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Differential effect of Periodontal pathogen *Fusobacterium nucleatum* and *Porphyromonas gingivalis* on modulation of specific partial-EMT genes in colorectal cancer cells

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ABSTRACT

Objective: While the epithelial-mesenchymal transition (EMT) is known to promote cancer stemness and metastasis, a hybrid partial EMT (p-EMT) state has recently been identified. This study examined the influence of *Fusobacterium nucleatum* (Fn) and *Porphyromonas gingivalis* (Pg) on p-EMT gene expression and their prognostic relevance in colorectal cancer (CRC).

Materials and methods: HCT 116 cells were infected with *Fn* or *Pg* at an MOI of 100. Expression of p-EMT markers (SPP1, EMP3, INHBA, THBS2, ITGA5, P4HA2) and EMT-markers (ZEB1, SNAI1) was analyzed via RT-PCR after 6 hours (h) and 24 h. Cell migration was assessed using a scratch assay. TCGA data were used to validate gene expression in CRC patients, and Kaplan-Meier analysis was used to evaluate prognostic significance.

Results: Pg infection induced a significant upregulation of EMP3 at the 24 h, while suppressing the expression of P4HA2, MXD1, and THBS2. The ZEB2 displayed a biphasic response, decreasing at 6 h before rising at 24 h, and SNAI1 was consistently suppressed. In contrast, *Fn.* infection resulted in the upregulation of ITGA5 and MMP3, accompanied by the downregulation of P4HA2, MXD1, MMP9, and SNAI1. Both enhanced cellular migration and wound closure capabilities. THBS2, CDH13, and P4HA2 were significantly elevated in tumor tissues compared to controls, while EMP3 and ZEB1 showed reduced expression. Elevated mRNA levels of EMP3, THBS2, and ITGA5 were significantly correlated with poorer overall survival.

Conclusion: Fn and *Pg* can promote invasive phenotypes in CRC through distinct mechanisms, each modulating a unique subset of p-EMT and without instigating a complete, coordinated EMT gene signature.

1. Introduction

Periodontal disease is a persistent inflammatory disorder caused by polymicrobial dysbiosis, with *Fusobacterium nucleatum (F. nucleatum)* and *Porphyromonas gingivalis (P. gingivalis)* acting as keystone pathogens.[1] *F. nucleatum* is a Gram-negative anaerobic bacterium commonly present in the oral cavity of both healthy and diseased persons. *F. nucleatum* expresses adhesins such as FadA, which attach to host

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epithelial cells and enhance biofilm formation, hence enabling the co-aggregation of other pathogens.[2] It also stimulates pro-inflammatory cytokines and activates oncogenic signaling pathways, including Wnt/β-catenin.[3] *P. gingivalis*, another Gram-negative anaerobic bacterium predominantly colonizing periodontal pockets, serves as a key constituent of the red complex and is strongly associated with both the initiation and progression of periodontal diseases. *P. gingivalis* synthesizes proteolytic enzymes that dismantle host tissues and immune modulators, while concurrently influencing autophagy and apoptosis.[4,5] Lipopolysaccharide (LPS) activates Toll-like receptors (TLRs), thereby initiating NF-κB-mediated inflammation.[6,7]

The "gut-oral axis" concept emphasizes the potential role of oral bacteria, especially periodontal infections, in the development of gastrointestinal disorders, including colorectal cancer (CRC).[8] Oral pathogens translocate along the oral-gut axis via enteral or hematogenous routes. Daily swallowing of 1.5 L saliva introduces oral microbiota into the gut.[9] Studies show specific oral bacteria colonize germ-free mice after fecal transplant gavage, surviving digestive transit and establishing colorectal populations.[10] Pathogens must overcome gastric acid, bile salts, and gut microbiota competition. Barrier disruption from proton pump inhibitors (PPIs) or antibiotics facilitates oral microbial translocation, enabling gut colonization and potential intestinal disease progression.[11,12] Beyond the enteral route, emerging evidence suggests that hematogenous dissemination may serve as the predominant mechanism underlying the ectopic colonization of oral pathogens in the distal gut.[13] Recent studies increasingly support circulatory transmission as a key pathway for oral microbiota to establish extraoral niches, particularly in gastrointestinal tissue.[14,15]

CRC continues to be one of the most common and lethal cancers globally, representing around 10% of all cancer-related fatalities.[16] Although hereditary and environmental variables, including food, smoking, and inflammatory bowel disease (IBD), are recognized contributors to CRC etiology, accumulating research indicates that the oral and gut microbiota may potentially significantly influence colorectal carcinogenesis.[17] Numerous investigations have shown *F. nucleatum* and *P. gingivalis* in colorectal cancer tissues at elevated levels compared to healthy controls [18–20]. Several possible mechanisms have been proposed for their plausible involvement in CRC mainly include chronic inflammation, immunomodulation and direct oncogenic signalling. These pathogens create a pro-inflammatory milieu by activating NF-κB and IL-6/STAT3 signaling, which are associated with tumor development.[21,22] *P. gingivalis* undermines T-cell responses,[23] whereas *F. nucleatum* attracts myeloid-derived suppressor cells (MDSCs), establishing an immunosuppressive environment.[18] *F. nucleatum*'s FadA directly binds to E-cadherin, hence activating β-catenin and upregulating oncogenes such as MYC.[24] *P. gingivalis* gingipains break tumor suppressor proteins and activate matrix metalloproteinases (MMPs), facilitating invasion.[25]

Epithelial-mesenchymal transition (EMT) is a dynamic process wherein epithelial cells lose their polarity and cell-cell adhesion, while acquiring mesenchymal traits that promote migration and invasion.[26] During EMT, there is a downregulation of epithelial markers such as E-cadherin, accompanied by an upregulation of mesenchymal markers like N-cadherin, vimentin and others.[27,28] Transcription factors including SNAI, TWIST, and ZEB are considered essential regulators of this process.[27,28] Numerous studies indicate that bacterial infections may induce EMT in epithelial cells. *Helicobacter pylori* induces epithelial-mesenchymal transition in gastric cancer by upregulating SNAI and TWIST.[29] *F. nucleatum* has been demonstrated to facilitate EMT in oral and colorectal cancer cells through the activation of the SNAI signaling pathway.[30] *P. gingivalis* can produce EMT-like alterations in gingival epithelial cells by upregulating mesenchymal markers.[25]

Traditionally, EMT was considered a binary switch between epithelial and mesenchymal states; however, recent research demonstrates that it occurs along a spectrum, where cells adopt a partial EMT (p-EMT) phenotype without fully shedding epithelial traits or acquiring complete mesenchymal characteristics [31–33]. Tumors exhibiting p-EMT display significant intratumoral heterogeneity, with coexisting epithelial, mesenchymal, and hybrid E/M cell populations, reflecting dynamic epithelial-mesenchymal plasticity.[33] The clinical importance of p-EMT is not yet completely elucidated; it has been strongly linked to aggressive tumor behavior, recurrence, and metastatic spread. This transitional phenotype, where tumor cells co-express epithelial and mesenchymal markers, has been documented in diverse cancers including colorectal cancers.[34,35] Whether *F. nucleatum* and *P. gingivalis* influence p-EMT genes is not well comprehended. This study seeks to examine the influence of *F. nucleatum* and *P. gingivalis* on p-EMT gene expression in colorectal cancer in CRC cell lines, offering novel insights into the potential role of periodontal infections in cancer advancement.

2. Material and methods

2.1. Bacteria culture

The bacterial strains *P. gingivalis* ATCC 53977 and *F. nucleatum* subsp. *nucleatum* ATCC 25586 were anaerobically cultured in brain heart infusion broth enriched with hemin (5 mg/mL) and menadione (5 mg/mL) (Himedia, India). Incubation was carried out at 37 °C for 48 h in a Whitley A20 anaerobic workstation with a gas mixture of 80% N_2, 20% CO_2, and 0.1% O_2. Following growth, the bacterial suspension was standardized to 0.5 McFarland turbidity and used at a multiplicity of infection (MOI) of 100 for subsequent cell infection assays.

2.2. Cell infections assays

HCT 116 cells were plated in 12-well plates at a density of 5×10^5 cells/mL and allowed to adhere overnight at 37 °C, reaching 70–80% confluence before infection. One hour prior to bacterial exposure, the medium was replaced with antibiotic-free DMEM. The cells were then infected separately with *P. gingivalis* or *F. nucleatum* subsp. *nucleatum* at an MOI of 100, following established protocols.[36,37] PBS- treated wells served as controls. All plates were maintained at 37 °C in a 5% CO_2 atmosphere. Following infection, supernatants and cell lysates were collected at 6 h and 24 h for subsequent RNA isolation.

2.3. Cell migration assay

HCT 116 cells were grown to 80–90% confluence and pretreated with mitomycin C (Sigma-Aldrich) for 1 h to inhibit proliferation. After washing with PBS, a uniform wound was created in the monolayer using a sterile 10 μL pipette tip. The cells were then exposed to *F. nucleatum* or *P. gingivalis*, while control groups received PBS. Each experiment was repeated independently (n = 3 times) and wound closure was quantified from 3 to 5 randomly selected fields per well. Migration was monitored by capturing images at 0 h, 6 h, and 24 h post-wounding using Image-Pro Plus software (v6.0, Media Cybernetics). Scratch closure was quantified using ImageJ and expressed as percentage wound closure relative to time 0. Data are presented as mean ± SEM from three independent experiments. Statistical comparisons between groups were performed using one-way ANOVA followed by Tukey's post hoc test. A p-value <0.05 was considered statistically significant. The percentage of wound closure was calculated using the formula: [(Initial scartch width - scratch width at time (t) / initial scratch width] x 100. (t =6 h or 24 h).

2.4. Gene expression analysis by qRT-PCR

Total RNA was extracted from harvested cells using the RNeasy kit, and RNA concentration was quantified using a NanoDrop ND-1000 spectrophotometer. cDNA synthesis was performed with a cDNA synthesis kit (Takara Bio, Shiga, Japan) following the manufacturer's protocol. Quantitative real-time PCR (qRT-PCR) was conducted on an Applied Biosystems QuantStudio 5 Real-Time PCR System (Waltham,

MA, USA). GAPDH served as the endogenous control for normalization, and relative gene expression levels were calculated using the 2−ΔΔCT method. The genes analyzed included p-EMT-related genes, Secreted phosphoprotein 1 (SPP1), Epithelial membrane protein 3 (EMP3), Inhibin subunit beta A (INHBA), Thrombospondin-2 (THBS2), Integrin alpha-5 (ITGA5), Prolyl 4-hydroxylase subunit alpha 2 (P4HA2). Additionally, EMT-related genes were analyzed: Zinc finger E-box binding homeobox 1 (ZEB1) Cadherin-13 (CDH13) and Snail family transcriptional repressor 1 (SNAI1). The primer sequences used for human genes are listed in Supplementary Table 1. Primer specificity was confirmed by melt curve analysis showing a single peak for each target. Amplification efficiency ranged between 95 and 105% (Supplementary Fig. 1).

All the comparisons are made between bacterial treatments at identical timepoints. The results are expressed as mean ± SD from a minimum of three separate experiments (n = 3). Statistical evaluations were conducted using SPSS 22.0 (SPSS, Inc., Chicago, IL, USA). For comparisons involving two groups, Student's t-test was employed, while one-way ANOVA was used for analyses across multiple groups. Statistical significance was defined as $P < 0.05$.

2.5. RNA-sequencing data acquisition and processing

Colorectal cancer RNA-sequencing (RNA-seq) data and corresponding clinical information were obtained from The Cancer Genome Atlas (TCGA) database, including the Colon Adenocarcinoma (COAD) and Rectal Adenocarcinoma (READ) projects. RNA-seq data were processed using the STAR alignment pipeline and normalized to transcript per million (TPM) values. Considering the high degree of similarity between colon adenocarcinoma and rectal adenocarcinoma with respect to histopathological characteristics, molecular subtypes, and clinical treatment strategies, as well as their frequent combined analysis in large-scale transcriptomic and prognostic studies, the TCGA-COAD and TCGA-READ cohorts were jointly analyzed to increase statistical power.

A total of 698 RNA-seq datasets were initially retrieved, including multiple samples derived from the same patient, and were curated according to the official TCGA sample annotations. Samples lacking survival outcome information or without matched RNA-seq data were excluded. Ultimately, samples with both RNA-seq expression profiles and complete clinical information were retained for downstream analyses, comprising 616 primary tumor tissues and 51 adjacent normal tissues.

2.6. Kaplan-Meier Plotter analysis

To evaluate the prognostic significance of partial epithelial–mesenchymal transition (p-EMT)-related genes in colorectal cancer, Kaplan–Meier survival analysis was performed using tumor samples with available overall survival (OS) information. A total of 654 eligible cases were included in the survival analysis. For each gene, patients were stratified into high-expression and low-expression groups based on the median mRNA expression level (or specified quantile thresholds where applicable), resulting in 327 patients per group. Kaplan–Meier survival curves were generated to estimate OS, and differences between groups were assessed using the log-rank test. A two-sided P value < 0.05 was considered statistically significant.

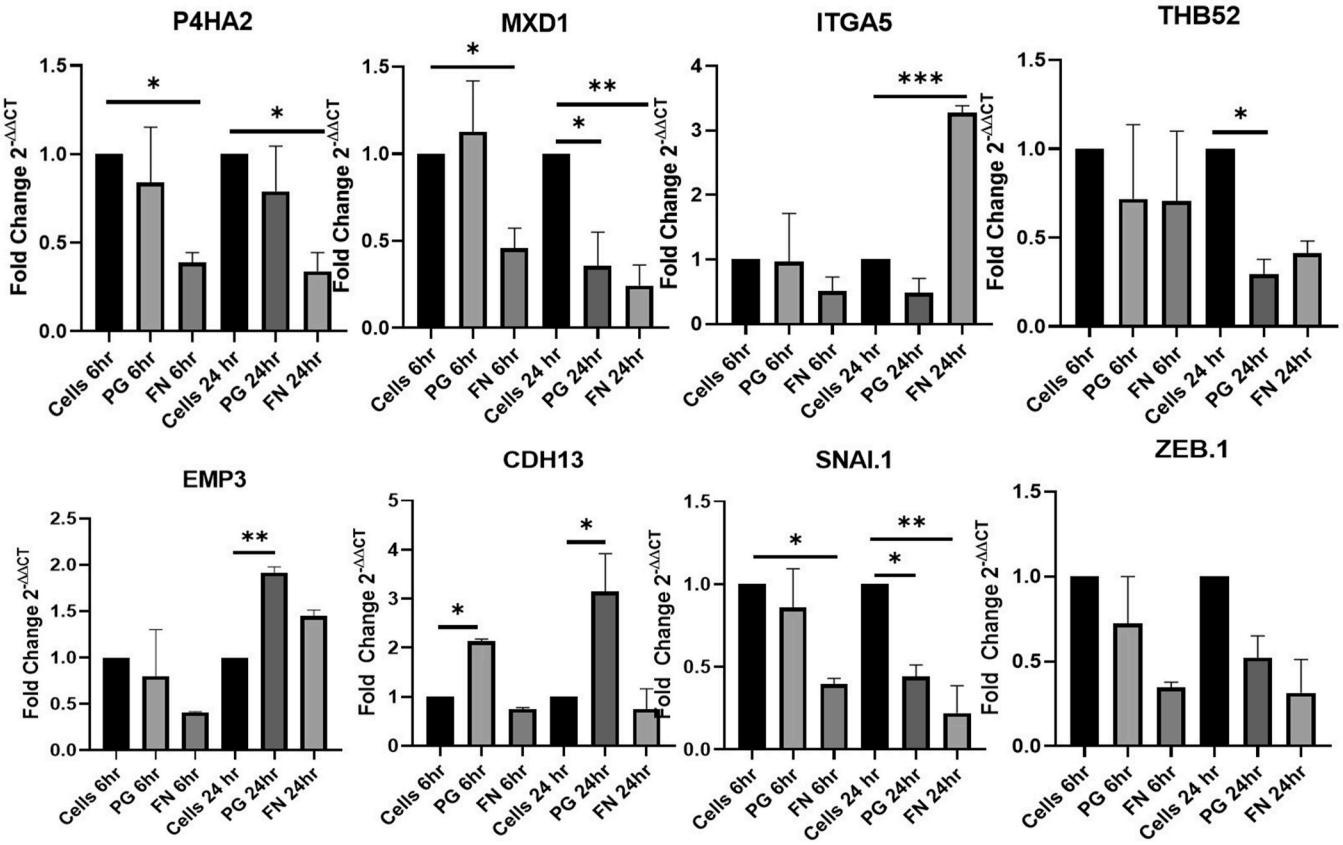

Fig. 1. Differential p-EMT gene expression in HCT116 cells following *P. gingivalis* and *F. nucleatum*. EMP3 expression increased at 24h, while P4HA2 (6h, 24h), MXD1, and THBS2 decreased at 24h. CDH13 and MMP3 were upregulated at both 6h and 24h. Expression of SPP1, INHBA, and ITGA5 was not significantly altered. *F. nucleatum* infection of HCT116 cells significantly downregulated P4HA2 and MXD1 at 6h and 24h, and upregulated ITGA5 and MMP3 at 24h. Expression of SPP1, INHBA, and EMP3 was unaffected.

3. Results

3.1. Effect of P. gingivalis infection on key p- EMT genes

Infection of HCT 116 cells with *P. gingivalis* resulted in differential expression of key genes associated with p-EMT. Notably, P4HA2 expression was downregulated at both 6 h and 24 h post-infection, while MXD1 and THBS2 exhibited reduced expression levels at the 24 h time point (Fig. 1). In contrast, EMP3 mRNA expression showed a significant increase at 24 h, despite remaining unchanged at the earlier 6 h mark. Among other p-EMT genes, including SPP1, INHBA, and ITGA5, no significant alterations in expression were observed following *P. gingivalis* infection, suggesting that these genes may not be directly influenced by bacterial exposure under the tested conditions. However, infection with *P. gingivalis* induced an upregulation in the expression of CDH13 and MMP3 at 6 h and 24 h. Interestingly, ZEB2 displayed a biphasic response, with an initial decrease in expression at 6 h followed by an increase at 24 h. Conversely, SNAI1 exhibited a sustained downregulation at the 24 h time point (Supplementary Fig. 2).

3.2. Effect of F. nucleatum infection on key p- EMT genes

F. nucleatum infection resulted in significant downregulation of P4HA2 and MXD1 at both time intervals. Interestingly, ITGA5 mRNA levels remained stable at 6 h but demonstrated marked upregulation by the 24 h mark (Fig. 1). Analysis of other p-EMT genes revealed that SPP1, INHBA, and EMP3 expression remained unaffected by bacterial infection at both time points, suggesting that these genes may not participate in *F. nucleatum*-mediated EMT modulation under these experimental conditions. Infection with *F. nucleatum* also stimulated increased expression of MMP3, whereas ZEB2 exhibited a suppression at 6 h. In contrast, MMP9 and SNAI1 expression showed progressive downregulation, reaching its lowest level at the 24 h time point (Supplementary Fig. 2).

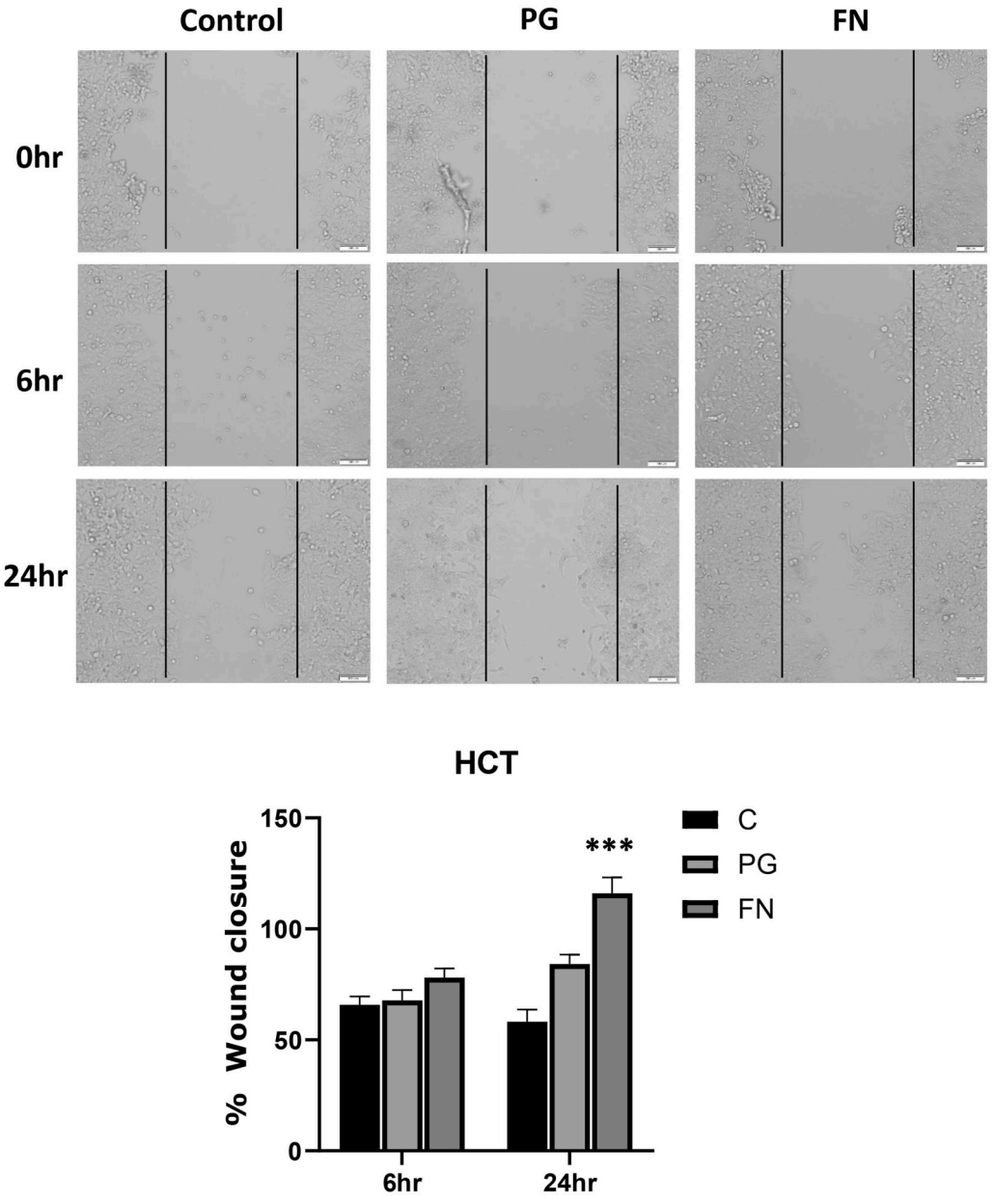

Fig. 2. *F. nucleatum* and *P. gingivalis* promote the migration of HCT cells.

3.3. F. nucleatum and P. gingivalis promote the migration of HCT cells

Infection of HCT 116 cells with either *F. nucleatum* or *P. gingivalis* significantly enhanced cell migration, as measured by a wound healing assay. The percentage of wound closure was significantly greater in both infected groups compared to the uninfected control at 24 h, indicating that both bacteria promote cancer cell motility ($p \leq 0.001$) (Fig. 2).

3.4. Gene expression analysis of key p-EMT genes

Using UALCAN, we analyzed the mRNA expression of p-EMT-associated genes in CRC and normal oral tissues. Notably, THBS2, CDH13, and P4HA2 showed significantly elevated expression in CRC patients compared to normal controls, whereas EMP3 and ZEB1 was downregulated (Fig. 3). Additionally, the EMT-linked genes and SNAI1 were also upregulated where as ZEB1 was downregulated in CRC tissues. There was no difference in expression levels of ITGA5 gene between cases and controls. The heat map of the differential gene expression is shown in Fig. 4.

3.5. Kaplan-Meier Plot analysis

To determine the prognostic value of p-EMT-related genes in CRC we performed Kaplan-Meier survival analysis based on gene expression levels. Elevated EMP3, THBS2, and ITGA5 mRNA expression was significantly associated with worse overall survival ($P < 0.05$) (Fig. 5). However, no notable survival differences were found for other p-EMT genes MXD1, P4HA2 and CDH13 between high and low expression groups. The high expression of EMT marker genes ZEB1 and SNAI1 were also significantly associated with worse overall survival.

4. Discussion

The oral microbiota consists of a diverse and complex community of microorganisms. More than 700 bacterial species naturally inhabit the oral cavity, and these commensal bacteria are essential for preserving a healthy oral environment while also influencing systemic diseases.[38,39] Among these, periodontal pathogens such as *F. nucleatum* and *P. gingivalis* are increasingly recognized for their roles beyond the oral cavity, with accumulating evidence implicating them in the initiation and progression of colorectal cancer (CRC)[18–22].

The results of the present study demonstrate that both *F. nucleatum* and *P. gingivalis* exert distinct and measurable effects on the expression of key p-EMT-related genes in colorectal cancer cell lines. The observed modulation of EMT and p-EMT markers suggests a direct bacterial influence on the molecular machinery governing tumor invasiveness and metastasis. Importantly, the concept of partial epithelial–mesenchymal transition (p-EMT), an intermediate cellular state wherein epithelial cells acquire mesenchymal properties without fully losing their epithelial identity, has gained prominence for its role in promoting cancer cell plasticity, invasion, and resistance to therapy [31–33].

Our findings indicate that *F. nucleatum* infection induces a transcriptional profile favouring p-EMT activation. Specifically, the upregulation of ITGA5,a critical receptor for fibronectin that is strongly linked to cancer cell invasion, metastasis, and stemness and MMP3, along with suppression of P4HA2, MXD1, and SNAI1, suggests an enhancement of invasive potential while partially restraining full EMT induction. The stability of other p-EMT markers (SPP1, INHBA, EMP3) further emphasizes that *F. nucleatum* does not enact a broad p-EMT shift. *F. nucleatum* has been shown to promote EMT and tumor aggressiveness in CRC through activation of oncogenic pathways such as EGFR-AKT-ERK and Wnt/β-catenin.[40,41] In oral cancer models, *F. nucleatum* has also been shown to drive cells toward a hybrid epithelial/mesenchymal phenotype and promote invasion, underscoring its role in modulating EMT plasticity.[42] Together, this supports the notion that *F. nucleatum* facilitates a tumor microenvironment conducive to cancer progression by inducing a subset of p-EMT pathways[30,43]

In contrast, *P. gingivalis* infection produced a different transcriptional footprint: EMP3 was elevated at 24 h, P4HA2 and THBS2 were downregulated, and EMT regulators like CDH13 and MMP3 were upregulated. Meanwhile, ZEB2 exhibited a biphasic response, and SNAI1 exhibited sustained downregulation. The gene expression profile induced by *P. gingivalis* reveals a targeted and time-dependent reprogramming of specific EMT-related pathways rather than a broad, coordinated shift.

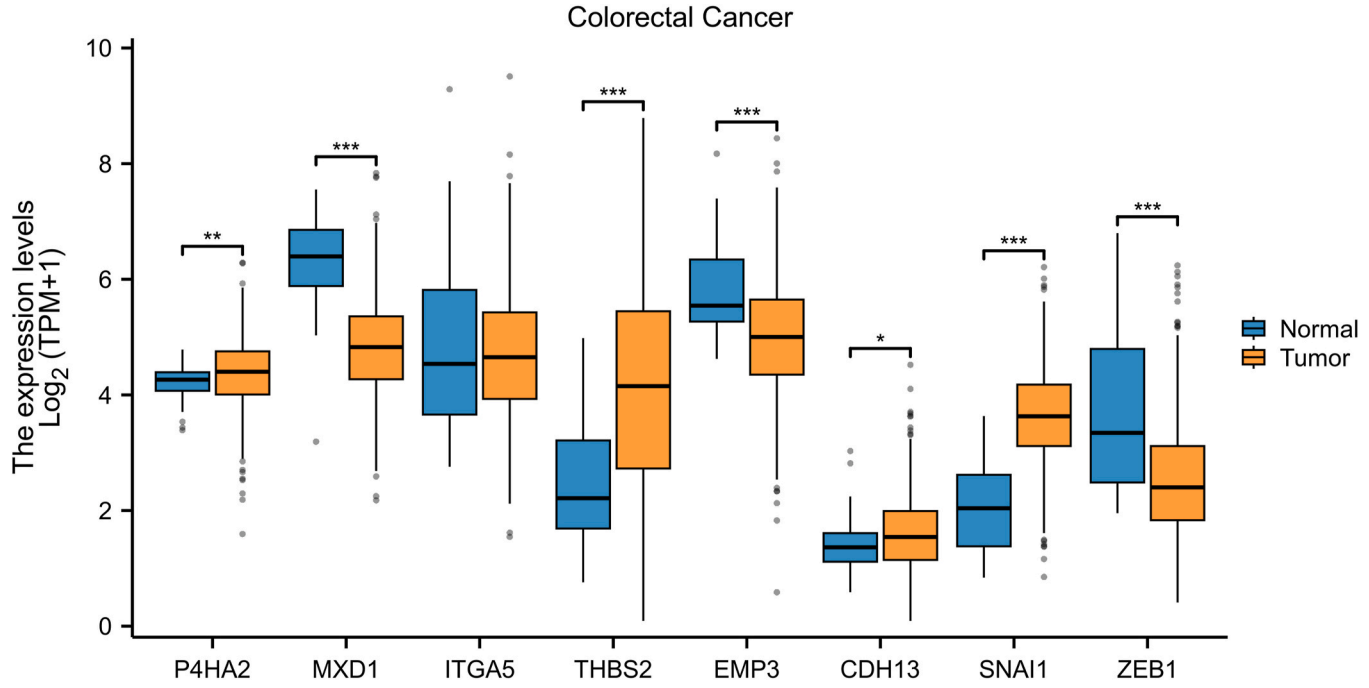

Fig. 3. Differential mRNA expression levels of p-EMT-related genes in CRC patients. Notably, THBS2, CDH 13, and P4HA2 showed significantly elevated expression in CRC patients compared to normal controls, whereas EMP3 and ZEB1 were downregulated.

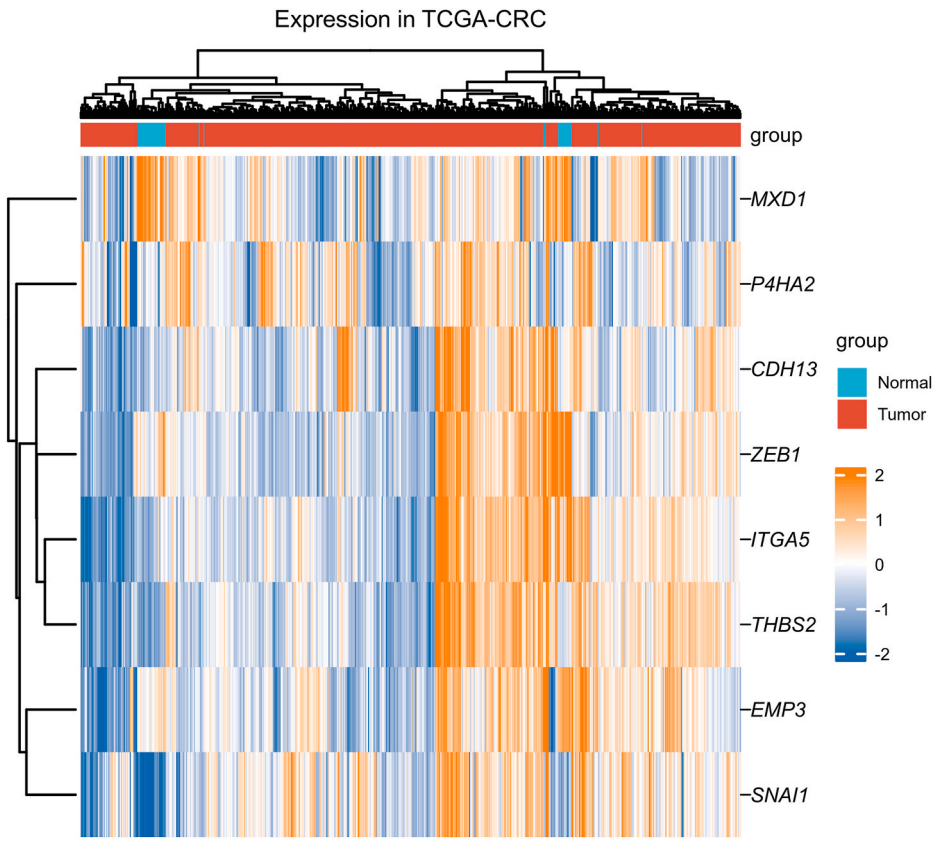

Fig. 4. Heatmap of differential mRNA expression levels of p-EMT genes.

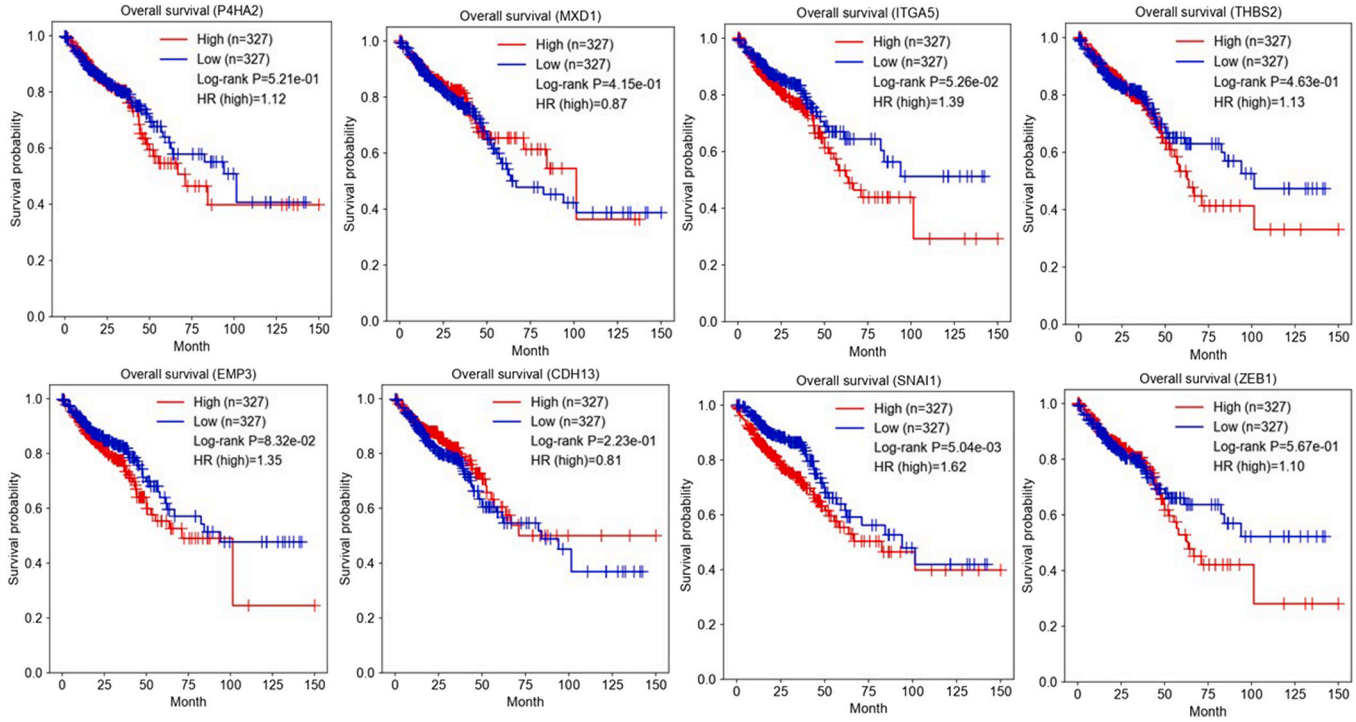

Fig. 5. Correlation between the mRNA expression levels of p-EMT-related genes and prognosis of colorectal cancer patients. Elevated mRNA expression of p-EMT genes EMP3, THBS2, and ITGA5, along with EMT markers ZEB1 and SNAI1, was significantly associated with worse overall survival. High expression of MXD1, P4HA2, and CDH13 showed no significant association with survival outcomes.

The significant downregulation of P4HA2, a collagen-modifying enzyme and THBS2, an adhesive glycoprotein suggests a focused disruption of extracellular matrix homeostasis and cell-matrix adhesion, conditions that can prime the microenvironment for migration. Concurrently, the

upregulation of CDH13, a non-classical cadherin associated with motility and MMP3 provides a direct mechanistic basis for the observed enhancement in cell migration. The biphasic regulation of the transcriptional repressor ZEB2 hints at a complex, dynamic adjustment of the epithelial plasticity network. Importantly, the stability of core p-EMT markers like SPP1 and INHBA indicates that *P. gingivalis* does not initiate a canonical partial EMT state. Instead, it appears to orchestrate a unique pro-migratory signature by selectively suppressing certain matrix-stabilizing genes (P4HA2, THBS2) while activating genes that help in cell motility (CDH13, MMP3). This pattern underscores the bacterium's ability to modulate discrete facets of the EMT machinery to promote an invasive phenotype. These complex dynamics parallel findings in other epithelial models: *P. gingivalis* is known to induce upregulation and nuclear localization of ZEB1, and heighten expression of vimentin and MMP9, promoting partial EMT and enhanced migratory capacity in gingival epithelial cells.[44] In primary oral epithelial cells, long-term *P. gingivalis* infection increased SNAI, SLUG, and ZEB1, while reducing E-cadherin, representing classical EMT progression.[45] Moreover, chronic *P. gingivalis* exposure in oral cancer models induced EMT-like changes with upregulation of stem-cell markers and multiple MMPs.[46] Although our model reflects CRC cells rather than oral epithelium, our data suggest that *P. gingivalis* may have a role in eliciting p-EMT and EMT. The observed gene expression dynamics, notably the downregulation of *SNAI1,* further indicate that this transition is subject to context-specific regulatory mechanisms in colorectal cancer.

Collectively, our findings demonstrate that *F. nucleatum* and *P. gingivalis* each promote CRC cell migration through distinct, non-canonical mechanisms. *F. nucleatum* drives a progressive and cohesive 'ITGA5-MMP3 axis', upregulating key mediators of matrix adhesion and remodeling while downregulating matrix-stabilizing factors a signature more directly aligned with established pro-invasive pathways. In contrast, *P. gingivalis* orchestrates a more complex, time-dependent response centered on SNAI1-independent motility effectors (CDH13, MMP3) and selective matrix disruption, suggesting a unique regulatory strategy. Unlike complete EMT, which is rarely observed in vivo, p-EMT states allow cells to retain epithelial adhesion while acquiring mesenchymal features, thus facilitating collective invasion and metastatic colonization.[31] Our data, showing pathogen-driven alterations in p-EMT-related genes, suggest that *F. nucleatum* and *P. gingivalis* may be instrumental in maintaining these hybrid states. The clinical correlation of key modulated genes (ITGA5, EMP3, THBS2) with patient survival further underscores the translational relevance of these microbial-gene signatures.[26]

While our results advance understanding of p-EMT regulation by periodontal pathogens in CRC, some limitations remain. While we utilized the rigorously curated TCGA datasets, our analyses are subject to potential technical artifacts, batch effects from multi-institutional data collection, undetected sample overlaps across platforms, and inaccuracies in clinical annotation that are beyond our control. Although our analysis provides strong transcriptomic evidence, this study lacks protein-level validation. Future work is needed to corroborate these mRNA findings at the proteomic level. While standardized conditions enable a comparative analysis at 6 h and 24 h, the lack of a $t = 0$ baseline measurement limits our ability to determine the exact fold-change relative to the pre-infection state. Further, the reliance on in vitro cell assays may not fully recapitulate in vivo tumor-microbiome interactions. Future work should examine these effects in in-vivo CRC models and patient-derived organoids. The distinct gene signatures induced by *F. nucleatum* suggest they may promote tumor progression through different mechanistic nodes. Dissecting signaling pathways linking exposure to *F. nucleatum* or *P. gingivalis* to p-EMT gene expression such as EGFR/AKT for *F. nucleatum* or NF-κB/ZEB for *P. gingivalis,* would strengthen mechanistic insights. Finally, evaluating functional consequences such as invasion, metastasis, and therapy resistance in relation to p-EMT modulation is critical.

5. Conclusion

In conclusion, this comparative study reveals that the periodontal pathogens *Fusobacterium nucleatum* and *Porphyromonas gingivalis* promote pro-migratory phenotypes in colorectal cancer cells through distinct and non-canonical transcriptional programs. While neither bacterium instigates a conventional p-EMT state, each elicits a unique pro-invasive gene signature characterized by the selective modulation of specific p-EMT and EMT-associated genes. The clinical relevance of these bacterium-associated signatures is underscored by the significant correlation of key modulated genes (EMP3, THBS2, ITGA5) with poorer overall survival in CRC patients. Together, our results strengthen the concept of the oral-gut axis in cancer biology and refine the mechanistic paradigm of microbiota-driven cancer progression, demonstrating that procarcinogenic bacteria do not operate through a universal pathways but rather use specific, targetable host pathways. Future work should focus on validating these targets in vivo and exploring causal relationships to completely exploit this emerging axis in microbiome-cancer research.

Patent consent

Not applicable.

Data availability statement

Not applicable.

Ethics approval statement

Not applicable.

Ethical clearance

Not applicable (Invitro study).

Declaration of AI use

DeepSeek AI was used to improve the sentence quality, structure, and readability of the overall manuscript. The authors take full responsibility for the content.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Nil.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jobcr.2026.101410.

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