Parvimonas Micra Promotes Intestinal Tumorigenesis in Conventional Apcmin/+ Mice and in Germ-Free Mice

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Research

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Abstract

Background: Large-scale meta-analysis of fecal shotgun metagenomic sequences revealed high abundance of *Parvimonas micra* in colorectal cancer (CRC) patients. We investigated the role of *P. micra* in colon tumorigenesis.

Results: *P. micra* was significantly enriched in 128 stool samplesfrom CRC patients compared with 181 samples from healthy controls(p<0.0001) and in 52 pairedtissue biopsies from CRC patientsthan 61 samples from healthy individuals (p<0.05). *P. micra* strain 512 was isolated from the feces of a CRC patient.Colon cell lines exposed to *P. micra*- conditioned medium significantly increased cell proliferation.*Apc*$^{min/+}$ mice gavaged with *P. micra* exhibited significantly higher tumor burden and load (both p<0.01). Consistently, cell proliferation was significantly higher in the colon tissues of germ-free mice gavaged with *P. micra*evidenced by increased Ki-67-positive cells and PCNA protein expression. Th2 and Th17 cells were markedly increased, while Th1 cells were reduced in the lamina propria of the colon tissues of mice gavaged with *P. micra* (all p<0.01). Moreover, *P. micra* colonization in germ-free mice was associated with increased expression of pro-inflammatory cytokines including Tnf-α, Il17a, Il6 and Cxcr1.

Conclusions: *P. micra* promoted intestinal carcinogenesis in *Apc*$^{min/+}$ mice and increased cell proliferation in germ-free mice. The tumor-promoting effect of *P. micra* was associated with altered immune responses and enhanced inflammation in the gut.

Introduction

Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths worldwide [1]. CRC is a malignant disease contributed by a variety of factors, including genetic mutations, epigenetic changes, chronic inflammation, diet, and lifestyle [2, 3]. Accumulating evidence suggests that gut microbiota contributes to CRC development [4–6]. Gut commensal microbiota plays multiple roles in maintaining host health and inducing diseases [7–9]. A balanced microbiota could produce essential nutrients, prompt efficient host nutrient absorption, aid development of a mature and competent host immune system, and prevent pathogen colonization [10–16]. Dysbiosis of the gut microbiota, however, could result in inflammation, barrier failure, mucosal tissue damage and altered microenvironment to favor the development of colon cancer [7, 17]. A number of studies have found that microbiota can drive colorectal carcinogenesis by causing DNA damage, oncogene expression and gene silencing [4, 9, 18, 19]. Realizing the importance of microbiota, new models of CRC development now take the function of microbiota into account.

In addition to the role of gut microbiota alterations in CRC, several individual microbes have been identified to contribute to CRC development. For instance, patients with *Streptococcus bovis*-induced endocarditis had a higher risk of developing colorectal adenomas or asymptomatic neoplasms [20]. *Fusobacterium nucleatum* [21] and *Peptostreptococcus anaerobius* [22] have also been studied as a CRC-promoting microbe in both human and animal models, by inducing mucin secretion and inflammatory

cytokine tumor necrosis factor (TNF)-α expression in direct contact with, or during invasion of colonic cells [23].

In light of the association of CRC pathogenesis with gut microbes [24–26], identifying bacterial pathogens that are drivers of colon tumorigenesis is imperative in the manipulation of the gut microbiota for CRC prevention and treatment. Previously, we performed a large-scale meta-analysis of fecal shotgun metagenomic sequences from CRC patients and control subjects from four cohorts of different ethnicities (Chinese, French & German, Austrian and American), and identified a higher abundance of *P. micra* in CRC compared to controls [27]. *P. micra* has also been reported to be associated with colon cancer consensus molecular subtype 1 [28]. *Parvimonas micra*, formerly known as *Peptostreptococcus micros* or *Micromonas micros*, is a gram-positive, anaerobic and opportunistic pathogen commonly found in the human oral cavity [29–31]. It is also frequently isolated from a wide range of human infections, including orofacial odontogenic infections, periodontitis lesions, endodontic abscesses and purulent pleurisy [32–35]. Moreover, increasing evidences show that oral microbes are associated with CRC [36]. We therefore speculated that *P. micra* might be involved in the development of CRC. As far as we know, there is no current report on its potential involvement in CRC. Herein, we investigated the effect of *P. micra* on colon tumor initiation and development using both conventional $Apc^{min/+}$ and germ-free mouse models.

Methods

Patient Recruitment And Samples Collection

Stool samples were retrieved from the research stool bank, collected from individuals undergoing colonoscopy at the Shaw Endoscopy Centre at the Prince of Wales Hospital, the Chinese University of Hong Kong (CUHK). The cohort included and excluded criteria were described previously [37]. Mucosal biopsies were obtained from individuals who had undergone standardized colonoscopy examinations at the Prince of Wales Hospital of the Chinese University of Hong Kong and Beijing Military General Hospital. All the samples stored at − 80 °C immediately after collection until further analysis. Ethics approval was provided by The Joint Chinese University of Hong Kong - New Territories East Cluster Clinical Research Ethics Committee and Beijing Military General Hospital.

Extraction of bacterial DNA from feces and tissues

Two hundred milligram of fecal samples were thawed on ice, and fecal DNA was extracted using the ZR Fecal DNA MiniPrep Kit (Zymo Research, CA) according to manufacturer's instructions. All DNA samples were stored at -80 °C, and the DNA quantity was determined using the Thermo Scientific NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific, PA). To extract bacterial DNA from human mucosal biopsies, glass beads (< 100 μm Sigma) and QIAamp DNA Mini Kit (QIAGEN) were used according to manufacturer's protocol.

Isolation of *P. micra* from fecal samples of CRC patients

Fecal samples from CRC patients with high relative abundance of *P. micra* were used for bacterial isolation. Samples were spread on blood agar plates and incubated in anaerobic chamber. The identities of the colonies were determined by gram staining and amplification of 16S rRNA gene using universal primers targeting hypervariable regions V1-V4 and V6 of the 16S rRNA gene, and confirmed by *P. micra*-specific primers and sanger sequencing.

Human CRC cell lines and normal epithelial cell line

Normal colonic epithelial cell line NCM460 and colon cancer cell lines (HT29 and Caco2) were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and grown in Dulbecco's Modified Eagle's Medium (DMEM) medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich, St Louis, MO). All three cell lines were cultured in 37 °C incubator with 95% oxygen and 5% CO_2. Culture media were renewed every 2–3 days.

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) Assay

Bacteria cultures were diluted 1:5 or 1:10 and cultured in anaerobic condition at 37 °C (2–3 days before experiments). Cells were seeded in 96-well plates (1000–2000 cells / well) with 10% FBS. Cultured *P. micra* and *E. coli* were centrifuged at 5000 rpm for 10 min, followed by the removal of supernatant and filtration using 0.22um filter. The filtered medium was diluted to 12.5% with cell culture medium (DMEM + 10% FBS). The cell culture medium was replaced with 100 μL of diluted 12.5% bacterial-conditioned medium and cultured under anaerobic condition. After 0 hours, 24 hours, 48 hours and 72 hours, 10 ul of MTT was added and replaced with 100 μL DMSO every 4 hours. Cell proliferation was estimated by measuring the OD using microplate reader (Multiskan GO, Thermo Scientific).

Animal model and treatment

Resident microbiota of 6 weeks old *Apc* $^{min/+}$ mice were depleted using a cocktail of broad-spectrum antibiotics (ampicillin 0.2 g/L, vancomycin 0.1 g/L, neomycin 0.2 g/L, and metronidazole 0.2 g/L) in drinking water for 2 weeks, before oral gavage with *P. micra, E. coli* or broth control 3 times per week for 8 weeks, and were sacrificed after 10 weeks. Eight weeks old germ-free mice were randomly assigned to 3 groups and gavaged with 1×10^8 colony forming unit (CFU) of *P. micra*, *E. coli*, or broth once. Five mice each at 8, 12, and 32 weeks were sacrificed. All animal experiments were performed in accordance with guidelines approved by the Animal Experimentation Ethics Committee of the Chinese University of Hong Kong.

Reverse-transcription PCR and quantitative PCR

Total RNA was isolated using TRIZOL reagent (Qiagen, Valencia, CAA). PrimeScript™ RT reagent Kit (Perfect Real Time) (Takara, Kusatsu, Shiga, Japan) were used for reverse transcription PCR. Gene

expression data was analyzed using relative quantification 2-ct method and normalized to the fold change detected in corresponding control cells, which was defined as 1.0. Primers sequences are listed in **S Table 1**.

PCR array

Mouse Cancer Finder PCR array PM033ZC (Qiagen), including 84 genes representative of 9 different biological pathways involved in transformation and tumorigenesis, and Mouse Inflammatory Response and Autoimmunity PCR Array PAMM-077 (Qiagen), including 84 key genes of inflammatory cytokines and chemokines as well as their receptors, were performed according to manufacturer's protocol.

Protein extraction and Western Blotting

Total protein was obtained using CytoBuster protein extraction reagent (Merch Chemicals, Nottingham, UK).The antibodies used in this study are proliferating cell nuclear antigen (PCNA) (ab29, Abcam) and GAPDH (SC-25778, Santa Cruz).

Colony formation assay

Colon epithelial cell line NCM460 and cancer cell lines HT-29 and Caco-2 (1000 cells/well) were plated in 6-well plates. After culturing for 5–7 days, cells were fixed with 70% ethanol and stained with 0.5% crystal violet solution. Colonies with more than 50 cells per colony were counted. All experiments were conducted three times in triplicates.

Flow cytometry

Flow cytometry cell sorting was performed as described previously [38]. Briefly, lamina propria leukocytes was profiled by flow cytometry after staining with surface markers CD4 (100528, Biolegend), intracellular markers IL4 (504119, Biolegend), IL17 (506903, Biolegend) and interferon (IFN)-γ (505806, Biolegend).

Statistical analysis

All statistical tests were performed using GraphPad or R Software. Multiple group comparisons were analyzed by one-way analysis of variance (ANOVA). Non-parametric data between two groups were computed by Mann-Whitney U test Data were presented as mean ± SD. *P*-value < 0.05 was considered statistically significant.

Results

P. micra was significantly enriched in fecal samples and tissue biopsies of CRC patients

Using large-scale shotgun metagenome sequences of fecal samples and 16S sequencing of tissue biopsies, we have recently demonstrated that *P. micra* was highly enriched in both stool and tissues of CRC patients as compared with healthy subjects in a meta-analysis (**Figure S1A**) [38, 39].

To validate the enrichment of *P. micra* in fecal samples of patients with CRC, we analysed the gut metagenome sequences of individual cohorts (France (control, n = 66; CRC, n = 89) and Austria (control, n = 63 and CRC, n = 46) (Fig. 1A). The abundance of *P. micra* in fecal samples of patients with CRC was significantly higher than in normal control subjects in both European cohorts ($P < 0.01$). Furthermore, a geographically-matched independent Chinese cohort (control, n = 110; CRC, n = 111) consistently confirmed that *P. micra* was significantly enriched in fecal samples of patients with CRC ($P < 0.001$) (Fig. 1B).

To further verify the results from metagenome sequencing data, the abundance of *P. micra* was detected by real-time quantitative PCR using additional 181 control and 128 CRC fecal samples as well as 61 mucosal samples of normal colon and 52 pairs of colorectal carcinoma-adjacent normal and cancerous mucosae from Beijing and Hong Kong. qPCR confirmed the enrichment of *P. micra* in both fecal and mucosal samples of patients with CRC compared with control samples ($P < 0.0001$) (Fig. 1C and 1D). Taken together, these results showed increased prevalence of *P. micra* in feces as well as tumor biopsy samples in colorectal neoplasms and suggest a potential functional role for this bacterium in tumorigenesis.

P. micra-conditioned medium promotes proliferation of colonic cells

A strain of *P. micra* was successfully isolated from the fecal sample of a CRC patient (**Figure S1B)**. Analysis of chromatograms showed nearly identical sequence match of its 16S rRNA gene sequence with those of *P. micra* strains deposited in the NCBI RefSeq database (**Figure S1C**). Growth dynamics and colony morphology of this *P. micra* strain were showed in **Figure S2**. To investigate the functional role of *P. micra*, we performed MTT assays on various colonic cell lines with the conditioned medium of *P. micra*. Interestingly, *P. micra*-conditioned medium significantly promoted the proliferation of both normal colonic epithelial cell line, NCM460 and cancer cell lines, HT29 and Caco2 compared with *E. coli*-conditioned medium and broth control groups (Fig. 2A). To verify the MTT viability assay, we performed colony formation assays on the colonic cell lines co-cultured with bacterial conditioned medium. We found that the *P. micra*-conditioned medium consistently increased the clonogenicities of normal colonic epithelial cell line NCM460 as well as cancer cell lines HT29 and Caco2 when compared with control groups (Fig. 2B).

*P. micra*promotes intestinal tumorigenesis in conventional *Apc*$^{min/+}$ mice by triggering inflammation

Next, we determined whether *P. micra* could drive tumorigenesis in a murine *Apc*$^{min/+}$ model of CRC. Before oral gavage with *P. micra*, the resident microbiota was depleted using a cocktail of broad-spectrum antibiotics (ampicillin 0.2 g/L, vancomycin 0.1 g/L, neomycin 0.2 g/L, and metronidazole 0.2 g/L) in

drinking water for 2 weeks. Mice were then orally gavaged with *P. micra*, *E. coli* or broth control 3 times per week for 8 weeks (Fig. 3A). Depletion of fecal bacterial DNA shown by a 10-fold reduction of total bacteria in the feces was confirmed by real-time quantitative PCR analyses (Fig. 3B). The abundance of *P. micra* was increased after *P. micra* introduction (Fig. 3B). Mice were sacrificed after 10 weeks. We observed significantly more tumors in mice inoculated with *P. micra* than the *E. coli* and PBS control groups ($p < 0.01$), suggesting that *P. micra* may play a pro-tumorigenic role *in vivo* (Fig. 3C and 3D); the tumor load was consistently higher in *P. micra*-gavaged group than control groups ($p < 0.01$) (Fig. 3D).

To gain insights into the potential mechanisms underlying the tumorigenic role of *P. micra*, we examined inflammation scores of proximal and distal colons and found that the inflammation scores were significantly higher in the mice gavaged with *P. micra* compared to *E. coli* and broth control groups (Fig. 3E). In the lamina propria of colonic tissues, flow cytometry analyses showed increased levels of Th2 and Th17 cell infiltration, and reduced number of Th1 cells (Fig. 3F). Together, these results suggest that the promotion of colonic tumor formation by *P. micra* in *Apc*$^{min/+}$ mice was associated with Th17 inflammatory response.

P. micra promotes colonic cell proliferation in germ-free mice

Germ-free mice model was used to further ascertain the function of *P. micra* in promoting CRC, Germ-free mice of 8-weeks-old were randomly assigned to 3 groups and gavaged with 1×10^8 CFU of *P. micra*, *E. coli*, or broth once. At three time points (8 weeks, 20 weeks, and 32 weeks), 5 mice from each group mice were sacrificed (Fig. 4A). To investigate whether *P. micra* could promote epithelial cell proliferation, we performed Ki-67 IHC staining on the colon tissues. The protein levels of Ki-67 were not significantly different among all groups at week 8. Interestingly, the protein levels of Ki-67 were higher in the *P. micra* group than the control group at week 20 and 32 (Fig. 4B). The ability of *P. micra* in promoting cell proliferation in the colonic epithelial tissues was confirm by increased PCNA protein expression, in *P. micra*-gavaged germ-free mice as compared with broth and *E. coli* control groups (Fig. 4C). These findings confirmed the results from *in vitro* MTT and colony formation assays and indicated that *P. micra* could promote colonic cell proliferation.

Altered expression of cell proliferation-related genes in *P. micra*-induced tumorigenesis in germ-free mice

The potential mechanisms underlying the tumorigenic role of *P. micra* in germ-free mice were determined by Cancer Gene expression analysis, which indicated that cell proliferation-related pathways were altered (Fig. 5A). We found downregulation of genes in apoptotic pathways; FasL, Casp7, cellular senescence; Map2k3 and DNA damage and repair; Gadd45g. Genes that are known to function in angiogenesis (Pgf, Tek, Angpt1, Fit1) and regulate cell proliferation (Tbx2, Mki67, Mcm2, Cdc20) were found to be

upregulated. In addition, stemness associated genes (Sirt1, Bmi1) and those involved with invasion and metastasis (Cdh2, Foxc2, Snai1) were observed to have more than 2 fold increased expression in *P. micra* induced tumorigenesis (Fig. 5B).

Altered expression of immune responses and inflammation related genes in *P. micra*-induced tumorigenesis in germ-free mice

The Mouse Inflammatory Response and Autoimmunity PCR Array was used to analyse potential contribution of inflammation to the role of *P. micra* in colon tumorigenesis in germ-free mice. Significant up-regulation (48 transcripts) and down-regulation (6 transcripts) of gene expression were observed at 32 weeks after gavage with *P. micra*. Differentially expressed genes included interleukin 17a (Il17a), Il22, and Il23a, which encode 3 cytokines secreted by Th17 lymphocytes. We found the upregulation of genes that function in chemotaxis of immune cells including neutrophil chemotaxis (Cxcl1, Cxcl2, Cxcl5, Cxcl9, Cxcr2, Cxcr4 and Ccl20), T-lymphocyte chemotaxis (CCr4, Ccl17, Ccl19, Ccl22, Ccl24, Cxcl9, Cxcl10 and Cxcl11), and monocyte chemotaxis (Ccl1, Ccl2, Ccl3, Ccl4, Ccl5, Ccl7 and Ccl8) (Fig. 5C and 5D). In addition, genes in pro-inflammatory response pathway (IL1α, IL1β, Il1rn, Tlr2, Tlr4, Il1rap, Ifnγ and Ltb), and humoral response pathway (Ccr7, Tnf-α, Il1b, Il6, Nfkb1 and Itgb2) demonstrated increased expression in *P. micra* gavaged mice compared to control. However, Ccl11, Ccl25, Kng1, Il9 and Crp were downregulated in *P. micra*-gavaged mice. Quantitative RT-PCRs using specific primer-probes were performed on genes identified in the microarray study and confirmed the changes in expression of Tnf-α, Il17a, Il6 and Cxcr1 (Fig. 5E). These genes corresponded to altered immune state involving chemotaxis, antigen presentation, pro-inflammatory response and the Th17 pathway. Taken together, these results suggest that tumor-promoting effect of *P. micra* is associated with altered immune responses and enhanced inflammation in the gut.

Discussion

We previously reported the enrichment of *P. micra* in fecal samples of CRC patients by next-generation sequencing technology [38]. In this study, increased abundance of *P. micra* in CRC patients were validated by additional metagenome sequencing analysis and *P. micra* specific qPCR of stool samples. Interestingly, *P. micra* was also shown to be enriched in the colonic mucosa of patients with CRC in two independent cohorts of Hong Kong and Beijing subjects. Our findings suggest that *P. micra* is associated with CRC and could be a potential driver in colonic carcinogenesis.

The potential of *P. micra* in driving colonic tumorigenesis was elucidated in normal colon epithelial and CRC cell lines, and confirmed in bacteria-depletion *Apc*$^{min/+}$ and germ-free mouse models. We found that *P. micra*-conditioned medium increased the proliferation of NCM460 and cancer cell lines HT29 and Caco2 when compared with *E.coli* MG1655 and broth control groups. *In vivo*, *P. micra* promoted tumor

number and load in *Apc*$^{min/+}$ mice and enhanced cell proliferation in germ-free mice. These results collectively demonstrate that *P. micra* could accelerate colonic tumorigenesis.

CRC associated bacteria evoke carcinogenesis via various means. *F. nucleatum* modulates host immunity and tumor microenvironment by inducing mucin secretion and inflammatory cytokine TNF-α expression, while *P. anaerobius* induces intracellular cholesterol biosynthesis to induce colon cell proliferation [21, 22]. Also, carcinogenic *E. coli* interferes with DNA repair as a mechanism of promoting colonic cell proliferation [40]. Given the diverse ways through which bacteria can promote tumorigenesis, we performed experiments to gain insight into the mechanisms employed by *P. micra* in CRC development. Our array analysis revealed that the tumor-promoting effect of *P. micra* is associated with altered immune responses and enhanced inflammation in the gut. *P. micra* evoked multiple inflammatory and oncogenic pathways in *Apc*$^{min/+}$ and germ-free mice. We observed significant increases in Il17a, Il22, and Il23a, implicating Th17 cell response as a major pathway activated by *P. micra*. Consistent with our results, multiple studies, exemplified by the observations that both IL22 and IL23 enhanced tumorigenesis, and that, blockade of IL17A inhibited tumor growth [41, 42], in colitis-associated cancer models have described the potential involvement of Th17 pathway in colorectal tumorigenesis [43, 44].

Furthermore, we observed up-regulation of genes involved in the chemotaxis of neutrophils, T-lymphocytes and monocytes in *P. micra*-gavaged mice highlighting a role for immune cell chemotaxis in *P. micra*-induced tumorigenesis. Increased levels of circulating and tumor-infiltrating myeloid cells have been reported in CRC patients [45, 46]. Elevation of immune cells chemotaxis in *P. micra*-induced CRC as observed in this study further underscores the importance of chemotaxis in the progression of colon carcinogenesis. As a defence mechanism, the host mounts antibody responses against tumor associated antigens including those capable of inducing CRC [47]. In addition to enhanced proliferation of cells co-cultured with *P. micra*-conditioned medium, we found upregulation of humoral immune response in *P. micra* gavaged mice compared to both *E.coli* and broth control groups, suggesting the secretion of carcinogenic antigens into the colon by *P. micra*, against which antibodies were produced. Taken together, these results suggests that *P. micr*a induces colonic cell proliferation via the secretion of carcinogenic antigens, immune cell chemotaxis and enhanced gut inflammation.

Conclusion

We found that enhanced colon colonization by *P. micra* may predispose the host to colorectal tumorigenesis. In addition to being a fecal biomarker, we characterized *P. micra* as a potential bacterial driver in colon carcinogenesis through enhanced inflammation and altered immune responses in the gut. These findings have potential impact in the prevention, diagnosis and treatment of CRC.

Declarations

Ethics Approval and Consent to Participate

All subjects had given written informed consent. The clinical study protocol was approved by The Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee and Beijing Military General Hospital. All animal studies were performed in accordance with guidelines approved by the Animal Experimentation Ethics Committee of The Chinese University of Hong Kong.

Consent for Publication

Not Applicable.

Availability of Data and Materials

All the data and materials supporting the findings of this article have been included in the manuscript and supplementary figures.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

LZ performed experiments, analyzed data and drafted the manuscript. YZ, RZ, XZ and ESHC performed experiments. OOC, WKKW, SHW and JJYS commented the study and revised the manuscript. HW provided germ-free mice. KFT supervised the study and revised the manuscript. JY designed, supervised the study and wrote the paper.

Abbreviations

CFU, colony forming unit;CRC, colorectal cancer; IFN, interferon; IL, interleukin; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-DiphenyltetrazoliumBromide; PCNA, proliferating cell nuclear antigen; TNF, tumor necrosis factor.

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Figures

Figure 1

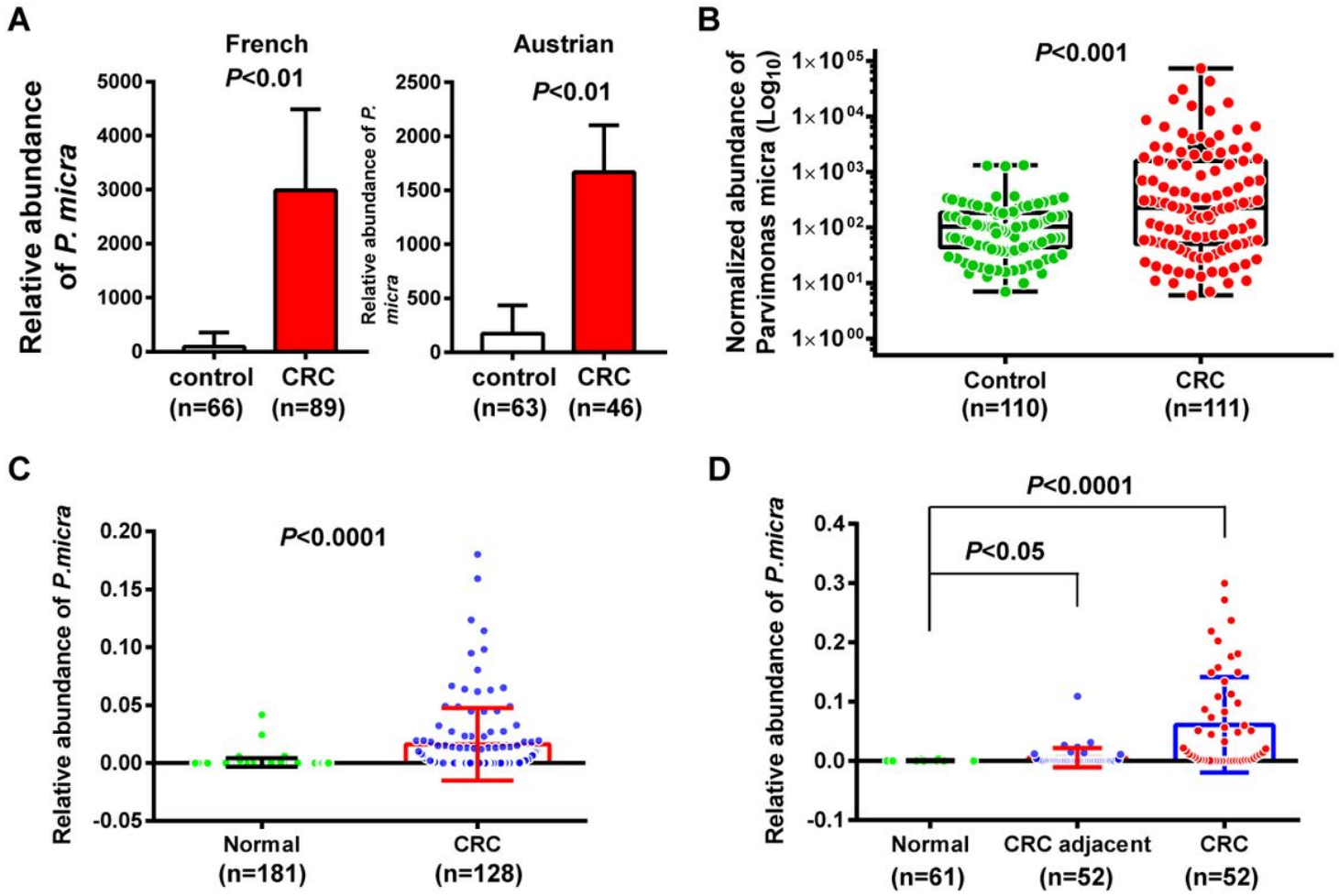

Figure 1

P. micrais enriched in stool and tissue samples of patients with CRC. (A) The level of P. micrain stool samples from two public cohorts of French control: n=66 and CRC: n=89) and France (control: n=63 and CRC: n=46). (B) Validation of P. micra level in independent Chinese metagenomic data (control: n=110 and CRC: n=111). Real time qPCR analysis of P. micraabundance in (C) fecal samples of control (n=181) and CRC patients (n=128), and (D) histological control (n=61) and patient-matched tumor and tumor-adjacent mucosal samples (n=52). Statistical significance was determined by Mann-Whitney U test and nonparametric one-way analysis of variance.

Figure 2

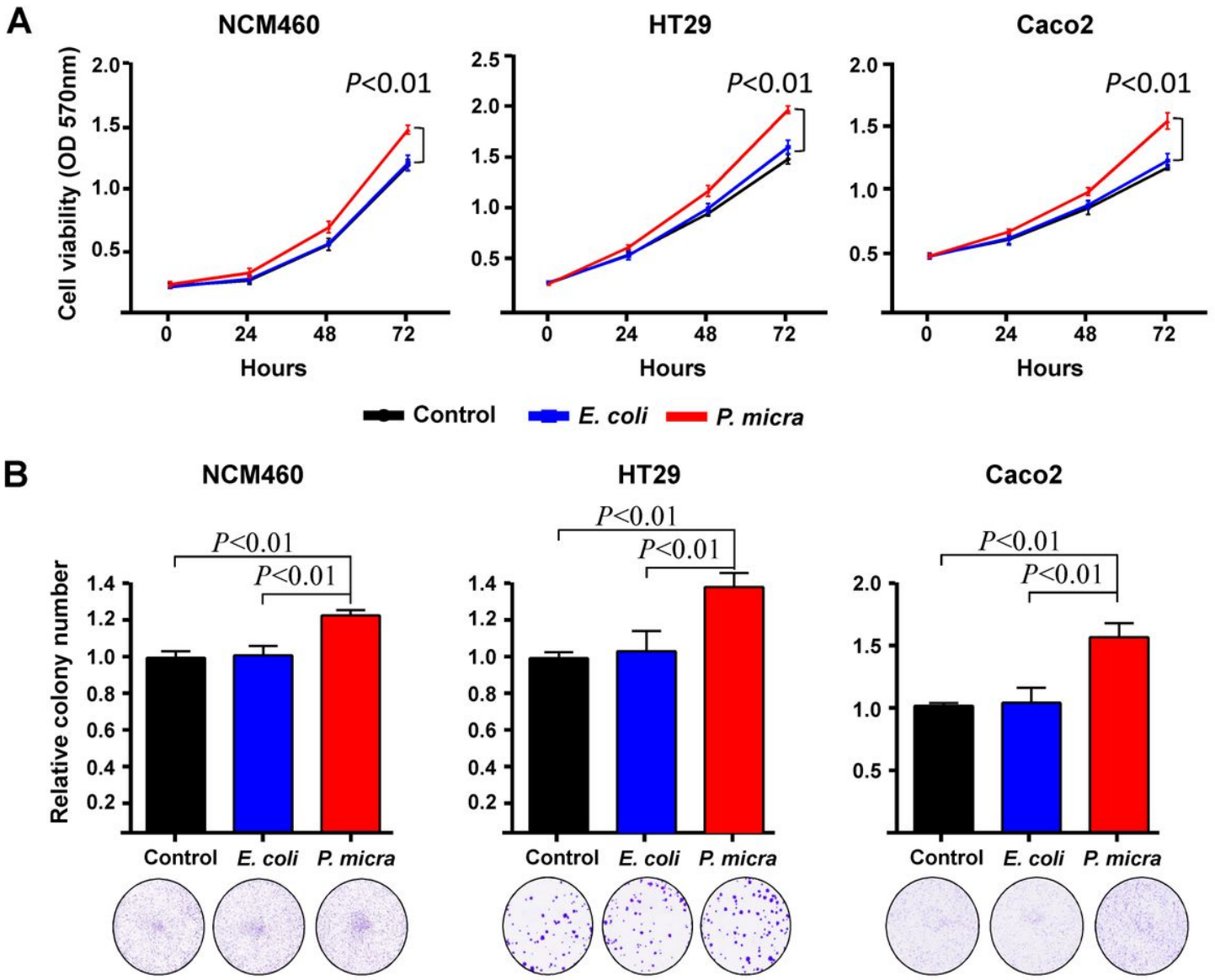

Figure 2

Conditional medium of P. micrapromotes colonic epithelial cell lines proliferation. (A) Cell viability at different time points after treatment with conditioned media in NCM460, HT29, and Caco2 cell lines. (B) Colony formation assays of colonic epithelial cells treated with P. micraconditioned medium. Semi-quantitative and qualitative analysis of clonogenicities in NCM460, HT29, and Caco2 cell lines.

Figure 3

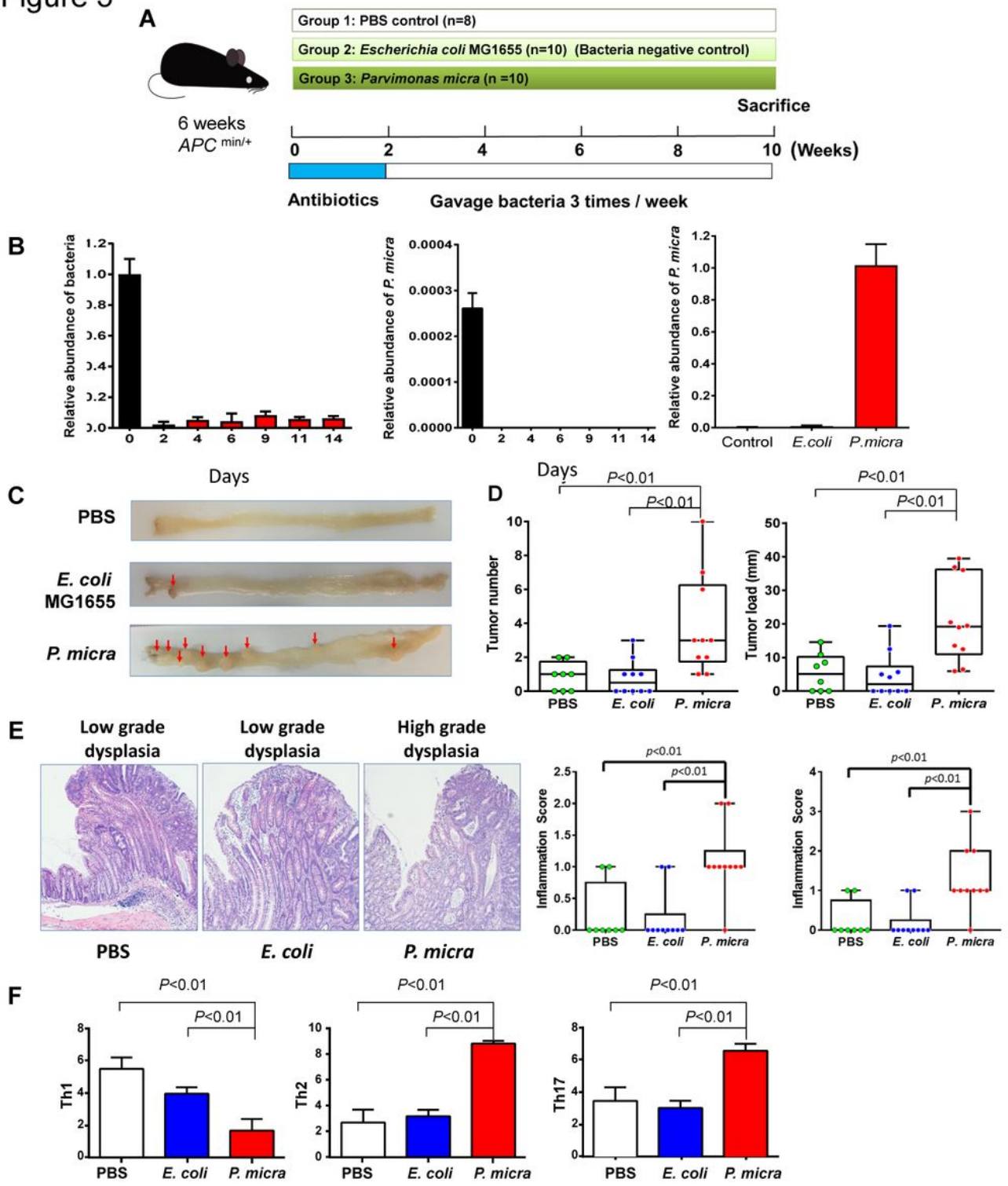

Figure 3

P. micraaccelerates colonic tumorigenesis in Apcmin/+mice. (A) Schematic diagram showing the experimental design and timeline of Apcmin/+mice model (PBS group n=8, E.coli MG1655 group n=10 and P. micragroup n=10). (B) Fecal quantitation of total bacteria and P. micra in Apcmin/+mouse model in after microbiota depletion by antibiotics and after P. micraintroduction.(C) Representative colonic morphologies of mice under different treatments. (D) The colonic tumor numbers and loads of

Apcmin/+mice under different treatments. (E) Representative histologic photos of colon tissues of mice by H&E staining. The inflammation score was significantly higher in P. micragroup than PBS and E. coli group in both proximal and distal colons. (F) Flow cytometry analysis showing increased Th2 and Th17 cells and reduced Th1 cells in the colon of Apcmin/+mice after P. micra treatment. Percentages of Th1, Th2 and Th17 cells were compared using the Mann-Whitney U test.

Figure 4

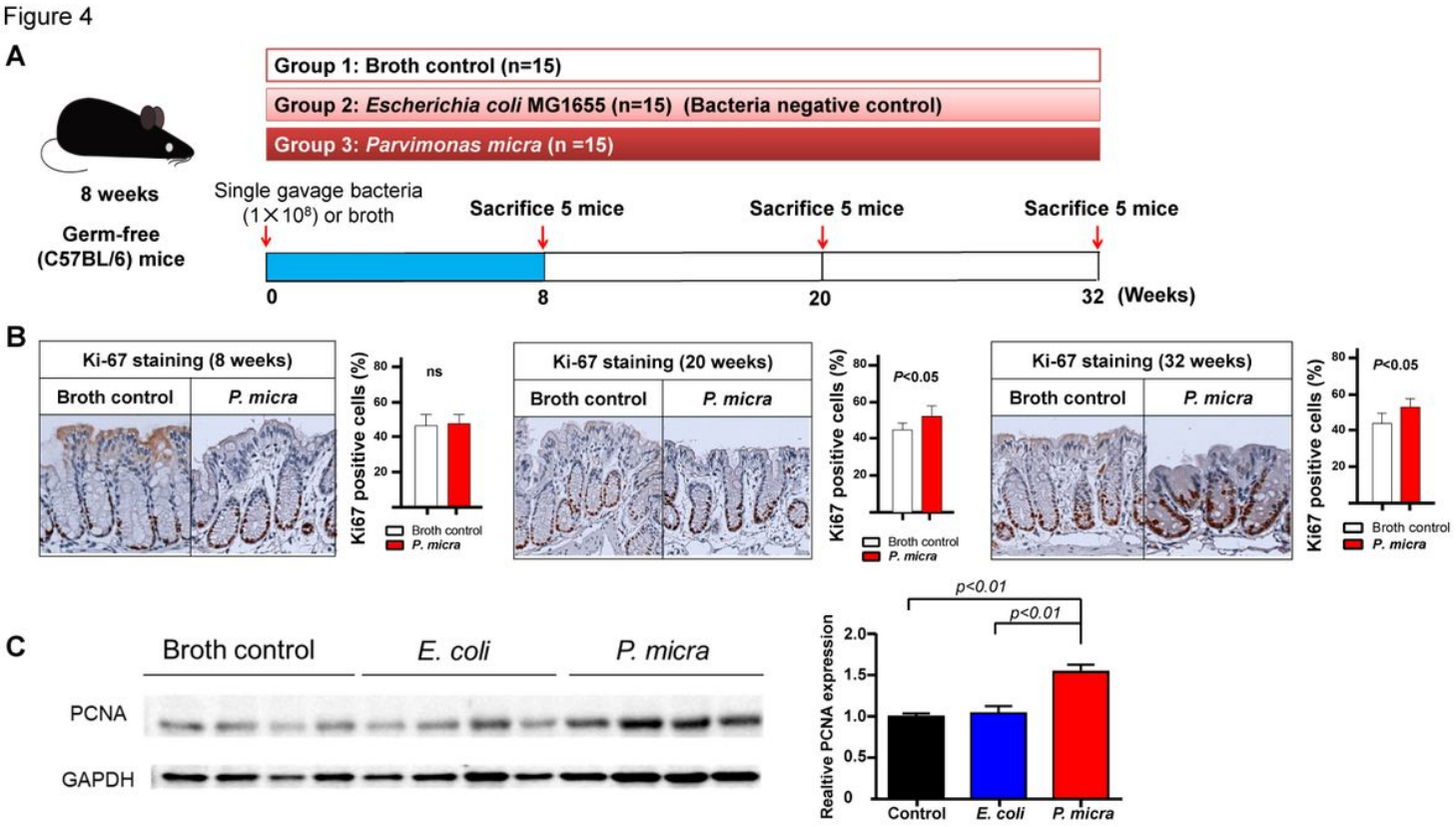

Figure 4

Effects of P. micraon cell proliferation and inflammatory factors in the germ-free mice model. (A) Design of P. micragavage experiment in Germ-free mice (broth control group n=15, E. coli MG1655 group n=15, and P. micragroup n=15). (B) Immunohistochemistry showing Ki-67-positive cells in the colon of germ-free mice at 8 weeks, 20 weeks, and 32 weeks after P. micra gavage, and the proportion of Ki-67-positive cells in the colon of Germ-free mice. (C) Western blot showing relative protein expression of PCNA in the colon of Germ-free mice at 32 weeks after bacteria gavage.

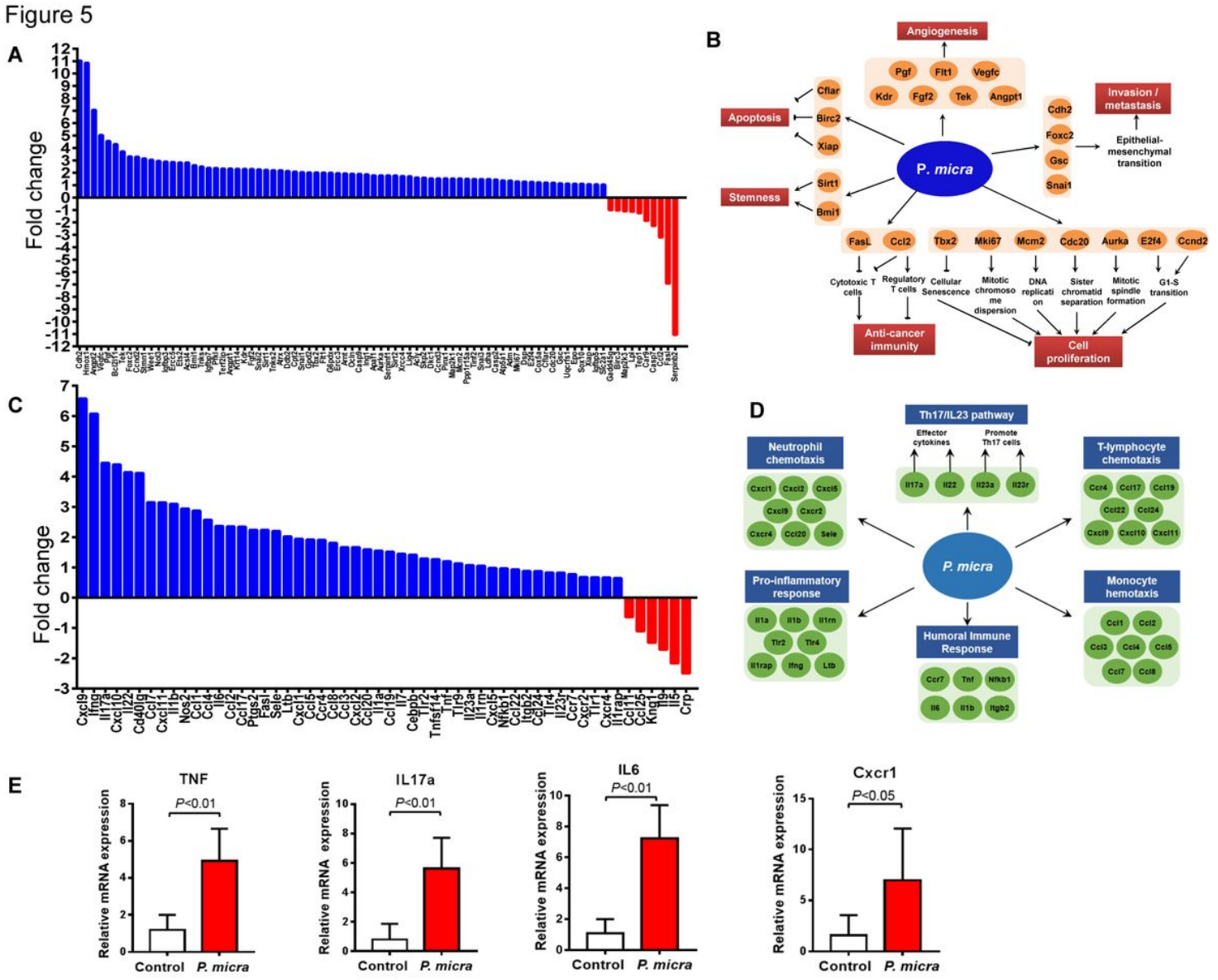

Figure 5

Altered expression of cell proliferation-related genes in P. micra-induced tumorigenesis(A) Significant up-regulation in the expression of 74 transcripts and down-regulation of 10 transcripts by the Mouse Cancer Pathway Finder PCR Array. (B) A systematic diagram showing major oncogenic pathways implicated by up-regulated genes identified by the PCR array, mostly focus on cell proliferation. (C) Significant up-regulation (48 transcripts) and down-regulation (6 transcripts) of gene expression by the Mouse Inflammatory Response and Autoimmunity PCR Array 32 weeks after gavage withofP. micra 32 weeks. (D) A systematic diagram showing major inflammatory pathways implicated by up-regulated genes identified by the PCR array. (E) qPCR validation was performed to confirm changes in expression of genes including TNF, Il17a, Il16, and chemokine(C-X-R motif) ligand 1 (Cxcl1). Relative expressions were compared using the Mann-Whitney U test.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS1.pdf
- STable1.pdf
- FigureS2.pdf