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Fusobacterium nucleatum increases CTGF expression through TLR2–YAP signaling axis in cancer-associated fibroblasts, thereby promoting colorectal cancer progression

Haoran Wang^{1,2†}, Baiqiang Lin^{3†}, Zhiyue Wang^{1,4†}, Jinhua Yang^{1,4}, Xiao Guo⁵, Wei Li⁶, Bowen Li^{1,4}, Shang Shi⁷, You Zhou², Rui Han², Yunwei Wei^{1,4*} and Yang Liu^{1,4*}

Abstract

Fusobacterium nucleatum (*F. nucleatum*) is increasingly recognized as a microbial driver of colorectal cancer (CRC) progression through immune modulation, inflammation dysregulation, and activation of oncogenic signaling cascades. Cancer-associated fibroblasts (CAFs), the predominant stromal constituents within the tumor microenvironment (TME), play pivotal roles in orchestrating tumorigenic processes. While *F. nucleatum*'s interactions with epithelial and immune compartments have been extensively characterized, its immunological crosstalk with CAFs remains elusive. Through multimodal experimental approaches including RNA sequencing and functional validation in xenograft models, we delineate a novel TLR2/YAP/CTGF signaling axis mediating *F. nucleatum*-driven CAFs activation. Specifically, *F. nucleatum* colonization induces TLR2-dependent YAP dephosphorylation, facilitating its nuclear translocation and transactivation of CTGF, a classic regulator of stromal-tumor crosstalk. Clinically, elevated CTGF expression correlates with *F. nucleatum* burden in human CRC specimens. These findings position CTGF as a potential therapeutic target in microbiota-driven colorectal carcinogenesis.

Introduction

Colorectal cancer (CRC) maintains its staggering global burden as the third most common malignancy and second leading cause of cancer-related mortality [1]. Beyond hereditary and dietary risk factors, emerging paradigms implicate gut microbiota dysbiosis as a critical microenvironmental driver of CRC pathogenesis [2].

Cancer-associated fibroblasts (CAFs) constitute the principal cellular constituents of the tumor microenvironment (TME) in the majority of solid tumors, exerting a crucial influence on tumor cell growth, proliferation, invasion, migration, and chemoresistance [3]. Numerous studies have documented the significant involvement of CAFs in various crucial processes such as extracellular matrix (ECM) maintenance, angiogenesis,

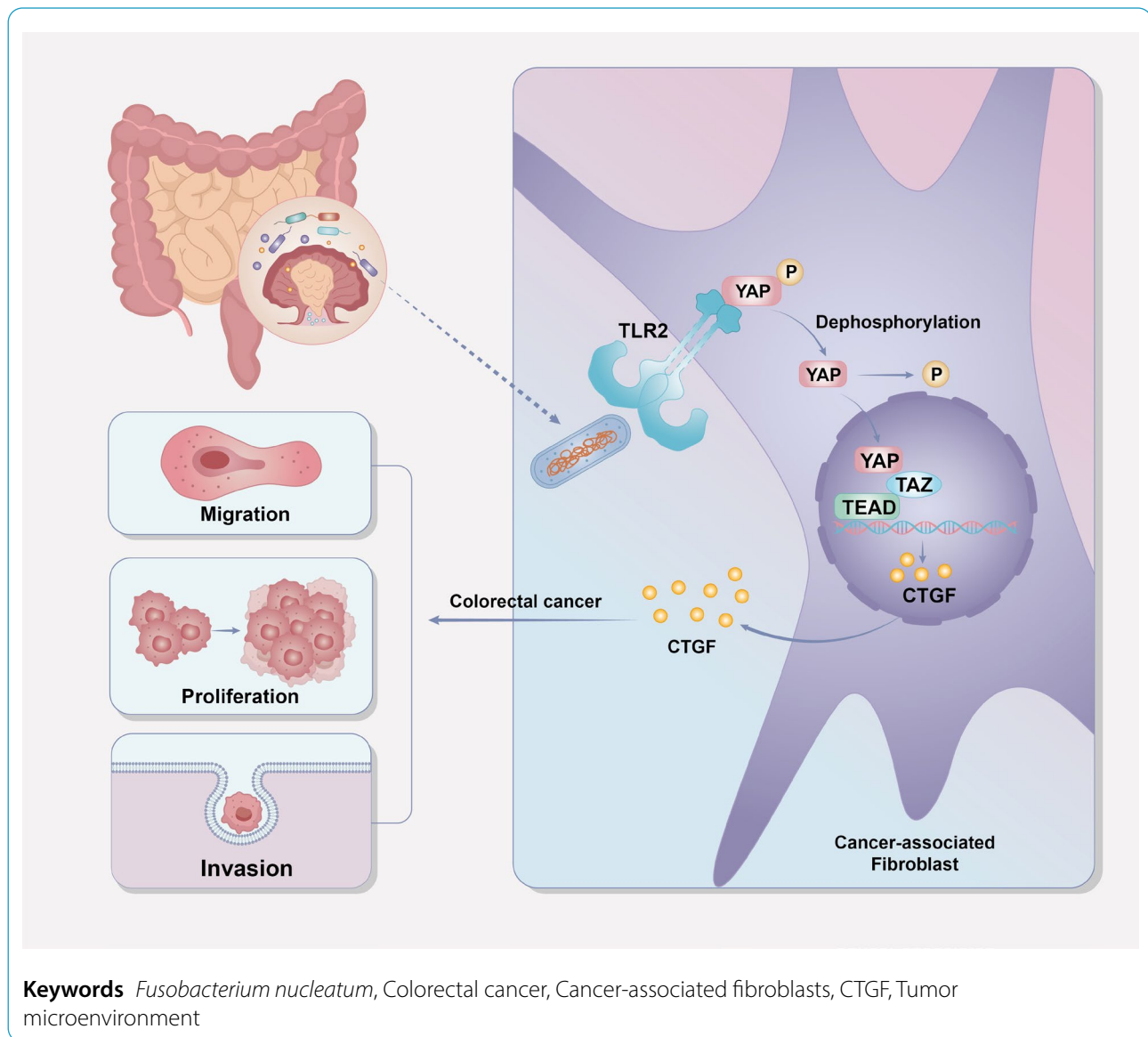
[†]Haoran Wang, Baiqiang Lin and Zhiyue Wang have contributed equally to this work.

*Correspondence:
Yunwei Wei
hydwyyw11@hotmail.com
Yang Liu
lyang712@icloud.com

Full list of author information is available at the end of the article



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immunosuppression, invasion, and chemotherapy resistance [4].

F. nucleatum, an oral anaerobic bacterium prevalent in human CRC tissue, has been linked to the progression of CRC [5]. Our previous studies indicate that *F. nucleatum* fosters the proliferation and chemotherapy resistance by modulating CRC cells' ferroptosis [6]. Emerging evidence highlights a critical interplay between gut microbiota and CAFs in the progression of CRC. Certain microbial metabolites, such as bile acids, have been shown to indirectly activate CAFs in CRC, thereby enhancing their tumor-promoting properties and contributing to a pro-tumorigenic microenvironment [7]. *Actinomyces* activates the TLR2/NF- κ B pathway and reduces CD8⁺ T lymphocyte infiltration in CRC [8]. Among the gut microbiota, *F. nucleatum* has garnered particular

attention due to its enrichment in CRC tissues and its role in promoting inflammation, immune evasion, and epithelial-to-mesenchymal transition [9–11]. However, whether and the precise mechanisms by which *F. nucleatum* interacts with CAFs, then promoting CRC progression, remain largely undefined.

In this study, we find that CAFs were infected with *F. nucleatum* then upregulating connective tissue growth factor (CTGF), a key regulator of tumor progression. Our findings reveal that *F. nucleatum* promotes CRC development by modulating CTGF expression in CAFs through the TLR2-YAP signaling pathway. This activation enhances tumor cell proliferation and migration, contributing to cancer progression. These results uncover a novel mechanism linking microbial influence to stromal reprogramming in CRC and highlight CTGF as a

potential therapeutic target in *F. nucleatum*-associated colorectal cancer.

Materials and methods

Culture of bacteria

Standard strain *F. nucleatum* (ATCC 25586) was inoculated in freshly configured BHI (Solarbio, China) medium and cultured for 48 h under 37°C anaerobic conditions (MITSUBISHI Gas Chemical Co, Japan). The turbidity of the bacterial solution indicated that the bacteria grew well and could be subcultured. After centrifugation at 8000 rpm for 10 min, the bacteria were collected, and the optical density at 600 nm was measured. Bacterial counts are based on standardized curves created for the photometer. Remaining bacteria were resuspended in a mixture of 50% BHI with 50% glycerol (Solarbio, China) and frozen in a -80°C cryogenic refrigerator (Thermo, USA) for use.

Isolation and culture of cells

Fresh CRC tissue samples were obtained from the First Hospital of Harbin Medical University. The separation method for CAFs involved excising 2–3 cm carcinoma tissues and 2–3 cm of normal intestinal tissue, followed by rinsing the specimens with normal saline solution for 2–3 times. To prevent necrotic tissue formation and minimize the duration of tissue exposure to room temperature, the samples were stored in a cell culture medium. The remaining tissue was then cut into 1 mm × 1 mm × 1 mm fragments using sterile ophthalmic scissors. After the cell culture medium was resuspended, large pieces of tissue were removed by 200-mesh sieve filtration. Then, the cells were seeded into the flask with DMEM medium containing 15% FBS, and purified CAFs and normal fibroblasts were obtained by the differential adherence method. Human colon cancer cells (LoVo, HCT-116) were purchased from the Cell Bank of the Type Culture Collection, Chinese Academy of Sciences (Shanghai, China). LoVo was cultured in Ham's F-12k medium (Procell, China) supplemented with 10% fetal bovine serum (FBS), while HCT-116 was cultured in Maccoy's 5 A (Procell, China) supplemented with 10% FBS. The use of these clinical samples was approved by the Harbin Medical University Ethics Committee (IRB-AF/SC-08/05.0).

Western blot analysis

PBS-washed cells were added to RIPA lysis buffer (Beyotime, China) with a phosphatase inhibitor (Beyotime, China) and a protease inhibitor (Beyotime, China) in a ratio of 100:10:1. The mixture was incubated on ice for 30 min and subsequently centrifuged to obtain the protein in the supernatant. The protein concentration was measured using the NARODROP2000 (Thermo, USA). After adjusting the protein concentration to 10 µg/ul,

SDS-PAGE protein loading buffer (5×) (Beyotime, China) was added. The samples were then boiled at 100°C and stored at -80°C for future use. The protein was isolated using the PAGE gel rapid preparation kit (Epizyme, China) and subsequently transferred to a nitrocellulose membrane (Meck, USA). The membrane was then blocked at room temperature with rapid blocking fluid (Beyotime, China). The following primary antibodies diluted with antibody diluent (Beyotime, China) were utilized: Vimentin (Proteintech, Wuhan), FAP (Proteintech, Wuhan), α-SMA (Proteintech, Wuhan), GAPDH (Proteintech, Wuhan), CTGF (Proteintech, Wuhan), TLR2 (Proteintech, Wuhan), YAP (Proteintech, Wuhan), p-YAP (Proteintech, Wuhan), E-cadherin (Proteintech, Wuhan), N-cadherin (Proteintech, Wuhan), the immunoreactive protein (IRP) bands were subsequently detected using enhanced chemiluminescence assay kit (Abbkine, Wuhan), the marker (Thermo Scientific PageRuler 26616) was applied to determine the molecular weights of the protein bands (Thermo, USA).

Cell Immunofluorescence assay

CAFs were seeded in six-well plates containing slides, allowing the cells to adhere and stretch overnight. The cells were subsequently fixed with 4% paraformaldehyde and permeabilized with 0.5% Triton X-100 (Beyotime, China). Following fixation, the cells were blocked with 2% BSA at room temperature for 1 h. They were then incubated with primary antibodies against Vimentin, FAP, and α-SMA at 4°C overnight. The next day, after rinsing the cells with PBS, the appropriate fluorescent secondary antibody (Proteintech, Wuhan) was added and incubated in a dark room for 1 h. The nuclei were stained with DAPI (Beyotime, China) for 2 min. Subsequently, the samples were examined and photographed using either an Olympus fluorescence microscope or a laser confocal microscope.

Bacteria Co-localize with cells

Fluorescent labeling of *F. nucleatum* was achieved using fluorescein isothiocyanate (FITC) dye (Qiyue, China) at an optimal concentration (2 µg/ml). The labeled *F. nucleatum* were subsequently mixed and incubated for 2 h under anaerobic conditions at 37°C, shielded from light. Rinse and centrifuge the cells to remove unconjugated FITC. *F. nucleatum* was then co-cultured with CAFs, which had been pre-seeded on coverslips (Shitai, Zhejiang) for 2 h. Following this incubation period, the cells were fixed, blocked, and incubated with specific antibodies. The co-localization of the bacteria and cells was then examined using confocal microscopy, and images were captured for further analysis.

Scanning electron microscopy

CAFs were seeded onto slides in a six-well plate and allowed to adhere for 24 h. Subsequently, the cells were incubated with *F. nucleatum* under anaerobic conditions at 37°C for varying durations of 1 h, 2 h, 6 h, 12 h, and 24 h. Following incubation, the cells were washed three times with PBS and then fixed with 2.5% glutaraldehyde (Solarbio, China) for 2 h. After PBS wash, the cells were fixed with 1% osmium tetroxide (Solarbio, China) pre-cooled to 4°C for 1 h. The cells underwent a series of dehydration steps using acetone and an alcohol gradient. The concentration gradient is 30% → 50% → 70% → 80% → 90% → 95% → 100% → 100%. The immersion time for 30% and 50% concentrations is 15 min, while the immersion time for the other concentrations is 20 min. Finally, the samples were dried using an acetonitrile drying method. The infiltration of *F. nucleatum* into CAFs at various time intervals was monitored and documented through photography following the application of electrical conduction to the samples.

High-throughput sequencing

To compare the transcriptome difference of *F. nucleatum* -positive and 5 *F. nucleatum* -negative CRC tissues were used for generating RNAseq data. To compare the transcriptome difference of *F. nucleatum* -CAF and CAF, 3 *F. nucleatum* -CAF (*F. nucleatum* co-culture) and 3 CAF isolates from 6 different patients at passage 1 were used for generating RNAseq data. The RNA-seq transcriptome library was prepared following Illumina® Stranded mRNA Prep, Ligation (San Diego, CA) using 1 µg of total RNA. The sequencing library was performed on NovaSeq X Plus platform (PE150) using NovaSeq Reagent Kit. The raw paired end reads were trimmed and quality controlled by fastp with default parameters. Then clean reads were separately aligned to reference genome with orientation mode using HISAT2 software. The mapped reads of each sample were assembled by StringTie in a reference-based approach.

Differential expression analysis and functional enrichment
To identify DEGs (differential expression genes) between two different samples, the expression level of each transcript was calculated according to the transcripts per million reads (TPM) method. RSEM was used to quantify gene abundances. DEGs with $|\log_2FC| \geq 1$ and $FDR < 0.05$ (DESeq2) or $FDR < 0.001$ (DEGseq) were considered to be significantly different expressed genes. In addition, functional-enrichment analysis including GO and KEGG were performed to identify which DEGs were significantly enriched in GO terms and metabolic pathways at Bonferroni-corrected P-value < 0.05 compared with the whole-transcriptome background. GO functional enrichment and KEGG pathway analysis were

carried out by Goatools and Python scipy software, respectively.

Real-time fluorescent quantitative PCR

As described in our previous studies [6], the cells were lysed using Trizol reagent in a 1.5 mL EP tube and mixed for 5 min at room temperature. Subsequently, chloroform was added, and the mixture was shaken for 2 min before being centrifuged for 10 min. Following centrifugation at 4°C, the supernatant was collected, and isopropanol was added. After 10 min, the supernatant was mixed with isopropanol again. The mixture was then subjected to high-speed centrifugation at 4°C, after which the supernatant was precipitated with 75% ethanol. To prevent genomic DNA contamination, complementary DNA (cDNA) was synthesized from the RNA using a reverse transcription kit containing a gDNA remover (Toyobo, Japan). The Ct values obtained from various samples were compared utilizing the 2-ΔΔCt method. GAPDH was utilized as the internal reference gene. To quantify the amounts of *F. nucleatum*, genomic DNA (gDNA) was isolated from fresh CRC tissue using the Genomic DNA Purification Kit (Tiangen, China). The SYBR® Green Real-time PCR Master Mix (Toyobo, Japan) was employed to quantify *F. nucleatum*, using PGT as the internal reference gene. We included no-sample negative controls throughout the experiments to monitor for potential contamination. The sequences of the gene primers are as follows:

Primer Name	Sequence 5'–3'
CTGF-F	TCTCCTTCTGTCCCATCTTGCTG CCCATCTTGCTG
CTGF-R	GATCGGGACGCGGTAGGA CGCGGTAGGA
TLR2-F	TGCTTTCCTGCTGGAGATTT
TLR2-R	TGTAAACGCAACAGCTTCAGG
GAPDH-F	AACCTTGGCATTGTGGAAGG
GAPDH-R	CACATTGGGGGTAGGAACAC

CCK-8 experiment

5000 HCT116 or Lovo cells were uniformly seeded into each well of 96-well plates and incubated for 24 h. According to the experimental protocol, conditioned media with multiplicities of infection (MOI) of 10:1, 20:1, 50:1, and 100:1 from CAFs infected with *F. nucleatum* for 24 h, as well as conditioned media from uninfected CAFs, were added to the wells. Each experimental condition was replicated in five wells. The 96-well plates were then incubated for an additional 24 h. At specified time points (0 h, 24 h, 48 h, and 72 h), the 96-well plates were removed from the incubator, and the cells were washed with PBS. Cell proliferation was assessed based on the optical density (OD) at 450 nm measured in different wells.

EDU experiment

Following the inoculation of HCT116 or Lovo cells in 24-well plates and allowing sufficient time for cell adherence, conditioned media from CAFs (cancer-associated fibroblasts) infected with *F. nucleatum* for 24 h and conditioned media from uninfected CAFs were added to the respective wells. Subsequently, 5-ethynyl-2'-deoxyuridine (EdU) (Beyotime, China) was introduced into the medium and incubated for 2 h at 37°C. Post incubation, the cells were washed with PBS and fixed with 4% paraformaldehyde for 30 min. After another PBS wash, the cells were incubated with 0.3% Triton X-100. Subsequently, the cells were stained with click working fluid (Beyotime, China) for 30 min at room temperature, followed by staining with DAPI for 5 min, ensuring that exposure to light was minimized throughout the process. Three fields of view (200×) were randomly selected using a fluorescence microscope. The number of EdU-labeled cells (proliferating cells) and DAPI-labeled cells (total cells) was counted. The cell proliferation rate (%) was calculated using the formula: number of proliferating cells/total number of cells×100%.

Wound-healing assay

HCT116 or Lovo cells were digested and resuspended in serum-free medium, when the cells adhere mixed with conditioned media from CAFs infected with *F. nucleatum* for 24 h, as well as conditioned media from uninfected CAFs. A sterile 200-μL pipette tip was used to create a scratch wound on the cell monolayer, followed by PBS washing to remove dislodged cells and replenishment with basal medium. Photographs (40×) were taken at 0 and 24 h after the scratch using an inverted microscope.

Transwell migration assay

HCT116 or Lovo cells were digested and resuspended in serum-free medium, When the cells adhere mixed with conditioned media from CAFs infected with *F. nucleatum* for 24 h, as well as conditioned media from uninfected CAFs. These mixtures were subsequently added to the upper chamber of a Transwell system (Corning, USA), while the lower chamber contained medium supplemented with 20% fetal bovine serum (FBS). After an incubation period of 24 h, the Transwell chamber was removed, fixed in formaldehyde for 20 min, stained with crystal violet for 30 min, and then photographed under a microscope for cell counting.

3D spheroid proliferation assay

Following the digestion of HCT116 or Lovo cells, the cell concentration was standardized to 1×10^4 cells and subsequently introduced into a 96-well ultra-low adsorption cell culture plate (Corning, USA). Globules are formed overnight. A 48-well plate was preheated in an incubator

set to 37°C. A mixture was prepared by combining 22 μL of PBS with 22 μL of 0.1 N NaOH (Solarbio, China) and 100 μL of rat tail collagen. The medium containing the cell spheroids was then aspirated from the 96-well plate and incorporated into the collagen mixture. The components were mixed thoroughly and distributed into preheated 48-well plate. The plate was incubated at 37°C to allow collagen solidification. After 20 min, we introduced the conditioned medium from CAFs infected with *F. nucleatum* for 48 h, as well as the conditioned medium from uninfected CAFs, into the solidified collagen. The microscope was used to capture the images of the cell spheres at 0, 24, and 48 h. Spheroid diameters were quantified at the start and at the end of the experiment to confirm no expansion and contraction of the spheroids during the duration of the experiment. Migration over 48 h is quantified by measuring the extent of spheroid spread and overall area of migration after normalization to spheroid size. The embedded spheroids were imaged at 0, 24, and 48 h, and the resulting migration spread was quantified using ImageJ.

Cell lentiviral transfection

The Control ShRNA, CTGF ShRNA, and TLR2 ShRNA lentiviruses were constructed by Shanghai Genechem. CAFs were cultured in 24-well plates, with approximately 2×10^5 cells per well at the time of transfection. The original medium was replaced with fresh medium containing 6 μg/ml Polybrene, and an appropriate amount of viral suspension was added. The treated cells were incubated at 37°C to facilitate viral infection. After 4 h, fresh medium was added to dilute the Polybrene, and after 24 h, the virus-containing medium was replaced with fresh medium. Transfection efficiency was assessed 3–4 days post-transfection.

Establishment of xenograft tumor model in nude mice

The athymic female BALB/c nude mice (8 weeks old, 19–21 g) were procured from Vitonex company. The conditioned medium of CAFs not infected with *F. nucleatum*, CAFs infected with *F. nucleatum*, shCTGF-CAFs infected with *F. nucleatum*, and shTLR2-CAFs infected with *F. nucleatum* were combined with HCT116 cells (5×10^6 cells) and injected subcutaneously into the posterior region of the mice. Tumor volumes were measured every three days to monitor tumor progression. On Day 21, the tumor-bearing mice were euthanized and subjected to autopsy. The tumors were excised and fixed in 4% paraformaldehyde (Solarbio, China). The 4-μm sections were prepared via paraffin embedding and subsequently utilized for immunohistochemical analysis. All mice were maintained under specific sterile conditions. The experimental procedures involving the mice were conducted in

compliance with the guidelines established by the Harbin Medical University Animal Ethics Committee.

Immunohistochemistry

Following the dewaxing and hydration of paraffin-embedded sections, 3% hydrogen peroxide solution (Solarbio, China) was applied to eliminate endogenous peroxidase activity. EDTA solution (ZS BIO, China) was utilized to prepare an EDTA antigen retrieval solution with a pH of 9.0. Antigen retrieval was then performed using a high-temperature method. The sections were subsequently blocked with a 1% BSA solution for 1 h. Primary antibodies, including CTGF (1:500), α -SMA (1:500), Ki67 (1:500), N-cadherin (1:500), and E-cadherin (1:500), were prepared at the specified concentrations and incubated with the sections. Following PBS washing, secondary antibodies were incubated for 1 h. Subsequently, the DAB color solution (ZS BIO, China) was applied to develop color, and hematoxylin (Solarbio, China) was used to stain the nuclei. After dehydration, the sections were sealed with neutral gum (Solarbio, China) and baked at 65°C for 12 h. The prepared sections were then examined under a microscope.

Immunofluorescence of tissue sections

Paraffin-embedded sections were subjected to dewaxing and hydration processes, followed by antigen retrieval using EDTA antigen retrieval solution at pH 9.0. The sections were then blocked with a 1% BSA solution and incubated overnight at 4°C with the appropriate concentrations of primary antibodies. Post-incubation, the sections were treated with the specific to the primary antibody fluorescent secondary antibody for 1 h, and subsequently stained with DAPI for 2 min. Each step was interspersed with washes using PBS. The sections were immediately sealed and subsequently observed and photographed under a microscope.

The cancer genome atlas analyses

Transcriptomic data from TCGA were analyzed by aligning clean reads to GRCh38 using Bowtie2 (v2.5.1), followed by mRNA quantification with featureCounts (Subread v2.0.6). Unmapped reads underwent microbial taxonomic classification via Kraken2 (v2.1.3) against the Standard database, with Bracken (v2.8.3) estimating species-level abundance (confidence threshold: 0.5), ultimately generating paired mRNA expression and microbial profiles for integrated analysis.

Statistical analyses

Statistical analyses were conducted using Prism 8.0 and SPSS version 22.0. Data following a normal distribution are presented as mean $\pm$ standard deviation, whereas non-normally distributed data are reported as

median $\pm$ interquartile range. Differences between the two groups with normally distributed data are evaluated using a two-tailed t-test, while the Mann-Whitney U test is employed for non-normally distributed data. Categorical variables are analyzed using Fisher's exact test. Significant differences among the three groups are assessed using one-way Analysis of Variance (ANOVA). A p-value of less than 0.05 is considered indicative of statistical significance.

Results

F. nucleatum-positive CRC tumors exhibit a higher degree of cancer-associated fibroblasts (CAFs) activation

First, we performed transcriptome sequencing on CRC tissue samples and stratified the patients into two groups based on the relative abundance of *F. nucleatum* in tumor tissues (Fig. 1A). GO functional annotation analysis revealed significant enrichment of biological functions related to CAFs activation in the *F. nucleatum*-positive group (Fig. 1B). KEGG pathway enrichment analysis revealed significant enrichment of signaling pathways associated with TGFBR and PDGFRA in the *F. nucleatum*-positive group (Fig. 1C). These results suggested that *F. nucleatum* colonization in tumor tissue may contribute to CAFs activation. Immunofluorescence analysis of CRC tissues revealed significantly elevated expression of CAFs-associated markers (Vimentin, FAP, and α -SMA) in *F. nucleatum*-positive specimens compared to *F. nucleatum*-negative specimens and normal fibroblast specimens (Fig. 1D and Fig. S1). Consistently, western blot analysis further confirmed the upregulation of Vimentin, FAP, and α -SMA expression in *F. nucleatum*-positive tumors (Fig. 1E). Together, these results indicated that CAFs activation is more pronounced in *F. nucleatum*-positive tumors.

Fusobacterium nucleatum-infected CAFs promote CRC cell proliferation and migration through paracrine effects

The intracellular localization of *F. nucleatum* within CAFs indicated that *F. nucleatum* was capable of adhering to and infecting CAFs (Fig. 2A). Subsequently, we further applied scanning electron microscopy (SEM) to directly show the various modes of binding and invasion of *F. nucleatum* into CAFs. After 2 h of co-culture with fibroblasts, *F. nucleatum* was observed adhering to the surface of CAFs. By 12 h of co-culture, internalized *F. nucleatum* was detected within the CAFs, indicating progressive bacterial invasion over time (Fig. 2B). CAFs acted as the main stromal cell in the TME, which can promote the proliferation and migration of colorectal cancer cells by cytokine secretion [12]. To further investigate whether this pro-tumorigenic function of CAFs could be modulated by *F. nucleatum*. We co-cultured CAFs with *F. nucleatum* at MOI = 10/20/50/100 for 24/48/72/96

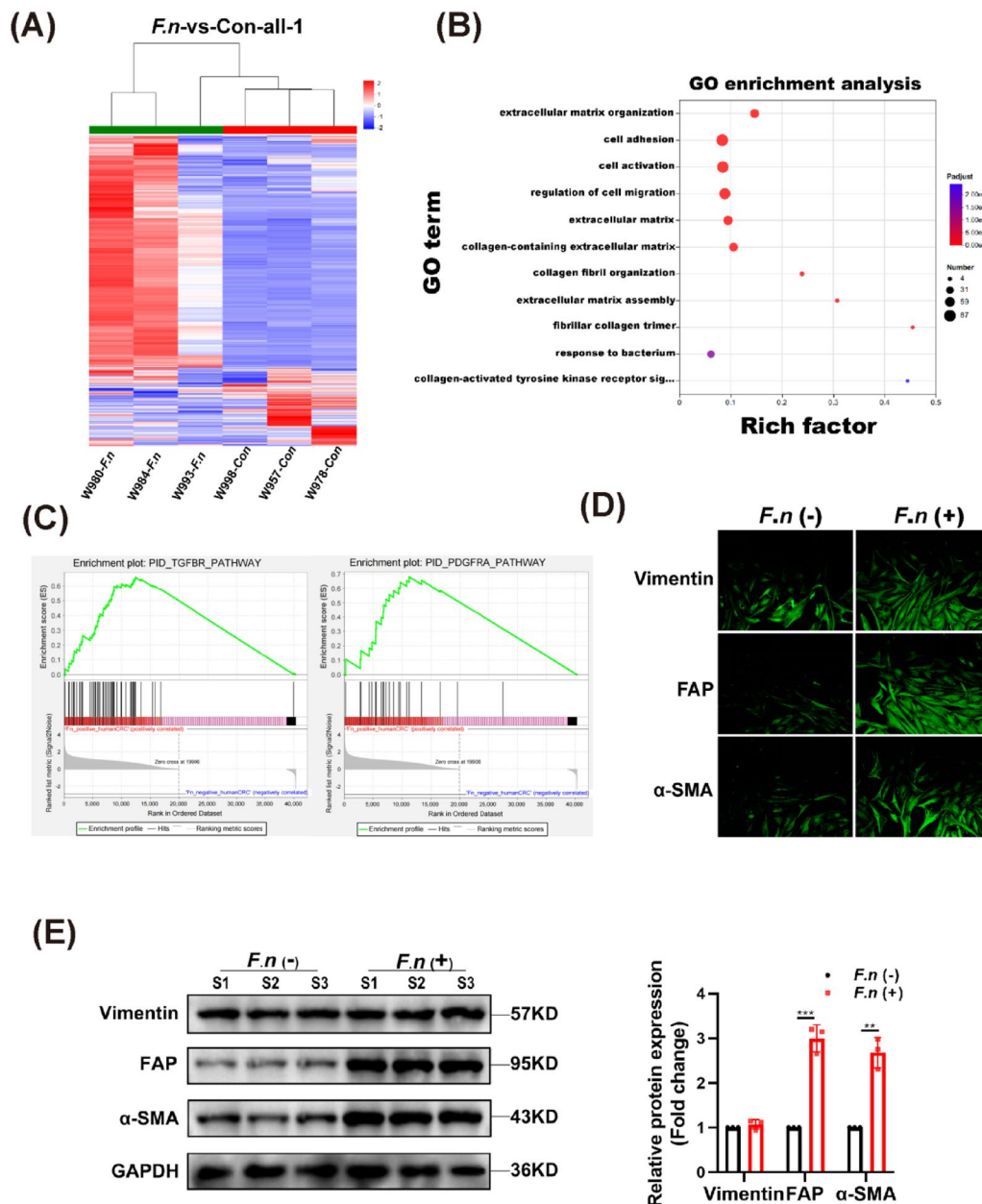


Fig. 1 *F. nucleatum* invaded the CAFs and subsequently activated it. **(A)** The heatmap illustrates differential gene expression between *F. nucleatum*-negative and *F. nucleatum*-positive groups in CRC tissues. **(B)** Gene Ontology (GO) enrichment analysis of differentially expressed genes in CRC tissues from two groups. **(C)** KEGG pathway analysis showing altered gene expression of major genes between the two groups. **(D)** Immunofluorescence staining of CRC tissues from two groups. **(E)** Immunoblot analysis of protein extracts from CAFs in two groups

h, respectively, to generate different conditioned media (CAF-*F.n*-CM). CCK8 assays showed that CAF-*F.n*-CM enhanced CRC cells' proliferation compared with control groups (Fig. 2C and Fig. S2). To further validate this finding, we performed EdU assays, which similarly demonstrated that CAF-*F.n*-CM promoted the proliferation of both HCT116 and LoVo cells compared to their respective controls (Fig. 2D). Moreover, wound healing and transwell assays demonstrated that CAF-*F.n*-CM significantly enhanced the migratory capacity of CRC cells (Fig.

2E-F). Western blot analysis also showed that CAF-*F.n*-CM enhances the expression of N-cadherin and reduces the expression of E-cadherin in HCT116 and LoVo cells (Fig. 2G). These findings indicated that the pro-migratory phenotype induced by CAF-*F.n*-CM in CRC cells can be recapitulated in a 3D co-culture model, supporting its physiological relevance (Fig. 2H and Fig. S3).

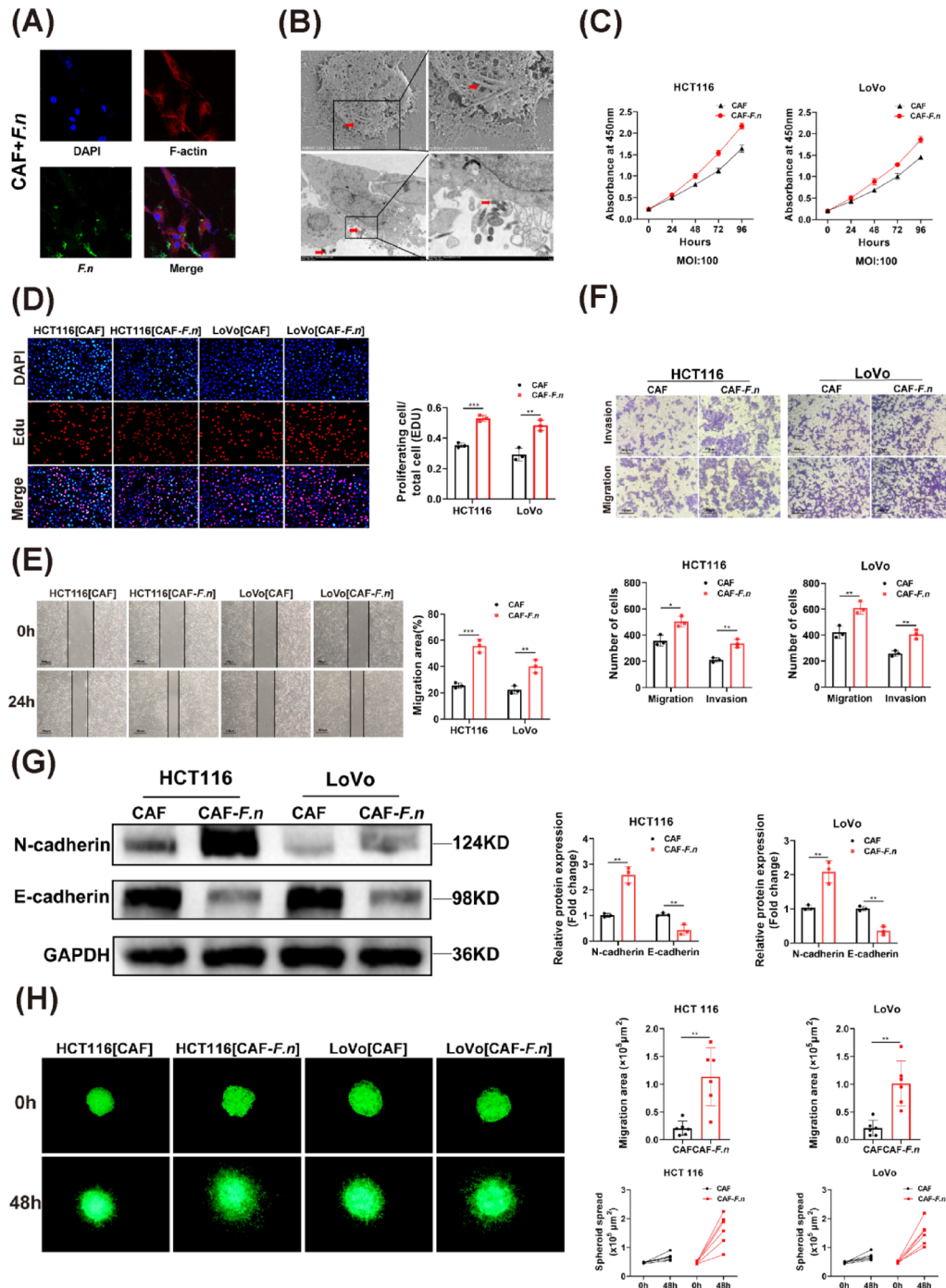


Fig. 2 Conditioned medium obtained from CAFs after co-culturing with *F. nucleatum* enhanced the proliferation and migration of HCT116 and LoVo cells in vitro. **(A)** Immunofluorescence staining of *F. nucleatum* (green), F-actin (red), and DAPI (blue) in CAFs co-cultured with *F. nucleatum* (63x oil immersion) **(B)** SEM images of *F. nucleatum* entering CAFs. *F. nucleatum* entering a CAFs exposure (arrows). TEM results show that *F. nucleatum* adheres to (arrows) and is partially internalized (arrows) in CAFs after co-culture. **(C)** CRC cell proliferation measured by CCK-8 assay. **(D)** Representative images and quantification of EdU assay of CRC cells. **(E)** Representative images and quantification of CRC cells migration in a wound healing assay. **(F)** Representative images and quantification of CRC cells migration and invasion were assessed using a Transwell system. **(G)** Immunoblot analysis of EMT-related protein extracts from CRC cells. **(H)** Representative images and quantification of depicting cell spheres composed of either 10,000 HCT116 cells or LoVo cells migrating within collagen mixtures

Fusobacterium nucleatum induces CAFs secreting CTGF

Compared to controls, 3,917 genes were upregulated and 3,770 genes were downregulated in *F. nucleatum*-treated CAFs (Fig. 3A). Previous studies have shown that the expression of CTGF in CAFs is associated with tumor microenvironment remodeling [13]. Notably, the

significant upregulation of CTGF among the differentially expressed genes was consistent with its established role in CAFs activation (Fig. 3B). RT-qPCR validation confirmed the upregulation of CTGF mRNA in *F. nucleatum*-treated CAFs (Fig. 3C). Western blot analysis confirmed that *F. nucleatum*-treatment upregulated

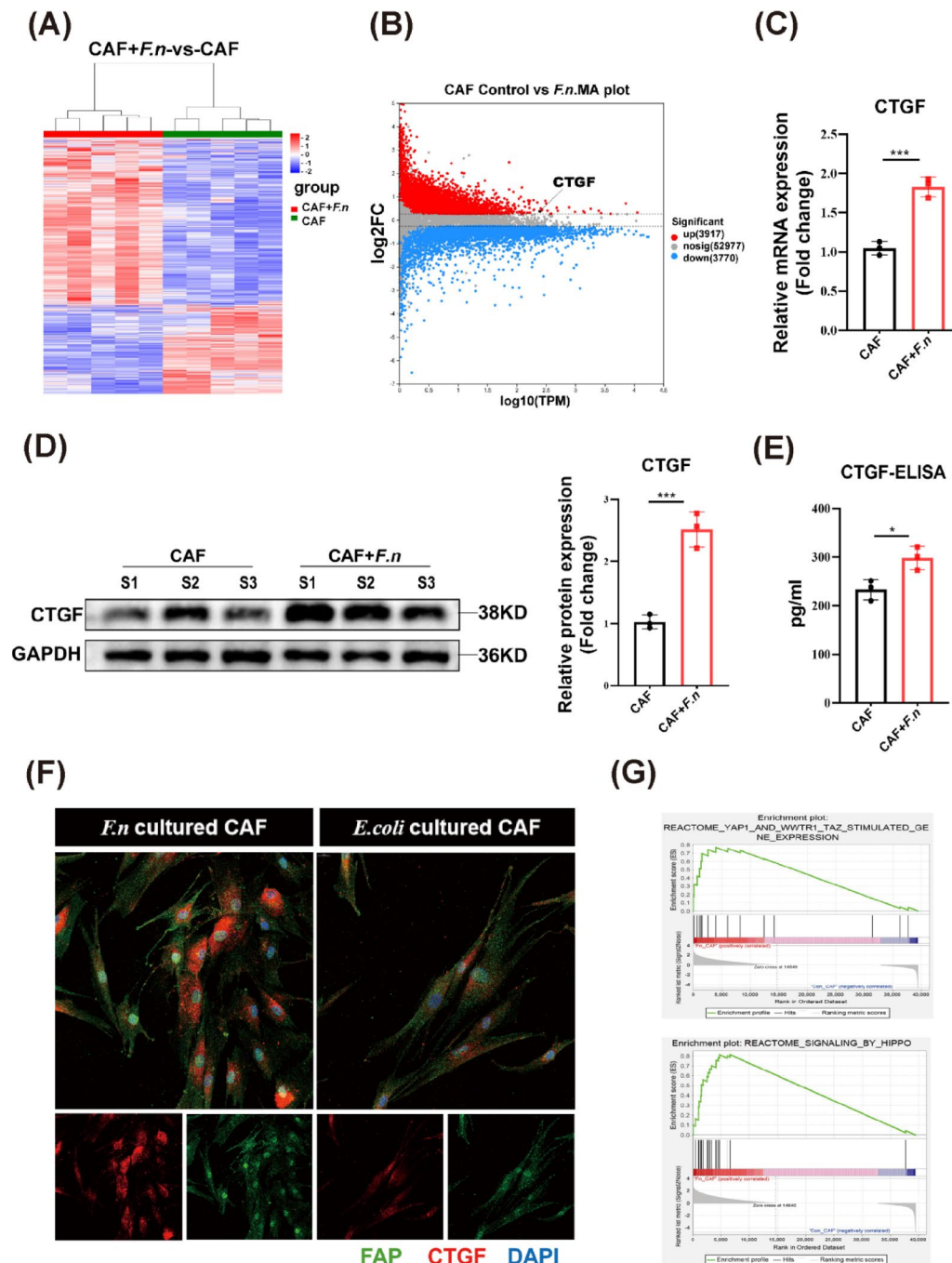


Fig. 3 CTGF is a key factor in the activation of CAFs by *F. nucleatum*. **(A)** Heatmap illustrating differential expression of key genes in CAFs upon *F. nucleatum* co-culture. **(B)** Volcano plot showing gene expression changes in CAFs co-cultured with *F. nucleatum*. **(C)** RT-qPCR analysis of mRNA levels for CTGF in CAFs induced by *F. nucleatum* infection. **(D)** Immunoblot analysis of CTGF extracts from CAFs. **(E)** ELISA quantification of CTGF secretion in CAFs following *F. nucleatum* infection. **(F)** Immunofluorescence staining of CTGF (red) and DAPI (blue) in CAFs cultured with or without *F. nucleatum* (63x oil immersion). **(G)** KEGG pathway analysis showing altered gene expression in CAFs induced by *F. nucleatum* infection

CTGF protein expression in CAFs (Fig. 3D), while ELISA further demonstrated enhanced CTGF secretion into the conditioned medium (Fig. 3E and Fig. S4). *F. nucleatum*-treated CAFs showed intensified CTGF and FAP signals by immunofluorescence, consistent with an activated state (Fig. 3F). We also revealed significant enrichment of TLR2 and YAP signaling pathways in *F. nucleatum*-treated CAFs, suggesting their potential role in mediating CTGF upregulation (Fig. 3G).

CTGF upregulation in *F. nucleatum*-infected CAFs contributes to CRC cells' proliferation and migration

To investigate the role of CTGF in *F. nucleatum*-infected CAFs on CRC cells, we knocked down CTGF and confirmed the decreased CTGF expression in CAF, even under *F. nucleatum* infection (Fig. 4A-B). The CCK-8 assay results showed that CTGF knockdown in CAFs attenuated the ability of CAF-*En*-CM to promote metabolic activity and consequently reduce the proliferative capacity of CRC cells in vitro (Fig. 4C). Similarly, the EdU assay results demonstrated that CTGF knockdown in CAFs impaired the ability of CAF-*En*-CM to stimulate DNA synthesis and thereby inhibit the proliferation of CRC cells in vitro (Fig. 4D). The Wound Healing Assay initially indicated, and the Transwell Assay further quantitatively confirmed, that CTGF knockdown in CAFs impaired the migrative-promoting effect of CAF-*En*-CM on CRC cells in vitro (Fig. 4E-F). E-cadherin and N-cadherin are both cell adhesion molecules (part of the cadherin family) that mediate calcium-dependent cell-cell adhesion. This shift from E-cadherin to N-cadherin expression is a key feature of tumor EMT and metastasis [14]. We also examined the expression of N-cadherin and E-cadherin in CRC cells to investigate the mechanism underlying their enhanced migratory ability. Notably, these effects were attenuated upon CTGF knockdown (Fig. 4G). This finding was further corroborated by similar results observed in a 3D co-culture model (Fig. 4H).

***F. Nucleatum* promoted colorectal cancer progression via TLR2–YAP–mediated CTGF upregulation in CAFs**

Previous studies have shown that nuclear localization of YAP in CAFs influences CTGF expression, with TLR2 acting as an upstream regulator of the YAP pathway. In conjunction with the findings from transcriptome sequencing, we postulated that *F. nucleatum* played a role in the advancement of CRC by modulating the TLR2–YAP pathway and enhancing the expression of CTGF in CAFs. Initially, the impact of *F. nucleatum* on YAP nucleation in CAFs was investigated. Western blot analysis revealed that co-culturing CAFs with *F. nucleatum* resulted in a decrease in cytoplasmic YAP content and an increase in nuclear YAP content, indicating enhanced nuclear translocation of YAP in CAFs following *F.*

nucleatum intervention (Fig. 5A). Immunofluorescence analysis further demonstrated *F. nucleatum* could adhere to CAFs and increased nuclear translocation of YAP after CAFs co-cultured with *F. nucleatum* (Fig. 5B and Fig. S5). Western blot analysis demonstrated that while the adhesion of viable *F. nucleatum* to CAFs resulted in increased TLR2 expression, decreased YAP phosphorylation, enhanced YAP nuclear translocation, and elevated CTGF expression, inactivated *F. nucleatum* failed to elicit these effects (Fig. 5C and Fig. S6). Following the down-regulation of TLR2 in CAFs, the expression of CTGF was significantly reduced (Fig. 5D-E). These findings suggested that *F. nucleatum* reduces YAP phosphorylation by upregulating TLR2 expression in CAFs, promotes its nuclear translocation, and thereby increases CTGF expression, ultimately driving colorectal cancer progression in vivo.

TLR2 signaling mediated by *F. nucleatum* induced upregulation in CAFs

To validate the mechanism by which *F. nucleatum*-infected CAFs promote CRC progression in vivo, we investigated the effects of conditioned medium derived from infected CAFs on tumor proliferation and migration. A nude mouse model bearing subcutaneously transplanted tumors was employed. Results indicated that the CAF+*En* group exhibited increased tumor volume and weight compared to the CAF-shNC group, whereas the CAF-shCTGF+*En* and CAF-shTLR2+*En* groups displayed decreased tumor volume and weight relative to the CAF+*En* group (Fig. 6A-C). The expression levels of Ki67 in the CAF+*En* group were found to be significantly higher compared to CAF-shCTGF+*En*, and CAF-shTLR2+*En* groups, suggesting a greater proliferative capacity in the CAF-shNC group (Fig. 6D). Furthermore, the CAF+*En* group exhibited elevated expression of N-cadherin, decreased expression of E-cadherin, indicating a heightened migratory potential (Fig. 6E). These findings suggested that *F. nucleatum* increases the expression of CTGF by up-regulating TLR2 expression in CAFs, thereby promoting the progression of CRC in vivo. Clinical CRC tissue analysis demonstrated enhanced CAFs activation and elevated CTGF expression in *Fusobacterium nucleatum*-positive tumors (Fig. 6F). Consistently, interrogation of TCGA colorectal cancer datasets revealed that tumors with higher *F. nucleatum* abundance exhibited significantly increased CTGF transcript levels, further supporting our findings (Fig. 6G).

Discussion

Recent studies have highlighted the close relationship between intestinal flora and the development of CRC, particularly focusing on the role of *F. nucleatum* in promoting CRC progression [15]. However, existing research

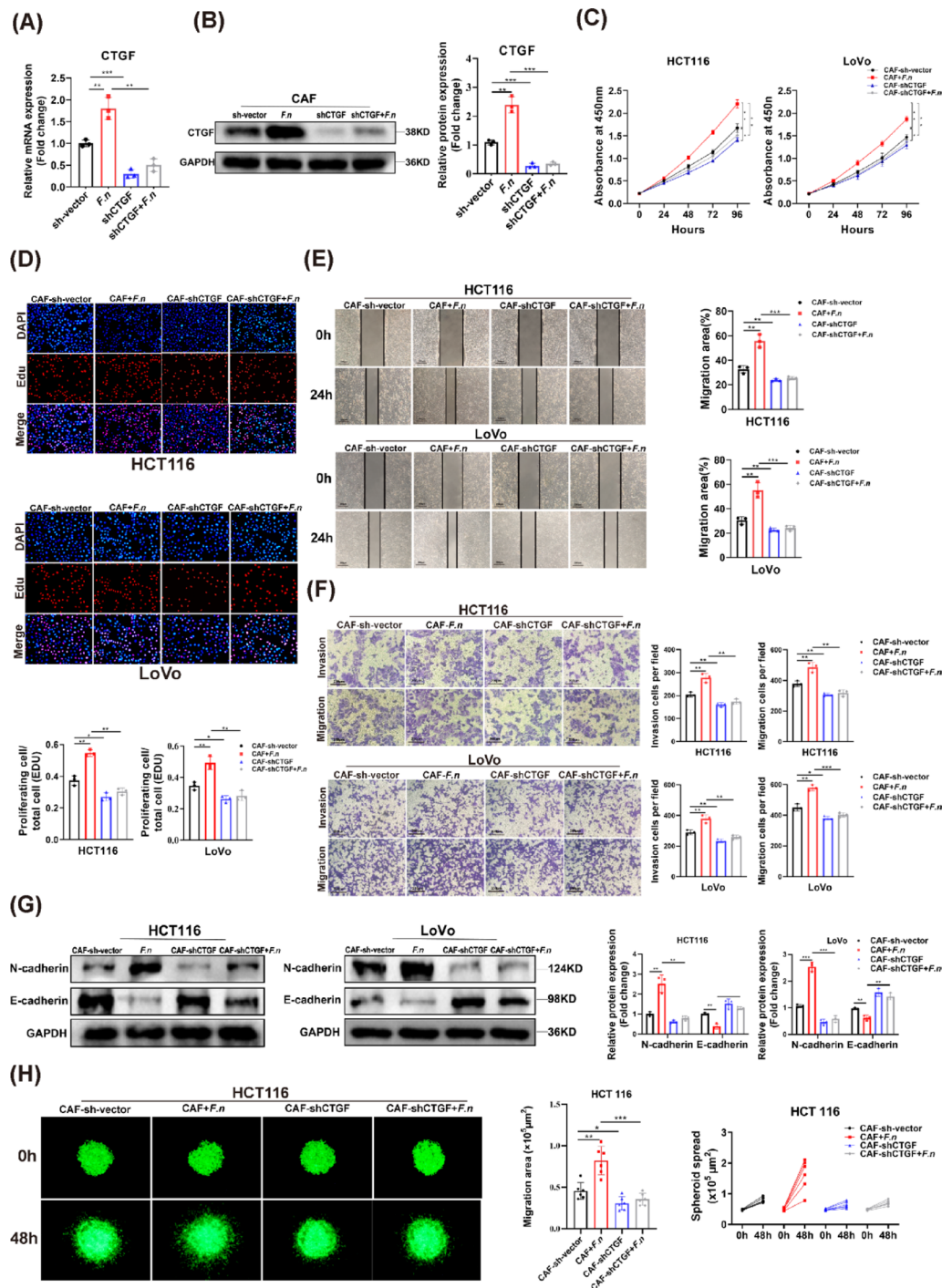


Fig. 4 *F. nucleatum* impacts the expression of CTGF in CAFs, promoting the proliferation and migration of CRC cells. **(A)** The mRNA expression levels of CTGF in CAFs, assessed by RT-qPCR. **(B)** Immunoblot analysis of protein extracts from CAFs using the indicated antibodies. **(C)** The proliferation of CRC cells assessed by CCK8 test. **(D)** Representative images and quantification of EdU assay of CRC cells. **(E)** Representative images and quantification of CRC cells migration in a scratch test. **(F)** Representative images and quantification of CRC cells migration and invasion in a Transwell system with an 8-micron pore size. **(G)** Immunoblot analysis of EMT related protein extracts from CRC cells. **(H)** Representative images and quantification of depicting cell spheres composed of either 10,000 HCT116 cells or LoVo cells migrating within collagen mixtures

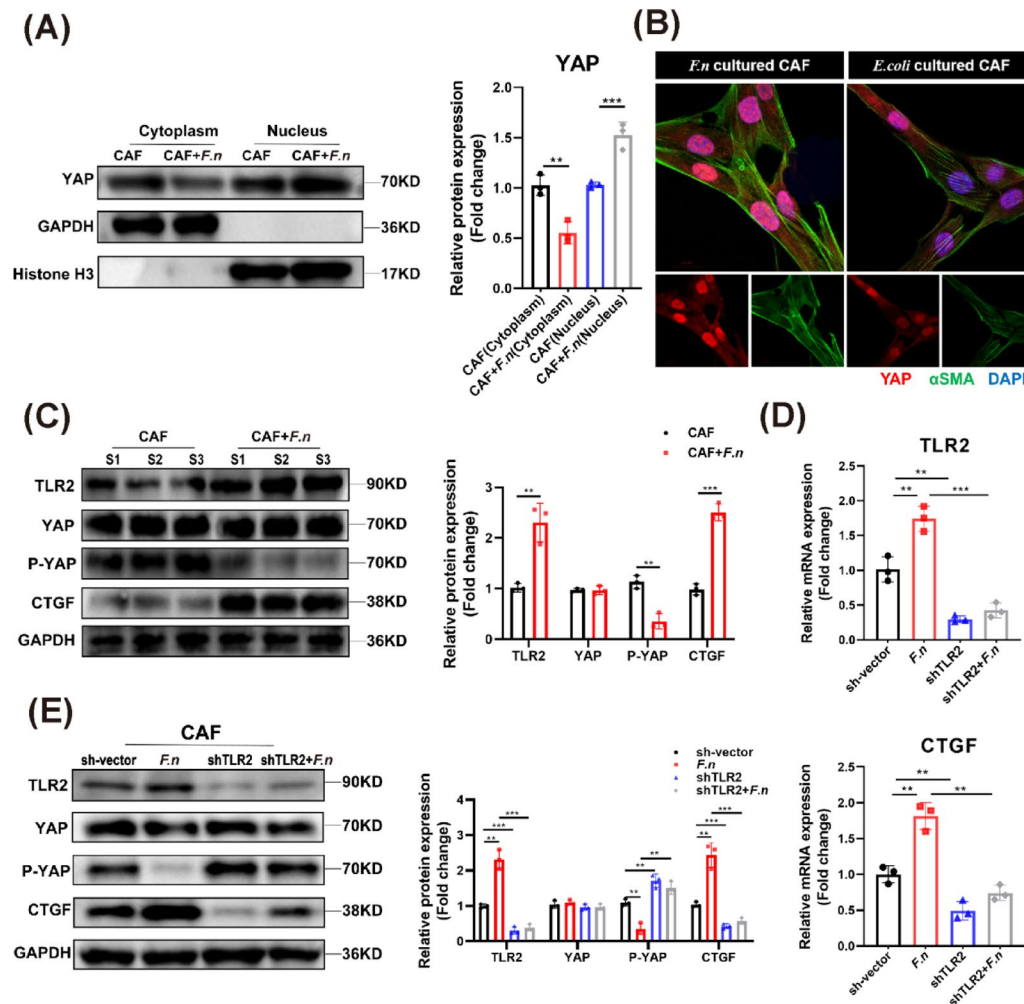


Fig. 5 *F. nucleatum* influences the progression of CRC by modulating the TLR2-YAP pathway to upregulate the expression of CTGF in CAFs. (A) Immunoblot analysis to quantify the levels of YAP extracts from CAFs. (B) Immunofluorescence staining of YAP (red) and DAPI (blue) in CAFs cultured with or without *F. nucleatum* (63x oil immersion). (C) Immunoblot analysis of protein extracts from CAFs following co-culture with *F. nucleatum*. (D) The mRNA expression levels of TLR2 and CTGF in CAFs were assessed by RT-qPCR following treatment with shTLR2. (E) Immunoblot analysis of protein extracts from CAFs following treatment with shTLR2.

has primarily focused on the direct impact of *F. nucleatum* on CRC cells. The regulation of the tumor microenvironment (TME) is crucial in the progression of CRC [16], necessitating a thorough examination of the interplay between these factors. This study primarily concentrates on *F. nucleatum*, which indirectly influences CRC progression by modulating the TME—a topic that has been infrequently addressed in CRC studies.

CAFs play a crucial role as a matrix component within the TME, resulting from the alteration of normal fibroblast properties upon stimulation and subsequent changes in cytokine secretion [17]. Recent research has revealed that these fibroblasts can impact colorectal cancer progression through various mechanisms, such as extracellular matrix (ECM) remodeling, influencing biomechanical signaling, facilitating the secretion of growth factors, enhancing angiogenesis, and suppressing

the recruitment of immune cells, among other effects [18–20]. The microbiota has been reported can modulate CAFs through its metabolites [21] or influence the immune system to indirectly regulate CAFs [22]. These mechanisms collectively contribute to the progression of various tumor types by modulating CAFs. *F. nucleatum* is well known to contribute to CRC progression and is associated with poor prognosis. In this study, we observe that *F. nucleatum*-positive tumors exhibit a higher profile of CAFs activation.

The adhesion of bacteria to host cells is critical for their pathogenic activity, primarily mediated by bacterial adhesins [23]. Current understanding suggests that bacteria modulate host cellular functions through interactions with membrane receptors or exert effects via intracellular receptors following cellular invasion [24]. The findings of this study demonstrate that *F. nucleatum*

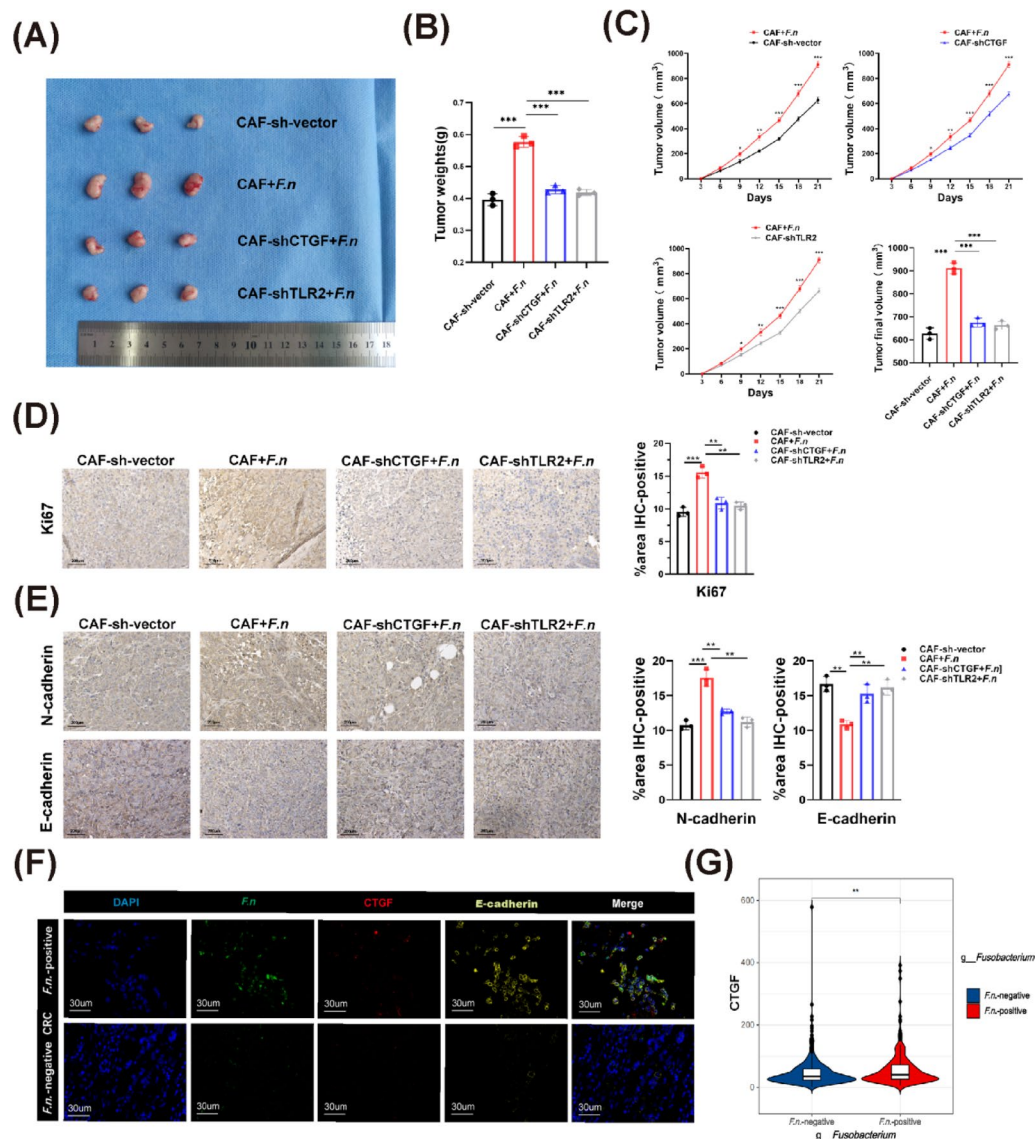


Fig. 6 *F. nucleatum* upregulates the expression of CTGF in CAFs by inducing the expression of TLR2, thereby facilitating the progression of CRC in an in vivo setting. **(A)** Representative photographs of subcutaneous xenograft tumors across four experimental groups (n=3). **(B)** Quantitative analysis of xenograft tumor mass across four experimental groups (n=3). **(C)** Longitudinal monitoring of xenograft tumor volume across four experimental groups (n=3). **(D)** Immunohistochemistry staining for Ki67 in xenograft tumors (n=3). **(E)** Immunohistochemistry staining for N-cadherin and E-cadherin in xenograft tumors (n=3). **(F)** Immunofluorescence staining of DAPI (blue), *F. nucleatum* (green), CTGF (red), and E-cadherin (yellow) in CAFs cultured with or without *F. nucleatum*. **(G)** The relationship of CTGF expression to *F. n*-negative (n=399) or *F. n*-positive (n=302)

interacts directly with CAFs. Using confocal microscopy, we observed colocalization of *F. nucleatum* and CAFs. After a 2-hour co-culture, *F. nucleatum* adhered to the surface of CAFs, and by 12 h, bacterial cell invasion into CAFs was evident. These results confirm the ability of *F. nucleatum* to bind to and invade CAFs. However, whether intracellular invasion is essential for the progression of CAFs-mediated CRC remains unclear and warrants further investigation.

Previous studies have demonstrated that CAFs enhance the angiogenesis capacity of colorectal cancer through elevated expression of vascular endothelial growth factor

A (VEGFA), validating the feasibility of this mechanistic paradigm [21]. This represents a classic model in which CAFs-derived cytokines facilitate cancer cell progression. Our study reveals that conditioned media from co-cultured CAFs and *F. nucleatum* can significantly enhance the proliferation and migration of HCT116 and Lovo cells in vitro. This implies that CAFs may exert indirect effects on CRC cells via secreted factors.

Subsequently, utilizing RNA-seq for genome-wide transcriptome analysis, our study identified that PLAT, CTGF, and FBN1 were significantly upregulated in CAFs co-cultured with *F. nucleatum*. Notably, CTGF is among

the significantly upregulated genes and is known to play a crucial role in the activation of CAFs [25]. Furthermore, elevated CTGF expression has been implicated in promoting tumor progression across multiple malignancies, including *Helicobacter pylori*-driven gastric cancer progression [13], modulation of glioma cell proliferation and apoptosis [26], and stimulation of breast cancer cell proliferation [27]. Notably studies have demonstrated that CAF-derived extracellular vesicles can promote liver cancer metastasis by delivering CTGF [28]. Moreover, autotaxin (ATX) derived from pancreatic cancer-associated fibroblasts drives autocrine CTGF expression to modulate pro-tumor signaling transduction [29].

In addition, transcriptome sequencing in this study reveals alterations in TLR-related pathways in CAFs following co-culture with *F. nucleatum*. *F. nucleatum* has been shown to promote cancer development through toll-like receptor 2 (TLR2)/toll-like receptor 4 (TLR4) signaling [30]. TLR2 is implicated in the conversion of stromal cells into CAFs [31]. For example, TLR2 activates NF- κ B, stimulates fibroblasts to increase the expression of VCAM-1 and ICAM-1, and promotes monocyte adhesion [32]. Subsequently, we conducted additional investigations into the mechanism by which *F. nucleatum* facilitates CRC progression following TLR2 knockdown in CAFs. Our findings indicate that depletion of TLR2 in CAFs results in a concomitant decrease in CTGF expression, leading to a diminished capacity of *F. nucleatum* to promote CRC advancement. These results align with the hypothesis that *F. nucleatum* may contribute to CRC progression by modulating the TLR2-YAP pathway to enhance CTGF expression in CAFs.

While our research elucidates how *F. nucleatum* may indirectly influence CRC progression by affecting CAFs, we recognize certain limitations in our study. It remains uncertain in our study whether *F. nucleatum* internalization is essential or whether adhesion alone is sufficient. We will further investigate this question in future studies. It remains to be determined whether the conditioned medium retains bacterial cells components of *F. nucleatum*, which could contribute to CRC progression, and these factors should be thoroughly investigated. However, although we performed bacterial filtration each time when collecting CM after co-culturing *F. nucleatum* with cells, it may still not be possible to completely exclude bacterial soma components. Additionally, current ways to block the effects of *F. nucleatum* on tumors include the use of metronidazole or β -lactam antibiotics and the blocking of FadA's interaction with the host. The potential for clinical translation of our findings requires further development. Looking forward, we aim to therapeutically target key genes on CAFs to modulate the tumor micro-environment of colorectal cancer, thereby potentially delaying disease progression in CRC patients.

Conclusion

In summary, our findings provide experimental evidence for a novel mechanism by which *F. nucleatum* promotes colorectal cancer progression through its modulation of CAFs. Mechanically, *F. nucleatum* promotes the expression of CTGF in CAFs via the TLR2/YAP pathway, which facilitates the proliferation and migration of colorectal cancer cells, ultimately driving CRC progression. Our study elucidates how the gut microbiota modulates CRC progression via the TME, uncovering potential therapeutic targets for both understanding CRC pathogenesis and developing novel treatments.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12935-025-04023-2>.

Supplementary Material 1

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Author contributions

Haoran Wang: writing- original draft, investigation, methodology, conceptualization, formal analysis, data curation and funding acquisition. Baiqiang Lin, Zhiyue Wang: writing- original draft, investigation, methodology, conceptualization, formal analysis and data curation. Jinhua Yang, Xiao Guo, Bowen Li, and You Zhou: investigation, visualization, and software. Wei Li, Shang Shi, and Rui Han: investigation. Yang Liu and Yunwei Wei: funding acquisition, project administration, resources, supervision, writing—review, validation. All authors read and approved the final version of the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics statement

Animal studies were conducted under the protocol approved by the First Affiliated Hospital of Harbin Medical University (2022037). The clinical trial was approved by the Institutional Review Board (IRB-AF/SC-08/05.0) of the First Affiliated Hospital of Harbin Medical University. All participants in this study provided written informed consent.

Competing interests

The authors declare no competing interests.

Author details

¹Department of Pancreatic and Gastrointestinal Surgery Division, Ningbo No.2 Hospital, Ningbo, China

²Department of Central Laboratory, The First Affiliated Hospital of Harbin Medical University, Harbin, China

³Department of Oncology and Laparoscopy Surgery, The First Affiliated Hospital of Harbin Medical University, Harbin, China

⁴Ningbo Key Laboratory of Intestinal Microecology and Human Major Diseases, Ningbo, China

⁵Department of General Surgery, The First Affiliated Hospital of Henan, University of Science and Technology, Luoyang, China

⁶Department of Neurology, Heilongjiang Provincial Hospital, Harbin, China

⁷Department of Breast Surgery, Ningbo Medical Center Lihuili Hospital, Ningbo, China

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References

- Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin*. 2022;72(1):7–33.
- Dong J, Tai JW, Lu L-F. MiRNA–microbiota interaction in gut homeostasis and colorectal cancer. *Trends Cancer*. 2019;5(11):666–9.
- Rimal R, Desai P, Daware R, Hosseinnajad A, Prakash J, Lammers T, et al. Cancer-associated fibroblasts: origin, function, imaging, and therapeutic targeting. *Adv Drug Deliv Rev*. 2022. <https://doi.org/10.1016/j.addr.2022.114504>.
- Ganguly D, Chandra R, Karalis J, et al. Cancer-Associated Fibroblasts: Versatile Players in the Tumor Microenvironment. *Cancers (Basel)*. 2020;12(9):2652.
- Haruki K, Kosumi K, Hamada T, Twombly TS, Vayrynen JP, Kim SA, et al. Association of autophagy status with amount of *Fusobacterium nucleatum* in colorectal cancer. *J Pathol*. 2020;250(4):397–408.
- Li B, Wei Z, Wang Z, Xu F, Yang J, Lin B, Chen Y, Wenren H, Wu L, Guo X, et al. *Fusobacterium nucleatum* induces oxaliplatin resistance by inhibiting ferroptosis through E-cadherin/beta-catenin/GPX4 axis in colorectal cancer. *Free Radic Biol Med*. 2024;220:125–38.
- Kim TY, Kim S, Kim Y, Lee YS, Lee S, Lee SH, et al. A high-fat diet activates the BAS-FXR axis and triggers cancer-associated fibroblast properties in the colon. *Cell Mol Gastroenterol Hepatol*. 2022;13(4):1141–59.
- Xu Z, Lv Z, Chen F, Zhang Y, Xu Z, Huo J, et al. Dysbiosis of human tumor microbiome and aberrant residence of actinomyces in tumor-associated fibroblasts in young-onset colorectal cancer. *Front Immunol*. 2022;13:1008975.
- Qu X, Yin F, Pei M, Chen Q, Zhang Y, Lu S, et al. Modulation of intratumoral *Fusobacterium nucleatum* to enhance sonodynamic therapy for colorectal cancer with reduced phototoxic skin injury. *ACS Nano*. 2023;17(12):11466–80.
- Tong Y, Lu G, Wang Z, Hao S, Zhang G, Sun H. Tubeimusiide I improves the efficacy of a therapeutic *Fusobacterium nucleatum* dendritic cell-based vaccine against colorectal cancer. *Front Immunol*. 2023;14:1154818.
- Lu X, Xu Q, Tong Y, Zhang Z, Dun G, Feng Y, et al. Long non-coding RNA EVADR induced by *Fusobacterium nucleatum* infection promotes colorectal cancer metastasis. *Cell Rep*. 2022;40(3):111127.
- Wang D, Wang X, Song Y, Si M, Sun Y, Liu X, Cui S, Qu X, Yu X. Exosomal miR-146a-5p and miR-155-5p promote CXCL12/CXCR7-induced metastasis of colorectal cancer by crosstalk with cancer-associated fibroblasts. *Cell Death Dis*. 2022;13(4):380.
- Chen B, Liu X, Yu P, Xie F, Kwan JSH, Chan WN, Fang C, Zhang J, Cheung AHK, Chow C, et al. H. pylori-induced NF-kappaB-PIEZO1-YAP1-CTGF axis drives gastric cancer progression and cancer-associated fibroblast-mediated tumour microenvironment remodelling. *Clin Transl Med*. 2023;13(11):e1481.
- Pastushenko I, Blanpain C. EMT transition states during tumor progression and metastasis. *Trends Cell Biol*. 2019;29(3):212–26.
- Wong CC, Yu J. Gut microbiota in colorectal cancer development and therapy. *Nat Rev Clin Oncol*. 2023;20(7):429–52.
- Al Zein M, Boukhoud M, Shammaa H, Mouslem H, El Ayoubi LM, Iratni R, et al. Immunotherapy and immunoevasion of colorectal cancer. *Drug Discov Today*. 2023;28(9):103669.
- Biffi G, Tuveson DA. Diversity and biology of Cancer-Associated fibroblasts. *Physiol Rev*. 2021;101(1):147–76.
- Najafi M, Farhood B, Mortezaee K. Extracellular matrix (ECM) stiffness and degradation as cancer drivers. *J Cell Biochem*. 2019;120(3):2782–90.
- Fang Z, Meng Q, Xu J, Wang W, Zhang B, Liu J, et al. Signaling pathways in cancer-associated fibroblasts: recent advances and future perspectives. *Cancer Commun*. 2023;43(1):3–41.
- Mao X, Xu J, Wang W, Liang C, Hua J, Liu J, Zhang B, Meng Q, Yu X, Shi S. Crosstalk between cancer-associated fibroblasts and immune cells in the tumor microenvironment: new findings and future perspectives. *Mol Cancer*. 2021;20(1):131.
- Wang H, Chen J, Chen X, et al. Cancer-associated fibroblasts expressing sulfatase 1 facilitate VEGFA-dependent microenvironmental remodeling to support colorectal cancer. *Cancer Res*. 2024;84(20):3371–87.
- Zhu Z, Huang J, Zhang Y, Hou W, Chen F, Mo YY, et al. Landscape of tumoral ecosystem for enhanced anti-PD-1 immunotherapy by gut *Akkermansia muciniphila*. *Cell Rep*. 2024;43(6):114306.
- Kline KA, Falke S, Dahlberg S, Normark S, Henriques-Normark B. Bacterial adhesins in host-microbe interactions. *Cell Host Microbe*. 2009;5(6):580–92.
- Wahlstrom A, Sayin SI, Marschall HU, Backhed F. Intestinal crosstalk between bile acids and microbiota and its impact on host metabolism. *Cell Metab*. 2016;24(1):41–50.
- Choi KM, Kim B, Lee SM, Han J, Bae HS, Han SB, Lee D, Ham IH, Hur H, Kim E, et al. Characterization of gastric cancer-stimulated signaling pathways and function of CTGF in cancer-associated fibroblasts. *Cell Commun Signal*. 2024;22(1):8.
- Zhang LX, Jin W, Zheng J, Dai YX, Song Y, Ni HB, et al. MicroRNA-375 regulates proliferation and apoptosis of glioma cancer cells by inhibiting CTGF-EGFR signaling pathway. *Bratisl Lek Listy*. 2018;119(1):17–21.
- Santolla MF, Lappano R, Cirillo F, Rigracciolo DC, Sebastiani A, Abonante S, et al. MiR-221 stimulates breast cancer cells and cancer-associated fibroblasts (CAFs) through selective interference with the A20/c-Rel/CTGF signaling. *J Exp Clin Cancer Res*. 2018;37(1):94.
- Zheng M, Liu L, Cui H, Zhao Y, Chen W, Bai S, Peng W, Wang Y, Li Y, Wang R, et al. Cancer-associated fibroblast-derived extracellular vesicles facilitate metastasis in hepatocellular carcinoma by delivering CTGF. *Cell Oncol*. 2025.
- Volat F, Medhi R, Maggs LZ, et al. Pancreatic CAF-derived autotaxin drives autocrine CTGF expression to modulate protumorigenic signaling. *Mol Cancer Ther*. 2025;24(2):230–41.
- Sun CH, Li BB, Wang B, Zhao J, Zhang XY, Li TT, et al. The role of *Fusobacterium nucleatum* in colorectal cancer: from carcinogenesis to clinical management. *Chron Dis Transl Med*. 2019;5(3):178–87.
- Wu H, Xiang Z, Huang G, He Q, Song J, Dou R, Yang C, Wang S, Xiong B. BGN/FAP/STAT3 positive feedback loop mediated mutual interaction between tumor cells and mesothelial cells contributes to peritoneal metastasis of gastric cancer. *Int J Biol Sci*. 2023;19(2):465–83.
- Cheng CY, Chen YH, Thuy Tien Vo T, Chui Hong Y, Wang CS, Canh Vo Q, et al. CORM-2 prevents human gingival fibroblasts from lipoteichoic acid-induced VCAM-1 and ICAM-1 expression by inhibiting TLR2/MyD88/TRAF6/PI3K/Akt/ROS/NF-kappaB signaling pathway. *Biochem Pharmacol*. 2022;201:115099.

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