**Lucat1** promotes colorectal cancer tumorigenesis by targeting the ribosomal protein L40-MDM2-p53 pathway through binding with **Uba52**

Qianwen Zhou1,2 | Zhibo Hou3 | Siyu Zuo1,2 | Xin Zhou4 | Yadong Feng5 | Yong Sun6 | Xiaoqin Yuan1,2

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in both men and women in the USA. However, the underlying molecular mechanisms that drive CRC tumorigenesis are still not clear. Several studies have reported that long noncoding RNAs (lncRNAs) have important roles in tumor development. Here, we undertook a transcriptome microarray analysis in 6 pairs of CRC tissues and their corresponding adjacent normal tissues. A total of 1705 differentially expressed lncRNAs were detected in CRC tissues at stages I/II and III/IV (fold change greater than or equal to 2 or less than or equal to 0.5). Among them, we found that the lncRNA lung cancer-associated transcript 1 (**Lucat1**) was upregulated in CRC tissues and was closely associated with poor overall survival of CRC patients, through analysis of clinical data and The Cancer Genome Atlas. Functional studies indicated that **Lucat1** promoted CRC cell proliferation, apoptosis, migration, and invasion in vitro and in vivo. Furthermore, knockdown of **Lucat1** rendered CRC cells hypersensitive to oxaliplatin treatment. Mechanistically, bioinformatic analysis indicated that low expression of **Lucat1** was associated with the p53 signaling pathway. Chromatin isolation by RNA purification followed by mass spectrometry and RNA immunoprecipitation revealed that **Lucat1** bound with **Uba52**, which encodes ubiquitin and 60S ribosomal protein L40 (Rpl40). We found that Rpl40 functions in the ribosomal protein-MDM2-p53 pathway to regulate p53 expression. Taken together, our findings indicate that suppression of **Lucat1** induces CRC cell cycle arrest and apoptosis by binding **Uba52** and activating the Rpl40-MDM2-p53 pathway. These results implicate **Lucat1** as a potential prognostic biomarker and therapeutic target for CRC.

**Keywords**
colorectal cancer, lung cancer-associated transcript 1, MDM2, p53, UBA52

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**Abbreviations:** 5-FU, 5-fluorouracil; CHX, cycloheximide; CRC, colorectal cancer; lncRNA, long noncoding RNA; Lucat1, lung cancer-associated transcript 1; PARP, poly(ADP-ribose) polymerase; qPCR, quantitative PCR; RP, ribosomal protein; Rpl40, ribosomal protein L40; TCGA, The Cancer Genome Atlas.

Zhou and Hou contributed equally to this work.

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1 | INTRODUCTION

Colorectal cancer is the third most commonly diagnosed cancer in both men and women in the USA. The annual age-standardized incidence rate for CRC (2009-2013) was 40.7 per 100 000 persons, and the mortality rate (2010-2014) was 14.8 per 100 000 persons.1,2 In China, CRC is the fourth most common malignant cancer with increasing incidence.3 While CRC patients with clinical stage I-II disease have relatively good prognosis, patients with stage III-IV have a poor therapeutic response and prognosis.4 The underlying molecular mechanisms that drive CRC tumorigenesis remain to be fully elucidated. Accumulating evidence has shown that IncRNAs have functions in tumor development, and these IncRNAs could provide new candidates for diagnostics and therapy.

Long noncoding RNA is a type of noncoding RNA transcript longer than 200 nucleotides.5 A large number of studies have indicated that IncRNAs regulate gene expression through chromatin modification, transcription, and post-transcriptional processing.6,7 Long noncoding RNAs have been shown to be differentially expressed in various cancers such as colorectal, breast, and lung cancer, suggesting that they might have roles in tumorigenesis and tumor metastasis,7 and could act as diagnostic biomarkers in a variety of cancers.8,9 Although some IncRNAs have been characterized in CRC with regards to their biological function and mechanisms,10,11 the expression pattern of IncRNAs in CRC progression remains unclear.

In this study, we attempted to identify CRC progression-associated IncRNAs. Using IncRNA microarray in clinical samples, we found that LUCAT1 IncRNA has an important role in the regulation of CRC progression and response to chemotherapy, and thus might serve as a potential prognostic marker and therapeutic target.

2 | MATERIALS AND METHODS

Colorectal cancer tissues and corresponding adjacent normal mucosa samples were obtained from 90 patients with CRC who underwent surgical resection at the First Affiliated Hospital of Nanjing Medical University (Nanjing, China). All samples were immediately snap-frozen in liquid nitrogen after collection and stored at −80°C until total RNA was extracted. None of the patients received chemotherapy or radiotherapy before surgical treatment. The clinical information of the CRC patients is summarized in Table 1. This project was approved by the Research Ethics Committee of Nanjing Medical University (approval ID: 2016/640). Other materials and methods are described in Appendix S1. All the primers used for real-time PCR are shown in Table S1, and all the Abs used in western blotting are shown in Table S2.

3 | RESULTS

3.1 Long noncoding RNA expression profile in CRC tissues

To identify potential IncRNAs involved in CRC progression, we collected primary tumors and the corresponding adjacent normal mucosal tissues from 3 patients with stage I/II and 3 patients with stage III/IV disease, and then undertook a transcriptome microarray in which protein-coding mRNAs and IncRNAs were included. With filtering conditions of fold change greater than or equal to 2 or less than or equal to 0.5 and P < 0.05, a total of 2043 protein-coding mRNAs were detected as differentially expressed between CRC and normal tissues (Figure 1A). Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis revealed that these differentially expressed genes were significantly enriched in the cell cycle, in cytokine-cytokine receptor interactions, RNA polymerases, DNA replication, and the p53 signaling pathway (Figure 1B). Abnormally expressed IncRNAs in CRC tissues are shown in Figure 1C. In total, 1705 differently expressed IncRNAs were detected in CRC tissues at both I/II and III/IV stages (fold change more than or equal to 2, or less than or equal to 0.5, P < 0.05). Among them, 896 were upregulated and 809 were downregulated in all CRC tissues.

3.2 LUCAT1 is upregulated in CRC and is associated with poor prognosis

Among the upregulated IncRNAs, we found that LUCAT1, which is reported to participate in the tumorigenesis of several cancers, was highly expressed in all CRC tissues at different stages. To validate the

| TABLE 1 Association of LUCAT1 expression with clinicopathological parameters in patients with colorectal cancer (n = 90) |
| Feature | Total | Low | High | P value |
|---------|-------|-----|------|--------|
| Age     |       |     |      |        |
| <60 years | 41    | 14  | 27   | .605   |
| >60 years | 49    | 13  | 36   |        |
| Sex     |       |     |      |        |
| Male    | 49    | 16  | 33   | .961   |
| Female  | 41    | 11  | 30   |        |
| T classification |       |     |      |        |
| 1       | 2     | 2   | 0    |        |
| 2       | 20    | 12  | 8    | .025   |
| 3       | 45    | 10  | 35   |        |
| 4       | 23    | 3   | 20   |        |
| N category |     |     |      |        |
| 0       | 45    | 25  | 20   | .928   |
| 1       | 18    | 1   | 17   |        |
| 2       | 27    | 1   | 26   |        |
| Clinical stage |       |     |      |        |
| I/II    | 46    | 26  | 20   | .031   |
| III/IV  | 44    | 1   | 43   |        |
| Metastasis |       |     |      |        |
| No      | 86    | 27  | 59   | .990   |
| Yes     | 4     | 0   | 4    |        |
| Tumor size |       |     |      |        |
| <5 cm   | 63    | 23  | 40   | .354   |
| >5 cm   | 27    | 4   | 23   |        |
microarray results, LUCAT1 expression was measured in 90 matched CRC samples using RT-qPCR. Our results showed that the levels of LUCAT1 in CRC tissues were nearly 4.09-fold increased as compared with the corresponding adjacent normal mucosa (Figure 2A). The correlation analysis of LUCAT1 expression revealed that increased LUCAT1 levels were significantly associated with advanced CRC TNM stage ($P = .0035$; Figure 2B). However, there was no significant association between LUCAT1 expression level and several other clinical parameters such as sex, age, and histological grade in our study (Table 1).

We further examined the clinical effect of LUCAT1 in CRC by analyzing TCGA database. We compared the data from 599 CRC tissues and 51 normal colon tissues and found that the expression levels of LUCAT1 were significantly increased in CRC tissues by 3.97-fold ($P < .0001$; Figure 2C) and were associated with tumor clinical stage (Figure 2D). Furthermore, Kaplan-Meier analysis indicated that patients with higher LUCAT1 expression had poorer overall survival than those with low expression of LUCAT1 (Figure 2E).

Taken together, these data indicated that LUCAT1 is highly expressed in CRC and is associated with cancer progression and poor prognosis.

### 3.3 LUCAT1 promotes cell proliferation and invasion in vitro

We next investigated the effect of LUCAT1 in CRC cells. We first screened the expression levels of LUCAT1 in CRC cell lines HCT116, HT29, SW480, RKO, Caco-2, and LoVo and in a normal colon epithelial cell line, FHC. LUCAT1 expression was found to be significantly higher in CRC cell lines compared with FHC (Figure 3A). Based on this result, we selected HCT116 and RKO cells in which to study the biological function of LUCAT1 through loss- and gain-of-function approaches.

Three specific siRNAs and pcDNA-LUCAT1 were transiently transfected into HCT116 and RKO cells. As shown in Figure 3B, siRNAs specifically targeting LUCAT1 decreased the expression of LUCAT1, whereas pcDNA-LUCAT1 transfection increased the expression of LUCAT1. The results from CCK-8 assays showed that siRNAs siLUCAT1-2 and siLUCAT1-3 reduced the viability of HCT116 and RKO cells. In contrast, overexpression of LUCAT1 significantly increased cell viability (Figure 3C). As siLUCAT1-2 showed the best capacity in silencing and growth inhibition, it was chosen to generate the lentivirus for further studies. Colony formation assays revealed that knockdown of LUCAT1 could significantly inhibit cell proliferation in both HCT116 and RKO cell lines, whereas LUCAT1-overexpressing cells showed the opposite effect (Figure 3D).

The effect of LUCAT1 on the cell cycle and apoptosis was then examined using flow cytometry. The results revealed that, compared with the control shRNA, knockdown of LUCAT1 arrested the cells in $G_0/G_1$ phase and decreased $S$ phase in both HCT116 and RKO cell lines (Figure 3E). In addition, LUCAT1 knockdown increased the proportion of apoptotic cells and induced PARP-1 expression in HCT116 and RKO cells (Figure 3F,G). Then the effects of LUCAT1 on migration and invasion were investigated. The results of Transwell assays showed...
that silencing of *LUCAT1* significantly impaired CRC cell migration and invasion compared with control cells (Figure 4).

Together, these data suggested that *LUCAT1* promotes CRC cell proliferation and invasion in vitro.

### 3.4 *LUCAT1* promotes CRC cell proliferation in vivo

To further determine whether *LUCAT1* mediates tumorigenesis in vivo, stable *LUCAT1*-knockdown HCT116 cells (sh-*LUCAT1*) and control HCT116 cells (sh-Ctrl) were s.c. injected into the flanks of 5-week-old male nude mice. As shown in Figure 5A, all the mice injected with sh-Ctrl cells developed tumors; however, only 3 of the 5 mice injected with sh-*LUCAT1* cells developed tumors. We also found that xenograft tumors grown from sh-*LUCAT1* HCT116 cells had smaller mean volumes, slower growth, and lighter tumor weight than xenograft tumors grown from control cells (Figure 5B,C). The results of RT-qPCR showed that the average expression levels of *LUCAT1* in xenograft tumors from the sh-*LUCAT1* group were lower than those from the control groups (Figure 5D).

We also found that the expression of proliferation marker Ki-67 was significantly decreased in tumors from sh-*LUCAT1* cells compared with tumors from control cells (Figure 5E). Likewise, the apoptosis marker cleaved PARP-1 was higher in xenograft tumors from the sh-*LUCAT1* group than those from the control group (Figure 5F). Collectively, these data implied that suppression of *LUCAT1* expression could repress CRC cell proliferation and induce apoptosis in vivo.

### 3.5 *LUCAT1* knockdown confers drug sensitivity in CRC cells

To investigate the role of *LUCAT1* in drug responsiveness, we assessed the effects of *LUCAT1* knockdown on cell proliferation and...
apoptosis in HCT116 cells treated with oxaliplatin and 5-FU. Stable LUCAT1-knockdown HCT116 and control cells were exposed to various concentrations of oxaliplatin or 5-FU for 24 and 48 hours, and cell viability was examined by CCK-8 assay. Figure 6A shows that LUCAT1 knockdown significantly accelerated the reduction in cell viability in response to 5-FU and oxaliplatin treatment for 24 hours or 48 hours, especially at 2.5 and 5.0 μg/mL. Treatment with both oxaliplatin and 5-FU at 5.0 μg/mL for 48 hours increased the rate of apoptotic cells in LUCAT1-knockdown cells compared with control cells (Figure 6B). Overall, knockdown of LUCAT1 rendered colon cancer cells hypersensitive to the cytotoxicity of the chemotherapeutic drugs oxaliplatin and 5-FU.

3.6 Low expression of LUCAT1 correlates with the p53 pathway

To investigate which biological pathways LUCAT1 might be involved in, we undertook Gene Set Enrichment Analysis (GSEA) with 478 CRC tumor samples in TCGA database. Notably, we found that low LUCAT1 expression had a high correlation with the p53 pathway and p53 target gene expression in colon cancer (with the filtering conditions of false discovery rate (FDR) <25% and a nominal P value <.001; Figure 7A), which was consistent with our previous KEGG analysis data showing that the p53 pathway is involved in CRC (Figure 1B). This suggested that LUCAT1 might regulate CRC proliferation and apoptosis by way of the p53 pathway. To validate this, we tested the expression of p53 and its associated genes involved in the cell cycle and apoptosis in LUCAT1-knockdown cells and found that LUCAT1 knockdown increased p53, p21, and Bax expression and decreased the expression of Bcl-2 (Figure 7B). LUCAT1 knockdown induced p53 expression at the protein level but not at the mRNA level, indicating that LUCAT1 might affect p53 stability. To verify this, treatment with CHX, which inhibits protein synthesis, was carried out. The results showed that stability of p53 was increased by LUCAT1 knockdown (Figure 7C), suggesting that LUCAT1 influences p53 protein stability. MG132 treatment showed LUCAT1 affected p53 stability in proteasome-dependent degradation (Figure 7D).
Given that HCT116 and RKO cells possess the WT TP53 gene, we used the SW480 cell line with mutant TP53 to test the effect of LUCAT1. As shown in Figure 7E,F, LUCAT1 knockdown did not decrease SW480 cell viability, nor did it alter the expression levels of p21 or p53. These results indicated that the effect of LUCAT1 on cell proliferation and apoptosis might occur through the p53 pathway.

3.7 | LUCAT1 binds UBA52 to affect its stability

Recent studies have suggested that many lncRNAs directly bind proteins to participate in molecular regulation pathways. To investigate whether LUCAT1 functioned in a similar manner, we undertook chromatin isolation by RNA purification (ChiRP) assays using biotin-labeled probes specific for LUCAT1 and analyzed the precipitated proteins by mass spectrometry. Sixteen proteins that might potentially bind to LUCAT1 were identified by mass spectrometry (Figure 8A). Among these proteins, UBA52 was selected for further verification due to its better mass spectrometric data and its association with CRC.12 We next undertook RNA immunoprecipitation (RIP) using anti-UBA52 in cell extracts from HCT116 cells. As shown in Figure 8B, LUCAT1 was enriched with anti-UBA52 Ab, but not with the IgG control. We also found that LUCAT1 knockdown upregulated

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**FIGURE 4** Lung cancer-associated transcript 1 (LUCAT1) promotes colorectal cancer cell invasion in vitro. Transwell assays were used to detect the migration (A) and invasion (B) of LUCAT1-knockdown and overexpressing cells after 36-48 h. Data represent mean ± SEM from 3 independent experiments. *P < .05, **P < .01
the expression of UBA52, whereas LUCAT1 overexpression downregulated UBA52 levels (Figure 8C). However, LUCAT1 knockdown or overexpression did not significantly affect the mRNA levels of UBA52 (Figure 8C) indicating that LUCAT1 might affect UBA52 protein stability. To verify this, CHX treatment was carried out, and the results showed that UBA52 stability was increased by LUCAT1 knockdown (Figure 8D). To determine whether LUCAT1 affected the proteasomal degradation of UBA52, we undertook in vivo ubiquitination experiments by transfecting pcDNA-Ub-HA and pcDNA-UBA52-His together with LUCAT1 siRNA or pcDNA-LUCAT1 into HCT116 cells, in which ubiquitin was immunoprecipitated with anti-HA Ab and detected by anti-His. The results showed overexpression of LUCAT1 increased polyubiquitination of UBA52, whereas knockdown of LUCAT1 diminished UBA52 polyubiquitination (Figure 8E). Together, these results suggested that LUCAT1 might physically bind with UBA52 and affect its stability in a proteasome-dependent manner.

3.8 Knockdown of LUCAT1 upregulates the RPL40-MDM2-p53 pathway

UBA52 is a hybrid gene encoding a fusion protein comprising ubiquitin at the N-terminus and 60S RPL40 at the C-terminus. Upon translation, ubiquitin and RPL40 are immediately cleaved from the translation product. Previous studies have shown that RPs can regulate p53 stability through the RP-MDM2-p53 pathway responsible
Our results showed that LUCAT1 knockdown significantly decreased the expression of rRNA, 18S RNA, and pre-rRNA (Figure 9A), which participate in ribosome biogenesis, indicating that LUCAT1 knockdown induced ribosomal stress. Previous studies showed that 5-FU treatment could trigger ribosomal stress and promote p53 accumulation by the RP-MDM2-p53 pathway. As illustrated in Figure 9B, overexpression of LUCAT1 compromised p53 and MDM2 expression initially induced by 5-FU treatment.
FIGURE 7 Low expression of lung cancer-associated transcript 1 (LUCAT1) is correlated with the p53 pathway and its target genes. A, Gene Set Enrichment Analysis (GSEA) indicated a significant correlation between low expression of LUCAT1 and the p53 pathway and its target genes. B, Expression levels of p53, p21, Bcl-2, and Bax by western blotting (left panel) and RT-quantitative PCR (right panel) in stable LUCAT1-knockdown (sh-LUCAT1) and control (sh-Ctrl) HCT116 cells. C, Stable sh-LUCAT1 and sh-Ctrl HCT116 cells were treated with 100 μg/mL cycloheximide (CHX) and harvested at different time points as indicated. p53 protein was detected by western blotting, quantified by densitometry, and plotted against time to determine p53 stability. D, Stable sh-LUCAT1 and sh-Ctrl HCT116 cells were treated with 5 μmol/L MG132 for 12 h, and p53 protein was detected by western blotting. E, Cell viability of SW480 transfected with LUCAT1 shRNA was measured by CCK-8 assay. F, Protein levels of p53, p21, Bax, and Bcl-2 in SW480 cells transfected with LUCAT1 shRNA were determined by western blot analysis. Error bars indicate means ± SEM. *P < .05, **P < .01

FIGURE 8 Lung cancer-associated transcript 1 (LUCAT1) binds with UBA52 to decrease its stability. A, LUCAT1 binding proteins were precipitated through chromatin isolation by RNA purification (ChirP) assay in HCT116 cells. Left panel is the relative LUCAT1 level with targeted LUCAT1 probes and control probes. Right panel shows the proteins identified by mass spectrometry. B, RNA immunoprecipitation (RIP) assay was carried out using anti-UBA52, IgG as a negative control in HCT116 cells. The enrichment of LUCAT1 was detected using RT-quantitative PCR (qPCR). GAPDH was a negative control. C, Expression of UBA52 was detected with western blotting (upper panel) and RT-qPCR (lower panel) in HCT116 cells with low expression and overexpression of LUCAT1. D, Stable sh-LUCAT1 and control (sh-Ctrl) HCT116 cells were treated with 100 μg/mL cycloheximide (CHX) and harvested at different time points as indicated. UBA52 was detected by western blotting and quantified by densitometry. E, HCT116 cells were transfected with pcDNA-UBA52 in combination with LUCAT1 siRNA or pcDNA-LUCAT1, in the presence of the HA-ubiquitin (HA-Ub) plasmid as indicated at the top. The cells were treated with MG132 (20 μmol/L) for 6 h before harvesting and cell lysates were subjected to immunoprecipitation (IP) using anti-HA Ab. Ubiquitinated proteins were detected by immunoblotting (IB) with the anti-His Ab. LUCAT1 expression level was detected by RT-qPCR. *P < .05, **P < .01. n.s, not significant
Next, we investigated whether RPL40 cleaved from UBA52 is involved in the RP-MDM2-p53 pathway by binding to MDM2. We transfected HCT116 cells with UBA52 plasmid, containing a His-tag at the C-terminus and a FLAG-tag at the N-terminus. We then undertook protein immunoprecipitation with anti-His Ab. Western blotting with anti-MDM2 showed that RPL40 bound to MDM2 (Figure 9C).
Intriguingly, we found that LUCAT1 knockdown decreased protein expression of MDM2 while still increasing its mRNA levels, and MG132 treatment rescued MDM2 protein expression (Figure 9D-E), suggesting LUCAT1 knockdown could also affect MDM2 stability. Consistent with this, CHX treatment revealed LUCAT1 knockdown induced MDM2 protein degradation (Figure 9F). To test whether the effect of LUCAT1 on MDM2 expression is proteasome-dependent, we carried out an in vivo ubiquitination assay in which ubiquitin was immunoprecipitated...
and detected by Ab against MDM2. Indeed, knockdown of LUCAT1 increased polyubiquitination of MDM2, whereas overexpression of LUCAT1 diminished MDM2 polyubiquitination (Figure 9G). The results showed LUCAT1 protects MDM2 from proteasome-mediated degradation.

As UBA52 is cleaved into RPL40 and ubiquitin, we speculated that, in addition to the binding of RPL40 to MDM2, ubiquitin from UBA52 might participate in MDM2 ubiquitination, thus leading to its degradation by the proteasome system. To confirm this, we co-transfected MDM2 and UBA52 overexpression plasmids into HCT116 cells and undertook immunoprecipitation assays. The results showed ubiquitin from UBA52 bound to MDM2 (Figure 9H), suggesting that LUCAT1 knockdown-induced UBA52 might provide the ubiquitin pool to accelerate the degradation of MDM2. To examine whether overexpressed UBA52 reduced the expression of MDM2, we constructed a plasmid encoding WT UBA52 (UBA52-WT) and a cleavage-resistant UBA52 (UBA52-CR) and transfected them into HCT116 cells. Consistently, we found that UBA52-WT overexpression reduced the expression of MDM2 and increased p53 expression, whereas UBA52-CR had no effect on MDM2 and p53 expression (Figure 9I). This indicated that RPL40, cleaved from UBA52, functions to regulate the MDM2-p53 pathway, and ubiquitin from UBA52 regulates the stability of MDM2. We also found that UBA52-WT overexpression rescued the expression levels of p53 initially downregulated by LUCAT1 overexpression (Figure 9J). Together these data indicated that LUCAT1 downregulated p53 expression by binding to UBA52 to induce its degradation, which impairs the RPL40-MDM2-p53 pathway and releases more MDM2 for p53 degradation (Figure 10).

4 | DISCUSSION

In the present study, we revealed that the lncRNA LUCAT1 was highly expressed in CRC tissues through a transcriptome microarray undertaken in 6 pairs of CRC tissues and their corresponding
adjacent normal tissues. Moreover, clinical and TCGA data showed that LUCAT1 was upregulated in the advanced stages of CRC and was closely associated with the poor overall survival of CRC patients. Functional investigation indicated that LUCAT1 promoted CRC cell proliferation, migration, and invasion, and inhibited CRC cell apoptosis. Together these findings suggest that LUCAT1 might have an important role in CRC carcinogenesis.

Lung cancer-associated transcript 1 is located on chromosome 5 and was first observed in the airway epithelium of smokers and in various cancer cell lines. In addition to lung cancer, LUCAT1 has also been reported to be involved in esophageal cancer, renal cell carcinoma, and cisplatin-resistant ovarian cancer. Like other IncRNAs, LUCAT1 participates in carcinogenesis and cancer progression by regulating gene expression through chromatin modification, transcription, and post-transcriptional procession. For example, LUCAT1 represses the expression of p21 and p57 in lung cancer by associating with polycomb repressor complexes (PRC2). LUCAT1 inhibits the expression of tumor suppressors in esophageal cancer through DNA methylation, by regulating the stability of the DNA methyltransferase DNMT1. LUCAT1 participates in methotrexate resistance regulation through the miR-200c-ABCf1 pathway. LUCAT1 has also been reported to be a liver metastasis-associated lncRNA through an analysis of CRC tissues from TCGA and Gene Expression Omnibus (GEO) databases; however, the underlying mechanisms are still unclear. Consistent with other studies, in which some lncRNAs including LUCAT1 are associated with chemotherapy resistance, our results revealed that LUCAT1 knockdown enhanced the apoptosis of CRC cells treated with oxaliplatin and 5-FU.

To reveal the mechanism of LUCAT1 in CRC, we undertook a bioinformatic analysis of GSEA with TCGA databases and found that LUCAT1 downregulation is associated with the p53 pathway. Experimental data also verified that LUCAT1 knockdown could affect p53 and its downstream pathway, including upregulating p53 and p21 expression and downregulating Bcl-2 expression. Some IncRNAs can participate in carcinogenesis through interactions with specific proteins. For instance, IncRNA-hPVT1 promotes cell proliferation, cell cycling, and stem cell-like properties in hepatocellular carcinoma cells through binding to NOP2 and enhancing its stability. P53RRA IncRNA induces cell cycle arrest, apoptosis, and ferroptosis by binding with RAS GTPase-activating protein-binding protein 1, resulting in p53 retention in the nucleus. In this regard, we undertook ChiRP and mass spectrometry followed by RIP and identified UBA52 as a binding protein to LUCAT1. UBA52, which is located on chromosome 19, is a hybrid gene encoding a fusion protein comprising ubiquitin at the N-terminus and RPL40 at the C-terminus. Upon translation, ubiquitin and RPL40 are immediately cleaved from the translated product. Ubiquitin is the source of the ubiquitin pool and RPL40 participates in ribosomal biogenesis. As a cellular "gatekeeper", p53 senses endo- and exogenous stressors and maintains cellular homeostasis by regulating cell proliferation, apoptosis, and senescence. p53 is largely post-translationally regulated by MDM2, which is an E3 ubiquitin ligase that binds p53 to promote its degradation. There are some regulatory factors that act on MDM2-p53. For example, p19ARF inhibits MDM2-mediated p53 ubiquitination and degradation by binding with MDM2. Recently, many studies have reported that a subset of ribosomal proteins including RPL5, RPL11, RPL26, RPS7, and RPS14 could bind to MDM2, inhibit its E3 ubiquitin ligase activity, and stabilize p53. This is the so-called RP-DMMD2-p53 pathway, which responds to ribosomal stress or nucleolar stress. Recent studies found that the RP-DMMD2-p53 pathway could be regulated by several proteins such as SPIN1, which is highly expressed in ovarian cancer, showing oncogenic potential and preventing RPL5 from interacting with MDM2, thereby alleviating the RPL5-DMMD2-p53 pathway.

In the present study, we found that RPL40, cleaved from UBA52 as a ribosomal protein, is involved in the RP-DMMD2-p53 pathway by interacting with MDM2, and therefore causing the accumulation of p53. This RPL40-DMMD2-p53 pathway accounts for LUCAT1 disruption-induced ribosomal stress characterized by the perturbation of ribosome biogenesis with decreased rRNA, 18S RNA, and pre-rRNA expression. In addition to the binding of RPL40 to MDM2, ubiquitin cleaved from UBA52 can also regulate MDM2 stability through ubiquitination. Our observation that overexpressed UBA52 abrogated LUCAT1-decreased p53 expression further supports this hypothesis.

In conclusion, this study found that LUCAT1 was significantly increased in CRC tissues and that suppression of LUCAT1 induced CRC cell cycle arrest and apoptosis, through binding UBA52 and activating the RPL40-DMMD2-p53 pathway. Our data indicated that LUCAT1 is involved in CRC progression, and could serve as a potential prognostic biomarker and a therapeutic target for CRC.

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CONFLICT OF INTEREST
There are no conflicts of interest to declare.

ORCID
Xiaoqin Yuan https://orcid.org/0000-0002-3084-5624

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.