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ORIGINAL PAPER

Preliminary study on the mechanism of POFUT1 in colorectal cancer

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Abstract

To analyse the effect of POFUT1 (Protein O-Fucosyltransferase 1) on the proliferation, migration and apoptosis of colorectal cancer (CRC) cells and to explore its potential mechanism. The effects of POFUT1 silencing in vitro on the proliferation, migration, and apoptosis of CRC cells were investigated using the SW480 and RKO cell lines. The effect of POFUT1 expression on cell phenotype was detected by cell proliferation assay (CCK8), colony formation assay, flow cytometry, wound healing assay, transwell assay, cell apoptosis assay, etc. In vitro, silencing of POFUT1 resulted in decreased proliferation, cell cycle arrest, reduced migration and increased apoptosis of CRC cells. In CRC cells, POFUT1 plays a tumour-promoting role by promoting cell proliferation and migration and inhibiting apoptosis.

Keywords Colorectal cancer · POFUT1 · Proliferation · Migration · Apoptosis

Introduction

Colorectal cancer (CRC) is one of the most common malignant tumours of the digestive system in the world. According to World Health Organization (WHO) statistics, with 1.93 million new CRC patients worldwide in 2020, the incidence rate was the 3rd highest, and the mortality rate was the 2nd highest (935,000 cases), second only to lung cancer. Meanwhile, in China, with 555,000 new colorectal cancer patients in 2020, the incidence rate ranked second, with 286,000 deaths, ranking fifth [1]. The incidence and mortality rate of colorectal cancer are on the rise year by year, which poses a serious threat to human health.

Previous studies have confirmed that high expression of the POFUT1 (Protein O-Fucosyltransferase 1) gene can be associated with a worse prognosis in multiple cancers [2, 3], and this gene is generally highly expressed in colorectal cancers [4, 5]. Immunohistochemistry of clinical tissue specimens has also demonstrated that POFUT1 is highly expressed in colorectal cancer but shows low expression in adjacent tissues and distant cancerous tissues. However, the effect of POFUT1 on the development and progression of colon cancer remains unclear. In this study, the effects of POFUT1 on the proliferation, migration, and apoptosis of CRC cells were investigated by silencing POFUT1 in vitro.

Materials and methods

Materials

RKO and SW480 cell lines were used for CRC research. All cells were purchased from Xiangya Medical College, Central South University (Hunan, China). Rabbit anti-human POFUT1 polyclonal antibody (A8338) was purchased from Boster Biological Technology Co., Ltd. (Wuhan, China). Sth-POFUT1-001/002/003 was purchased from RiboBio Co., Ltd. (Guangzhou, China). POFUT1 primers were purchased

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from Tsingke Biotechnology Co., Ltd. (Beijing, China). Matrigel glue was purchased from Becton, Dickinson and Company (New Jersey, USA). The CCK-8 kit and Crystal Violet were purchased from Biological Industries (Ra'anana, Israel).

Methods

Cell culture and transfection

SW480 and RKO cells were cultured with Dulbecco's modified Eagle's medium (DMEM) + 10% foetal bovine serum + 1% penicillin/streptomycin (Gibco, Carlsbad, CA, USA) in a 37 °C + 5% CO_2 incubator (Thermo Fisher Scientific, Waltham, MA, USA). After adhesion growth of cells, the next day, the culture medium could be changed to a new medium, and the growth conditions were observed.

The cultured SW480 and RKO cell lines were transfected. Solution A consisted of the following: 245 μL OpTI-MEM/well + 5 μL Lipofectamine 2000/well. Solution B consisted of the following: 245 μL OpTI-MEM/well + 5 μL siRNA/well placed at room temperature for 5 min. Liquid B was added to liquid A and mixed. After 20 min at room temperature, 500 μl of the mixture was added to each well. After 48 h and 72 h, RNA and protein were extracted to detect cell transfection efficiency. The target sequences of siRNA used in the experiment are shown in Table 1.

q-PCR

According to the operation steps of the RNA extraction kit, total RNA in cells was extracted, and RNA was transcribed into cDNA using a reverse transcription system. The relative expression of POFUT1 mRNA was detected by a real-time PCR kit (GAPDH was used as an internal reference), and the primer sequences are shown in Table 2.

Table 1 siRNA target sequences

The name of the primer	Target sequences
SiPOFUT1-A	GGATCAGTTTCATGTGATG
SiPOFUT1-B	GGCATTTCCTTCAGTGCTT
SiPOFUT1-C	GGGTGGCATACTGCTTTGA

Table 2 qPCR primer names and sequences

The name of the primer	Target sequences
GAPDH	F: 5′-CCAGCAAGAGCACAAGAGGAA-3′
	R: 5′-ATGGTACATGACAAGGTGCGG-3′
POFUT1	F:5′-CCAGATTCATGCCCACCTTG-3′
	R:5′-TCAGGCAGGCACATAGTCAT-3′

Western blot method

Cells were lysed with protein lysis buffer on ice to extract total protein, and the protein concentration was determined by the BCA method. After SDS−PAGE, the sample was transferred to PVDF membranes. After the membrane transfer was completed, the PVDF membrane was placed in 5% skimmed milk (TBST dilution) and blocked at room temperature for 1.5 h. The primary antibody and secondary antibody were incubated. After washing, the corresponding volume of developer (covering the surface of the membrane) was added according to the strip size and developed on the imager. The pictures are stored on the computer for backup.

Cell proliferation assay (CCK8)

Cells were seeded in 96-well plates, CCK-8 solution was added, and the cells were placed in the incubator for 4 h. OD values were measured at 490 nm with a microplate reader. The data were saved and used as starting values for standard curves. The number of cells at 24 h, 48 h, 72 h and 96 h was observed. After the measurement, all the data were taken with time as the horizontal coordinate and OD value as the vertical coordinate, and the cell proliferation curve was plotted.

Colony formation assay

Cells in a good growth state were selected for the colony formation assay. Clone formation was observed daily in six-well plates with a density of 500 cells per well. After approximately 14 days of culture, the cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet solution. Clones with more than 50 cells were observed under a microscope and photographed, and the proliferation capacity of the cells was calculated.

Flow cytometry

The cell samples to be tested were cleaned, digested and centrifuged. The cells were fixed with precooled 70% ethanol at 4 °C. After fixation overnight, the samples were centrifuged and cleaned again. Appropriate PI was added into each tube and treated at a low temperature for 30 min away from light. Flow cytometry was used for detection and analysis.

Wound healing assay

Cells in a good growth state were added to a six-well plate at 2×10^6 cells per well. After the cells were overgrown, the cells were washed twice with PBS. Serum-free medium was added, and the cells were photographed under an inverted microscope and labelled for 0 h. After 48 h of culture, the

cells were observed and photographed under an inverted microscope, and the migration distance and migration ability of the cells were calculated.

Transwell assay

The cells were plated in 6-well plates and resuspended in 200 µL serum-free medium after transfection. The cell suspension was added to the upper chamber of the Transwell chamber and the lower chamber with 10% FBS medium. After culture, the number of cells passing through the membrane in each group was observed under a fluorescence microscope, and the field of view was randomly selected under the microscope to take photos, record and analyse the number of cells.

Cell apoptosis assay

The cultured cells were washed twice with PBS, 5 µl FITC Annexin-V + 5 µl propidium iodide (PI) was added, and the two dyes were evenly mixed together and kept away from light for 15–30 min at room temperature. Finally, the data were analysed by flow cytometry.

Statistical analysis

Data were imaged with GraphPad Prism v5.0 software, and differences between the two groups were expressed as the mean ± standard deviation. SPSS 22.0 software was used for statistical analysis. Measurement data were analysed by t-test. Two-sided a = 0.05 was used as the test level, and $p < 0.05$ was considered statistically significant, where * means $p < 0.05$, ** means $p < 0.01$, and *** means $p < 0.001$.

Results

Silencing POFUT1 inhibits the proliferation of CRC cells in vitro

Silencing POFUT1 in vitro

To investigate the cellular effects in SW480 and RKO cells after POFUT1 silencing in vitro, we used transient transfection siRNA to inhibit POFUT1 expression. First, RKO cells were transfected with siPOFUT1-A, siPOFUT1-B, or siPOFUT1-C, and the silencing efficiency of POFUT1 was detected. Q-PCR and Western blot analysis showed that all three siRNAs had obvious silencing effects, and siPOFUT1-A had the best silencing effect (Fig. 1a, b). Based on the above results, siPOFUT1-A was transfected into SW480 cells, and POFUT1 expression was detected. The results showed that POFUT1 expression was significantly decreased in SW480 cells, consistent with the results observed in RKO cells (Fig. 1c, d). Therefore, siPOFUT1-A, which had the best silencing effect, was selected for further experiments.

Silencing POFUT1 inhibits the proliferation of CRC cells in vitro

RKO and SW480 cells were selected for the cell proliferation assay to study the effect of POFUT1 silencing on the proliferation ability of CRC cells. After POFUT1 silencing, the number of cells at 24 h, 48 h, 72 h and 96 h was observed, and the proliferation curve was plotted. The results showed that the cell proliferation rate of the POFUT1 silencing group was significantly decreased, and the number of cells in the four periods was much less than that in the NC control group (Fig. 1e, f). These findings suggest that POFUT1 silencing can inhibit the proliferation of CRC cells.

Silencing POFUT1 inhibits clonogenesis of CRC cells in vitro

To further investigate the inhibitory effect of POFUT1 silencing on the proliferation of human CRC cells, RKO and SW480 cells were used in colony formation assays. The results showed that the number of cell colonies in the POFUT1 silencing group was significantly reduced compared with that in the normal group (Fig. 1g, h), which suggested that POFUT1 silencing could inhibit the colony formation of colorectal cancer cells.

Silencing of POFUT1 affects the cell cycle of CRC cells in vitro

The effect of POFUT1 silencing on the cell cycle of human CRC cells was detected by flow cytometry. In RKO and SW480 cells, the number of G0/G1 phase cells increased significantly after POFUT1 silencing (Fig. 1i, j). The results indicated that POFUT1 silencing led to cell cycle arrest in the G0/G1 phase and thus affected the cell cycle of CRC cells.

Silencing POFUT1 inhibits the migration of CRC cells in vitro

We evaluated the biological effects of POFUT1 on the migration behaviour of CRC cells using wound healing and transwell assays. In RKO and SW480 cells, the results of the wound healing assay showed that compared with the control cells, the POFUT1 silencing group showed a reduced wound healing speed (Fig. 2a, b, d). The results of the transwell assay showed that the number of cells penetrating the membrane in the POFUT1 silencing group was significantly reduced compared with that in the control group (Fig. 2c, e).

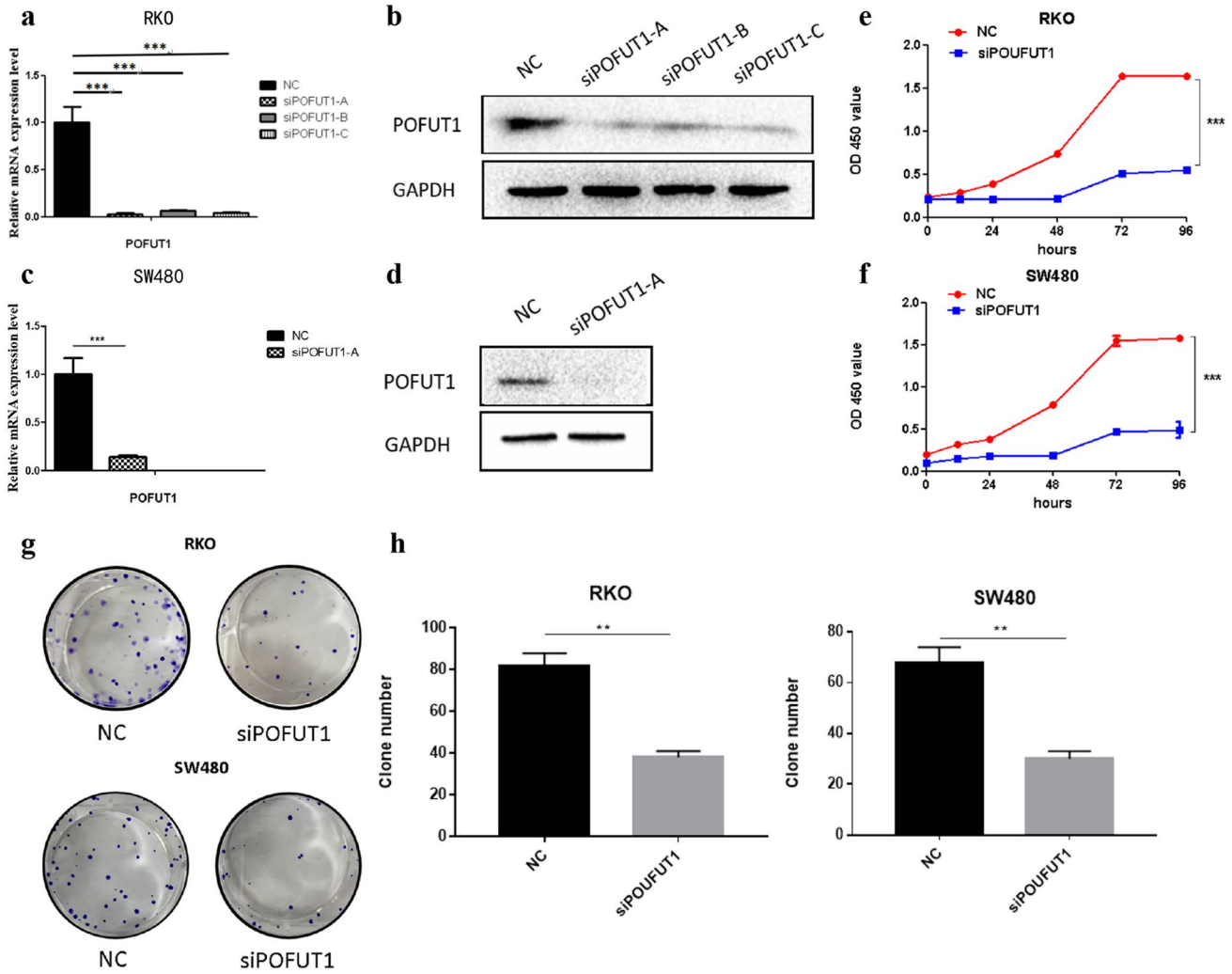


Fig. 1 Silencing POFUT1 inhibits the proliferation of CRC cells in vitro. **a** Relative mRNA levels of POFUT1 were measured by real-time q-PCR assays after RKO cells were transfected with different siRNAs (siPOFUT1-A, siPOFUT1-B and siPOFUT1-C) (***, siPOFUT1-A group, siPOFUT1-B group and siPOFUT1-C group compared with NC group, $p < 0.001$); **b** Protein levels of POFUT1 were measured by Western blot assays after RKO cells were transfected with different siRNAs (siPOFUT1-A, siPOFUT1-B and siPOFUT1-C); **c** Relative mRNA levels of POFUT1 were measured by real-time q-PCR assays after SW480 cells were transfected with siPOFUT1-A (***, siPOFUT1-A group compared with NC group, $p < 0.001$); **d** Protein levels of POFUT1 were measured by Western blot assays after RKO cells were transfected with siPOFUT1-A. **e, f** Effect of siPOFUT1 on the proliferation of RKO cells and SW480 cells (***, siPOFUT1 group compared with NC group, $p < 0.001$); **g** Effect of siPOFUT1 on colony formation of RKO cells and SW480 cells; **h** Quantification of the colony formation results (**, siPOFUT1 group compared with NC group, $p < 0.01$); **i, j**. Effect of siPOFUT1 on the cell cycle of RKO cells and SW480 cells (**, siPOFUT1 group compared with NC group, $p < 0.01$)

The results indicated that the silencing of POFUT1 expression could inhibit the migration ability of CRC cells.

Silencing POFUT1 promotes apoptosis of CRC cells in vitro

To investigate the effect of POFUT1 silencing on the apoptosis of tumour cells, annexin V-FITC staining was used to evaluate the apoptosis rate. The results indicated that the apoptosis rate of CRC cells after POFUT1 silencing was higher, especially that of the RKO cell line (Fig. 3a, b). Therefore, the above experiments indicated that POFUT1 silencing could induce the apoptosis of CRC cells.

Discussion

Extensive evidence suggests that many molecules play a crucial role in CRC tumorigenesis and development, such as Bcl-3 [6], Claudin-3 [7], and CDK1 [8]. These abnormally expressed signalling molecules cause activation of downstream promoting tumour signalling pathways, which causes

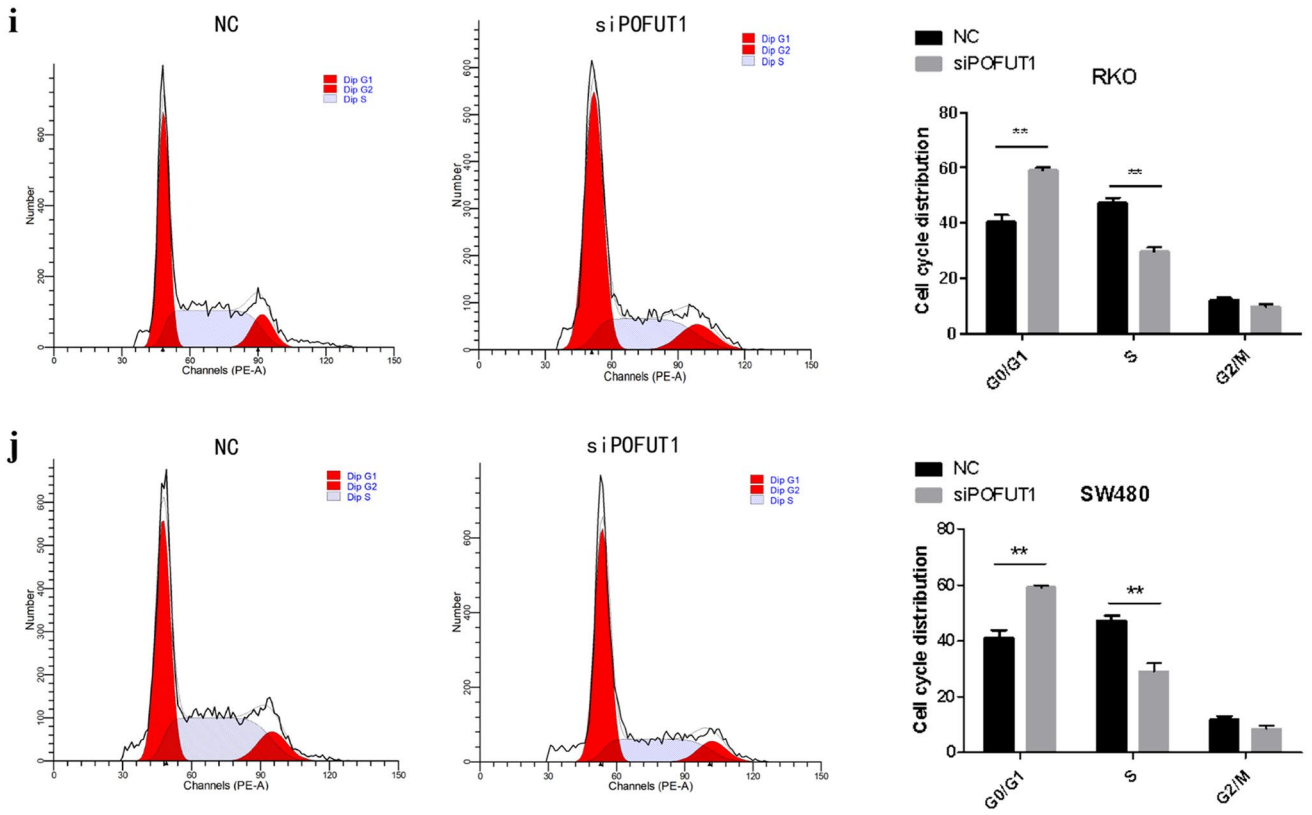


Fig. 1 (continued)

abnormal tumour cell proliferation, invasion, and migration. Recent studies have shown that glycosyltransferases, such as FUT5 and FUT6, play a role in the progression of tumour cells, which can mediate the proliferation, invasion and migration of CRC cells. The structures of POFUT1 and POFUT2 are very similar, and both belong to the FUT family. Using GDP-Fuc as a glycosyl donor, L-Fuc was transferred to the side hydroxyl group of the Ser/Thr peptide chain and catalysed the synthesis of the O-fucoside bond [9, 10]. POFUT1, a key kinase that catalyses O-fucose synthesis, encodes O-fucosyltransferase, which adds O-focuse to serine/threonine residues of EGF-like repeat C2XXXX(S/T)C3. Studies have shown that O-fucosylation is essential for the function of some EGF-like repeats [11]. POFUT1 is widely expressed in cells, but its role in various cells and its mechanism of action remain unclear.

Human POFUT1 is encoded by a stretch of the gene located between PLAGL2 and KIF3B on the long arm of chromosome 20 and consists of 393 amino acids. In mice, knockout of the homologous gene POFUT1 is fatal. POFUT1 is essential for the structure, function, and survival of mouse podocytes [11]. POFUT1 promotes the invasion and migration of trophoblast cells at the maternal-foetal interface. The expression level of POFUT1 in different states of pregnant women showed significant differences. The expression level of POFUT1 in the placental villi formed in pregnant women with threatened abortion was decreased but increased in the placental villi formed in normal pregnant women. POFUT1 also promotes uterine angiogenesis and vascular remodelling by enhancing the O-fucosylation of uPA [12]. POFUT1 deficiency has also been associated with other abnormalities in animals and humans, including skin and cardiovascular diseases, microcephaly, and myogenic fusion [13–16]. Knockdown of POFUT1 can effectively inhibit the proliferation of oral squamous cell carcinoma [17]. Studies have found that POFUT1 is overexpressed in a variety of cancers and promotes tumour development. POFUT1 plays an extremely important role in many diseases, especially in malignant tumours, and is involved in tumour proliferation, invasion, metastasis and apoptosis [4, 18–20]. Although POFUT1 has been found to play a role in some solid cancers during cancer development, little is known about its role in CRC. Studies have shown that POFUT1-silenced CRC cells have reduced proliferation, cell cycle arrest, reduced migration, and higher levels of apoptosis [4]. The importance of POFUT1 in promoting CRC tumorigenesis in vivo was confirmed by a mouse model of CRC development, indicating that POFUT1 is an important CRC-related oncogene, and its overexpression is inherently associated with CRC tumorigenesis [4].

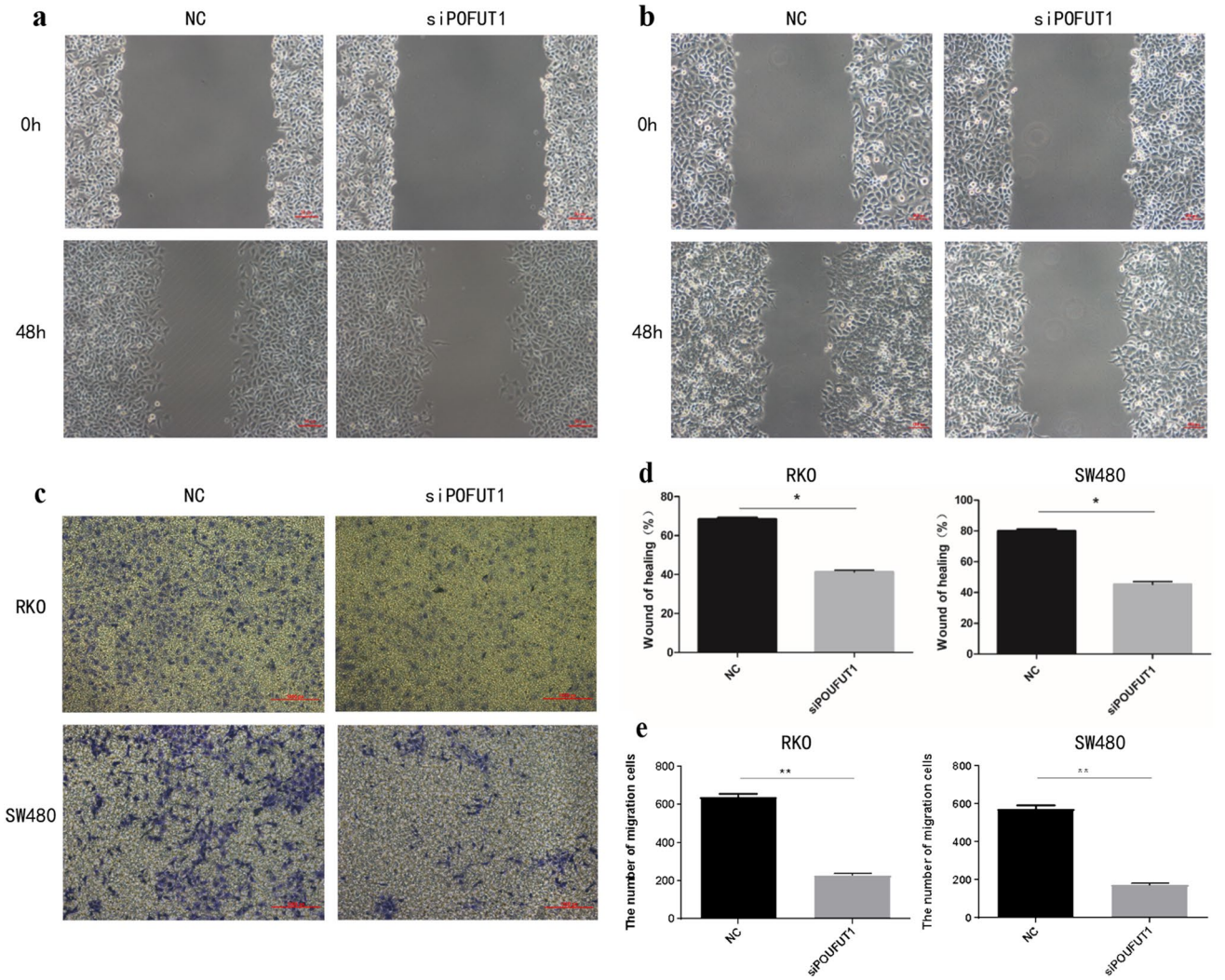


Fig. 2 Silencing POFUT1 inhibits the migration of CRC cells in vitro. **a**,**b**. Wound healing assay. Effect of siPOFUT1 on the cell migration ability of RKO cells and SW480 cells; **c** Transwell assays. Effect of siPOFUT1 on the migration ability of RKO cells and SW480 cells. **d** Quantification of the wound healing assay (*, siPOFUT1 group compared with NC group, $p < 0.05$); **e** Quantification of the Transwell assays (**, siPOFUT1 group compared with NC group, $p < 0.01$)

In this study, two CRC cell lines (SW480 and RKO) were selected for cytological experiments. Through cell proliferation assay (CCK8), colony formation assay, flow cytometry, wound healing assay, transwell assay and cell apoptosis assay, it was found that in vitro silencing of POFUT1 results in decreased proliferation, cell cycle arrest, reduced migration and increased apoptosis of CRC cells. At present, the main known target of POFUT1 is the Notch receptor. Notch is a widely existing and highly conserved signalling pathway in mammals that can regulate tumour proliferation and metastasis and has become an important signalling pathway in tumorigenesis [21, 22]. Therefore, to further explore the mechanism of POFUT1 in CRC, the relationship between POFUT1 and the Notch signalling pathway and its possible signalling pathway deserves further study.

In conclusion, in vitro silencing of POFUT1 can inhibit proliferation and migration and induce apoptosis of CRC cells. POFUT1 is expected to become a novel biological marker for the clinical diagnosis of CRC. This study provides new insights for the development of novel treatment strategies for CRC and the assessment of patient prognosis.

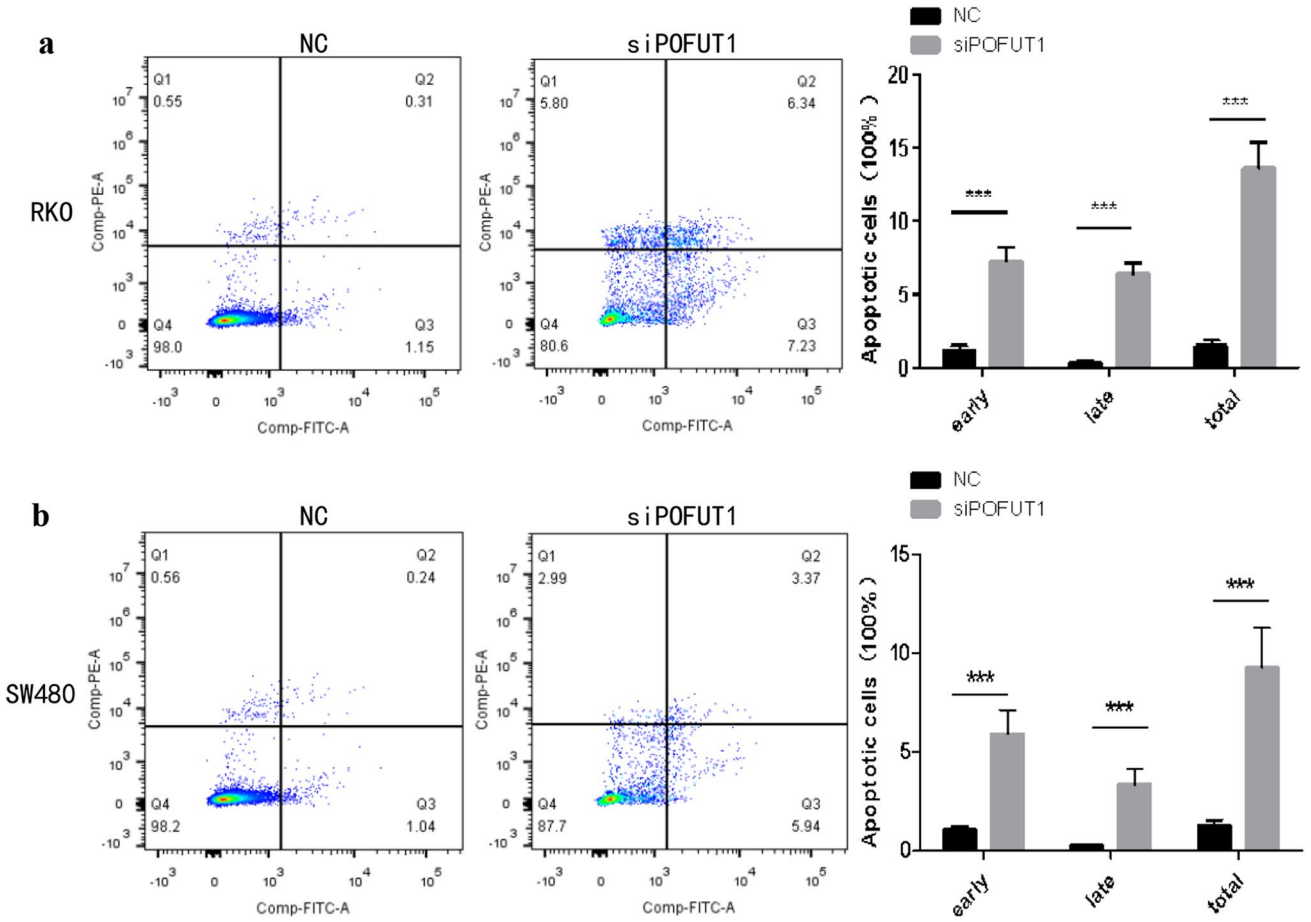


Fig. 3 Silencing POFUT1 promotes apoptosis of CRC cells in vitro. **a** Flow cytometry. Effect of siPOFUT1 on apoptosis in RKO cells; **b** Effect of siPOFUT1 on apoptosis in SW480 cells (***, siPOFUT1 group compared with NC group, $p < 0.001$)

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Data availability The data that support the findings of this study are available from the corresponding author, He Huang, upon reasonable request.

References

1. Siegel R L, Miller K D, Jemal A. Cancer statistics, 2020 . CA: a cancer journal for clinicians, 2020, 70(1): 7–30.
2. Li Q, Wang J, Ma X, et al. POFUT1 acts as a tumor promoter in glioblastoma by enhancing the activation of Notch signaling. J Bioenerg Biomembr. 2021;53(5):621–32.
3. Wan G, Tian L, Yu Y, et al. Overexpression of Pofut1 and activated Notch1 may be associated with poor prognosis in breast cancer. Biochem Biophys Res Commun. 2017;491(1):104–11.
4. Du Y, Li D, Li N, et al. POFUT1 promotes colorectal cancer development through the activation of Notch1 signaling. Cell Death Dis. 2018;9(10):995.
5. Komor MA, de Wit M, van Den Berg J, et al. Molecular characterization of colorectal adenomas reveals POFUT1 as a candidate driver of tumor progression. Int J Cancer. 2020;146(7):1979–92.
6. Chen X, Wang C, Jiang Y, et al. Bcl-3 promotes Wnt signaling by maintaining the acetylation of β-catenin at lysine 49 in colorectal cancer. Signal Transduct Target Ther. 2020;5(1):52.
7. Ahmad R, Kumar B, Chen Z, et al. Loss of claudin-3 expression induces IL6/gp130/Stat3 signaling to promote colon cancer malignancy by hyperactivating Wnt/β-catenin signaling. Oncogene. 2017;36(47):6592–604.
8. Tong Y, Huang Y, Zhang Y, et al. DPP3/CDK1 contributes to the progression of colorectal cancer through regulating cell proliferation, cell apoptosis, and cell migration. Cell Death Dis. 2021;12(6):529.
9. Chen CI, Keusch JJ, Klein D, et al. Structure of human POFUT2: insights into thrombospondin type 1 repeat fold and O-fucosylation. EMBO J. 2012;31(14):3183–97.
10. Li Z, Han K, Pak JE, et al. Recognition of EGF-like domains by the Notch-modifying O-fucosyltransferase POFUT1. Nat Chem Biol. 2017;13(7):757–63.
11. Zhang S, Yang Q, Liu Z, et al. POFUT1 is dispensable for structure, function and survival of mouse podocytes. Am J Transl Res. 2020;12(5):2212–24.
12. Zhang D, Yang Y, Liang C, et al. poFUT1 promotes uterine angiogenesis and vascular remodeling via enhancing the O-fucosylation on uPA. Cell Death Dis. 2019;10(10):775.
13. Der Vartanian A, Chabanais J, Carrion C, Maftah A, Germot A. Downregulation of POFUT1 impairs secondary myogenic

fusion through a reduced NFATc2/IL-4 signaling pathway. Int J Mol Sci. 2019;20(18):4396.
14. Li M, Cheng R, Liang J, et al. Mutations in POFUT1, encoding protein O-fucosyltransferase 1, cause generalized dowling-degos disease. Am J Hum Genet. 2013;92(6):895–903.
15. Takeuchi H, Wong D, Schneider M, et al. Variant in human POFUT1 reduces enzymatic activity and likely causes a recessive microcephaly, global developmental delay with cardiac and vascular features. Glycobiology. 2018;28(5):276–83.
16. Wang Y, Wu B, Lu P, et al. Uncontrolled angiogenic precursor expansion causes coronary artery anomalies in mice lacking Pofut1. Nat Commun. 2017;8(1):578.
17. Yokota S, Ogawara K, Kimura R, et al. Protein O-fucosyltransferase 1: a potential diagnostic marker and therapeutic target for human oral cancer. Int J Oncol. 2013;43(6):1864–70.
18. Leng Q, Tsou JH, Zhan M, et al. Fucosylation genes as circulating biomarkers for lung cancer. J Cancer Res Clin Oncol. 2018;144(11):2109–15.
19. Li D, Lin C, Li N, et al. PLAGL2 and POFUT1 are regulated by an evolutionarily conserved bidirectional promoter and are collaboratively involved in colorectal cancer by maintaining stemness. EBioMedicine. 2019;45:124–38.
20. Zhang C, Huang H, Zhang J, et al. Caveolin-1 promotes invasion and metastasis by upregulating Pofut1 expression in mouse hepatocellular carcinoma. Cell Death Dis. 2019;10(7):477.
21. Guo J, Li P, Liu X, et al. NOTCH signaling pathway and noncoding RNAs in cancer. Pathol Res Pract. 2019;215(11): 152620.
22. Nowell CS, Radtke F. Notch as a tumour suppressor. Nat Rev Cancer. 2017;17(3):145–59.

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