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ACSL3 Regulates CRC Cell Proliferation through ACSL3-LPIAT1 Signaling Pathway

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ABSTRACT

Background: Colorectal cancer (CRC) is a common cancer in China, with a high mortality rate. Lipid metabolism disorders can promote cancer metabolism reprogramming, and lipid metabolism related genes are prognostic biomarkers of cancer.

Objective: To investigate whether ACSL3 is involved in EMT and metastasis of CRC, and to explore the molecular mechanism of ACSL3 involvement in CRC.

Method: Establish CRC models with high expression of ACSL3 and knockdown of ACSL3. CCK8, flow cytometry, scratch assay, and Transwell assay were used to measure the effect of ACSL3 on CRC cells. In vivo experimental analysis of changes in tumor volume and weight caused by overexpression or knockdown of ACSL3. In addition, we also studied whether ACSL3 affects the change of Prostaglandin through participating in ACSL3-LPAIAT1 signal axis, and then affects the occurrence and progression of tumors.

Results: The apoptosis rate of cell lines with high expression of ACSL3 is significantly reduced. Overexpression of ACSL3 significantly enhances the migration and invasion ability of CRC cells. Silencing ACSL3 can partially reverse the increased invasiveness caused by overexpression of ACSL3. Overexpression of ACSL3 can increase the expression levels of LPIAT1 and cPLA2, which can be partially reversed by silencing ACSL3. In vivo experiments have also found that upregulation of ACSL3 has the effect of accelerating tumor volume and weight.

Conclusion: ACSL3 plays an important role in the progression of CRC, and may be a prognostic biomarker, becoming a new potential therapeutic target for the treatment of CRC.

INTRODUCTION

The incidence rate of colorectal cancer (CRC) is the fourth in the world, and the mortality rate is the second. It is also one of the most common cancers in China Chen et al. (2016). Surgery is still the main treatment for early colorectal cancer patients, and the 5-year survival rate after surgery can reach 55% Andreou et al. (2013). However, most patients are already in the advanced stage at the time of diagnosis. Colorectal liver metastasis (CRCLM) is the main cause of death in patients. Early detection of meaningful biomarkers is crucial for improving the survival of colorectal cancer patients.

Cancer can cause energy metabolism disorders and evade immune damage Hanahan et al. (2011). Fatty acids (FA) are involved in energy metabolism and cellular signaling pathways to maintain normal physiological functions Tabuchi et al. (2021). FA metabolic disorders can lead to excessive fat synthesis and deposition, ultimately leading to metabolic disorders and tumor development. Long chain acyl CoA synthase (ACSL) is a family of enzymes responsible for converting FA with chain lengths of 8-22 into fatty acyl CoA esters, which is a prerequisite step for fatty acid oxidation (FAO). In turn, fatty acyl coenzyme A can be transported into Mitochondrial matrix and undergo FAO Han et al. (2019). ACSL enzyme is involved in metabolic reprogramming of tumor cells. This is also the idea behind the development of many cancer prevention or treatment drugs Sánchez-Martínez et al. (2015), Mark et al. (2023), Wang et al. (2021). ACSL stimulates the progression of colon cancer, and increases the metabolic activity, invasion, and migration characteristics of malignant cells Heyer et al. (2017). ACSLs are widely involved in Endoplasmic reticulum stress, Ferroptosis, drug resistance and tumor inflammation microenvironment by regulating FA metabolism Liu et al. (2018). When ACSLs lose control, they can alter the

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distribution, type, and quantity of intracellular FA, leading to tumors and other metabolic diseases Tang et al. (2018), Yan et al. (2015). Especially in tumors, the number and size of lipid droplets are related to the invasiveness of cancer.

There are five subtypes of ACSL, namely ACSL1, ACSL3, ACSL4, ACSL5 and ACSL6. Although each subtype catalyzes similar Enzyme catalysis, their intracellular functions and metabolic results are different. Acyl CoA synthase 3 (ACSL3) is mainly present in lipid droplets within cells Poppelreuther et al. (2018).

Lipid droplets are the main Organelle for storing neutral lipids, which play an important role in storing energy and providing lipid molecules. ACSL3 promotes the formation of lipid droplets and is beneficial for maintaining lipid homeostasis. The high expression of ACSL3 has been detected in various cancers. The overexpression of ACSL3 promotes the absorption of oleic acid and enhances the cell's resistance to lipid oxidative stress. Oleic acid protects Melanoma cells from Ferroptosis in an ACSL3 dependent manner, and promotes cell long-distance metastasis Ubellacker et al. (2020). ACSL3 interacts with CDCP1 to promote the metastasis of triple negative breast cancer cells Wright et al. (2017).

Lysine demethylase 3A and Androgen receptor (AR) synergistically enhance the transcription of ACSL3 and participate in the development of prostate cancer Wilson et al. (2017).

In addition, ACSL3 can mediate the increase of FA uptake and oxidation in Mutant KRAS lung cancer Padanad et al. (2016). The high expression of ACSL3 is associated with poor prognosis in non-small cell lung cancer patients Fernandez et al. (2020).

Therefore, metabolic pathways targeting ACSL3 and FAO may be used to treat CRC and other FA addicted cancers. However, it is currently unclear how dysregulation of ACSL3 expression affects the occurrence and development of colorectal cancer.

Therefore, this study explores the relationship between ACSL3 and CRC infiltration and migration, in order to provide assistance in predicting the prognosis of colorectal cancer.

MATERIALS AND METHODS

Cell culture

The human intestinal epithelial cell line FHC cells (ATCC CRL-1831) were used as the control. Six human colon cancer cell lines with different mutation backgrounds were selected as experimental subjects, including SW480 (ATCC CCL-228), SW620 (ATCC CCL-227), LoVo (ATCC CCL-229), HT29 (ATCC HTB-38), Caco-2 (ATCC HTB-37), and HCT116 (ATCC CCL-247). DMEM medium containing 10% Fetal bovine serum was used for culture at 37 ℃ and 5% CO_2. It is passed down every 2-3 days. Take logarithmic growth stage cells for subsequent experiments. Cell culture were purchased from ATCC (https://www.atcc.org/).

Mice

BALB/c-nu nude mice without thymus were used as control, 4-6 weeks of age. The tumor was formed by cell injection. ACSL3-SW480, SW480, sh-ACSL3, and sh-NC-HT29 cells were injected into the armpit of mice, and fed at 26 to 28℃ and 40% to 60% humidity. After 28 days, the neck was removed and the tumor was dissected. All mice were purchased from Guangdong Medical Laboratory Animal Center（https://gdmlac.com.cn/）.

RT-PCR

Total RNA was extracted from cells with an RNA extraction kit (Qiagen). PrimeScript ® RT (Takara) kit for synthesizing cDNA. RT qPCR reaction conditions: 94 ℃, 5 minutes; 94 ℃, 30s, 60 ℃, 30s, 72 ℃, 60s, 40 cycles; Extend at 72 ℃ for 5 minutes. Normalize using GAPDH as an internal reference.

ACSL3-F: ACCAAAGACCAACATCGCCA

ACSL3-R: TCGGTGGCTTTCCATCAACA

GAPDH-F: AATGACCCCTTCATTGAC

GAPDH-R: TCCACGACGTACTCAGCGC

CCK8

The experiment consists of four groups, including overexpressing FOXP3 cells, oe normal control, knockdown FOXP3 cells, and sh normal control. Inoculate 1x10 ^ 6 cells per well in a 96-well plate and culture for 24 hours; Add 10 μL CCK-8 solution; Incubate for 4 hours; Measure the absorbance value at 450 nm using an enzyme-linked immunosorbent assay.

Transwell invasion experiment

Dilute the coating matrix adhesive with 50mg/L Matrix in a ratio of 1:8, then add it to the upper chamber surface at the bottom of the chamber and air dry at 4 degrees. After 12 hours of cell starvation, use resuspension. Add 4 × 10 ^ 7 cells to the small chamber, and add 200ul of 1640 culture medium containing 10% FBS to the lower chamber. After 24 hours, remove the small chamber and wipe off the noninvasive cells in the upper chamber with an alcohol containing cotton swab. Fix for 10-15 minutes (4% Paraformaldehyde), dye with Crystal violet dye for 5 minutes, rinse with water and air dry.

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Randomly select 4 fixed cell positions and count cells under a 20× field of view under a microscope.

Scratch experiment

Lay 4 × 10 ^ 6 cells on a 6-well plate and culture for 24 hours. Use the gun head to match the ruler, and mark the line vertically with the gun head. Wash the cells with PBS three times, remove the scratched cells, add serum-free medium, and continue cultivation. Take samples from 0-48 hours and take photos.

Plasmid transfection

Design and synthesis of ACSL3 specific shRNA. Add 4 × 10 ^ 5 cells to a 6-well plate and culture for 24 hours. Replace serum free culture medium. Add shRNA and lipo 3000 mixed with 1640 medium in proportion, let stand at room temperature for 20 minutes, then add 6-well plates and incubate for 4 hours; Replace the serum containing 1640 medium and incubate for 48 hours.

Construction of stable transgenic cell lines with slow viruses

PCDNA3.1-FLAG-ACSL3 overexpression lentivirus was synthesized by Guangzhou Aiji Biosynthesis. Inoculate 10 ^ 5 cells into a six-well plate. After 24 hours, add 5 μL/mL polybrene and lentivirus particles (50MOI), incubate for 24 hours, and replace the culture medium. Evaluate the transfection efficiency of ACSL3 in cells using Real-time PCR. Cells overexpressing ACSL3 are labeled oe-ACSL3. The control group cells were transfected with empty vectors labeled as oe NC.

Western blot analysis

We use primary antibodies targeting ACSL3 and GAPDH. Second antibody: anti-mouse and anti-rabbit antibodies conjugated with Horseradish peroxidase. Extract total protein from CRC cells. Measure protein concentration using BCA method and add an appropriate amount of 5 × SDS loading buffer, protein lysis denaturation at 100 ℃ for 10 minutes. The protein samples were separated by 10% SDS-PAGE gel electrophoresis and transferred to PVDF. 5% skimmed milk was sealed at room temperature for 1.5 hours, and TBST membrane was washed 3 times. The diluted primary antibody (dilution ratio, 1:1000) was immersed in the PVDF membrane and incubated overnight at 4 ℃. TBST washing film 3 times. Add the corresponding secondary antibody (dilution, 1:2000), incubate at room temperature for 1 hour, and rinse the membrane 3 times with TBST. Chemiluminescence method for development.

Oil Red O Staining

Oil Red O (Sigma Aldrich) staining was used to measure lipid accumulation. 1 × 10 ^ 5 CRC cells were added to a 6-well plate and transfected with shRNA plasmid for 48 hours. The cells were washed with PBS for three times and fixed with 10% Paraformaldehyde for 30 minutes. Then stain with 1.5% oil red solution at room temperature for 30 minutes. Add 60% isopropanol and mix for 20 seconds, then wash with PBS 3 times.

Immunohistochemical analysis

Fix the tissue with 4% formaldehyde, embed it in paraffin, and slice it. Dewaxing agent dewaxing, ethanol aqueous solution rehydration. Endogenous peroxidase activity is blocked by hydrogen peroxide (3%). The first antibody (anti-ACSL3) was incubated overnight at 4 ℃ and then incubated with the anti-rabbit second antibody. Re-dye with Haematoxylin.

Establishment of the mouse model

Purchase female BALB/c mice, select mice aged 4-6 weeks, and randomly divide them into two groups, with 5 mice in each group. Transfer stable cell lines shNC and HT29 shACSL3 cells (5 × 10 6/200 μL PBS) were injected subcutaneously into the mouse near the Scapula. Measure the size of subcutaneous implanted tumors every 3 days. According to the guidelines of the Animal Care and Use Committee of China Medical University, when the tumor diameter reaches 2.0 cm, mice are euthanized through cervical dislocation. This experiment was approved by the Animal Protection Committee.

Statistical analysis

SPSS version 20.0 (IBM) is used for statistical analysis. The Student's test was selected for continuous variable analysis between the two groups, and a p-value of<0.05 indicates a statistically significant difference. All data in this experiment need to be measured independently and repeated 3 times.

RESULTS

Expression of ACSL3 in colon cancer cell lines

To further detect the expression of ACSL3 in colon cancer cell lines, qPCR was used to detect the mRNA expression level of ACSL3 in human intestinal epithelial cell line FHC and colon cancer cell lines, including SW480, SW620, LoVo, HT29, Caco-2, and HCT116. The results showed that the mRNA expression of ACSL3 in the HT29 cell line was significantly higher than that in FHC, while the mRNA expression of ACSL3 in the SW480 cell line was significantly higher than that in FHC (Figure 1) (P<0.05).

SW480 was the least expressed in 6 colon cancer cell lines. Two cell lines SW480 and HT29 and FHC were selected for subsequent biological experiments.

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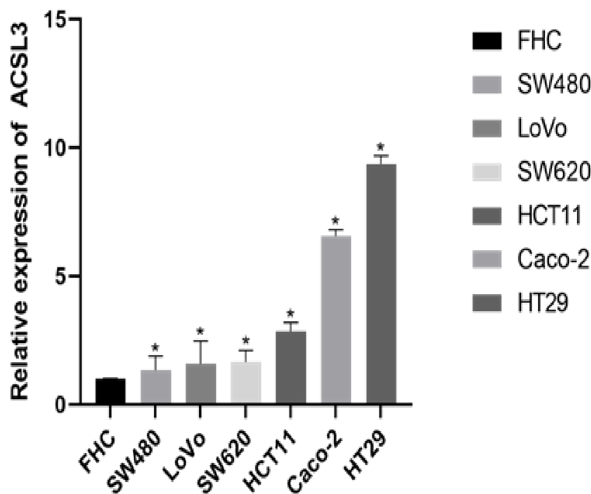
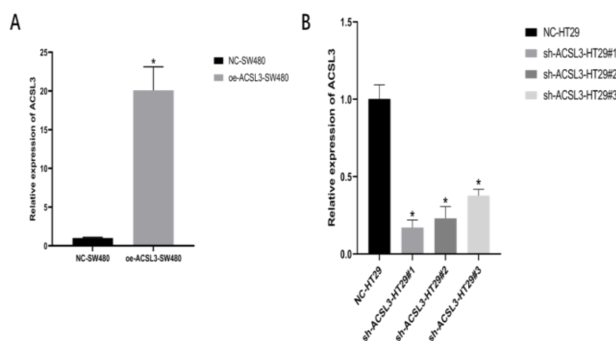
Figure 1: ACSL3 expression in the human intestinal epithelial cell line FHC cells and different cell lines. (* P < 0.05)



Establishment of ACSL3 high expression model and knockdown model

Based on the above experiment, the SW480 cell line was selected to establish an ACSL3 high expression model. Select the HT29 cell line to establish an ACSL3 knockdown model. Before conducting functional experiments, we constructed an ACSL3 overexpression vector and transfected it into SW480 cells. The results showed a significant increase in the mRNA expression level of ACSL3 in the experimental group (Figure 2A), indicating the successful establishment of the overexpression model. We also tested the efficiency of lentiviruses such as sh-ACSL3#1, sh-ACSL3#2, and sh-ACSL3#3, and the results showed that sh-ACSL3#1 had the highest silencing efficiency (Figure 2B). Subsequently, sh-ACSL3#1 was chosen to continue the experiment.

Figure 2: Establishment of ACSL3 overexpression model and knockdown model.



(A) ACSL3 was overexpressed in SW480 cells, and the relative expression of ACSL3 was significantly increased (*P<0.05). (B) ACSL3 was silenced in HT29 cells, the relative expression of ACSL3 was significantly decreased, and the silencing efficiency of sh-ACSL3#1 was the highest. (* P < 0.05).

Upregulation of ACSL3 promotes proliferation and invasion of CRC cells

Enhanced cell proliferation, migration, and invasion are the three fundamental characteristics of cancer progression and metastasis formation. To elucidate how overexpression of ACSL3 affects the carcinogenic characteristics of CRC cells, we evaluated whether overexpression of ACSL3 can promote the proliferation and migration ability of CRC cells. Evaluate the relationship between ACSL3 expression and SW480 proliferation using Cell Count Kit 8 (CCK-8) assay. Compared with the control group, overexpression of ACSL3 promoted the proliferation rate of proliferating cells (P<0.05) (Figure 3A). The results of scratch and transwell experiments showed that overexpression of ACSL3 significantly enhanced the migration and invasion ability of CRC cells (P<0.05) (Figures 3B, 3C). Therefore, ACSL3 plays an important role in the proliferation and migration of CRC. Using flow cytometry to measure apoptosis, compared with the negative control group (si-NC), the high expression of ACSL3 significantly inhibited the apoptosis rate (P<0.05) (Figure 3D).

In order to verify whether the overexpression of ACSL3 is related to the EMT phenotype, we tested E-cadherin as an epithelial marker, N-cadherin and Vimentin as mesenchymal markers, and performed Western blot analysis. The results showed that, compared with the control cells, the overexpression of ACSL3 increased the expression of N-cadherin, Snail and vimentin in SW480 cells, and decreased the expression of E-cadherin (Figure 3E). This is consistent with the loss of epithelial features and the increase in EMT phenotype. The oil red O staining results also support that overexpression of ACSL3 reduces FA accumulation (Figure 3F). The CEA level in the cell supernatant of the overexpression ACSL3 group was also significantly higher than that of the control group (Figure 3G). Therefore, the upregulation of ACSL3 can promote the proliferation and invasion of CRC cells.

Knocking down ACSL3 can inhibit CRC cells

We also verified that knocking down the expression of ACSL3 can inhibit CRC cell activity. Compared with the control group, the cck-8 detection results showed that knocking down ACSL3 resulted in a decrease in the proliferation rate of HT29 cells (P<0.05) (Figure 4A). Scratch and transwell experiments showed that knocking down ACSL3 can reduce the migration and invasion ability of CRC cells (P<0.05) (Figures 4B, 4C). Flow cytometry analysis revealed a significant increase in apoptosis rate in the low expression group of ACSL3 (Figure 4D).

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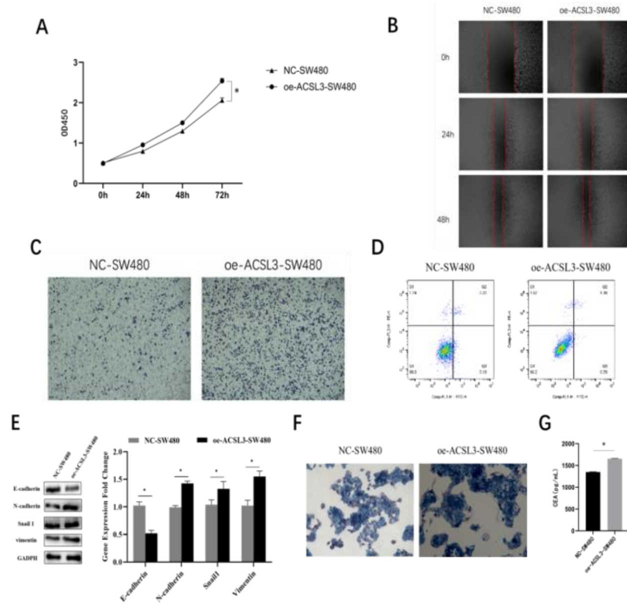
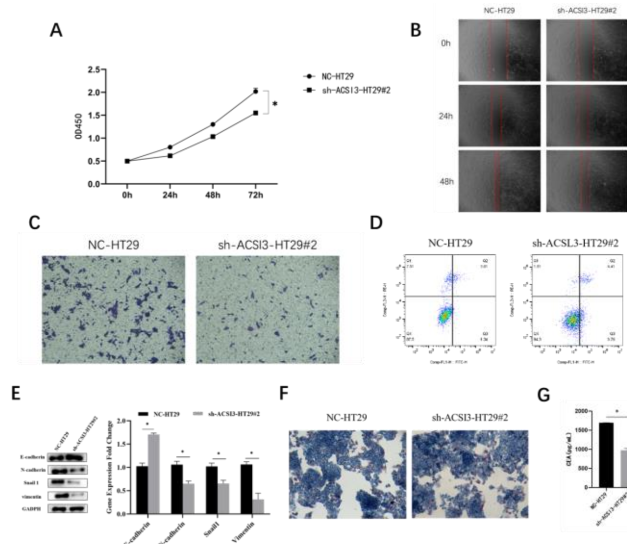
Figure 3: Overexpression of ACSL3 significantly promoted cell proliferation and invasion, and decreased cell apoptosis, and had a certain relationship with EMT.



(A) CCK-8 assay showed that overexpression of ACSL3 gene improved cell proliferation efficiency compared with control group. (* P < 0.05) (B, C) The results of scratch and transwell experiments showed that overexpression of ACSL3 significantly enhanced the migration and invasion ability. (D) Flow cytometry showed that overexpression of ACSL3 decreased the apoptosis rate. (E) WB experiments show that overexpression of ACSL3 increased the expression of N-cadherin, Snail and vimentin in SW480 cells, and decreased the expression of E-cadherin. (F)The oil red O staining shows that overexpression of ACSL3 reduces FA accumulation. (G) ELISA results show overexpression of ACSL3 increased the CEA level significantly (*P<0.05).

Figure 4: Silencing ACSL3 significantly inhibited colon cancer cell formation.



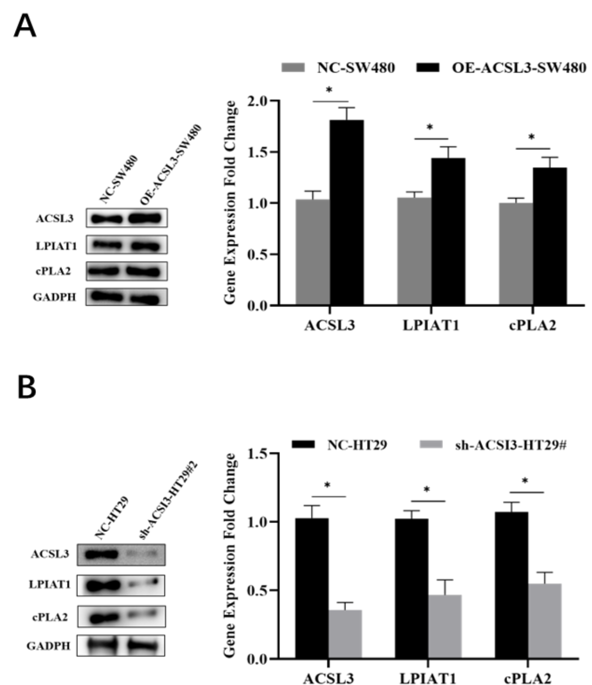
(A) CCK-8 assay showed that overexpression of ACSL3 gene reduced cell proliferation efficiency compared with control group. (* P < 0.05) (B, C) Scratch and transwell experiments showed that knocking down ACSL3 can reduce the migration and invasion ability. (D) Flow cytometry showed that silencing ACSL3 increased the apoptosis rate (*P<0.05). (E) WB experiments show that the sh-ACSL3 experimental group had low expression of N-cadherin, Snail and vimentin, and increased expression of E-cadherin. (F) Oil red O staining results showed that knocking down ACSL3 increased the accumulation of FA. (G) ELISA results showed that silencing ACSL3 significantly reduced CEA levels (* P < 0.05).

The results of EMT phenotypic factor verification found that, compared with the control group, the sh-ACSL3 experimental group had low expression of N-cadherin, Snail and vimentin, and increased expression of E-cadherin (Figure 4E). This also confirms once again that knocking down ACSL3 can slow down the metastasis of CRC cells. Oil red O staining results showed that knocking down ACSL3 increased the accumulation of FA (Figure 4F). After silencing ACSL3, the CEA level in the cell supernatant was lower than that in the control group (Figure 4G).

Regulating CRC cells through the ACSL3-LPIAT1 signaling axis

Maria Wilson et al. (2017). found that ACSL3-LPIAT1 axis is a necessary condition for the continuous synthesis of Prostaglandin in lung cancer, and the enhancement of Prostaglandin production will promote the occurrence and progression of cancer.

Figure 5: ACSL3 functions through ACSL3-LPIAT1 signaling axis.



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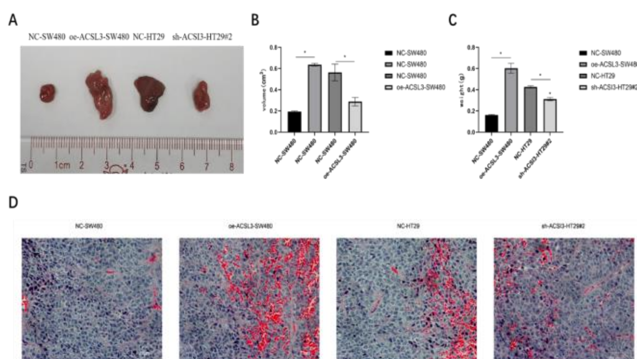
(A) Overexpression of ACSL3 increased the expression of LPIAT1 and cPLA2. (B) Silencing ACSL3 decreased the expression of LPIAT1 and cPLA2.

So we also investigated the role of ACSL3-LPIAT1 signaling axis in CRC cells. When overexpressing ACSL3, the expression levels of LPIAT1 and cPLA2 also showed an increasing trend compared to the control group (Figure 5A). However, silencing ACSL3 partially reversed the increased expression of LPIAT1 and cPLA2 in CRC cells induced by overexpression of ACSL3 (Figure 5B). This indicates that ACSL3 promotes the occurrence of CRC EMT, and this process is regulated by LPIAT1.

The tumor promoting effect of ACSL3 gene in vivo

To verify that ACSL3 can promote the proliferation of colon cancer cells in vivo, we subcutaneously injected the oe ACSL3-SW480, SW480, sh-ACSL3, and sh-NC-HT29 cell lines transfected with lentivirus into the armpits of mice. After 28 days, the mice were euthanized. Compared with the control group, the subcutaneous tumor volume of mice in the overexpression ACSL3 group were significantly higher than those in the control group, while the ACSL3 knockdown group was significantly lower and statistically significant (Figure 6A). These results strongly confirm that ACSL3 can promote the proliferation of colon cancer cells in vivo. The overexpression of ACSL3 significantly increased the subcutaneous tumor volume and weight compared to the NC group, while knocking down ACSL3 reduced the tumor volume and weight of colon cancer cells (Figure 6B, C). The results of H&E staining are also consistent with the above (Figure 6D). This indicates that ACSL3 can promote the proliferation and metastasis of colon cancer cells in vivo.

Figure 6: The expression of ACSL3 promotes the growth of colon cancer cells.



(A, B) During the 28-day mouse tumor volume measurement, overexpression of ACSL3 increased the subcutaneous tumor volume, while silencing ACSL3 reduced the tumor volume. (C) The trend of tumor weight was consistent. (D) The results of H&E show overexpression of ACSL3 promotes the complete morphology of tumor cells with clear boundaries, uniform cell size and uniform morphology, while silencing is the opposite.

DISCUSSION

Despite extensive research in the diagnosis and treatment of colorectal cancer in the past, the mortality rate still ranks second in the world. Liver metastasis is the main cause of death in CRC patients. Although the Targeted therapy of VEGF and EGFR has achieved results Saliakoura et al. (2020), Singh et al. (2016), He et al. (2014), the overall survival of colorectal cancer patients with liver metastasis has not been significantly prolonged. Lipid metabolism has been proposed as a potential target for cancer diagnosis, prognosis, and treatment [22]. The ACSL (Acetyl CoA Synthase) family is a key enzyme in the synthesis of lipid compounds. By regulating Fatty acid metabolism, it can lead to metabolic disorders of tumors and other metabolic diseases.

ACSL3 is located in lipid droplets generated by cell metabolism. Because of its hydrophobic hairpin like N-terminal, it also exists on the cytoplasm surface of Endoplasmic reticulum et al. (2016). ACSL3 is also related to the transport of Golgi apparatus body backward to the plasma membrane Kassan et al. (2013). The gene is also used in prognostic drug approaches for liver cancer treatment and lung cancer patients Xiaoding et al. (2023), Ye et al. (2023). ACSL3 mainly acts on the activation reaction of fatty acids. ACSL1, ACSL3, and ACSL4 are highly expressed in liver cancer, and the combination of ACSL3 and ACSL4 can be used as combined biomarkers for the diagnosis of liver cancer Ansari et al. (2017), Ndiaye et al. (2020). ACSL3 and ACSL4 are upregulated in prostate cancer. The expression of ACSL3 in prostate cancer tissue is 1.5 times higher than that in normal prostate tissue Zhan et al. (2024). ACSL3 is up-regulated in KRAS Mutant lung cancer Padanad et al. (2016). In non-small cell lung cancer, ACSL3 increases the synthesis of Prostaglandin and promotes the occurrence and development of tumors Saliakoura et al. (2020). However, the overexpression of ACSL3 is related to the poor survival rate of patients with Melanoma Migita et al. (2017). In this study, we found that the expression level of ACSL3 is inconsistent in different types of CRC cells, and the apoptosis rate of cell lines with high ACSL3 expression is significantly lower than that of low ACSL3 expression lines.

Overexpression of ACSL4 increases the migration and invasion of colon cancer cells Heyer et al. (2017). We found that overexpression of ACSL3 significantly enhanced migration and invasion ability in cell line SW480. Silencing ACSL3 can partially reverse the increased invasiveness caused by overexpression of ACSL3. Therefore, ACSL3 plays an important role in the proliferation and migration of CRC, and the impact of ACSL3 upregulation on EMT is explored by detecting E-cadherin, vimentin, and snail. The results indicate that the upregulation of ACSL3 plays an important role in promoting EMT in SW480 cells. In vivo experiments,

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compared with the control group, upregulation of ACSL3 has the effect of accelerating tumor volume and weight. However, the mechanism by which the upregulation of ACSL3 activates EMT in colon cancer cells is unknown.

Maria Saliakoura et al. (2020). found that ACSL3-LPIAT1 axis is a necessary condition for continuous synthesis of Prostaglandin in lung cancer. The increase of Prostaglandin level is closely related to the survival rate of cancer cells and the enhancement of tumor growth, migration, invasion and immunosuppression Chen et al. (2016). ACSL3-LPIAT1 metabolic axis drives Prostaglandin synthesis and promotes CRC tumorigenesis Saliakoura et al. (2020). We hypothesized that the ACSL3-LPIAT1 metabolic axis also affects CRC progression by driving Prostaglandin synthesis. And cPLA2 is highly expressed in NSCLC and CRC Wang et al. (2010). In this study, the expression levels of LPIAT1 and cPLA2 also showed an increasing trend when overexpressing ACSL3. However, silencing ACSL3 partially reversed the increased expression of LPIAT1 and cPLA2 in CRC cells induced by overexpression of ACSL3. This indicates that ACSL3 and LPIAT1 regulate the proliferation of CRC cells by acting at least partially on the same metabolic axis.

The tumor marker CEA is considered a glycoprotein that can be detected in blood and adenocarcinoma cancer cells Yoo et al. (2011), Hammarstrom et al. (1999). It is produced by fetal intestinal tissue and epithelial tumor cells and contributes to angiogenesis Sisik et al. (2013). Serum CEA levels in colorectal cancer, breast cancer, gastric cancer, lung cancer, ovarian cancer, pancreatic cancer and other malignant tumors increased Hammarstrom et al. (1999). Research has shown that the CEA sensitivity of CRC patients ranges from 65% to 74% Yakabe et al. (2010). In this study, we also tested the CEA levels in the cell culture supernatant of the high expression ACSL3 group and the silent ACSL3 group, and found that the CEA levels in the cell culture supernatant of the overexpression ACSL3 group were significantly higher than those of the control group. However, the CEA levels in the cell culture supernatant of the silent ACSL3 group were significantly reduced, which is consistent with the prediction.

In summary, we have studied for the first time that ACSL3 is closely related to EMT and can promote the migration and invasion of colorectal cancer cells. The upregulation of ACSL3 mediates the mechanism of EMT, which is that ACSL3-LPIAT1 metabolic axis drives the synthesis of Prostaglandin, promotes the occurrence of CRC tumors, and also provides a theoretical basis for the mechanism of CRC EMT.

CONCLUSION

In summary, we determined that ACSL3 promotes the proliferation, invasion and migration of colorectal cancer cells through the ACSL3-LPAIAT1 pathway.

CURRENT & FUTURE DEVELOPMENTS:

This study verified and demonstrated the function of ACSL3 gene in colorectal cancer cells and the signal transduction pathway involved, providing a theoretical basis for the mechanism of CRC EMT, providing a new genetic marker for the prognosis of colorectal cancer and a new target for the treatment of colorectal cancer.

DECLARATIONS

Ethics approval and consent to participate

The present study was authorized by the Ethics Committee of the Shenzhen People's Hospital (Shenzhen, China; No.SOP-QT-031-01), and the animal experiments were performed in the accordance with ARRIVE guidelines (https://arriveguidelines.org).

Cell line catalog number

Cell line were purchased by ATCC (https://www.atcc.org/) website.SW480 (ATCC CCL-228), SW620 (ATCC CCL-227), LoVo (ATCC CCL-229), HT29 (ATCC HTB-38), Caco-2 (ATCC HTB-37), and HCT116 (ATCC CCL-247).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no confict of interest.

Funding

Not applicable.

Authors' contributions

Liu designed the study and wrote the manuscript. Chen analysed the data and prepared figures. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

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