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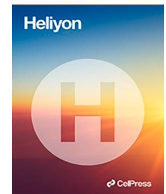

Research article

Contribution of PKS+ *Escherichia coli* to colon carcinogenesis through the inhibition of exosomal miR-885-5p

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A R T I C L E I N F O

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A B S T R A C T

Objectives: About 90 % of all colorectal cancer (CRC) fatalities are caused by the metastatic spread of primary tumors, which is closely correlated with patient survival and spreads by circulating tumor cells (CTCs). The epithelial-mesenchymal transition (EMT) that characterizes CTCs is associated with a poor prognosis. Organotropic metastasis is dictated by the transmission of miRNAs by cancer-derived exosomes. The purpose of this research is to examine PKS + E's function. Coli in CRC metastases and exosomal miR-885-5p suppression.
Methods: A cohort of 100 patients (50 CRC, 50 healthy) underwent colonoscopy screenings from February 2018 to August 2021. Exosomes were isolated using ultracentrifugation, and exosomal miRNA was analyzed using sequencing and qPCR.
Results: Among the patients, 40 tested positive for *E. coli* (12 CRC, 23 healthy). Serotyping revealed that 68.57 % harbored the PKS gene. Exosomal miR-885-5p levels were significantly altered in CRC patients with PKS + *E. coli*. Intriguingly, our findings indicate that exosomes derived from EMT-CRC cells did not affect miR-885-5p synthesis in HUVECs. Moreover, we observed that the levels of miR-885-5p in both exosomes and the total CRC-conditioned medium were comparable upon isolation of exosomes from CRC cells. What's more, an increased expression of miR-558-5p within the tumors, and the group that received exosome treatment, as well as the EMT-HCT116 group, exhibited a higher occurrence of distant metastasis.
Conclusion: PKS + E. By inhibiting exosomal miR-885-5p, coli is linked to CRC metastases, offering a possible target for therapeutic intervention.

1. The strength of this study

1. *E. coli* patients showed a clear correlation between the onset of CRC and the presence of PKS + strains.
2. Exosomal miR-885-5p, derived from CRC cells undergoing epithelial-mesenchymal transition.

2. Introduction

Colorectal cancer (CRC) ranks third in terms of frequency of diagnosis and is the second most lethal cancer type worldwide [1].

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Abbreviation

CTCs:	Circulating tumor cells
EMT:	Epithelial-mesenchymal transition
CRC:	Colorectal cancer
PKS+	Polyketide synthase-positive
E. coli:	*Escherichia coli*

Distant metastases of colorectal cancer pose a serious challenge to therapy [2]. 15 %–25 % of patients with colorectal cancer (CRC) had liver metastases at the time of diagnosis. In addition, 15 %–25 % of patients had liver metastases after surgical resection of the primary CRC site [3]. The total incidence of colorectal and distal colon cancer, particularly rectal and distal colon cancer, has grown in those under 50 years old but dropped in those over 50 in recent years. Regretfully, 80%–90 % of these liver metastases are original causes of death [4]. Although CRC treatment has a high death rate, there has been notable success with both surgical resection and chemotherapy [5]. Twenty percent of patients with recently diagnosed colorectal malignancies had metastases at the time of diagnosis, and an additional twenty-five percent acquired metastases after initially presenting with limited disease [6]. Compared to patients with other isolated sites of metastases, patients with peritoneal metastatic CRC had noticeably lower overall survival. Poor survival is a result of both peritoneal involvement and an increased number of metastatic sites in individuals with multiple sites of metastasis [7]. Common modalities of treatment include radiation therapy and chemotherapy, administered both before surgery (neoadjuvant) and after surgery (adjuvant). Even while it has been shown that chemotherapy and radiation therapy may lessen tumor burden, a major disadvantage of these treatment modalities is the increased risk of patient recurrence [8,9]. However, the incidence of CRC is expected to rise annually due to factors such as the westernization of food, ongoing stress, and the extensive use of antibiotics to modify the gut microbiota. *E. coli* also has a significant role in colon cancer [10]. E. The intestines are home to the gram-negative, anaerobic bacteria known as *E. coli* [11]. Even though they are not normally harmful, some toxic E. Coli that grow in the human gut are harmful and may lead to illness in a number of ways. an increased E abundance. Coli was found in CRC patient mucosal samples [12]. *E. coli*, a polyketone peptide that is genotoxic and generated by PKS + E. Coli activates cell cycle arrest and DNA damage signaling pathways, which results in double-stranded DNA damage and tumor formation [13]. E. Coli also secretes cell-lethal dilatory toxins that cause apoptosis, DNA damage, and cell cycle arrest, in addition to additional virulence factors such cyclooxygenase 2, cytotoxic necrosis factor, and cycle inhibitors [14]. It is crucial to investigate novel approaches for treating metastatic CRC. Investigating the pathophysiology and potential causes of CRC is crucial to understanding the disease.

As is often known, colorectal cancer (CRC) is a complicated illness impacted by both hereditary and environmental factors [9,15]. While age, sex, and genetic mutations are unmodifiable risk factors, certain factors like smoking, excessive alcohol consumption, obesity, sedentary lifestyles, diabetes, red meat consumption, high-fat diets, and inadequate fiber intake can be altered to mitigate the risk [16]. Current developments in fundamental research suggest that the gut microbiota plays a critical role in the development, expansion, and spread of colorectal cancer [17]. Metagenomics confirms that the normal crypts of colon cancer patients exhibit a high incidence of pks + *E. coli* mutational and indel signatures, in contrast to those of healthy persons [18]. A unique mutational signature in CRC suggests that prior exposure to bacteria with the colibactin-producing pks pathogenicity island + directly causes the underlying mutational process [19]. Research suggests that pks + E infection affects the course of colorectal cancer (CRC). Pks + E levels are significantly elevated in CRC patients, especially in normal tissues close to the cancer site. Still unclear, nevertheless, is the precise process behind metastasis [20]. According to epidemiologic data, pks E. Stool or tissue samples from CRC patients had more coli + than those from inflammatory bowel disease patients or healthy controls. Numerous pieces of evidence point to pks E. Coli + promotes mutagenesis that starts tumors. First, a rise in tumor frequency rather than tumor size is the primary characteristic of the tumorigenic phenotype shown in preclinical models [21]. Comparing the frequency of PKS-associated mutational characteristics to the number of colonized CRC patients with pks + E, significant differences were found. coli+ (~55–60 %), indicating that these microorganisms can encourage the growth of tumors by non-mutagenic means [22]. Different kinds of cells produce small vesicles called exosomes that are attached to lipids. They are widely dispersed and are increasingly being acknowledged in the area of regenerative medicine due to their potential use as disease indicators and therapeutic agents [23], for example, CRC tumors [24]. Tumor cell-released exosomes are known to have distinct RNA and protein contents, and they are being recognized as a key player in the communication network that facilitates the growth of tumors [22].

MicroRNAs (miRNAs) modulate gene expression post-transcriptionally, serving as important regulators of tumor development. They do this by preventing the translation of target mRNAs and causing them to become unstable [25]. Since miRNAs play a critical role in regulating gene expression, there has been much research done on them as possible biomarkers. In addition, researchers are creating miRNA inhibitors and mimics as novel treatment approaches [26]. Exosomes are vesicles that vary in size from 40 to 100 nm and are formed by different kinds of cells. The union of the cell membrane and the outer membrane of the multivesicular body eventually releases these entities into the extracellular matrix [27]. Exosomes produced by tumor-associated macrophages during carcinogenesis may serve as novel therapeutic targets and diagnostic indicators for the prevention of cancer [28]. Exosomes generated from cancer cells have a considerable potential to modify the local and distant environments, and they are often produced in greater quantities by cancer cells than by normal cells [29]. Proteins, lipids, and nucleic acids transported by exosomes are being investigated as possible targets for cancer therapy as well as as promising biomarkers for cancer diagnosis and prognosis. Furthermore, exosomes from various origins act differently in cancer applications [30,31]. Given that their patterns are different from those of their parent

cells, exosomal miRNAs could have an active sorting mechanism [32]. Exosomal miRNAs have also been shown to have a major effect on the spread of cancer cells and angiogenesis, which both accelerate the course of the illness [33].

Our results lead to the hypothesis that pks + E is much more common in people with CRC (CRC) than in other populations. As a result, pks + E may benefit from miRNA being transferred by exosomes to cells in nearby healthy tissues that are close to malignant cells. Coli bacteria proliferate in tumor tissues, which aids in the development of CRC.

3. Materials and methods

The First Affiliated Hospital and College of Clinical Medicine at Henan University of Science and Technology's institutional review boards (IRBs) and hospital ethics committees approved the work reported here (DE44232342).

All participants willingly gave their informed permission in accordance with the CONSORT principles and the Helsinki Declaration. Respect for the UK Animals (Scientific Procedures) Act 1986 and its rules, the EU Directive 2010/63/EU for animal research, and the National Research Council's Guide for the Care and Use of Laboratory Animals were all given high priority when it came to animal experiments.

3.1. Isolation of E. coli from clinical specimens

Pieces of evidence of *E. coli* presence were discovered in fecal samples, tumor specimens, and tissue biopsies collected from both individuals in good health and those diagnosed with CRC (CRC). A cohort of 100 patients, consisting of 50 CRC patients and 50 healthy individuals, underwent routine colonoscopy screenings followed by either colon or rectal resections for research purposes from February 2018 to August 2021. We designated early- and late-onset colorectal cancer, respectively, as those that manifest at <50 and ≥ 50 years of age [34]. Patients aged 18–75 years, undergoing routine colonoscopy, with no history of other cancers or severe psychological diseases. Participants who fulfilled the following eligibility requirements were also chosen from our hospital's two medical centers' proctology departments, colorectal surgical clinics, and patient wards. The exclusion criteria included: 1) a life expectancy of less than three months; 2) a diagnosis of serious psychiatric disorder; 3) a history of cancer; 4) an Eastern Cooperative Oncology Group (ECOG) performance score of less than three; and 5) the incapacity to communicate either orally or in writing [35].

Processing, isolation, and culture of all clinical specimens were conducted by the First Affiliated Hospital, and College of Clinical Medicine of Henan University of Science and Technology. The samples underwent homogenization, followed by culturing in Luria Bertani broth overnight. Subsequently, the cultured samples were streaked onto MacConkey Agar to obtain pure colonies of *E. coli*. All colonies exhibiting a mucoid appearance were identified as *E. coli* using the Bruker Biotyper MALDI-TOF MS technique, following the guidelines provided by the manufacturer. To strengthen the species identification, the 16s rRNA gene sequencing method was employed. Samples of feces were frozen at −80 °C. Following the manufacturer's instructions from Qiagen, Hilden, Germany, we isolated the fecal samples using the Qiagen QIAamp DNA Mini Kit. Basic E. Coli colonies were kept until later usage at −80 °C with 30 % glycerol added. The removal of E. Utilizing the Qiagen DNeasy Blood & Tissue Kit and according to the manufacturer's guidelines, coli DNA was extracted. The IMPLEN NanoPhotometer was used to evaluate and measure the extracted DNA's purity and concentration. For further examination, a DNA purity range of 1.8–2.0 (A260/A280 ratio) was chosen. Any extra DNA was separated into aliquots and kept for later use at −20 °C.

3.2. Cell culture and reagents

The Chinese Academy of Sciences provided five CRC cell lines (LOVO, HCT-116, DLD-1, SW620, and SW480) for the study [36,37]. Furthermore, the NCM460 cell line was acquired, which is a representative of a healthy intestinal epithelium. These cell lines were grown using the Roswell Park Memorial Institute (RPMI) 1640 media supplemented with 10 % fetal bovine serum in an incubator set at 37 °C with 5 % CO2. The American Type Culture Collection provided the Human umbilical vein endothelial cells (HUVECs), which were cultured in M199 medium (Invitrogen, Shanghai, China) with 10 % FBS (Gibco, USA) added as a supplement. In order to get a final concentration of 50 ng/ml, recombinant IL6 produced from human sources (R&D Systems) was properly diluted. The dilution was done in phosphate-buffered saline solution that had 0.1 % BSA (bovine serum albumin) added to it. Before the HUVECs from Biosolar in China were co-cultured, exosomes underwent a pre-incubation stage with annexin V at a concentration of 2 g/ml. Two hours were spent on this pre-incubation [24].

3.3. Isolation, analysis, and treatment of exosomes

To support the growth of CRC c ells, RPMI-1640 media was employed, and it was supplemented with exosome-free fetal bovine serum at a concentration of 10 %. To isolate CRC cells from exosomes, the FBS used was subjected to ultracentrifugation. This process was carried out overnight using the Beckman Optima L-100XP, with centrifugal forces reaching 110,000 g.

The cell culture medium was removed after a 72-h incubation period, and it was then centrifuged twice: once at 2000 g for 20 min and once at 25000 g for 40 min at 4 °C. After that, the resultant supernatant was filtered using a 0.22 m filter. Moreover, the BCA Protein Assay kit (provided by Millipore, Billerica, MA, USA) and a transmission electron microscope (HT7700, made by Hitachi in Japan) were used to carefully inspect, characterize, and quantify the isolated exosomes.

Exosomes were extracted by ultracentrifugation at 110,000 g for 90 min. Following this, the exosomes were resuspended in a phosphate-buffered saline solution and tagged with PKH67 (Sigma, St. Louis, MO, USA). Fluorescence microscopy pictures were then

taken after exosomes were cultured with human umbilical vein endothelial cells (HUVEC) for 240 min. Lastly, to aid in cell breakdown, exosomes and recipient cells were co-cultured for 72 h. Ultracentrifugation was used to separate the exosomes, then qPCR and sequencing were used to measure the miRNA. At 2 x 105, the number of recipient cells did not change. Furthermore, the same processing procedures were applied to epithelial-mesenchymal transition (EMT)-HCT116/SW620 cell-conditioned media that were only treated with Triton X-100 (0.1 %) or RNase (2 mg/ml) [38]. Zetasizer ano ZS (Malvern Instruments Ltd., Malvern, UK) was used to measure the dynamic light scattering (DLS) detection and zeta potential of nanovesicles [39,40].

3.4. Animal experiment

The instructions issued by the Hubei Provincial Key Laboratory of Tumor Biological Behavior were adhered to while conducting animal experiments. The Animal Health and Ethics Committee of Henan University of Science and Technology's College of Clinical Medicine gave its permission for all animal experiments. CRC cells were injected into BALB/c nude mice for in vivo research. For the orthogonal heterosexual transfer experiment, five times as many CRC cells were subcutaneously implanted into female BALB/c nude mice, aged 4–6 weeks. After 14 days, the xenografts were aseptically dissected, and the fibrotic tissue was extracted. The tumor tissue was then minced, removed, and cut into 1 mm3 pieces. Under general anesthesia, the naked mice's caecum and ascending colon were exteriorized, allowing the 1 mm3 xenograft pieces to be implanted sub-serosally. After that, the colon was sutured back into the belly. To evaluate permeability, mice were given intravenously 100 mg/kg of rodamine-dextran in their tail veins. The nude mice had their hearts punctured to extract 1 mL of blood, which was then centrifuged with ethylene diamine tetraacetic acid. Finding CTCs later on was made simpler by separating the cells from the plasma. After that, the cells were once again suspended in phosphate-buffered saline. Exosomes were isolated from plasma using the exoRNeasy Serum/Plasma MaxiKits (QIAGEN, Germany), and the RNA was then extracted from the exosomes using the Trizol reagent. Using the quantitative reverse transcription-polymerase chain reaction (qRT-PCR) amplification technique, the quantity of miR-885-5p in the exosomes was determined. After being paraffin-fixed, the liver and lungs were repeatedly sectioned, stained with hematoxylin-eosin (HE), and inspected under a microscope.

3.5. PCR

The Trizol reagent was used in the RNA extraction process (Vazyme, China). Then, using the RT Master kit (Vazyme, China), the RNA was translated into complementary DNA (cDNA). Real-time PCR analysis was carried out utilizing the generated cDNA and the SYBR-Green PCRMasterMix (Vazyme, China) to measure the expression of the genes.

3.6. Western blot

The proteins were separated for protein analysis using SDS-PAGE gel electrophoresis, and then they were moved onto a polyvinylidene fluoride membrane (Millibo, USA) for Western blot examination. The membrane was blocked with 5 % skim milk and then incubated with the primary antibody for a whole night at 4 °C. The cleaned membrane was then allowed to sit at room temperature for 60 min while the secondary antibody was incubated. The Bio-Rad ChemiDoc XRS hardware and the Bio-Rad Image Lab software were used for the protein detection and density analysis, respectively.

3.7. Assay for transendothelial invasion

A Transwell system with 24-well plates (Corning) covered with Matrigel (Falcon 354480; Corning) and featuring an 8 μm pore size was used to study transendothelial invasion. After being incubated at 4 °C for a whole night, the Matrigel (5 mg/ml) from Corning was diluted 1:10 in RPMI1640 and kept at −20 °C. 50 μl of the diluted Matrigel was added to the top chamber of the Transwell system, and it was left to harden for 2 h at 37 °C. 1 x 105 cells suspended in 500 μl of RPMI 1640 with 1 % FBS were added to the top compartment, and 750 μl of RPMI 1640 with 10 % FBS was added to the bottom chamber. The cells were counted and photos were taken using five optical microscope fields at a 100× magnification after being incubated for 48 h. Every experiment was conducted thrice. There were three separate runs of the experiment. Transendothelial invasion was used with and without exosome treatment to assess if GFP-expressing HCT-116 cells had pierced the HUVEC monolayer.

3.8. Purification of extracellular vesicles

Extracellular vesicles were purified by cultivating EMT tumor cells to about 100 % confluence. They were then incubated for 48 h in an EV-depleted medium, which was made comprised of the supernatants of the whole media following an overnight centrifugation at 100,000 g. Using a differential centrifugation procedure, exosomes were separated from the supernatants at 4 °C: 300×*g* for 10 min, 3000×*g* for 10 min, 10,000×*g* for 20 min, and 100,000×*g* for 70 min. Following a PBS wash, the separated exosomes underwent a second centrifugation stage at 100,000×*g* for 70 min. As previously reported [41], the size distribution of exosomes was ascertained by means of dynamic light scattering (DLS) using a Nanosizer device (ZEN3790, Malvern Instruments, UK). To see the isolated exosomes placed on copper mesh, transmission electron microscopy was used. Prior to TEM imaging, the samples were dried, cleaned with distilled water, and dyed with phosphotungstic acid [42]. Using the Pierce BCA Protein Assay (23225, Thermo Fisher Scientific, USA), the concentration of total proteins was measured in order to quantify exosomes. Lastly, PKH26 was used to label the exosomes in accordance with the manufacturer's instructions (MINI26, Sigma-Aldrich, USA). The OME chemical (PHR1059, Sigma Aldrich, USA)

was produced in DMSO and administered at a concentration of 20 μg mL-1 to grown cells for a duration of 24 h in order to suppress exosomal release [43].

3.9. Check for the healing of wounds

Cells were grown in a six-well plate for the wound healing experiment until they achieved 90%–100 % confluence. After that, a wound was made with plastic tips. After the incision was made, the cells underwent three PBS washes in order to remove any leftover cellular debris. Two days after the incision was formed, pictures of the migrating cells near the front of the wound were taken. To assure accuracy, each experiment was conducted three times.

3.10. Isolation and characterization of CTCs, transfection of miR-27b-3pmimic, inhibitor, and target gene siRNA

In this investigation, miR-27b-3p mimic, inhibitor, and siRNA targeting relevant genes were transfected into isolated, identified, and circulating tumor cells (CTCs). Conventional techniques [44] were used to improve and identify the CTCs. Guangzhou, China-based RiboBio provided the Hsa-miR-885-5p mimic, inhibitor (also known as anti-miR-885-5p), and matching negative control. The cells were rinsed with PBS (pH 7.4) before to transfection. Lipofectamine 2000 (Invitrogen, USA) at a final concentration of 50 nM was used to promote transfection.

3.11. RIP assay and ChIP assay

The RNA immunoprecipitation experiments were conducted using an RNA immunoprecipitation kit obtained from Millibo, Massachusetts. In brief, 5 x 107 cells were collected and lysed in a lysis buffer. The resulting supernatant was then subjected to Trizol digestion and RNA extraction after being combined with a specific antibody. Following reverse transcription, the isolated miRNA was detected using real-time PCR. The investigation for chromatin immunoprecipitation (ChIP) analysis utilized the Cell Signaling SimpleChIP Enzyme Chromatin IP kit (#9003, USA). Cross-links were eliminated and eluted, and the precipitated DNA was quantified using qRT-PCR.

3.12. Statistical analysis

Every cell culture experiment was run through a statistical analysis after being completed in triplicate with independent duplicates. Unless otherwise stated, the means of all experimental results were provided. The statistical analyses were conducted using IBM SPSS (version 22.0, United States) and GraphPad Prism (version 6.0, GraphPad Software, United States). When comparing more than two groups, a one-way analysis of variance was used to assess the quantitative means of the groups using the two-tailed Student's tests. A p-value of 0.05 was considered to demonstrate statistical significance. Each in vivo and in vitro experiment was run in three separate runs.

Table 1
Serotyping and PKS gene prevalence in *E. coli* isolates.

	Total (100)	clbA+	P-value	blf+	P-value
Cohort					
Control	50 (50 %)	23 (46 %)	NS	7 (14 %)	NS
CRC	50 (50 %)	12 (46 %)		15 (30 %)	
sex (%)					
Female	30 (60 %)	12 (40 %)	NS	5 (16.6)	NS
male	20 (40 %)	11 (55 %)		2 (10 %)	
CRC					
onset (%)					
Early	14 (28 %)	3 (21.42 %)	NS	4 (28.4 %)	NS
late	36 (72 %)	17 (47.2 %)		11 (30.5 %)	
sex (%)					
Female	29 (58 %)	13 (44.8 %)	NS	11 (39.2 %)	NS
male	21 (42 %)	10 (47.61 %)		7 (33.3 %)	
Location (%)					
proximal	11 (22 %)	4 (36.36 %)	NS	5 (45.45 %)	NS
distal	39 (78 %)	10 (25.64 %)		11 (28.20 %)	
stage (%)					
i	11 (22 %)	2 (18.18 %)	NS	3	NS
ii	12 (24 %)	3		3	
iii	19 (38 %)	4		6	
iv	8 (16 %)	2		1	

4. Results

4.1. PKS + E. Coli is strongly linked to the development of CRC

To ascertain the PKS colibactin genotoxin gene's frequency in *E. coli* isolates and their correlation with virulence traits in both healthy people and those suffering from colorectal cancer (CRC), this research looked at patients in both groups. Merely 40 people among them had positive E tests. coli, consisting of 23 healthy people and 12 CRC patients. Interestingly, serotyping analysis showed that 24 (68.57 %) of the 35 clinical isolates harbored the PKS colibactin genotoxin gene, indicating their membership in the six most common serotypes of *E. coli*. It is noteworthy that all eight PKS-positive isolates of the K1 serotype originated from clinical samples of patients with colorectal cancer (CRC), while the remaining seven PKS-positive isolates were obtained from healthy individuals. K20, K54, K57, K1, K2, and K5 coli. Interestingly, the K1 and K2 serotypes, which are very virulent, showed the greatest degree of pathogenicity. Ten PKS-positive isolates were examined, and the results showed that two of them fit the K1 serotype, four the K2 serotype, and two the K57 serotype—all of which are thought to be the serotypes associated with colorectal cancer patients. (Tables 1–2).

Within this study, we investigated the clbA gene primers in the presence of colibactin-producing bacteria. The encoding for this gene was found on the pks island. Moreover, we employed universally compatible primers for *E. coli* as an effective control during the PCR process. Notably, our findings revealed a higher proportion of pks + bacteria in late-onset CRC (17 out of 35) compared to early-onset CRC (3 out of 35; $P < 0.05$) (Fig. 1A). These results suggest that colibactin-producing bacteria are less prevalent in early-onset CRC than in the late-onset cases. To gain insights into the functioning of these genes, their presence will be assessed in the following section. Exosomes derived from tumor cells undergoing an EMT contain MiR-27b-3p, which regulates inflammation.

4.2. MiR-27b-3p communication via exosomes regulates the development of colorectal cancer

Several cues from the tumor microenvironment are involved in the start and control of the EMT process. Interleukin-6 (IL-6) is one of the pro-inflammatory cytokines that is most prevalent in the TME among these signals. The HCT116 cell line, a colorectal cancer cell line with an epithelial-like phenotype, was used in our investigation. We treated the cells with IL-6 at a dose of 50 ng/ml, according to conventional methods, in order to mimic the tumor microenvironment. Exosomes were extracted from the cell culture medium of HCT116 cells that experienced EMT as well as control HCT116 cells in our experiment. These exosomes (Fig. 1B–F) possessed a characteristic disc-shaped morphology with an average diameter of around 100 nm.

We used a Western blot analysis to validate the effective extraction of exosomes and found that exosomal membrane markers, such as TSG101 and CD63, were present. These markers functioned as gauges for successful exosome extraction. We also obtained size distribution data from NanoSight analysis, which showed that the median diameters of the EMT-HCT116 and control HCT116 exosomes were 130.3 nm and 127.0 nm, respectively.

We then carried out an experiment to evaluate the EMT-HCT116 exosomes' functionality. We used PKH67, a green fluorescent dye, to mark the exosomes from EMT-HCT116 cells before delivering them to human umbilical vein endothelial cells (HUVECs). The effective transfer of exosomes from EMT-HCT116 to HUVECs was shown by the green fluorescence that was present in the recipient HUVECs. Building on these discoveries, we also gave HUVECs exosomes derived from a combination of EMT-HCT116 and control HCT116 cells.

Comparing exosomes from control cells to those from EMT-HCT116 cells, our findings showed that the exosomes from the former considerably improved vascular permeability. Through the use of a transendothelial cell invasion experiment, we were able to verify this conclusion by seeing tumor cells that had been tagged with green fluorescent protein (GFP) infiltrating the HUVEC monolayer. Further evidence came from Western blot analysis, which showed that treating HUVECs with EMT-HCT116 exosomes significantly lowered the amounts of MUC and IL-6.

We used Annexin V, an inhibitor of exosome uptake, to verify the exosome internalization process. The increase in permeability brought on by exosomes from EMT-HCT116 cells in HUVECs was inhibited by Annexin V treatment. All of these data point to the same conclusion: via certain molecular pathways, exosomes secreted by EMT-HCT116 cells increase vascular permeability. Immunofluorescence and Western blot analyses were performed to verify the recovery of MUC and IL-6 expression. According to our research, EMT-HCT116 cells' exosome-mediated communication may play a part in controlling inflammation in HUVECs (Fig. 2A–G).

Table 2
E. coli presence of PKS colibactin gene and capsular serotype.

Virulence Gene		Colorectal Cancer (n = 12)		Healthy (n = 23)		Total
		PKS + ve	PKS –ve	PKS + ve	PKS –ve	
Colibactin (PKS)		8	4	16	7	35
Klebsiella pneumoniae	K1 serotype	2	1	2	0	5
	K2 serotype	4	1	0	0	5
	K5 serotype	0	1	0	5	6
	K20 serotype	0	0	3	1	4
	K54 serotype	0	0	3	0	3
	K57 serotype	2	0	0	1	3
	Unknown *	0	1	8	0	9
	Total	8	4	16	7	35

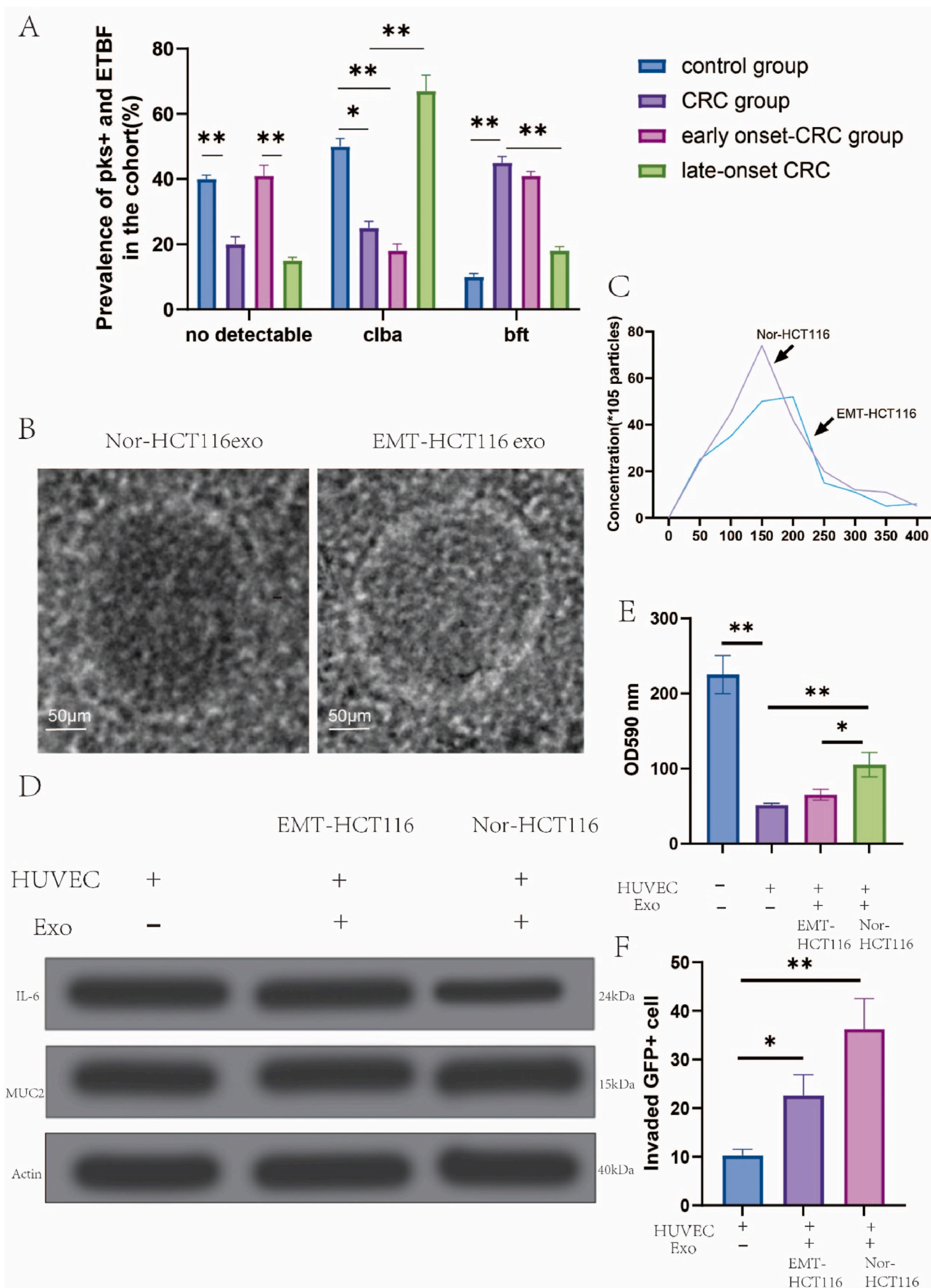

Fig. 1. PKS-Positive *E. coli* Isolates Harboured More Virulence Genes Compared to PKS-Negative Isolates. A, Prevalence of pks+ and ETBF in the cohort. B, Exosome results. C, concerntration of exosome between Nor-HCT116 and EMT-HCT116. C, Westren blot of IL-6, MUC2 between Nor-HCT116 and EMT-HCT116. D, OD590nm. F, Invaded GFP + cell. *: $p < 0.05$; **:$p < 0.01$. All experiments were validated three times.

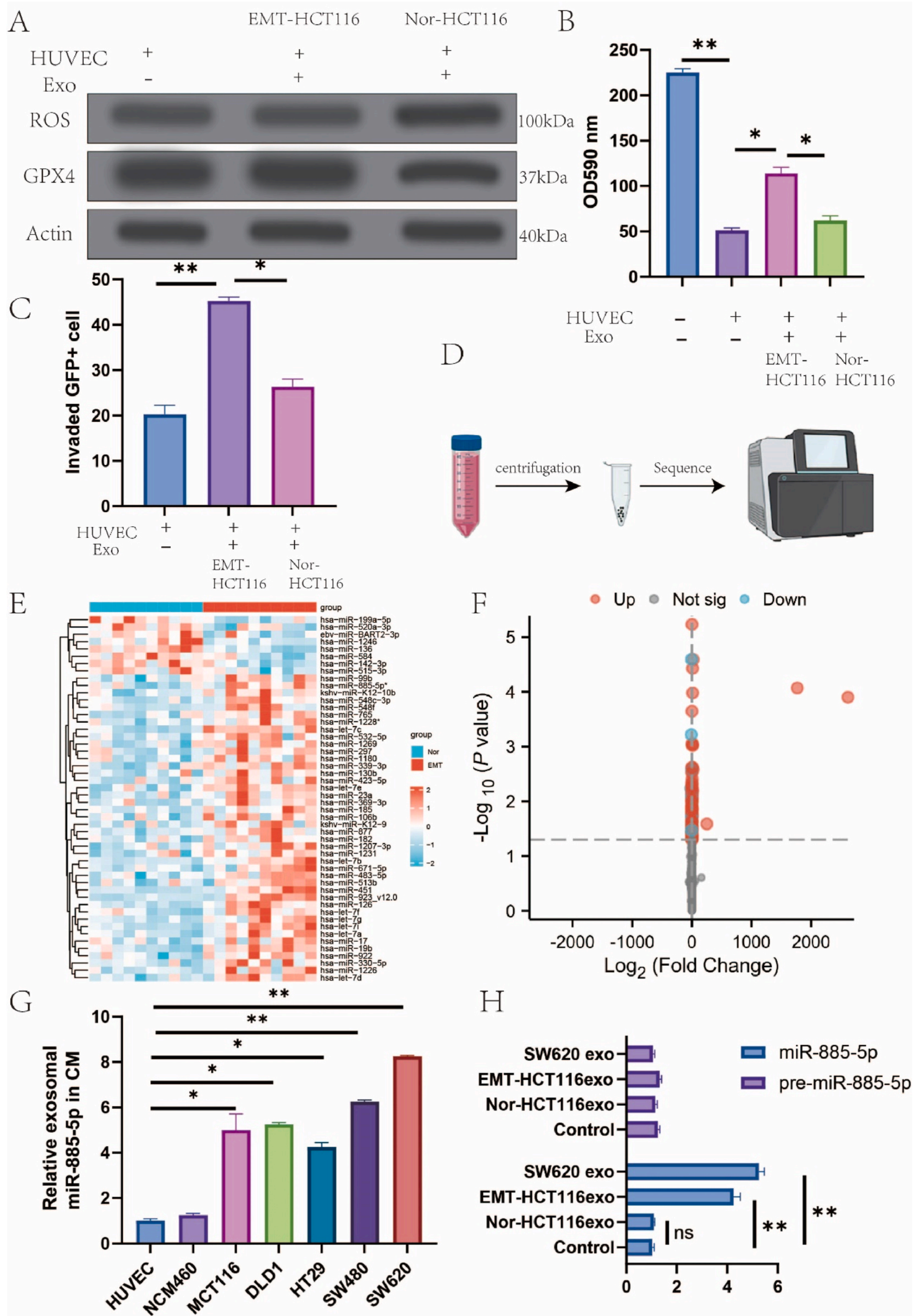

Fig. 2. Exosomal miR-27b-3p derived from EMT tumor cells modulates inflammation. A, Western blot of ROS and GPX4 between Nor-HCT116 and EMT-HCT116. B, OD590nm. C, Invaded GFP + cell. D, RNA sequence protocol. E, mi-RNA expression heat map. F, volcano map. G, Relative exosomal miR-885-5p in CM. H, Difference expression of miR-885-5p and pre miR-885-5p treated with Nor-HCT116 and EMT-HCT116. *: $p < 0.05$; **:$p < 0.01$. All experiments were validated three times.

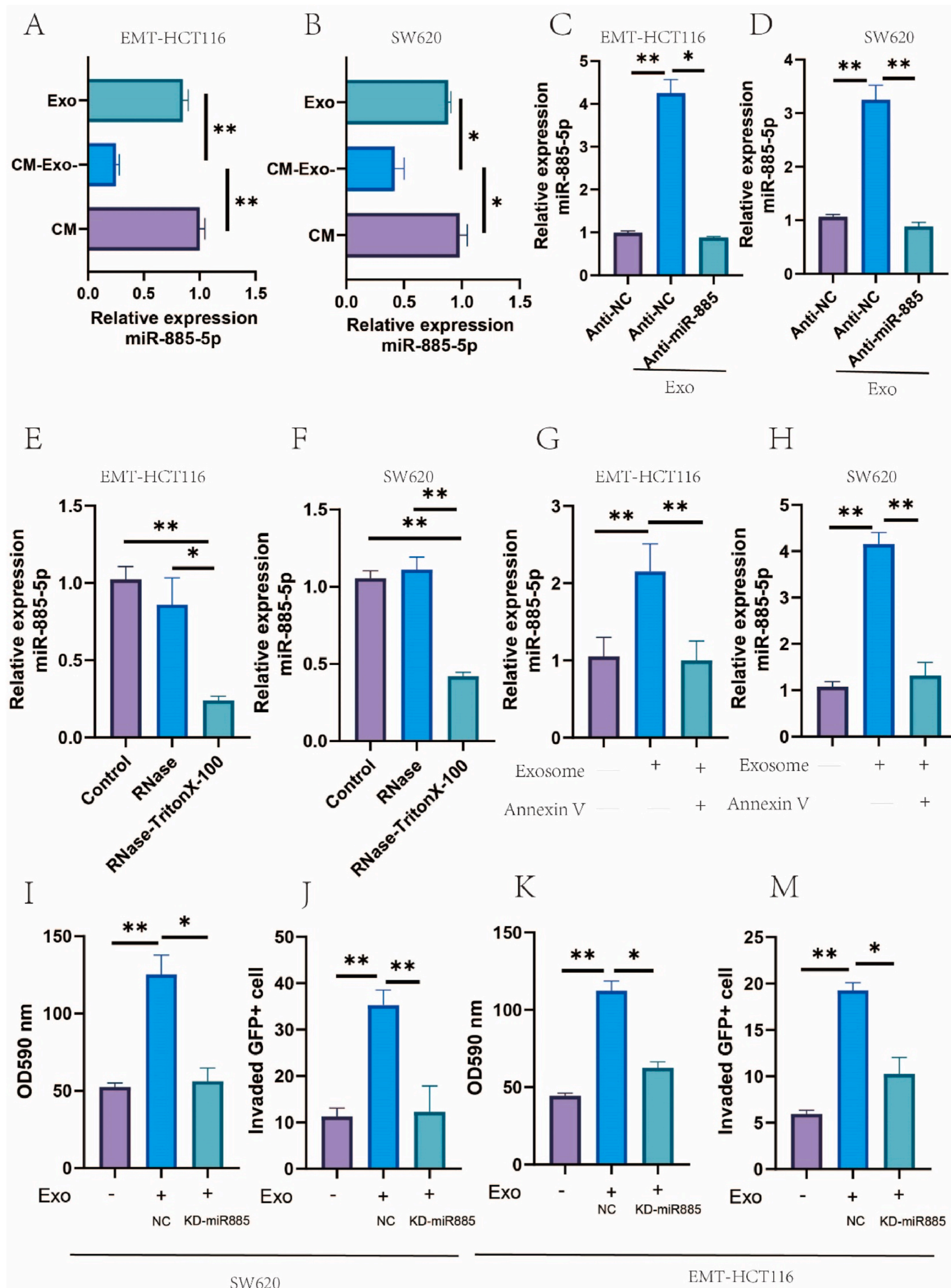

Fig. 3. The impact of exosomal miR-885-5p on tumour cell transendothelial invasion. A,B. Relative expression of miR-885-5p between EMT-HCT116 and SW620. C,D Anti-miR-885 treated with exosomal in EMT-HCT116 and SW620. E,F RNase-TritonX-100 treated with exosomal in EMT-HCT116 and SW620. G,H expression of miR-885-5p treated with exosome and annexin V in EMT-HCT116 and SW620. I-M, OD590 and invaded GFP + cell treated with KD-miR885 in EMT-HCT116 and SW620. *: $p < 0.05$; **:$p < 0.01$. All experiments were validated three times.

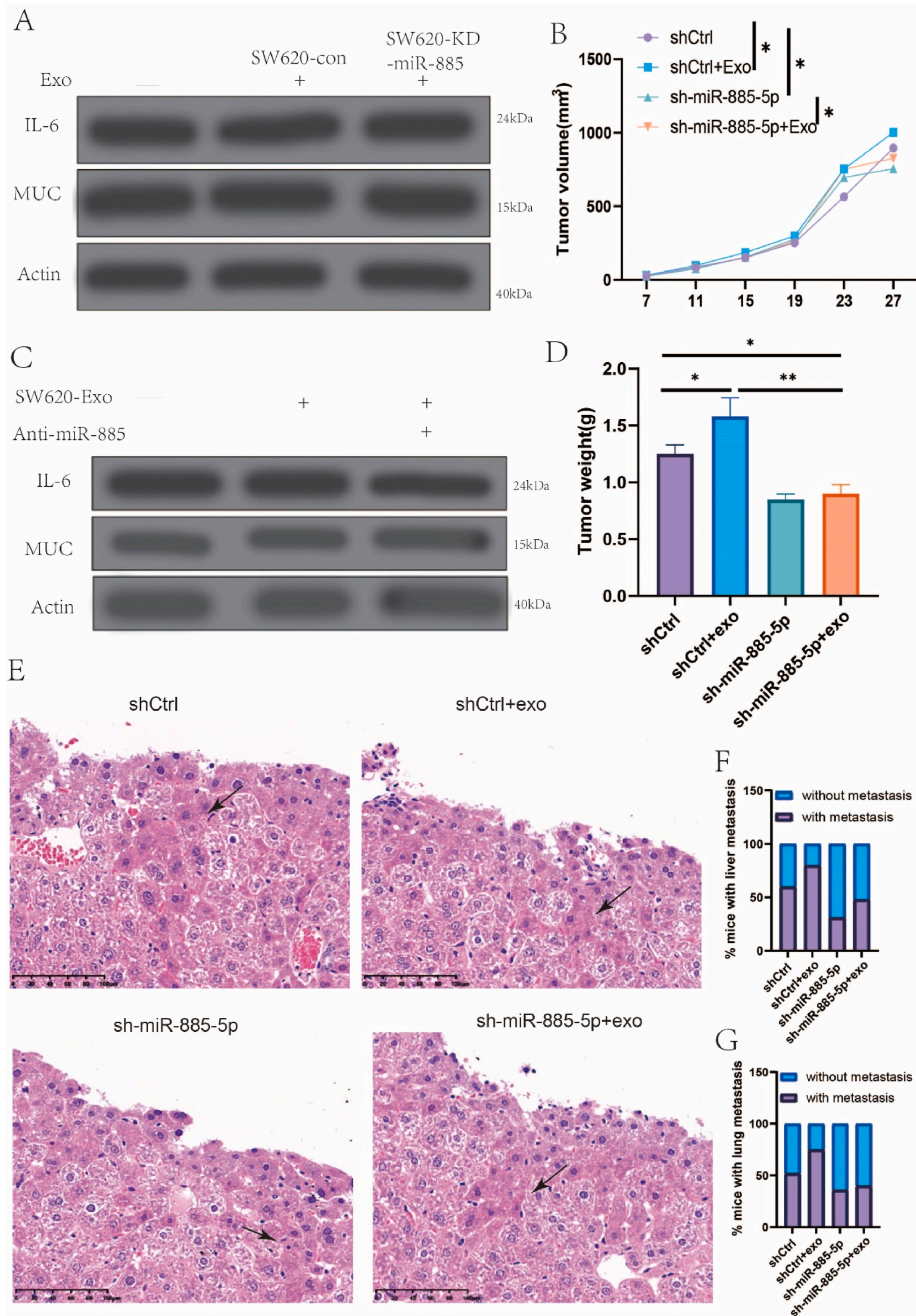

Fig. 4. Colorectal cancer cell-secreted miR-885-5p enhanced tumour metastasis in vivo. A, Western blot of IL-6 and MUC between control and KD-miR-885. B, Tumor volume. C, Western blot of IL-6 and MUC between SW620-exo and anti-miR-885. D, Tumor weight. E. HE stain among shCtrl, shCtrl + exo, sh-miR-885-5p and sh-miR-885-5p + exo. F. precent of mice with liver metastasis. G. precent of mice with LUNG metastasis. *: $p < 0.05$; **:$p < 0.01$. All experiments were validated three times.

4.3. Exosomal miR-885-5p's function in controlling cancer cells' penetration of the endothelium

The Illumina sequencing investigation was performed on the Nor-HCT116-exosomes and EMT-HCT116-exosomes due to the importance of exosomal miRNAs in intercellular signaling. After a thorough analysis using a number of databases, including as miRWalk, Starbase, and miRmap, miR-558-5p was shown to be the microRNA that was markedly up-regulated in EMT-HCT116 exosomes. As a result, this remarkable discovery enhanced HUVEC permeability. In order to learn more, miR-885-5p expression in a variety of human CRC cell lines (DLD-1, SW620, HT29, HCT-116, SW480, and NCM460) was evaluated by qRT-PCR in contrast to HUVEC and NCM460 cells. Remarkably, compared to HUVEC and NCM460 cells, two different mesenchymal CRC cell lines (SW620 and SW480) showed significantly greater amounts of secreted miR-885-5p. We next investigated whether exosomal miR-885-5p is released by CRC cells and transferred to endothelial cells. Interestingly, our results show that exosomes from EMT-CRC cells had no effect on HUVECs' ability to synthesize miR-885-5p. Furthermore, upon isolating exosomes from CRC cells, we found that the amounts of miR-885-5p in both exosomes and the whole CRC-conditioned media (CM) were similar (Fig. 3A–M).

Our analysis of this experiment showed that recipient HUVEC cells treated with exosomes/cell CM produced from EMT-HCT116 cells and SW620 cells transfected with a miR-885-5p inhibitor had significantly lower levels of miR-885-5p. Furthermore, a noteworthy finding was observed, indicating that extracellular miR-885-5p levels in CRC-CM were unaffected by RNase A treatment. However, combining RNase A with Triton X-100 resulted in a significant decrease. These results imply that extracellular miR-885-5p is bundled into membrane-enclosed exosomes rather than being released directly from CRC cells. Our study's main goal was to investigate how exosomal miR-885-5p controls cancer cells' ability to penetrate the endothelium barrier.

We performed in vitro tests to measure the permeability of endothelial cells by measuring the amount of rhodamine-labeled dextran that penetrated a monolayer of HUVECs co-cultured with exosomes. The transfection effectiveness of the lentiviral approach was

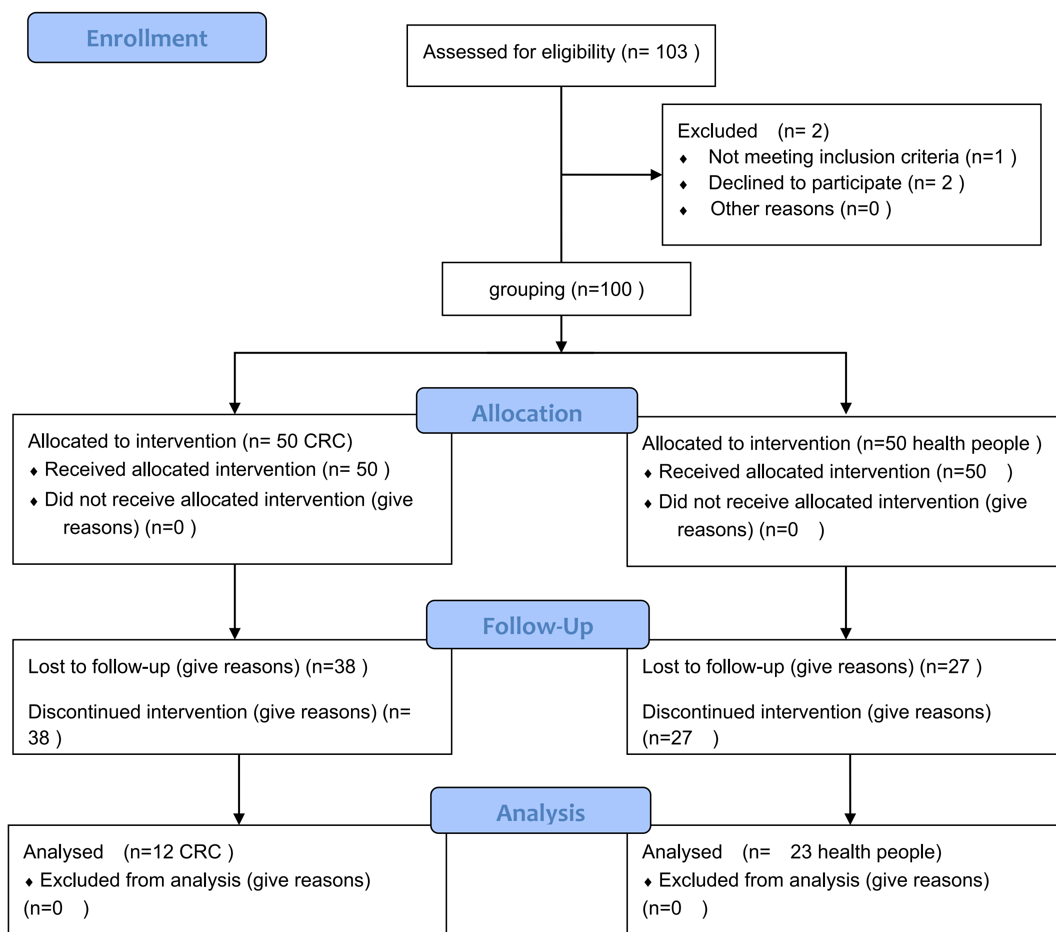

Fig. 5. Flow diagram.

evaluated by fluorescence analyses and qRT-PCR after SW620-KD-miR-885-5p cells were created. Interestingly, our results showed that exosomes produced from SW620-KD-miR-885-5p cells might prevent cancer cells from invasively lining their transendothelium. The next experiment we undertook was called transendothelial cell invasion, and it required identifying the cancer cells tagged with GFP that had entered the HUVEC monolayer and survived. Our previous findings were further supported by this investigation. Surprisingly, we discovered that exosomes released by EMT-HCT116 cells increased rhodamine penetration; on the other hand, exosomes produced by EMT-HCT116-KDmiR-885-5p cells decreased transendothelial invasion. Notably, in transfected HUVEC cells, the lowering of miR-885-5p impeded endothelial cell invasion and rhodamine penetration. According to our research, CRC cells produce exosomal miR-885-5p, which is thought to be the cause of the decline in MUC and IL-6 protein levels in HUVECs. Exosomes produced from EMTHCT116/SW620 cells were shown to reduce MUC and IL-6 protein levels in HUVECs, whereas miR-885-5p suppression increased MUC and IL-6 expression, according to Western blot and immunofluorescence assays. In the rescue experiment, HUVECs were exposed to exosomes generated by SW620 cells, which caused HUVEC permeability to be restored at the same time as MUC and IL-6 were upregulated.

4.4. Through CTCs, CRC cells' exosomal miR-885-5p promotes metastasis

We implanted control and SW620-KD-miR-885-5p cell lines into the mouse caecum to investigate the possible function of exosomal miR-885-5p in rupturing blood vessel barriers and causing cancer propagation in vivo for investigation. The control + exosome group had bigger tumors than the control group, according to an analysis of tumor weight and volume. On the other hand, compared to the control group, the tumor volume was much reduced after treatment with sh-miR-885-5p.

Evaluations were also conducted on distant metastases in the liver and lung parts. Compared to mice with the SW620-KD-miR-885-5p mutation, mice with the SW620-NC mutation showed a greater prevalence of distant metastases. In HCT116 xenografts, we intratumorally injected exosomes from EMT-HCT116 cells and parental cells to examine the effect of exosomal miR-27b-3p on tumor metastasis in vivo. The results showed that there was a greater expression of miR-558-5p inside the tumors, and that there was a higher incidence of distant metastasis in both the EMT-HCT116 group and the exosome-treated group. In conclusion, our findings suggest that CRC cells' exosomal miR-885-5p promotes metastasis via CTCs (Fig. 4A–G) (see Fig. 5).

5. Discussion

They develop CRC later in life, and the number of strains with PKS genes is increasing [45]. In the gut microbiota of CRC patients, enterotoxigenic Bacteroides fragilis and polyketide synthase-positive bacteria (pks +) are two of the most common pathogens [46]. Our research has shown that the location of virulence genes in E. Coli isolates from people with colorectal cancer (CRC) are associated with PKS. Compared to the isolates that tested negative for PKS, the E. PKS-positive Col isolates exhibited a greater prevalence of virulence genes. Moreover, exosomes generated from CRC cells undergoing an EMT significantly increased the expression of miR-885-5p. This specific microRNA has shown the ability to lower MUC and IL-6 production, which in turn alters inflammation and encourages CRC cell intravasation, ultimately leading to metastasis.

Strains of *E. coli* have been linked to a number of variables that may ultimately cause CRC. coli strains that produce colibactin from their polyketide synthesis (pks) locus have been introduced, specifically [47]. There have been rumors that E. Colibactin, a polyketide-peptide genotoxin, is produced by bacteria carrying polyketide synthetase (pks) island, which aids in the development of colorectal cancer [48]. Through rigorous validation, a metastasis-specific miRNA signature, such as miR-885-5p in pCRCs, revealed new tissue- and serum-based CRC metastasis-specific miRNA biomarkers [49]. Although miR-885-5p has not been well studied, it has a function in the initiation and spread of cancer [50]. The functional and therapeutic significance of PKS and E has been emphasized by our work. Coli in CRC. Research indicates that when CRC metastasizes, E. Coli secretes miR-885-5p, which promotes the intravasation of cancer cells and initiates EMT. Metastasis is largely dependent on the interaction of cancer cells with the tumor microenvironment (TME) [51,52]. It has been shown that IL-6, one of the most common inflammatory mediators in the TME, encourages EMT, subsequent tumor development, and metastasis [53].

Complex tissue conditions are necessary for the development, invasion, and metastasis of cancers [54]. Since stromal cell types in the TME are genetically stable, they provide an appealing therapeutic target with a lower chance of drug resistance and tumor recurrence than tumor cells [55,56]. It has been clear in recent years that inflammation has a role in the pro-invasive and pro-metastatic environment that exists inside tumors [57]. Various cytokines and chemokines secreted by infiltrating immune cells influence stromal cells as well as tumor cells in the tumor microenvironment [58]. Tumor cells have malignant characteristics, such the capacity to spread, as they get larger, endangering the lives of those around them. The epithelial-mesenchymal transition (EMT), which allows epithelial cancer cells to take on mesenchymal characteristics and increase their ability to migrate and motulate, is one of the most significant events in this phase [59,60]. Tumor cells migrate and metastasize to certain distant organs under the guidance of chemokine gradients, which are detected by chemokine receptors [61,62]. VEGF family members and other angiogenesis and lymphatic genesis stimulators are found in activated macrophages. Inflammatory mediators secreted by immune cells facilitate the survival and colonization of metastatic cancer cells in target organs after they have entered the circulatory system [63]. Macrophages start the premetastatic location during monocytes and/or metastasis and encourage tumor cell extravasation, survival, and further growth [64,65]. Additionally, macrophages inhibit the immune system, which stops T lymphocytes and natural killer cells from attacking tumor cells both during the course of the tumor and after the tumor has recovered from chemotherapy or immunotherapy [66]. Consistent with our findings, we have also shown that the pro-inflammatory cytokine IL-6 preferentially facilitates the packing of miR-885-5p into exosomes generated by cancerous cells.

In the context of CRC, this study aims to investigate the interactions between the host and intestinal microbiota, focusing on the mechanisms involved in intestinal barrier breakdown, genotoxicity, and inflammatory responses. Existing literature has established a strong association between genotoxicity and CRC, which is supported by the substantial presence of mucosa-associated pks (+) *E. coli* in individuals with CRC and inflammatory bowel disease [67]. Research has demonstrated that PKS + *E. coli* can induce 8-oxoguanine DNA damage, which is closely linked to the occurrence of colon cancer. However, it is important to note that other variables may also contribute to the development of CRC. Notably, individuals with early precancerous lesions and those with metastatic CRC exhibit distinct compositions of gut microbiota [21]. Consistent with these findings, our study reveals that the supplementation of *E. coli* and the presence of PKS significantly accelerate the development of CRC tumors [68]. PKS + *E. coli* specifically causes 8-oxoguanine DNA damage, which displays a high correlation with the prevalence of colon cancer. Moreover, our research affirms previous observations indicating that individuals with early precancerous lesions and those with metastatic CRC have different compositions of gut microbiota [69]. Additionally, animal models have shown that pks + E, according to Daiki Watanabe et al. The development of colorectal cancer (CRC) and the correlation between food consumption and pks E prevalence are both influenced by coli in the gut. Coli that was identified from Japanese people's microbiota [70]. Chemotherapy is a crucial component of colon cancer treatment at every stage. The development and progression of colon cancer have been linked to the gut microbiome, which may also change the toxicity and effectiveness of treatment drugs. The gut microbiome's equilibrium may be impacted by antibiotics either directly or indirectly, which can have an impact on clinical outcomes [71]. Overall, our results emphasize the importance of *E. coli* supplementation and PKS positivity in the development of CRC.

In recent years, the vital role of miRNAs in mediating the interaction between bacterial pathogens and host cells has gained significant attention. Exosomal miRNAs undergo significant alterations following infection with pathogenic bacteria, as evidenced by extensive research [72]. In colon cancer tissues, for instance, research has shown that miR-92a targets the anti-apoptotic protein BCL-2-interacting mediator of cell death [73]. For instance, miR-885-5p is downregulated in a number of cancers, including as osteosarcoma, renal cancer, hepatocellular carcinoma, and pancreatic cancer [74–78]. miR-885-5p targets mRNAs such CDK2, MCM5 to prevent the growth of tumors [75]. Exosome-delivered microRNAs (miR) like miR-885-5p are also essential in controlling the course of certain illnesses [79–81]. Moreover, Su et al.'s study demonstrates that miR-885-5p overexpression promotes CRC cell migration and proliferation by focusing on the cytokine signaling suppressor [82]. Statistical analysis has revealed that elevated levels of miR-885-5p hold promise as metastasis-specific biomarkers since they are observed in primary CRC (pCRC) and corresponding liver metastases (LMs) [49]. Exosome-delivered MiR in CRC tumors was also found in our study.

The PKS + E is present. Coli has a high correlation with colorectal cancer (CRC) linked to colitis, as well as inflammatory bowel disease (IBD). Regarding the function of exosomal miRNAs in PKS + E, there is still uncertainty [83]. Recent investigations have focused on exosomes derived from oncogenic intestinal flora, specific miRNAs associated with exosomes, and CRC metastases. These miRNAs hold potential as therapeutic targets for CRC treatment [77]. It is crucial to explore the miRNAs associated with PKS + *E. coli* exosomes and other gut flora linked to CRC for future research endeavors [84]. Moreover, studies have demonstrated an increase in the secretion of exosomes by CRC cells upon *E. coli* infection, where exosomes derived from infected cells promote CRC cell motility, thereby accelerating cancer progression [20].

The reversible transformation of a polarized epithelial cell into a mesenchymal cell phenotype is the hallmark of the dynamic process known as the epithelial to mesenchymal transition (EMT) [85]. Tumor growth and metastasis are caused in part by abnormal activation of EMT and therapeutic resistance resulting from the deregulation of miRNA expression [86]. Exosomal miR-27b-3p expression is indicative of the EMT in CRC cells, and it promotes the development of circulating tumor cells (CTCs). This implies that exosomal miR-27b-3p may be a useful biomarker for the spread of CRC [51]. Given that improving gut barrier function, preventing pathogen colonization, modifying the innate immune system, modifying the composition of the microbiota, and selectively cytotoxically attacking tumor cells can all contribute to the success of clinical treatments, the development of personalized microbiome therapies may be essential [87].

When formulating a personalized treatment plan for CRC, it is essential to consider the bacteria associated with the tumor to mitigate their adverse effects [11]. Modulating the microbiota, referred to as "microbiota-targeted therapy" or "cancer bacterial therapy," holds promise in developing novel targeted medications, as different bacterial species play distinct roles in the development of CRC [88]. This approach encompasses the utilization of antibiotics, dietary modifications to modulate the gut flora, probiotics, and fecal transplants. The gastrointestinal environment changes when the levels of harmful bacteria decrease, leading to increased survival of beneficial bacteria. This shift represents the ultimate therapeutic objective [89]. It is worth noting that aside from its negative impact on CRC, the gut microbiota may also exert positive effects [90].

6. Limitation

The research we conducted has several limitations. First, the sample size was limited, and future studies should include larger cohorts. Second, the function of the intestinal flora barrier is impacted by the impacts of intestinal flora and metabolites. Although this aspect of the effect is not included in our study, we want to include this fundamental research in a future study. Ultimately, it was discovered that there were not enough CRC patient cases included in our study and that additional cases should be included in further research. Future research must deepen our understanding of the connection between miR and exosomes. In addition, considering the complexity and particularity of the intestinal microbiota, it is of great significance to explore other possible therapeutic targets and predictive targets in future studies for the diagnosis and treatment of CRC. What's more, further studies are needed to validate these findings in larger and more diverse populations.

7. Conclusion

Our study suggests that PKS + *E. coli* plays a significant role in CRC metastasis through the inhibition of exosomal miR-885-5p, offering a potential target for therapeutic intervention. The above results not only find PKS as a target for potential CRC patients but also provide important directions for how to influence CRC progression.

Implications

Targeting PKS + *E. coli* or modulating exosomal miR-885-5p could provide new strategies for preventing CRC metastasis.

Ethics approval and consent to participate

The First Affiliated Hospital and College of Clinical Medicine at Henan University of Science and Technology's institutional review boards (IRBs) and hospital ethics committees approved the investigational research detailed below (DE44232342).

Consent for publication

We all agree to publication.

Data availability statement

The article contains the data that this research utilized to support its conclusions.

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Informed consent

All patients were informed and signed informed consent.

CRediT authorship contribution statement

Xiaoming He: Visualization, Validation, Supervision, Software, Resources, Project administration, Formal analysis, Data curation, Conceptualization. **Enbo Ren:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources. **Lujia Dong:** Visualization, Validation, Supervision, Software, Resources. **Pengfei Yuan:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation. **Jiaxin Zhu:** Investigation, Funding acquisition, Data curation, Conceptualization. **Dechun Liu:** Writing – review & editing, Writing – original draft, Visualization, Validation. **Jianguang Wang:** Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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