laboratory Animals, Nanjing Medical University.

2.15 | Immunohistochemistry (IHC)

Xenograft tumor tissue samples were immunostained with H&E and Ki67. Anti-Ki67 was from Santa Cruz Biotechnology (Dallas, TX, USA). The IHC staining results were independently scored by the author and a pathologist to minimize subjectivity and then compared, and the final comprehensive results were obtained.

2.16 | Statistical analysis

Statistical analysis was carried out using SPSS software (SPSS, Chicago, IL, USA). The χ² precision tests were used to analyze clinical pathology data. To compare MAPKAP5-AS1 expression in CRC tissue and matched normal tissue, a paired t test was used. To compare 2 samples, an unpaired two-tailed t test was used. A P value <0.05 was considered statistically significant. The results are expressed as mean ± SD. Statistical significance was assigned at *P < .05 or **P < .01. All experiments were carried out at least 3 times, in triplicate samples.

3 | RESULTS

3.1 | MAPKAP5-AS1 is upregulated in human CRC tissues and is positively correlated with larger tumor size, advanced TNM stage, and lymph node metastasis

In order to determine whether IncRNAs play an important role in CRC, we compared case-control microarray data of paired CRCs in The Cancer Genome Atlas CRC database and the Gene Expression Omnibus database (GSE24514). We carried out a differential expression analysis and found that MAPKAP5-AS1, an IncRNA with a high differential expression, had not been reported. To further verify the results of the data analysis, we detected the expression of MAPKAP5-AS1 in 50 cases of tumor tissue and adjacent tissues by qRT-PCR (Figure 1A). The results showed that the expression of MAPKAP5-AS1 in tumor tissues was significantly higher than adjacent tissues. Further analysis of clinical data revealed a correlation between the expression level of MAPKAP5-AS1 and the clinicopathological features of patients with colorectal cancer. High expression of MAPKAP5-AS1 in CRC tissues was closely related to higher pathological stage (P = .007; Figure 1B), larger tumor (P = .004; Figure 1C), and positive lymph node status (P = .001; Figure 1D). Kaplan-Meier survival analysis and log-rank tests show survival time for cases with high MAPKAP5-AS1 expression was below those with low MAPKAP5-AS1 expression (Figure 1E). However, several other clinical parameters were found not to be significantly associated with MAPKAP5-AS1 expression (Table 1). Therefore, we speculate that the expression of MAPKAP5-AS1 could be related to CRC.

3.2 | MAPKAP5-AS1 promotes CRC cell proliferation in vitro and downregulation of MAPKAP5-AS1 promotes G₀/G₁ arrest

To investigate the biological function of MAPKAP5-AS1 in colorectal cancer, we first detected the expression level of MAPKAP5-AS1 in 4 CRC cell lines and normal colon epithelium cell line (HCoEpiC). Quantitative RT-PCR results showed that MAPKAP5-AS1 was significantly upregulated in SW480 (P < .01) and DLD-1 (P < .01) cells compared to normal colon epithelium cells (Figure 1F). Next, the siRNAs of MAPKAP5-AS1 and its blank control si-NC were transfected into CRC cells. The results showed that the expression of 2# and 3# siRNAs of MAPKAP5-AS1 was significantly reduced, but the 1# interference sequence showed poor transfection efficiency (Figure 1G). Subsequent experiments knocked down MAPKAP5-AS1 with 2# and 3# interference sequences. The CCK-8 and colony formation assay results showed knockdown of MAPKAP5-AS1 can significantly inhibit CRC cell proliferation (Figure 2A,B). To investigate whether MAPKAP5-AS1 promotes proliferation partly by inhibiting apoptosis, we used flow cytometry to detect apoptosis. The results showed that, compared with the control group, the apoptosis rate was significantly increased after knocking down MAPKAP5-AS1 (Figure 2C). To assess whether MAPKAP5-AS1 affects CRC cell proliferation through MAPKAP5-AS1-mediated cell cycle changes, we examined cell cycle progression in CRC cells by flow cytometry. Results showed that MAPKAP5-AS1 knockdown increased the percentage of cells in G₀/G₁ phase and decreased the percentage of cells in S and G2/M phase compared with control cells (Figure 3A). These studies indicate that MAPKAP5-AS1 has an important effect on CRC cells by affecting the cell cycle. Cell proliferation ability, tested with EdU, also showed similar results. The results suggest that abnormally high expression of MAPKAP5-AS1 in CRC cells can promote cell proliferation (Figure 3B). The TUNEL assay for detecting apoptosis was also consistent with the above results (Figure 3C). These findings suggest that MAPKAP5-AS1 might be closely related to the proliferation of CRC cells.
FIGURE 1 MAPKAP5-AS1 is upregulated in human colorectal cancer (CRC) tissues and is significantly correlated with larger tumor size, advanced TNM stage, lymph node metastasis, and poor prognosis. A, Relative expression of MAPKAP5-AS1 in CRC tissues (n = 50) compared with the corresponding non-tumor tissues (n = 50); the patients were classified into two groups according to the median level of MAPKAP5-AS1. MAPKAP5-AS1 expression was examined by quantitative RT-PCR (qRT-PCR) and normalized to GAPDH expression. B-D, MAPKAP5-AS1 expression was significantly higher in patients with larger tumor size, higher pathological stage, and lymph node metastasis. E, Kaplan-Meier overall survival curves according to MAPKAP5-AS1 expression level. Cum., cumulative. F, MAPKAP5-AS1 expression was assessed by qRT-PCR analysis in CRC cells (DLD-1, HCT116, HT29, and SW480) and the normal human colonic epithelial cell line (HCoEpiC). G, Quantitative PCR analysis of MAPKAP5-AS1 expression levels following transfection of DLD-1 and SW480 cells with siRNAs against MAPKAP5-AS1. Representative images and data based on 3 independent experiments. Bars: ± SD. **P < .01. siNC, negative control.
TABLE 1 Relationships between MAPKAP5-AS1 expression and clinicopathological characteristics of colorectal cancer patients

| Characteristics       | MAPKAP5-AS1 Low expression (n = 25) | MAPKAP5-AS1 High expression (n = 25) | chi-squared test P-value |
|-----------------------|-------------------------------------|-------------------------------------|--------------------------|
| Age (y)               |                                     |                                     |                          |
| ≤60                   | 12                                  | 10                                  | .56700                   |
| >60                   | 13                                  | 15                                  |                          |
| Gender                |                                     |                                     |                          |
| Male                  | 11                                  | 15                                  | .25800                   |
| Female                | 14                                  | 10                                  |                          |
| Tumor size            |                                     |                                     |                          |
| ≤5 cm                 | 17                                  | 4                                   | .00020**                 |
| >5 cm                 | 8                                   | 21                                  |                          |
| Histological differen|                                     |                                     |                          |
| Middle or high        | 19                                  | 7                                   | .00068**                 |
| low or undiffer       | 6                                   | 18                                  |                          |
| TNM stage             |                                     |                                     |                          |
| I, II                 | 17                                  | 7                                   | .00460**                 |
| III, IV               | 8                                   | 18                                  |                          |
| Lymph node metastasis|                                     |                                     |                          |
| Positive              | 9                                   | 15                                  | .02450*                  |
| Negative              | 16                                  | 8                                   |                          |

*P < .05.  **P < .01.

3.3 | Knockdown of MAPKAP5-AS1 inhibits CRC tumorigenesis in vivo

The statistical results of tumor volume changes showed that the tumor formation ability of the mice in the MAPKAP5-AS1 knockdown group was significantly reduced and the growth rate was significantly slower compared to the control group (Figure 4A). As shown, qPCR confirmed that the MAPKAP5-AS1 expression was lower in tumor tissues derived from sh-MAPKAP5-AS1-transfected cells (Figure 4B), accurate measurement calculations prove that the knockdown of MAPKAP5-AS1 tumor volume is clearly smaller (Figure 4C) and the average weight of tumors in the MAPKAP5-AS1 knockdown group was lower than the control group (Figure 4D). Moreover, IHC analysis confirmed that the tumors formed from DLD-1/sh-MAPKAP5-AS1 cells displayed weaker Ki-67 staining than those formed from control cells (Figure 4E). Our results indicate that silencing MAPKAP5-AS1 expression can inhibit tumor growth in CRC cells in vivo.

3.4 | MAPKAP5-AS1 overexpression induces promotion of CRC cell proliferation

To further explore the function of MAPKAP5-AS1, we carried out an overexpression experiment in HCT116 cells. Both CCK-8 and EdU assays revealed that the viability of HCT116 cells transfected with pcDNA-MAPKAP5-AS1 was significantly impaired compared with empty vector-treated cells (Figure 5A-C). The colony formation assays results showed that overexpression of MAPKAP5-AS1 in HCT116 cells significantly promoted cell colony formation (Figure 5D). These findings indicated that MAPKAP5-AS1 promoted CRC cell proliferation.

3.5 | MAPKAP5-AS1 promotes CRC cell proliferation by partially binding histone EZH2 and subsequently inhibiting expression of target gene p21

To investigate the mechanism of MAPKAP5-AS1 in CRC, we first analyzed its distribution in CRC cells and found that MAPKAP5-AS1 is mainly located in the nucleus (Figure 6A,B), which means that MAPKAP5-AS1 might be involved in transcriptional regulation. We used qRT-PCR to detect the 8 classical mRNA levels of proliferation-related genes after knocking down MAPKAP5-AS1 (p15, p21, p27, p57, Bcl-2, Bax, KLF-2, and Trail; Figure 6C). The results showed that, compared with the control group, p21 mRNA levels were changed to varying degrees after knockdown of MAPKAP5-AS1 in SW480 and DLD-1 cells (Figure 6D, p21, which was reported to be closely related to proliferation, was selected as a downstream target for further studies. We then predicted the possible binding proteins of MAPKAP5-AS1 and found that it could bind to histone modification enzymes. Given that approximately 20% of IncRNAs have been reported to exercise regulatory functions in the form of binding to polycomb repressive complex2 (PRC2) and it has been reported that lncRNA often forms a complex with AGO2 protein to silence the target. Based on this, we reasonably suspect that MAPKAP5-AS1 could associate with the PRC2 core subunit EZH2 and RNA-induced silencing complex (RISC) core subunit AGO2 to regulate downstream target gene p21. The above prediction results were further verified by RIP experiments. The results showed that MAPKAP5-AS1 can bind to histone EZH2 rather than RISC AGO2 in SW480 and DLD-1 cell lines. IncRNA DUXAP10 has been reported as not bound to EZH2,17 we chose it as a negative control (Figure 6E,F). Subsequently, in order to investigate whether MAPKAP5-AS1 is involved in histone EZH2 enrichment in p21 promoter region. The p21 promoter-specific primers were designed for ChIP-qPCR in SW480, DLD-1, and HCT116 cells. The results showed that, compared with the control group, knockdown of MAPKAP5-AS1 significantly reduced the enrichment of EZH2 and H3K27me3 in the p21 promoter region; overexpression works just the opposite, suggesting that MAPKAP5-AS1 and EZH2 can be enriched in the p21 promoter region and inhibit transcription (Figure 6G,H). We then decreased EZH2 expression in DLD-1 cells by transfection with si-EZH2. The qRT-PCR results suggested that the expression level of p21 increased following si-EZH2 transfection (Figure 6I). Taken together, the experimental results indicate that MAPKAP5-AS1 promotes CRC cell proliferation by partly binding histone EZH2 and subsequently inhibiting the expression of the downstream target gene p21.
3.6 | p21 is potentially involved in the oncogenic function of MAPKAPK5-AS1

To verify the effect of p21 in CRC cells, p21 was knocked down in DLD-1 cells (Figure 7A,B). The CCK-8 and colony formation assays showed a significant change of CRC cell viability after p21 changed (Figure 7C). Edu shows that si-p21 transfects DLD-1 cells (Figure 7D). These data indicate that inhibition of p21 promotes CRC cell proliferation. We also undertook colony formation assays to determine whether p21 is involved in MAPKAPK5-AS1-mediated CRC cell proliferation. DLD-1 cells were cotransfected with MAPKAPK5-AS1 and p21 shRNA. Colony formation assays showed that proliferation of DLD-1 cells cotransfected with sh-MAPKAPK5-AS1 and sh-p21 was increased compared to that in HUCCT1 cells treated with sh-MAPKAP5-AS1 alone (Figure 7E).
Collectively, these data indicate that MAPKAPK5-AS1 partially promotes proliferation of CRC cells by p21 downregulation.

4 | DISCUSSION

In recent years, more and more tumor-associated IncRNAs have been discovered. A large number of published works have reported that these IncRNAs play an important role in multiple tumors. Our previous research shows that IncRNA SNHG17 can promote the proliferation of CRC cells by inhibiting the expression of p57. The high expression of IncRNA LL22NC03-N64E9.1 could predict poor prognosis in patients with colon cancer. Long ncRNA SH3PXD2A-AS1 inhibits p21 expression by binding EZH2 to promote CRC cell proliferation. Therefore, the discovery of new CRC-related IncRNAs is of great significance for further understanding the molecular mechanisms of CRC progression and providing clinical therapeutic targets. In this study, we analyzed 2 sources
of high-throughput microarray data of CRC and found IncRNA MAPKAPK5-AS1. The expression of IncRNA MAPKAPK5-AS1 in two CRC tissue microarrays was significantly upregulated and has not been previously reported. The above analysis results were further validated in 50 CRC tissues. Statistical analysis of clinical pathological data found that high expression of MAPKAPK5-AS1 predicts poor prognosis in CRC patients.

Subsequently, we knocked down MAPKAPK5-AS1 in 2 high-expression CRC cell lines and overexpressed it in the low-expression cells, through a series of in vitro and in vivo functional assays. MAPKAPK5-AS1 was found to promote the proliferation of CRC cells and inhibit its apoptosis. In summary, MAPKAPK5-AS1 plays an important role in the malignant phenotype of CRC.
Many lncRNAs can regulate the expression of downstream target genes by forming complexes with RNA-binding proteins, then acts as an oncogene or a suppressor oncogene. In this study, we found that knockdown of MAPKAPK5-AS1 in CRC cells could lead to changes in related downstream genes, including p15, p21, p27, p57, KLF2, and Trail. Among them, p21 has high basal expression abundance in CRC cells, which aroused our interest. We examine published reports and find that some lncRNAs can play an oncogene function by inhibiting p21 at the transcriptional level, such as lncRNA LUCAT1 and lncRNA TUG1. In this study, we used bioinformatics analysis to predict possible binding proteins of MAPKAPK5-AS1 and further confirmed by RIP experiments that MAPKAPK5-AS1 binds to EZH2. Subsequently, qRT-PCR results showed that knockdown of MAPKAPK5-AS1 significantly

**FIGURE 5** MAPKAPK5-AS1 overexpression induces promotion of colorectal cancer cell proliferation. A, Level of MAPKAPK5-AS1 in HCT116 cells transfected with pcDNA-MAPKAPK5-AS1 detected by quantitative PCR analysis. B-D, MTT, 5-ethynyl-2′-deoxyuridine (EdU) staining, and colon formation assays were used to determine cell viability. Representative images and data based on 3 independent experiments. Bars: ± SD, *P < .05, **P < .01
upregulated the expression of p21. The ChIP-qPCR experiments confirmed that MAPKAPK5-AS1 can bind to EZH2 to inhibit the expression of p21 at the transcriptional level. In summary, we discovered for the first time that MAPKAPK5-AS1 regulates the biological function of CRC by binding to EZH2 to silence the downstream target gene p21 at the transcriptional level.

We found that high expression of MAPKAPK5-AS1 in CRC tissue predicts poor prognosis in patients, and the activation
of the MAPKAPK5-AS1/EZH2/p21 regulatory axis plays an important role in the progression of CRC. However, this conclusion still needs to be expanded to verify clinical samples. MAPKAPK5-AS1 could play another role in CRC and further research is needed.

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

ETHICAL STANDARDS
All institutional and national guidelines for the care and use of laboratory animals were followed.
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