Intestinal Stem and Progenitor Cells Exhibit Distinct Adaptive Responses to Inflammatory Stress in IBD

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Abstract

Background

Intestinal epithelial stem cells (SCs) and their transit-amplifying (TA) progeny are critical for mucosal repair and regeneration. However, their behaviour under chronic inflammatory conditions, such as those observed in Inflammatory Bowel Disease (IBD), remains incompletely understood.

Methods

We investigated the impact of chronic inflammation on intestinal stem/progenitor cells by integrating bulk RNA sequencing from the largest IBD biopsy cohort to date with single-cell transcriptomic analysis and experimental assays using patient-derived intestinal organoids.

Results

Active inflammation was associated with a reduction in canonical LGR5$^+$ intestinal stem cells and a concurrent expansion of OLFM4$^+$ populations, consistent with an inflammation-induced epithelial repair program. Notably, SC/TA cells from both inflamed and non-inflamed IBD tissues exhibited persistent transcriptional changes that were distinct from those in healthy controls. Single-cell analysis identified transcriptionally heterogeneous SC/TA subpopulations, including a previously uncharacterized inflammation-associated cluster enriched in immune signalling pathways. Pseudotime trajectory analysis demonstrated a shift in differentiation toward deep crypt secretory (Paneth-like) cell lineages under inflammatory conditions.

Conclusions

Chronic intestinal inflammation reshapes the epithelial stem and progenitor cell compartment, promoting altered differentiation and the emergence of immune-responsive epithelial states. These findings highlight the plasticity of the human intestinal epithelium in IBD and point to new avenues for therapeutic strategies aimed at maintaining epithelial integrity during chronic inflammation.

53 Introduction

Inflammatory Bowel Disease (IBD) is increasingly recognized by the World Health Organization

as a major global health concern, especially given its rising prevalence in high-income

countries and growing incidence among younger populations [1-3]. IBD encompasses chronic,

relapsing-remitting inflammatory disorders of the gastrointestinal tract, with Crohn's disease

(CD) and Ulcerative Colitis (UC) being the primary subtypes. These conditions are driven by

an abnormal immune response directed at the intestinal lining, resulting in symptoms such as

abdominal pain, diarrhea, fatigue, and weight loss. Over time, approximately 70% of individuals

with CD and 20–30% of those with UC require surgical intervention due to complications like

strictures, fistulas, abscesses, or failure of medical therapy and malignancy [2,3]. Although the

exact aetiology of IBD remains unresolved, current evidence supports a multifactorial origin

involving genetic susceptibility, environmental triggers, and immune system dysregulation.

During chronic inflammation, epithelial cells are persistently exposed to pro-inflammatory

cytokines and oxidative stress, leading to the activation of signalling pathways such as Hippo-

YAP1 and Wnt/β-catenin, which are crucial for maintaining intestinal homeostasis[4-6]. This

inflammatory environment compromises the gut barrier by altering epithelial function and

increasing tissue permeability, thereby intensifying immune responses [7,8]. These changes

negatively impact the proliferation of intestinal stem cells (SC) and transit-amplifying

progenitors (TA), while also promoting cell death, ultimately weakening epithelial integrity [9].

SC, which reside at the intestinal crypts, are essential for sustaining epithelial renewal by

generating TA that divide and differentiate into the diverse mature cell types lining the intestine

[10-12]. It is unclear how SC and TA (SC/TA) attempt to repair or adapt to the inflammatory

environment, or what specific signals drive their response in a timely manner in healthy and

IBD tissue. It is particularly relevant to know how these cells adapt under stress: whether they

shift towards rapid proliferation to replace damaged cells, lean towards self-renewal to

preserve the stem cell pool or undergo differentiation.

Bestrophin-4 (BEST4)[+] cells, are a subpopulation of mature absorptive epithelial cells

characterized by the expression of the ion channel BEST4[13]. These cells have an important

physiological relevance because their unique gene expression profile shows that they have a

specialized functions in pH regulation, electrolyte transport, antimicrobial defense, and

immune communication[14]. BEST4^{+} cells are emerging as an important cell type in IBD and

their loss in inflammation has been reported[14–16], Disruptions among BEST4^{+} cells could impact

barrier function, antimicrobial defense, and local immune environments, processes highly

relevant to IBD pathophysiology. However, the underlying mechanisms, functional

consequences, and potential for barrier permeability and regeneration remains unclear. One

of the main reasons for limited progress in this area is the absence of BEST4^{+} cells in mice[17–]

[20]. Integrating them into our human studies of SC/TA populations is relevant for better

understanding the origins of BEST4^{+} as they may arise from distinct stem or transit-amplifying

cells and understanding their role during chronic inflammation. Overall, the functional

relevance of SC/TA and BEST4^{+} cells and its dynamic changes in IBD could reveal key insights

into tissue repair, novel therapeutical approaches and the understanding of IBD progression.

In this study, we set out to identify the dynamic molecular response of intestinal SC, TA and

BEST4[+] epithelial cell types during inflammation by comparative analysis of omics data and

experimental approaches using IBD patients tissue data, DSS-treated UC mouse models and

IBD patient-derived organoids (PDOs). Using our multimodal approach, we assessed changes

in cell type markers, identified biological processes, and pathway modulations associated with

inflammation. Our approach offers the first evidence in a controlled setting to trace molecular

signatures and adaptive responses of distinct epithelial molecular clusters in relation to

inflammation severity, providing valuable insights into how inflammatory processes disrupt

SC/TA epithelial function and contribute to IBD progression. Modulation of these cell types

reflects a shift in stemness and differentiation pathways, where specific OLFM4$^+$ SC/TA cells

increasingly remain in a proliferative state under heightened inflammation, rather than

progressing toward full epithelial maturation. Moreover, BEST4$^+$ mature cells showed a slight

increase in low-inflammation tissue, however, their numbers were significantly downregulated

under high inflammatory conditions. These cells may play a regulatory role in maintaining

epithelial homeostasis and their reduction during severe inflammation could contribute to

disease progression in IBD. This epithelial remodelling highlights a potential pathogenic

imbalance between stemness and differentiation in both inflamed and non-inflamed IBD

epithelium. Furthermore, we detected that the differentiation trajectory of IBD stem cells is

skewed toward the deep crypt secretory cell lineage, also referred to as colonic Paneth-like

cells, indicating an aberrant differentiation process. Collectively, our findings demonstrate for

the first time how intestinal stem cell and progenitor epithelial cell types actively engages in

adaptive responses to inflammation, balancing proliferation and differentiation processes that

are critical for tissue repair and epithelial.

118 Results

Profiling epithelial cell heterogeneity based on inflammation severity

To understand the epithelial cell heterogeneity under varying degrees of inflammation, we

investigated the cell type distribution based on macroscopic appearance of IBD tissue. To that

end, we utilized bulk-RNA sequencing (RNAseq) data from the largest cohort of IBD patients'

biopsies analysed to date (IBD Plexus Study of a Prospective Adult Research Cohort, SPARC

IBD) (UC=1,097, CD=2,138) of the Crohn's & Colitis Foundation IBD Plexus program which

includes clinical, genetic, molecular, environmental, and patient-reported outcomes from

individuals with CD or UC[21] (Figure S1A).

We employed deconvolution analysis using CIBERSORTx to quantitatively estimate the

epithelial, immune, and stromal cells type proportion with a signature matrix constructed using

single cell-RNAseq (sc-RNAseq) data from human intestine as reference[15] (Figure 1A and

S1B). The overall quantitative relative abundance of cycling TA, and immature enterocytes (2)

pool involved mainly in metabolic processes, decreased with increasing inflammation severity

reflecting an altered capacity within the epithelial lining. However, there was an increase in

immature enterocytes (1), which are involved in tight junction repair during inflammation[22].

This rise may be a compensatory mechanism, aiming to restore barrier integrity despite the

diminished support from the TA pool. In parallel, M cells, key players in mucosal immune

surveillance, were significantly enriched in inflamed tissues, consistent with heightened

immune activity (Figure 1B and S1C). Well-characterized SC, TA, BEST4+ cell type markers

and/or Wnt pathway target genes, such as *LGR5*, *AXIN2*, *EPHB2, ASCL2, PROM1*, *LRIG1* and

BEST4, were significantly lower expressed in inflammation vs normal or possible inflammation

of UC (Figure 1C, D) and CD (Figure S1D, E). In contrast, *OLFM4*, a key SC/TA marker strongly

associated with cell proliferation and rapid cellular renewal [23], increased as inflammation

intensified (Figure 1C, D and S1D, E). Clusterin (*CLU*) 'revival stem cell' pool marker involved

in tissue regeneration after injury[24] increased with inflammation severity (Figure 1C and S1D).

This suggested an adaptive response, where OLFM4+ proliferative cells in IBD tissue aim to

counterbalance the loss of epithelial integrity caused by inflammation.

To understand better the healthy SC/TA response to an initial signal of injury and compare it

to IBD tissue response, we analysed the DSS-mouse model bulk-RNAseq[25] (GSE22307), and

the sc-RNAseq [26](GSE264408) data where EPCAM+ epithelial cells were extracted from the

total cell population (Figure 1F). SC/TA and Wnt gene expression markers as *Lgr5*, *Sox9*,

Ephb2 and *Axin2* decreased over the course of inflammation, indicating a general decline

within the intestinal tissue *in vivo* that are altered by an inflammatory stimulus. Lgr5^{+} stem cells

tend to recover at day 4, reflecting an initial response to restore this pool in healthy epithelium.

We identified an upregulation *of Clu* expression correlated with rising levels of S100 Calcium-

Binding Protein A9 (*S100A9*), a biomarker commonly upregulated in inflammatory diseases[27]

(Figure 1E-G). Interestingly, Olfm4 increased at the protein level as inflammation intensified in

the small intestine of DSS-treated mice (Figure 1H). These findings show how SC/TA pools

balance regeneration and barrier integrity during inflammation in healthy and IBD tissue.

Characterization of epithelial cell types in IBD vs healthy tissue using a 3D patient-

derived organoid model

To investigate epithelial cell heterogeneity in IBD cells in absence of active inflammatory

conditions, we derived 3D-intestinal epithelial organoids from IBD patient's biopsies that retain

the unique genetic and phenotypic characteristics of patient's tissue that had been affected

by chronic inflammation[28–30] (Figure 1A). The expansion of these cells allowed us to create

simplified *in vitro* IBD models that maintain active stem cell niches, and ongoing differentiation

characteristic of non-inflamed (IBD normal) patients' cells (Figure 2A). We performed bulk-

RNAseq of healthy (hPDOs) derived from healthy individuals and IBD-PDOs derived from IBD

patient's tissue: IBD patient 89 (P89), P90 and P99. All the IBD-PDOs exhibited similar gene

expression profiles, with P99 displaying a more pronounced inflammatory phenotype

(Supplementary Figure 2A-C). Healthy and IBD-P99 organoids of early (passage 3) and late

passages (passage 10) were sequenced to assess potential differences in molecular

characteristics over time in culture. This approach allowed us to determine that passage

number did not have a major influence on their gene expression profile (Figure 2B-I and S2A).

The RNA-seq data was first validated by analysing previously reported IBD-PDOs

biomarkers[31]: downregulated genes (*AQP8*, *CD177*, *PIGR*, *PLA2G2A*, and *ZNF257)* (Figure

2B) and upregulated genes (*FLNA*, *HLA-DRA*, *HLA-DRB1*, *KCNJ3*, *LYZ*, *MSN*, and *SLITRK6)*

of IBD- vs hPDOs (Figure 2C). We identified 80 upregulated and 20 downregulated DEGs in

IBD- vs hPDOs (fold change cutoff=5, p-value<0.05), where IBD-PDOs exhibited upregulation

of genes involved in inflammatory pathways, such as cytokines and chemokines as IL-1β and

IL-6 (Figure 2D, E). By Signalling Pathway Impact Analysis, we found other enriched pathways

such as Wnt, PI3K-Akt and Ras, and downregulated pathways such as TGFβ signalling in IBD-

PDOs vs hPDOs reflecting the impact of chronic inflammation on intestinal non-inflamed IBD

epithelial tissue which included metabolic shifts, epithelial barrier function and, key signalling

pathways involved in regeneration (Figure 2F and S2B).

To quantify and compare cell type content in both type of PDOs, we employed deconvolution

analysis using CIBERSORTx with a signature matrix constructed using sc-RNAseq data from

healthy human intestinal epithelial cells as reference[32]. We found that TA and Paneth cells

were increased in IBD- compared to hPDOs while BEST4+ and enterocyte pools were

decreased (Figure 2G). By qPCR we detected a significant increase of *OLFM4* in IBD-

compared to hPDOs, however we found no significant change in *LGR5 and CLU* expression

(Figure 2H and S2C). Moreover, the bulk-RNAseq gene expression analysis confirmed the

significant upregulation of *OLFM4* and *EPHB2* (TA and Wnt target gene, respectively) in IBD-

vs hPDOs (Figure 2I).

IL-22 is a cytokine that has a pivotal role in enhancing epithelial proliferation and stem cell-

driven regeneration [33,34] which are essential processes disrupted during IBD. So, we analysed

IL-22 pathway activation in human hPDOs to study OLFM4+ proliferative cells function in repair

and regenerative intestinal mechanisms[35,36]. To that end we extracted EPCAM+ epithelial cells

from the total population of sc-RNAseq data[35] (GSE189423) and analysed the DEGs (Figure

2D-G). Interestingly, one of the top upregulated genes was *OLFM4* (Figure 2I) which we further

validated by qPCR in our IL-22 treated vs control hPDOs. In parallel, IL-22 treatment reduced

goblet cells detected by the decrease of *MUC2* expression marker (Figure 2J, K). Our findings

identify OLFM4$^+$ pool as key players of IBD epithelium and in epithelial repair mechanisms

induced in healthy epithelium.

Characterization of SC/TA and BEST4$^+$ pools in healthy and IBD non-inflamed and

inflamed tissue.

We next analysed the heterogeneity of intestinal SC/TA and BEST4$^+$ cell pools as these are

key epithelial cells involved in regeneration by using publicly available sc-RNAseq data[15,37]. We

extracted EPCAM$^+$ epithelial cells from IBD non-inflamed (IBD normal) and inflamed tissues,

and from healthy individual biopsies (UC=18, healthy=12 and, CD=46, healthy=25). Cell type

annotations were standardized by using epithelial reference dataset (Figure 3A) (GSE185224)

and labels of the cell types were transferred into the selected sc-RNAseq data using SingleR,

ensuring unbiased labelling across datasets[38] (Figure 3B and S3A). We then extracted SC/TA,

and BEST4$^+$ cell populations data, the latter being part of the enterocyte lineage directly

derived from progenitors. We separated the data into healthy, non-inflamed (or normal), and

inflamed groups, and observed a decrease in the SC/TA and BEST4$^+$ pools in IBD inflamed

tissue compared to healthy samples (Figure 3C and S3B).

Next, we performed principal component analysis on the variable features in a total of 11,268

cells for UC and 9,894 cells for CD datasets. We identified clusters using the Louvain algorithm

with results visualised via Uniform Manifold Approximation and Projection (UMAP). This

analysis resulted in 19 transcriptionally distinct UC clusters, and 13 CD clusters highlighting

the functional heterogeneity and roles of SC/TA and BEST4$^+$ cell types (Figure 3D and S3C).

Clusters were further classified based on the cell type (BEST4$^+$, SC and TA) (Figure 3E and

S3D) and health status of the source tissue (healthy, non-inflamed and inflamed) (Figure 3F

and S3E). The relative abundance of these clusters was evaluated based on cell types and

health status (Figure 3G, H and S3F, G). We used the top 50 gene markers of each cluster to

identify representative pathways, cell types and gene ontologies enrichment analyses (Table

S1). Of all the computed clusters, UC-C1, -3, -4, -7, -11, -12, -17, -18 and CD-C1, -5, -8, -12, -

13, aligned clearly with known biological processes so we further refined the markers specific

to these clusters (Figure 3I, J and S3H, I). Interestingly, we identified six gene clusters (UC-

C1, -3, -12 and CD-C1, -5, -8) that predominantly consisted of TA cells (Figure 3G and S3F)

highly enriched in MKI67$^+$ proliferative states, showing high expression of proliferative markers

like *PTTG1*, and *UBE2C* (Figure 3I-K and Table S1). The gene clusters UC-C1, -3 and CD-C1,

-5 reflected enrichment of genes associated with cell division, M-phase of cell cycle, and

microtubule cytoskeleton organization in mitosis. UC-C12 and CD-C8 were enriched in

markers of S-phase, DNA synthesis and replication (Figure 3L and S3H).

Furthermore, we observed that IBD-PDOs vs hPDOs had higher expression levels of the top

50 proliferative markers, likely highlighting these cells' role in adaptive proliferative

mechanisms (Figure S3I). We further analysed the top 50 markers from these clusters in the

DSS-mouse model (GSE22307) and observed that the MKI67$^+$ cluster markers showed a

biphasic proliferative response of healthy cells to cellular damage. This response was

characterized by an initial decrease in proliferation, followed by a subsequent replenishment

of proliferative cells, surpassing the original gene expression levels (Figure S3J, K). We further

validated increased expression of *OLFM4* in IBD inflamed and non-inflamed vs healthy tissue

in most of the clusters (Figure S3L). Together these findings highlight relevant and unique

dynamics of these clusters in the SC/TA and BEST4$^+$ pools during inflammation in healthy and

IBD epithelium.

An immune-enriched gene cluster present exclusively in SC/TA and BEST4$^+$ pools of IBD

inflamed tissues

We further investigated clusters UC-C18 and CD-C12 as these were predominantly composed

of IBD cells and were absent in healthy tissue (Figure 3H and S3G). Enrichment analysis of the

top 50 gene markers from each cluster showed their involvement in immune-related pathways

such as IL-17, TNFα, NF-kB and Oncostatin M (Figure 4A and S4A). Notably, top marker genes

such as *ANXA1*, *REG4*, *TFF1*, and *TIMP1,* previously linked to mucosal healing, epithelial

restitution, and regeneration, were highly expressed within these clusters[39–42]. Markers for UC:

ANXA1, *REG4*, *TFF1*, *CD55*, *TIMP1*, and *PI3; and* for CD: *PI3, IGGL5, REG4, DUOXA2, TIMP1,*

and CXCL1) were chosen for further analysis as their expression was specific to UC-C18 and

CD-C12, respectively (Figure 4B and S4B). These markers were present in SC, TA and

BEST4[+] cell types (Figure 4C, D and S4C, D). In SPARC IBD UC and CD data, the top genes

of UC-C18 and CD-C12 were able to stratify patients according to their macroscopic

appearance. Moreover, the average expression of these genes was significantly upregulated

in inflammation and possible inflammation groups when compared to the normal group.

Interestingly, this upregulation was also statistically significant in inflammation vs possible

inflammation group (Figure 4E, F and S4E, F). We observed that the average gene expression

of this signature increased with inflammation status from day 0 to 6 of DSS-treated mice

(GSE22307) (Figure 4G and S4G). In IL-22 treated hPDOs sc-RNAseq data (GSE189423) we

found that *ANXA1*, *CD55*, *PI3* and *TIMP1* were increased in IL-22 treated human organoids vs

control and all of them were expressed in LGR5^{+} stem cells (Figure 4H, I). Hence, the UC-C18

and CD-C12 gene signature, represent inflammatory biomarkers of the epithelial

compartment, and show promise in molecularly stratifying IBD patients based on their

macroscopic appearance.

Inflammation-Dependent Adaptive Dynamics of Epithelial Cells Categorized by a Novel

Inflammation-Linked Gene Signature Associated with Disease Severity in IBD

We aimed to identify a reliable inflammation-associated gene signature to molecularly stratify

patient samples according to the degree of inflammation and understand epithelial dependent

inflammation dynamics. We curated an inflammation-related gene list (n=476) (Table S2) and

compared their average gene expression in different clinical classifications of inflammation

severity. We categorized the samples by clinical disease activity and found not significant

differences between the groups defined by Mayo score for UC (mild, moderate, severe, and

remission) and short Crohn's Disease Activity Index (sCDAI) for CD (Figure 5A and S5A).

However, when samples were categorized by macroscopic appearance recorded during

endoscopy of IBD patients (normal or non-inflamed, possible inflammation, and inflammation)

they formed three significantly distinct groups (Figure 5B and S5B). We then confirmed that

S100A9 gene expression was significantly upregulated in the inflamed samples when

compared to possible inflammation or normal ones (Figure 5C and S5C). We further confirmed

S100A9 protein upregulation in inflamed vs non-inflamed regions of IBD patient's tissue

samples (Figure 5D).

We validated the categorization of these groups by using an inflammation-related gene

signature (n=42) from a prior UC study[15] (Figure S5D, E). In addition to the expected

upregulation of inflammatory genes in the inflamed vs normal group, we found that possible

inflammation category also showed elevated expression of inflammatory markers compared

to the normal group suggesting an underlying genetic predisposition to inflammation that is

not clear in tissue macroscopic assessments. Overall, we found that the macroscopic

appearance is a reliable classifier of inflammation severity, hence, it was maintained for further

analyses.

We then performed differential gene expression analysis to compare the inflammatory profile

across these groups using the curated inflammation-related gene signature (n=478). In UC,

we found 112 differentially expressed genes (DEGs) between inflammation vs normal samples,

38 DEGs between possible inflammation vs normal samples, and 29 DEGs between

inflammation vs possible inflammation samples (Table S3). A Venn diagram illustrated that 26

genes (26-GIN-UC) were consistently differentially expressed across all three comparisons

(Figure 5E). In addition, multiple dimensionality (MDS) analysis showed that this gene

signature was effective in clustering the samples according to the clinical classifier of their

macroscopic appearance highlighting their potential in distinguishing inflammation (Figure 5F).

In CD, we found 380 DEGs between inflammation vs normal samples, 304 DEGs between

possible inflammation vs normal samples and 35 DEGs between inflammation vs possible

inflammation samples A Venn diagram illustrated those 30 genes (30-GIN-CD) shared among

the three comparisons and MDS plots demonstrated effective sample stratification (Table S3

and Figure S5F, G). To further evaluate both gene signatures (26-GIN-UC and 30-GIN-CD),

we performed unsupervised hierarchical clustering analysis on the SPARC IBD cohort which

aligned with macroscopic appearance effectively clustering the samples according to the

inflammation category. Enrichment analysis of these two gene signatures showed strong

representation of inflammatory response pathways as TNFα and IL-6/JAK/STAT (Figure 5G, H

and S5H, I). Comparison of inflamed vs normal samples from the proteomics SPARC IBD

dataset (UC and CD) confirmed that members of inflammatory pathways such as interleukins

IL-10, -24, -17 were amongst the significantly differentially expressed proteins (*p*-value < 0.05)

(Figure 5I and S5J). Protein-protein interaction analysis showed that proteins related to focal

adhesion, extracellular matrix reorganisation were downregulated, whereas proteins related to

pro-inflammatory and profibrotic mediators were upregulated (data not shown).

We further validated these gene signatures in three different transcriptomic datasets of dextran

sulphate sodium (DSS)-treated UC mouse model that tracks inflammatory responses and

disease progression over time[43]. We identified the upregulation of the 26-GIN-UC and 30-GIN-

CD signatures with increasing inflammation, observing the onset, peak, and resolution of

inflammation in a time-dependent manner (Figure 5J-L, and S5K).

We then evaluated the effectiveness of the new identified gene signatures in stratifying IBD

samples according to inflammation degree. First, we performed unsupervised hierarchical

clustering on SPARC IBD UC samples, identifying five distinct groups based on their UC-C18

gene expression profiles. This clustering reflected the underlying biological patterns

(endoscopic assessment/macroscopic appearance) thereby providing a novel unbiased

approach for IBD samples stratification. We found that these groups corresponded to varying

inflammation potential grades, ranging from 1 to 5, with 1 representing low inflammation and

5 representing severe inflammation. We validated this classification with the average

expression of the 26-GIN-UC (Figure 5) and UC-C18 gene signatures, which showed a

progressive increase with the degree of inflammation, confirming the appropriate stratification

of the samples (Figure 6A-C). Notably, *S100A9*, showed a significant upregulation in the most

inflamed samples, reinforcing the robust and unbiased framework for classifying IBD samples

based on inflammation degree (Figure 6D).

We overlaid the average expression of specific SC/TA and differentiated cell markers with both

inflammatory signatures. *LGR5* expression, showed varying levels in different inflammation

degrees, with an overall decrease in the highest inflammation degree 5 and weak negative

correlation with (r = -0.085) with UC-C18 signature. The spline curve model shows the dynamic

shift in *LGR5* expression in the different inflammation degrees (Figure 6E and S6). *OLFM4*

expression exhibited a subsequent upregulation in degrees 3 to 5 with an overall positive

correlation (r = 0.579) with the UC-18 signature (Figure 6F and S6). Similarly, the revival stem

cell marker *CLU* increased with severity of inflammation with positive correlation (r = 0.542)

(Figure 6G and S6), while *BEST4* was upregulated in degree 2 but its expression gradually

decreased in degree 3 and 4 (Figure 6H). *VIL1*, gene expressed in enterocytes lining the

intestinal villi and crypts, showed a dynamic expression from degree 2 to degree 4 (Figure 6I).

AVIL, gene marker for brush border integrity and actin dynamics and *FABP1* enterocyte

marker were both decreased upon severity of inflammation (Figure 6J, K). We observed that

proliferation markers such as *PCNA* exhibited a general increase when compared degree 5 vs

degree 1 (Figure 6H) that reflects the balance between inflammation-induced epithelial injury

and the subsequent regenerative response. Moreover, we observed a similar trend of negative

correlation of *BEST4*, *VIL1*, *AVIL* and *FABP1* with UC-18 expression (r = -0.3, -0.35, -0.536, -

0.496, respectively). (Figure S6). We obtained very similar results for CD dynamic shifts (CD-

C12, and 30-GIN-CD) (Figure S7). We evaluated the robustness of these signature using

patient level hold out test our models performed very well for "Normal" samples, while

classification of inflamed states was more modest and the intermediate "Possible

inflammation" cases were not well predicted as it likely represents a heterogeneous mix of

early, partial, or ambiguous lesions, and may also capture endoscopic uncertainty (Figure

S7p,q). To address this, we also applied unsupervised clustering of the gene signature

followed by dendrogram-based cutting to derive an alternative, data-driven ordering (Figure

6). This approach produced a molecularly defined progression of five groups that may better

reflect underlying biology. This complementary analysis highlights that transcriptomic ordering

can resolve heterogeneity within inflamed states. In addition, we also evaluated whether

clinical parameters had an effect on the ability of this gene signature to stratify inflammation.

We found that the gene signature score remained strongly associated with macroscopic

appearance after adjusting for sex, race, and smoking status, with direction and magnitude

consistent across models for both UC and CD. None of the covariates materially altered the

effect of the signature, indicating that the association is independent of demographic or

smoking-related factors (Supplementary Table S5, S6).

The combination of scatter plots and spline curve model provided a comprehensive approach

in understanding how the average expression of UC-C18 and CD-C12 gene signature reflects

dynamic changes in different cell type markers. While scatter plots offer a more rigid, point-

by-point correlation, spline curves capture smoother, more continuous transitions in

expression patterns within our patient stratifications. Together, these methods show

inflammation-dependent SC/TA and BEST4$^+$ cells dynamics and demonstrate that these pools

derived UC-C18 and CD-C12 gene signatures reflect both broad and subtle dynamic shifts

across different inflammation degrees.

Exploring molecular mechanisms in IBD by data integration of UC and CD sc-RNAseq

datasets

We then performed integration of the epithelial cell compartments of SCP259 (UC) and

SC1884 (CD) to ensure comparability across both IBD subtypes, UC and CD. This revealed

overlapping epithelial cell populations (Figure S8A-D). We applied standardised automated

cell annotations with CellTypist, referencing the pan-gastrointestinal (pan-GI) atlas, with minor

manual adjustments (Figure S8E). This analysis revealed a marked depletion of colonocytes

in inflamed samples, accompanied by a notable expansion of deep crypt secretory cells (DCS),

which are thought to represent the colonic equivalent of small intestinal Paneth cells (Figure

S8F).

To confirm the combined changes associated with inflammation in both IBD subtypes, we

performed unsupervised clustering of gene expression profiles based on disease status,

grouping inflamed UC and CD samples into "inflamed", "non-inflamed" category and "healthy"

samples (Figure S8G). This analysis identified a core set of 20 top differentially expressed

genes that were consistently upregulated in inflamed UC/CD but exhibited minimal expression

in non-inflamed and healthy samples (Figure S8H). We used these genes to create an

inflammatory gene score to assess cell-type-specific expression patterns, which revealed that

they are predominantly expressed in the DCS population, with limited expression across other

cell types (Figure S8I). Transcription factor activity mapping across various cell populations

further identified NFκB activation in DCS as a key feature of inflamed state, consistent with

findings from the differential expression analyses comparing IBD inflamed vs non-inflamed

cells in DCS and stem cells (Figure 7A-D). Trajectory analysis indicated that the differentiation

trajectory of intestinal SCs is disrupted during inflammation, with a skewing toward DCS

lineages (Figure 7E). Genes related to DCS trajectory were expressed higher in inflamed tissue

relative to the healthy samples (Figure 7F).

Together, these findings suggest that inflammation disrupts normal epithelial differentiation,

driving SCs toward a DCS-like fate characterized by expression of pro-inflammatory genes.

Notably, the data identifies biomarkers of inflammation-associated stem cell reprogramming in

the colonic epithelium.

Discussion

Accurately evaluating inflammation severity in IBD remains a clinical challenge. Existing

approaches such as symptom-based scores and biomarker assessments, frequently lack the

sensitivity and specificity needed to reliably reflect the extent of inflammation. Endoscopy,

often followed by histological examination, remains the gold standard but is invasive, costly,

and not readily accessible in all settings. This underscores the urgent need for robust, non-

invasive, and reproducible molecular methods to assess inflammation in IBD. Such tools are

crucial for improving patient stratification, guiding treatment decisions, and deepening our

understanding of epithelial and immune-driven tissue pathology[44–46]. Traditional clinical indices

obtained by endoscopies, such as the sCDAI for CD and the Mayo score for UC, are widely

used but often fail to reflect the molecular and cellular heterogeneity of inflammation observed

at the tissue level[47,48]. In this study, we found that macroscopic appearance assessed during

endoscopy closely aligned with inflammation-associated gene expression, suggesting its

possible future potential to serve as a biologically meaningful classifier of inflammation severity

rather than overall disease activity. Through differential gene expression analysis based on

macroscopic appearance, we identified distinct inflammation-associated gene signatures for

UC and CD. These signatures offer an unbiased approach for stratifying patient samples and

provide valuable insights into epithelial cell diversity, regenerative responses, and barrier

function across varying degrees of inflammation in IBD disease subtypes. These findings are

preliminary and require prospective validation before they can be proposed as clinical tools.

Understanding how different epithelial cell populations, including stem and progenitor cells,

react to varying levels of inflammation is essential for unravelling the processes of mucosal

injury and healing. Our comprehensive profiling of epithelial cell heterogeneity across different

inflammation stages reveals significant and coordinated shifts in the different epithelial cell

pools. Through a multimodal analytical approach, we observed a reduction in TA cells as

inflammation severity increased, a trend previously associated with impaired proliferative

capacity during chronic inflammation or skewed differentiation dynamics[16,49]. In contrast, we

observed that LGR5$^+$ stem cells varied across inflammation duration and intensity, which

possibly suggests a constant compensatory mechanism aimed at restoring epithelial barrier

integrity. Interestingly, OLFM4$^+$ proliferative cells exhibited sustained upregulation during

inflammation, reflecting a regenerative response to counterbalance epithelial damage, and this

expression remained elevated even in non-inflamed IBD tissue further highlighting the

expansion of proliferative stem cells as a hallmark of IBD. Our findings revealed that these

dynamics were substantiated in mouse healthy tissue[50], where inflammation led to the

decrease of TA pool along with an increase in Clu$^+$ and Olfm4$^+$ stem-like populations. This new

identified epithelial functional plasticity driven by inflammation has potential implications for

barrier dysfunction and regeneration.

Under inflammatory conditions, the proliferation of SC/TA cells is predominantly regulated by

pathways such as Wnt/β-catenin and Ras signalling. These pathways are essential for

sustaining the equilibrium between self-renewal and differentiation within the stem cell

niche[6,10,51,52]. Inflammatory cytokines such as TNFα and IL-6 are known to enhance the

proliferation of intestinal stem cells by activating signalling pathways like STAT3. This

activation can amplify the inflammatory milieu and contribute to an excessive proliferative

response within the tissue[53–56]. Our mechanistic insights and SC/TA dynamics data indicate

that while enhanced proliferation may initially facilitate tissue repair, it can also lead to

dysregulation in chronic inflammatory settings. This may result in depletion of the stem cell

reservoir or inappropriate differentiation, ultimately weakening the intestinal barrier and

contributing to the sustained epithelial injury seen in IBD[36]. Targeting specific proliferation-

related pathways within this epithelial clusters may offer therapeutic potential by fine-tuning

regenerative responses to improve barrier integrity while avoiding the overactivation that

contributes to disease pathology.

We found that IBD tissue classified as non-inflamed still exhibits subtle transcriptional and

cellular alterations compared to healthy control, suggesting a baseline state of immune

activation and epithelial stress even in macroscopically normal regions[16,57]. To explore

epithelial cell-type alterations between IBD and healthy tissue in the absence of overt

inflammation, we leveraged 3D PDO models, which we confirmed that it recapitulates the

cellular composition and regenerative dynamics of the intestinal epithelium[29–31]. IBD-PDOs

displayed a distinct epithelial profile compared to hPDOs, including upregulation of

inflammatory mediators and SC/TA proliferative markers such as OLFM4. These changes

were accompanied by a reduction in differentiated epithelial cell types, such as enterocytes

and BEST4$^+$ cells, and an increase in Paneth cell populations, suggesting a shift toward a

regenerative or stress-associated state. This reinforces the concept that epithelial cells in IBD

retain a molecular imprint of inflammation, even in histologically normal areas[16,58,59].

Additionally, IL-22 pathway activation in hPDOs showed that OLFM4$^+$ cells are activated during

epithelial repair, supporting their role as mediators of regeneration in both homeostatic and

disease settings. We highlight the utility of PDO models to dissect functionally epithelial

alterations in IBD offering insights into therapeutic response.

We further characterized SC/TA and BEST4^{+} epithelial clusters uniquely enriched in inflamed

IBD tissues but absent in healthy samples. These clusters were significantly enriched for

immune-related pathways that are well-established drivers of chronic intestinal inflammation

and epithelial remodelling in IBD[60,61]. This enrichment suggests the emergence of

inflammation-specific epithelial transcriptional states during active disease. We report here, for

the first time, an inflammation-associated epithelial gene signature specific to stem and

progenitor cells. This signature successfully stratified patients based on their macroscopic

inflammation degree in both UC and CD. The observed enrichment of SC/TA signatures in

inflamed tissue aligns with earlier reports of crypt hyperplasia and stem cell activation as

hallmark features of IBD pathology[56,62]. Thus, these transcriptional states likely represent

inflammation-induced epithelial remodelling programs, offering a refined molecular framework

for patient stratification and potential therapeutic targeting of regenerative responses in IBD.

Our data suggest that inflammation alters the differentiation trajectory of intestinal stem cells,

skewing them toward DCS cell lineages, which are implicated in epithelial barrier maintenance

and immune regulation [16]. This shift occurred alongside a relative depletion of colonocyte

differentiation and was also evident in non-inflamed IBD-PDOs when compared to healthy

controls. This adaptability can be crucial in chronic inflammatory conditions as stem cells must

maintain tissue integrity despite continuous stress. However, it also raises questions about

long-term consequences, as chronic inflammation can lead to exhaustion or altered

differentiation patterns in stem cells, potentially impacting their regenerative capabilities over

time[63,64]. These findings reflect a broader epithelial remodelling driven by disrupted lineage

commitment. Notably, we identified a core set of inflammation-associated genes highly

expressed in DCS, with NF-κB signalling emerging as a prominent regulator that requires

direct experimental follow-up for functional validation. This aligns with previous studies

highlighting the central role of the NF-κB pathway in mediating epithelial responses after injury

and sustaining chronic inflammation[65,66]. Furthermore, NF-κB activation has been shown to

modulate Wnt pathway in colonic mouse tissue and specific ablation of RelA/p65 retards crypt

stem cell and progenitors expansion[67,68]. Future investigations may help to determine whether

modulating this pathway in DCS could lead to new therapeutic opportunities for IBD. However,

selectively targeting NF-κB signalling in DCS is technically challenging due to the complex

cellular composition of the intestinal mucosa, intestinal region-specific identity, and the broad

physiological roles of NF-κB in maintaining epithelial integrity and immune balance[65,66,69].

Furthermore, several studies have demonstrated the complex interactions between individual

NF-κB subunits in regulating colonic susceptibility to inflammation[69–71]. In addition, off-target

modulation could disrupt homeostatic mechanisms, potentially leading to adverse

consequences such as impaired wound healing, or dysregulated inflammatory responses.

Overcoming these barriers will require advances in targeted delivery systems, and rigorous

preclinical validation to ensure safety and specificity for future therapeutic applications.

In summary, our findings highlight how inflammation-induced alterations in epithelial stemness,

and differentiation contribute to the dysfunction of the intestinal barrier in IBD. The capacity of

stem and progenitor cells to adapt under inflammatory stress offers valuable insights for

enhancing tissue repair strategies. Targeting these adaptive mechanisms may support the

development of novel therapies that sustain epithelial regeneration, minimize disease flares,

and foster long-term remission. Understanding the link between epithelial plasticity and

specific inflammatory cues may ultimately guide precision treatments aimed at improving

clinical outcomes and patient well-being.

527   Methods

528   Ethical Review

The research complies with all relevant ethical regulations and guidelines. Intestinal biopsy

samples were obtained from the Queen's Medical Centre of Nottingham (UK) with patient

consent from IBD patients and histologically healthy individuals in accordance with ethical

guidelines (17/EM/0126).

Animal DSS-experiments were performed in accordance with protocols approved by the

Service de la Consommation et des Affaires Vétérinaires of Canton Vaud, Switzerland

(VD1052/VD1053). The work has been reported in line with the ARRIVE guidelines 2.0. Section

expanded in the "Declarations" section.

IBD Plexus Cohort

The patient cohort included in this study was derived from the Study of a Prospective Adult

Research Cohort with IBD (IBD Plexus Study of a Prospective Adult Research Cohort, SPARC

IBD) (UC=1,097, CD=2,138)), a part of the Crohn's & Colitis Foundation IBD Plexus program[21].

This includes a geographically diverse research cohort of patients with IBD using standardized

data and biosample collection methods and processing techniques. We included patients from

the SPARC IBD cohort in our analysis, who had a history of UC and CD and had available bulk-

RNAseq of biopsies. We also included plasma proteomics, where applicable, for patients within

the cohort. SCDAI for CD and 6-point MAYO for UC patients scores were calculated for

patients with sufficient data at enrolment and assigned a disease severity classifier:

Disease Activity	sCDAI	6-point MAYO
Remission	<150	0-1
Mild	150 – 219	2-3
Moderate	220-450	4-5
Severe	>450	6

548 Table 1. SCDAI for CD and 6-point MAYO for UC patients scores for disease activity

549 classification.

Data acquisition for bioinformatic analysis

All the data processing and analysis was done in RStudio (Version 4.2.0 and 4.3.0) or Python.

IBD Plexus data

The raw counts of bulk-RNAseq of UC and CD patients derived from biopsy samples within the SPARC IBD cohort were batch corrected using *Combat_seq*, followed by normalisation and log transformation. Multi-dimensionality Scaling (MDS) analysis was performed to check the sample distribution. SPARC IBD samples were compared against different levels of histological inflammation: erosions or ulcers (or inflammation) vs normal, erosions or ulcers vs possible inflammation (or non-inflamed) and erosions or ulcers vs possible inflammation, for a curated inflammatory gene list (n=476) (Supplementary Table 2). Differential gene expression analysis for the different groups in IBD Plexus Cohort was conducted using *edgeR*[72] and *limma*[73]. P-values threshold was set at 0.05 and adjusted using the Benjamini-Hochberg method.

Human sc-RNAseq data

Pre-processed and annotated sc-RNAseq data containing EPCAM+ epithelial cells from human tissue was obtained from Single Cell Portal of the accession numbers SCP259, SCP1884 and the reference dataset from Gene Expression Omnibus (GEO) under the accession number GSE185224. SCP259 contained cells from both non-inflamed and inflamed tissues data from UC, and healthy individuals' biopsies (UC=18, healthy=12). SCP1884 was a comparable dataset with CD patients and normal individuals' biopsies (CD=46, healthy=25). Cells with mitochondrial gene content exceeding 5%, features fewer than 200 and more than 2,500 were excluded to retain the highest quality cells. Each dataset was processed according to standard workflow using *Seurat*[74] (version 5, package in R. Cell annotation was uniformly performed using SingleR to predict labels using the reference dataset. The counts matrix from both the

datasets was used as the query, while the GSE185224 reference counts were used for the

reference labels. Cell type labels were predicted using the Wilcoxon differential expression

method. The predicted labels were added to the Seurat object and was subset to include only

cells with "TA", "BEST4+" and "SC" labels and this was used for further analysis.

For single-cell dataset integration studies, SCP259 and SCP1884 raw matrices were imported

using scanpy (v1.10.4) and cells were filtered to exclude cells with greater than 20%

mitochondrial counts and greater than 6000 features. Next, cells were processed through

scrublet and predicted doublets excluded. Counts per cell were normalised to a target of 1×10^4

and then log-transformed. Highly variable genes were selected followed by principle-

component analysis (PCA), nearest-neighbour estimation (n_neighbours = 5, n_pcs = 17,

knn=True) and UMAP projection followed by leiden clustering (resolution, 0.3). To correct for

batch-effects across the two datasets, batch-balanced k-nearest neighbours (bbknn)

correction was performed using the 'Subject' as batch key and leiden cluster as confounder.

PCA, nearest neighbour, UMAP projection and leiden clustering were repeated using the same

settings. Automated cell-type annotation was performed using CellTypist majority voting using

the   Pan-GI   cell   atlas   model   (:

https://cellgeni.cog.sanger.ac.uk/gutcellatlas/celltypist_models/2_full_healthy_reference_AP_

all_organs_finalmodel.pkl). Each leiden cluster was annotated with the predicted cell label. To

check the validity of annotation, cluster specific gene expression was ranked, using the

Wilcoxon method. In most cases, marker genes accorded with known markers of each subset,

but in a few cases (e.g. Stem_2), gene expression suggested misclassification (e.g. Paneth

cell) and therefore these labels were manually corrected. In addition, some clusters were

merged (e.g. Goblet_1, Goblet_2) where leiden clustering had separated cells of the same

type. Trajectory analysis of specific subsets was performed using scfates and decoupler was

used for pseudobulk analysis of cell populations across health states to determine differential

gene expression and transcription factor activity. Finally, genemania (genemania.org) was

used for network and gene-set functional analysis of differentially expressed genes.

Cluster UC-C18 and CD-C12

The subset data with progenitors' cells for each dataset were handled individually and

processed according to standard workflow from the Seurat package in R. Dimensionality

reduction and Louvain Leiden clustering (resolution 0.1 -1.5) were carried out and cell lineages

were annotated based on enrichment analysis and defined marker gene expression of each

cluster.

DSS-treated mouse models sc-RNAseq data

Single-cell RNAseq data was obtained from GSE264408 was utilised to study the cell type

changes in normal, acute and chronic mouse models. Raw matrices were loaded for each

sample using the Seurat package. Each sample was processed into a Seurat object, applying

filters for quality control: cells with 200–4,000 detected genes and mitochondrial content <10%

were retained. Metadata including sample identifiers and condition types were added to Seurat

objects. Seurat objects for all samples were merged into a single dataset and standard

workflow was applied. Data were further harmonized using the Harmony algorithm to correct

batch effects, and integrated clusters were identified using shared nearest neighbour graphs

and clustering algorithms. To focus on epithelial S/P cells, $Epcam^+$ cells were subset (Epcam

> 0.5). Dimensionality reduction (UMAP) and clustering were repeated. $Epcam^+$ cells were

categorized based on "Lgr5" expression into "Lgr5_Positive" and "Lgr5_Negative" groups.

Wilcoxon tests assessed differential expression significance across conditions. Visualizations

included violin plots and feature plots for key markers.

DSS--treated mouse models microarray transcriptomic data

Raw expression data of GSE22307 and GSE42768 was downloaded from NCBI-GEO platform

and log2 normalised using limma package. Similarly, GSE131032 and GSE214600 raw counts

data downloaded from same platform. To process raw counts, we used deseq function of

Deseq2 package. Z-score was calculated from normalised data to visualize gene expression

in either heatmap or spline curve using pheatmap and ggplot2.

PDOs sc-RNAseq data

Raw sc-RNAseq of IL-22 treated and untreated intestinal human organoids were retrieved

GSE189423, merged, and batch effects were corrected using Harmony integration. EPCAM$^+$

cells were identified and further analyzed through dimensionality reduction (UMAP),

clustering, and marker gene expression profiling. To focus on epithelial stem/progenitor cells,

Epcam$^+$ cells were subset (Epcam > 0.5). UMAP and clustering were repeated. Epcam$^+$ cells

were categorized based on "Lgr5" expression into "Lgr5_Positive" and "Lgr5_Negative"

groups. Wilcoxon tests assessed differential expression significance across conditions.

Visualizations included violin plots and feature plots for *ANXA1* and *REG4*.

PDOs bulk-RNAseq data

RNA collected from healthy (P12) and IBD organoids (P89, P90, P99) and their mRNA was

sequenced. A custom pipeline with standard bulk-RNAseq workflow was implemented for

quality control, trimming and alignment. Aligned reads were quantified using FeatureCounts.

Pathway Enrichment Analysis

Key pathways of the DEGs were performed in MSigDB database using the web tool, Enrichr.

Pathways enriched for the differentially expressed proteins in plasma proteomics of SPARC

IBD cohort was identified using KEGG database using protein-protein-interaction networking

using the web tool, StringDB. Gene Ontology analysis for the cluster makers was performed

using web tool, Enrichr. DEGs with an unadjusted p-value < 0.05 were considered significant.

For downstream pathway analysis, we further filtered for DEGs with an adjusted p-value (padj)

< 0.05 and an absolute log2 fold change > 5. Pathway enrichment was performed using

Signaling Pathway Impact Analysis (SPIA) implemented via the iLINCS platform. Non-relevant

pathways, such as those associated with viral responses (e.g., COVID-19), were excluded.

Pathways related to epithelial biology were retained for interpretation.

Deconvolution Analysis

sc-RNAseq dataset from[15] was used to generate a signature matrix which was employed to

derive cell proportions in SPARC IBD samples using CIBERSORTx

(https://cibersortx.stanford.edu). Cell proportion chart was made using *ggplot2* in R version

(4.3.0).

Ordinal Logistic Regression Analysis

An ordinal logistic regression model (proportional odds model, polr function in the R package

MASS) was used to assess the relationship between a transcriptomic signature score and

macroscopic appearance in SPARC IBD samples. The signature score was derived as the

mean z-scored expression of differentially expressed genes. Models were adjusted for clinical

and demographic covariates (e.g., sex, race, smoking status), as well as an epithelial marker–

based score to account for tissue composition. Odds ratios (OR) and 95% confidence intervals

(CI) were calculated by exponentiating regression coefficients.

Spline curve analysis

The relationship between the degree of inflammation in SPARC IBD data or DSS treatment in

mouse model data and gene expression (expressed as the Z score of CPM) was visualized

using a generalized additive model (GAM). we used the geom_smooth() function in the R

package ggplot2 with the following parameters:

$$y \sim s(x, bs="cs", k=4)$$

where y represents the Z score (derived from log2 normalized CPM values) and x represents

the degree of inflammation. A cubic spline basis (bs = "cs") was used with k=4 to control the

smoothness of the fitted curve.

DSS-mouse model

To model UC in mice, DSS or placebo was administered in the drinking water to induce acute

colitis. C57BL/6 male mice aged 6–10 weeks were used. A solution of 2.5% DSS (w/v) was

prepared using autoclaved water and provided to the mice for 6 days. Throughout the

experiment, mice were monitored daily for body weight, stool consistency, and rectal bleeding.

At the end-point, animals were euthanized, and colonic tissues were collected for

histopathology assays. Adult mice were euthanized by inhalation of carbon dioxide (CO_2) for

6 minutes, delivered at a displacement rate of 20% of the cage volume per minute, in

accordance with approved ethical protocols. No anaesthesia was used prior to euthanasia.

PDOs establishment and culture

Human intestinal crypts were isolated from adult tissues using 25 µg/mL of liberase (Roche),

embedded into Matrigel (Corning, Cat 356231) and grown into organoids following previous

publications[75]. Organoids were routinely tested for mycoplasma contamination and resulted

negative. The details of organoid donors used in each experiment are listed in Supplementary

Table 4. Human intestinal organoids were cultured in IntestiCult™ Organoid Human Growth

Medium or in-house media prepared following previous publications[75,76] and were split once a

week by mechanical dissociation and with TryPLE every two weeks.

RNA extraction and sequencing of PDOs

RNA was collected from healthy individuals and IBD patient derived organoids embedded in

Matrigel in 48-well plates. RNA was extracted using Qiagen RNeasy kit and RNA quality was

assessed with the NanoDrop and the RIN score was checked. RNA samples were sequenced

by Novogene, Cambridge, UK.

Real-time PCR (qPCR)

250 ng of total RNA isolated from healthy and IBD-PDOs was converted to cDNA using

Superscript II (Invitrogen). 20 µL of cDNA was diluted in 200 µL of nuclease free water. For

analysing gene expression SYBR green (Applied biosystem) was used according to

manufacturer's protocol. All genes CT values were normalised with *GAPDH* (housekeeping

gene) of each sample independently.

Gene	Forward (5'->3')	Reverse (5'->3')
LGR5	CTTCCAACCTCAGCGTCTTC	TTTCCCGCAAGACGTAACTC
OLFM4	GACCAAGCTGAAAGAGTGTGAGG	CCTCTCCAGTTGAGCTGAACCA
MUC2	ACCCGCACTATGTCACCTTC	GGACAGGACACCTTGTCGTT
CLU	GAGCAGCTGAACGAGCAGTTT	CTTCGCCTTGCGTGAGGT
GAPDH	CTTGAAGGGTGGAGCCCAAAAG	ATGGATGACTTGGCCAG

Table 2. List of primers

Immunostaining

Paraffin processing from intestinal biopsies

Biopsy tissues were fixed in 4% PFA, placed into individual cassettes, and labelled accordingly.

Tissue processing was performed using a Leica TP1020, enabling efficient handling of multiple

samples. Tissues were exposed to a graded series of methanol solutions (50% to 100%

concentration), with each step lasting 1 hour at RT under vacuum and agitation. Following

dehydration, tissues were transferred to xylene for 1 hour at RT, again with vacuum and

agitation, to remove residual alcohol and prepare the samples for paraffin infiltration.

Embedding was carried out in molten paraffin using disposable molds (Embedding machine)

while avoiding bubble formation. The embedded tissues were then cooled on the machine's

cooling surface until solidification, and the resulting paraffin blocks were subsequently stored

at 4°C.

Tissue sectioning

A water bath was prepared with distilled water at 45°C for tissue sectioning. Meanwhile, the

formalin-fixed paraffin-embedded (FFPE) blocks and forceps were chilled on ice. A microtome

blade was installed onto the cutting bed with the correct orientation, and the FFPE block was

secured in the block holder. Trimming was performed at 10-µm intervals until the desired

depth was reached. Sections were then cut at 4 µm, forming a continuous ribbon that was

gently transferred to the water bath using forceps. After briefly floating to separate the

sections, slides (Superfrost) were used to mount the individual sections for subsequent

Immunofluorescence (IF) staining.

Immunofluorescence staining

Paraffin-embedded tissue sections were placed on slides and dewaxed in xylene to remove

residual paraffin. The sections were then rehydrated through sequential ethanol washes

(100%, 90%, 80%, and 70%), each for 5 minutes, followed by distilled water. A 0.5 M sodium

citrate buffer (pH 6.0) was freshly prepared, brought to a boil in a microwave, and the slides

were submerged for 15 minutes before cooling to room temperature. Permeabilization was

carried out using 0.5% Triton X-100 for 1 hour, and blocking solution was prepared by

dissolving 0.1 g BSA in 10 ml of wash solution (0.1% Triton X-100 in PBS). Primary antibodies,

diluted in blocking solution at the below described concentrations, were applied overnight at

4°C. Slides were washed three times, then incubated for 1 hour in the dark with secondary

antibodies and DAPI (1:500 in blocking solution). After three additional washes using wash

solution, coverslips were mounted using 100% glycerol. Imaging was performed using a Leica

confocal SPE microscope.

Whole mount PDOs immunostaining

For immunofluorescence staining of organoids, we followed protocol from[77,78] with slight

modifications. Briefly, organoids were harvested in PBS for staining in 1.5 mL Protein Lowbind

tube (Eppendorf) coated with 1% w/v BSA solution. Organoids were then fixed with 4% PFA

for 1 hour at 4°C followed by centrifugation at 500 xg for 5 minutes. Permeabilization was

performed using a solution of PBS with 0.5% Triton X-100 for 30 minutes at room temperature.

Organoids were then blocked with a PBS solution containing 0.5% BSA for 30–60 minutes at

4°C. Primary antibody incubation was carried out overnight at 4°C on rotating platform using

described concentration in table below. The following day, samples were washed three times

with PBS and incubated with a secondary antibody mix containing DAPI for 1 hour at room

temperature in the dark. After washing secondary antibody (dilution 1:500 of Alexa 488 anti-

mouse or Alexa 568 anti-rabbit), organoids were plated in 96-well plate with black wall and

clear bottom and imaged using Lecia Confocal SPE.

Antibodies and dyes	Company	Catalog	Dilution
S100A9	Proteintech	26992-1-AP	1:200
DAPI	Scientific Laboratory	D9542-1MG	1:500
OLFM4	Cell Signalling	D6Y5A	1:200
β-catenin	Santacruz Biotech	sc-7963	1:500

Table 3. List of antibodies.

Data & code availability

The SPARC IBD data are available upon approved application to Crohn's & Colitis Foundation

IBD Plexus study (https://www.crohnscolitisfoundation.org/research/plexus). PDOs bulk-

RNAseq data and the code done for this manuscript is deposited in Github Repository

(https://github.com/brinda-bala/IBDstemcell.git).

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Declarations

Ethics approval and consent to participate:

Human tissue samples were collected in accordance with the Declaration of Helsinki and with approval from the East Midlands - Leicester Central Research Ethics Committee under reference number [17/EM/0126, protocol 17015]. Written informed consent was obtained from all participants prior to sample collection. *Approval date: 02/07/2020*. Study title: Modelling Intestinal iNflammation and predIctinG response to therapies Using human enTeroids

All animal experiments were performed in accordance with institutional guidelines and approved by the Cantonal Veterinary Office Vaud, Switzerland under license number [VD1052/VD1053]. Approval date: 1/11/2015. Study title: Production and analysis of mouse models of cancer stem cells

Consent for publication: Not applicable

Clinical trial number: Not applicable.

Availability of data and material

The SPARC IBD data are available upon approved application to Crohn's & Colitis

Foundation IBD Plexus study (https://www.crohnscolitisfoundation.org/research/plexus).

PDOs bulk-RNAseq data and the code done for this manuscript is deposited in Github

Repository (https://github.com/brinda-bala/IBDstemcell.git).

The bulk RNA-seq data can be accessed here (GSE310845):

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE310845

Competing interests:

C.P. is an AstraZeneca employee and AstraZeneca shareholder. P.O.M., G.M., and N.H.

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Authors' contributions

P.O.M conceived, designed the study and wrote the manuscript with the help of B.B. P.O.M,

B.B., and S.P. analysed and interpret data. B.B. carried out the modelling work, model-data

comparison, RNAseq (single-cell and bulk) analyses, IBD Plexus analysis, experimental

approaches and produced the figures together with S.P. W.D performed sc-RNAseq analyses

by using Python. S.P performed mathematical tests, experimental approaches and

transcriptomic (RNAseq and microarrays) analyses. G.M. compiled and helped with patients'

stratification, samples and clinical input. P.O.M, B.B, S.P., L.G., W.D., L.G., N.H., J.H., C.P., and

G.M contributed to the discussions, interpretations and writing of the manuscript.

The authors declare that they have not use AI-generated work in this manuscript.

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Materials & Correspondence.

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Tables

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Figure legends

Figure 1. Epithelial cell heterogeneity profile varies with inflammation severity in IBD.

(A) Bar plots showing the relative proportions of epithelial cell types stratified by endoscopic

inflammation scores in SPARC IBD-UC samples.

(B) Violin plots depicting significant differences in cell type proportions across macroscopic

appearance groups (normal, possible inflammation, inflammation).

(C) Violin plots of representative epithelial gene markers across the same macroscopic

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(D) Heatmap showing unsupervised clustering of epithelial marker genes based on

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(E) Heatmap (left) and spline curve (right) illustrating dynamic expression of epithelial

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(F) Violin plots of selected gene markers in the DSS-treated mouse model from the same

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(G) Dot plot of epithelial marker expression in single-cell RNA-seq data from DSS-treated

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(H) Representative immunofluorescence images of mouse small intestine sections from

control and DSS-treated animals stained for OLFM4 (red) and DAPI (blue). Yellow arrows

indicate crypts. Images represent three independent biological replicates. Scale bar =

100 µm.

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Figure 2. Characterization of epithelial cell alterations in IBD using a patient-derived

organoid (PDO) model.

(A) Representative brightfield microscopy images of healthy (hPDOs) and IBD-derived PDOs

(IBD-PDOs). Scale bar = 100 µm. Bulk RNA-seq analysis of hPDOs (n = 7, results of 2

independent batches with early passage (n = 3, 3 independent wells) and late passage (n =

4, 4 independent wells) and IBD-PDOs (n = 13, results of 3 independent donors, P89 (n = 4,

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independent batches with early passage (n = 3, 3 independent wells) and late passage (n =

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(B–C) Heatmaps showing expression of previously reported IBD-related markers (Dotti et al.

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(D) Volcano plot of differentially expressed genes (DEGs) between hPDOs and IBD-PDOs

(adjusted p < 0.05, $\log_2$ fold change > 5). Red dots represent significant DEGs; grey dots are

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(G) Bar plot indicating estimated epithelial cell-type proportions in hPDOs and IBD-PDOs by

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(J) Brightfield images of hPDOs treated with IL-22 or vehicle control. Scale bar = 500 μm.

Representative images from three independent biological replicates.

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Figure 3. SC, TA and BEST4+ epithelial subpopulations in healthy, non-inflamed, and

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(A) UMAP embedding of epithelial cells from the reference dataset (Burclaff et al., 2022),

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(E–F) UMAPs annotated by (E) predicted cell type and (F) tissue health status.

(G) Bar plot showing the distribution of UC clusters across epithelial cell types.

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(L) Bar plot showing enriched pathways from Gene Ontology (GO-Biological Processes

2023) for the top 50 genes in cluster UC-C1 (left), -C3 (middle) and –C12 (right).

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Figure 4. Immune-enriched gene clusters within SC, TA and BEST4+ epithelial

populations in inflamed UC.

(A) Bar plot showing enriched Hallmark gene sets from MSigDB for the top 50 genes in

cluster UC-C18.

(B) UMAP plots showing expression of selected UC-C18 marker genes (*ANXA1*, *REG4*,

TFF1, *CD55*, *TIMP1*, and *PI3*) in SC/TA and BEST4+ cells from the UC dataset (SCP259).

(C) Dot plot showing expression of the selected UC-C18 markers across epithelial cells

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(E) Heatmap showing average expression of UC-C18 marker genes across SPARC IBD-UC

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(F) Violin plots showing UC-C18 gene expression patterns in SPARC IBD-UC samples

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(G) Spline curve showing average expression dynamics of UC-C18 marker genes in DSS-

treated mouse colitis dataset (GSE22307).

(H) Dot plot showing UC-C18 marker expression in SC, TA, and BEST4+ populations from

hPDOs treated with IL-22 or vehicle control (GSE189423).

(I) Dot plot showing expression of UC-C18 markers stratified by LGR5 expression status in

epithelial cells (GSE189423).

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Figure 5. A novel inflammation-linked gene signature associated with inflammation

severity in IBD.

(A–C) Violin plots showing average expression of curated inflammatory genes (n = 476) in

SPARC IBD-UC samples grouped by (A) disease activity, (B) macroscopic appearance, and

(C) S100A9-based classification into normal (n = 747), possible inflammation (n = 141), and

inflammation (n = 219) groups.

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(J–L) Heatmaps and spline curves showing dynamic expression of the 26-GIN-UC signature

in DSS-treated mice from datasets: (J) GSE22307, (K) GSE214600, (L) GSE42768.

$P < 0.01$ (**), $P < 0.0001$ (****).

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Figure 6. Adaptive epithelial responses in UC stratified by degree of inflammation.

(A) Spline curve showing the average expression of the 26-GIN-UC and UC-C18 gene

signatures across increasing inflammation scores.

(B–C) Box plots showing expression levels of (B) 26-GIN-UC and (C) UC-C18 gene

signatures across low to high inflammation categories.

(D) Spline curve (top) and box plot (bottom) of *S100A9* expression stratified by inflammation

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(I–L) Spline curves (top) and box plots (bottom) for epithelial differentiation and proliferation

markers (I) *VIL1*, (J) *AVIL*, (K) *FABP1*, and (L) *PCNA* across inflammation categories.

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Figure 7. Integration of UC and CD sc-RNAseq data reveals distinct epithelial

mechanisms of inflammation.

Differential gene expression and transcription factor pathway activation in DCS (A, B) and

SCs (C, D) in Inflamed vs non-Inflamed groups.

(E) Basic trajectory pseudotime analysis of the route from SCs to DCS. Taking the middle

and late expressed genes in this trajectory (*KRT8*-early, *SCG5*-middle and *ANXA1*-late).

(F) Bubble plot showing the expression of DCS trajectory related genes according to health

status.

a

TNF-alpha Signaling via NF-kB
Apical Junction
Epithelial Mesenchymal Transition
Angiogenesis
IL-6/JAK/STAT3 Signaling
Coagulation
UV Response Up
Apoptosis
p53 Pathway
Estrogen Response Early

b

ANXA1 *REG4* *TFF1*

MDFI *PI3* *CD55*

c

Health
Inflamed / Non-inflamed / Healthy

% expressing
0 38 75

id
ANXA1
REG4
TFF1
CD55
TIMP1
PI3

Scaled mean expression
0 0.5 1

d SCP259 - Ulcerative Colitis

ANXA1 *REG4* *CD55*
TFF1 *TIMP1* *PI3*

Health Status
Non-inflamed
Inflamed
Healthy

SingleR Labels
BEST4+ ISC TA

Avg Expression
0.0 0.5 1.0 1.5

e

group
Normal
Possible inflammation
Erosions or Ulcers

Z-score
2
1
0
-1
-2

f C18 expression in IBD Plexus (UC cohort)

Expression (log(CPM + 1), Batch-corrected)

Normal Possible Inflammation Inflammation
Group

** **** ****

h IL22-Treated Organoids

Genes
TIMP1
TFF1
REG4
PI3
CD55
ANXA1

Control Treated

Cell Count
400
800
1200
1600

Avg Expression
0.4
0.0
-0.4

i IL22-Treated Organoids

Genes
TIMP1
TFF1
REG4
PI3
CD55
ANXA1

Lgr5_Positive Lgr5_Negative

Cell Count
500
1000
1500
2000

Avg Expression
0.4
-0.4

g (GSE22307)

Normalised Z-score

Day
0 2 4 6

A

Differential gene expression in DCS cells in
Inflamed vs. NonInflamed

B

Differential transcription factor activation and pathway activity in
Inflamed vs. NonInflamed (DCS cells)

C

Differential gene expression in Stem cells in
Inflamed vs. NonInflamed

D

Differential transcription factor activation and pathway activity in
Inflamed vs. NonInflamed (Stem cells)

E

Stem -> DCS trajectory analysis

Early

Middle

Late

F

Expression of DCS trajectory related genes according to health status