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Research Article

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Posted Date: March 23rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8753984/v1

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Additional Declarations: No competing interests reported.

Establishment of a Gene Expression Signature for Colitis-Associated Colorectal Cancer to Assess Cancer-Promoting Effects of Standard IBD Treatments

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Abstract

Background: Inflammatory bowel disease, particularly ulcerative colitis, is a chronic inflammatory disorder of the gastrointestinal tract that can progress to colitis-associated colorectal cancer. In addition to disease-related risk, certain pharmacological therapies used in UC management may further increase the likelihood of malignancies, including colorectal cancer. This study was conducted to identify novel biomarkers associated with carcinogenesis and early detection of CAC in patients with ulcerative colitis, using integrative bioinformatics approaches.

Methods: Differential gene expression analysis was performed on microarray datasets. Genes commonly dysregulated in UC, sporadic colorectal cancer, and CAC, as well as CAC-specific genes, were identified as potential biomarkers. The expression levels of selected candidate genes were subsequently quantified by quantitative real-time PCR in colonic tissue samples obtained from UC patients receiving the studied pharmacological treatments, untreated UC patients, and healthy control individuals.

Results: The genes *SERPINE1*, *COL1A1*, *MMP9*, *IL1B*, *ANXA5*, *CAV1*, *TLR2*, and *VCAM1* were selected as candidate biomarkers. Long-term use of 5-aminosalicylic acid for more than five years resulted in a significant upregulation of *COL1A1*, *ANXA5*, and *VCAM1*. Treatment with corticosteroids and immunomodulatory agents significantly increased the expression of *IL1B* and *SERPINE1*, whereas anti-TNF therapy led to a significant increase only in *CAV1* expression.

Conclusions: Anti-TNF therapy was associated with significant upregulation of *CAV1*, a gene involved in membrane signaling, endothelial integrity, and inflammatory regulation, and uniquely showed reduced expression of pro-inflammatory and adhesion-related genes such as *IL1B and VCAM1* compared with untreated patients. In contrast, long-term treatment with 5-ASA, corticosteroids, and immunomodulatory agents did not reduce the expression of any analyzed genes and instead increased the expression of multiple candidates. These treatments were characterized by upregulation of *IL1B*, *SERPINE1*, *COL1A1*, *MMP9*, *ANXA5*, *TLR2*, and *VCAM1* suggest amplified inflammatory cell infiltration and epithelial damage, creating a microenvironment conducive to chronic inflammation, disease recurrence, and progression toward colitis-associated colorectal cancer. Overall, prolonged use of 5-ASA, corticosteroids, and immunomodulatory therapies appears to promote inflammation-driven carcinogenic pathways, limiting their suitability for long-term UC management, whereas anti-TNF therapy demonstrates a comparatively protective molecular profile by attenuating key inflammatory and immune pathways and may represent reduced contribution to CAC progression.

Keywords: Inflammatory bowel disease, Ulcerative colitis, Colitis-associated colorectal cancer, Differential gene expression analysis, IBD treatment

Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are the primary forms of inflammatory bowel diseases (IBDs), which are chronic, idiopathic conditions marked by alternating periods of remission and flare-ups (1, 2). In the diagnostic work-up of IBD, colonoscopy with direct visualization and targeted biopsies is considered the gold standard as it enables assessment of mucosal inflammation, extent of disease, and histopathologic evaluation (3).

A clear and well-documented connection exists between chronic inflammation and the progression of colorectal cancer (CRC), the second leading cause of cancer-related deaths in Western nations. Colitis-associated colorectal cancer (CAC) accounts for about 15% of IBD-related deaths, though only 1–2% of IBD patients develop CAC (4). The incidence of CRC in UC patients has decreased over time due to improved medical therapies and endoscopic surveillance programs. However, individuals with UC still face a higher likelihood of developing CRC compared to those without chronic colitis (5). Risk factors for CAC include a family history of CRC, disease duration, severity and extent of UC, age at UC diagnosis, and the presence of concurrent primary sclerosing cholangitis (PSC). Persistent inflammation and extensive disease involvement are the primary drivers of CAC development (6). Certain medications used to treat UC may also heighten the risk of malignancies, including CRC, small bowel cancers, intestinal lymphoma, anal cancer, and extraintestinal cancers (7).

The pharmacological treatments of UC are divided into several groups: aminosalicylates (5-ASA), corticosteroids, immunomodulators, biologics, and small molecules. 5-ASA drugs are critical for

managing mild to moderate UC by reducing colon inflammation and promoting remission. Mesalamine and sulfasalazine are two commonly used drugs from the 5-ASA group (8). Research indicates that consistent use of 5-ASA drugs significantly lowers the risk of CAC, with evidence from meta-analyses and a large cohort study highlighting their protective role, particularly in UC patients compared to those with Crohn's disease. The 2017 ECCO Consensus (European Crohn's and Colitis Organization) supports mesalamine's use for chemoprevention in UC patients to decrease long-term cancer risk (9).

Corticosteroids are used to induce remission in moderate to severe UC but are not suitable for maintaining remission due to their side effects. Examples of corticosteroid medications include Prednisone and Budesonide(10). There is limited direct evidence linking corticosteroids to malignancy, but their immunosuppressive effects may indirectly increase the risk of lymphoma or virus-related cancers (such as HPV). Long-term use is not recommended due to side effects, including an increased risk of infection, and they are only suitable for short-term treatment (11).

Immunomodulators, such as Azathioprine, Mercaptopurine, and Cyclosporine, suppress immune activity and are employed to sustain remission in challenging cases of UC Azathioprine, a purine analog prodrug, is converted into 6-mercaptopurine and is commonly used for moderate to severe UC, often in combination with biologic therapies. These agents reduce lymphocyte proliferation by blocking DNA synthesis in immune cells but carry risks of adverse effects, including pancreatitis, hepatotoxicity and lymphoma (12). Thiopurines elevate the risk of lymphoma by 5- to 6-fold, especially in individuals with EBV exposure, and are associated with rare but severe hepatosplenic T-cell lymphoma (HSTCL) in young males, typically within two years of initiating therapy. Long-term use also increases the incidence of non-melanoma skin cancer (NMSC) and has been linked to other cancers, such as acute myeloid leukemia (AML) and severe myelodysplastic syndrome. Although effective for controlling IBD, thiopurines should be used cautiously in high-risk groups, such as young men prone to HSTCL, and are contraindicated in patients with active malignancies. Intermittent or cyclic thiopurine regimens may help lower the risk of developing malignancies (13-16).

Biologics are engineered proteins prescribed for moderate to severe UC, targeting inflammatory proteins (17). Anti-tumor necrosis factor (anti-TNF) agents are widely utilized in the management of IBD. A study identified a modest but statistically significant increase in the risk of malignancies, particularly lymphomas, including HSTCL, especially when used in combination with thiopurines. Additionally, an elevated risk of skin cancers, such as melanoma (predominantly associated with biologic therapies) and non-melanoma skin cancer (primarily linked to thiopurine use), has been observed (11). Newer biologic agents, such as vedolizumab (an anti-integrin) and Ustekinumab (an anti-IL-12/23), may reduce systemic immunosuppression, potentially offering a safer malignancy profile. However, long-term data remain limited, as clinical trials frequently exclude patients with recent malignancies, and real-world studies often have relatively short follow-up periods (18).

Despite colonoscopy being regarded as the diagnostic gold standard for UC, this approach is limited by its invasiveness, operator dependency, and imperfect ability to predict long-term complications such as CAC. Consequently, important gaps remain in our understanding of the molecular mechanisms linking chronic inflammation in UC to carcinogenesis. Moreover, existing evidence regarding the oncogenic potential of pharmacological therapies used in UC management is largely derived from epidemiological and observational studies, which are inherently limited by confounding, heterogeneous treatment exposures, and a lack of mechanistic insight. To address these limitations, the present study aims to investigate the long-term malignancy risks associated with commonly used UC therapies through integrative bioinformatic analyses and in vitro validation. By adopting this combined approach, we seek to provide a more comprehensive evaluation of the comparative oncogenic safety profiles of UC treatments and to contribute mechanistic evidence that may inform risk stratification and therapeutic decision-making in patients with UC.

Methodology

1. Data selection and processing

   From approximately 40 datasets identified through filtered searches (criteria: sample size, publication date, species [Homo sapiens], and study type [expression by array, expression by sequencing]), no datasets utilizing expression by sequencing were found for CAC. Consequently, all selected datasets were of the expression by array type. Selected datasets underwent quality control analysis using R packages ggplot2 (https://ggplot2.tidyverse.org/) and grid (https://cran.r-project.org/package=grid) to perform principal component analysis (PCA). Microarray datasets were retrieved from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo) using the following search keywords: “Inflammatory Bowel Disease” for ulcerative colitis (UC), “Colorectal Cancer” for sporadic colorectal cancer (sCRC), and “Ulcerative Colitis–Associated Colorectal Cancer” for colitis-associated cancer (CAC). The selected datasets included are listed in table 1. All datasets were merged, batch effects were corrected using the sva package (https://cran.r-project.org/package=SmartSVA) and quality control was reapplied to the integrated dataset to ensure data integrity.

Table 1. Selected datasets

GEO accession	Platforms	Samples	Country of origin	Date
GSE87466	GPL13158	21 normal 87 colitis	United States	2018
GSE75214	GPL6244	11 normal 74 colitis	Belgium	2017
GSE44076	GPL13667	98 tumor	Spain	2014

		50 healthy controls		
GSE41568	GPL570	39 primary tumor	United States	2016
GSE37283	GPL13158	5 normal 11 colitis-associated neoplasia	United States	2012

2. Differential expression analysis

The merged datasets for each disease were subjected to differential expression analysis using R packages, including limma, with criteria set at an adjusted p-value < 0.05 and |logFC| > 1. VennDiagram was used via the Venny web tool (https://bioinfogp.cnb.csic.es/tools/venny/) to identify common DEGs among UC, sCRC, and CAC, as well as DEGs unique to CAC.

3. Enrichment analysis

Functional enrichment analysis was performed for both common DEGs and CAC-specific DEGs ($|logFC| \geq 1$ and adjusted p-value ≤ 0.05). Enrichment analysis was conducted using the Gene Ontology (GO) database, implemented through R software with the packages clusterProfiler (https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html) and org.Hs.eg.db (https://www.bioconductor.org/packages/org.Hs.eg.db/\) to identify relevant biological processes, molecular functions, and cellular components.

4. Construction of PPI protein interaction network

Protein–protein interaction (PPI) networks for common DEGs (across UC, sCRC, and CAC) and CAC-specific DEGs were constructed separately using the STRING plugin within Cytoscape (19), a freely available software platform for visualizing and analyzing protein interaction networks to investigate functional relationships between genes and proteins. Confidence thresholds were set at 0.7 for common DEGs and 0.6 for CAC-specific DEGs. Nodes in the networks were color-coded based on logFC values, with their sizes scaled according to node degree. The cytoHubba plugin in Cytoscape was utilized to identify and select DEG nodes that ranked highly across multiple topological parameters (e.g., degree, closeness, betweenness) or a subset thereof, while also representing candidate biomarkers for UC and CAC.

5. Tissue collection

Tissue samples were collected during routine colonoscopy procedures from the research institute for gastroenterology and liver diseases of Taleghani hospital and were immediately preserved in RNAlater solution to ensure RNA integrity until further processing. The ethics approval code was obtained prior to study initiation and all participants provided written informed consent in accordance with ethical guidelines and agreed to participate in the study. Tissue samples were obtained from individuals listed in table 2.

Table 2. Summary of clinical and demographic characteristics of patients and healthy controls

Group	Healthy controls	5-ASA	Corticosteroids/ Immunomodulators	Anti-TNF	Treatment-naïve
Number	20	14	10	10	11
Age	48.05±15.22	48.64±15.92	43.6±7.64	34.6±5.69	41.36±15.35
Men	8	7	5	4	8
Women	12	7	5	6	3

5-ASA, 5-aminosalicylates

6. RNA extraction

Total RNA was extracted from tissue samples using the The FavorPrep™ Blood/Cultured Cells Total RNA Extraction Mini Kit (FAVORGEN, Taiwan, cat #FABRK001) according to the manufacturer's protocol. For RNA purification, a DNase I RNase-free kit (Sinaclon, Iran, cat #MO5401) was utilized for the purpose of eliminating DNA contamination. The synthesis of complementary DNA (cDNA) was carried out using the AddScript cDNA Synthesis Kit (Korea, cat. #22701), following the manufacturer's instructions.

7. Real-time PCR

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using the Roche LightCycler®, Germany to analyze the expression of eight genes (*MMP9*, *IL1B, COL1A1*, *SERPINE1*, *CAV1*, *ANXA5*, *TLR2*, and *VCAM1).* The housekeeping gene *EEF2*, identified through the Ucamp website (http://www.housekeeping.unicamp.br/) as the optimal reference gene based on the target species and tissue, was used as an internal control. Real-time PCR was conducted on synthesized cDNA using the RealQ Plus 2x Master Mix Green (Amplicon, Denmark). Primers were designed using the online Primer-BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/), In order to ensure the

most accurate results, the specificity, secondary structures, sequences, and potential dimer formation of the primers were thoroughly evaluated. The primer sequences are provided in Table 3. The PCR protocol consisted of an initial denaturation step at 95°C for 15 minutes, followed by 40 cycles of amplification Each cycle consisted of a denaturation step at 95°C for 20 seconds, an annealing step at 60°C for 30 seconds, and an extension step at 72°C for 30 seconds Annealing temperatures for each primer pair were optimized using gradient PCR.

Table 3. Primers

Gene	Sequence (5′ ---- > 3′)	Product Length	Primer Length
MMP9	F: TGACAGCGACAAGAAGTGGG R: TTCAGGGCGAGGACCATAGA	208	F: 20 R: 20
IL1B	F: CAGAAGTACCTGAGCTCGCC R: AGATTCGTAGCTGGATGCCG	153	F: 20 R: 20
COL1A1	F: TGACCTCAAGATGTGCCACT R: CCTCTTGTCCTTGGGGTTCT	187	F: 20 R: 20
SERPINE1	F: CCGCCTCTTCCACAAATCAG R: AATGTTGGTGAGGGCAGAGA	202	F: 20 R: 20
VCAM1	F: GGCAGAGTACGCAAACACTT R: ACAGGATTTTCGGAGCAGGA	141	F: 20 R: 20
TLR2	F: TTATCCAGCACACGAATACACAG R: AGGCATCTGGTAGAGTCATCAA	160	F: 23 R: 20
CAV1	F: AACCAACAACCTCAACTGCC R: ACTTGTACACTCCATGGCCA	88	F: 20 R: 20
ANXA5	F: ACCTTCCCCTGACCTGAGTA R: GCCTTCCGAAGAGTTTCTGC	106	F: 20 R: 20
EEF2	F: CATCATCTCCACCTACGGCG R: GCTGGGTCAAAGTACCTGTCA	246	F: 20 R: 21

EEF2;

8. Statistical analyses

For data analysis, the ΔΔCt value was first calculated (using Livak method). This method is used to calculate relative changes in gene expression between different samples compared to the control samples. Data analysis was performed using GraphPad Prism software. After applying the paired t-test and subsequently on-way ANOVA test (normality of the data was evaluated and confirmed), the significance threshold was set at set at $p < 0.05$.

Results

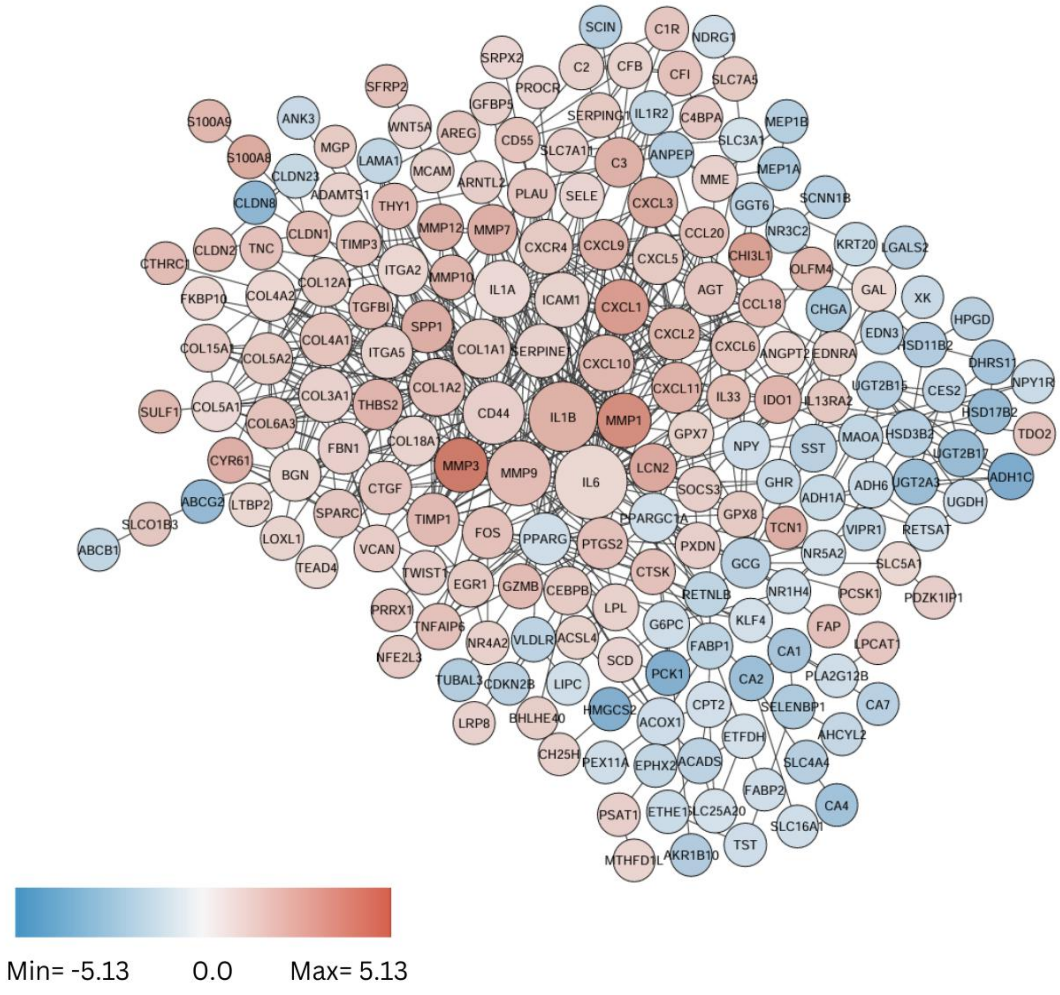
1. Common and Distinct DEGs were obtained from microarray datasets.

   After the integration of raw data from GSE87466, GSE75214, GSE44076, GSE41568 and GSE37283, normalization and batch effect correction were performed on the combined raw data. Principal component analysis (PCA) plots were generated before and after these steps for the final merged dataset. Differential expression analysis of the microarray data was conducted using the limma package in R applying a threshold of $|logFC| > 1$ and an adjusted p-value < 0.05. The final merged dataset comprised 87 control samples, 161 UC samples, 137 CRC samples, and 11 CAC samples. Based on this analysis and the applied filters, 268 upregulated and 294 downregulated genes were identified in UC tissues, 234 upregulated and 314 downregulated genes in sCRC tissues, and 328 upregulated and 293 downregulated genes in CAC tissues. Venn diagram was utilized to identify DEGs shared across all diseases and those unique to CAC. In total, 461 genes (60.8% of DEGs) were identified as common DEGs, and 153 genes (20.2% of DEGs) were identified as CAC-specific DEGs.

2. Common and Distinct DEGs were visualized as Protein-Protein Interaction (PPI) network

   The STRING plugin within Cytoscape software was utilized to construct and visualize PPI networks for 461 common DEGs between UC, sCRC, and CAC (Figure 1A) as well as for 153 CAC-specific DEGs, to further investigate their interactions (Figure 1B). Nodes were color-coded based on their logFC values, with node sizes reflecting the degree of connectivity. Using Cytoscape, the PPI network for common DEGs comprised 459 nodes and 2632 edges. Additionally, 198 singleton nodes were excluded from the common DEG network to refine the analysis. The CAC-specific PPI network consisted of 152 nodes and 201 edges, with 87 singleton nodes removed to refine the analysis.

A


SCIN
C1R
NDRG1
SRPX2
C2
CFB
CFI
SLC7A5
SFRP2
PROCR
GFBP5
SERPING1
IL1R2
C4BPA
MEP1B
S100A9
ANK3
WNT5A
AREG
CD55
SLC7A11
SLC3A1
S100A8
MGP
LAMA1
MCAM
C3
ANPEP
MME
MEP1A
CLDN23
ARNTL2
PLAU
SELE
CXCL3
GGT6
SCNN1B
CLDN8
ADAMTS1
THY1
MMP12
MMP7
CXCR4
CXCL9
CCL20
NR3C2
CLDN2
TNC
CLDN1
TIMP3
CXCL5
CHI3L1
KRT20
LGALS2
CTHRC1
ITGA2
MMP10
IL1A
ICAM1
AGT
OLFM4
GAL
XK
FKBP10
COL4A2
COL12A1
TGFBI
CXCL1
CCL18
CHGA
HPGD
SPP1
CXCL2
EDN3
HSD11B2
COL15A1
COL5A2
COL4A1
ITGA5
COL1A1
SERPINE1
CXCL6
ANGPT2
EDNRA
DHRS11
CXCL10
NPY1R
COL3A1
COL1A2
CXCL11
IL33
IDO1
IL13RA2
UGT2B15
CES2
SULF1
COL5A1
THBS2
CD44
IL1B
MMP1
HSD17B2
COL6A3
GPX7
NPY
SST
MAOA
HSD3B2
TDO2
CYR61
FBN1
COL18A1
MMP3
MMP9
LCN2
UGT2B17
BGN
IL6
SOCS3
GHR
ADH6
UGT2A3
ADH1C
ABCG2
CTGF
LTBP2
ADH1A
UGDH
SLCO1B3
SPARC
TIMP1
FOS
PPARGC1A
GPX8
TCN1
VIPR1
RETSAT
ABCB1
LOXL1
VCAN
PPARG
PTGS2
PXDN
NR5A2
SLC5A1
TEAD4
TWIST1
EGR1
GZMB
CEBPB
CTSK
GCG
RETNLB
NR1H4
PCSK1
PDZK1IP1
PRRX1
LPL
G6PC
KLF4
TNFAIP6
NR4A2
VLDLR
ACSL4
FAP
FABP1
LPCAT1
NFE2L3
SCD
PCK1
CA1
CA2
PLA2G12B
TUBAL3
CDKN2B
LIPC
CPT2
SELENBP1
CA7
HMGCS2
ACOX1
LRP8
BHLHE40
ETFDH
AHCYL2
CH25H
PEX11A
EPHX2
ACADS
SLC4A4
FABP2
CA4
PSAT1
ETHE1
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SLC16A1
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AKR1B10
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Max= 5.13

B

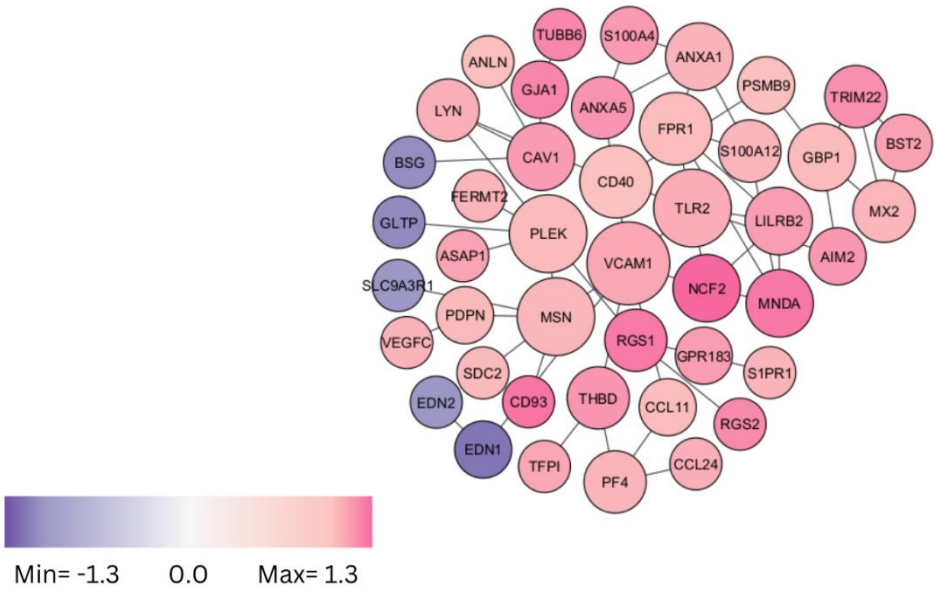


Figure 1| A. Common genes of UC, CRC and CAC co-expression network. Nodes are colored by logFC (varies from -5.13 to 5.13) and their size are associated with amount of degree they own. 198 singletons were deleted manually. 459 nodes and 2632 edges were identified. *IL6* (Degree= 46), has the highest degree among common DEGs. **B. CAC-exclusive genes co-expression network.** Nodes are colored by logFC (varies from -1.3 to 1.3). Size of nodes are associated with amount of degree they own. 87 singletons were deleted manually in the network. *VCAM1*(Degree=7) has the highest degree among exclusive DEGs.

3. Candidate genes were elected according to the specific ranking factors

   Common and CAC-specific nodes were screened using eleven topological ranking metrics. *SERPINE1*, *COL1A1*, *MMP9,* and *IL1B* as common genes among UC, sCRC and CAC, *ANXA5*, *CAV1*, *TLR2*, and *VCAM1* as CAC-exclusive genes were ultimately selected as candidate biomarkers using the CytoHubba plugin in Cytoscape. The ranking metrics included betweenness, closeness, bottleneck, eccentricity, radiality, clustering coefficient, stress, maximum neighborhood component (MNC), density of maximum neighborhood component (DMNC), maximal clique centrality (MCC), and edge percolated component (EPC).

4. Enrichment analysis was implemented for achieved DEGs.

   Functional enrichment analysis of common DEGs was conducted using the GO database and R software, employing the packages clusterProfiler and org.Hs.eg.db. Key enriched pathways included collagen degradation, extracellular matrix breakdown, and extracellular matrix organization (Figure 2A). Additionally, CAC-specific DEGs were analyzed using

the same R packages and database, revealing enrichment in distinct pathways such as leukocyte chemotaxis and leukocyte migration (Figure 2B).

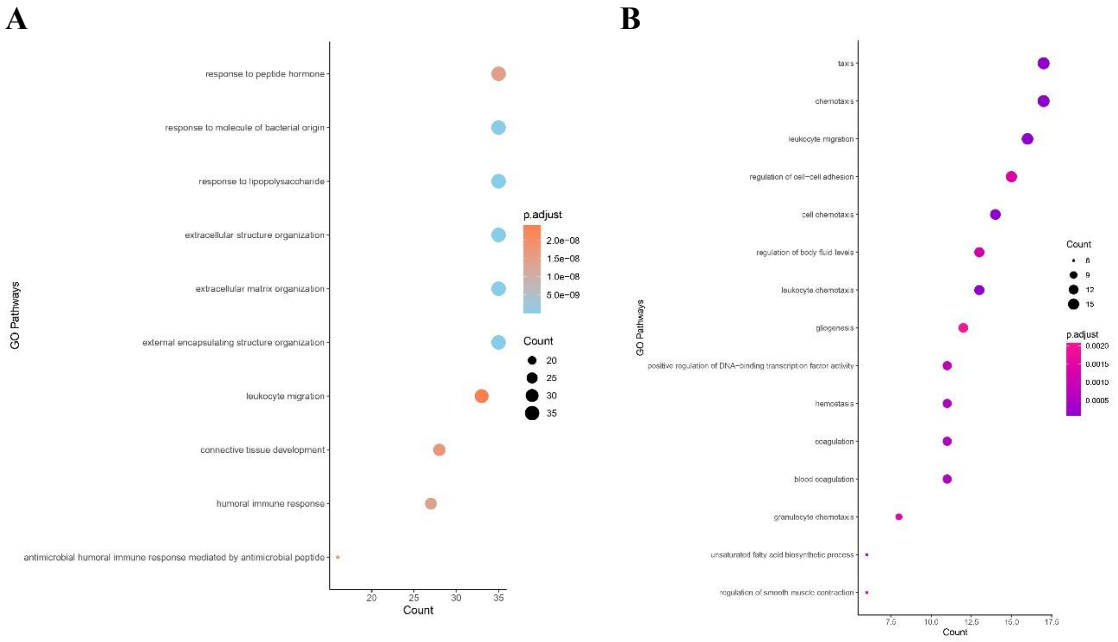


Figure 2| Enrichment analysis of common and exclusive genes. (A) Key enriched pathways of common DEGs analyzed with Gene Ontology (GO) database included collagen degradation, extracellular matrix breakdown, and extracellular matrix organization. **(B)** Key enriched pathways of CAC-exclusive DEGs, revealing enrichment in distinct pathways such as leukocyte chemotaxis, leukocyte migration, and chemotaxis.

5. Total RNA was extracted.

The concentration of RNA extracted from cultured cells derived from tissue samples of patients and healthy controls was quantified using a NanoDrop spectrophotometer. The

mean RNA concentrations were determined approximately 98 ng/μL for UC samples and 76 ng/μL for the control group. The 260/280 absorbance ratios of the extracted RNAs ranged from 1.2 to 1.8, and the 260/230 ratios ranged from 2.0 to 2.2, indicating that the RNAs were of good quality and suitable for performing qPCR.

6. Expression of candidate genes in tissues of treated patients and controls

The genes *SERPINE1*, *COL1A1*, *MMP9*, *IL1B*, *ANXA5*, *CAV1*, *TLR2*, and *VCAM1* were selected as candidate biomarkers. The expression of candidate genes was measured in tissue samples from patients receiving different treatment regimens and from treatment-naïve patients and compared to healthy control subjects.

***SERPINE1*:** In treatment-naïve UC patients, *SERPINE1* expression was markedly elevated (92.55-fold, p-value = 0.0006) compared to healthy controls. Long-term (>5 years) use of 5-ASA increased *SERPINE1* expression by 4.20-fold (Adjusted p-value = 0.7725), while corticosteroids and immunomodulators produced a much stronger upregulatory effect (15.2-fold, Adjusted p-value = 0.0053). Anti-TNF therapy also increased *SERPINE1* levels (5.25-fold, Adjusted p-value = 0.6578) (Figure 3).

***COL1A1*:** *COL1A1* expression increased 18.4-fold (p-value = 0.0094) in treatment-naïve UC patients relative to controls. Long-term 5-ASA use caused a substantial rise in *COL1A1* expression (47.04-fold, Adjusted p-value < 0.0001). Corticosteroids and immunomodulators produced a moderate increase (10.12-fold, adjusted p-value = 0.7035), while anti-TNF therapy also elevated *COL1A1* expression (10.83-fold, Adjusted p-value = 0.6548) (Figure 4).

***MMP9*:** Treatment-naïve UC patients showed a 4.08-fold increase in *MMP9* expression (p-value = 0.0020). Long-term 5-ASA therapy further increased *MMP9* expression (5.77-fold, Adjusted p-value = 0.2687). Corticosteroid and immunomodulator treatment produced a modest rise (3.06-fold, adjusted p-value = 0.8656), and anti-TNF therapy resulted in a 6.47-fold increase (Adjusted p-value = 0.2222) (Figure 5).

***IL1B*:** *IL1B* expression was strongly upregulated in treatment-naïve UC patients (10.95-fold, p-value < 0.0001). Long-term 5-ASA use increased *IL1B* expression by 6.22-fold (Adjusted p-value = 0.1565). Corticosteroids and immunomodulators produced a significant rise (8.49-fold, adjusted p-value = 0.0365), while anti-TNF therapy had a minimal effect (1.58-fold, Adjusted p-value = 0.9956) (Figure 6).

***ANXA5*:** *ANXA5* expression increased 7.07-fold in treatment-naïve UC patients (p-value = 0.0003). Long-term 5-ASA use caused a moderate increase (2.10-fold, Adjusted p-value = 0.0236). Corticosteroids and immunomodulators slightly elevated *ANXA5* levels (1.85-fold, adjusted p-value = 0.1489), whereas anti-TNF therapy reduced expression (0.58-fold, Adjusted p-value = 0.6767) (Figure 7).

***CAV1*:** *CAV1* showed a mild increase in treatment-naïve UC samples (1.37-fold, p-value = 0.2374). Long-term 5-ASA therapy produced a notable elevation (8.61-fold, Adjusted p-value = 0.9780). Corticosteroids and immunomodulators resulted in a moderate increase (2.74-fold, adjusted p-value = 0.9998), while anti-TNF treatment caused a dramatic rise in *CAV1* expression (61.09-fold, Adjusted p-value = 0.0479) (Figure 8).

***TLR2*:** *TLR2* expression was reduced in treatment-naïve UC patients (0.56-fold, p-value = 0.0390). In contrast, long-term 5-ASA therapy increased *TLR2* expression (5.93-fold, Adjusted p-value = 0.1365). Corticosteroid and immunomodulator treatment caused a similar rise (7.14-fold, adjusted p-value = 0.0763), and anti-TNF therapy also increased expression (6.12-fold, Adjusted p-value = 0.1686) (Figure 9).

***VCAM1*:** Treatment-naïve UC patients exhibited a strong increase in *VCAM1* expression (18.9-fold, p-value = 0.0032). Long-term 5-ASA therapy further elevated *VCAM1* levels (6.75-fold, Adjusted p-value = 0.0011). Corticosteroids and immunomodulators induced a mild, non-significant increase (1.90-fold, adjusted p-value = 0.9161), while anti-TNF therapy substantially reduced *VCAM1* expression (0.45-fold, Adjusted p-value = 0.9786) (Figure 10).

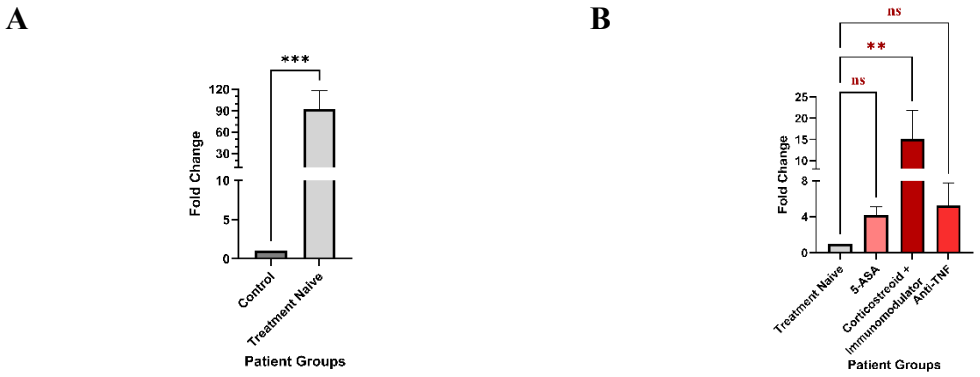


Figure 3| Expression of *SERPINE1* in Tissues of Treated Patients and Controls. (A) Expression of *SERPINE1* in treatment naïve group vs control group: In UC patients, *SERPINE1* gene expression increases 92.55-fold compared to the control group (p-value = 0.0006). **(B)** Expression of *SERPINE1* in treatment groups vs treatment naïve group: Long-term use (>5 years) of 5-ASA increases *SERPINE1* expression by approximately 4.195-fold (Adjusted p-value = 0.7725). Corticosteroids and immunomodulators, when used for more than 5 years, increase *SERPINE1* expression by about 15.2-fold (Adjusted p-value = 0.0053). Anti-TNF drugs, with usage of 5 years or more, increase *SERPINE1* expression by approximately 5.248-fold compared to healthy controls (Adjusted p-value = 0.6578)**;** *SERPINE1*: serpin family E member 1; Error bars in the charts represent the standard error of the mean (SEM); * P = 0.05, ** P = 0.01, ** P < 0.001, **** P < 0.0001

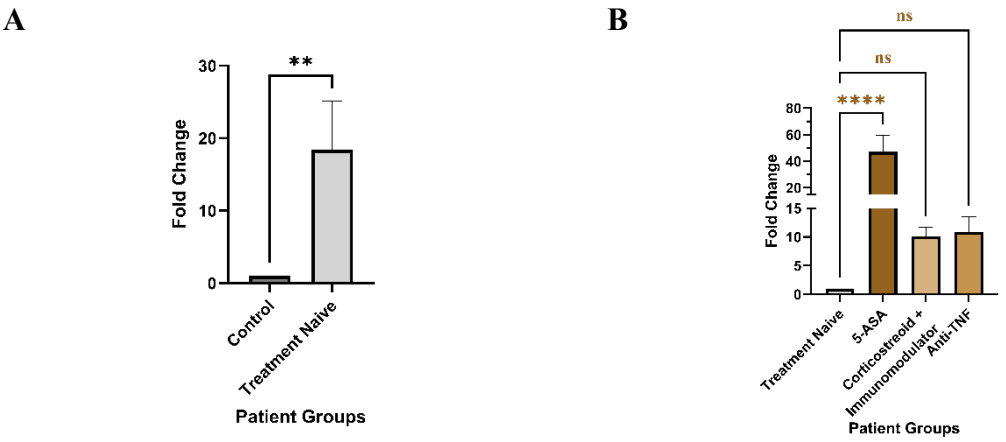


Figure 4| Expression of *COL1A1* in Tissues of Treated Patients and Controls. (A) Expression of *COL1A1* in treatment naïve group vs control group: In UC patients, *COL1A1* gene expression increases 18.4-fold compared to the control group (p-value = 0.0094). **(B)** Expression of *COL1A1* in treatment groups vs treatment naïve group: Long-term use (>5 years) of 5-ASA increases *COL1A1* expression by approximately 47.04-fold (Adjusted p-value < 0.0001). Corticosteroids and immunomodulators, when used for more than 5 years, increase *COL1A1* expression by about 10.118-fold (Adjusted p-value = 0.7035). Anti-TNF drugs, with usage of 5 years or more, increase *COL1A1* expression by approximately 10.831-fold compared to healthy controls (Adjusted p-value = 0.6548). *COL1A1*: collagen type I alpha 1 chain; Error bars in the charts represent the standard error of the mean (SEM); * P = 0.05, ** P = 0.01, ** P < 0.001, **** P < 0.0001

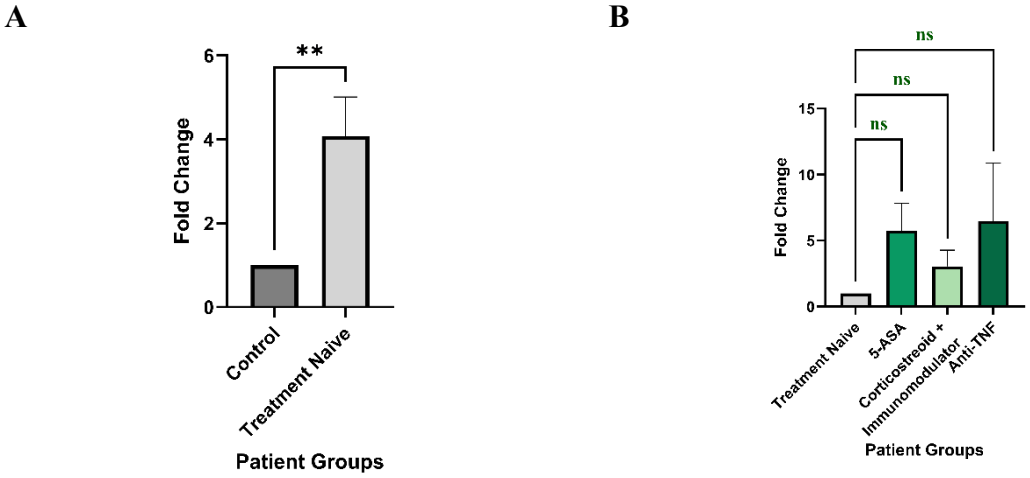


Figure 5| Expression of *MMP9* in Tissues of Treated Patients and Controls. **(A)** Expression of *MMP9* in treatment naïve group vs control group: In UC patients, *MMP9* gene expression increases 4.077-fold compared to the control group (p-value = 0.0020). **(B)** Expression of *MMP9* in treatment groups vs treatment naïve group: Long-term use (>5 years) of 5-ASA increases *MMP9* expression by approximately 5.772-fold (Adjusted p-value = 0.2687). Corticosteroids and immunomodulators, when used for more than 5 years, increase *MMP9* expression by about 3.055-fold (Adjusted p-value = 0.8656). Anti-TNF drugs, with usage of 5 years or more, increase *MMP9* expression by approximately 6.466-fold compared to healthy controls (Adjusted p-value = 0.2222). *MMP9*: matrix metallopeptidase 9; Error bars in the charts represent the standard error of the mean (SEM); * P = 0.05, ** P = 0.01, ** P < 0.001, **** P < 0.0001

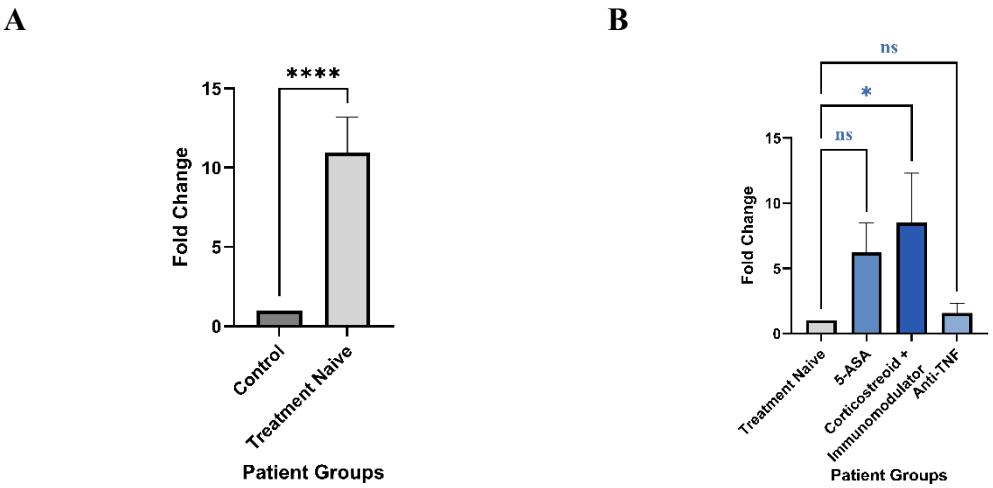


Figure 6| Expression of *IL1B* in Tissues of Treated Patients and Controls. (A) Expression of *IL1B* in treatment naïve group vs control group: In UC patients, *IL1B* gene expression increases 10.95-fold compared to the control group (p-value < 0.0001). **(B)** Expression of *IL1B* in treatment groups vs treatment naïve group: Long-term use (>5 years) of 5-ASA increases *IL1B* expression by approximately 6.223-fold (Adjusted p-value = 0.1565). Corticosteroids and immunomodulators, when used for more than 5 years, increase *IL1B* expression by about 8.494-fold (Adjusted p-value = 0.0365). Anti-TNF drugs, with usage of 5 years or more, increase *IL1B* expression by approximately 1.579-fold compared to healthy controls (Adjusted p-value = 0.9956). *IL1B*: interleukin 1 beta; Error bars in the charts represent the standard error of the mean (SEM); * P = 0.05, ** P = 0.01, ** P < 0.001, **** P < 0.0001

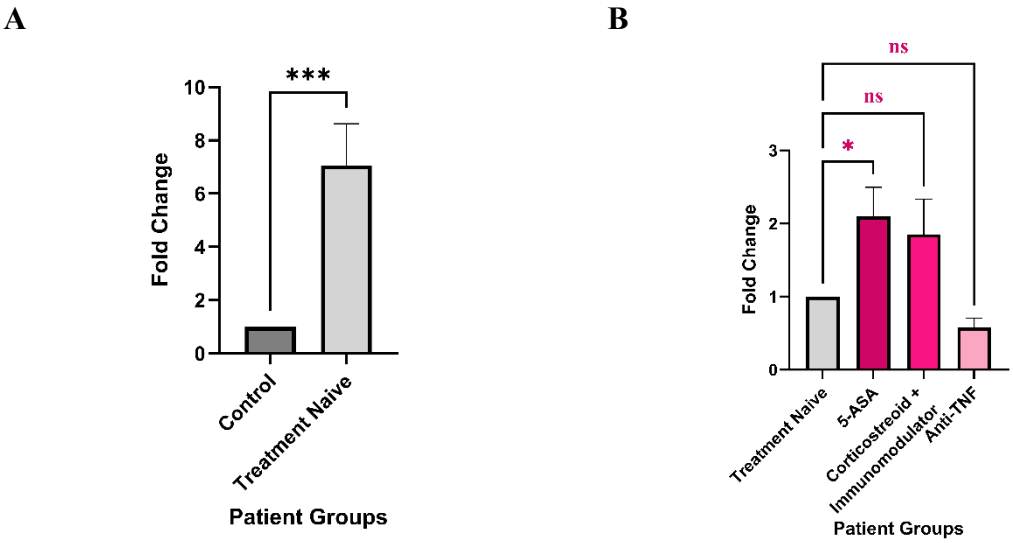


Figure 7| Expression of *ANXA5* in Tissues of Treated Patients and Controls. (A) Expression of *ANXA5* in treatment naïve group vs control group: In UC patients, *ANXA5* gene expression increases 7.065 -fold compared to the control group (p-value = 0.0003). **(B)** Expression of *ANXA5* in treatment groups vs treatment naïve group: Long-term use (>5 years) of 5-ASA increases *ANXA5* expression by approximately 2.096-fold (Adjusted p-value = 0.0236). Corticosteroids and immunomodulators, when used for more than 5 years, increase *ANXA5* expression by about 1.8545-fold (Adjusted p-value = 0.1489). Anti-TNF drugs, with usage of 5 years or more, increase *ANXA5* expression by approximately 0.578-fold compared to healthy controls (Adjusted p-value = 0.6767). *ANXA5*: annexin A5; Error bars in the charts represent the standard error of the mean (SEM); * P = 0.05, ** P = 0.01, ** P < 0.001, **** P < 0.0001

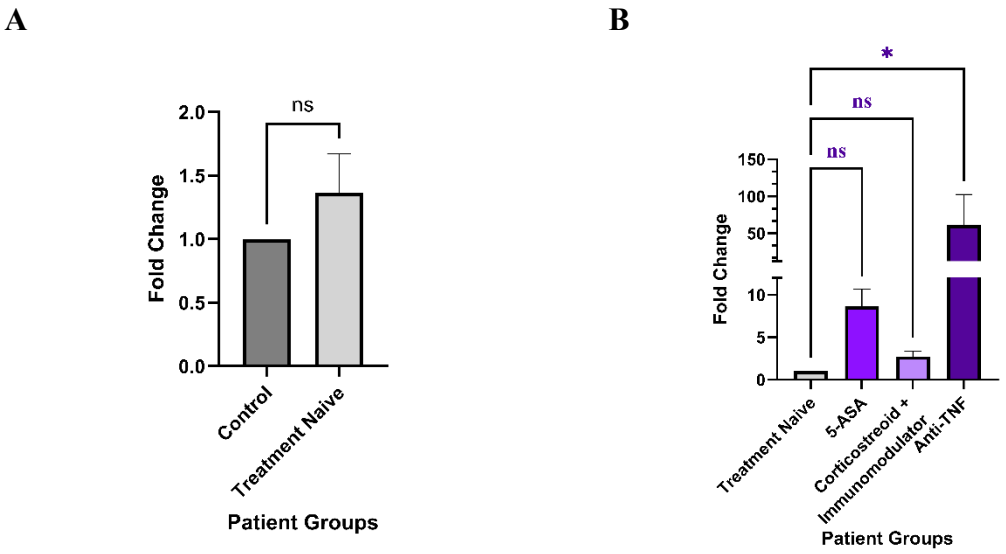


Figure 8| Expression of *CAV1* in Tissues of Treated Patients and Controls. **(A)** Expression of *CAV1* in treatment naïve group vs control group: In UC patients, *CAV1* gene expression increases 1.367-fold compared to the control group (p-value = 0.2374). **(B)** Expression of *CAV1* in treatment groups vs treatment naïve group: Long-term use (>5 years) of 5-ASA increases *CAV1* expression by approximately 8.606-fold (Adjusted p-value = 0.9780). Corticosteroids and immunomodulators, when used for more than 5 years, increase *CAV1* expression by about 2.742-fold (Adjusted p-value = 0.9998). Anti-TNF drugs, with usage of 5 years or more, increase *CAV1* expression by approximately 61.09 -fold compared to healthy controls (Adjusted p-value = 0.0479). *CAV1*: caveolin 1; Error bars in the charts represent the standard error of the mean (SEM); * P = 0.05, ** P = 0.01, ** P < 0.001, **** P < 0.0001

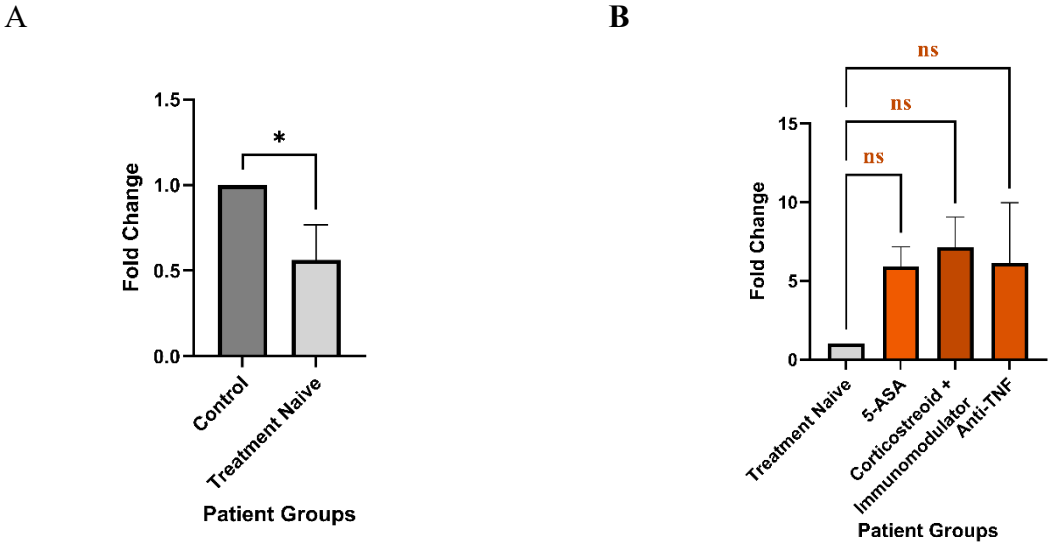


Figure 9| Expression of *TLR2* in Tissues of Treated Patients and Controls. (A) Expression of *TLR2* in treatment naïve group vs control group: In UC patients, *TLR2* gene expression increases 0.5624-fold compared to the control group (p-value = 0.0390). **(B)** Expression of *TLR2* in treatment groups vs treatment naïve group: Long-term use (>5 years) of 5-ASA increases *TLR2* expression by approximately 5.933-fold (Adjusted p-value = 0.1365). Corticosteroids and immunomodulators, when used for more than 5 years, increase *TLR2* expression by about 7.136-fold (Adjusted p-value = 0.0763). Anti-TNF drugs, with usage of 5 years or more, increase *TLR2* expression by approximately 6.117-fold compared to healthy controls (Adjusted p-value = 0.1686). *TLR2*: toll-like receptor 2; Error bars in the charts represent the standard error of the mean (SEM); * P = 0.05, ** P = 0.01, ** P < 0.001, **** P < 0.0001

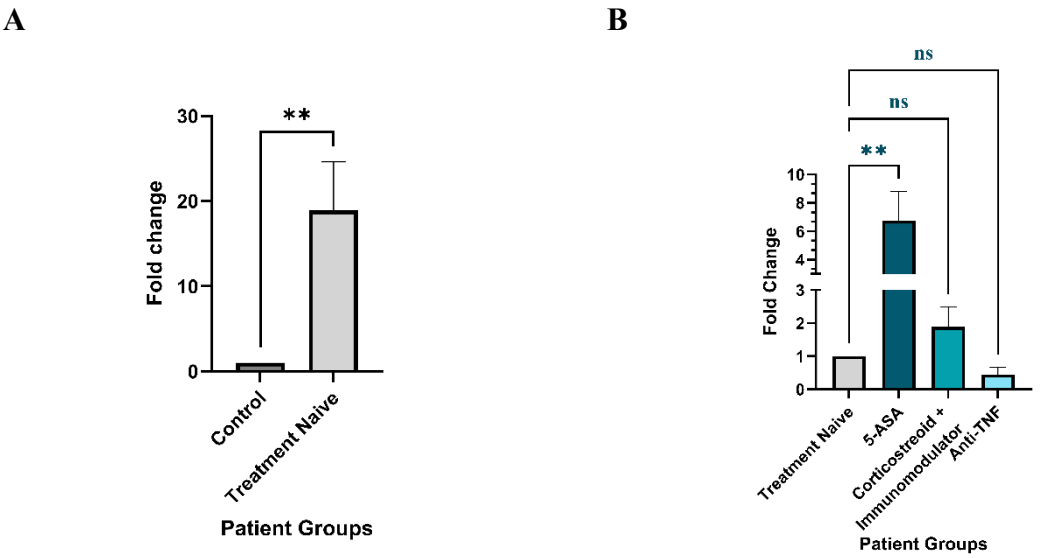


Figure 10| Expression of *VCAM1* in Tissues of Treated Patients and Controls. (A) Expression of *VCAM1* in treatment naïve group vs control group: In UC patients, *VCAM1* gene expression increases 18.9-fold compared to the control group (p-value = 0.0032). **(B)** Expression of *VCAM1* in treatment groups vs treatment naïve group: Long-term use (>5 years) of 5-ASA increases *VCAM1* expression by approximately 6.747-fold (Adjusted p-value = 0.0011). Corticosteroids and immunomodulators, when used for more than 5 years, increase *VCAM1* expression by about 1.896-fold (Adjusted p-value = 0.9161). Anti-TNF drugs, with usage of 5 years or more, reduce *VCAM1* expression by approximately 0.454-fold compared to healthy controls (Adjusted p-value = 0.9786) *VCAM1*: vascular cell adhesion molecule; Error bars in the charts represent the standard error of the mean (SEM); * P = 0.05, ** P = 0.01, ** P < 0.001, **** P < 0.0001

Table 4. Expression and logFC of candidate genes

Candidate Genes	Microarray Data Analysis			Expression Mean			
	CAC	CRC	UC	Treatment Naive	5-ASA	corticosteroids / immunomodulators	anti-TNF
SERPINE1	1.296 *	1.230 *	1.228 *	92.55 *	4.195	15.02 *	5.248
COL1A1	1.508 *	1.578 *	1.591 *	18.4 *	47.04 *	10.118	10.831
MMP9	2.006 *	1.714 *	1.919 *	4.077 *	5.772	3.055	6.466
IL1B	2.402 *	1.871 *	2.095 *	10.95 *	6.223	8.494 *	1.579
ANXA5	1.150 *	0.898	0.979	7.065 *	2.096 *	1.854	0.578
CAV1	1.124 *	0.739	0.888	1.367	8.606	2.742	61.09 *
TLR2	1.074 *	0.801	0.838	0.562 *	5.933	7.136	6.117
VCAM1	1.090 *	0.579	0.884	18.9 *	6.747 *	1.896	0.454

Abbreviations: *SERPINE1*: serpin family E member 1; *COL1A1*: collagen type I alpha 1 chain; *MMP9*: matrix metallopeptidase 9; *IL1B*: interleukin 1 beta; *ANXA5*: annexin A5; *CAV1*: caveolin 1; *TLR2*: toll-like receptor 2; *VCAM1*: vascular cell adhesion molecule; 5-ASA: 5-Aminosalicylates; UC: Ulcerative Colitis; CRC: Colorectal Cancer; CAC: Colitis-Associated Colorectal Cancer; *: significant

Discussion

CAC, a serious complication of IBD, poses a global health challenge and imposes a heavy burden on healthcare systems. Chronic intestinal inflammation leads to cellular changes and increases CAC risk, necessitating regular screening and anti-inflammatory therapies. This issue is expanding

in both developed and developing regions, highlighting the need for global policies to control inflammation and monitor complications (20). Recent genetic studies on CAC have elucidated its molecular mechanisms. Studies have identified specific mutations and chromosomal abnormalities in CAC, distinguishing it from sCRC under the influence of chronic inflammation, emphasizing the importance of biomarkers for early detection and targeted therapies (21). CAC risk rises significantly with disease duration and extent of colonic involvement, laying the foundation for routine surveillance (22).

The present study aimed to predict the effects of related therapies on CAC by examining DEGs through bioinformatics and laboratory methods. A key difference from prior studies is the focus on the impact of UC medications by evaluating molecular biomarkers on CAC, which has received less attention. We utilized multiple datasets for more comprehensive analyses and extracted genetic overlaps between UC, sCRC, and CAC, opening new avenues for prevention. Some of the previous studies have assessed the clinical effects of different drugs on UC patients, studied epidemiologically. According to these studies, 5-ASA (especially mesalazine) significantly reduces CAC risk, with greater efficacy at higher doses (23). Another study found that long-term thiopurines (>5 years) increase malignancy risk (skin cancer and lymphoma) 1.9-fold in IBD, in a dose- and duration-dependent manner (24). Small molecules (JAK inhibitors and S1PR modulators) in elderly IBD patients may elevate malignancy risk, but evidence is limited and appear to carry a lower risk than thiopurines (25). Biologics like anti-TNF indirectly reduce CAC risk by alleviating chronic inflammation, the key driver of neoplastic changes, though immunosuppression may raise other malignancies (lymphoma, NMSC) (26). Prior investigations are subject to certain limitations such as scarce data on long-term drug effects on key UC/CAC gene expression, often confined to diagnostic aspects.

For prediction of the effects of related therapies on carcinogenesis, eight biomarker genes identified in this study: shared among UC/sCRC/CAC (*SERPINE1*, *COL1A1, MMP9, IL1B*) and CAC-exclusive (*ANXA5, CAV1, TLR2, VCAM1*). These genes drive inflammation, fibrosis, EMT, angiogenesis, and cell migration, facilitating tumorigenesis and CAC pathways progression (27-41). Bioinformatics analysis revealed significant upregulation of *SERPINE1*, *COL1A1*, *MMP9*, and *IL1B* in UC tissues relative to healthy controls. These findings were corroborated in the in vitro assessments in which increased expression was observed in drug-naïve UC patients. *ANXA5*, *TLR2* and *VCAM1* also exhibited significant differential expression in in vitro results between UC patients and controls, yet bioinformatic evaluations demonstrated no significant differences between UC and control groups. In contrast bioinformatics and in vitro findings for CAV1 gene were aligned. Discrepancies for *ANXA5, TLR2* and *VCAM1* expression in bioinformatics and in vitro likely reflect differences in dataset composition, statistical thresholds, and biological variability across cohorts. Bioinformatics pipelines often apply stringent cutoffs that may mask subtle but biologically relevant changes, whereas experimental assays can detect expression differences under diverse conditions. Moreover, the close pathological relationship between colitis and CAC supports the notion that some CAC-exclusive genes may participate in both

inflammatory and carcinogenic processes. Thus, while bioinformatics provides valuable hypotheses, experimental validation remains essential to uncover the broader biological roles of candidate genes. Building on these insights into gene-drug interactions, the following analysis contrasts our molecular findings with established evidence on pharmacologic modulation of CAC risk, while acknowledging study limitations and proposing avenues for future research.

Previous studies have shown that 5-ASA (especially mesalamine) significantly reduces CAC risk in a dose-dependent manner, while in our study, it failed to reduce expression of all candidate genes of this study, maintained any of them near control and upregulated *COL1A1*, *ANXA5*, and *VCAM1* significantly suggesting that these drugs may influence multiple biological pathways beyond their intended anti-inflammatory effects. Increased *COL1A1* expression is linked to enhanced epithelial–mesenchymal transition, blood-vessel development, and heightened cellular responsiveness to TNF-α, all of which may contribute to tissue remodeling and a pro-inflammatory microenvironment (29). Upregulation of *ANXA5*, which negatively regulates apoptosis and coagulation, may promote cell survival and persistence of damaged or dysplastic cells (34, 35). Similarly, elevated *VCAM1* expression can augment B-cell differentiation, T-cell proliferation, cell adhesion, and chronic inflammatory signaling (40, 41). Collectively, these expression changes raise the possibility that, under certain conditions, 5-ASA could inadvertently potentiate inflammatory pathways and microenvironmental alterations that are associated with carcinogenic processes. however further mechanistic studies are required.

Evidence from previous studies suggests that prolonged exposure to corticosteroids and immunomodulators, including thiopurines, particularly beyond five years of use, may elevate the risk of malignancy whereas in this study, corticosteroid and immunomodulatory treatments were associated with a broad upregulation of all eight candidate genes, with particularly pronounced and significant increases observed for *SERPINE1* and *IL1B*. Elevated *SERPINE1* expression is known to enhance angiogenesis, fibrinolysis, cell migration, and monocyte chemotaxis, processes that collectively contribute to tissue remodeling and a pro-inflammatory microenvironment (27, 28). Similarly, increased *IL1B* expression promotes inflammatory signaling and leukocyte recruitment, both of which can sustain or amplify chronic inflammation (32, 33). The simultaneous induction of these pathways, together with the upregulation of the other genes in our panel, suggests that corticosteroids and immunomodulatory agents may exert complex, context-dependent effects on mucosal biology. The observed gene expression profile raises the possibility that, in certain settings, they could reinforce inflammatory activity and microenvironmental dynamics associated with early carcinogenic processes. Although causality cannot be inferred from gene expression alone, these findings underscore the need for further investigation into the long-term molecular consequences of these therapies.

Anti-TNF therapy did not lead to uniform upregulation of all eight genes assessed in this study, decreased expression of some genes such as *ANXA5* and *VCAM1* and the magnitude of expression changes was generally milder compared with those observed following treatment with other drug classes. Notably, only *CAV1* exhibited a significant increase in expression in response to anti-TNF

agents. Given that *CAV1* downregulates the MAPK cascade and JAK–STAT signaling while enhancing gap-junction assembly and reducing cellular proliferation, its selective induction may reflect a beneficial modulation of pathways involved in inflammation and tissue homeostasis (36, 37). Importantly, emerging evidence identifies *CAV1* as a key regulator of gut barrier integrity, a function that is particularly relevant in UC, where epithelial barrier dysfunction is a hallmark of disease pathogenesis (42). The observed expression pattern suggests that anti-TNF drugs may exert protective effects against inflammation-driven carcinogenic processes rather than contributing to malignancy risk. These findings align with clinical data indicating that anti-TNF therapy does not increase and may potentially reduce the long-term risk of malignancy in patients with chronic inflammatory conditions.

Acknowledging these comparative insights and their implications for CAC risk modulation, it is essential to address the inherent limitations of the present study that may temper the generalizability and interpretive depth of our findings. Study limitations included reduced sample size due to the 5-year drug threshold and anti-TNF resistance (lowering statistical power), lack of RNA-Seq (using less sensitive microarray), and absence of proteomics due to technical/cost constraints, and the lack of complementary protein data is considered a limitation of this study. To mitigate these limitations and advance the translational relevance of our findings, the following recommendations are proposed for future investigations. 1) Larger multicenter/international cohorts; 2) Blood analysis alongside tissue; 3) RNA-Seq implementation; 4) Colorectal cancer in Crohn's disease; 5) Proteomics integration; 6) Animal models (DSS-induced UC/CAC); 7) AI-driven multi-omics integration.

Conclusion

In summary, this study provides an integrated bioinformatic and experimental assessment of eight key genes at the intersection of UC, sCRC, and CAC, offering new insights into how commonly used UC therapies may modulate molecular pathways involved in inflammation-driven carcinogenesis. Our findings highlight that, despite the well-established clinical benefits of 5-ASA, corticosteroids, immunomodulators, and anti-TNF agents in controlling intestinal inflammation, these drugs exert distinct and sometimes divergent effects on CAC-related gene expression. While 5-ASA and immunosuppressive therapies markedly upregulated several genes involved in angiogenesis, EMT, immune activation, and tissue remodeling (changes that may, under specific conditions, contribute to a pro-tumorigenic microenvironment), anti-TNF treatment demonstrated a more favorable molecular profile. Notably, anti-TNF agents significantly induced only *CAV1*, a gene increasingly recognized for its crucial role in restoring gut barrier integrity and mitigating inflammation-associated neoplastic risk. These differential patterns underscore the complexity of drug–gene interactions in UC and emphasize that therapeutic modulation of inflammation does not uniformly translate to reduced carcinogenic potential. Future research using larger, multicenter cohorts and multi-omics approaches, including RNA-Seq, proteomics, and integrative AI-driven

analyses, will be essential to refine these observations and clarify their translational significance. Ultimately, a deeper understanding of therapy-gene interactions may enhance personalized medicine strategies, improve risk stratification, and contribute to more effective prevention of CAC.

Abbreviations: IBD, inflammatory bowel disease; CD, crohn's disease; UC, ulcerative colitis; CRC, colorectal cancer; CAC, colitis-associated colorectal cancer; DEG, differentially expressed gene; PSC, primary sclerosing cholangitis; 5-ASA, 5-aminosalicylates; ECCO, european crohn's and colitis organization; HSTCL, hepatosplenic T-cell lymphoma; NMSC, non-melanoma skin cancer; AML, acute myeloid leukemia; Anti-TNF, anti-tumor Necrosis factor; PCA, principal component analysis; GEO, gene expression omnibus; GO, gene ontology; PPI, protein–protein interaction; cDNA, complementary DNA; qRT-PCR, quantitative real-time polymerase chain reaction; MNC, maximum neighborhood component; DMNC, density of maximum neighborhood component; MCC, maximal clique centrality; EPC, edge percolated component

Declarations

Ethics and consents

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Isfahan University of Medical under the approval code IR.MUI.MED.REC.1402.451. Written informed consent was obtained from the patients for the participance and for the publication of any identifying images or clinical details that could potentially compromise their anonymity.

Data availability statement

All data generated or analysed during this study are available from the corresponding author on reasonable request.

Conflict of Interests

The authors declare there are no competing interests.

Funding sources

This work was supported by Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences Tehran Iran.

Author Contributions

MK and NK designed the project. SP conducted the data analysis, performed the in vitro experiments, and drafted the initial manuscript. MK, ShB and NK reviewed and revised the manuscript.

Acknowledgements

We acknowledge the use of Chat-GPT (GPT-5) for assisting in refining the content of this article to enhance clarity and conciseness.

References

1. Muller M, Hansmannel F, Arnone D, Choukour M, Ndiaye NC, Kokten T, et al. Genomic and molecular alterations in human inflammatory bowel disease-associated colorectal cancer. United European Gastroenterol J. 2020;8(6):675-84.
2. Olfatifar M, Zali MR, Pourhoseingholi MA, Balaii H, Ghavami SB, Ivanchuk M, et al. The emerging epidemic of inflammatory bowel disease in Asia and Iran by 2035: A modeling study. BMC Gastroenterol. 2021;21(1):204.
3. Kaenkumchorn T, Wahbeh G. Ulcerative Colitis: Making the Diagnosis. Gastroenterol Clin North Am. 2020;49(4):655-69.
4. Shahgoli VK, Noorolyai S, Ahmadpour Youshanlui M, Saeidi H, Nasiri H, Mansoori B, et al. Inflammatory bowel disease, colitis, and cancer: unmasking the chronic inflammation link. Int J Colorectal Dis. 2024;39(1):173.
5. Sato Y, Tsujinaka S, Miura T, Kitamura Y, Suzuki H, Shibata C. Inflammatory Bowel Disease and Colorectal Cancer: Epidemiology, Etiology, Surveillance, and Management. Cancers (Basel). 2023;15(16).
6. Fantini MC, Guadagni I. From inflammation to colitis-associated colorectal cancer in inflammatory bowel disease: Pathogenesis and impact of current therapies. Dig Liver Dis. 2021;53(5):558-65.
7. Axelrad JE, Hashash JG, Itzkowitz SH. AGA Clinical Practice Update on Management of Inflammatory Bowel Disease in Patients With Malignancy: Commentary. Clin Gastroenterol Hepatol. 2024;22(7):1365-72.
8. Aslam N, Lo SW, Sikafi R, Barnes T, Segal J, Smith PJ, Limdi JK. A review of the therapeutic management of ulcerative colitis. Therap Adv Gastroenterol. 2022;15:17562848221138160.
9. Dan WY, Zhou GZ, Peng LH, Pan F. Update and latest advances in mechanisms and management of colitis-associated colorectal cancer. World J Gastrointest Oncol. 2023;15(8):1317-31.
10. Mason M, Siegel CA. Do inflammatory bowel disease therapies cause cancer? Inflamm Bowel Dis. 2013;19(6):1306-21.
11. Greuter T, Rieder F, Kucharzik T, Peyrin-Biroulet L, Schoepfer AM, Rubin DT, Vavricka SR. Emerging treatment options for extraintestinal manifestations in IBD. Gut. 2021;70(4):796-802.
12. AlAmeel T, AlMutairdi A, Al-Bawardy B. Emerging Therapies for Ulcerative Colitis: Updates from Recent Clinical Trials. Clin Exp Gastroenterol. 2023;16:147-67.
13. Nazerai L, Willis SC, Yankilevich P, Di Leo L, Bosisio FM, Frias A, et al. Thiopurine 6TG treatment increases tumor immunogenicity and response to immune checkpoint blockade. Oncoimmunology. 2023;12(1):2158610.

14. Long MD, Martin CF, Pipkin CA, Herfarth HH, Sandler RS, Kappelman MD. Risk of melanoma and nonmelanoma skin cancer among patients with inflammatory bowel disease. Gastroenterology. 2012;143(2):390-9.e1.
15. Jussila A, Virta LJ, Pukkala E, Färkkilä MA. Malignancies in patients with inflammatory bowel disease: a nationwide register study in Finland. Scand J Gastroenterol. 2013;48(12):1405-13.
16. Noor NM, Lee JC, Bond S, Dowling F, Brezina B, Patel KV, et al. A biomarker-stratified comparison of top-down versus accelerated step-up treatment strategies for patients with newly diagnosed Crohn's disease (PROFILE): a multicentre, open-label randomised controlled trial. Lancet Gastroenterol Hepatol. 2024;9(5):415-27.
17. Kayal M, Shah S. Ulcerative Colitis: Current and Emerging Treatment Strategies. J Clin Med. 2019;9(1).
18. Yang C, Huang J, Huang X, Huang S, Cheng J, Liao W, et al. Risk of Lymphoma in Patients With Inflammatory Bowel Disease Treated With Anti-tumour Necrosis Factor Alpha Agents: A Systematic Review and Meta-analysis. J Crohns Colitis. 2018;12(9):1042-52.
19. Ono K, Fong D, Gao C, Churas C, Pillich R, Lenkiewicz J, et al. Cytoscape Web: bringing network biology to the browser. Nucleic Acids Research. 2025;53(W1):W203-W12.
20. Ekbom A, Helmick C, Zack M, Adami HO. Ulcerative colitis and colorectal cancer. A population-based study. N Engl J Med. 1990;323(18):1228-33.
21. Chatila WK, Walch H, Hechtman JF, Moyer SM, Sgambati V, Faleck DM, et al. Integrated clinical and genomic analysis identifies driver events and molecular evolution of colitis-associated cancers. Nat Commun. 2023;14(1):110.
22. Zhou RW, Harpaz N, Itzkowitz SH, Parsons RE. Molecular mechanisms in colitis-associated colorectal cancer. Oncogenesis. 2023;12(1):48.
23. Qiu X, Ma J, Wang K, Zhang H. Chemopreventive effects of 5-aminosalicylic acid on inflammatory bowel disease-associated colorectal cancer and dysplasia: a systematic review with meta-analysis. Oncotarget. 2017;8(1):1031-45.
24. Zheng KYC, Guo CG, Wong IOL, Chen L, Chung HY, Cheung KS, Leung WK. Risk of malignancies in patients with inflammatory bowel disease who used thiopurines as compared with other indications: a territory-wide study. Therap Adv Gastroenterol. 2020;13:1756284820967275.
25. Strigáč A, Caban M, Małecka-Wojciesko E, Talar-Wojnarowska R. Safety and Effectiveness of Thiopurines and Small Molecules in Elderly Patients with Inflammatory Bowel Diseases. J Clin Med. 2024;13(16).
26. Williams CJ, Peyrin-Biroulet L, Ford AC. Systematic review with meta-analysis: malignancies with anti-tumour necrosis factor-α therapy in inflammatory bowel disease. Aliment Pharmacol Ther. 2014;39(5):447-58.
27. Chen TY, Zhou M, Lin MQ, Liang ST, Yan Y, Wang SM, et al. Research Progress on the SERPINE1 Protein and Chronic Inflammatory Diseases of the Upper Respiratory Tract: A Literature Review. Int Arch Allergy Immunol. 2021;182(11):1097-102.
28. Lopez-Legarrea P, Mansego ML, Zulet MA, Martinez JA. SERPINE1, PAI-1 protein coding gene, methylation levels and epigenetic relationships with adiposity changes in obese subjects with metabolic syndrome features under dietary restriction. J Clin Biochem Nutr. 2013;53(3):139-44.

29. Li X, Sun X, Kan C, Chen B, Qu N, Hou N, et al. COL1A1: A novel oncogenic gene and therapeutic target in malignancies. Pathol Res Pract. 2022;236:154013.
30. Regalado CR, Balogh M. MMP9: Link between neuropathy and colorectal cancer? Front Mol Biosci. 2024;11:1451611.
31. Van den Steen PE, Dubois B, Nelissen I, Rudd PM, Dwek RA, Opdenakker G. Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9). Crit Rev Biochem Mol Biol. 2002;37(6):375-536.
32. Jo EK, Kim JK, Shin DM, Sasakawa C. Molecular mechanisms regulating NLRP3 inflammasome activation. Cell Mol Immunol. 2016;13(2):148-59.
33. Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. Annu Rev Immunol. 2009;27:519-50.
34. Jin M, Zhang J, Sun Y, Liu G, Wei X. ANXA5: related mechanisms of osteogenesis and additional biological functions. Front Cell Dev Biol. 2025;13:1553683.
35. Hua R, Qiao G, Chen G, Sun Z, Jia H, Li P, et al. Single-Cell RNA-Sequencing Analysis of Colonic Lamina Propria Immune Cells Reveals the Key Immune Cell-Related Genes of Ulcerative Colitis. J Inflamm Res. 2023;16:5171-88.
36. Simón L, Campos A, Leyton L, Quest AFG. Caveolin-1 function at the plasma membrane and in intracellular compartments in cancer. Cancer Metastasis Rev. 2020;39(2):435-53.
37. Song Y, Xie F, Ma S, Deng G, Li Y, Nie Y, et al. Caveolin-1 protects against DSS-induced colitis through inhibiting intestinal nitrosative stress and mucosal barrier damage in mice. Biochem Pharmacol. 2020;180:114153.
38. Shuto T. [Regulation of expression, function, and inflammatory responses of innate immune receptor Toll-like receptor-2 (TLR2) during inflammatory responses against infection]. Yakugaku Zasshi. 2013;133(12):1401-9.
39. Frolova L, Drastich P, Rossmann P, Klimesova K, Tlaskalova-Hogenova H. Expression of Toll-like receptor 2 (TLR2), TLR4, and CD14 in biopsy samples of patients with inflammatory bowel diseases: upregulated expression of TLR2 in terminal ileum of patients with ulcerative colitis. J Histochem Cytochem. 2008;56(3):267-74.
40. Singh V, Kaur R, Kumari P, Pasricha C, Singh R. ICAM-1 and VCAM-1: Gatekeepers in various inflammatory and cardiovascular disorders. Clin Chim Acta. 2023;548:117487.
41. Pickett JR, Wu Y, Ta HT. VCAM-1 as a common biomarker in inflammatory bowel disease and colorectal cancer: unveiling the dual anti-inflammatory and anti-cancer capacities of anti-VCAM-1 therapies. Cancer Metastasis Rev. 2025;44(2):40.
42. Yu M, Zhang J, Lin B, Zhu W, Song X, Pan J, et al. DJ-1 counteracts Caveolin-1-mediated necroptosis to inhibit epithelial barrier dysfunction in colitis. Cell Death Dis. 2025;16(1):657.

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