Balkan Med J 2024;41:491-8

Exploring the Effect of Fidgetin-Like 1 on Colorectal Cancer Through Tissue Chip and In Vitro Experiments

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Background: Fidgetin-like 1 (FIGNL1) is extensively overexpressed in a variety of cancers. It facilitates non-small cell lung cancer tumor cell proliferation and hepatocellular carcinoma formation due to abnormal DNA repair. Clinically relevant data indicates that its high expression is linked with the poor prognosis of patients with renal clear-cell carcinoma, low-grade gliomas, and hepatocellular carcinoma. Nevertheless, the scope of FIGNL1's involvement in cancer, particularly colorectal cancer (CRC), remains unclear.

Aims: To investigate the function of FIGNL1 in CRC.

Study Design: Cell culture study

Methods: The TCGA database and immunohistochemistry analysis were employed to investigate FIGNL1 expression in CRC tissue. A cell viability assay was performed using the Cell Counting Kit-8. The cell migration and invasion were evaluated using the transwell assay. Small interfering RNA (siRNA) transfection was conducted to knockdown FIGNL1 expression. Infection with FIGNL1 overexpression lentivirus was performed to promote FIGNL1 overexpression. The STRING database was employed for predicting protein interaction.

Results: FIGNL1 was substantially upregulated in human CRC tissues and was associated with TNM stages and lymph node metastasis in patients. The inhibition of CRC cell proliferation, migration, and invasion in Caco-2 cells was achieved by silencing FIGNL1 using siRNA. Additional investigations suggested that FIGNL1 overexpression could promote CRC cell proliferation, migration, and invasion via P38 signaling pathway activation in Colo-205 cells. Subsequent experiments demonstrated that FIGNL1-mediated P38 phosphorylation was contingent upon SPIDR interaction.

Conclusion: These results implied that FIGNL1 was a potential anticancer drug target, which also offered a novel strategy for future CRC treatment.

INTRODUCTION

Colorectal cancer (CRC), a common digestive tract malignancy, is the third most lethal form of cancer worldwide.[1] Its etiology is complex, owing primarily to the synergistic effect of environment, diet, lifestyle, and genetic factors. The development of CRC is relatively insidious and gradual, as it typically takes 10-15 years for normal intestinal epithelial cells to undergo neoplastic transformation. By the time the patients experience clinical symptoms, CRC has often progressed to the middle and advanced stages.[2] With the development of novel targeted therapies and technological innovation, the use of immunotherapy for tumors is progressively becoming more prevalent in clinics. For instance, the immunosuppressant epidermal growth factor receptor antibody exhibits remarkable efficacy in 50-60% of patients who lack *RAS* and *BRAF* gene mutations.[3] However, CRC-associated mortality rates continue to be elevated. Given the current state of clinical detection and treatment, it is imperative to explore novel clinical markers or drug targets to provide additional benefits to CRC patients.

The Fidgetin-like 1 (FIGNL1) protein is a member of the ATPase subgroup, which is associated with cellular active proteins. It is recruited to DNA damage sites to repair double-strand breaks of DNA, which are dependent on homologous recombination. A previous study has reported that FIGNL1 is a novel inhibitor for the formation of super-hybridized rice as it regulates DMC1

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Received: July 11, 2024 **Accepted:** August 22, 2024 **Available Online Date:** October 31, 2024 • **DOI:** 10.4274/balkanmedj.galenos.2024.2024-7-9

Available at www.balkanmedicaljournal.org

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Cite this article as: Xu Y, Shen Y, Zhang C, Zheng L, Ji F, Chen J, Cheng S, Zheng Y. Exploring the Effect of Fidgetin-Like 1 on Colorectal Cancer Through Tissue Chip and In Vitro Experiments. Balkan Med J.; 2024; 41(6):491-8.

function based on homologous chromosome recombination.[4] In mammalian cell experiments, FIGNL1 overexpression enhanced the expression of alkaline phosphatase and osteocalcin, resulting in the reduced proliferation of osteoblasts without causing cell apoptosis.[5] Researchers discovered a correlation between cancer and FIGNL1 in pan-cancer analyses as the investigation advanced.[6,7] A clinic-based study demonstrated a link between FIGNL1 and a poor prognosis in non-small cell lung cancer.[8] In vitro experiments have demonstrated that FIGNL1 overexpression in A549 and H1299 cells significantly accelerated cell proliferation by reducing the cell number in the G1 phase of the cell cycle and through apoptosis, while simultaneously increasing cell fission and migration.[9] Nonetheless, the broader function of FIGNL1 in cancer, especially in CRC, remains unclear. In this study, FIGNL1 was discovered to be upregulated in human CRC tissues and exhibited a positive correlation with cancer progression. Further in vitro experiments revealed that FIGNL1 promoted CRC cell proliferation, migration, and invasion by activating the P38 pathway. These results implied that FIGNL1 was a potential anticancer drug target, which could aid the development of novel strategies for future CRC treatment.

MATERIALS AND METHODS

Human tissue samples

Clinical specimens consisting of 206 tumor samples and 30 paracancerous tissue were acquired from the department of pathology between January 2022 and December 2023. All the patients provided consent to participate in the study, agreed to the publication of the study, and signed the informed consent form before the study. This study was conducted in accordance with the Declaration of Helsinki and approved by the Hai'an City People's Hospital Ethical Committee (approval number: 2022-K032-01, date: 06.05.2022).

Immunohistochemistry

The clinical specimens were processed into tissue microarrays. Following embedding, slicing, dewaxing, and hydration, the slides were stained with the primary antibody against FIGNL1 (1:200, 17604-1-AP, Proteintech, Wuhan, China). Two professional pathologists independently reviewed the results and determined the immunohistochemistry (IHC) score based on the staining intensity and staining area of positive cells. The score of 0 was regarded as negative, while 1 was weakly positive, 2 was medium positive, and 3 was strongly positive. A low expression group was designated by an IHC score of 0 or 1, while a high expression group was designated by an IHC score of 2 or 3.

Cell culture

The Caco-2 and Colo-205 cell lines were procured from Procell (Wuhan, China). NCM460 cells were acquired from American Type Culture Collection. The Caco-2 cells were cultured in minimum essential medium (MEM, A1451801, Gibco, New York, USA) containing 20% fetal bovine serum (FBS, 13011-8611, Tianhang, Hangzhou, China) and 1% penicillin-streptomycin (PS, C100C5, NCM Biotech, Suzhou, China). The Colo-205 cells and NCM460 cells were cultured in Roswell Park Memorial Institute-1640, which contained 10% FBS and 1% PS. Incubation of both cell lines was conducted at 37 °C with 5% CO_2.

Small interfering RNA transfection

The cells were seeded in 24-well plate up to 60% density. The small interfering RNA (siRNA) was mixed with 100 µl of non-FBS medium followed by transfection using GP-transfect-Mate (G04008, GenePharma, Shanghai, China). The cells were cultured in a total of 500 µl of medium for 48 hours after the mixture solution was added to a 24-well plate. SiRNAs targeting FIGNL1 and SPIDR were synthesized using GenePharma (Shanghai, China). The detailed sequences of siRNAs were as follows: FIGNL1 siRNA1: 5'-GGAUCAAGUUCGACCCAUAGC-3'; FIGNL1 siRNA2: 5'-GGAUCUCAACAAACAGAUAGU-3'; FIGNL1 siRNA3: 5'-GGAAACAGAUAGUAAUUAAUC-3'. The SPIDR siRNA sequence was determined from a previous study as 5'-GCUGAAGAGA AGACGAUUA-3'.[10]

FIGNL1 overexpression lentivirus infection

FIGNL1 overexpression lentivirus and control lentivirus were generated and synthesized by HanBio (Shanghai, China). The serum-free culture medium was used to innoculate the cells in a 24-well plate, and the lentivirus was incorporated into the serum-free medium with polybrene (MOI: 5). Following a 24-hour infection, the cells were cultured in a normal medium that was replaced and followed by additional treatment.

SB203580 administration

P38 inhibitor SB203580 was procured from MedChemExpress (New Jersey, USA) and dissolved in dimethyl sulfoxide. The proliferation, migration, and invasion of the cells were assessed after they were pretreated with 30 µM SB203580 for 1 hour.

Cell proliferation

Cell viability was determined using the Cell Counting Kit-8 (CCK-8) kit in accordance with the manufacturer's specifications (GK10001, GLPBIO, California, USA). Briefly, 10 µl of CCK-8 was added to each well of the 96-well plate and incubated for 30 min at 37 °C. Subsequently, absorbance was tested at 450 nm using a microplate reader.

Real-time PCR

Total RNAs were extracted from cells and cropped tissues using Trizol reagent (R401-01, Vazyme, Nanjing, China). RNA concentration was assessed using an ultramicro spectrophotometer, and equal RNAs were reverse transcribed to cDNA using the HiScript III 1st Strand cDNA Synthesis Kit (R312-01, Vazyme, Nanjing, China). Next, target mRNAs were detected using a Taq Pro Universal SYBR qPCR Master Mix (Q712-02, Vazyme, Nanjing, China). The GAPDH mRNA was used as an internal reference. The quantitative expression level was analyzed using the $2^{-\Delta\Delta}$Ct method. The primers were acquired from Sangon Biotech (Shanghai, China). The sequence information is as follows:

FIGNL1 forward primer: 5'-TACTTCGCAATTACATCTGGCAT-3';

FIGNL1 reverse primer: 5'-GGGAAATCTCAGAGTTTGCCC-3'.

GAPDH forward primer: 5'-ACAACTTTGGTATCGTGGAAGG-3';

GAPDH reverse primer: 5'-GCCATCACGCCACAGTTTC-3'.

Immunoprecipitation

The immunoprecipitation assay was performed referring to the manufacturer's instructions (P2179S, Beyotime, Shanghai, China). In brief, cell lysate was added to the cells, and the supernatant was obtained by centrifugation. The protein A/G magnetic beads were pre-incubated with FIGNL1 antibody, thoroughly mixed with the cell lysate, and the mixture was incubated overnight at 4 °C. After the magnetic beads were washed, samples were prepared for Western blot analysis.

Western blot

Western blot analysis was conducted as described in previous studies.[11,12] The samples were lysed in RIPA lysis buffer (P0013C, Beyotime, Shanghai, China) with a protease inhibitor cocktail (P1005, Beyotime, Shanghai, China) to extract the total proteins. This was followed by protein quantification using an enhanced BCA protein assay kit (P0010S, Beyotime, Shanghai, China). Proteins of different molecular weights were segregated using polyacrylamide gel electrophoresis and then transferred to polyvinylidene difluoride (PVDF) membranes. The PVDF membranes were incubated with primary antibody in 5% non-fat milk at 4 °C overnight. After incubation with a second antibody labeled with horseradish peroxidase (SA00001-1 and SA00001-2, Proteintech, Wuhan, China) at room temperature, an enhanced chemic luminescence solution was employed to record the band signal by chemiluminescence imaging system (Tanon, Shanghai, China). Details of primer antibodies used in this study are as follows: FIGNL1 (17604-1-AP, Proteintech, Wuhan, China), GADPH (60004-1-Ig, Proteintech, Wuhan, China), p-JNK (80024-1-AP, Proteintech, Wuhan, China), T-JNK (24164-1-AP, Proteintech, Wuhan, China), p-ERK (28733-1-AP, Proteintech, Wuhan, China), T-ERK (11257-1-AP, Proteintech, Wuhan, China), p-P38 (28796-1-AP, Proteintech, Wuhan, China), T-P38 (14064-1-AP, Proteintech, Wuhan, China), SPIDR (HPA041582, Sigma-Aldrich, Missouri, USA).

Cell invasion and migration assay

A ranswell assay was conducted to assess the cell invasion and migration using cell chambers (724321, NEST, Suzhou, China). In the migration assay, the cells were seeded in the upper chambers without FBS and incubated for 48 hours. The lower chambers were filled with FBS-supplemented medium. The upper chambers were covered with Matrigel for the invasion assay, and the subsequent operations were identical. The diaphragms were fixed with methanol and then stained with crystal violet solution (C0121, Beyotime, Shanghai, China). Subsequently, images were acquired using a microscope.

Statistical analysis

We used the GraphPad Prism 6 software to perform statistical analysis. Data were presented as mean ± standard deviation. A two-tailed Student's t-test was used to compare the difference between two groups, and one-way analysis of variance was employed to determine the difference among multiple groups. Each experiment was conducted at least three times.

RESULTS

FIGNL1 was upregulated in human CRC samples

We initially explored FIGNL1 expression in the TCGA database and detected increased FIGNL1 expression in CRC cells (Figure 1a). This finding was corroborated by IHC analysis on the tissue microarray (Figure 1b). In addition, the IHC-score-based division of the samples into low and high expression groups revealed that FIGNL1 overexpression was closely associated with TNM stages and lymph node metastasis. However, no correlation was observed between FIGNL1 and other clinicopathological parameters (Table 1).

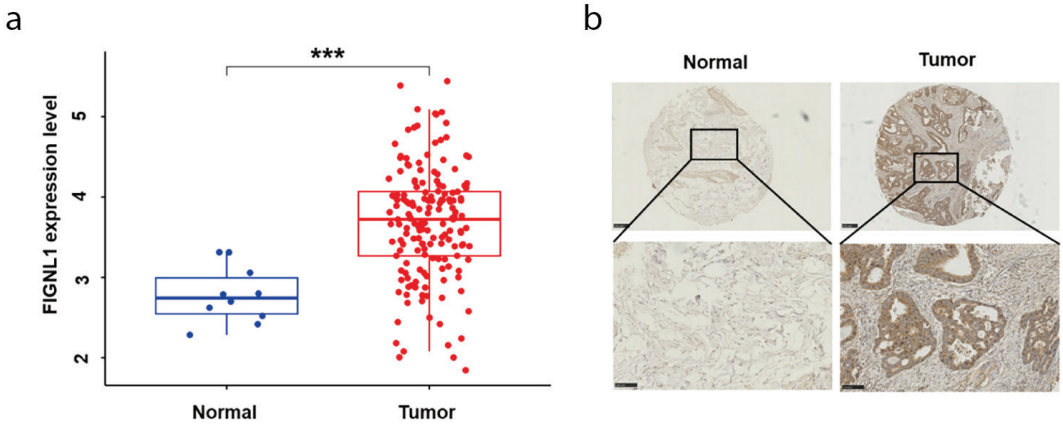

FIG. 1. High FIGNL1 expression in human CRC samples. (a) The expression of FIGNL1 in the TCGA database. (b) Immunohistochemical detection of FIGNL1 expression in clinical CRC samples.
FIGNL1, fidgetin-like 1; CRC, colorectal cancer.

Silencing FIGNL1 suppressed CRC cell proliferation, migration, and invasion

To determine the impact of FIGNL1 on CRC progression, we evaluated the FIGNL1 expression in Colo-205 and Caco-2 cells, with NCM460 cells as a health control. As depicted in Figure 2a, Colo-205 cells demonstrated a reduced FIGNL1 expression compared to Caco-2 cells. Caco-2 cells were transfected with four siRNAs that targeted FIGNL1 mRNA. The FIGNL1 siRNA 1 demonstrated optimal knockdown efficiency in FIGNL1 mRNA expression (Figure 2b) and protein expression (Figure 2c). FIGNL1 siRNA 1 was employed in the subsequent functional experiment. The proliferation of Caco-2 cells

TABLE 1. Relationship Between FIGNL1 and Clinicopathology in the CRC Cohort.

Characteristics	n	FIGNL1		χ^2	*p* value
		Low expression	High expression		
Total	206	43	163		
Age				0.0273	0.869
≤ 60	62	12 (27.9)	50 (30.7)		
> 60	144	31 (72.1)	113 (69.3)		
Sex				1.0229	0.312
Male	89	22 (51.2)	67 (41.1)		
Female	117	21 (48.8)	96 (58.9)		
TNM stage				8.1642	0.004*
1 and 2	123	17 (39.5)	106 (65.0)		
3 and 4	83	26 (60.5)	57 (35.0)		
Node stage				10.091	0.001*
Negative	127	17 (39.5)	110 (67.5)		
Positive	79	26 (60.5)	53 (32.5)		

CRC, colorectal cancer; FIGNL1, Fidgetin-like 1.

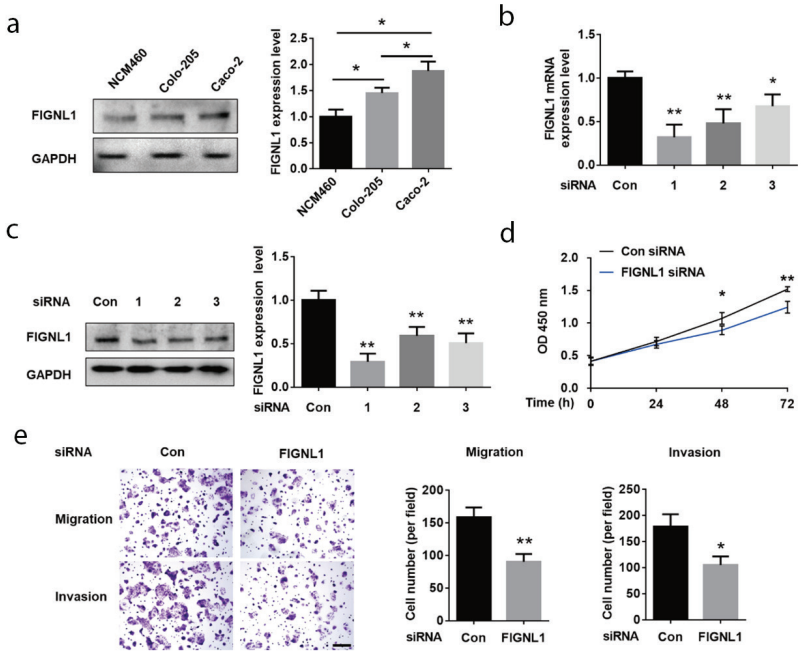

FIG. 2. FIGNL1 knockdown inhibited Caco-2 cell proliferation, migration, and invasion. (a) Representative blot demonstrating FIGNL1 expression in NCM460, Colo-205, and Caco-2 cells. (b) Bar chart illustrating FIGNL1 mRNA expression following FIGNL1 siRNA treatment in Caco-2 cells. (c) Representative blot depicting FIGNL1 expression following FIGNL1 siRNA treatment in Caco-2 cells. (d) Line chart exhibiting Caco-2 cell proliferation with FIGNL1 siRNA treatment. (e) Cell migration and invasion were examined using transwell assay after FIGNL1 siRNA transfection in Caco-2 cells. Scale bar: 500 μm. $^{*}p < 0.05$ and $^{**}p < 0.01$ versus control siRNA group.
Con, control; OD, optical density; FIGNL1, fidgetin-like 1; CRC, colorectal cancer; siRNA, Small interfering RNA.

was significantly inhibited at 48 h and 72 h time points following FIGNL1 knockdown (Figure 2d). Moreover, FIGNL1 knockdown significantly inhibited Caco-2 cell migration and invasion (Figure 2e). These results suggested that inhibition of FIGNL1 expression may suppress CRC progression in vitro.

FIGNL1 overexpression enhanced CRC cell proliferation, migration, and invasion

The Colo-205 cells were infected with the lentivirus vector for FIGNL1 overexpression. Compared with the control lentivirus group, FIGNL1 overexpressive lentivirus increased the FIGNL1 mRNA (Figure 3a) and protein expression (Figure 3b). FIGNL1 overexpression facilitated Colo-205 cell proliferation at time points of 48 h and 72 h (Figure 3c). Moreover, FIGNL1 overexpression prominently induced Colo-205 cell migration and invasion (Figure 3d). These results implied that FIGNL1 promotes CRC progression in vitro.

FIGNL1 affected CRC progression by activating the P38 pathway

Previous studies have revealed that the ERK/JNK/P38 MAPK signaling pathways were involved in the regulation of several cancers.[13,14] Thus, we explored whether FIGNL1 relied on the ERK/JNK/P38 MAPK signaling pathways to facilitate CRC progression. Transfection of FIGNL siRNA into the Caco-2 cells decreased P38 phosphorylation, while the phosphorylation level of JNK and ERK was minimally affected (Figure 4a). FIGNL1 overexpression in the Colo-205 cells increased P38 phosphorylation accompanied by unaltered phosphorylation of JNK and ERK (Figure 4b). Proliferation, migration, and invasion assays were conducted with the P38 inhibitor sb203580 to confirm the role of P38 in FIGNL1-meadiated CRC's progression. Pretreatment with sb203580 inhibited the elevated Colo-205 cell proliferation caused by the FIGNL1 overexpression-inducing lentivirus infection (Figure 4c). Furthermore, sb203580 also inhibited Colo-205 cell migration and invasion in the presence of FIGNL1 overexpression-inducing lentivirus (Figure 4d). These results indicated that FIGNL1 induced CRC progression by activating the P38 pathway in vitro.

FIGNL1 activated P38 pathway by interaction with SPIDR

Our study also aimed to investigate the mechanism by which FIGNL1 activates the P38 pathway. By employing the STRING database to predict protein interactions, we discovered that the proteins interacting with FIGNL1 were primarily associated with DNA function, specifically double-strand break repair via homologous recombination, as indicated by gene ontology analysis (Figure 5a, b). The biological functions of FIGNL1 may be significantly influenced by SPIDR, as indicated by the collective scores of a variety of indicators (Figure 5c). Immunoprecipitation assays revealed that FIGNL1 overexpression augmented the binding between FIGNL1 and SPIDR (Figure 5d). P38 phosphorylation was suppressed in cells that overexpressed FIGNL1 because of SPIDR knockdown (Figure 5e). These results suggested FIGNL1 activated P38 phosphorylation by interaction with SPIDR (Figure 6).

DISCUSSION

Globally, CRC is the third most prevalent and lethal malignancy, and its incidence is increasing annually.[15] Additionally, the incidence of early-onset CRC among young individuals is on the rise, which presents an increasingly severe challenge to global public health.[16,17] The diverse subtypes of CRC present substantial obstacles to early

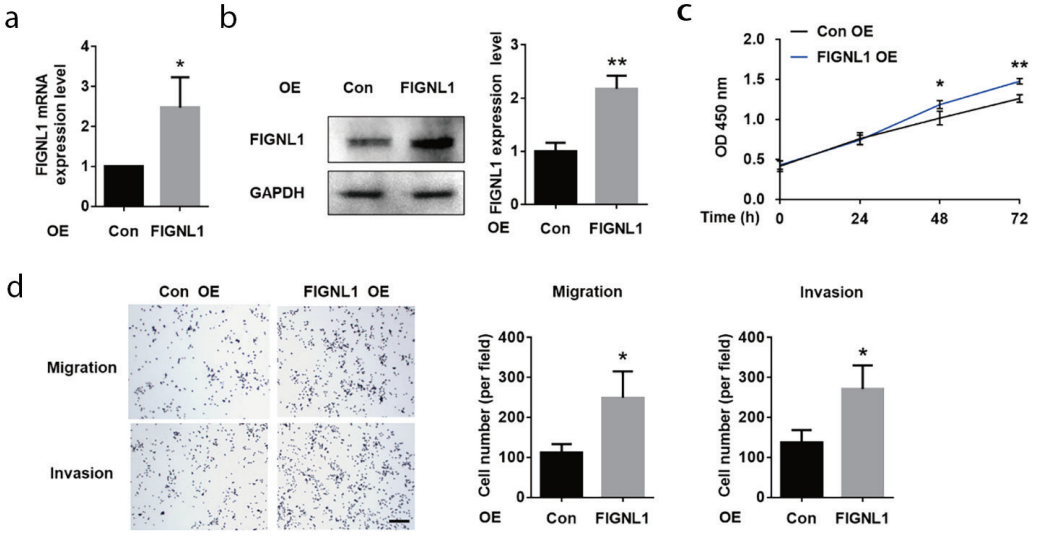

FIG. 3. FIGNL1 overexpression promoted Colo-205 cell proliferation, migration, and invasion. (a) Bar chart displaying FIGNL1 mRNA expression following FIGNL1 overexpression lentivirus infection in Colo-205 cells. (b) Representative blot depicting FIGNL1 expression after FIGNL1 overexpression-inducing lentivirus infection in Colo-205 cells. (c) Line chart exhibiting the Colo-205 cell proliefration after FIGNL1 overexpression-inducing lentivirus infection. (d) Cell migration and invasion were assessed using transwell assay after FIGNL1 overexpression-inducing lentivirus infection in Colo-205 cells. Scale bar: 200 μm. $^{*}p < 0.05$ and $^{**}p < 0.01$ versus control overexpression group.
FIGNL1, fidgetin-like 1; Con, control; OD, optical density; OE, overexpression.

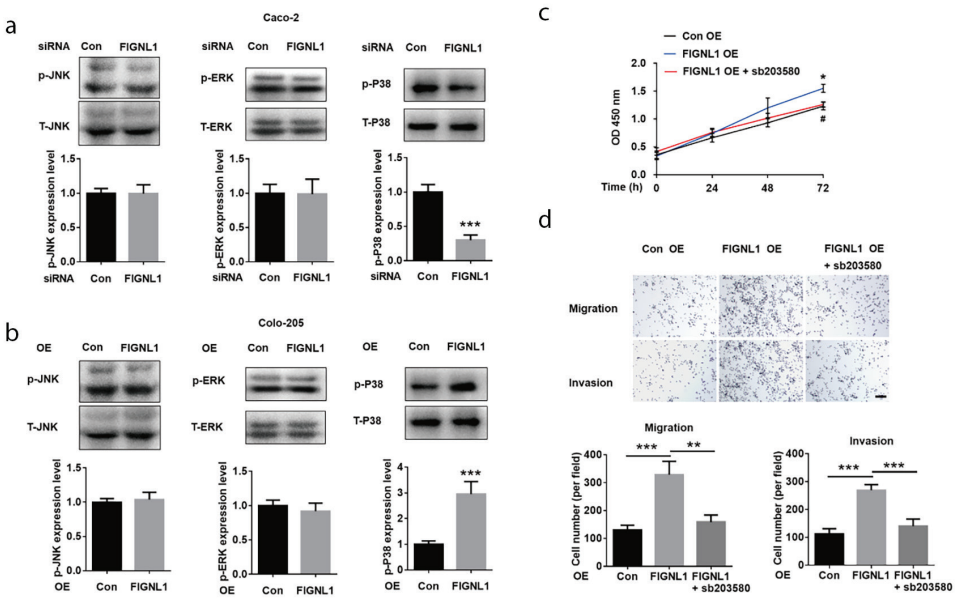

FIG. 4. FIGNL1 promoted CRC progression by activating the P38 pathway in vitro. (a) Representative blots demonstrating ERK/JNK/P38 MAPK expression following FIGNL1 siRNA treatment in Caco-2 cells. (b) Representative blots depicting ERK/JNK/P38 MAPK expression with lentivirus infection-related FIGNL1 overexpression in Colo-205 cells. (c) Line chart illustrating the Colo-205 cell proliferation following sb203580 treatment after FIGNL1 overexpression and control lentivirus infection. (d) Cell migration and invasion were examined by transwell assay after FIGNL1 overexpression-inducing lentivirus infection and sb203580 treatment in Colo-205 cells. Scale bar: 200 μm. $^{*}p < 0.05$ versus control overexpression group. $^{\#}p < 0.05$ versus FIGNL1 overexpression group.

FIGNL1, Fidgetin-like 1; Con, control; OD, optical density, OE, overexpression; siRNA, Small interfering RNA

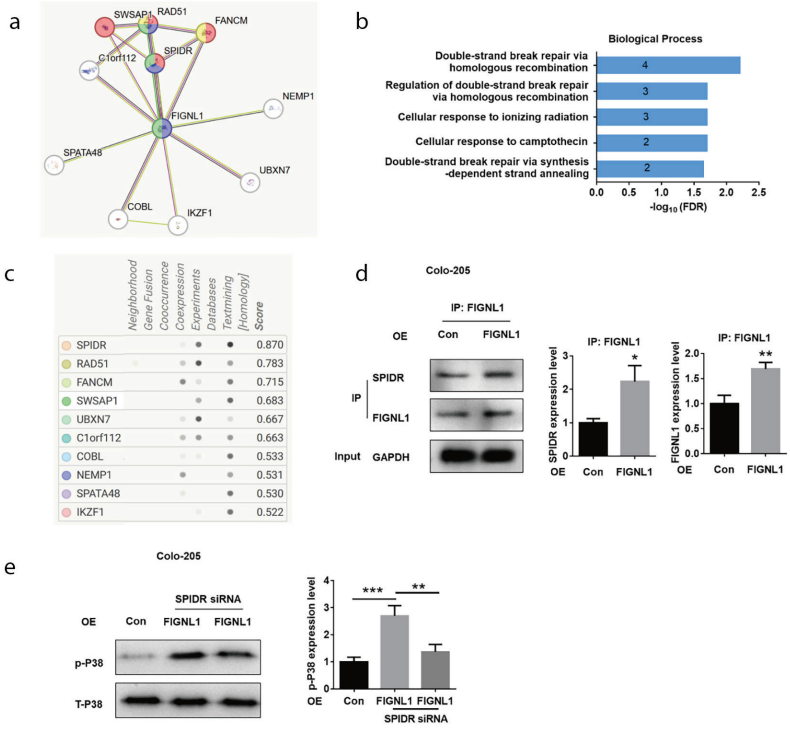

FIG. 5. FIGNL1 activated P38 phosphorylation via SPIDR. (a) Prediction of proteins interacting with FIGNL1. (b) Gene enrichment was attained through gene ontology analysis. (c) The collective scores of various indicators regarding the interaction between FIGNL1 and the targeted proteins. (d) Representative blots depicting the binding between FIGNL1 and SPIDR. (e) Representative blots demonstrating P38 phosphorylation in FIGNL1-overexpression Colo-205 cells after SPIDR siRNA treatment.

FIGNL1, Fidgetin-like 1; Con, control; OE, overexpression; IP, immunoprecipitation.

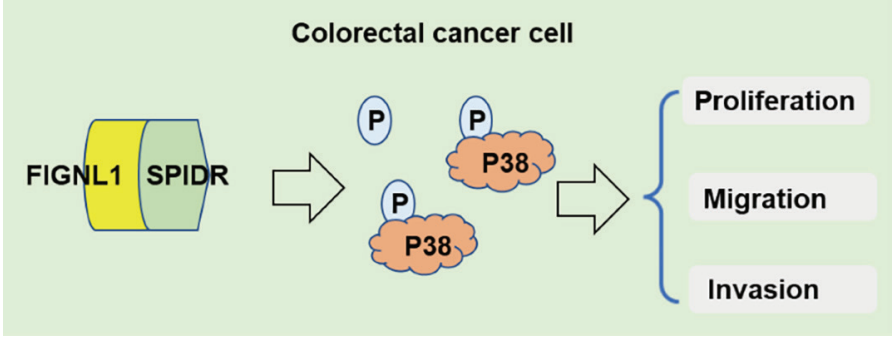

FIG. 6. FIGNL1 interacting with SPIDR and facilitating CRC cell proliferation, migration, and invasion by activating the P38 pathway.
FIGNL1, Fidgetin-like 1; CRC, colorectal cancer.

diagnosis and proper treatment.[18] Exploring novel disease biomarkers or prognostic evaluation indicators is essential to decreasing CRC-related incidence and mortality. In this investigation, FIGNL1 expression was found to be exceedingly abundant in CRC tissues from patients and exhibited a negative correlation with patient survival time and prognosis. Previous studies have reported that increased FIGNL1 protein levels were detected in lung cancer tissues, which were associated with increased resistance of the cancer cells to anticancer drugs.[9,19] These findings are consistent with the FIGNL1 results in CRC tissues in this study. Moreover, numerous studies have revealed that FIGNL1 is a promising tumor biomarker that aids in cancer diagnosis and in the evaluation of patient prognosis.[6-8] Our results demonstrated a correlation between FIGNL1 expression and TNM stage as well as lymph node metastasis.

Previous study has emphasized the relationship between FIGNL1 and diverse malignancies including renal clear-cell carcinoma, low-grade gliomas and hepatocellular carcinoma. These cancers are also strongly associated with promoter methylation and immune infiltration.[7] Although this study primarily relies on online databases for data analysis, it provides compelling evidence that FIGNL1 is closely related to human cancers. In lung cancer cells, FIGNL1 knockdown suppressed H1299 and A549 cell proliferation, and promoted their apoptosis. Also, the study findings demonstrated that FIGNL1 was essential for the progression of lung cell migration and division.[9] In our study, FIGNL1 was detected in multiple CRC cell lines. To achieve optimal overexpression and knockdown efficiency, FIGNL1 overexpression experiment was conducted in Colo-205 cells, which exhibit low basal expression levels of FIGNL1. The FIGNL1 knockdown experiments were performed in Caco-2 cells which are characterized by high basal expression levels of FIGNL1. Silencing of FIGNL1 inhibited proliferation, migration, and invasion in Caco-2 cells, as evidenced by subsequent analyses. Conversely, FIGNL1 overexpression enhanced proliferation, migration, and invasion in Colo-205 cells. These findings validate the pivotal role of FIGNL1 in promoting tumorigenesis and progression in vitro. Although our study did not include subcutaneous tumorigenesis experiments to verify the role of FIGNL1 in CRC in vivo, the current evidence is sufficient to substantiate the potential role of FIGNL1 in CRC.

Most studies focus solely on the potential link between FIGNL1 and various cancers. However, the mechanism by which FIGNL1 contributes to the initiation and progression of cancer is unclear. MAPKs are serine/threonine-protein kinases that regulate biofunction through the phosphorylation process, which can be activated by numerous exogenous stimulations or intracellular stress.[20,21] Previous research has revealed that MAPK signaling pathway activation plays a crucial role in the development of multiple cancers.[22-24] Abnormal MAPK signaling may possess therapeutic potential in oral squamous cell and ovarian carcinomas.[25,26] Similarly, the role of the MAPK signaling pathway in colon cancer is widely recognized, and its activation can further promote cancer progression.[27-29] To determine whether FIGNL1 induced CRC progression via the MAPK signaling pathway, total and phosphorylated ERK/JNK/P38 protein levels were evaluated in CRC cells exhibiting FIGNL1 knockdown and FIGNL1 overexpression. Our study revealed that FIGNL1 enhanced CRC cell proliferation, migration, and invasion by activating the P38 MAPK pathway. The significance of the MAPK signaling pathway in malignancy is further substantiated by our findings. These results suggested that FIGNL1 was a potential target for anticancer drugs, thus offering novel strategies for future CRC treatment.

Homologous DNA recombination is a critical mechanism for DNA damage repair and an important component of carcinogenesis. A prior investigation has demonstrated the involvement of SPIDR, a scaffolding protein, in DNA damage repair processes. The examination of the interaction between FIGNL1 and SPIDR holds potential for advancing cancer diagnostic and therapeutic strategies.[30] Furthermore, SPIDR exhibits the ability to form stable complex structures with the diverse proteins that play a role in DNA damage repair mechanisms.[31] However, there is limited evidence to suggest that SPIDR is a factor in the pathogenesis of CRC. The bioinformatics analysis conducted in our study demonstrated a physical interaction between FIGNL1 and SPIDR, which was further confirmed by the cell experiment in Colo-205 cells. Our research findings suggest that the downregulation of SPIDR expression inhibits the P38 phosphorylation induced by FIGNL1 overexpression, potentially implicating DNA homologous recombination pathways.

FIGNL1 expression was upregulated in human CRC tissues and exhibited a positive correlation with cancer progression. FIGNL1 facilitated CRC cell proliferation, migration, and invasion by activating the P38 pathway.

Ethics Committee Approval: This study was conducted in accordance with the Declaration of Helsinki and approved by the Hai'an City People's Hospital Ethical Committee (approval number: 2022-K032-01, date: 06.05.2022).

Informed Consent: All the patients provided consent to participate in the study, agreed to the publication of the study, and signed the informed consent form before the study.

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

Authorship Contributions: Concept- Y.Z.; Design- Y.Z.; Supervision- Y.Z.; Fundings- Y.X.; Data Collection or Processing- Y.X., Y.S.; Analysis and/or Interpretation- C.Z., L.Z., F.J., J.C., S.C.; Writing- Y.X., Y.S.; Critical Review- Y.Z.

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: This study was supported by Project of Hai'an City People's Hospital (no: 202226) and Clinical Medicine Special Project of Nantong University (no: 2023JQ018).

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