

A transcriptomic score to classify the inflammation-dysplasia-cancer sequence lesions in Inflammatory Bowel Disease

Anneline Cremer MD, PhD^{1,2}, Nicolas Rosewick PhD², Maxfield Kelsey³, Eric Trépo MD, PhD^{1,2}, Frédéric Libert PhD⁴, Martine De Vos MD, PhD⁵, Filip Baert MD, PhD⁶, Tom Moreels MD, PhD⁷, Edouard Louis MD, PhD⁸, Jean-François Rahier MD, PhD⁹, Pieter Demetter MD, PhD², John M. Sedivy PhD³, Séverine Vermeire MD, PhD¹⁰ and Denis Franchimont MD, PhD^{1,2}.

1. Department of Gastroenterology, HUB Erasme University Hospital, Université Libre de Bruxelles, Brussels, Belgium
2. Laboratory of Experimental Gastroenterology, Université Libre de Bruxelles, Brussels, Belgium
3. Center on the Biology of Aging, and the Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, United States
4. Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire (IRIBHM), Université Libre de Bruxelles, Brussels, Belgium.
5. Department of Gastroenterology, University Hospital Ghent, Ghent, Belgium
6. Department of Gastroenterology, AZ Delta, Roeselare, Belgium
7. Department of Gastroenterology, University Hospital Antwerp, Edegem, Belgium
8. Department of Gastroenterology, University Hospital Liège, Liège, Belgium
9. Department of Gastroenterology, CHU UCL Namur site Mont-Godinne, Université catholique de Louvain, Yvoir, Belgium
10. Department of Gastroenterology, University Hospital Leuven, Leuven, Belgium

Correspondence:

Anneline Cremer, MD, PhD

Department of Gastroenterology and Hepatology, Erasme University Hospital

Route de Lennik 808, 1070 Brussels, Belgium

Tel: +32 25555773

Mail: anneline.cremer@hubruxelles.be

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Abbreviations:

AIC	Akaike Information Criterion
BIRD	Belgian Inflammatory Bowel Disease Research Group
BMP5	Bone Morphogenetic Protein 5
CA	Carbonic Anhydrase
CAC	Colitis-Associated Colorectal Cancer
CD	Crohn's Disease
cGAS	Cyclic GMP-AMP Synthase
ChIP-seq	Chromatin Immunoprecipitation Sequencing
CMS	Consensus Molecular Subtypes
CRC	Colorectal Cancer
DESeq2	Differential Expression Analysis using Sequence Counts version 2
DNA	Deoxyribonucleic acid
ENCODE	Encyclopedia of DNA Elements
FFPE	Formalin-Fixed Paraffin-Embedded
GSEA	Gene Set Enrichment Analysis
HERVK	Human Endogenous Retrovirus type K
HGD	High-Grade Dysplasia
IBD	Inflammatory bowel disease
IDC	Inflammation-Dysplasia-Cancer
IFN	Interferon
IRF3	Interferon Regulatory Factor 3
KCNQ1OT1	CDKN1C/KCNQ1 Opposite Strand/Antisense Transcript 1
KRAS	Kirsten rat sarcoma virus
L1HS	LINE-1 element from Homo sapiens
LGD	low grade dysplasia
lncRNA	long non-coding RNA
miRNAs	microRNAs
MSI	microsatellite instability
MSigDB	Molecular Signatures Database
NES	Normalized Enrichment Score
NF- κ B	Nuclear Factor kappa-light-chain-enhancer of activated B cells
NOS	Not Otherwise Specified
olm	Ordinal Logistic Regression
PCA	Principal Component Analysis
PSC	Primary sclerosing cholangitis
RefSeq	Reference Sequence
RNA	Ribonucleic Acid
RTEs	Retrotransposable Elements
SSL	Sessile Serrated Lesion

STING	Stimulator of Interferon Genes
T2T-HS1	Telomere-to-Telomere Human Reference Genome version 1
TCGA	The Cancer Genome Atlas
TCGAbiolinks	The Cancer Genome Atlas Bioinformatics Links R package
TCGA-COAD	The Cancer Genome Atlas Colon Adenocarcinoma
TNF- α	Tumor necrosis factor- α
TSA	Traditional Serrated Adenoma
UC	Ulcerative colitis
vst	Variance Stabilizing Transformation

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ABSTRACT

Background and aims:

Inflammatory bowel disease (IBD) is associated with a higher risk of developing colorectal cancer, according to the inflammation-dysplasia-cancer (IDC) sequence from inflammation to colitis-associated colorectal cancer (CAC). The objective of this study was to identify and generate a transcriptomic signature and score, related to the IDC sequence, that could ultimately classify dysplasia and cancer in IBD.

Methods:

Demographics, clinical parameters, histological characteristics and RNA-sequencing data were evaluated on 134 formalin-fixed paraffin-embedded lesions from 2 independent cohorts of IBD patients with low- or high-grade dysplasia (LGD, HGD) and/or CAC. An ordinal logistic regression screened for significant IDC sequence-associated genes that were computed in a transcriptomic signature score.

Results:

Principal component analysis and unsupervised clustering on 1% of the most variable genes showed a good clustering between the 4 lesion groups (Normal Mucosa, Inflamed Mucosa, LGD/HGD, and CAC). A gene signature was identified on 27 genes that correlated with the lesion groups in the exploratory cohort. The most weighted gene in this transcriptomic signature was the long non-coding regulatory RNA KCNQ1OT1, a gate keeper against genomic instability and transposon activation. Based on these 27- genes expression, we built and validated a transcriptomic signature score to classify dysplasia and CAC. The overall accuracy of the transcriptomic signature score was 85.71% in the exploratory cohort and 90.91% in the validation cohort.

Conclusion: We identified a tissue-based transcriptomic score to classify IDC lesions in IBD patients and uncovered some of the pivotal genes in the carcinogenesis related to inflammation in IBD.

Keywords: RNA expression; carcinogenesis; inflammatory bowel disease; dysplasia; colorectal cancer

INTRODUCTION

Patients with inflammatory bowel disease (IBD) (Crohn's disease (CD) and ulcerative colitis (UC)) have an increased risk of developing dysplasia and colorectal cancer (CRC), namely colitis-associated colorectal cancer (CAC) compared to the general population[1]. Carcinogenesis related to IBD follows the inflammation-dysplasia-cancer (IDC) sequence from inflammation, to low-grade (LGD), high-grade dysplasia (HGD), and cancer. Chronic mucosal inflammation is the obvious trigger as disease extent, duration, and activity are the most prominent clinical risk factors for CAC[2].

The management of patients with dysplasia, more specifically with LGD lesions, remains a challenge for most clinicians as some patients/LGD lesions are associated with a higher risk of progression to CAC. Lesion (flat or polypoid, multifocal lesions) or patient (sclerosing cholangitis) characteristics are the only markers to guide therapeutic decisions towards endoscopic resection or surgery[1]. A signature of the IDC sequence could potentially help stratify lesions at risk for developing CAC and adapt follow-up and treatment decisions accordingly. This requires in the first place the description of molecular features of these mucosal lesions before further prognostic validation in prospective cohorts.

This IDC progression results from the transformation and progressive accumulation of chromosomal abnormalities, somatic mutations, and epigenetic modifications that is distinct from the adenoma-carcinoma sequence in the general population[3]. The same driver genes of the canonical pathways of carcinogenesis are present and may contribute to the development of CAC[4], but their occurrence has been reported repeatedly to differ in timing and frequency compared to sporadic CRC[5–7]. Most notably *TP53* mutations are typically early events during tumour progression in CAC, whereas *TP53* mutations rarely occur in the adenomatous precursors of sporadic CRC. On the contrary, *APC* and *KRAS* mutations are reported to be less prevalent and occur later in CAC than in sporadic CRC[8–10]. Mutations may not be the first event predisposing to cancer development in IBD. The IDC sequence seems to result more from a transcriptional rewiring, through non genetic events, such as epigenetic changes, early during tumorigenesis[11]. Indeed, careful analysis of the clonality of genomic changes of the surrounding mucosa of associated early lesions and/or CAC indicates substantial genetic heterogeneity and the absence of a clear genetic field effect for cancer risk[12]. Today, it is difficult to reconcile all these reported findings of the IDC sequence in a comprehensive model.

Early and late transcriptional changes during the IDC sequence can highlight some of the transcriptional rewiring pathways that are dysregulated and participate to the tumorigenesis related to inflammation. The best illustration is the transcriptome-based classification of CRC with the large-scale international effort resulting in amalgamation of 4 consensus molecular subtypes (CMS)[13]. To the same extent, some transcriptomic changes during the IDC sequence must help the classification of dysplasia and CAC with some having a mechanistic input in the transformation of the colonocytes while others only reflecting the consequences of dedifferentiated colonocytes and their environment.

The objectives of this study were to detect in the IDC sequence genes which expression is correlated with lesion groups to identify/generate a transcriptomic gene signature and score that best classify IDC lesions in a large multicentre retrospective cohort of IBD patients.

MATERIALS AND METHODS

This is a large national cohort study conducted across 7 Belgian tertiary centres within the Belgian Inflammatory Bowel Disease Research and Development (BIRD) Group. We retrospectively evaluated all patients with histologically confirmed IBD, diagnosed with at least 1 episode of dysplasia (LGD or HGD) and/or CAC between January 1, 1990, and December 31, 2016. Ethics Committee reference number approval: P2013/331 (February 25, 2014). We have built one of the world's largest cohorts of dysplasia and CAC in IBD patients with 1183 lesions in 541 patients between 1990 and 2016. See characterization of the Dysplastic/Colitis-associated Colorectal Cancer lesions in Cremer et al[14]. Among those, 150 formalin-fixed paraffin-embedded (FFPE) samples underwent RNA sequencing and 134 were analysed (flowchart in **Suppl Figure1**). Clinical characteristics and demographic details of the patients were collected by electronic chart review. Two cohorts of patients were analysed. An exploratory cohort to build the transcriptomic score which was validated on the samples of our validation cohort. Normal Mucosa and Inflamed Mucosa are defined in supplementary materials. Methodology about histological classification of the lesions, consensus molecular subtypes analysis and Gene set enrichment analysis (GSEA) are described in supplementary materials.

RNA-seq preprocessing

RNA extraction, quality assessment of RNA, library preparation, and RNA-sequencing methodology are described in supplementary materials. Sequence quality was assessed using FastQC to explore quality distribution and other sequence characteristics. Raw reads were aligned on human hg38 genome using STAR aligner (v2.7.5a)[15]. Read counts per gene (ENSEMBL v100) were extracted using feature Counts[16]. Gene expression analysis was performed using Differential Expression Analysis using Sequence Counts version 2 (DESeq2) R package[17]. Samples with high levels of rRNA read count (>25% of total read count) were further discarded from analysis. Only genes expressed (>10reads) in minimum 10 samples were used for further analysis (36,193 out of 60,668). DESeq2's normalization using Variance Stabilizing Transformation (vst) was used for clustering, visualization and gene signature construction. Unsupervised hierarchical clustering was performed using Euclidean distance and complete linkage on DESeq2's vst read counts. Analysis of retrotransposon expression is described in supplementary materials.

Gene signature and transcriptomic score

In order to detect genes which expression is correlated with lesion groups (Normal Mucosa→Inflamed Mucosa→LGD/HGD→CAC) in the IDC sequence, we performed an ordinal logistic regression (olm) for each 36,193genes (expression(vst)~group). The construction of the gene signature and the transcriptomic score is described in **Figure 2** and in supplementary materials.

The Cancer Genome Atlas

The Cancer Genome Atlas Colon Adenocarcinoma (TCGA-COAD) data were retrieved using TCGAbiolinks R package[18]. Data processing is described in supplementary materials.

RESULTS

Study population

A total of 95 neoplastic lesions inside diseased areas (56LGD, 7HGD and 32CAC), 25 histologically Inflamed Mucosa on biopsies taken during follow-up colonoscopy of IBD patients, and 14 histologically Normal Mucosa on biopsies taken during routine screening colonoscopy of healthy individuals were included in the study. Exploratory cohort included 73 lesions (45LGD, 3HGD and 25CAC) from the same number of

IBD patients, while validation cohort includes 22 lesions (11LGD, 4HGD and 7CAC) from 16 IBD patients (**Suppl Table 1**). Demographics and clinical parameters of the study population are summarized in **Table 1**. Histological and transcriptional classification of dysplasia and CAC are described in supplementary data.

Global gene expression profiling according to stages of neoplasia

After alignment on human genome, on average 22,768,770 (min:625,082–max:63,056,801) and 14,879,093 (min:553,740–max:40,332,689) RNA-seq reads aligned on ENSEMBL genes for exploratory and validation cohorts respectively and were further used in gene expression analysis. On the entire cohort, 36,193 genes were identified in the sequence (Normal Mucosa-Inflamed Mucosa-LGD/HGD-CAC). Principal component analysis (PCA) (**Figure 1A**) and unsupervised clustering of the top 1% (362genes) of the most variable genes from 36,193 genes and 134 samples (**Figure 1B, Suppl Table 3**) showed an overall good clustering between the 4 lesion groups (Normal Mucosa-Inflamed Mucosa-LGD/HGD-CAC). PCA and unsupervised clustering of CAC samples highlighted a good segregation especially between CMS4 and CMS 1, 2 and 3 (**Suppl Figure 2**). Gene set enrichment analyses are described in supplementary data.

A transcriptomic signature-score specific of the inflammation-dysplasia-cancer sequence

Using an ordinal logistic regression (**Figure 2A**), a gene signature of 27 genes that correlated with lesion groups was identified based on our exploratory lesions (**Figure 3**). A gene signature weight for the 27 genes was computed using a backward elimination strategy (**Suppl Table 4**). Among these 27 genes, 23 were downregulated and 4 were upregulated. Heatmap showed that 4 groups were clustered together and very well separated from each other when considering only the 27 genes of the gene signature (**Figure 4**). Based on the 27 genes expression, we generated a transcriptomic score to classify dysplasia (LGD/HGD) and CAC in IBD patients. Coefficients for all genes are available in supplementary data (**Suppl Table 5**). The overall correct classification rate based on olm probabilities of the transcriptomic score was 85.71% in our exploratory cohort. We then validated this transcriptomic score in an independent validation cohort with an overall correct classification rate of 90.91% (**Table 2, Suppl Figure 5**). Sensitivity and specificity of the score are available in supplementary data.

Replication of the transcriptomic signature score in the TCGA-COAD cohort

The transcriptomic signature score was applied to the publicly available TCGA-COAD cohort with an overall correct classification of 16.00% (**Suppl Table 6, Suppl Figure6**). Interestingly TCGA normal samples were perfectly classified as normal samples, while TCGA-COAD samples were misclassified highlighting transcriptomic differences between CAC and sporadic CRC.

KCNQ1OT1 and retrotransposable element expression in the inflammation-dysplasia-cancer sequence

The gene signature weight for the 27 genes using a backward elimination strategy (**Suppl Table 4**) showed that the most weighted gene in this transcriptomic signature is KCNQ1OT1 (KCNQ1 opposite strand/antisense transcript 1 of CDKN1C (cyclin-dependent kinase inhibitor 1C), an un-spliced long non-coding regulatory RNA (lncRNA). KCNQ1OT1 is a paternally expressed lncRNA that is involved, in *cis*, in the transcriptional silencing of eight to ten nearby protein-coding genes and, in *trans*, in heterochromatin reorganisation and repression of transposon activation and retrotransposition[19,20]. KCNQ1OT1 guards against genomic instability and senescence through sequence-specific DNA methylation and hence repression of RTEs, in particular evolutionarily young LINE-1 (L1HS) and Alu subfamilies (AluY)[19]. Indeed, RTEs promote genomic instability, insertional mutagenesis and therefore tumorigenesis, as has been reported in a wide variety of cancers[21,22]. In order to evaluate the potential relationship of KCNQ1OT1 with the expression of RTEs in the IDC sequence, we first looked at the expression of RTEs according to lesion group. Interestingly, there was a significant increase of L1HS, AluY, and Human Endogenous Retrovirus type K (HERVK) subfamily expression in LGD/HGD and CAC groups compared to the control group (**Suppl Figure7A**). HERVK expression was significantly increased as early as in the inflammation group compared to the control group. To demonstrate a direct association between KCNQ1OT1 and these RTEs, we then looked at the expression of KCNQ1OT1 and RTEs in the different lesion groups (**Suppl Figure7B**) and in each of the samples (**Suppl Figure7C**) and found a direct correlation in the expression of KCNQ1OT1 and RTEs across the lesion groups of IDC sequence.

Since KCNQ1OT1 is a negative regulator of RTEs, a repressor being positively correlated with its target is counter intuitive. One possible explanation is that the concomitant increase of RTEs and KCNQ1OT1 results from a positive regulatory feedback loop of RTEs on KCNQ1OT1 expression. Inflammation has been associated with RTE de-repression[23]; indeed, as found here with HERVK expression. This would suggest that a primary increase of RTEs during inflammation and later in tumorigenesis might secondarily induce

KCNQ1OT1 expression. Inversely, it is well known that LINE1 expression can trigger a type I interferon (IFN-I) response and promote inflammation[24]. The L1 retro-transposition cycle produces cytosolic cDNA which the cell takes as evidence of an invading viral pathogen via sensing by Cyclic GMP-AMP Synthase (cGAS)[24]. The cGAS-Stimulator of Interferon Genes (STING) axis then orchestrates an IFN-I response through the activation of Interferon Regulatory Factor 3 (IRF3) and Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF- κ B)[25]. We thus interrogated the Encyclopaedia of DNA Elements (ENCODE) Consortium (National Human Genome Research Institute) for transcription factor binding to the KCNQ1OT1 promoter (**Suppl Table 7**). Examining transcription factor Chromatin Immunoprecipitation Sequencing (ChIP Seq) data, we found that both IRF3 and NF- κ B bind the KCNQ1OT1 promoter in the majority of cell lines assayed (**Suppl Figure 8**)[26]. Thus, our data, together with publicly available TF ChIP results, suggests that RTEs may promote KCNQ1OT1 expression through its IFN-responsive promoter, resulting in the concomitant increase of RTEs and KCNQ1OT1 along the IDC progression sequence.

DISCUSSION

In this study, we evaluated the transcriptome of 134 different lesions of the IDC sequence in IBD. We identified a gene signature of 27 genes of which the expression was correlated with the IDC sequence in an exploratory cohort, from normal and inflamed-mucosa, to dysplastic lesions and CAC. This transcriptomic signature helped generate a transcriptomic score that showed a good prediction rate in reclassifying the lesions in a small validation cohort. It is important to point out that this score is not intended to predict future progression from inflammation to dysplasia and CAC. It is a class prediction score that helps the correct classification of the lesion. Remarkably, this IDC signature score failed to reclassify the sporadic CRC lesions from the publicly available TCGA cohort suggesting that the IDC gene score is specific or only representative of the inflammation-induced tumorigenesis. Yet, some of these 27 genes have already been reported in separate studies as relevant prognostic marker for disease progression and/or overall survival in sporadic CRC and in cancer overall [19,20,27–31].

This 27 gene signature may reveal some of the relevant players in the IDC sequence. All these genes are related to colonocytes or infiltrated immune cells in the dysplasia or adenocarcinoma specimens. Obviously, it is uncertain whether they reflect causes or consequences of the tumorigenesis process. Among the 27 genes in the signature, several have notable biological relevance. For example, CD163L1 is

likely associated with macrophage activity [32], CD177 may relate to neutrophil infiltration [33], and PYY could be linked to SCFAs and the GLP-1 axis [34]. The most weighted gene in this transcriptomic signature is KCNQ1OT1, an un-spliced lncRNA, that is involved in transposon activation and heterochromatin reorganization[19]. KCNQ1OT1 was initially identified in the imprinting control region 2 on human chromosome 11p15[35], that is the most epi-mutated region of the congenital imprinting disorder, Beckwith-Wiedemann Syndrome, associated with an increased tumour risk[36,37]. KCNQ1OT1 seems clinically deleterious as it represents a poor prognostic marker for disease progression in these cancers[20]. A recent study highlights the specific role of KCNQ1OT1 in guarding against genomic instability and senescence through sequence-specific DNA methylation and hence repression of RTEs, particularly evolutionarily young LINE1 (L1HS) and Alu subfamilies (AluY)[19]. Indeed, RTEs promote genomic instability, insertional mutagenesis and therefore tumorigenesis, as has been reported in a wide variety of cancers[21,22]. Widespread LINE1 insertions have been documented as an early event in gastrointestinal cancer evolution[21,38]. In this study, we showed an increase in the expression of RTEs along the IDC sequence, and a direct correlation between KCNQ1OT1 and RTE expression across lesions of the IDC sequence. We speculate that KCNQ1OT1 may initially be able to restrain RTE de-repression and expression early on during chronic inflammation, but as insults to epigenomic stability accrue throughout the IDC sequence, despite the increased expression of KCNQ1OT1, RTEs can ultimately surpass this defense mechanism and promote inflammation-induced tumorigenesis[12,39]. Thus, the predominant weight assigned to KCNQ1OT1 in this transcriptomic signature, may in fact make it a surrogate marker of global retrotransposon activation, suggesting in turn a pathogenic role for RTEs in the IDC sequence.

In parallel, the transcriptomic signature identified genes that code for structural and/or functional proteins in various cell systems. Interestingly, most transcripts, except 3 of them, in the signature are inversely associated with CAC, suggesting that the downregulation of key pathways is a hallmark of tumorigenesis. Claudin-1, a tight junction specific protein, is one of the 3 upregulated genes in the signature. Claudin-1 overexpression has already been reported in active IBD and in IBD-associated dysplasia and CAC[30] and was correlated with inflammation. Several of the downregulated genes have already been reported so far in IBD and IBD-related dysplasia and cancer such as the carbonic anhydrases CA2 and CA4 genes[27]. Interestingly, CA2 and CA4 may reflect functional interrelationships that are relevant to the IDC sequence. The observed downregulation of CA2 in our study contrasts with findings in sporadic CRC, where CA2 overexpression has been linked to poor prognosis[40]. This discrepancy may reflect differences between colitis-associated cancer (CAC) and sporadic CRC or variations in gene versus

protein expression. Humoral immune response, immunoglobulin complex and antigen binding were the most enriched pathways in the top 1% mostly regulated genes. ADAMDEC1 is an anti-inflammatory secreted peptidase belonging to the disintegrin metalloproteinase family, that plays a role in the crosstalk between dendritic cells and germinal center T-helper cells, and is almost exclusively expressed in the gastrointestinal tract[31,41]. TMIGD1 is part of immunoglobulin domain containing cell adhesion molecules involved in several processes, including cell differentiation and apoptosis. TMIGD1 expression is lower in active CD, progressively lost in sporadic CRC, and associated with poor CRC overall survival[29]. SCNN1B, a sodium transporter and known target of amiloride included in the signature, is notable for its therapeutic implications. While amiloride has been reported to inhibit colon cancer cell growth in vitro [42], there are conflicting data regarding diuretic use and colon cancer mortality [43]. These findings highlight the need for further research into its potential role in CAC progression and its utility as a therapeutic target. BMP5 gene modulates epithelial-mesenchymal transition and its loss of expression correlates with recurrence and poor prognosis in human CRC[28].

One limitation of our study is the use of FFPE-based sequencing, which can introduce biases; however, rigorous quality control and consistent processing across samples ensured robust differential expression, and future validation in fresh or Trizol-preserved specimens will confirm its reproducibility. Another limitation is the possibility of overfitting when deriving a 27-gene signature from the analysed sample size. However, the robust performance of the signature in the validation cohort supports its generalizability. It is important to note that we observed significant differences in gene expression between normal control tissue and inflamed mucosa, which underscores the relevance of including inflamed mucosa in our analysis. This choice was made to capture the full spectrum of transcriptional changes in the IDC sequence, reflecting the natural history of CAC [44]. While inflammation introduces variability in gene expression, driven by immune cell infiltration and widespread transcriptional disturbances, this variability represents a critical early step in the IDC sequence [45,46]. Excluding inflammation could lead to a different set of genes with more linear expression patterns but would fail to represent the early events initiated by inflammation that drive tumorigenesis in IBD

In conclusion, we have identified a transcriptomic 27 gene signature associated with the IDC sequence and generated promising hypotheses about pivotal genes and underlying mechanisms involved in its progression, providing a basis for future functional validation studies. The most discriminant top gene in this signature, the non-coding RNA KCNQ10T1, is consistent with the activation of RTEs that may be

involved in the progression of the IDC sequence. The transcriptomic score generated here helps the classification of the lesions of the IDC sequence but should be further evaluated for its prognostic value in prospective independent cohorts.

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TABLES

Table 1. Demographics and clinical parameters of the study population (most advanced grade) (n= Number of patients)

Variables	LGD/HGD (n=50)	CAC (n=27)	Normal Mucosa(n=14)	Inflamed Mucosa(n=25)
Type of IBD, n (%)				
- CD	16 (32%)	14 (52%)	NA	6 (24%)
- UC	33 (66%)	12 (44%)		18 (72%)
- Unclassified IBD	1 (2%)	1 (4%)		1 (4%)
Male, n (%)	35 (70%)	14 (52%)	4 (29%)	11 (44%)
Age (yr) at IBD diagnosis, median (IQR)	50 (36-65) (n=47)	33 (25-49) (n=27)	NA	24 (22-39) (n=25)
Follow-up duration (yr) after IBD diagnosis, median (IQR)	15 (8-22) (n=47)	28 (16-33) (n=27)	NA	NA
Deceased, n (%)	3 (6%)	10 (37%)	NA	NA
Smoking status, n (%)				
- Active	2 (4%)	4 (15%)		
- Stopped	16 (32%)	5 (18,5%)		
- No	17 (34%)	13 (48%)		
- Unknown	15 (30%)	5 (18,5%)	14 (100%)	25 (100%)
Age (yr) at diagnosis of the index lesion, median (IQR)	57 (48-71) (n=50)	56 (46-63) (n=27)	49 (34-58)	27 (23-46) (n=25)
Duration (yr) of IBD at time of index lesion diagnosis, median (IQR)	8 (1-14) (n=47)	18 (9-26) (n=27)	NA	1 (0-5)
Follow-up duration (mo) after diagnosis of the index lesion, median (IQR)	5 (2-8) (n=50)	4 (2-9) (n=27)	NA	NA
Family history of CRC, n (%)				
- Yes	3 (6%)	1 (4%)	0	0
- No or unknown	47 (94%)	26 (96%)	14	25
Associated PSC, n (%)	4 (8%)	3 (11%)	0	0

*p value for two-sided Chi-squared test or Kruskal-Wallis test. Bold values are significant. Patients were classified according to the most advanced lesion during colonoscopy or at surgery performed at follow-up.

LGD: low-grade dysplasia; HGD: high-grade dysplasia; CAC: colitis-associated colorectal cancer; CRC: colorectal cancer; PSC: primary sclerosing cholangitis; IQR: interquartile range; IBD: inflammatory bowel disease; CD: Crohn disease; UC: Ulcerative colitis

Table 2. Classification rates of the transcriptomic signature score in the exploratory and validation cohorts						
Exploratory cohort		Lesion groups classification based on ordinal logistic probabilities				% correct classification
		Normal Mucosa	Inflamed Mucosa	LGD/HGD	CAC	
Real group	Normal Mucosa	13	1	0	0	92.86 %
	Inflamed Mucosa	0	18	7	0	72.00 %
	LGD/HGD	0	7	40	1	83.33 %
	CAC	0	0	0	25	100.00 %
Total correct classification						85.71 %
Validation cohort		Lesion groups classification based on ordinal logistic probabilities				% correct classification
		Normal Mucosa	Inflamed Mucosa	LGD/HGD	CAC	
Real group	LGD/HGD	0	0	13	2	86.67 %
	CAC	0	0	0	7	100 %
Total correct classification						90.91 %

FIGURE LEGENDS

Figure 1. Unsupervised transcriptomic profile of the IDC sequence. **A)** PCA bi-plot of all samples coloured by lesion groups and **B)** heatmap of the top 1% of most variable genes (n=362genes) within all samples reflects lesion groups of the IDC sequence.

Figure 2. Schematic description of the transcriptomic score formula. **A)** For each gene a p-value and a beta regression are computed using an ordinal logistic regression. The beta represents the expression trend of the associated gene relative to IDC sequence (i.e., if positive the gene follow a positive trend from Normal Mucosa towards CAC e.g. Gene2; in opposite if negative the gene follows a negative trend from Normal Mucosa towards CAC e.g. Gene1). Non-significant p-value (i.e., $p > 0.05$) represents an absence of expression trend (e.g., Gene3). **B)** Selection of the best gene signature based on Akaike information criterion (AIC). Each point (x,y) represents a gene list of length x with an AIC of y. Gene lists were build based on genes ordered by olm p-value computed on the IBD-IDC sequence (Normal Mucosa→Inflamed Mucosa→LGD/HGD→CAC). Transcriptional signature score was computed for 100 incremental gene lists (from n=1gene to n=100genes in the signature). An AIC for each gene lists assessing the quality of the model (i.e., the transcriptional signature score reflects the IBD-IDC sequence) was computed. The gene list with the lowest AIC (first 27 genes ordered by olm p-value) was retained as IBD-IDC gene signature. **C)** To assess the selected 27-gene signature robustness we computed an empirical p-value for the N=27genes in the IBD-disease signature by counting the number of size-matched sampled gene list (n=100,000) with a lower AIC than the 27-gene IBD-disease signature. **D)** In order to build a transcriptomic score using a list of genes for a sample of interest, we multiply for each gene its associated beta with the gene expression value in the sample of interest. Hereby two examples for Sample A and B using Gene 1, 2 and 3.

Figure 3. Expression of the 27 genes included in the IBD-IDC gene expression signature in the exploratory cohort. Expression scale is in DESeq2's vst read count.

Figure 4. Transcriptional signature score based on the 27-gene signature. **A)** Heatmap of the 27genes of the gene signature in the exploratory cohort. Top bar shows the signature score and the lesion groups of the IDB-IDC sequence. **B)** Transcriptional signature score of the exploratory cohort stratified by IBD-IDC sequence lesion groups. **C)** Transcriptional signature score of the validation cohort stratified by IBD-IDC sequence lesion groups.

Authors' contribution

AC: conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article. DF: conception and design of the study, analysis and interpretation of data, drafting the article. NR, MK: analysis and interpretation of data, revising the article critically for important intellectual content. Other acquisition of data, revising the article critically for important intellectual content. All final approval of the version to be submitted.

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Data transparency statement: Data and analytic methods underlying this article will be made available to researchers upon reasonable request to the corresponding author.

Profiling Accession Numbers: Data will be available via the NCBI's Gene Expression Omnibus after approval of the manuscript.

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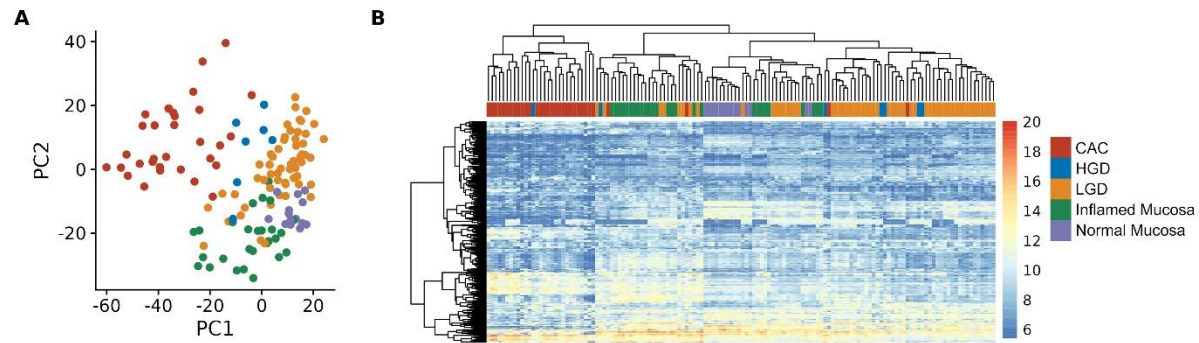
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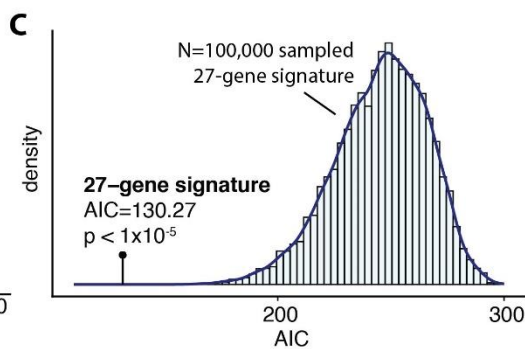
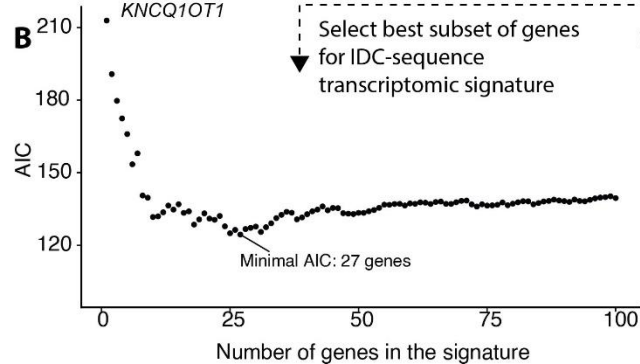
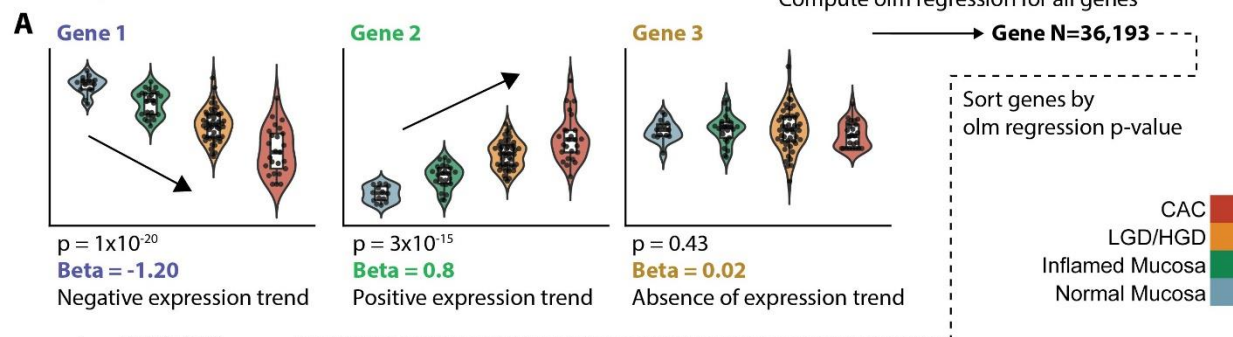
Figure 1



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Figure 2

Transcriptomic score formula



D Score for list of genes

- Example with genes 1, 2 and 3 in **Sample A**:

Sample A expression
gene1 = 3.2
gene2 = 7.1
gene3 = 10.4

$$\begin{aligned} & \frac{-1.20}{0.80} \times \frac{3.2}{7.1} \text{ Beta(gene 1) x expression gene 1 in sample A} + \\ & \frac{0.80}{0.02} \times \frac{7.1}{10.4} \text{ Beta(gene 2) x expression gene 2 in sample A} + \\ & \frac{0.02}{10.4} \times \frac{10.4}{10.4} \text{ Beta(gene 3) x expression gene 3 in sample A} \end{aligned}$$

$$\begin{aligned} & -3.84 \\ & + 5.68 \\ & + 0.208 \\ & \text{Transcriptomic score} \\ & = 2.048 \end{aligned}$$

- Example with genes 1, 2 and 3 in **Sample B**:

Sample B expression
gene1 = 1.3
gene2 = 12.1
gene3 = 5.7

$$\begin{aligned} & \frac{-1.20}{0.80} \times \frac{1.3}{12.1} \text{ Beta(gene 1) x expression gene 1 in sample B} + \\ & \frac{0.80}{0.02} \times \frac{12.1}{5.7} \text{ Beta(gene 2) x expression gene 2 in sample B} + \\ & \frac{0.02}{5.7} \times \frac{5.7}{5.7} \text{ Beta(gene 3) x expression gene 3 in sample B} \end{aligned}$$

$$\begin{aligned} & -1.56 \\ & + 9.68 \\ & + 0.114 \\ & \text{Transcriptomic score} \\ & = 8.234 \end{aligned}$$

Figure 3

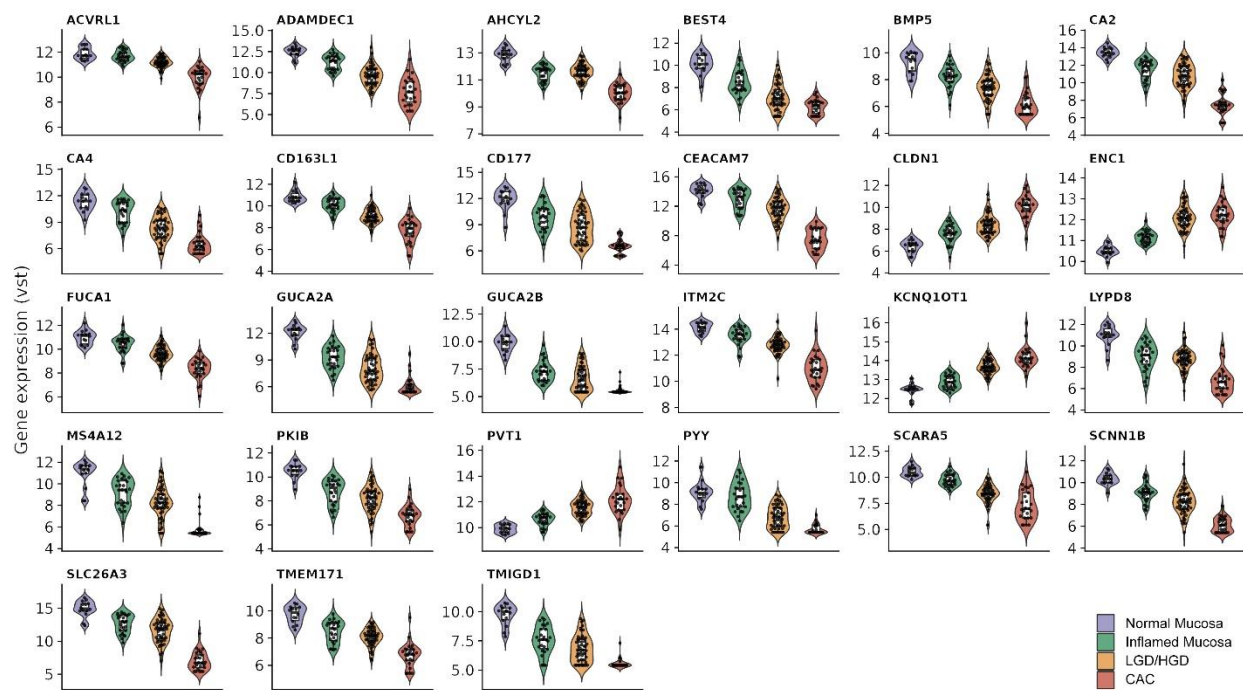


Figure 4

