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RESEARCH ARTICLE

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ABHD6 suppresses colorectal cancer progression via AKT signaling pathway

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Abstract

Colorectal cancer (CRC) continues to be a prevalent malignancy, posing a significant risk to human health. The involvement of alpha/beta hydrolase domain 6 (ABHD6), a serine hydrolase family member, in CRC development was suggested by our analysis of clinical data. However, the role of ABHD6 in CRC remains unclear. This study seeks to elucidate the clinical relevance, biological function, and potential molecular mechanisms of ABHD6 in CRC. We investigated the role of ABHD6 in clinical settings, conducting proliferation, migration, and cell cycle assays. To determine the influence of ABHD6 expression levels on Oxaliplatin sensitivity, we also performed apoptosis assays. RNA sequencing and KEGG analysis were utilized to uncover the potential molecular mechanisms of ABHD6. Furthermore, we validated its expression levels using Western blot and reactive oxygen species (ROS) detection assays. Our results demonstrated that ABHD6 expression in CRC tissues was notably lower compared to adjacent normal tissues. This low expression correlated with a poorer prognosis for CRC patients. Moreover, ABHD6 overexpression impeded CRC cell proliferation and migration while inducing G0/G1 cell cycle arrest. In vivo experiments revealed that downregulation of ABHD6 resulted in an increase in tumor weight and volume. Mechanistically, ABHD6 overexpression inhibited the activation of the AKT signaling pathway and decreased ROS levels in CRC cells, suggesting the role of ABHD6 in CRC progression via the AKT signaling pathway. Our findings demonstrate that ABHD6 functions as a tumor suppressor, primarily by inhibiting the AKT signaling pathway. This role establishes ABHD6 as a promising prognostic biomarker and a potential therapeutic target for CRC patients.

KEYWORDS

ABHD6, AKT signaling, clorectal cancer

1 | INTRODUCTION

Colorectal cancer (CRC) remains one of the most common cancers globally, ranking third in incidence among all tumors. Changes in lifestyle and dietary habits, such as increased consumption of animal-based foods and a more sedentary lifestyle, have led to decreased physical activity and increased body weight, resulting in an increased incidence of CRC.[1] CRC is also a leading cause of cancer-related deaths, accounting for 10% of cancer deaths in women and 9.4% in men.[2] Therefore,

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CRC seriously endangered human health and caused a heavy economic burden of disease.

Monoacylglycerol lipase (MAGL), a serine hydrolase that plays a crucial role in catalyzing the hydrolysis of monoglycerides, is reported as a potential therapeutic target for the treatment of several neurodegenerative diseases.[3] Also, MAGL is highly expressed in various cancers,[4–6] and promotes tumor migration and growth by increasing free fatty acid levels.[7,8] In CRC, the knockdown of MAGL manipulates tumor cell proliferation and apoptosis via the downregulation of Cyclin D1 and Bcl-2,[9] and the MAGL inhibitor URB602 can reduce the xenograft tumor volume, by downregulating the VEGF, FGF-2 and cyclin D1.[4] In contrast, alpha/beta hydrolase domain 6 (ABHD6) is a member of the serine hydrolase family that has the same function as MAGL in degrading 2-AG. However, the exact roles of ABHD6 in 2-AG metabolism and signaling are still unclear.[5,10]

ABHD6 is a newly discovered post-genomic protein and was identified as a bona fide member of the endocannabinoid signaling system.[11,12] Until now, some studies have focused on the role and potential therapeutic target of ABHD6 in demyelinating disease,[13,14] inflammatory processes,[15] physiological processes,[16,17] and even the regulation of bladder function.[18] However, the role of ABHD6 in tumors, unlike MAGL, remains controversial and was found differentially expressed among various tumor cell lines.[19] It is reported that ABHD6 is highly expressed in Ewing family tumors (EFT) but knockdown of it did not inhibit the growth of EFT cells.[20] However, in non-small cell lung cancer (NSCLC), ABHD6 was associated with a negative prognosis and advanced tumor stage.[21] Conversely, ABHD6 could be a potential anti-oncogene in hepatocellular carcinoma.[22]

This study aimed to explore the expression and role of ABHD6 in CRC. In this study, we identified that the expression of ABHD6 is lower in colorectal cancer compared with normal tissues. The results indicate that low expression of ABHD6 is associated with lymph node metastasis and a worse prognosis in colorectal cancer. ABHD6 silencing limits CRC development by suppressing proliferation, migration, and affecting the cell cycle. Downregulation of ABHD6 significantly induces intracellular reactive oxygen species (ROS) accumulation and activates AKT signaling.

2 | MATERIALS AND METHODS

2.1 | Data acquisition and processing

The expression matrix of CRC samples and corresponding normal samples were obtained from The Cancer Genome Atlas (TCGA) database. Overall survival (OS) and disease-specific survival (DSS) analyses were conducted using the R survival package (v 3.3.1). The cProSite (https://cprosite.ccr.cancer.gov/) was used to evaluate the protein expression levels of ABHD6 in CRC.

2.2 | Cell culture and reagents

Human CRC cell lines SW480, RKO, DLD1, and HT29 were obtained from the Cell Bank of the Chinese Academy of Science (Shanghai, China). RKO cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco), while SW480, DLD1, and HT29 were cultured in RPMI-1640 medium (Gibco). All culture media were supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% penicillin and streptomycin (PS; Gibco). The cells were incubated at 37°C in a humidified chamber containing 5% CO2.

2.3 | Lentivirus and viral infections

The lentiviruses for knockdown and overexpression of ABHD6 were purchased from GeneChem (Shanghai, China). Empty vector lentiviruses expressing GFP were used as NCs only. The short hairpin RNA (shRNA) sequences were as follows: sh-ABHD6 #1, CCGCATC CCTCATAACAACTT; sh-ABHD6 #2, CCTGGCATTTGTGGCTTCATT; sh-ABHD6 #3, CCTTCCAAAGAACCTGCACTT; sh-ABHD6 #4 GCAG TACTCAACTGACAATCA; sh-ABHD6 #5, GCATGCAAGTCCGCTA TGTTC. Cells were infected with lentivirus particles containing polybrene and selected with puromycin. On the third day after transfection, the cells were harvested and the transfection efficiency was evaluated by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and western blotting.

2.4 | qRT-PCR

Total RNA Kit (Tiangen, China) was utilized to obtain total RNA from CRC cells according to the manufacturer's protocol, and CFX96 Touch Real-time PCR System (BioRad) was used to conduct the qRT-PCR. The expression of the target transcript was normalized to that of GAPDH through the ΔΔCT method. The primers were as follows: ABHD6 forward: 5′-TGGTACTGGCGGAGGACATTGG-3′, ABHD6 reverse: 5′-GAGCATGAGGATGGAGGGTTTGTG-3′, GAPDH forward: 5′-GGAGCGAGAT CCCTCCAAAAT-3′, and GAPDH reverse: 5′-GGCT GTTGTCATACTTCTCATGG-3′.

2.5 | Western blotting

Total protein was extracted from the samples using lysis buffer (P0013; Beyotime BioTech Inst) with protease and phosphatase inhibitors. Equal amounts of protein were separated by 10%

SDS-PAGE (EpiZyme Biotechnology) and transferred onto polyvinylidene difluoride membranes (Millipore). The membranes were blocked with 5% non-fat milk for 1 h at room temperature and then incubated with the following antibodies overnight at 4°C: anti-ABHD6 (1:500; 20494-1-AP; Proteintech), anti-AKT (1:500; ab179463; Abcam), anti-phosphorylated AKT(S472 + S473 + S474) (1:500; ab192623; Abcam), anti-phosphorylated AKT(Thr308) (1:500; 13038 T; Cell Signaling Technology), GAPDH (1:1,000; 2118 S; Cell Signaling Technology). The membranes were subsequently incubated with an anti-rabbit secondary antibody (1:5000; ZB-2306; ZSGB-BIO), and protein signals were visualized by enhanced chemiluminescence detection (Mei5Bio) with a Bio-Rad gel imaging system (Bio-Rad).

2.6 | Cell proliferation assay

Cell proliferation was assessed by Cell Counting Kit-8 (CCK-8) assay and colony formation assay. For CCK-8 (Dojindo) assay, cells were seeded at 2000 cells/well into 96-well plates with 100ul culture medium. 10 μl CCK-8 solution was added to the cells at specific time points. After 2 h of incubation at 37°C and with a 5% CO_2 atmosphere, the reaction product was quantified by spectrophotometry according to the absorbance at 450 nm by a microplate reader (Sunrise, Tecan, Switzerland). For colony formation assays, cells were seeded into 6-well plates at a density of 1000 cells/well and cultured at 37°C in a humidified 5% CO_2 atmosphere for 10–14 days. The colonies formed were fixed with methyl alcohol and stained

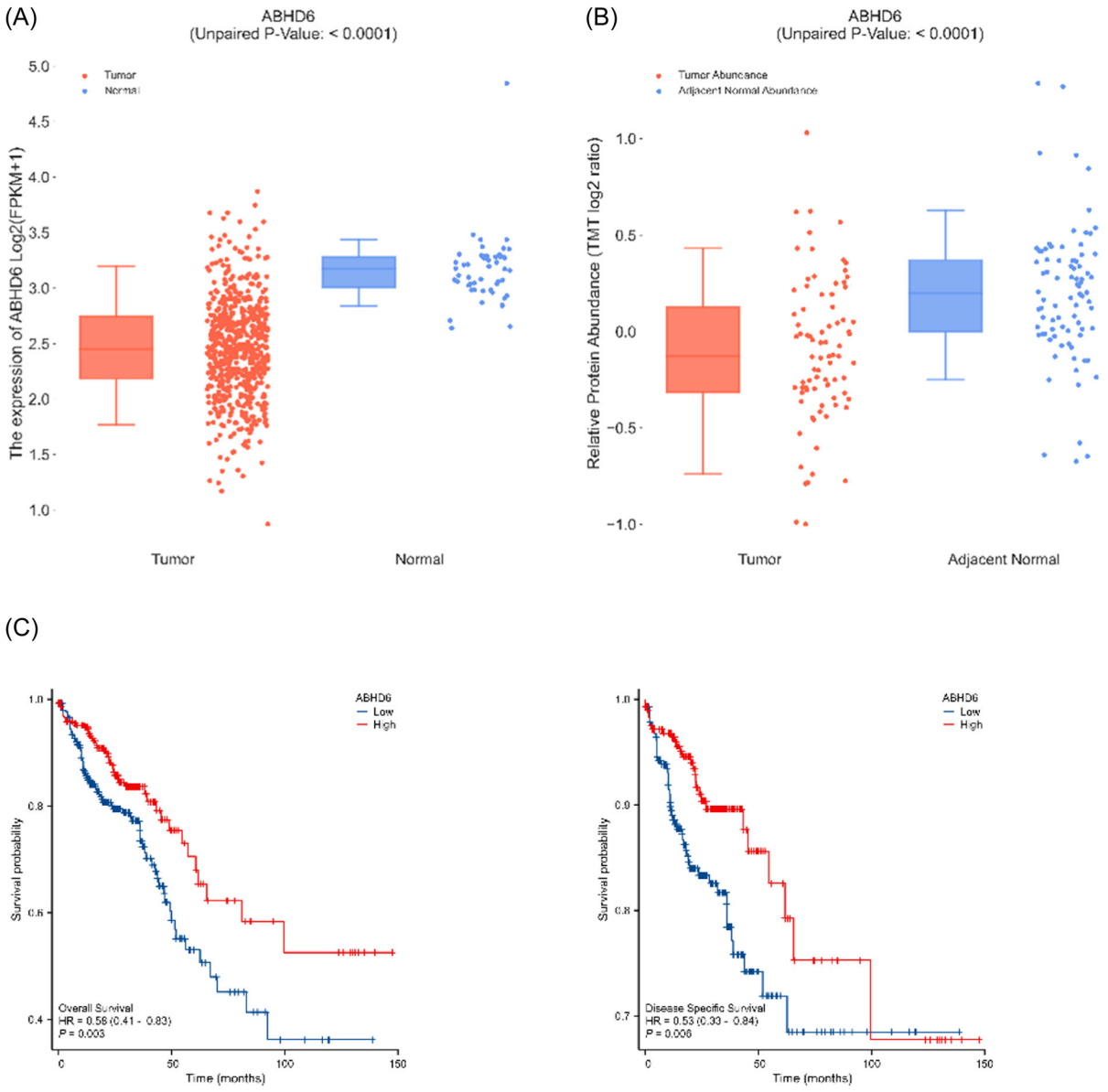

FIGURE 1 ABHD6 was under-expressed in CRC tissues and correlated with the poor prognosis of CRC patients. (A) Analysis of ABHD6 mRNA expression in CRC ($N = 619$) and normal tissues ($N = 51$) from TCGA database. (B) Analysis of ABHD6 protein expression in colon cancer tissues and paired adjacent normal tissues from cProSite database ($N = 96$). (C) Kaplan-Meier analysis showed the correlation of low expression of ABHD6 with poor OS and DSS in CRC patients. [Color figure can be viewed at wileyonlinelibrary.com]

with 0.1% crystal violet for 20 min. The number of colonies was counted manually. All experiments were performed in triplicate.

2.7 | Transwell migration assay

Cell migration assay was performed using a 24-well plate with an 8.0 μm pore polycarbonate filter (Corning Costar, Corning). The lower chamber was filled with 600 μl RPMI-1640 supplemented with 20% FBS. CRC cells (1×10^5 cells/well) were suspended in 200 μl of serum-free medium and were added to the upper chamber. After incubation for 36–48 h, the migrated CRC cells were fixed with methanol, stained with 0.1% crystal violet solution, and counted. Five random fields were observed by microscopy, and the average number of cells in the five fields was used for quantitative analysis. To investigate the dependence of ABHD6-mediated suppression of CRC proliferation and migration on Akt signaling, we treated SW480 sh-ABHD6 #1 cells with an Akt inhibitor Capivasertib (Synonyms: AZD5363) (MedChem Express, CAS No:1143532-39-1) at a concentration of 2.5 μM.

2.8 | Flow cytometric analysis of the cell cycle and apoptosis analysis

For cell-cycle studies, transfected cells and corresponding control cells were seeded in 6-well plates and cultured for 24 h. After washing with PBS, the cells were digested using 0.25% trypsin and then collected and washed two times with 1 ml PBS. Cells (1×10^6) were stained by adding 1 ml DNA staining solution and 10 μL permeabilization solution according to the manufacturer's protocol. Mix them by vortex for 5–10 s and incubate at room temperature in the dark for 30 min. The distribution of cell-cycle phases with different DNA contents was determined by flow cytometry (LSRFortessa; BD Biosciences) and analyzed using FlowJo analysis software (v.10.8.1). Cells were treated with the Oxaliplatin for 36 h and the apoptosis analysis was carried out by flow cytometry (Cytoflex S, Beckman) using the PE Annexin V Apoptosis Detection Kit (559763, BD Biosciences). The percentages of apoptotic cells were analyzed using FlowJo analysis software (v.10.8.1). Experiments were repeated three times.

2.9 | ROS measurement

For cellular ROS detection, cells were incubated in a humidified chamber at 37°C with 5% CO2 for 45 min with Cellular Reactive Oxygen Species Detection Assay Kit (Deep Red) (Abcam, ab186029) according to the manufacturer's protocol. After incubation, the fluorescence signal from the APC channel was monitored by flow cytometry (Cytoflex S, Beckman) within 1 h. The mean fluorescent intensity was analyzed using FlowJo analysis software (v.10.8.1).

TABLE 1 Relationship between ABHD6 expression levels and clinicopathologic characteristics in CRC patients.

Characteristics	Low expression of ABHD6 (*N* = 309)	High expression of ABHD6 (*N* = 310)	*p*
Age, n (%)			0.835
<= 65	133 (21.5%)	136 (22%)	
> 65	176 (28.4%)	174 (28.1%)	
Gender, n (%)			0.715
Male	167 (27%)	163 (26.3%)	
Female	142 (22.9%)	147 (23.7%)	
Pathologic T stage, n (%)			0.279
T1&T2	57 (9.2%)	68 (11%)	
T3&T4	251 (40.7%)	241 (39.1%)	
Pathologic N stage, n (%)			**0.004**
N0	157 (25.5%)	194 (31.5%)	
N1&N2	150 (24.4%)	115 (18.7%)	
Pathologic M stage, n (%)			0.095
M0	219 (40.1%)	240 (44%)	
M1	50 (9.2%)	37 (6.8%)	
Pathologic stage, n (%)			**0.005**
Stage I &Stage II	149 (24.9%)	183 (30.6%)	
Stage III & Stage IV	151 (25.2%)	116 (19.4%)	
Histological type, n (%)			**0.003**
Adenocarcinoma	257 (42.3%)	276 (45.4%)	
Mucinous adenocarcinoma	50 (8.2%)	25 (4.1%)	
Perineural invasion, n (%)			0.122
No	95 (40.9%)	77 (33.2%)	
Yes	40 (17.2%)	20 (8.6%)	

2.10 | Tumor xenografts

SW480 cells were infected with lentivirus carrying the target genes. 5×10^6 viable SW480 cells in 100 μl PBS were subcutaneously implanted into the right flank of 6-week-old male nude mice, which got from Charles River, Beijing (n = 6 per group). Tumor size was measured every 4 or 5 days using Vernier calipers and the volumes were calculated using the formula length × width^2/2. At the end of the experiment, all the animals were euthanized and included in the analysis. The study was approved by the Animal Care Committee of Peking University.

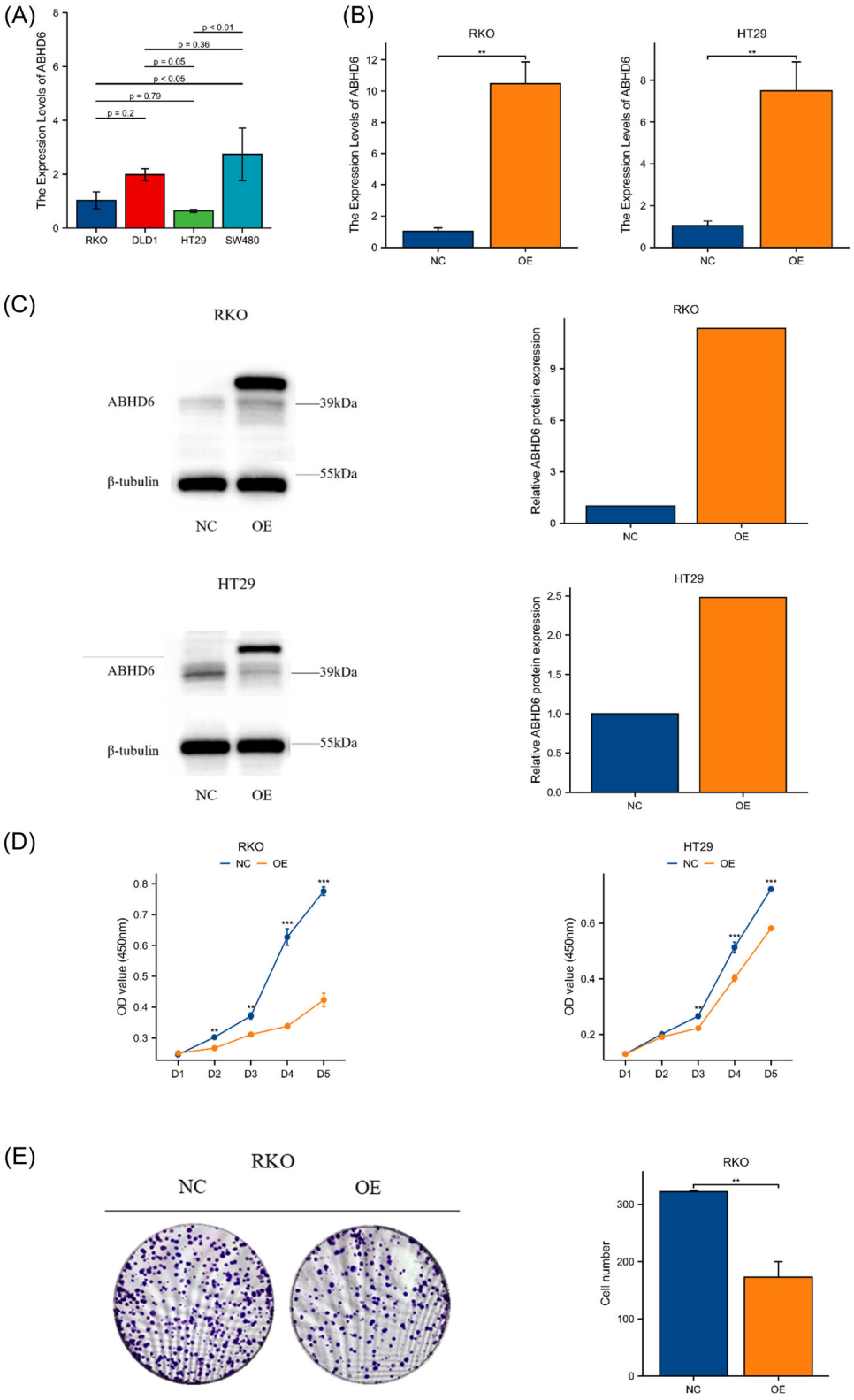

FIGURE 2 Overexpression of ABHD6 inhibits the proliferation, colony formation and migration of CRC cell lines. (A) mRNA expression levels of ABHD6 were evaluated by RT-qPCR in CRC cell lines. (B)RT-qPCR and (C) Western blot analysis of transfection efficiency in RKO and HT29 cell lines. (D) CCK-8 assays of ABHD6 overexpression and control RKO and HT29 cell lines. (E) Colony formation capacity of ABHD6 overexpression and control RKO cell lines. (F) Transwell migration assay in ABHD6 overexpression and control RKO cell lines. $^{**}p < 0.01$, $^{***}p < 0.001$. [Color figure can be viewed at wileyonlinelibrary.com]

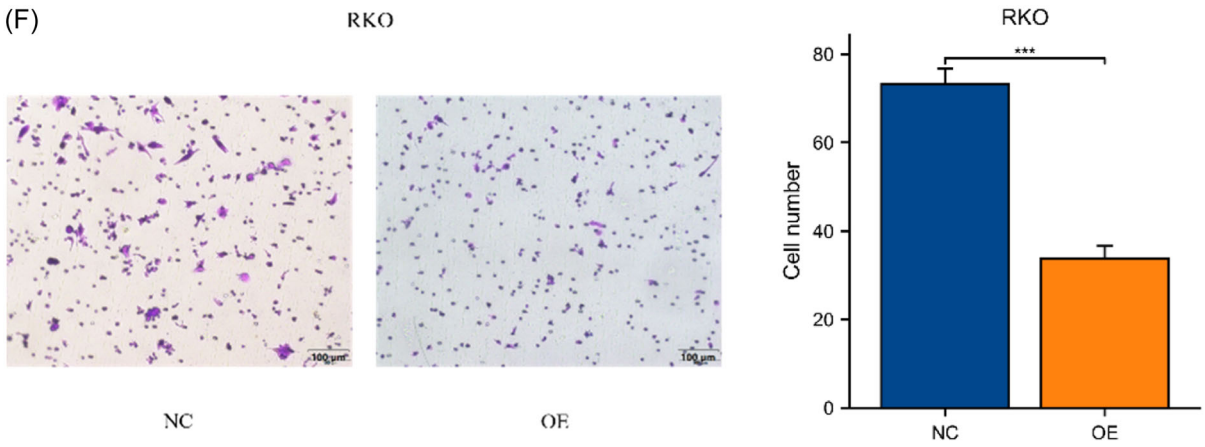

FIGURE 2 (Continued)

2.11 | Statistical analysis

Quantitative variables were analyzed by *t*-test between groups. One-way ANOVA was used for multiple group comparisons. The association between ABHD6 expression and clinicopathological features of CRC patients was assessed by chi-square test. Log-rank test using the Kaplan–Meier method was used to assess patients' survival outcomes. Tumor growth in vivo was compared using two-way repeated-measures analysis of variance. $P < 0{\cdot}05$ was considered statistically significant.

3 | RESULTS

3.1 | ABHD6 is downregulated in CRC tissues and associated with poor prognosis in CRC

To investigate the expression of ABHD6 in CRC, we analyzed the TCGA and cProSite databases and found that both ABHD6 mRNA (Figure 1A) and protein expression (Figure 1B) were significantly decreased in CRC tissues compared to normal tissues. Furthermore, we analyzed the relationship between ABHD6 expression and major clinicopathological characteristics. Low ABHD6 expression in CRC tissues was significantly correlated with N stage, pTNM stage, and histological type (Table 1). Additionally, low ABHD6 expression was found to be associated with worse OS and DSS in CRC patients (Figure 1C).

3.2 | ABHD6 overexpression inhibits CRC cell proliferation and migration

To further explore the role of ABHD6 in CRC, we examined ABHD6 expression in CRC cell lines (Figure 2A). Then we exhibited stable ABHD6-overexpressing RKO and HT29 cell lines and confirmed the overexpression of ABHD6 via RT-qPCR (Figure 2B) and western blotting (Figure 2C). Results from CCK-8 and colony formation assays demonstrated that ABHD6 overexpression led to a significant reduction in cell viability (Figure 2D) and colony formation efficiency (Figure 2E). Furthermore, transwell migration assay revealed that ABHD6 overexpression inhibited the migration of RKO cells compared with the control group (Figure 2F).

3.3 | ABHD6 knockdown promotes CRC cell proliferation and migration

We conducted stable ABHD6 knockdown in SW480 and DLD1 cell lines to explore the role of ABHD6 in CRC. The knockdown efficiency of ABHD6 was confirmed by qRT-PCR (Figure 3A) and western blotting (Figure 3B). Subsequently, we selected sh-ABHD6 #1 and sh-ABHD6 #4 for further study based on their high knockdown efficiency. To assess the effect of ABHD6 on the proliferative ability of CRC cells, CCK-8 and colony formation assays were performed. The results demonstrated that ABHD6 knockdown significantly increased the cell viability (Figure 3C) and colony formation efficiency (Figure 3D). To examine the effect of ABHD6 on cell migration, a transwell migration assay was employed. The results demonstrated that ABHD6 knockdown increased the migration of cells compared with the control group (Figure 3E).

3.4 | ABHD6 regulated the CRC cell cycle and susceptibility to oxaliplatin

To determine the inhibitory effect of ABHD6 on cell proliferation, we performed a cytometric analysis of the cell cycle. As shown in Figure 4A, ABHD6 silencing led to an increased cell cycle progression to the S phase in CRC cells. Conversely, ABHD6 overexpression arrested CRC cells in the G0/G1 phase (Figure 4B). We then investigated the ability of ABHD6 to affect apoptosis in response to treatment with oxaliplatin. Our results demonstrated that the downregulation of ABHD6 expression increased the sensitivity of cells to oxaliplatin-induced apoptosis (Figure 4C).

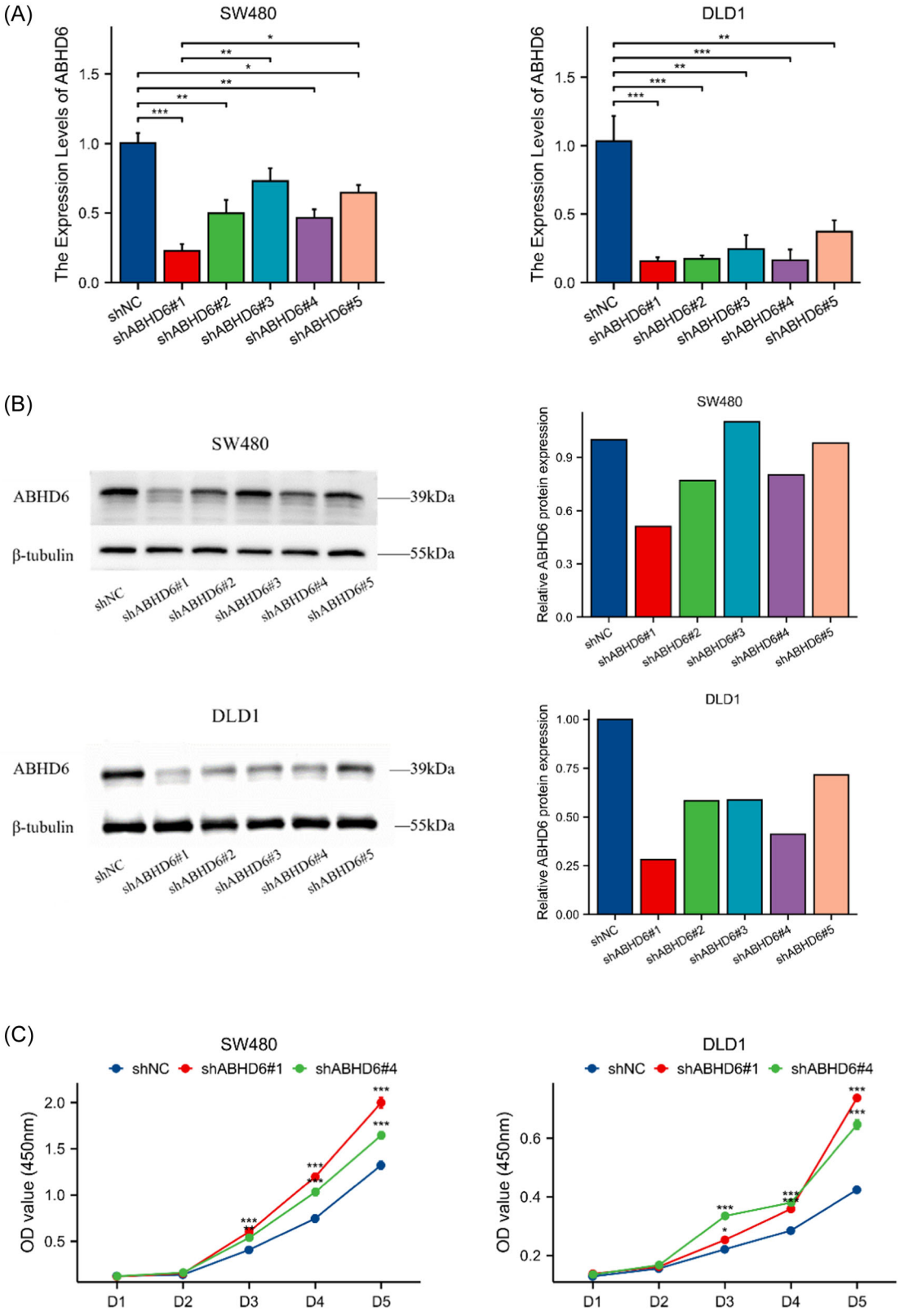

FIGURE 3 Knockdown of ABHD6 promotes the proliferation, colony formation and migration of CRC cell lines. (A) RT-qPCR and (B) Western blot analysis of transfection efficiency of shRNA-ABHD6 in SW480 and DLD1 cell lines. (C) CCK-8 assays of transfected SW480 and DLD-1 cells. (D) Colony formation capacity of transfected SW480. (E) Transwell migration assay in transfected DLD1 cells and control cells. $^{**}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. [Color figure can be viewed at wileyonlinelibrary.com]

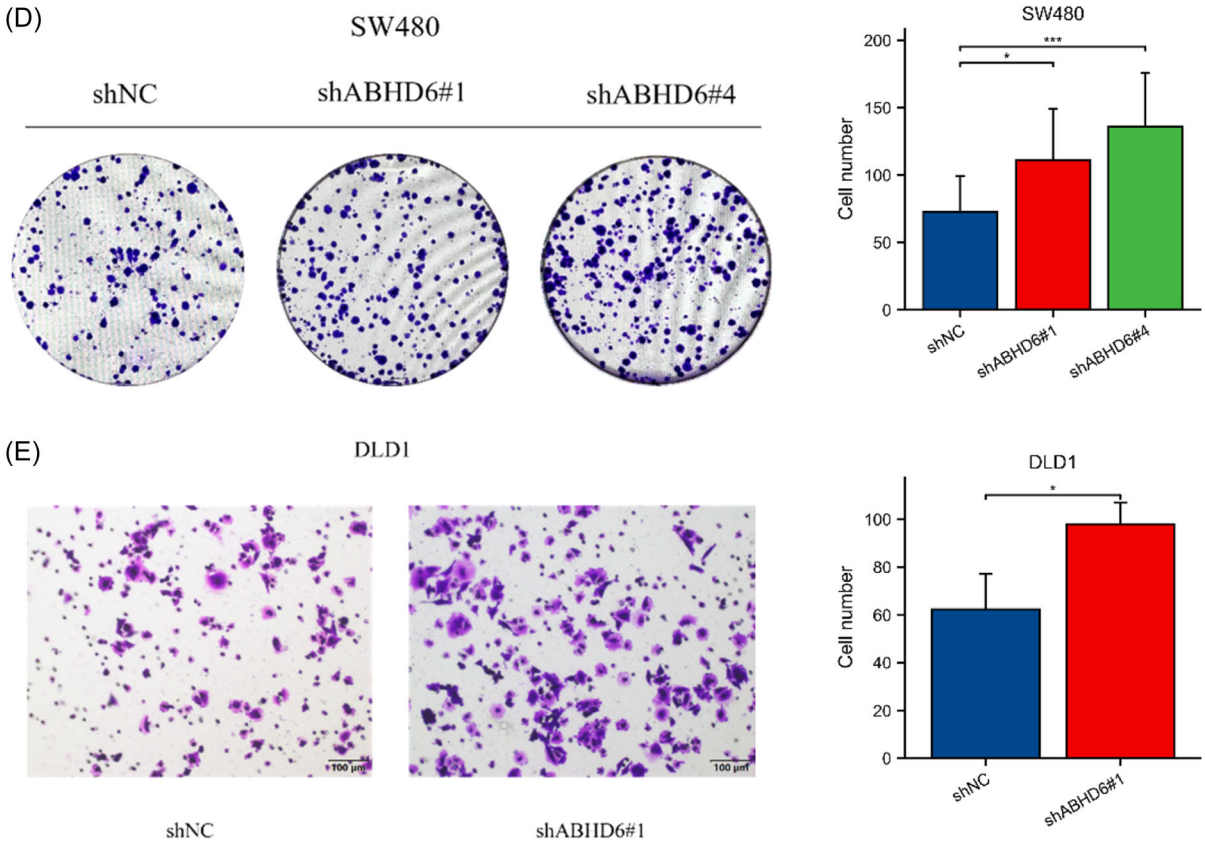

FIGURE 3 (Continued)

3.5 | Knockdown of ABHD6 promotes carcinogenesis of CRC in vivo

We developed a subcutaneous xenograft model to assess the effects of ABHD6 on proliferation and tumorigenesis in vivo. The tumor volume and weight generated by SW480 cells transfected with sh-ABHD6 #1 plasmids was larger than that of the controls (Figure 5A–C). These results indicate that ABDH6 inhibits the progression of CRC by increasing CRC cell proliferation in vivo.

3.6 | ABHD6 may exert biological function in tumor cells by PI3K-AKT and ROS pathway

To further elucidate the mechanisms of ABHD6 play in CRC cells, we performed RNA sequencing on stable transfected SW480 and RKO cell lines. The heatmap analysis revealed the presence of differentially expressed genes (DEGs), and the results indicated that ABHD6 can modulate the mRNA levels of molecules involved in migration and cell cycling (Figure 6A–B). Furthermore, our KEGG analysis results suggest that ABHD6 is involved in various signaling pathways associated with the PI3K/Akt signaling pathway and oxidative phosphorylation. These findings imply that ABHD6 is closely related to the PI3K/AKT signaling pathway and oxidative stress (Figure 6C–D).

3.7 | Overexpression of ABHD6 decreases the content of ROS in CRC cells

To verify the above conjecture, we tested the ROS level in cells stably knocked down and overexpressing ABHD6. The results revealed that ABHD6 is highly negatively correlated with the level of intracellular ROS (Figure 7).

3.8 | ABHD6 regulates the level of AKT protein phosphorylation in CRC cells

To further explore the molecular mechanisms by which ABHD6 mediates biological behavior, the PI3K/AKT pathways were assessed using western blot analysis in CRC cell lines with ABHD6 knockdown or overexpression. The results obtained from Western blot analysis indicated that the overexpression of ABHD6 resulted in a decreased AKT phosphorylation in RKO cells (Figure 8A). Conversely, down-regulation of ABHD6 in SW480 cells yielded contrasting outcomes, with an increase in AKT phosphorylation (Figure 8B). However, total

AKT expression was not affected, indicating that ABHD6 may inhibit CRC proliferation, migration by inhibiting the activation of the AKT signaling pathway. Furthermore, Capivasertib, a pan-AKT inhibitor, could suppress the proliferation and migration of SW480 shABHD6 cells, demonstrating that the suppressing role of ABHD6 was dependent on AKT signaling (Figure 8C–D).

4 | DISCUSSION

More than 10 genes containing α/β-hydrolase domain (ABHD) have been identified, with varying substrate specificity and catalytic activity, their functional annotations are not comprehensive.[23–27] Among them, ABHD6 is an integral membrane protein belonging to

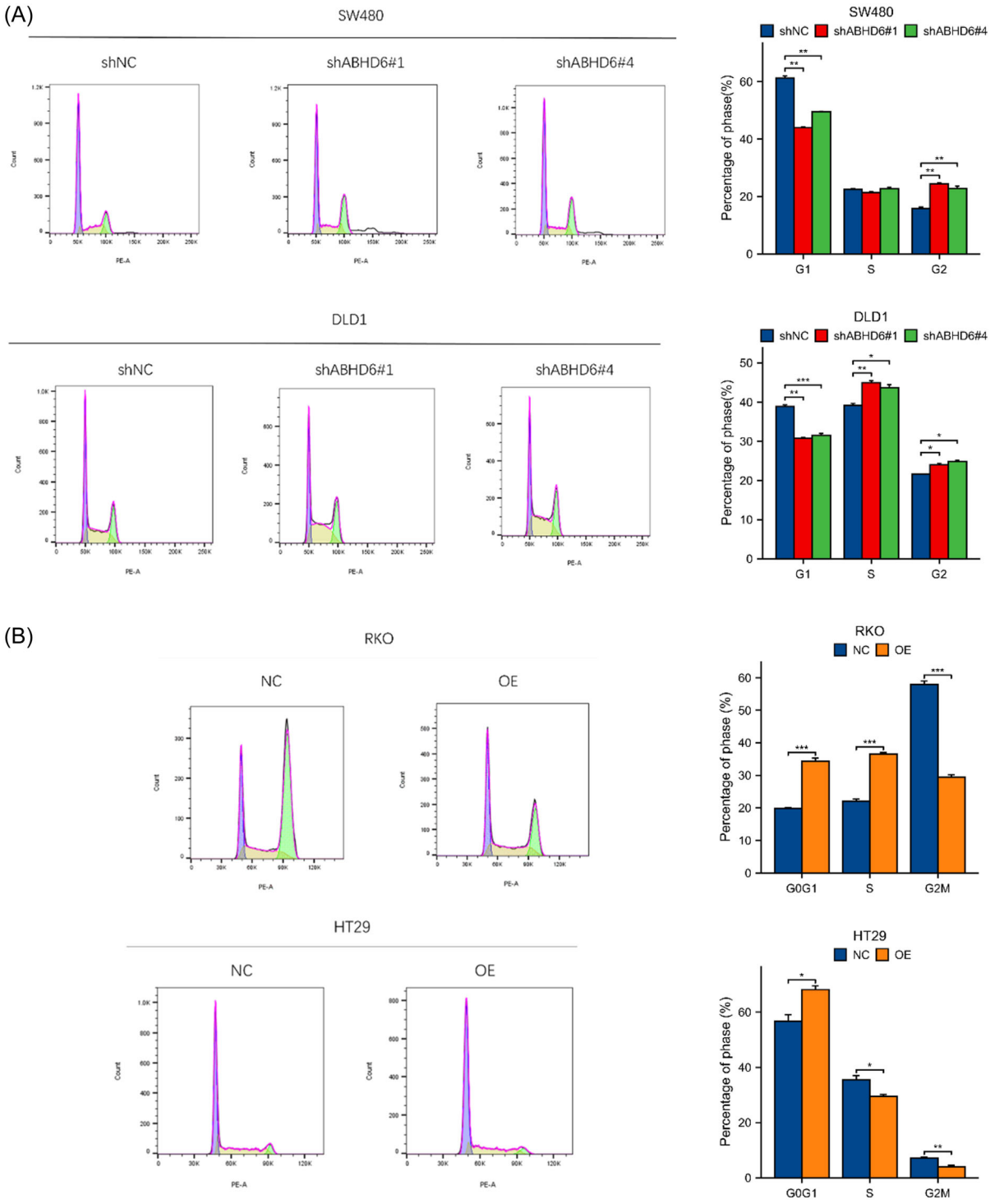

FIGURE 4 ABHD6 affects the cell cycle and sensitivity to Oxaliplatin of CRC cell lines. (A) The cell cycle of SW480 and DLD1 cell lines after downregulating ABHD6 expression. (B) The cell cycle of RKO and HT29 cell lines after upregulating ABHD6 expression. (C) The proportion of apoptotic cells was detected by flow cytometry. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. [Color figure can be viewed at wileyonlinelibrary.com]

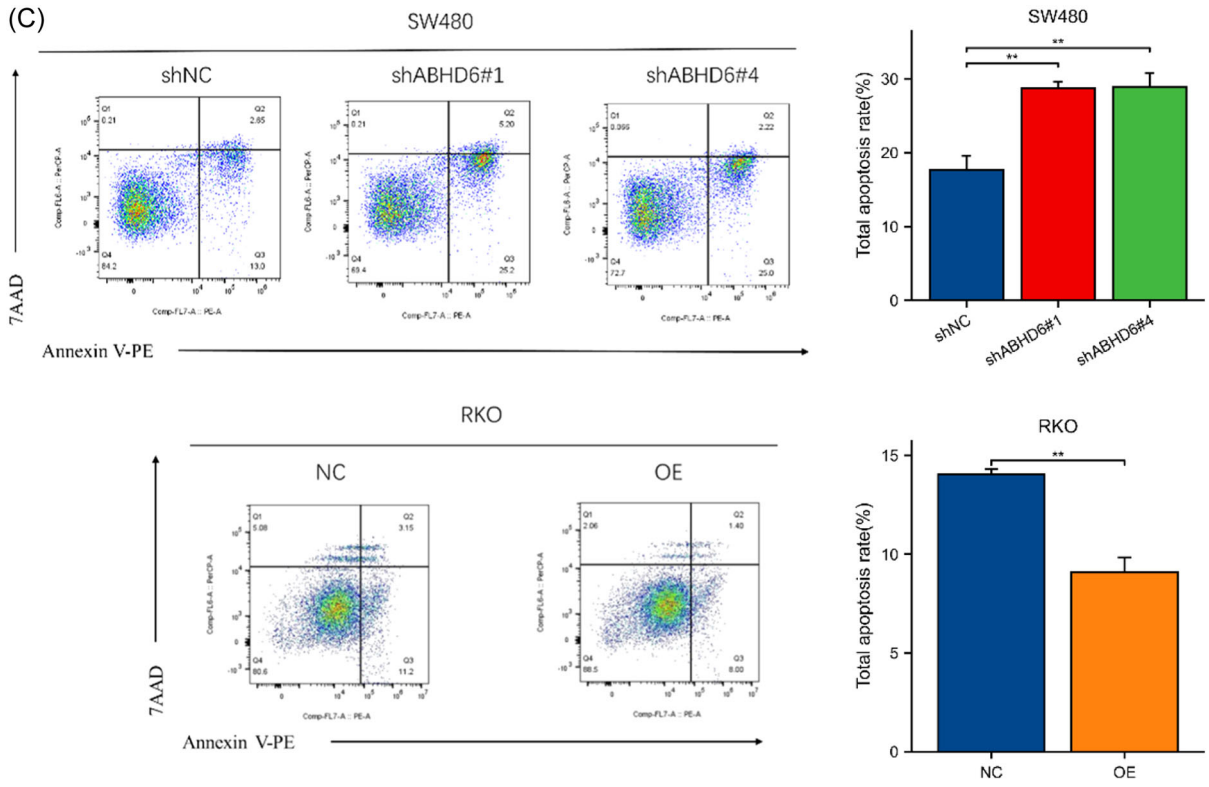

FIGURE 4 (Continued)

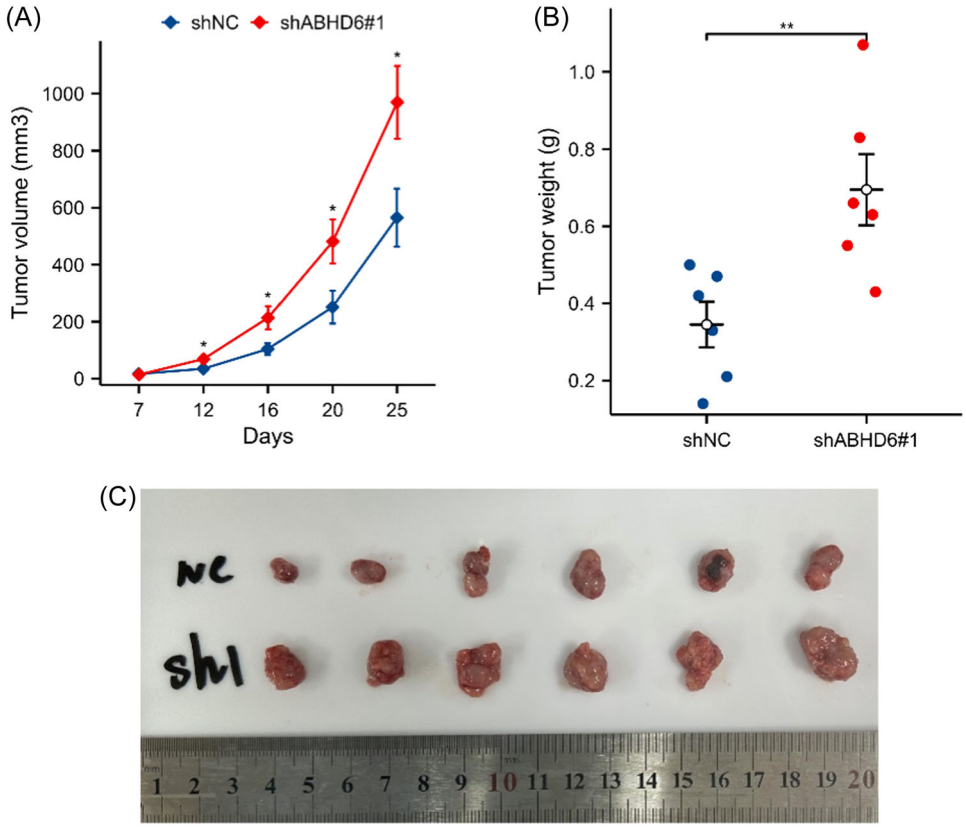

FIGURE 5 Knockdown of ABHD6 promotes CRC growth in vivo. (A) Effect of ABHD6 knockdown on CRC tumorigenesis in vivo. The volume of subcutaneous tumors was measured ($n = 6$). (B) Tumor weights were weighed and plotted. (C) The image of the removed tumors. *$p < 0{\cdot}05$, **$p < 0.01$. [Color figure can be viewed at wileyonlinelibrary.com]

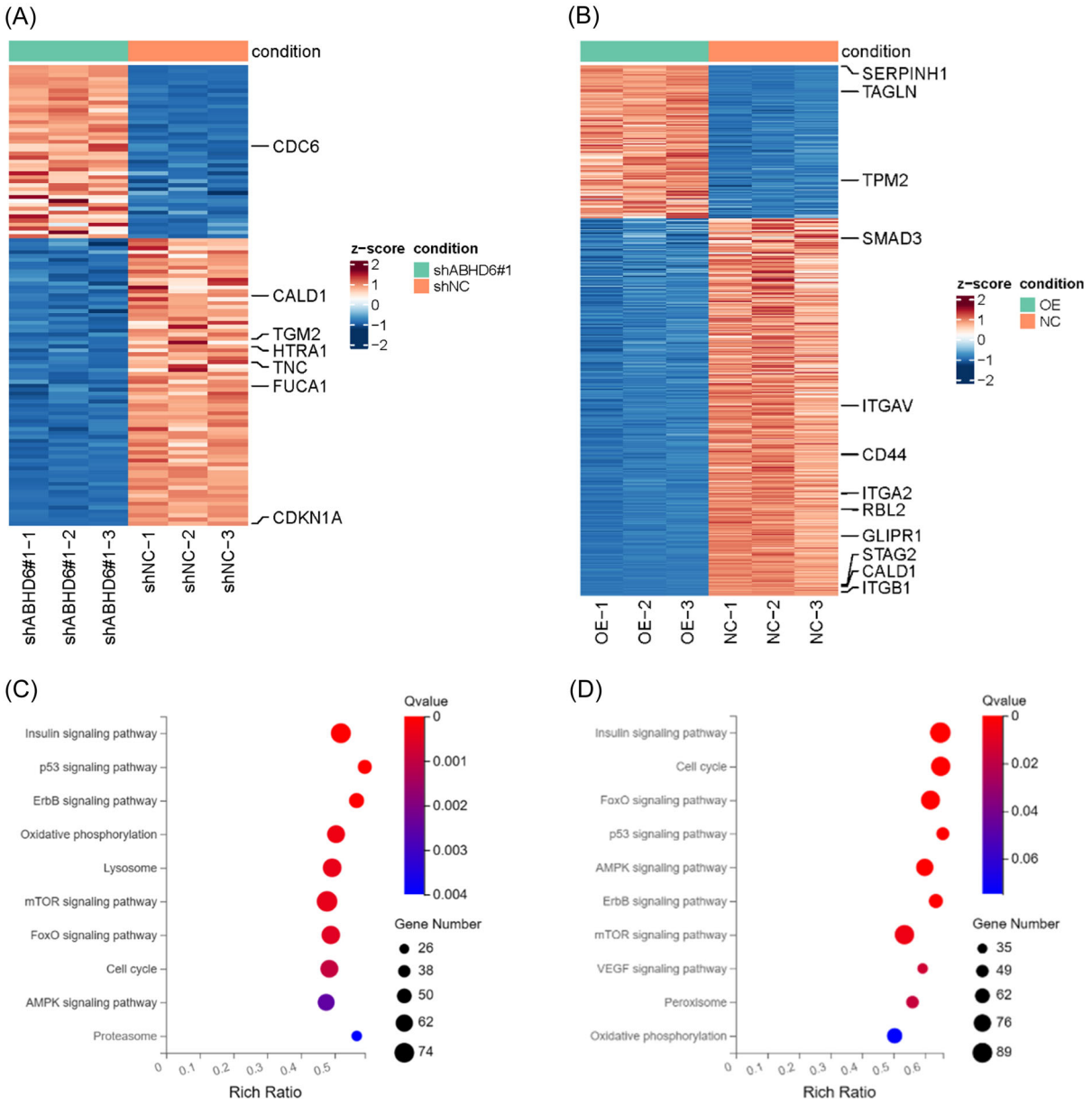

FIGURE 6 Differential gene expression and gene enrichment analysis of genes related to ABHD6 in CRC. Heatmap showing the changes of the differential expressed genes (A) between sh-ABHD6 #1 and NC groups in SW480 cells and (B) between OE and NC groups in RKO cells. KEGG enrichment analysis of differential expression genes (C) between sh-ABHD6 #1 and NC groups in SW480 cells and (D) between OE and NC groups in RKO cells. [Color figure can be viewed at wileyonlinelibrary.com]

the serine hydrolase family, functioning as a lipase that hydrolyzes various monoacylglycerols (MAGs).[10] It shares similar substrates with monoacylglycerol lipase (MAGL), the major enzyme responsible for hydrolyzing 2-arachidonoylglycerol (2-AG) in the nervous system. However, its contribution to this process appears limited unless MAGL expression is lacking.[28,29] 2-AG, a MAG containing an esterified arachidonic acid chain at the sn-2 position of the glycerol backbone, belongs to a class of signaling lipids that can activate the endocannabinoid (eCB) signaling system. ABHD6 is a new member of the eCB signaling system, which contains two cannabinoid receptors, CB1 and CB2, that can be activated by lipid signals, thereby affecting central and peripheral cell function. Previous studies have shown that ABHD6 is highly expressed in brain tissue, particularly in certain neurological disorders, making it a potential therapeutic target for a variety of diseases,[12,30] such as epilepsy[31] and multiple sclerosis,[14] although the results of related studies are still controversial.[32] Tchantchou et al.[33] reported that chronic treatment of WWL70, an ABHD6 inhibitor, improved motor coordination and working memory deficits in traumatic brain injury mice. The ABHD6 inhibitor relieved blood-brain barrier dysfunction and neurodegeneration by activating CB1 and CB2 receptors, and reducing the production of pro-inflammatory mediators. Furthermore, Alhouayek et al.[15] demonstrated the

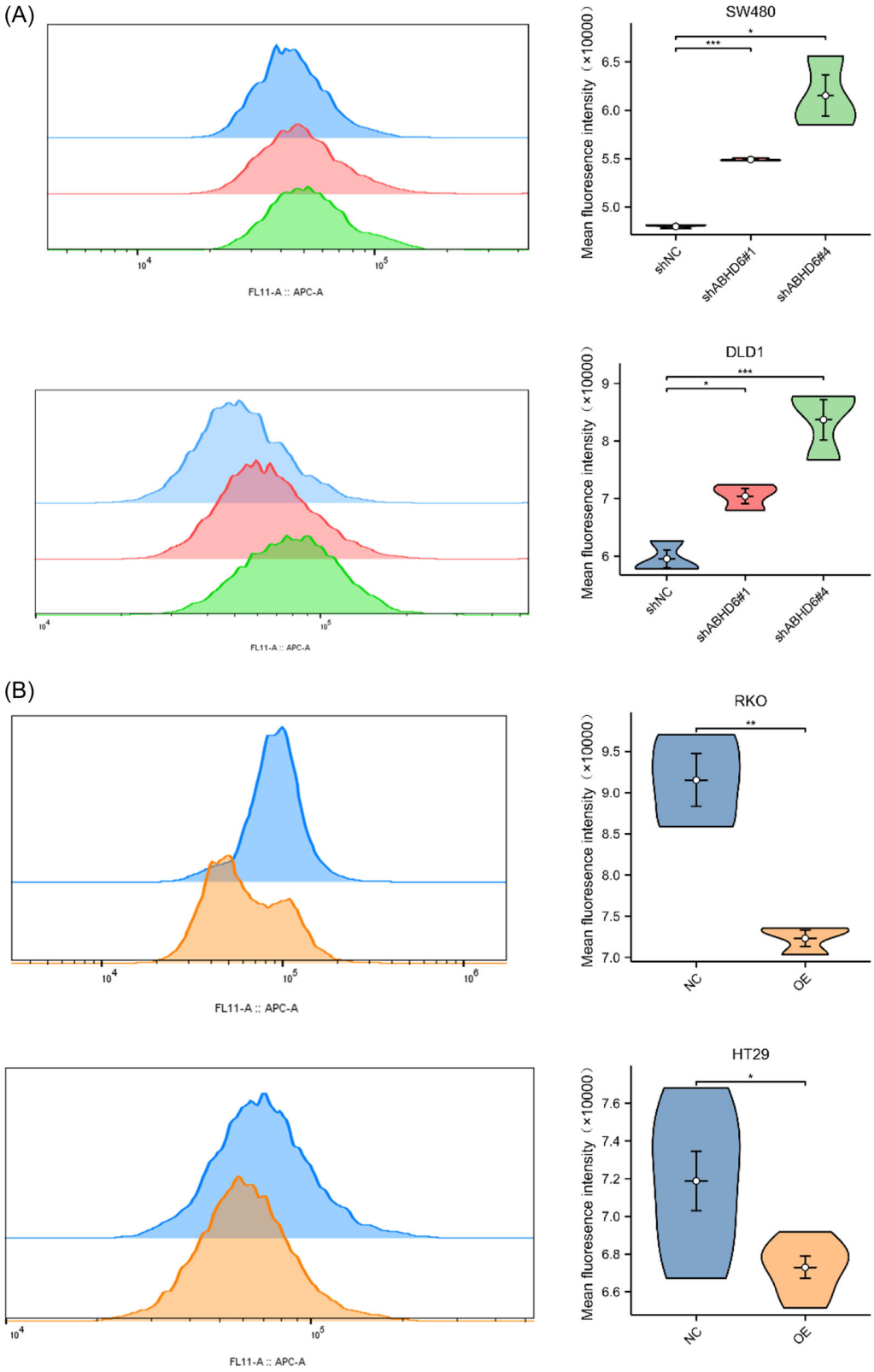

FIGURE 7 Reactive oxygen species assay in cells and the average fluorescence intensity of the ROS deep red probe were quantified. (A) Level of intracellular ROS in ABHD6 knockdown and control cell lines. (B) Level of intracellular ROS in ABHD6 overexpression and control cell lines. $^{*}p < 0{\cdot}05$, $^{**}p < 0.01$, $^{***}p < 0.001$. [Color figure can be viewed at wileyonlinelibrary.com]

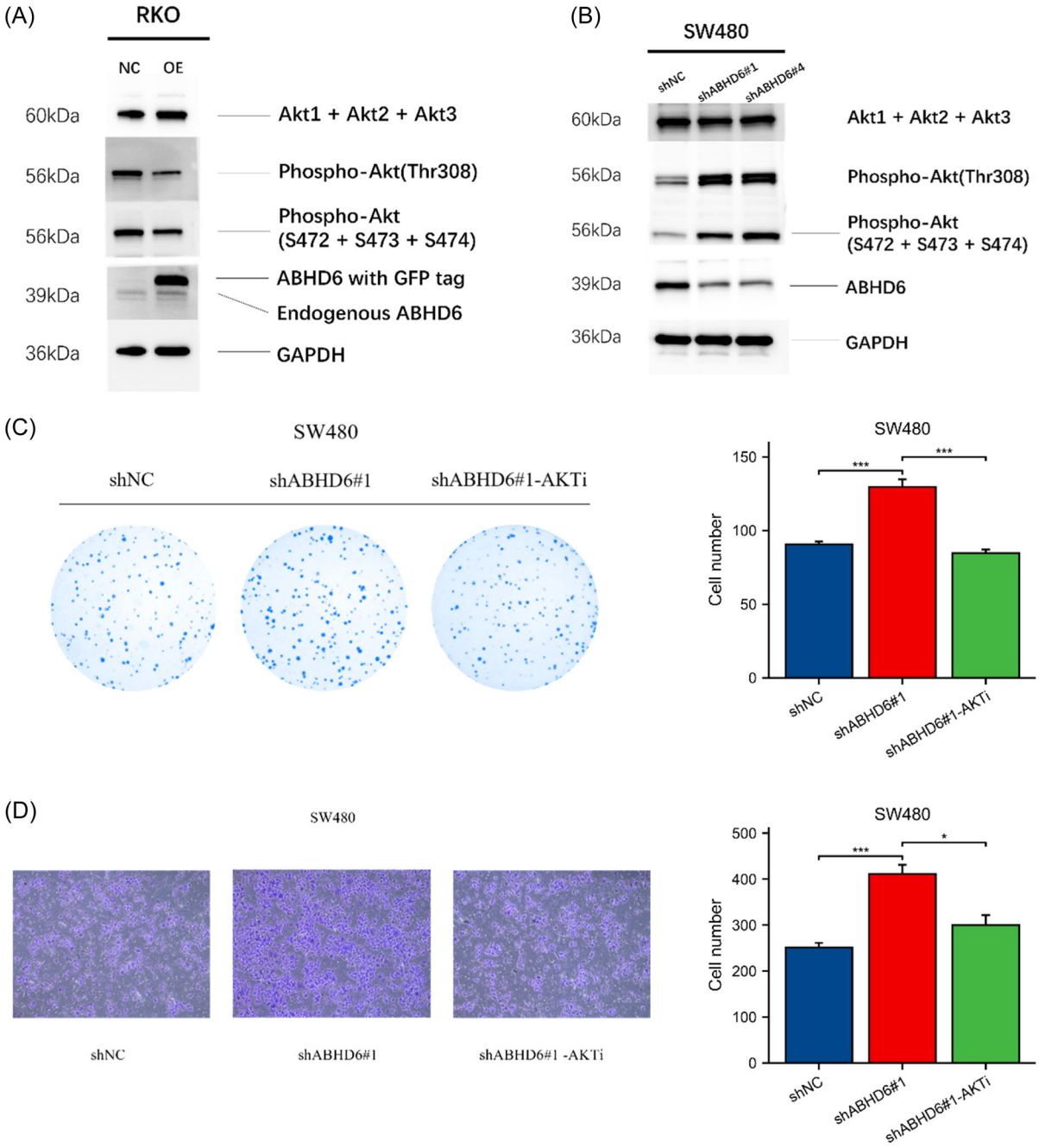

FIGURE 8 ABHD6 regulates CRC progression through AKT signaling pathway. Western blot analysis demonstrated that ABHD6 regulates the activation of AKT signaling pathway (A) Overexpression of ABHD6 effectively reduced protein levels of Phospho-AKT and (B) Downregulation of ABHD6 effectively increased protein levels of Phospho-AKT, but not protein levels of total AKT. Representative images and graphical representation show that Capivasertib, a pan-AKT inhibitor, can suppress the proliferation (C) and migration (D) of SW480 sh-ABHD6 #1 cells. $^{*}p < 0{\cdot}05$, $^{***}p < 0.001$. [Color figure can be viewed at wileyonlinelibrary.com]

interaction between the endocannabinoid and prostaglandin systems in macrophages, and blocking ABHD6 by drugs could potentially exert therapeutic effects on inflammation by controlling 2-AG levels. ABHD6 has also been shown to be associated with insulin secretion, with high expression in beta cells, where it appears to be the primary MAG hydrolase. In beta cells, glucose stimulates the production of long-chain saturated monoacylglycerols derived from lipolysis. Inhibition of ABHD6 enhances this process, glucose-stimulated insulin secretion in beta cells is inversely proportional to ABHD6 expression. In vivo experiments have also shown that knocking out ABHD6 can restore glucose-stimulated insulin secretion and improve glucose tolerance in diabetic mice.[17]

MAGL promotes migration, invasion and survival of tumor cells via regulating a fatty acid network enriched in oncogenic signaling lipids, and MAGL inhibition was disclosed to have the potential for cancer treatment.[5,7] Although ABHD6 has similar binding site

properties with MAGL, the function of ABHD6 in cancer remains controversial with varying effects observed in different cancer types. For example, in Ewing's sarcoma, although ABHD6 is significantly upregulated in tumor cells, its knockdown does not inhibit tumor growth and there is no correlation between ABHD6 expression in EFT samples and the prognosis of these patients.[20] Conversely, in NSCLC cells, silencing or pharmacological inhibition of ABHD6 reduces cell migration, invasion and tumor growth via activation of PPARα/γ signaling.[21] However, in hepatocellular carcinoma, high expression of ABHD6 is positively correlated with a favorable prognosis.[22] In our study, we observed that ABHD6 exerts an anticancer effect in CRC, and its low expression is closely associated with lymph node metastasis (LNM) and poor prognosis. Upregulating ABHD6 expression slows the proliferation rate of CRC cells, induces G0/G1 cell cycle arrest, and decreases cell migration ability.

AKT, also known as protein kinase B (PKB), is a serine/threonine kinase that is a key mediator of growth factor-induced cell survival.[34] The function of AKT involves a series of steps, including activation by survival factors, translocation to the plasma membrane, phosphorylation, and activation of downstream effectors.[35] AKT phosphorylation can occur through two primary mechanisms, the classical method involves phosphorylation at the Thr308 site by PDK1, while the other mechanism is mediated by the mammalian rapamycin complex 2 (mTORC2), which phosphorylates Ser473/Ser477/Thr479 site.[36] The activation of AKT leads to the activation of multiple downstream effectors that play a crucial role in cellular metabolic reprogramming, such as mTORC1, GSK3, and FOXO transcription factor family members.[37] mTORC1 promotes anabolism and limits autophagy, and is upregulated in most human cancers and genetic tumor syndromes.[38,39] GSK3 is also a vital regulator of cellular metabolism and can regulate NF-κB activity, which can be phosphorylated and inactivated by AKT.[40] FOXO is another essential downstream transcription factor affected by AKT. The transcriptional activity of FOXO is regulated by its shuttling between the nucleus and cytoplasm, and AKT-mediated phosphorylation of FOXO leads to its nuclear export while hindering its re-localization to the nucleus, thereby linking AKT-mediated repression of FOXO to multiple aspects of cancer progression.[41] AKT activation has been shown to play a pivotal role in tumorigenesis and the progression of various tumors.[42,43] The phosphorylation level of AKT is positively associated with cell proliferation, as well as Ki-67 expression, tumor invasion depth, venous vascular invasion, LNM, and tumor stage.[44] It is reported that PI3K/Akt signaling pathway is involved in the neuroprotective actions of 2-AG.[45] Phosphorylation of Akt and mTOR is required for oligodendrocyte progenitor cell proliferation stimulated by CB1/CB2 agonists.[46] In our study, we observed that the downregulation of ABHD6 expression promotes the proliferation and migration of CRC cells, as well as upregulating the phosphorylation level of AKT protein.

Accumulating evidence suggests that moderate levels of ROS support the proliferation and survival of cancer cells, while high levels of ROS damage macromolecular components, including DNA and proteins.[47] PTEN is an important gene for maintaining cell homeostasis and its mutation is related to the pathogenesis of various cancers and also an important regulator of PI3K/AKT. It regulates many cellular processes by antagonizing PI3K signaling.[48] Elevated ROS levels also promote the phosphoinositide-3,4,5-trisphosphate (PIP3) signaling pathway by oxidizing PTEN and activating PI3K.[49,50] Furthermore, the transcription factor FOXO, which is negatively regulated by the AKT pathway, can scavenge ROS by inducing several ROS scavenging systems.[51,52] Thus, a complex interplay exists between the PI3K/AKT signaling pathway and the antioxidant and biosynthetic activities of cancer cells in tumors. Our study suggests that ABHD6 exerts its effect on CRC by modulating the level of ROS. Overexpression of ABHD6 led to a significant reduction in intracellular ROS levels while knocking down ABHD6 expression resulted in a significant increase in ROS levels. These findings, combined with the results of cell function experiments and literature review, support the tumor suppressor role of ABHD6 in CRC. Further investigation is necessary to determine whether ABHD6 plays a coordinating role in inhibiting the AKT pathway and maintaining appropriate ROS levels in cells.

In conclusion, our study showed the downregulation of ABHD6 expression in CRC, which is associated with poor prognosis in CRC patients. We also demonstrated that ABHD6 regulates CRC cell proliferation, cell cycle, and migration through the AKT signaling pathway. These findings provide a new perspective on the diagnosis and treatment of CRC. Further studies are needed to fully elucidate the underlying mechanisms of ABHD6 in CRC and to explore its potential as a therapeutic target.

AUTHOR CONTRIBUTIONS

Zhidong Gao, Zexian Zeng and Yingjiang Ye initiated the project, supervised the study and revised the manuscript. Xiaoyu Xiong, Changjiang Yang conducted the in vitro and in vivo experiments, analyzed the data. Yiteng Jin conducted bioinformatic analysis and interpreted results. Xiaoyu Xiong, Changjiang Yang and Yiteng Jin wrote the manuscript. Rui Zhang and Shuo Wang supervised molecular studies. Lin Gan, Sen Hou and Yudi Bao contributed to the experiment and interpretation of the data. All authors read and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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