Experimental and Molecular Pathology 145 (2026) 105013

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

miR-21-5p dysregulation is associated with gut microbiota dysbiosis and pro-oncogenic markers in primary sclerosing cholangitis with concomitant inflammatory bowel disease

André Anastácio Santos [a,*], David Pires [b], Vanda Marques [a], Nicole Alesina [a], Pedro Miguel Rodrigues [g,h,i], Ana Catarina Bravo [c], Catarina Gouveia [c], Susana Saraiva [c], Luís Correia [d], Ricardo Crespo [e], João Pereira da Silva [e], Marília Cravo [f], Jesus Maria Banales [g,h,i,j], Joana Torres [c,f,k], Cecília Maria Pereira Rodrigues [a]

[a] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal
[b] CIIS - Centro de Investigação Interdisciplinar em Saúde, Faculdade de Medicina, Universidade Católica Portuguesa, Lisboa, Portugal
[c] Division of Gastroenterology, Hospital Beatriz Ângelo, Loures, Portugal
[d] Division of Gastroenterology, ULS Santa Maria, Lisboa, Portugal
[e] Division of Gastroenterology, Hospital Lusíadas, Lisboa, Portugal
[f] Division of Gastroenterology, Hospital da Luz, Lisboa, Portugal
[g] Department of Liver and Gastrointestinal Diseases, Biogipuzkoa Health Research Institute, Donostia University Hospital, University of the Basque Country (UPV/EHU), San Sebastian, Spain
[h] Ikerbasque, Basque Foundation for Science, Bilbao, Spain
[i] Center for the Study of Liver and Gastrointestinal Diseases (CIBERehd), Carlos III National Institute of Health, Madrid, Spain
[j] Department of Biochemistry and Genetics, School of Sciences, University of Navarra, Pamplona, Spain
[k] Faculdade de Medicina, Universidade de Lisboa, Portugal

ARTICLE INFO

Keywords:
PSC-IBD
Gut microbiota
Immune response
Gut-liver axis
miR-21

ABSTRACT

Background: Primary sclerosing cholangitis (PSC) is a chronic liver disease frequently associated with inflammatory bowel disease (IBD) and increased risk of colorectal cancer (CRC). Despite the strong association, the underlying mechanisms linking PSC-IBD, gut inflammation, and neoplastic potential remain unclear. This study explores the role of miR-21-5p dysregulation, gut microbiota dysbiosis, and pro-oncogenic markers in shaping the inflammatory and neoplastic microenvironment in PSC-IBD patients.

Methods: A case-control study was conducted, including PSC patients with concomitant IBD ($n = 14$) and control individuals without diagnosed PSC and IBD ($n = 20$). miR-21-5p levels were evaluated in serum, fecal samples, and colonic biopsies *via* qPCR. Gut microbiota composition was analyzed using 16S rRNA sequencing. Pro-oncogenic and inflammatory markers in colonic tissue were assessed *via* qPCR. *In vitro* studies were performed using cholangiocyte (H69), colorectal cancer (HCT116), and primary monocyte models to investigate the role of miR-21-5p.

Results: miR-21-5p was significantly upregulated in the right colon, serum, and fecal samples of PSC-IBD patients compared to controls. Gut microbiota analysis revealed dysbiosis, characterized by an increased *Bacteroidota/Bacillota* ratio and reduction in bile acid-metabolizing bacteria, including *Clostridium sensu stricto 1*, *Ruminococcaceae UCG-002*, and *Christensenellaceae_R7_group*. Colonic tissue analysis showed increased expression of EMT-related transcription factors *TWIST1* and *SNAIL*, inflammatory cytokines *IL-8*, *CCL2*, and *COX2*, and the stem cell marker *LGR5*. *In vitro* studies confirmed miR-21-5p role in upregulating *does* markers in monocytes and CRC cells.

Abbreviations: ACN, acetonitrile; CAC, colon adenocarcinoma; (CCA, cholangiocarcinoma; CCL2, C-C motif chemokine ligand 2; CRC, colorectal cancer; CD, Crohn's disease; COX2, cyclooxygenase-2; IBD, inflammatory bowel disease; IFN-γ, interferon-gamma; IL-8, interleukin-8; LGR5, Leucine-rich repeat-containing G protein-coupled receptor 5; miR-21, microRNA-21; MUC2, mucin-2; SNAIL, snail family transcriptional repressor 1; PSC, primary sclerosing cholangitis; TNF-α, Tumor Necrosis Factor-α; TWIST1, twist family bHLH transcription factor 1.

* Corresponding author at: Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal.
E-mail address: afasantos@ff.ulisboa.pt (A.A. Santos).

https://doi.org/10.1016/j.yexmp.2025.105013
Received 17 September 2025; Received in revised form 26 November 2025; Accepted 28 November 2025
Available online 5 December 2025

Conclusion: This study found a link between miR-21-5p dysregulation and gut microbiota dysbiosis, colonic inflammation, and pro-oncogenic signaling in PSC-IBD patients. These findings highlight miR-21-5p as a potential modulator of disease progression and neoplastic risk.

1. Introduction

Primary sclerosing cholangitis (PSC) is a rare, long-term liver condition characterized by progressive cholestasis due to inflammation, fibrosis, and narrowing of the intrahepatic and extrahepatic bile ducts. The exact cause of PSC remains unknown (Chazouilleres et al., 2022). Despite its rarity, PSC is the fifth leading cause of liver transplantation in the United States and is associated with a poor prognosis (Emek et al., 2019; Tabibian and Bowlus, 2017). Up to 80 % of PSC patients have inflammatory bowel disease (IBD), a chronic disorder of the gastrointestinal tract, which includes Crohn's disease (CD) and ulcerative colitis (UC) (Tabibian and Bowlus, 2017), often with unique features such as pancolitis, rectal sparing, and an increased risk of colorectal cancer (CRC) (Ananthakrishnan et al., 2018). In PSC-IBD, inflammation and colitis-related neoplasia are more commonly observed in the right colon than in IBD alone (Munster et al., 2024).

The gut microbiota plays a key role in IBD development. Evidence links imbalanced gut microbiota (dysbiosis), disrupted intestinal permeability, and abnormal T-cell activity to PSC-IBD and its distinct features (Qiu et al., 2022; Shaw et al., 2023; Torres et al., 2018; Torres et al., 2016). However, the high prevalence of IBD in PSC-IBD patients and its specific clinical traits remain poorly understood (D'Amico et al., 2025).

Non-coding RNAs have been described to regulate inflammation (Tang et al., 2021), and among them is microRNA-21-5p (miR-21-5p), whose overexpression is linked to intestinal inflammation and CRC (Jenike and Halushka, 2021; Peng et al., 2017). miR-21-5p has already been shown to promote tumor progression by downregulating tumor suppressors such as PTEN, PDCD4, and RECK, thereby enhancing cell proliferation, invasion, and resistance to apoptosis (Bautista-Sánchez et al., 2020). Indeed, a recent meta-analysis concluded that tissue levels of miR-21-5p could be a promising biomarker for CRC detection, with a summary receiver operating characteristic curve (SROC) of 0.93 (95 % CI: 0.91–0.95) (Li et al., 2023). Our group and others have shown that miR-21-5p loss ameliorates liver disease and prevents intestinal dysbiosis (Afonso et al., 2018; Rodrigues et al., 2015; Santos et al., 2020). Elevated miR-21-5p modulates gut microbiota by reducing *Lactobacillaceae abundance*, while miR-21-5p knockout (miR-21KO) models show increased microbiota diversity, including the probiotic *Lactobacillus reuteri* DSM 17938 (now renamed *Limosilactobacillus reuteri* DSM 17938), which may mitigate liver disease (Santos et al., 2020). Nevertheless, the influence of circulating miR-21-5p in PSC-IBD during inflammation remains unexplored.

Considering the complexity of PSC-IBD, this pilot study sought to investigate how elevated levels of miR-21 and alterations to gut microbiota composition alterations contribute to the unique inflammatory environment of PSC-IBD, potentially driving its characteristic gut inflammation.

2. Methods

2.1. Patient enrollment and ethics

This pilot case-control study involved PSC-IBD patients and control participants undergoing colonoscopy for CRC screening or polyp monitoring. Eligibility criteria included individuals aged 18 years or older, capable of providing informed consent, with confirmed PSC and IBD diagnosis (Chazouilleres et al., 2022). Sample collection was conducted over a three-year period (2019–2021), following written informed consent and adhered to the WMA Declaration of Helsinki for Medical Research Involving Human Subjects, Portuguese law DL 43/04 (August 19, DR I Série), and Ethical Commission approval (Ref. 0028/2014_RMEB). Demographic and clinical data were recorded. In the absence of any risk for disease exacerbation, PSC-IBD patients were asked to discontinue ursodeoxycholic acid (UDCA) treatment two weeks before sample collection to prevent confounding effects from bile acid interference. During colonoscopy, two biopsies were taken from both the right and left colon and stored in RNAlater solution (Thermo Fisher Scientific) and latter stored at −80 °C. Additionally, serum samples were collected the same day and preserved at −80 °C.

2.2. Patient fecal sample collection

Patients collected fecal samples immediately after evacuation using EasySampler® Stool Collection Kit (ALPCO Diagnostics). Samples were divided in two distinct tubes one for miRNA analysis (standard 50 mL cylinder) and bacterial DNA analysis (preserved with OMNIgene•GUT, DNAgenotek). All samples were collected always before colonoscopy to avoid the effects of bowel preparation. Samples were preserved at 4 °C for up to 24 h before preservation at −80 °C.

2.3. Serum miR-21-5p extraction and evaluation

To evaluate serum hsa-miR-21-5p expression, total miRNA was extracted from serum using miRNeasy Serum/Plasma Advanced Kit (Qiagen). Cel-miR-39 was spike-in into the serum before RNA isolation and used as an internal control. Total miRNA was converted into cDNA using the TaqMan MicroRNA Reverse Transcription kit (Thermo Fisher Scientific), and both hsa-miR-21-5p and cel-miR-39 serum levels were quantified by qPCR using TaqMan Universal Master Mix II, no UNG, and TaqMan MicroRNA assay hsa-miR-21-5p and cel-miR-39 (Thermo Fisher Scientific), all according to the manufacturer's instructions. The relative amounts of hsa-miR-21-5p were determined in duplicates using the threshold cycle ($2^{-\Delta\Delta Ct}$) method and normalized to cel-miR-39 expression.

2.4. Fecal miR-21-5p extraction and evaluation

Total RNA was extracted from feces using Trizol Reagent (Thermo Fisher Scientific) and converted into cDNA using the TaqMan MicroRNA Reverse Transcription kit (Thermo Fisher Scientific), all according to the manufacturer's instructions. Due to the lack of a proper miRNA control, four random samples from each group were used to search for a miR that could serve as a control. Using TaqMan™ Advanced miRNA Human Endogenous Controls Card (Thermo Fisher Scientific) and NormFinder software (MOMA), miR-320a-3p was selected as the best fit control miRNA. TaqMan Universal Master Mix II, no UNG, and TaqMan MicroRNA assay hsa-miR-21-5p and hsa-miR-320a-3p (Thermo Fisher Scientific) were used for qPCR. The relative amounts of hsa-miR-21-5p were determined in duplicates using the threshold cycle ($2^{-\Delta\Delta Ct}$) method and normalized to hsa-miR-320a-3p expression.

2.5. Bacterial 16S sequencing and analysis

Bacterial DNA was extracted from patient fecal samples using a QIAamp PowerFecal Pro DNA Kit (Qiagen), following the manufacturer's instructions. The gut microbiota composition of each sample was determined by sequencing the V4 region of the 16S rRNA gene (primers 515F-806R) using a 280-multiplex approach on a 2x250bp PE MiSeq run (Illumina, Inc.) and analyzed with QIIME2 software (Bokulich et al.,

2018; Caporaso et al., 2010). Demultiplexed paired-end reads (fastq files) were denoised with DADA2 (Bolyen et al., 2019). Taxonomy assignment of ASVs (amplicon sequence variant) was done against the Silva 138 database using Classify-sklearn (Ii et al., 2021). ASVs sequences were aligned, and phylogenetically uninformative positions were masked, before creating a maximum-likelihood phylogenetic tree with FastTree. For all analyses, samples were rarefied to the minimum coverage. Beta diversity analysis was performed to evaluate dissimilarities in microbial communities between groups using Bray–Curtis distances (quantitative community dissimilarity). Distances matrices were then clustered using principal coordinate analysis (PCoA) and PERMANOVA to test for differences between groups. MAASLIN2 analysis was performed in Rstudio for the identification of microbial associations either for microbial signatures as for the predicted bacterial metabolic pathways (Mallick et al., 2021).

2.6. MiR-21-5p online database analysis

The UALCAN database, the portal for tumor gene expression analysis (Chandrashekar et al., 2022; Chandrashekar et al., 2017), was used to assess the expression of miR-21-5p in cholangiocarcinoma and in colon adenocarcinoma.

2.7. Gene expression analysis

Total RNA was extracted from left and right colon tissue using Trizol Reagent (Thermo Fisher Scientific) and converted into cDNA using NZY Reverse Transcriptase (NZYTech), all according to the manufacturer's instructions. qPCR was performed in a QuantStudio™ 7 Flex Real-Time PCR System (Thermo Fisher Scientific). Primer sequences are listed in Table S1. Two independent reactions for each primer set were performed in a total volume of 12.5 μL containing 2× sensiFAST SYBR Hi-ROX kit (Bioline) and 0.6 μM of each primer (Stabvida). The relative amounts of each gene were calculated based on $2^{-\Delta\Delta Ct}$ method normalized to the level of β-actin and expressed as fold change from the control group.

2.8. HCT116 culture and in vitro assays

HCT116 cells were grown in McCoy's 5 A medium supplemented with 10 % of fetal bovine serum and 1 % of antibiotic-antimycotic (all from Gibco, Thermo Fisher Scientific), and kept at 37 °C, 5 % CO_2. To test the direct effect of miR-21, HCT116 cells were cultured for in a 12-well plate with an initial seeding density of 1×10^5 cells per well. After 48 h of plating, cells were transfected with 15 pmoL/well miR-21-5p mimic (Thermo Fisher Scientific) using Lipofectamine 3000 transfection kit (Invitrogen) following the manufacturer's instructions and exposed to either 20 ng/mL human-TNF-α (Peprotech) or 100 ng/mL of LPS (Thermo Fisher Scientific), as a pro-inflammatory stimulus. After 24 h of incubation, cells were recovered, total RNA extracted, and the gene expression analysis performed as previously described.

2.9. H69 cholangiocytes culture and in vitro assays

Non-tumoral SV40-immortalized human bile duct epithelial cells H69 cells were cultured in collagen-coated plates in fully supplemented DMEM/F-12 (Gibco) medium at 37 °C in a 5 % CO_2 environment, as previously described (Banales et al., 2012; Erice et al., 2018). Briefly, 3.0×10^5 cells were seeded in thin collagen-coated 6-well plates and incubated overnight. In the next day, after attachment, H69 cells were incubated with either 20 ng/mL human-TNF-α (Peprotech) or 100 ng/mL of LPS (Thermo Fisher Scientific) (Thermo Fisher Scientific) or vehicle control (PBS 1×) for 24 h in FBS-depleted fully supplemented DMEM/F-12 medium. Cell supernatant was then collected to proceed with extracellular vesicles isolation, while cells were harvested and processed for miRNA isolation.

2.10. Exosome isolation and RNA extraction

H69 culture media was harvested and centrifuged at 2000 ×*g* for 30 min to remove cells and debris. Then the supernatant was collected. To extract the exosome medium content, 1 mL of cell-free culture media was transferred to a new tube containing 500 μL of the Total Exosome Isolation reagent (Thermo Fisher Scientific). Samples were incubated at 4 °C overnight, and then centrifuged at 10,000 ×*g* for 1 h, at 4 °C. The resulting pellet was resuspended in 50 μL of 1× PBS and stored at −80 °C. For RNA extraction, Trizol Reagent (Thermo Fisher Scientific) was used as previously described.

2.11. Primary cell isolation and culture conditions

Human monocytes were obtained by isolating $CD14^+$ monocytes from buffy coats of healthy blood donors provided by the national blood institute (Instituto Português do Sangue e da Transplantação, Lisbon, Portugal) using the MojoSort magnetic cell separation system (Biolegend) and following a previously described procedure with minor alterations (Pires et al., 2021). Briefly, peripheral blood mononuclear cells were first isolated by density gradient centrifugation with Ficoll-Paque Plus (Cytiva), and then treated with Human TruStain FcX (Biolegend) and incubated with CD14-specific magnetic beads (Biolegend). $CD14^+$ monocytes bound to the beads were magnetically recovered and cultivated in Roswell Park Memorial Institute (RPMI)-1640 medium with 10 % (*v*/v) fetal bovine serum, 1 mM of sodium pyruvate, 10 mM of HEPES and 0.1 % β-mercaptoethanol (all from Gibco). To induce monocyte differentiation, 20 ng/mL recombinant human M-CSF (Thermo Fisher Scientific) was added to the medium to obtain macrophages. To determine the impact of miR-21-5p in monocyte differentiation, cells were transfected with miR-21-5p mimic or non-targeting miRNA mimic control (Thermo Fisher Scientific) on days 0, 3 and 6 post-isolation. Fresh cell culture medium was added every 3 days until day 6 post-isolation. On day 6, cells were harvested and targeted gene expression was evaluated by qPCR.

2.12. Statistical analysis

Statistical analysis was performed with GraphPad Prism version 9 software (La Jolla). The ROUT (Q = 1 %) method was used to identify outliers. D'Agostino & Pearson test normality and Lognormality test were used to evaluate sample distribution for each analysis performed. According to data normality distribution, differences between two groups were assessed with *t*-test or Mann-Whitney *U* test. Differences between three or more groups were evaluated using Kruskal-Wallis or one-way analysis of variance (ANOVA), followed by Dunn's or Tukey multiple comparison tests, respectively. A p value <0.05 was considered statistically significant. Results were presented as mean ± standard error of the mean (SEM).

3. Results

3.1. Patient characterization

From 2020 to 2023 a total of 34 individuals were recruited from Hospital Beatriz Ângelo (Loures), ULS Santa Maria (Lisboa), Hospital Lusíadas (Lisboa), and Hospital da Luz (Lisboa), from Portugal. This pilot study grouped 14 PSC-IBD patients with concomitant IBD and 20 controls without diagnosed PSC-IBD and/or IBD, detailed in Table 1. Among the PSC-IBD group, 8 had UC, 5 had CD, and 1 had undetermined IBD. The control group consisted of patients that underwent colonoscopy for evaluation of gastro-intestinal symptoms or for colorectal screening, and in whom no evidence of macroscopic or microscopic inflammation was found. Three PSC-IBD patients had cirrhosis at recruitment and active disease was documented in 4 PSC-IBD patients. No colorectal neoplasia was detected in either group. The median IBD

Table 1
Clinical characteristics of participants included in PSC-IBD and control groups.

Characteristics	PSC-IBD (n = 14)	Control (n = 20)	*p-value*
Age (median, IQR)	42 (38–47)	62 (42–67)	0.0333
Male sex, n (%)	9 (56)	7 (35)	0.2023
IBD, n (%)			
CD	5 (31)		
UC	8 (50)	NA	
Unclassified	1 (6)		
Calprotectin (median, IQR)	321 (99–746)	45 (39–205)	0.0196
Disease duration (PSC-IBD), y (median, IQR)	9 (5–12)		
Disease duration (IBD), y (median, IQR)	14 (9–17)		
Treatment, n (%)			
5-ASA	9 (56)		
Steroids	2 (13)		
IMM (Tracrolimus, MMF, AZA, or MTX)	3 (19)		
Biologic (IFX, ADA, or UST)	2 (13)		
Endoscopic inflammation at colonoscopy, n (%)		NA	
Terminal ileum	4 (25)		
Right colon	6 (38)		
Left colon	3 (19)		
UDCA, n (%)			
Permanent	3 (21)		
Stopped 15 days before colonoscopy	7 (44)		
No treatment	4 (29)		
Cirrhosis, n (%)	3 (19)		
History of liver transplant, n (%)	2 (13)		

CD, Crohn's disease; IBD, inflammatory bowel disease; 5-ASA, 5-aminosalicylic acid; IMM, immunomodulator; MMF, mycophenolate mofetil; AZA, azathioprine; MTX, methotrexate; IFX, infliximab; ADA, adalimumab; UST, ustekinumab; IQR, interquartil range; PSC-IBD, primary sclerosing cholangitis; UC, ulcerative colitis; UDCA – ursodeoxycholic acid.

duration in PSC-IBD was 14 years (IQR 9–17). PSC-IBD patients were treated with 5-ASA (56 %), steroids (13 %), IMM (19 %), or biologics (13 %), with 3 patients being treated with more than one specific treatment. As expected, fecal calprotectin levels were significantly elevated in PSC-IBD patients (median 321 ng/μL, IQR 99–746) when compared to controls ($p = 0.0196$).

3.2. miR-21-5p is increased in PSC-IBD patients

According to the UALCAN tumor gene expression portal, miR-21-5p is overexpressed in both cholangiocarcinoma and colon cancer ($p < 0.0001$) compared to non-tumoral surrounding tissue (Fig. 1A). In PSC-IBD group, miR-21-5p was upregulated in the right colon ($p = 0.0087$), with no differences observed in the left colon compared to controls (Fig. 1B). Circulating levels of miR-21-5p were significantly increased in both fecal ($p = 0.0374$) and serum ($p = 0.0108$) samples form PSC-IBD patients compared to controls (Fig. 1C, D). These findings further highlight miR-21-5p as a potential modulator of the disease phenotype in PSC-IBD.

3.3. Altered gut microbiota and predicted changes in microbial metabolic pathways in PSC-IBD patients

In a previous mouse model of elevated liver bile acids, we demonstrated an association between increased miR-21-5p levels and gut microbial dysbiosis (Santos et al., 2020). In the present cohort, gut microbiota composition analysis revealed a significant separation between PSC-IBD patients and controls in the unweighted UniFrac analysis ($p = 0.001$) (Fig. 2A). This was accompanied by a significant reduction in alpha diversity, as indicated by decreased Faith's phylogenetic diversity ($p = 0.0002$) and observed features ($p = 0.0003$) in PSC-IBD patients compared to controls (Fig. 2B).

Further analysis of microbial composition revealed a significant increase in the *Bacteroidota/Bacillota* (formerly *Bacteroidetes/Firmicutes*) ratio ($p = 0.0111$) (Fig. 2C, D), along with a decrease in the relative abundance of Gram-positive bacteria (Fig. 2E, F).

Functional pathway prediction using PICRUSt2 indicated that microbial dysbiosis was linked to significant alterations in key metabolic pathways. In particular, reductions were observed in pathways involved in amino acid metabolism (ARGSYN.PWY, ILEUDEG.PWY, and TRYPTOPHAN metabolism), short-chain fatty acid production (CENTBENZCOA.PWY), polyamine biosynthesis (PUTRESCINE and POLYAMINE biosynthesis), NAD biosynthesis (NADSYN.PWY), and phenolic compound degradation (PROTOCATECHUATE.ORTHO. CLEAVAGE.PWY and GALLATE degradation) were identified (Fig. 2G).

3.4. Pro-oncogenic and proinflammatory colonic markers in PSC-IBD patients

Given the observed dysbiotic effects of the gut microbiota, a panel of colonic tissue damage markers, including *MUC2* and *LGR5,* was evaluated. While *MUC2* mRNA expression showed no significant alterations, the intestinal stem cell marker *LGR5* was significantly upregulated in PSC-IBD group compared to controls ($p = 0.0365$) (Fig. 3A). As *LGR5* overexpression has been associated with colon cancer, additional markers of tumor evasion, epithelial-mesenchymal transition (EMT), and immune response were examined.

EMT-related transcription factors *TWIST1* and *SNAIL* were significantly elevated in PSC-IBD group ($p = 0.0316$ and $p = 0.0159$, respectively) (Fig. 3B). Markers associated with the inflammatory tumor

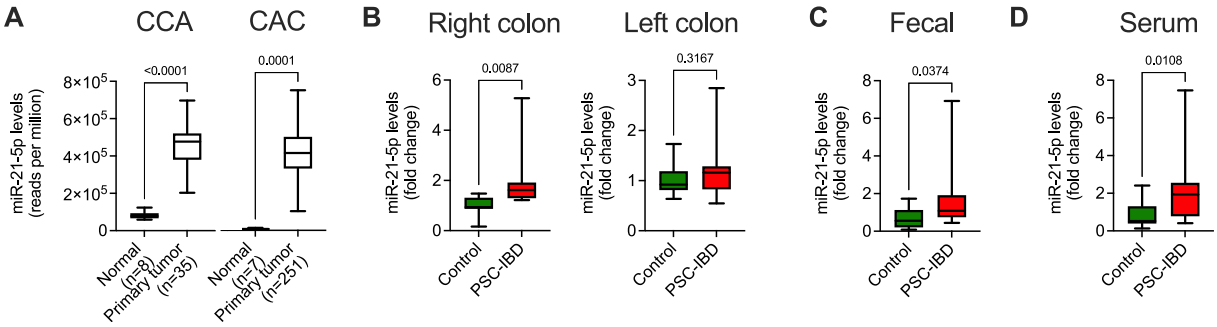

Fig. 1. PSC-IBD patients show a unique miR-21-5p profile in serum, feces and colon tissue.
A) Publicly available TCGA data analyzed *via* the UALCAN portal demonstrate significantly elevated miR-21-5p expression in tumor tissues compared to adjacent non-tumor tissues in both cholangiocarcinoma (CCA) and colon adenocarcinoma (CAC). B) Right and left colonic tissues miR-21-5p expression levels normalized to snRNA U6. C) Fecal miR-21-5p expression, normalized to miR-320a-3p. D) Serum miR-21-5p expression, normalized to spike-in *C. elegans* miR-39a. Results are presented as fold change, with mean values and error bars representing ± SEM.

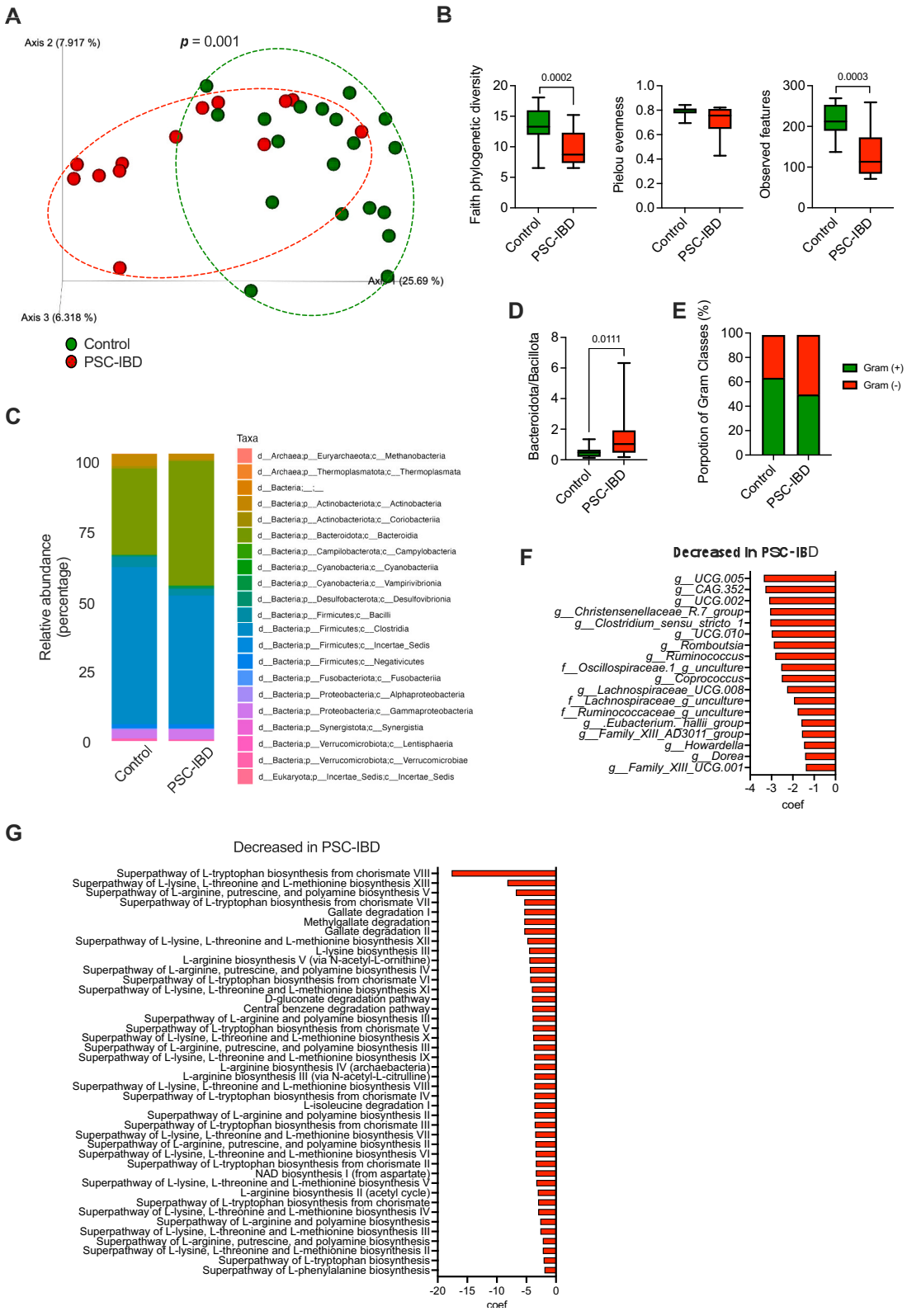

Fig. 2. Fecal microbiota analysis in PSC-IBD patients reveals a dysbiotic profile characterized by a reduction in beneficial Gram-positive bacteria.
A) Principal coordinate analysis (PCoA) of beta diversity based on unweighted UniFrac distances (ellipses are included for visual guidance). B) Alpha diversity metrics, including Faith phylogenetic diversity, Pielou evenness, and Observed features. C) Taxonomic *Class* stacked bar-plot comparing Control and PSC-IBD groups. D) *Bacteroidota/Bacillota* phylum ratio. E) Proportion of Gram posivite (+) and Gram negative (−) Bacterial per group. F) MAASLIN2 analysis highlighting the most significantly altered bacteria taxa in PSC-IBD *versus* Control groups. G) Picrust2 metabolic pathway prediction using MAASLIN2, identifying key microbial pathways altered in PSC-IBD. Results are presented as fold change, with mean values and error bars representing ± SEM.

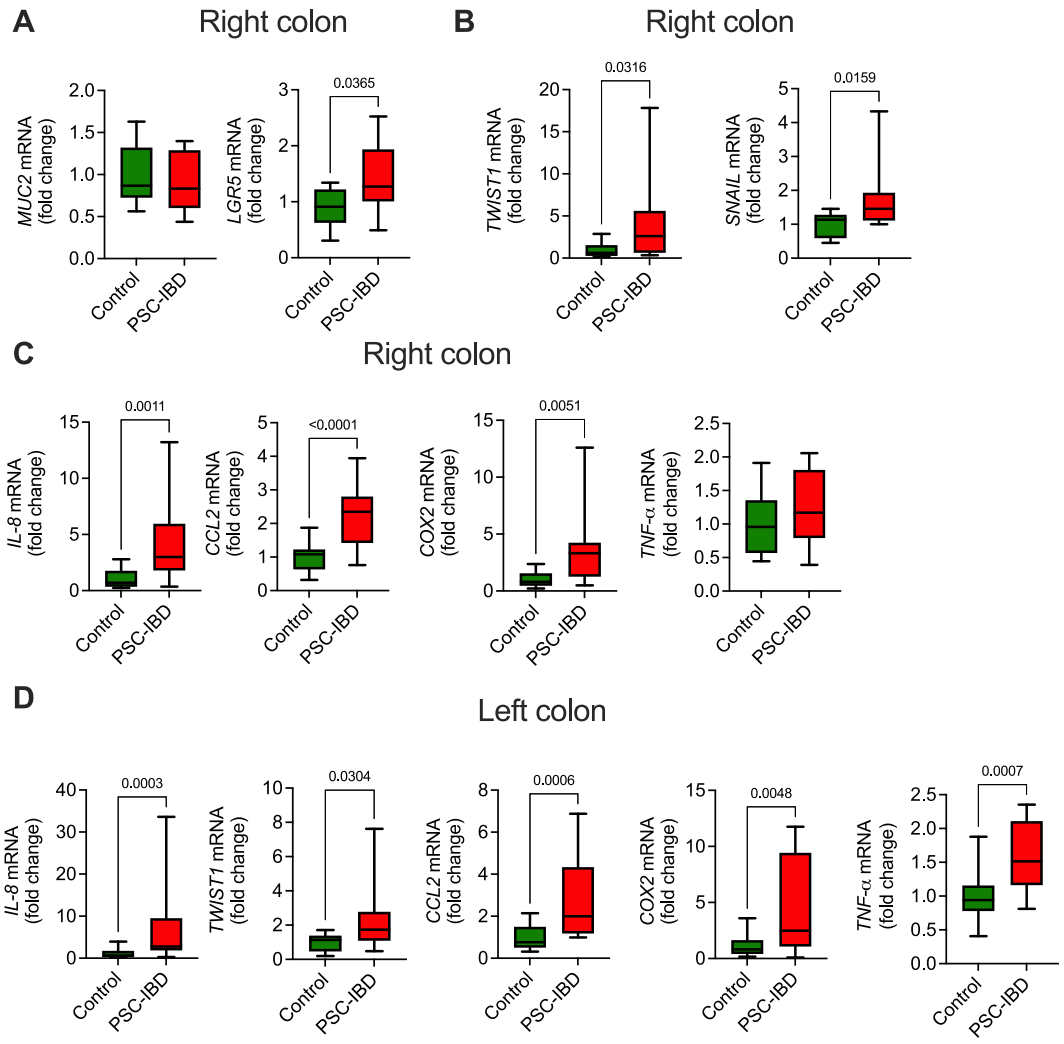

Fig. 3. Altered colon immune system response in PSC-IBD patients.
A) mRNA expression of intestinal integrity markers *MUC2* and *LGR5* in the right colon. B) mRNA expression of epithelial-mesenchymal transition markers *TWIST1* and *SNAIL* in the right colon. C) mRNA expression of immune-related markers *IL-8*, *CCL2*, *COX2,* and *TNF-α* in the right colon. D) mRNA expression of *IL-8*, *TWIST1*, *CCL2*, and *COX2,* and *TNF-α* in the left colon. qPCR results are presented in fold change, with mean values and error bars representing ± SEM.

microenvironment, including *IL-8*, *CCL2*, and *COX2,* showed increased expression in the right colon of PSC-IBD patients compared to controls ($p = 0.0011$, $p < 0.0001$, and $p = 0.0051$, respectively) (Fig. 3C). Similarly, in the left colon, significant increases in *IL-8, TWIST1, CCL2, COX2*, and *TNF-α* ($p = 0.0003$, $p = 0.0304$, $p = 0.0006$, $p = 0.0048$, and $p = 0.0007$, respectively) (Fig. 3D). These findings highlight the upregulation of pro-oncogenic and pro-inflammatory markers in PSC-IBD colonic tissues, reinforcing the elevated risk for colon cancer in PSC-IBD patients (Trivedi et al., 2020).

3.5. *miR-21 drives pro-tumorigenic environmental markers in in vitro models*

Given the increase in circulating miR-21-5p in PSC-IBD patients, *in vitro* studies were performed using human immortalized cholangiocytes H69, CRC cells HCT116, and primary monocytes isolated from blood. LPS or TNF-α exposure were used to mimic inflammatory responses induced by host or bacterial mediators. Interestingly, H69 exposure to LPS significantly increased miR-21-5p levels in exosome content ($p = 0.0003$; Fig. 4A), whereas no effect was observed under TNF-α exposure. In HCT116 cells, transfection with a miR-21 mimic resulted in a trend toward increased *IL-8* and *TWIST1* expression in the absence of stimuli. This effect was potentiated after incubation with TNF-α, leading to a significant upregulation of *IL-8, TWIST1,* and *CCL2* ($p = 0.0027$, $p = 0.0333$ and $p < 0.0001$, respectively; Fig. 4B). Similar effect was observed in differentiated monocytes transfected with miR-21-5p, which exhibited significantly increased expression of *IL-8* ($p = 0.0268$), *TWIST1* ($p = 0.0305$), and *CCL2* ($p = 0.0059$ (Fig. 4C). These findings underscore the role of miR-21-5p in promoting a pro-tumorigenic and inflammatory microenvironment by modulating key inflammatory and EMT-related markers across multiple cell types.

4. Discussion

In this study, we provide novel insights into the pathophysiology of PSC with concomitant IBD, emphasizing the role of miR-21-5p, gut microbiota dysbiosis, and pro-tumorigenic colonic alterations. Our findings shed light in the complex interplay between inflammatory mediators, microbial communities, and oncogenic pathways in PSC-IBD.

Our results indicate that miR-21-5p is significantly upregulated in the right colon, as well as in fecal and serum samples of PSC-IBD patients. This is consistent with prior reports identifying miR-21-5p as a critical regulator of inflammatory and oncogenic pathways in IBD and PSC (Jenike and Halushka, 2021; Peng et al., 2017). The differential expression of miR-21-5p between colonic segments suggests region-specific alterations, which may contribute to the variable neoplastic

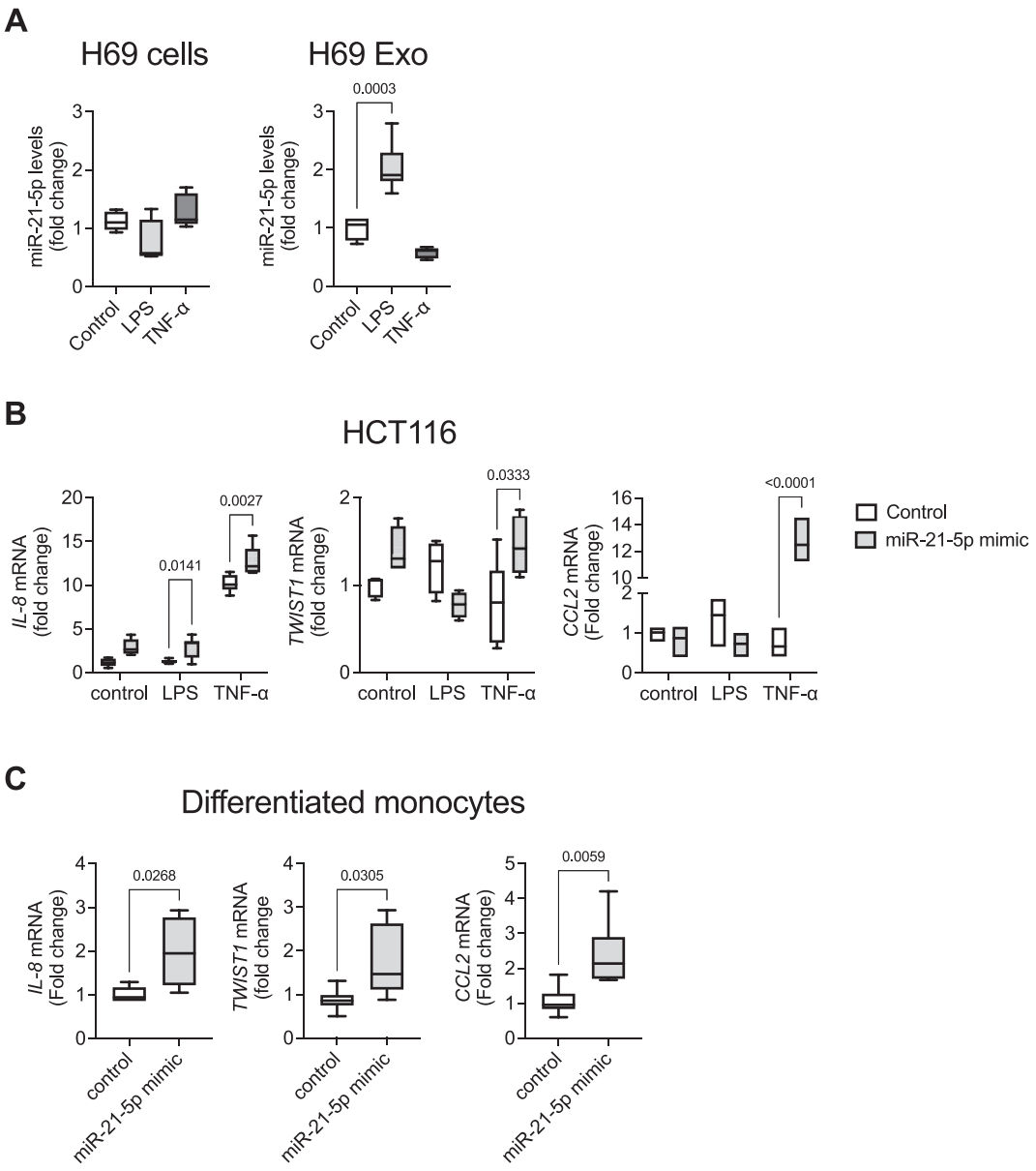

Fig. 4. miR-21-5p *in vitro* incubation partially recapitulates the PSC-IBD profile.
A) miR-21 expression in cell and exosome extracts from non-tumoral H69 cholangiocytes incubated with lipopolysaccharide (LPS; 100 ng/mL), TNF-α (20 ng/mL), or vehicle control for 24 h. B) mRNA expression analysis of *IL-8*, *TWIST1*, and *CCL2* in HCT116 colorectal cancer cells transfected with miR-21-5p mimic (15 pmoL/well) and exposed to TNF-α (20 ng/mL) or LPS (100 ng/mL). C) Expression of *IL-8*, *TWIST1*, and *CCL2* in differentiated monocytes transfected with miR-21-5p mimic or non-targeting miRNA control. Results are presented as fold change, with mean values and error bars representing ± SEM.

potential observed in PSC-IBD patients (Cordes et al., 2019; Zhang et al., 2022).

Fecal miRNAs have been implicated in modulating gut microbiota dynamics (Liu et al., 2016). Notably, we have previously demonstrated that miR-21-5p is associated with gut microbiota dysbiosis in a liver bile duct ligation model (Santos et al., 2020). Alterations in gut microbiota composition further strengthen the evidence for the involvement of microbial dysbiosis in PSC-IBD. In the present study, we observed a significant shift in microbial diversity, characterized by an increased Bacteroidota/Bacillota ratio and a relative depletion of Gram-positive bacteria, suggesting that gut microbiota alterations may have significant metabolic consequences, potentially contributing to the gut inflammation phenotype observed in PSC-IBD patients This aligns with findings from a miR-21KO mouse model, where miR-21-5p depletion led to an enrichment of *Bacillota* species (Johnston et al., 2018).

Importantly, PSC-IBD patients exhibited a reduction in bacteria involved in secondary bile acid metabolism, such as *Clostridium sensu stricto 1* (Li et al., 2020), *Ruminococcaceae UCG-002* (Hu et al., 2021), and *Christensenellaceae_R7_group* (Liu et al., 2024). Moreover, functional prediction further revealed a decline in key metabolic processes crucial for maintaining intestinal homeostasis, such as amino acid metabolism and short-chain fatty acid production (Gonçalves et al., 2018; Hole et al., 2023). The alterations likely contribute to the chronic inflammatory state observed in PSC-IBD, promoting epithelial dysfunction and pro-oncogenic signaling.

Our analysis of colonic tissue markers revealed a significant upregulation of *LGR5*, a stem cell marker associated with colon cancer (Morgan et al., 2018). The elevated expression of EMT-related transcription factors *TWIST1* and *SNAIL*, as well as inflammatory cytokines *IL-8*, *CCL2*, and *COX2*, further supports the hypothesis that PSC-associated colonic inflammation creates a microenvironment conducive to neoplastic transformation. Notably, increased *IL-8* expression has

previously been associated with *TWIST1* and *CCL2* upregulation during tumor development (Ha et al., 2017).

We had previous shown in an animal model of bile acid accumulation that gut microbiota dysbiosis and gut permeabilization were associated to an increase in miR-21-5p (Santos et al., 2020). Here, our experimental studies demonstrated that upon LPS stimulation, cholangiocytes preferentially secreted miR-21-enriched exosomes without a corresponding increase in total cellular miR-21 expression. These findings suggest that, under inflammatory conditions, cholangiocytes may selectively release miR-21-5p *via* exosomes, potentially affecting distant organs. Interestingly, exosomal miR-21-5p has been previously implicated in cancer progression in both the liver and colon (He et al., 2021; Zhou et al., 2018). Further *in vitro* studies using differentiated monocytes and CRC cells, reinforced the pro-tumorigenic role of miR-21-5p, as its transfection resulted in upregulation of key inflammatory markers, including *IL-8*, *TWIST1*, and *CCL2*. Although a miRBase search for hsa-miR-21-5p targets did not reveal a direct interaction with these markers, previous studies indicate that miR-21-5p inhibits PDCD4, a known regulator of TWIST1 expression (Luo et al., 2015). Also, *miR-21-5p* overexpression has been associated with increased *IL-8* levels (Pace et al., 2019), which in turn is targeted by *TWIST1* to promote cell-autonomous invasion (Li et al., 2012). Finally, a potential link between miR-21-5p and *CCL2* has been suggested in neuronal models (Zeboudj et al., 2023).

Despite the valuable insights provided by this study, several limitations must be acknowledged. First, the heterogeneity within the PSC-IBD group, combined with the relatively small cohort size, may limit the generalizability of our findings and preclude meaningful stratification by sex, age, treatment status, or IBD activity, underscoring the need for validation in a larger, independent cohort. Second, our analysis focused on a limited panel of tumor-related markers, which may not fully capture the full spectrum of oncogenic alterations in PSC-IBD-associated colonic tissues. Expanding the panel to include additional biomarkers could provide a more comprehensive understanding of disease progression. Third, our study lacks deeper molecular insights into the mechanisms underlying miR-21-5p-mediated effects. Lastly, it was already reported that UDCA does not contribute to significantly alter the gut microbiota of PSC-IBD patients (Sabino et al., 2016). Nevertheless, due to small cohort size we were unable to detect the influence of UDCA treatment either in gut microbiota or in miR-21-5p serum or fecal expression. Increasing the cohort and evaluating the effects of UDCA on miR-21-5p levels together in a longitudinal study would be crucial to understand their effects on the progression to CRC of patients with PSC-IBD. Future studies employing transcriptomic approaches, such as RNA sequencing, could help identify other miRNAs and signaling pathways involved in gut dysfunction, identifying novel therapeutic targets.

miR-21-5p exerts cell-specific functions that reflect its broad regulatory network. In cholangiocytes, miR-21 has been associated with enhanced fibrogenic and proliferative responses through suppression of PTEN and activation of the PI3K/AKT pathway, contributing to cholangiopathy and biliary remodeling (Chawra et al., 2024). In colorectal epithelial and cancer cells, miR-21 promotes tumorigenesis by downregulating PDCD4 and RECK, facilitating invasion, angiogenesis, and resistance to apoptosis (Asangani et al., 2008; Qin and Luo, 2014). In monocytes and macrophages, miR-21 acts as a key immunomodulator, dampening pro-inflammatory responses through inhibition of TLR4/NF-κB signaling and IL-12 production, but paradoxically promoting a tumor-supportive, M2-like phenotype in chronic inflammation (An and Yang, 2020; Sheedy et al., 2010).

Supporting these pleiotropic effects, our miRTARGET database analysis (Supplementary Fig. S1) identified over 24,000 potential gene targets of miR-21-5p across multiple signaling pathways, including those involved in inflammation, fibrosis, and oncogenesis. This extensive target landscape underscores the complex and context-dependent regulatory role of miR-21, consistent with our findings in PSC-IBD, where chronic inflammatory and dysbiotic stimuli may drive its upregulation and link immune dysregulation to pro-oncogenic signaling.

Collectively, our findings highlight the complex interplay between miR-21-5p dysregulation, gut microbial imbalances, and inflammatory reprogramming in PSC-IBD pathogenesis. Our results provide evidence that cholangiocyte damage, aggravated by bacterial dysbiosis and increased LPS production, may lead to secretion of extracellular vesicles containing miR-21-5p, which in turn may act as a key modulator of disease phenotype. This process not only influences colonic inflammation and oncogenic potential but also affects peripheral immune system differentiation. Given the elevated expression of pro-oncogenic and pro-inflammatory markers identified in this study, close surveillance strategies for PSC-IBD patients are warranted to mitigate their heightened risk for colon neoplasia.

Mechanistically, the up-regulation of EMT drivers such as TWIST1 and SNAIL1 in PSC-IBD may reflect a shift toward epithelial plasticity, mediated by AKT/β-catenin and NF-κB signaling (*e.g.*, as described in CRC models) (Oh et al., 2016). Concurrently, heightened IL-8 expression supports angiogenesis and neutrophil-driven microenvironmental remodeling in colonic epithelium (Lee et al., 2012). The elevated CCL2/CCR2 axis likely drives recruitment of monocytes/macrophages that further support epithelial transformation and immune suppression in the mucosa (Ozga et al., 2021). Finally, increased COX2 supports pro-survival prostaglandin signaling, epithelial proliferation and dysplasia from the chronic inflammatory (Yang et al., 2025). Together, the EMT-activation, chemokine/immune-cell recruitment and prostaglandin-driven proliferation provides a plausible mechanistic framework linking miR-21-5p with increased neoplasia risk in PSC-IBD.

The pathological progression of PSC-IBD likely involves the interplay of bile acid dysregulation, chronic inflammation, and activation of oncogenic and epithelial–mesenchymal transition (EMT) pathways. We have recently shown that, under PSC-IBD conditions, circulating primary bile acids, particularly glycochenodeoxycholic acid (GCDCA), modulate peripheral immune responses, contributing to intestinal inflammation and potentially promoting cancer progression (Santos et al., 2025). In the present study, we further observed that circulating miR-21-5p levels were increased in PSC-IBD, contributing also to similar tissue activation of oncogenic and EMT modulation. Thus, suggesting a model in which bile acid–driven immune modulation and miR-21-5p overexpression converge on EMT and inflammatory mediators to promote epithelial remodeling and neoplastic susceptibility in PSC-IBD.

Although miR-21-5p and gut microbiota dysbiosis represent attractive therapeutic targets, their direct modulation in PSC-IBD remains speculative. miR-21-5p inhibition has shown anti-inflammatory and anti-fibrotic effects in experimental models of liver injury and colitis, while microbiota-directed interventions such as probiotics, antibiotics, or fecal microbiota transplantation have demonstrated variable efficacy in conventional IBD (Hou et al., 2025). However, the complex interplay between bile acid signaling, immune regulation, and miRNA networks in PSC-IBD suggests that interventions targeting a single pathway are unlikely to be sufficient. Our pilot data reinforce that PSC-IBD should be considered a distinct clinical entity characterized by simultaneous dysregulation of miRNAs, immune mediators, and microbial composition. Further studies in larger, longitudinal cohorts are needed to evaluate whether modulation of miR-21-5p or gut microbiota could translate into reduced colorectal cancer risk in these patients.

Future research should aim to elucidate the mechanistic underpinnings of miR-21-5p-mediated oncogenesis and explore targeted strategies to counteract its pathogenic effects. Moreover, larger cohort studies are essential to validate our findings and assess the translational potential of miR-21-5p as a modulator of disease progression and malignancy risk in PSC-IBD.

CRediT authorship contribution statement

André Anastácio Santos: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition,

Formal analysis, Data curation, Conceptualization. **David Pires:** Writing – review & editing, Investigation, Formal analysis. **Vanda Marques:** Writing – review & editing, Formal analysis. **Nicole Alesina:** Formal analysis. **Pedro Miguel Rodrigues:** Writing – review & editing, Funding acquisition, Formal analysis. **Ana Catarina Bravo:** Resources, Methodology, Formal analysis. **Catarina Gouveia:** Resources, Methodology, Investigation, Formal analysis. **Susana Saraiva:** Project administration, Methodology. **Luís Correia:** Investigation. **Ricardo Crespo:** Investigation. **João Pereira da Silva:** Investigation. **Marília Cravo:** Investigation. **Jesus Maria Banales:** Writing – review & editing, Funding acquisition. **Joana Torres:** Writing – review & editing, Validation, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Cecília Maria Pereira Rodrigues:** Writing – review & editing, Supervision.

Ethics approval

This study was approved by the Ethics Committee of Beatriz Ângelo Hospital (Ref. 0028/2014_RMEB) and conducted in accordance with the ethical principles of the Declaration of Helsinki and Portuguese law (Decree-Law No. 43/2004 of August 19, DR I Série).

Funding

This work was supported by Fundação para a Ciência a Tecnologia (FCT) CEECIND/04663/2017, GEDII Project Award 2019, and EASL Daniel Alagille Award 2019 (to AAS). Additional funding was from Spanish Carlos III Health Institute (ISCIII) [FIS PI21/00922, PI18/01075, and PI23/01850, and Miguel Servet CPII19/00008 cofinanced by "Fondo Europeo de Desarrollo Regional" (FEDER) and Miguel Servet Program CP22/00073]; Sara Borrell CD19/00254; Núcleo de Gastroenterologia dos Hospitais Distritais (to JT and CG); "Fundación Científica de la Asociación Española Contra el Cáncer" ("AECC Lab call 2023" LA-BAE235286RODR to PMR).

Declaration of competing interest

On behalf of all authors, I declare that there are no conflicts of interest related to the manuscript entitled "miR-21-5p Dysregulation is Associated with Gut Microbiota Dysbiosis and Pro-oncogenic Markers in Primary Sclerosing Cholangitis with concomitant Inflammatory Bowel Disease", submitted to Experimental and Molecular Pathology for consideration. We confirm that none of the authors have any financial, personal, or professional relationships that could influence or appear to influence the work reported in this manuscript.

Should any conflicts arise in the future, we will promptly disclose them in accordance with the journal's policies.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yexmp.2025.105013.

Data availability

Data will be made available on request.

References

Afonso, M.B., Rodrigues, P.M., Simão, A.L., Gaspar, M.M., Carvalho, T., Borralho, P., Bañales, J.M., Castro, R.E., Rodrigues, C.M.P., 2018. miRNA-21 ablation protects against liver injury and necroptosis in cholestasis. Cell Death Differ. 25, 857–872. https://doi.org/10.1038/s41418-017-0019-x.

An, Y., Yang, Q., 2020. MiR-21 modulates the polarization of macrophages and increases the effects of M2 macrophages on promoting the chemoresistance of ovarian cancer. Life Sci. 242, 117162. https://doi.org/10.1016/j.lfs.2019.117162.

Ananthakrishnan, A.N., Bernstein, C.N., Iliopoulos, D., Macpherson, A., Neurath, M.F., Ali, R.A.R., Vavricka, S.R., Fiocchi, C., 2018. Environmental triggers in IBD: a review of progress and evidence. Nat. Rev. Gastroenterol. Hepatol. 15, 39–49. https://doi.org/10.1038/nrgastro.2017.136.

Asangani, I.A., Rasheed, S.A.K., Nikolova, D.A., Leupold, J.H., Colburn, N.H., Post, S., Allgayer, H., 2008. MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. Oncogene 27, 2128–2136. https://doi.org/10.1038/sj.onc.1210856.

Banales, J.M., Sáez, E., Úriz, M., Sarvide, S., Urribarri, A.D., Splinter, P., Tietz Bogert, P. S., Bujanda, L., Prieto, J., Medina, J.F., LaRusso, N.F., 2012. Up-regulation of microRNA 506 leads to decreased cl−/HCO3− anion exchanger 2 expression in biliary epithelium of patients with primary biliary cirrhosis. Hepatology 56, 687–697. https://doi.org/10.1002/hep.25691.

Bautista-Sánchez, D., Arriaga-Canon, C., Pedroza-Torres, A., De La Rosa-Velázquez, I.A., González-Barrios, R., Contreras-Espinosa, L., Montiel-Manríquez, R., Castro-Hernández, C., Fragoso-Ontiveros, V., Álvarez-Gómez, R.M., Herrera, L.A., 2020. The promising role of miR-21 as a Cancer biomarker and its importance in RNA-based therapeutics. Molecular Therapy - Nucleic Acids 20, 409–420. https://doi.org/10.1016/j.omtn.2020.03.003.

Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., Huttley, G. A., Gregory Caporaso, J., 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 6, 90. https://doi.org/10.1186/s40168-018-0470-z.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E.K., Da Silva, R., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons, S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang, K.B., Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M.G.I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., van der Hooft, J.J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K. C., Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J.G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857. https://doi.org/10.1038/s41587-019-0209-9.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336. https://doi.org/10.1038/nmeth.f.303.

Chandrashekar, D.S., Bashel, B., Balasubramanya, S.A.H., Creighton, C.J., Ponce-Rodriguez, I., Chakravarthi, B.V.S.K., Varambally, S., 2017. UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia 19, 649–658. https://doi.org/10.1016/j.neo.2017.05.002.

Chandrashekar, D.S., Karthikeyan, S.K., Korla, P.K., Patel, H., Shovon, A.R., Athar, M., Netto, G.J., Qin, Z.S., Kumar, S., Manne, U., Creighton, C.J., Varambally, S., 2022. UALCAN: An update to the integrated cancer data analysis platform. Neoplasia 25, 18–27. https://doi.org/10.1016/j.neo.2022.01.001.

Chawra, H.S., Agarwal, M., Mishra, A., Chandel, S.S., Singh, R.P., Dubey, G., Kukreti, N., Singh, M., 2024. MicroRNA-21's role in PTEN suppression and PI3K/AKT activation: implications for cancer biology. Pathology - Research and Practice 254, 155091. https://doi.org/10.1016/j.prp.2024.155091.

Chazouilleres, O., Beuers, U., Bergquist, A., Karlsen, T.H., Levy, C., Samyn, M., Schramm, C., Trauner, M., 2022. EASL clinical practice guidelines on sclerosing cholangitis. J. Hepatol. 77, 761–806. https://doi.org/10.1016/j.jhep.2022.05.011.

Cordes, F., Laumeyer, T., Gerß, J., Brückner, M., Lenze, F., Nowacki, T., Rijcken, E., Tepasse, P., Schmidt, H., Kucharzik, T., Bettenworth, D., 2019. Distinct disease phenotype of ulcerative colitis in patients with coincident primary Sclerosing cholangitis: evidence from a large retrospective study with matched cohorts. Dis. Colon Rectum 62, 1494. https://doi.org/10.1097/DCR.0000000000001496.

D'Amico, F., Allocca, M., Lusetti, F., Parigi, T.L., Rusconi, F., Hernandez, G., Segovia-Hilara, A., Solitano, V., Zilli, A., Furfaro, F., Fiorino, G., Invernizzi, P., Peyrin-Biroulet, L., Jairath, V., Danese, S., 2025. Primary Sclerosing cholangitis worsens prognosis in patients with inflammatory bowel disease: a propensity-matched cohort study. United European Gastroenterol J 00, 1–11. https://doi.org/10.1002/ueg2.70058.

Emek, E., Serin, A., Sahin, T., Yazici, P., Yuzer, Y., Tokat, Y., Bozkurt, B., 2019. Experience in liver transplantation due to primary Sclerosing cholangitis: a single center experience. Transplant. Proc. 51, 2439–2441. https://doi.org/10.1016/j.transproceed.2019.01.156.

Erice, O., Munoz-Garrido, P., Vaquero, J., Perugorria, M.J., Fernandez-Barrena, M.G., Saez, E., Santos-Laso, A., Arbelaiz, A., Jimenez-Agüero, R., Fernandez-Irigoyen, J., Santamaria, E., Torrano, V., Carracedo, A., Ananthanarayanan, M., Marzioni, M., Prieto, J., Beuers, U., Oude Elferink, R.P., LaRusso, N.F., Bujanda, L., Marin, J.J.G., Banales, J.M., 2018. MicroRNA-506 promotes primary biliary cholangitis–like

features in cholangiocytes and immune activation. Hepatology 67, 1420. https://doi.org/10.1002/hep.29533.
Gonçalves, P., Araújo, J.R., Di Santo, J.P., 2018. A cross-talk between microbiota-derived short-chain fatty acids and the host mucosal immune system regulates intestinal homeostasis and inflammatory bowel disease. Inflamm. Bowel Dis. 24, 558–572. https://doi.org/10.1093/ibd/izx029.
Ha, H., Debnath, B., Neamati, N., 2017. Role of the CXCL8-CXCR1/2 Axis in Cancer and inflammatory diseases. Theranostics 7, 1543–1588. https://doi.org/10.7150/thno.15625.
He, Q., Ye, A., Ye, W., Liao, X., Qin, G., Xu, Y., Yin, Y., Luo, H., Yi, M., Xian, L., Zhang, S., Qin, X., Zhu, W., Li, Y., 2021. Cancer-secreted exosomal miR-21-5p induces angiogenesis and vascular permeability by targeting KRIT1. Cell Death Dis. 12, 1–14. https://doi.org/10.1038/s41419-021-03803-8.
Hole, M.J., Jørgensen, K.K., Holm, K., Braadland, P.R., Meyer-Myklestad, M.H., Medhus, A.W., Reikvam, D.H., Götz, A., Grzyb, K., Boberg, K.M., Karlsen, T.H., Kummen, M., Hov, J.R., 2023. A shared mucosal gut microbiota signature in primary sclerosing cholangitis before and after liver transplantation. Hepatology 77, 715. https://doi.org/10.1002/hep.32773.
Hou, S., Yu, J., Li, Y., Zhao, D., Zhang, Z., 2025. Advances in fecal microbiota transplantation for gut Dysbiosis-related diseases. Adv. Sci. 12, 2413197. https://doi.org/10.1002/advs.202413197.
Hu, J., Wang, C., Huang, X., Yi, S., Pan, S., Zhang, Y., Yuan, G., Cao, Q., Ye, X., Li, H., 2021. Gut microbiota-mediated secondary bile acids regulate dendritic cells to attenuate autoimmune uveitis through TGR5 signaling. Cell Rep. 36, 109726. https://doi.org/10.1016/j.celrep.2021.109726.
Ii, M.S.R., O'Rourke, D.R., Kaehler, B.D., Ziemski, M., Dillon, M.R., Foster, J.T., Bokulich, N.A., 2021. RESCRIPt: Reproducible sequence taxonomy reference database management. PLoS Comput. Biol. 17, e1009581. https://doi.org/10.1371/journal.pcbi.1009581.
Jenike, A.E., Halushka, M.K., 2021. miR-21: a non-specific biomarker of all maladies. Biomark. Res. 9, 18. https://doi.org/10.1186/s40364-021-00272-1.
Johnston, D.G.W., Williams, M.A., Thaiss, C.A., Cabrera-Rubio, R., Raverdeau, M., McEntee, C., Cotter, P.D., Elinav, E., O'Neill, L.A.J., Corr, S.C., 2018. Loss of MicroRNA-21 influences the gut microbiota, causing reduced susceptibility in a murine model of colitis. J. Crohn's Colitis 12, 835–848. https://doi.org/10.1093/ecco-jcc/jjy038.
Lee, Y.S., Choi, I., Ning, Y., Kim, N.Y., Khatchadourian, V., Yang, D., Chung, H.K., Choi, D., LaBonte, M.J., Ladner, R.D., Nagulapalli Venkata, K.C., Rosenberg, D.O., Petasis, N.A., Lenz, H.-J., Hong, Y.-K., 2012. Interleukin-8 and its receptor CXCR2 in the tumour microenvironment promote colon cancer growth, progression and metastasis. Br. J. Cancer 106, 1833–1841. https://doi.org/10.1038/bjc.2012.177.
Li, S., Kendall, S.E., Raices, R., Finlay, J., Covarrubias, M., Liu, Z., Lowe, G., Lin, Y.-H., Teh, Y.H., Leigh, V., Dhillon, S., Flanagan, S., Aboody, K.S., Glackin, C.A., 2012. TWIST1 associates with NF-κB subunit RELA via carboxyl-terminal WR domain to promote cell autonomous invasion through IL8 production. BMC Biol. 10, 73. https://doi.org/10.1186/1741-7007-10-73.
Li, M., Liu, S., Wang, M., Hu, H., Yin, J., Liu, C., Huang, Y., 2020. Gut microbiota Dysbiosis associated with bile acid metabolism in neonatal cholestasis disease. Sci. Rep. 10, 7686. https://doi.org/10.1038/s41598-020-64728-4.
Li, J., Chen, H., Sun, G., Zhang, X., Ye, H., Wang, P., 2023. Role of miR-21 in the diagnosis of colorectal cancer: Meta-analysis and bioinformatics. Pathol. Res. Pract. 248, 154670. https://doi.org/10.1016/j.prp.2023.154670.
Liu, S., da Cunha, A.P., Rezende, R.M., Cialic, R., Wei, Z., Bry, L., Comstock, L.E., Gandhi, R., Weiner, H.L., 2016. The host shapes the gut microbiota via fecal MicroRNA. Cell Host Microbe 19, 32–43. https://doi.org/10.1016/j.chom.2015.12.005.
Liu, C., Du, M.-X., Xie, L.-S., Wang, W.-Z., Chen, B.-S., Yun, C.-Y., Sun, X.-W., Luo, X., Jiang, Y., Wang, K., Jiang, M.-Z., Qiao, S.-S., Sun, M., Cui, B.-J., Huang, H.-J., Qu, S.-P., Li, C.-K., Wu, D., Wang, L.-S., Jiang, C., Liu, H.-W., Liu, S.-J., 2024. Gut commensal Christensenella minuta modulates host metabolism via acylated secondary bile acids. Nat. Microbiol. 9, 434–450. https://doi.org/10.1038/s41564-023-01570-0.
Luo, F., Ji, J., Liu, Y., Xu, Y., Zheng, G., Jing, J., Wang, B., Xu, W., Shi, L., Lu, X., Liu, Q., 2015. MicroRNA-21, up-regulated by arsenite, directs the epithelial–mesenchymal transition and enhances the invasive potential of transformed human bronchial epithelial cells by targeting PDCD4. Toxicol. Lett. 232, 301–309. https://doi.org/10.1016/j.toxlet.2014.11.001.
Mallick, H., Rahnavard, A., McIver, L.J., Ma, S., Zhang, Y., Nguyen, L.H., Tickle, T.L., Weingart, G., Ren, B., Schwager, E.H., Chatterjee, S., Thompson, K.N., Wilkinson, J. E., Subramanian, A., Lu, Y., Waldron, L., Paulson, J.N., Franzosa, E.A., Bravo, H.C., Huttenhower, C., 2021. Multivariable association discovery in population-scale meta-omics studies. PLoS Comput. Biol. 17, e1009442. https://doi.org/10.1371/journal.pcbi.1009442.
Morgan, R.G., Mortensson, E., Williams, A.C., 2018. Targeting LGR5 in colorectal Cancer: therapeutic gold or too plastic? Br. J. Cancer 118, 1410–1418. https://doi.org/10.1038/s41416-018-0118-6.
Munster, K.N., Bergquist, A., Ponsioen, C.Y., 2024. Inflammatory bowel disease and primary sclerosing cholangitis: one disease or two? J. Hepatol. 80, 155–168. https://doi.org/10.1016/j.jhep.2023.09.031.
Oh, B.Y., Kim, S.-Y., Lee, Y.S., Hong, H.K., Kim, T.W., Kim, S.H., Lee, W.Y., Cho, Y.B., 2016. Twist1-induced epithelial-mesenchymal transition according to microsatellite instability status in colon cancer cells. Oncotarget 7, 57066–57076. https://doi.org/10.18632/oncotarget.10974.
Ozga, A.J., Chow, M.T., Luster, A.D., 2021. Chemokines and the immune response to cancer. Immunity 54, 859–874. https://doi.org/10.1016/j.immuni.2021.01.012.
Pace, E., Di Vincenzo, S., Di Salvo, E., Genovese, S., Dino, P., Sangiorgi, C., Ferraro, M., Gangemi, S., 2019. MiR-21 upregulation increases IL-8 expression and tumorigenesis program in airway epithelial cells exposed to cigarette smoke. J. Cell. Physiol. 234, 22183–22194. https://doi.org/10.1002/jcp.28786.
Peng, Q., Zhang, X., Min, M., Zou, L., Shen, P., Zhu, Y., 2017. The clinical role of microRNA-21 as a promising biomarker in the diagnosis and prognosis of colorectal cancer: a systematic review and meta-analysis. Oncotarget 8, 44893–44909. https://doi.org/10.18632/oncotarget.16488.
Pires, D., Calado, M., Velez, T., Mandal, M., Catalão, M.J., Neyrolles, O., Lugo-Villarino, G., Vérollet, C., Azevedo-Pereira, J.M., Anes, E., 2021. Modulation of cystatin C in human macrophages improves anti-mycobacterial immune responses to mycobacterium tuberculosis infection and coinfection with HIV. Front. Immunol. 12.
Qin, J., Luo, M., 2014. MicroRNA-221 promotes colorectal cancer cell invasion and metastasis by targeting RECK. FEBS Lett. 588, 99–104. https://doi.org/10.1016/j.febslet.2013.11.014.
Qiu, P., Ishimoto, T., Fu, L., Zhang, J., Zhang, Z., Liu, Y., 2022. The gut microbiota in inflammatory bowel disease. Frontiers in cellular and infection. Microbiology 12.
Rodrigues, P.M., Afonso, M.B., Simão, A.L., Borralho, P.M., Rodrigues, C.M.P., Castro, R. E., 2015. Inhibition of NF-κB by deoxycholic acid induces miR-21/PDCD4-dependent hepatocellular apoptosis. Sci. Rep. 5, 17528. https://doi.org/10.1038/srep17528.
Sabino, J., Vieira-Silva, S., Machiels, K., Joossens, M., Falony, G., Ballet, V., Ferrante, M., Assche, G.V., Merwe, S.V. der, Vermeire, S., Raes, J., 2016. Primary sclerosing cholangitis is characterised by intestinal dysbiosis independent from IBD. Gut 65, 1681–1689. doi:https://doi.org/10.1136/gutjnl-2015-311004.
Santos, A.A., Afonso, M.B., Ramiro, R.S., Pires, D., Pimentel, M., Castro, R.E., Rodrigues, C.M.P., 2020. Host miRNA-21 promotes liver dysfunction by targeting small intestinal Lactobacillus in mice. Gut Microbes 12, 1840766. https://doi.org/10.1080/19490976.2020.1840766.
Santos, A.A., Pires, D., Marques, V., Alesina, N., Herraez, E., Roudnický, P., Rodrigues, P. M., Godinho-Santos, A., Bravo, A.C., Gouveia, C., Saraiva, S., Correia, L., Crespo, R., da Silva, J.P., Cravo, M., Potesil, D., Zdráhal, Z., Banales, J.M., Marin, J.J.G., Torres, J., Rodrigues, C.M.P., 2025. Primary bile acid shapes peripheral immunity in inflammatory bowel disease-associated primary sclerosing cholangitis. Clin. Sci. (Lond.) 139, 703–716. https://doi.org/10.1042/CS20256078.
Shaw, D.G., Aguirre-Gamboa, R., Vieira, M.C., Gona, S., DiNardi, N., Wang, A., Dumaine, A., Gelderloos-Arends, J., Earley, Z.M., Meckel, K.R., Ciszewski, C., Castillo, A., Monroe, K., Torres, J., Shah, S.C., Colombel, J.-F., Itzkowitz, S., Newberry, R., Cohen, R.D., Rubin, D.T., Quince, C., Cobey, S., Jonkers, I.H., Weber, C.R., Pekow, J., Wilson, P.C., Barreiro, L.B., Jabri, B., 2023. Antigen-driven colonic inflammation is associated with development of dysplasia in primary sclerosing cholangitis. Nat. Med. 29, 1520–1529. https://doi.org/10.1038/s41591-023-02372-x.
Sheedy, F.J., Palsson-McDermott, E., Hennessy, E.J., Martin, C., O'Leary, J.J., Ruan, Q., Johnson, D.S., Chen, Y., O'Neill, L.A.J., 2010. Negative regulation of TLR4 via targeting of the proinflammatory tumor suppressor PDCD4 by the microRNA miR-21. Nat. Immunol. 11, 141–147. https://doi.org/10.1038/ni.1828.
Tabibian, J.H., Bowlus, C.L., 2017. Primary sclerosing cholangitis: a review and update. Liver Research 1, 221–230. https://doi.org/10.1016/j.livres.2017.12.002.
Tang, Y., Zong, S., Zeng, H., Ruan, X., Yao, L., Han, S., Hou, F., 2021. MicroRNAs and angiogenesis: a new era for the management of colorectal cancer. Cancer Cell Int. 21, 221. https://doi.org/10.1186/s12935-021-01920-0.
Torres, J., Bao, X., Goel, A., Colombel, J.-F., Pekow, J., Jabri, B., Williams, K.M., Castillo, A., Odin, J.A., Meckel, K., Fasihuddin, F., Peter, I., Itzkowitz, S., Hu, J., 2016. The features of mucosa-associated microbiota in primary sclerosing cholangitis. Aliment. Pharmacol. Ther. 43, 790–801. https://doi.org/10.1111/apt.13552.
Torres, J., Palmela, C., Brito, H., Bao, X., Ruiqi, H., Moura-Santos, P., Pereira da Silva, J., Oliveira, A., Vieira, C., Perez, K., Itzkowitz, S., Colombel, J., Humbert, L., Rainteau, D., Cravo, M., Rodrigues, C., Hu, J., 2018. The gut microbiota, bile acids and their correlation in primary sclerosing cholangitis associated with inflammatory bowel disease. United European Gastroenterol J 6, 112–122. https://doi.org/10.1177/2050640617708953.
Trivedi, P.J., Crothers, H., Mytton, J., Bosch, S., Iqbal, T., Ferguson, J., Hirschfield, G.M., 2020. Effects of primary Sclerosing cholangitis on risks of Cancer and death in people with inflammatory bowel disease, based on sex, race, and age. Gastroenterology 159, 915–928. https://doi.org/10.1053/j.gastro.2020.05.049.
Yang, L., Akanyibah, F.A., Yao, D., Jin, T., Mao, F., 2025. The role of COX-2 and its use as a therapeutic target in IBD and related colorectal cancer. Arch. Biochem. Biophys. 771, 110516. https://doi.org/10.1016/j.abb.2025.110516.
Zeboudj, L., Sideris-Lampretsas, G., Silva, R., Al-Mudaris, S., Picco, F., Fox, S., Chambers, D., Malcangio, M., 2023. Silencing miR-21-5p in sensory neurons reverses neuropathic allodynia via activation of TGF-β–related pathway in macrophages. J. Clin. Invest. 133. https://doi.org/10.1172/JCI164472.
Zhang, R., Lauwers, G.Y., Choi, W.-T., 2022. Increased risk of non-conventional and invisible Dysplasias in patients with primary Sclerosing cholangitis and inflammatory bowel disease. Journal of Crohn's and Colitis 16, 1825–1834. https://doi.org/10.1093/ecco-jcc/jjac090.
Zhou, Y., Ren, H., Dai, B., Li, J., Shang, L., Huang, J., Shi, X., 2018. Hepatocellular carcinoma-derived exosomal miRNA-21 contributes to tumor progression by converting hepatocyte stellate cells to cancer-associated fibroblasts. J. Exp. Clin. Cancer Res. 37, 324. https://doi.org/10.1186/s13046-018-0965-2.