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ORIGINAL ARTICLE

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LUCAT1 promotes colorectal cancer tumorigenesis by targeting the ribosomal protein L40-MDM2-p53 pathway through binding with *UBA52*

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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

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.

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 lncRNAs have functions in tumor development, and these lncRNAs 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 lncRNAs 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 lncRNAs have been characterized in CRC with regards to their biological function and mechanisms,[10,11] the expression pattern of lncRNAs in CRC progression remains unclear.

In this study, we attempted to identify CRC progression-associated lncRNAs. Using lncRNA microarray in clinical samples, we found that *LUCAT1* lncRNA 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 lncRNAs 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 lncRNAs 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 lncRNAs in CRC tissues are shown in Figure 1C. In total, 1705 differently expressed lncRNAs 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.

TABLE 1 Association of *LUCAT1* expression with clinicopathological parameters in patients with colorectal cancer (n = 90)

Feature	Total	*LUCAT1* expression		*P* value
		Low	High	
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	

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

Among the upregulated lncRNAs, 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

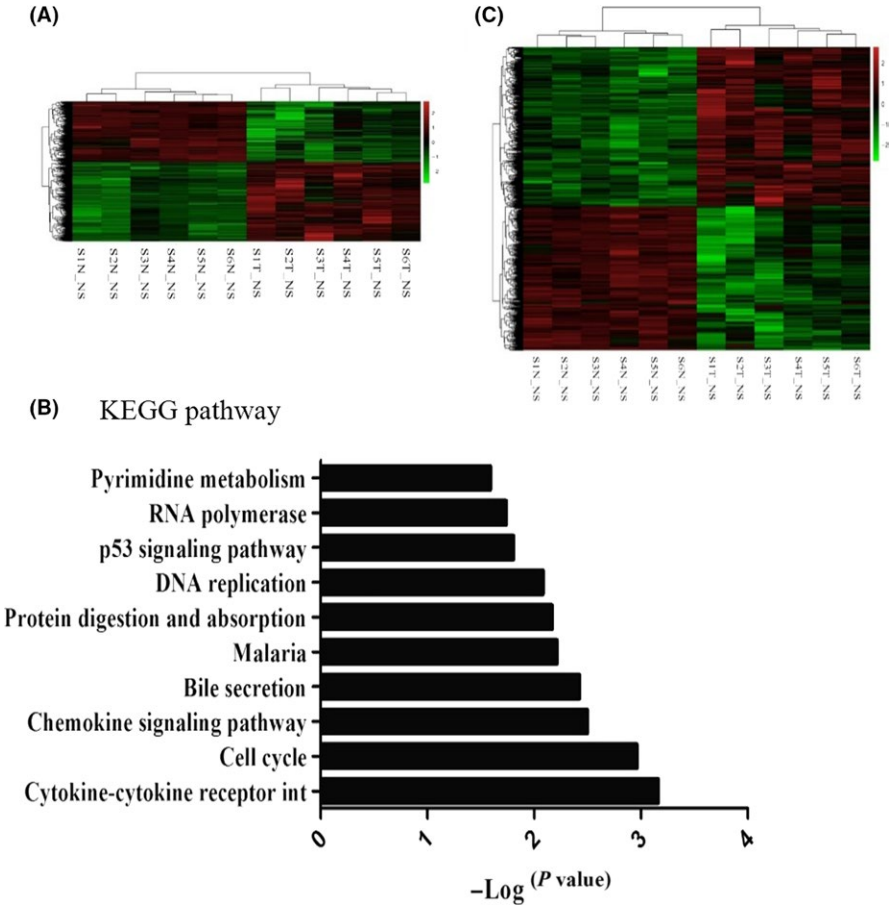


FIGURE 1 Transcriptome profiling in colorectal cancer (CRC) tissues and corresponding adjacent normal mucosal tissues. A, Hierarchical clustering heatmap of dysregulated mRNA expression in CRC and normal tissues. B, Top 10 pathways of Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis with differentially expressed mRNAs. C, Heatmap of dysregulated long noncoding RNA expression in CRC and normal tissues. In (A) and (C), the *x*-axis indicates sample names and *y*-axis indicates probe names. N, normal; T, tumor

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 colonic 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

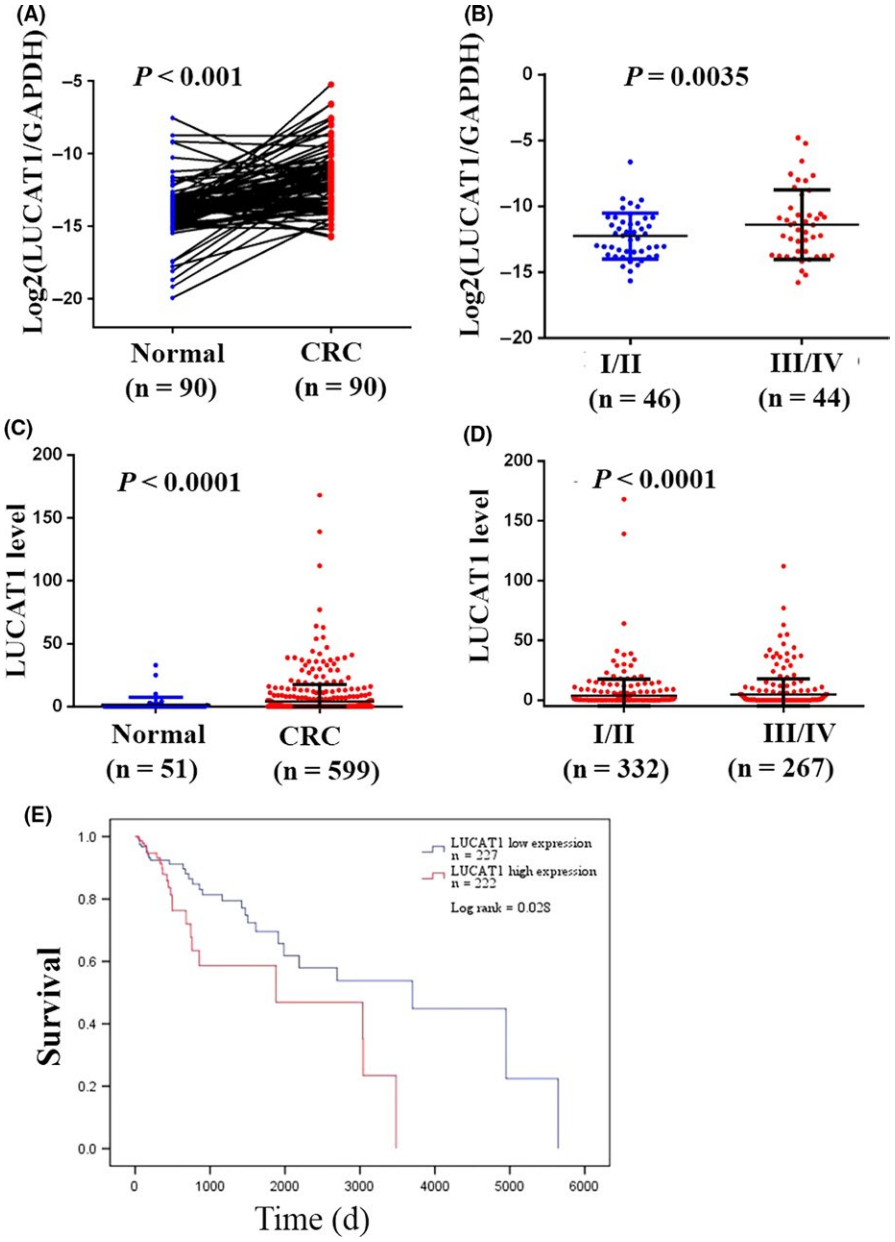


FIGURE 2 Lung cancer-associated transcript 1 (*LUCAT1*) is upregulated in colorectal cancer (CRC) tissues and high expression of *LUCAT1* is associated with poor prognosis in CRC patients. A, *LUCAT1* expression was increased significantly in CRC tissues compared with the corresponding nontumor tissues (n = 90). *LUCAT1* expression was detected by quantitative real-time PCR and plotted as $\log_2$(*LUCAT1*/*GAPDH*). B, *LUCAT1* expression was correlated with advanced CRC stage. C, Higher expression of *LUCAT1* was detected in 599 CRC tissues compared with 51 normal tissues from The Cancer Genome Atlas (TCGA) database. D, *LUCAT1* expression was correlated with advanced CRC stage in TCGA database analysis. E, Kaplan-Meier survival curves revealed an association of higher *LUCAT1* levels with short overall survival in CRC patients

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

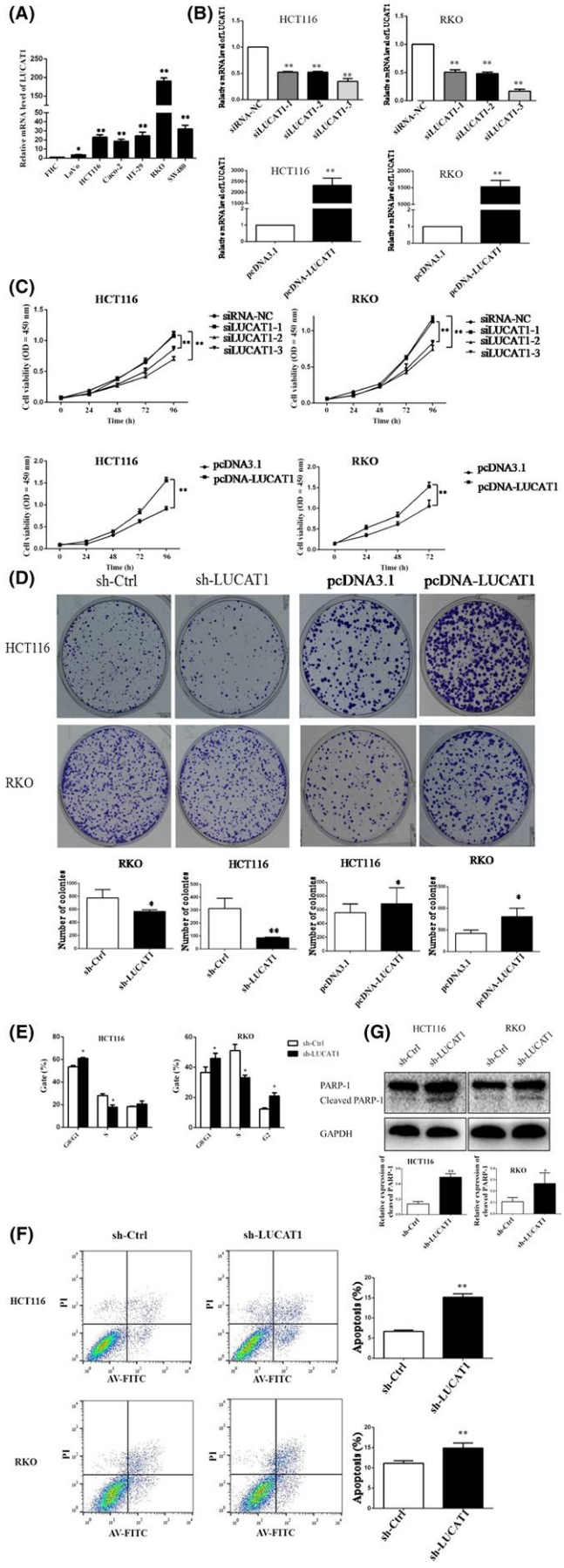


FIGURE 3 Lung cancer-associated transcript 1 (*LUCAT1*) promotes colorectal cancer (CRC) cell proliferation in vitro. A, RT-quantitative PCR analysis of *LUCAT1* in 6 CRC cell lines compared with FHC normal colonic epithelial cell line. B, *LUCAT1* expression was detected by RT-quantitative PCR in HCT116 and RKO cells transfected with the indicated siRNAs or pcDNA-LUCAT1. C,D, CCK-8 viability (C) and colony-forming growth assays (D) were measured in HCT116 and RKO cells transfected with *LUCAT1* siRNAs or pcDNA-LUCAT1 or *LUCAT1* shRNA. E,F, After 48 h of plasmid transfection, cell cycle distribution (E) and apoptotic cells (F) were examined by flow cytometry. G, Western blot of the expression levels of apoptosis-related poly(ADP-ribose) polymerase (PARP-1) in cells treated as indicated. Data represent mean ± SEM from 3 independent experiments. $^{*}P < .05$, $^{**}P < .01$

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).

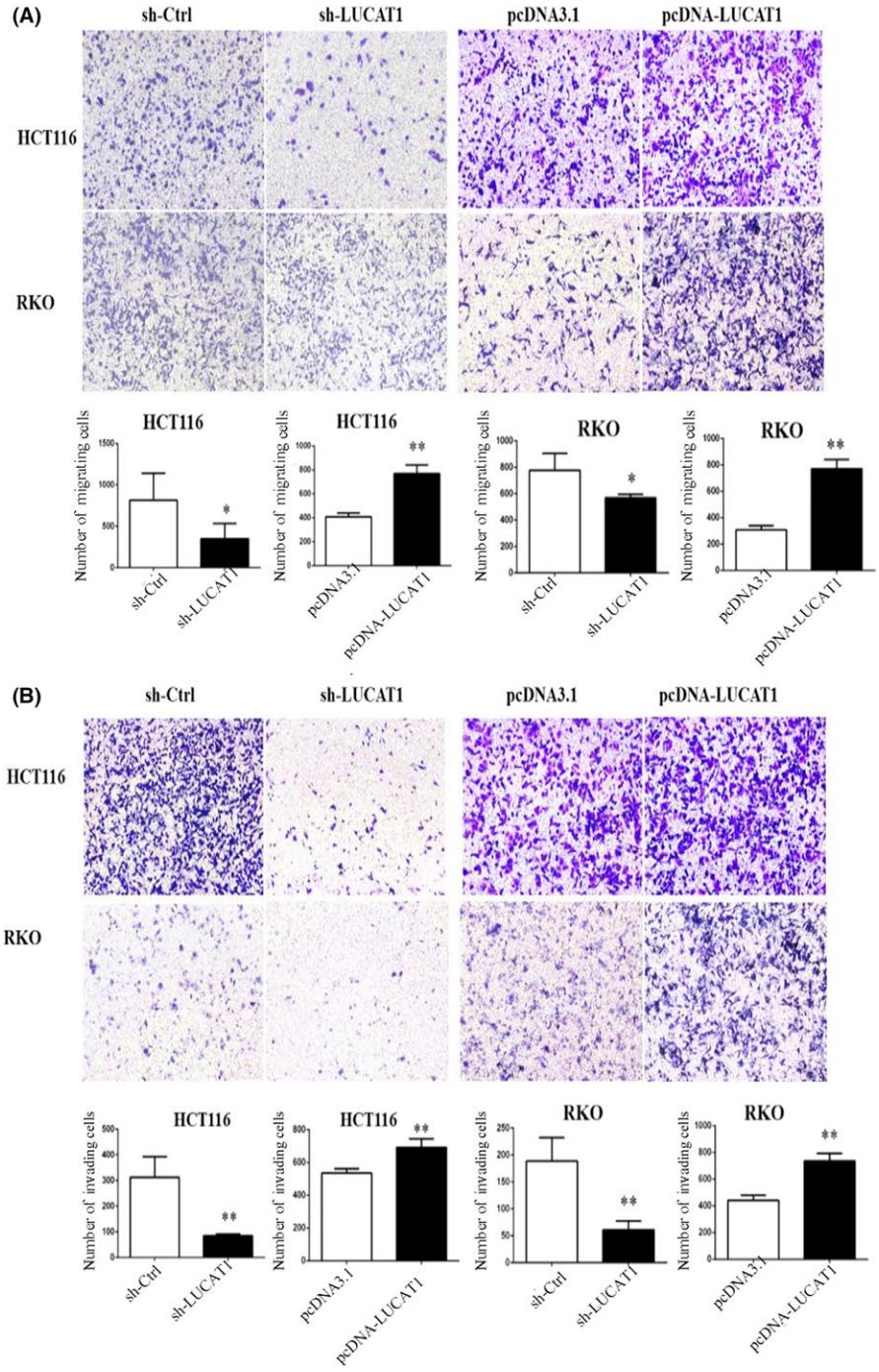


FIGURE 4 Lung cancer-associated transcript 1 (*LUCAT1*) promotes colorectal cancer cell invasion in vitro. Transwell assays were used to detect the migration (A) and invasion (B) of *LUCAT1*-knockdown and overexpressing cells after 36-48 h. Data represent mean ± SEM from 3 independent experiments. $^{*}P < .05$, $^{**}P < .01$

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

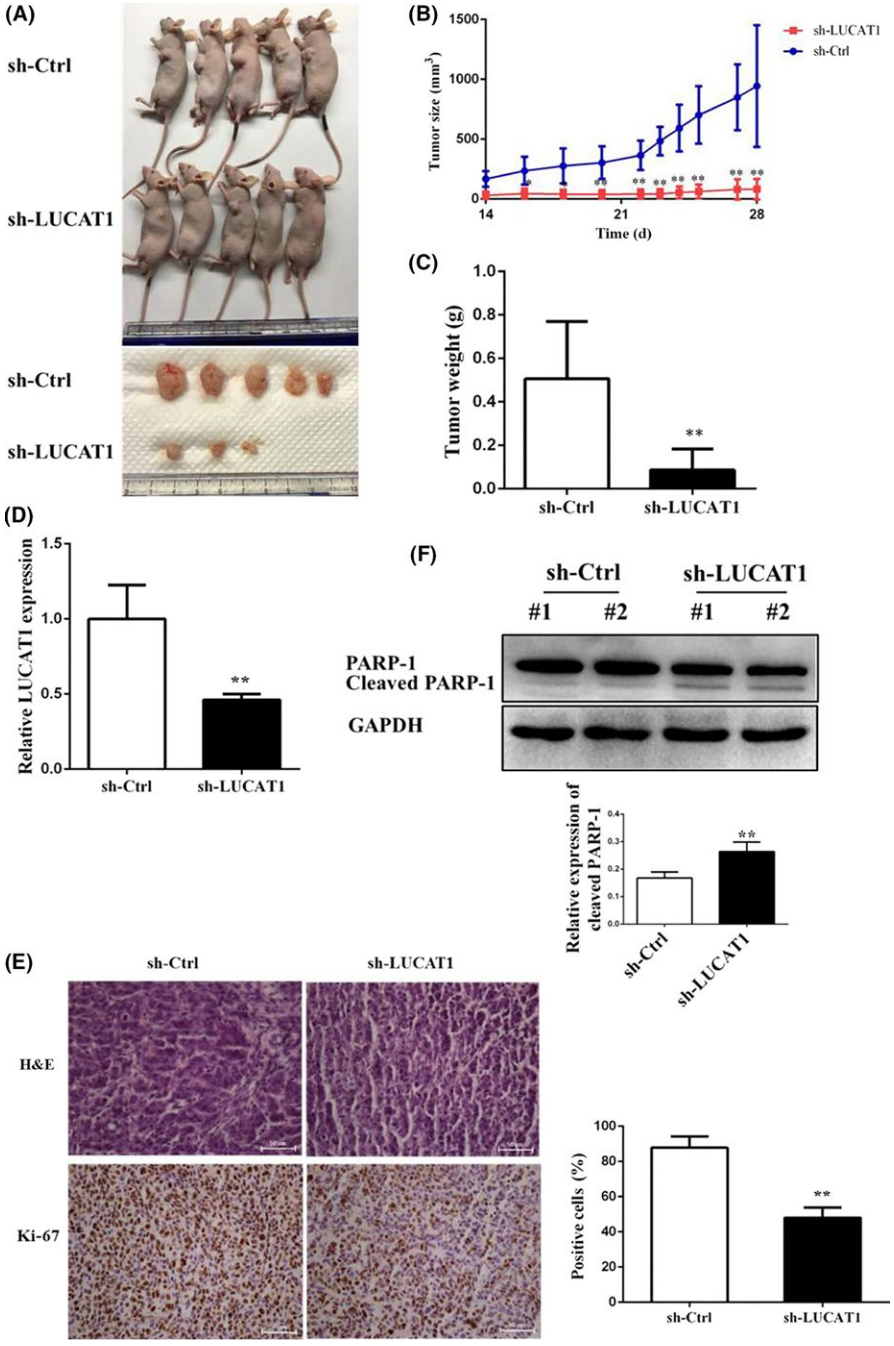


FIGURE 5 Lung cancer-associated transcript 1 (*LUCAT1*) accelerated the proliferation of colorectal cancer cells in vivo. A, Images of xenograft-transplanted nude mouse models (n = 5) and dissected tumors 4 wk after s.c. injection with stable *LUCAT1*-knockdown HCT116 cells (sh-LUCAT1) or control HCT116 cells (sh-Ctrl). B, Tumor size was calculated as the length × width^2 × 0.5 every 2-3 d after 2 wk of injection. C, Weights of xenograft tumors were measured. D, RT-quantitative PCR analysis of *LUCAT1* expression in xenograft tumor tissues. E, H&E staining and immunohistochemistry for Ki-67 of xenograft tumors. Original magnification, ×200. F, Western blot of cleaved poly(ADP-ribose) polymerase (PARP-1) in xenograft tumors. Error bars indicate means ± SEM. $^*P < 0.05$, $^{**}P < 0.01$

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.[13] Previous studies have shown that RPs can regulate p53 stability through the RP-MDM2-p53 pathway responsible

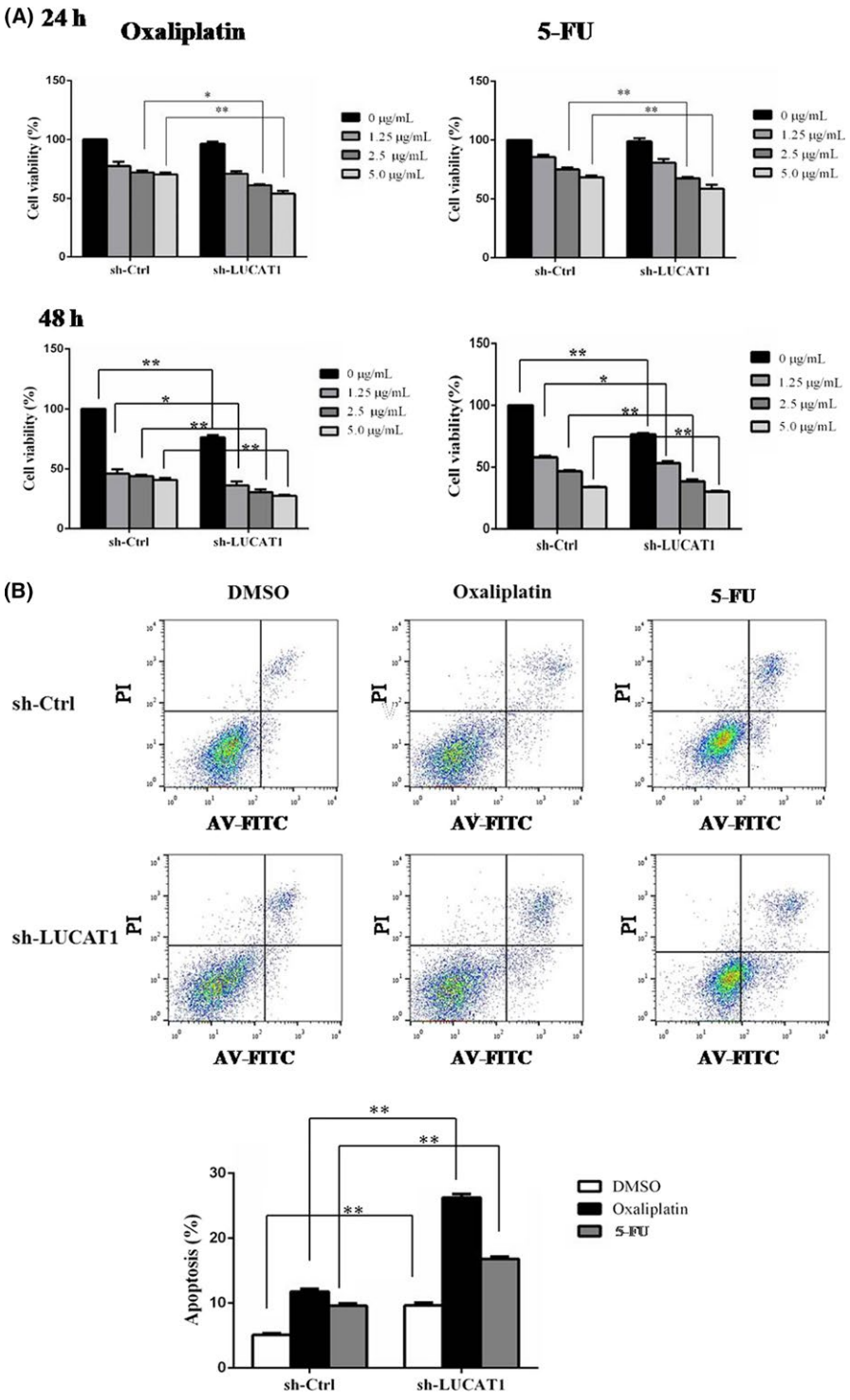


FIGURE 6 Lung cancer-associated transcript 1 (*LUCAT1*) knockdown hypersensitizes colorectal cancer cells to chemotherapeutic drugs. A, Stable *LUCAT1*-knockdown HCT116 cells (sh-LUCAT1) and control HCT116 cells (sh-Ctrl) were exposed to various concentrations (0, 1.25, 2.5, and 5.0 μg/mL) of oxaliplatin (left panel) and 5-fluorouracil (5-FU) (right panel) for 24 or 48 h. Cell viability was measured by CCK-8 assay. B, Cells treated with 5 μg/mL oxaliplatin or 5-FU for 48 h were evaluated by flow cytometry for apoptotic cells. Error bars indicate means ± SEM. **P* < .05, ***P* < .01. AV, annexin V; PI, propidium iodide

for ribosomal stress.[14] 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.[15] As illustrated in Figure 9B, overexpression of *LUCAT1* compromised p53 and MDM2 expression initially induced by 5-FU treatment.

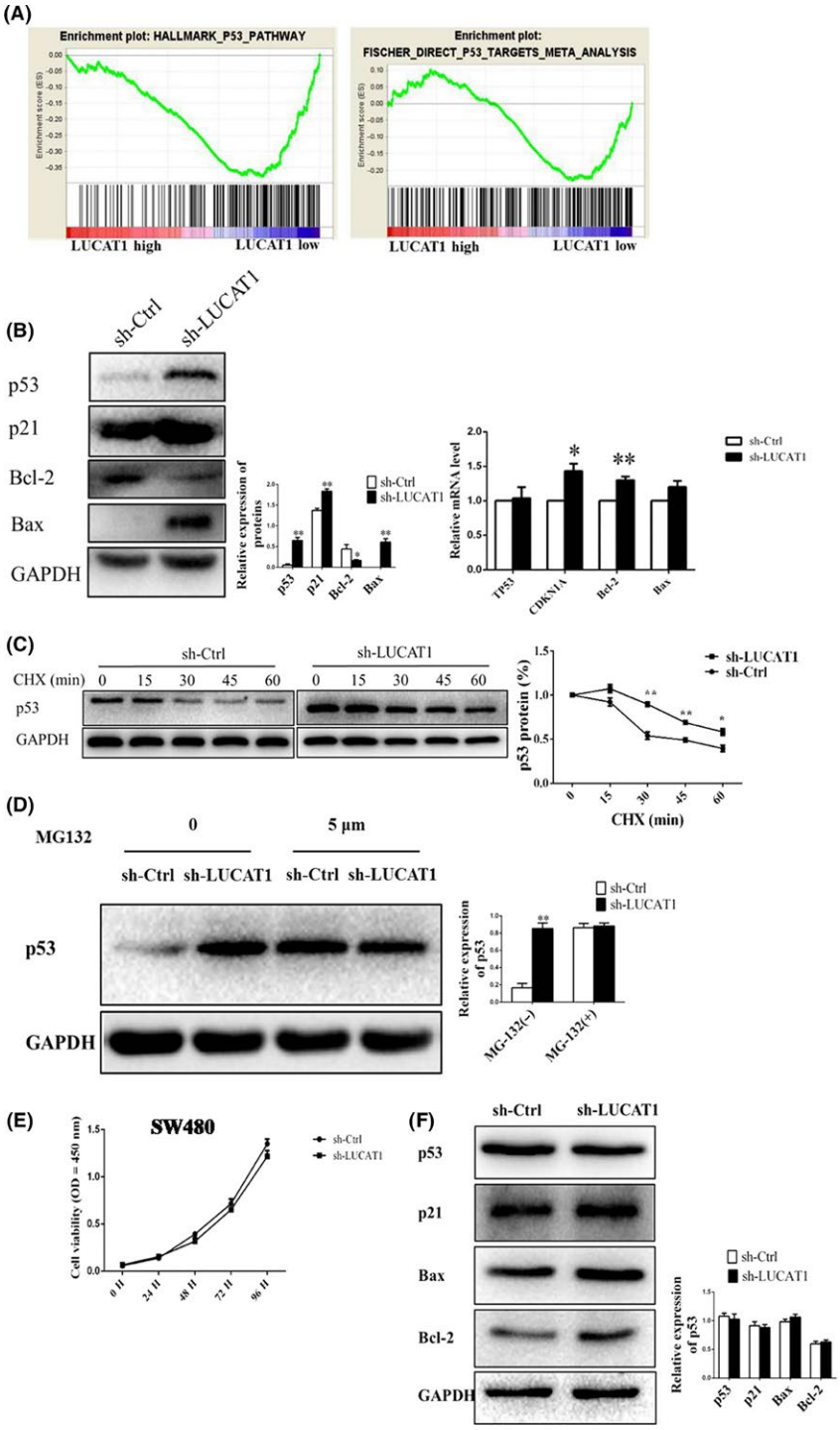
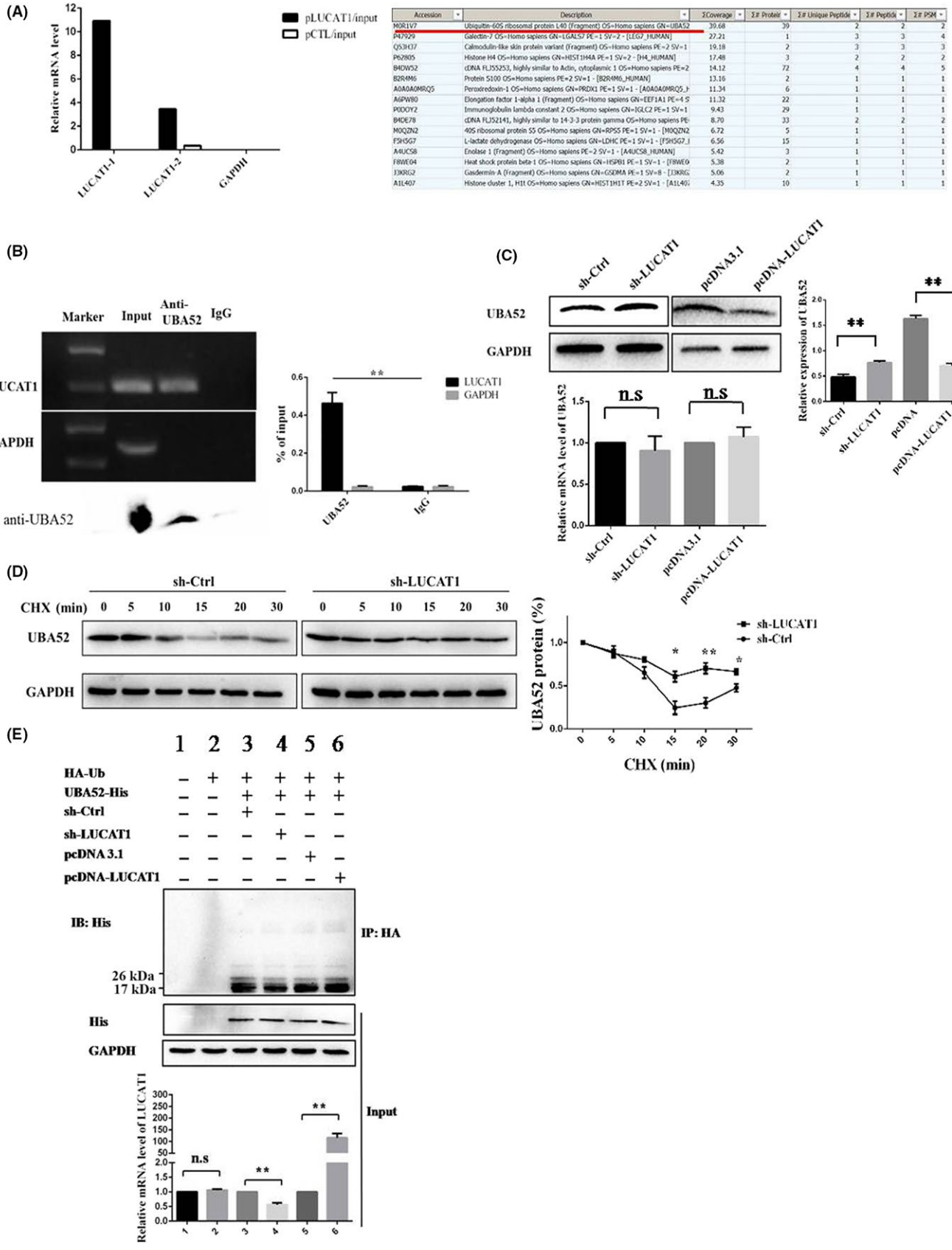


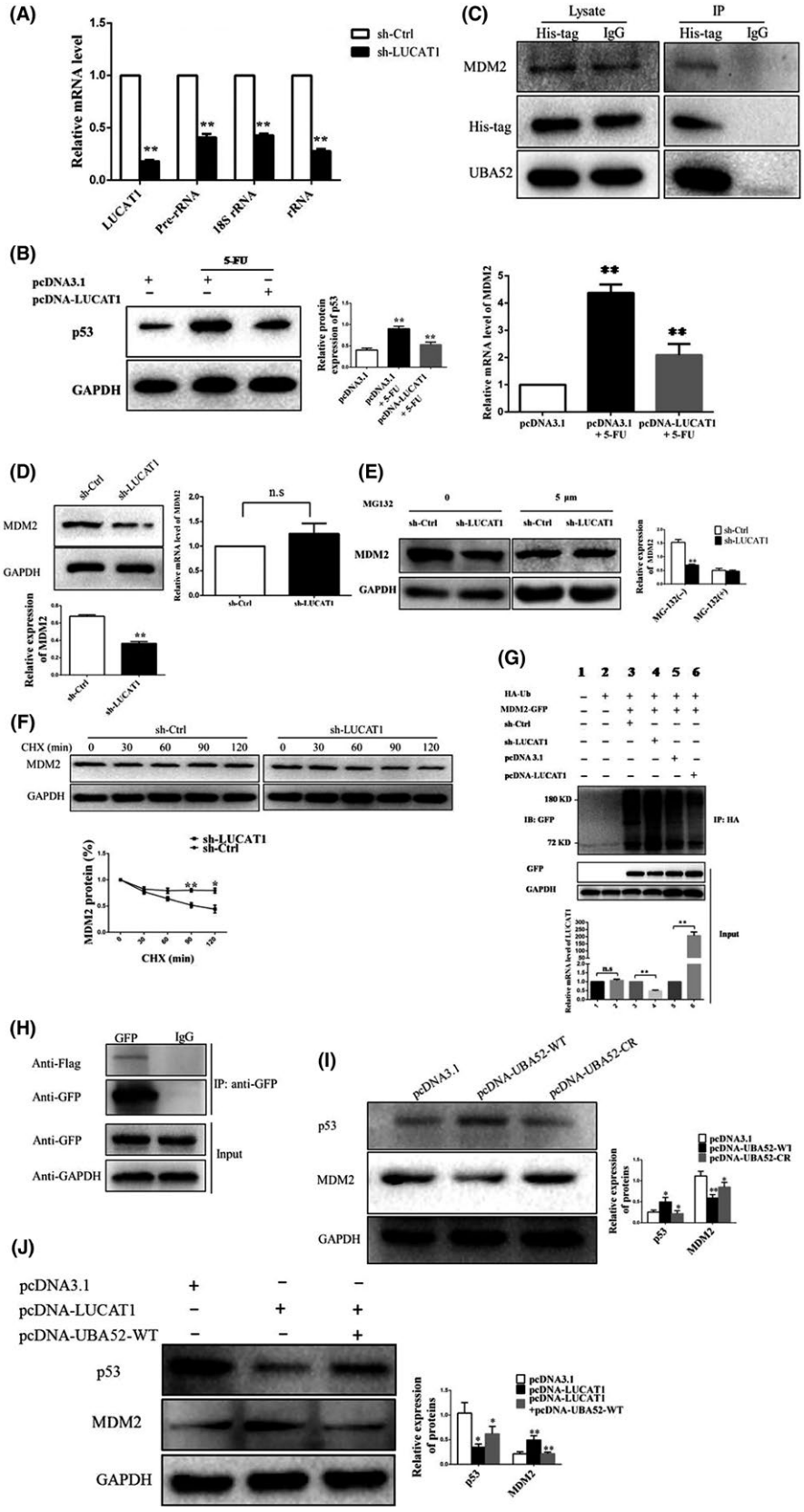
FIGURE 7 Low expression of lung cancer-associated transcript 1 (*LUCAT1*) is correlated with the p53 pathway and its target genes. A, Gene Set Enrichment Analysis (GSEA) indicated a significant correlation between low expression of *LUCAT1* and the p53 pathway and its target genes. B, Expression levels 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



Accession	Description	Coverage	# Protein	# Unique Peptide	# Peptide	# PSM
M0R1V7	Ubiquitin-60S ribosomal protein L40 (Fragment) OS=Homo sapiens GN=UBA52	39.68	39	2	2	2
P47929	Galectin-7 OS=Homo sapiens GN=LGALS7 PE=1 SV=2 - [LEG7_HUMAN]	27.21	1	3	3	4
Q53HJ7	Calmodulin-like skin protein variant (Fragment) OS=Homo sapiens PE=2 SV=1	19.18	2	3	3	3
P62805	Histone H4 OS=Homo sapiens GN=HIST1H4A PE=1 SV=2 - [H4_HUMAN]	17.48	3	2	2	2
B4DW52	cDNA FLJ55253, highly similar to Actin, cytoplasmic 1 OS=Homo sapiens PE=2	14.12	72	4	4	5
B2R4M6	Protein S100 OS=Homo sapiens PE=2 SV=1 - [B2R4M6_HUMAN]	13.16	2	1	1	1
A0A0A0MRQ5	Peroxiredoxin-1 OS=Homo sapiens GN=PRDX1 PE=1 SV=1 - [A0A0A0MRQ5_H	11.34	6	1	1	1
A6PW80	Elongation factor 1-alpha 1 (Fragment) OS=Homo sapiens GN=EEF1A1 PE=4 S	11.32	22	1	1	1
P0DOY2	Immunoglobulin lambda constant 2 OS=Homo sapiens GN=IGLC2 PE=1 SV=1	9.43	29	1	1	1
B4DE78	cDNA FLJ52141, highly similar to 14-3-3 protein gamma OS=Homo sapiens PE	8.70	33	2	2	2
M0QZN2	40S ribosomal protein S5 OS=Homo sapiens GN=RPS5 PE=1 SV=1 - [M0QZN2	6.72	5	1	1	1
F5H5G7	L-lactate dehydrogenase OS=Homo sapiens GN=LDHC PE=1 SV=1 - [F5H5G7_I	6.56	15	1	1	1
A4UCS8	Enolase 1 (Fragment) OS=Homo sapiens PE=2 SV=1 - [A4UCS8_HUMAN]	5.42	3	1	1	1
F8WE04	Heat shock protein beta-1 OS=Homo sapiens GN=HSPB1 PE=1 SV=1 - [F8WE0	5.38	2	1	1	1
I3XRG2	Gasdermin-A (Fragment) OS=Homo sapiens GN=GSDMA PE=1 SV=8 - [I3XRG	5.06	2	1	1	1
A1L407	Histone cluster 1, H1t OS=Homo sapiens GN=HIST1H1T PE=2 SV=1 - [A1L40	4.35	10	1	1	1

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

FIGURE 9 Knockdown of lung cancer-associated transcript 1 (*LUCAT1*) promotes the ribosomal protein L40 (RPL40)-MDM2-p53 pathway. A, Expression of rRNA, 18S RNA, and pre-rRNA detected with RT-quantitative PCR (qPCR). B, *LUCAT1*-overexpressing HCT116 were treated with 5-fluorouracil (5-FU) for 18 h and examined by western blotting with anti-p53 Ab and by RT-qPCR with specific primers. C, HCT116 cells were transfected with His-tagged *UBA52* plasmid for 48 h, immunoprecipitated (IP) with anti-His or control IgG, and detected by western blotting with the indicated Abs. D, Western blotting and RT-qPCR were used to detect MDM2 expression in *LUCAT1* knocked-down HCT116 cells. E, *LUCAT1*-knockdown HCT116 cells were treated with MG132 for 12 h and examined by western blotting with anti-MDM2 Ab. F, HCT116 cells with sh-LUCAT1 or control (sh-Ctrl) were treated with 200 μg/mL cycloheximide (CHX) and harvested at different time points as indicated. MDM2 was detected by western blotting and quantified by densitometry. G, HCT116 cells were transfected with pcDNA-MDM2-GFP in combination with *LUCAT1* siRNA or pcDNA-LUCAT1 in the presence of the HA-ubiquitin (HA-Ub) plasmid as indicated. 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 anti-GFP Ab. *LUCAT1* expression level was detected by RT-qPCR. H, HCT116 cells were co-transfected with pcDNA-UBA52 and pcDNA-MDM2 for 48 h, and immunoprecipitated with anti-GFP or control IgG and detected by western blotting with anti-FLAG. I, Western blotting was used to detect p53, MDM2, and ubiquitin expression in UBA52 WT or cleavage-resistant UBA52 (UBA52-CR)-expressing HCT116 cells. J, HCT116 cells were transfected with *LUCAT1* and *UBA52* plasmids to detect MDM2 and p53 expression by western blotting. $^{*}P < .05$, $^{**}P < .01$. n.s, not significant

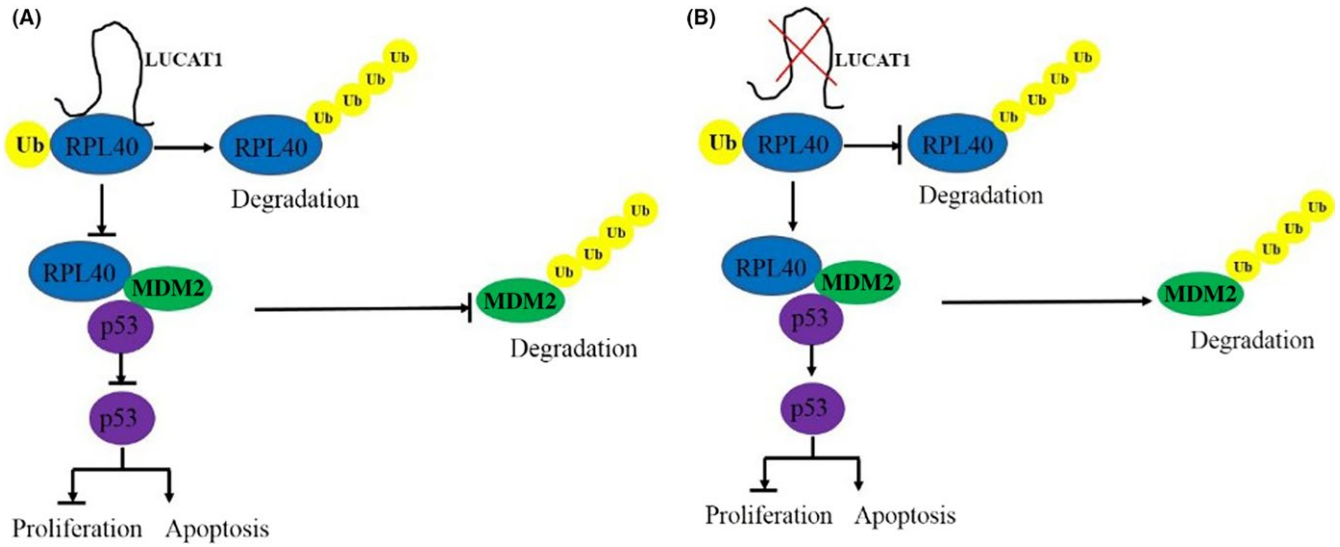


FIGURE 10 Proposed model of lung cancer-associated transcript 1 (*LUCAT1*) regulation of the ribosomal protein L40 (RPL40)-MDM2-p53 pathway. A, Effects of *LUCAT1* on UBA52 (Ub) and MDM2 and p53 expression. B, Effects of *LUCAT1*-knockdown on UBA52 and the RPL40-MDM2-p53 pathway

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.[16] In addition to lung cancer, *LUCAT1* has also been reported to be involved in esophageal cancer, renal cell carcinoma, and cisplatin-resistant ovarian cancer.[17-21] Like other lncRNAs, *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).[17] *LUCAT1* inhibits the expression of tumor suppressors in esophageal cancer through DNA methylation, by regulating the stability of the DNA methyltransferase DNMT1.[18] *LUCAT1* participates in methotrexate resistance regulation through the miR-200c-ABCB1 pathway.[22] *LUCAT1* has also been reported as a liver metastasis-associated lncRNA through an analysis of CRC tissues from TCGA and Gene Expression Omnibus (GEO) databases;[21] however, the underlying mechanisms are still unclear. Consistent with other studies, in which some lncRNAs including *LUCAT1* are associated with chemotherapy resistance,[19,23-25] 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 lncRNAs can participate in carcinogenesis through interactions with specific proteins. For instance, lncRNA-hPVT1 promotes cell proliferation, cell cycling, and stem cell-like properties in hepatocellular carcinoma cells through binding to NOP2 and enhancing its stability.[26] *P53RRA* lncRNA induces cell cycle arrest, apoptosis, and ferroptosis by binding with RAS GTPase-activating protein-binding protein 1, resulting in p53 retention in the nucleus.[27] 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.[13] 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.[28] p53 is largely post-translationally regulated by MDM2, which is an E3 ubiquitin ligase that binds p53 to promote its degradation.[29] There are some regulatory factors that act on MDM2-p53. For example, $p19^{ARF}$ inhibits MDM2-mediated p53 ubiquitination and degradation by binding with MDM2.[30] 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.[29,31-35] This is the so-called RP-MDM2-p53 pathway, which responds to ribosomal stress or nucleolar stress.[29] Recent studies found that the RP-MDM2-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-MDM2-p53 pathway.[36]

In the present study, we found that RPL40, cleaved from UBA52 as a ribosomal protein, is involved in the RP-MDM2-p53 pathway by interacting with MDM2, and therefore causing the accumulation of p53. This RPL40-MDM2-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-MDM2-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.

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SUPPORTING INFORMATION

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

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