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A prediction of the CRNDE role by modulating NF-κB pathway in inflammatory bowel disease (IBD)

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ABSTRACT

Long non-coding RNAs (lncRNAs) regulate multiple pathways and cellular mechanisms. Recent research has emphasized their involvement in the pathogenesis of complex diseases, such as Inflammatory Bowel Disease (IBD) which is characterized by chronic inflammation of the intestines. The two most common types of IBD are ulcerative colitis and Crohn's disease. CRNDE lncRNA was initially detected in colorectal cancer (CRC) and found to be involved in the tumorigenesis pathways. Further studies revealed the role of CRNDE in activating inflammation and promoting the release of inflammatory cytokines. This study utilizes the RNA-seq data analysis and bioinformatics tools to clarify the role of CRNDE in the IBD pathogenesis and confirms its expression in inflamed HT-29 and Caco-2 cell lines and also colonic and blood samples of UC patients and controls ex vivo. Based on our results, *CRNDE* was significantly upregulated in IBD samples compared to controls in RNA-seq data analysis and Real-time PCR of inflamed HT-29 cell line and colonic biopsies from UC patients. Additionally, predicted that its expression is positively correlated with the pro-inflammatory cytokines production. CRNDE interactions was investigated with several inflammation-related miRNAs and regulatory proteins computationally. Thus, *CRNDE* upregulation in the colon of IBD patients could be involved in IBD pathogenesis by promoting inflammatory pathways and targeting anti-inflammatory miRNAs.

1. Introduction

Inflammatory bowel disease (IBD) is a chronic intestinal disease that is mainly classified as ulcerative colitis (UC) and Crohn's disease (CD) [1]. The pathogenesis of IBD involves genetic susceptibility, environmental factors, and an imbalance in the gut microbiome, leading to dysregulated innate and adaptive immune system responses [2]. UC is described as chronic inflammation restricted to the colon. Symptoms include fever and fatigue, loss of appetite, abdominal pain, diarrhea with bleeding, and unintended weight loss [3]. On the other hand, CD is characterized by skip lesions and transmural inflammation that can affect the entire gastrointestinal tract from the mouth to the anus and its presenting symptoms are often variable and common with UC [4].

Long non-coding RNAs (lncRNAs) are a group of noncoding RNAs with >200 bp length and lack an open reading frame that encodes proteins [5]. Recent studies display that lncRNAs are involved in various biological processes in the human body, such as transcriptional activation of cells, intracellular transport, heat shock response, cell proliferation, apoptosis, metabolism, and drug resistance [6–8]. LncRNA can interact with proteins and can also act as an endogenous molecular sponge to competitively interfere with the biological activity of miRNA, thereby reducing the transcriptional inhibition function of miRNA on its

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downstream target genes [9,10]. Studies indicated a strong connection between various lncRNAs and IBD. Furthermore, lncRNAs have been suggested as a personalized therapy for IBD with the potential for accurate diagnosis, prognosis, and the prediction of therapeutic responses [11].

As a main pathway in IBD pathogenesis, the nuclear factor κB (NF-κB) transcription factor complex has an important function in the immune response and cell survival by targeting pro-inflammatory genes, anti-apoptotic proteins, and angiogenesis regulators [12,13]. Bacterial products, such as lipopolysaccharide (LPS) or host cytokines such as tumor necrosis factor α (TNFα), triggers NF-κB–related gene transcription and pro-inflammatory cytokine production such as Interleukin (IL)-1B and IL-6 [14]. Studies indicate that TNF-α and NF-κB pathways are strongly involved in initiating inflammation in the intestinal mucosa of IBD patients [15]. Therefore, TNF-α monoclonal antibody (infliximab) is used clinically to treat IBD and has achieved an efficacy in inflammation reduction [16,17].

The Colorectal neoplasia Differentially Expressed (CRNDE) lncRNA initially was found to be highly expressed in colorectal adenomas and carcinomas and was located on the long arm of human chromosome 16 [18]. Further studies indicate that CRNDE is involved in multiple tumor processes and shows high sensitivity/-specificity within plasma and tumor tissues [19]. CRNDE could have oncogenic activity and has been demonstrated to be upregulated in various cancers such as hepatic cancer [20], cervical cancer [21], clear-cell-renal-cell carcinoma [22], pancreatic cancer [23], and more. Also, based on previous studies, CRNDE was found to activate inflammation through the toll-like receptor 3-nuclear factor-kappa B (TLR3-NF-κB) -cytokine signaling pathway and promote the downstream release of inflammatory cytokines [24]. Knockdown of *CRNDE* lncRNA promotes the inactivation of the TLR3/NF-κB pathway and promotes inflammation and organ damage [25]. Based on this evidence, we hypothesized that CRNDE might be implicated in disease progression and inflammation in IBD patients by regulating inflammatory pathways such as NF-kB. We designed a comprehensive bioinformatics study for predicting target miRNAs and proteins and their roles in the pathogenesis of IBD. Furthermore, we aimed to validate CRNDE expression in inflamed cell lines such as HT-29 and Cancer coli-2 (Caco-2) (Human adenocarcinoma colorectal cell lines); and also, colonic tissue and blood samples of UC patients compared to controls ex vivo.

2. Material and method

2.1. RNA-seq data analysis

The RNA-seq raw data counts were downloaded from the GEO repository (www.ncbi.nlm.nih.gov/geo) with the accession number GSE83687 including samples of 134 patients undergoing bowel resection. Among these samples, colonic tissue samples were used (N = 89) containing 42 IBD and 47 controls. Control samples were harvested more than 10 cm away from the tumor and normal non-inflamed condition from patients undergoing bowel resection. Samples were sequenced by Illumina HiSeq 2500 (GPL16791) [26]. Log 2 transformation and normalization were performed for count data. For obtaining differentially expressed genes (DEGs) between IBD and control samples, the DEseq2 package was used in R, and the principal component analysis (PCA) plot was drawn by the ggplot 2 package. For filtering significant DEGs, an adjusted p-value <0.05 and $|\log \text{Fold Change (logFC)}| > 1$ was selected as the threshold.

2.2. Gene-miRNA network

For predicting CRNDE-miRNA interactions, we take advantage of LncBase from DIANA Tools (Diana.e-ce.uth.gr/lncbasev3). Furthermore, the mirWalk database (mirwalk.umm.uni-heidelberg.de/) was used to predict selected miRNA targets. Among target genes, only significant and experimentally validated interactions (By reporter assay, Western blot, microarray and next-generation sequencing experiments reported in miRTarBase www.mirtarbase.cuhk.edu.cn/~miRTarBase/miRTarBase_2022/php/index.php) were filtered. Selected target genes were compared with IBD DEGs, and shared genes were obtained. With Cytoscape [27] software (3.9.1 version), a network has been constructed between CRNDE target miRNAs and IBD DEGs.

2.3. LncRNA interactions

For evaluating CRNDE-Protein interactions, NPInter v4.0 (bigdata.ibp.ac.cn/npinter4) has been used to gather CRNDE interactions based on previously published literature and high-throughput data. Protein targets of CRNDE were enriched and protein-protein interaction network were constructed using the STRING online database (www.string-db.org). Networks visualization and analysis were performed by the Cytoscape tool. As an enrichment tool, the STRING database was used, and Reactome cellular pathways with adjusted p-value <0.05 were selected as significant pathways.

2.4. Pathway enrichment

To investigate biological pathways controlled by CRNDE indirectly, miRNA-targeted DEGs were enriched in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database using Enrichr (maayanlab.cloud/Enrichr/), an open access and trusted online tool with up-to-date gene-set libraries for visualizing and ranking enrichment results [28]. Only significant pathways with adjusted p-value <0.05 were selected.

2.5. Regression analysis

To evaluate the role of CRNDE in controlling IBDs' main inflammatory pathway, NF-kB, another pathway enrichment was performed for IBD DEGs in the KEGG database by the Enrichr, and NF-kB pathways DEGs were obtained. Regression analysis was performed for *CRNDE* expression and differentially expressed NF-kB pathway genes by the ggpubr package in R. Significant results had a p-value <0.05.

2.6. Cell culture

HT-29 and Caco-2 cell lines were purchased from the Iranian biological resource center (IBRC) and cultured in the Dulbecoo's modified eagles medium (DMEM) high glucose (Biosera, LM-D1111) with 10 % fetal bovine serum (FBS) (Gibco, Brazil) at 37 °C with 5 % CO_2 humidified incubator. Cells were seeded into 6 well cell culture plates for treatment. HT-29 cells were inflamed using enterotoxigenic *E. coli* (ETEC) with Multiplicity of Infection (MOI) 10 for 4 h. Caco-2 cell lines were also inflamed by 0.5 ng/ml IL-1B (BioLegend, cat #579402) for 24 h. Inflammation induction was measured using *RELA* gene expression by Real-time PCR. All the treatments were performed on duplicates and untreated cells were used as controls. After treatment, all cells were harvested using Trypsin-EDTA (0.25 %) (Gibco, cat #25200056) and stored at −80 ° C freezer.

2.7. Tissue and blood sample collection

Matched colonoscopic biopsies and blood samples were obtained from 18 UC patients and 18 controls at gastroenterology and hepatology clinic, Taleghani Hospital, Tehran, Iran. Based on our inclusion criteria, adult UC patients were confirmed by a gastroenterologist based on pathology and colonoscopy results and tissue samples were taken from patients in severe and active phase from inflamed sites of the colon. These patients were newly diagnosed and were naive to biological and immunosuppressive therapies. Patients with no detectable inflammation and no history of inflammatory bowel disease were selected as the control group. Conditions such as malignancy in the gastrointestinal

Table 1
Primer sequences and their properties.

Gene name	Sequence	Tm	Length	Product length
CRNDE F	ACCGGAAGGAACCATCTCAC	60 °C	20	100
CRNDE R	CCTTCTTCTGCGTGACAACTG	60 °C	21	
RELA F	TCACTCGGCAGATCTTGAGC	60 °C	20	197
RELA R	CCAGACCAACAACAACCCCT	60 °C	20	
GAPDH F	CTCAAGATCATCAGCAATGCCT	60 °C	22	134
GAPDH R	ACAGTCTTCTGGGTGGCAGT	60 °C	20	

tract or the history of autoimmune diseases were considered as exclusion criteria and excluded from the study. Sample collection and experimental protocols were approved by the Isfahan University of Medical Science Ethical Committee (IR.MUI.MED.REC.1399.1143). Blood samples were collected in EDTA tubes and centrifuged at 3000 rpm for 10 min for buffy coat separation.

2.8. RNA extraction and cDNA synthesis

Peripheral Blood Mononuclear Cell (PBMC) samples, tissue biopsies, and cell-cultured cells' RNA was extracted with the FAVORGEN total RNA Extraction kit (Taiwan, cat #FABRK001) based on manufacturers protocol. DNase I (Sinaclon, Iran, cat #MO5401) was used to purify the extracted RNA during the extraction protocol. RNA integrity was checked by nanodrop and gel electrophoresis. cDNA was synthesized by BIO FACT 2× RT Pre-Mix cDNA synthesis kit (Korea, cat #BR441-096) and random hexamer primers according to the instruction manual.

2.9. Real-time PCR for gene expression

Real-time PCR was carried out for cDNA from collected cells, tissue, and buffy coat samples using RealQ Plus 2× Master Mix Green (AMPLIQON, Denmark, cat # A323402) and Polymerase Chain Reaction (PCR) amplification by Mic Real-Time PCR System (Bio molecular systems, Switzerland). The primers were designed by the Primer-BLAST online tool and their specificity, secondary structures, and dimers were checked using BLAST and Oligo analyzer online tools (www.ncbi.nlm.nih.gov/tools/primer-blast/) (https://eu.idtdna.com/pages/tools/oligoanalyzer). Their sequences were as follows: human *CRNDE* sense: 5′- ACCGGAAGGAACCATCTCAC -3′, antisense: 5′- CCTTCTTCTGCGTG ACAACTG -3′; Human *RELA* sense: 5′- TCACTCGGCAGATCTTGAGC -3′, Human *RELA* antisense: 5′- CCAGACCAACAACAACCCCT -3′ human *GAPDH* sense: 5′- CTCAAGATCATCAGCAATGCCT -3′, antisense: 5′- ACAGTCTTCTGGGTGGCAGT -3. *GADPH* was used as endogenous control (Table 1). The PCR amplification settings were 15 min at 95 °C, followed by 40 cycles of denaturation for 20 s at 95 °C, annealing for 30 s at 60 °C, and extension for 30 s at 72 °C. All Real-time PCR experiments were performed in triplicate.

2.10. Statistical analysis

Statistical analysis was performed by REST © (Relative Expression Software Tool) (Qiagen, Hilden, Germany), a reliable and reproducible tool, using Pair Wise Fixed Reallocation Randomization Test for Real-time PCR results analysis [29]. P value < 0.05 was considered statistically significant. Statistical data visualization was done by the GraphPad-Prism 9 software.

3. Results

3.1. CRNDE is upregulated in RNA-seq data

For obtaining the CRNDE expression in the IBD samples, an analysis on the GSE83687 RNA-seq dataset performed and resulted in 2071 upregulated and 1055 downregulated DEGs with $|\text{logFC}| \geq 1$ and adjusted p-value <0.05. PCA plot showed a separation in expression profiles between IBD group and controls. However, some of the IBD samples were not separated from controls due to various stages of disease activity in different patients (Fig. 1). Among upregulated genes, CRNDE was upregulated in the IBD group compared to controls by log fold change 1.4 and adjusted p-value 1.24e-6 (Fig. 2). Also, significant disregulated genes in IBD patients enriched in KEGG pathways and 11 NF-kB related genes were extracted which were significantly upregulated in IBD patients compare to controls.

3.2. CRNDE regulates various inflammation and proliferation pathways by sponging miRNAs

LncRNAs can control cell function by sponging miRNAs. Therefore, by using DIANA tools online database (Using high-throughput experiments including microarrays and proteomics data), miRNA targets of

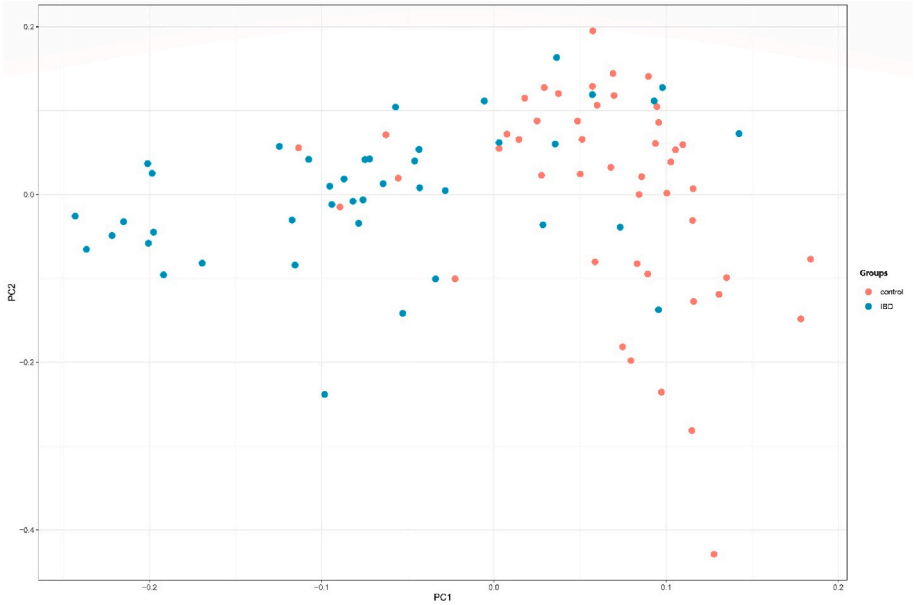

Fig. 1. PCA plot for IBD group (Blue) and control (Red) colonic samples. Partially separation of sample groups indicates a difference between expression profiles.

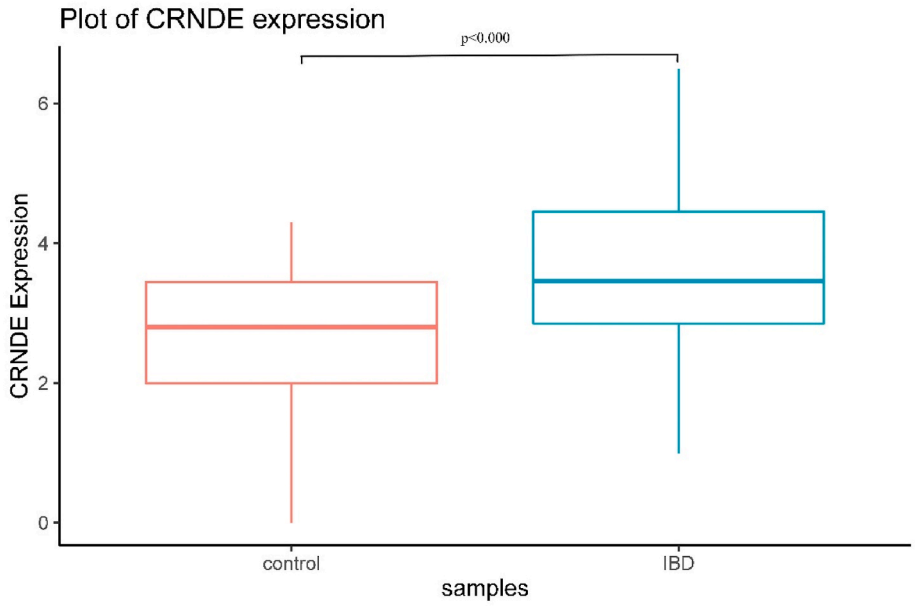

Fig. 2. Expression of CRNDE lncRNA in IBD and control colonic samples in RNA-seq data. Log fold change = 1.4, adjusted p value = 1.24E-6.

CRNDE were predicted. CRNDE was involved in the regulation of 59 miRNAs presented in Table 2. Validated gene targets for selected miRNAs were gathered from the miRwalk online tool (2525 genes), and shared genes between IBD DEGs and target genes (212 genes) were used to construct a gene-miRNA network in the Cytoscape tool (Fig. 3). Furthermore, these genes were enriched in Kyoto encyclopedia of genes and genomes (KEGG) pathways such as Toll-like receptor signaling pathway, Hippo signaling pathway, Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathway, proteoglycans in cancer, cytokine-cytokine receptor interaction, phosphatidylinositol-3 kinase-Ak strain transforming (PI3K-Akt) signaling pathway, NF-kappa B signaling pathway, TNF signaling pathway and Rat sarcoma virus (RAS) signaling pathway using EnrichR online tool.

3.3. *CRNDE is involved in gene regulation by targeting RNA-binding proteins*

Proteins also could change their function by interacting with lncRNAs. 73 protein targets for CRNDE were predicted using NPInter online tool and protein-protein interaction network with STRING online

Table 2
Predicted miRNA targets for CRNDE based on DIANA tools database. Abbreviations, IP: Immunoprecipitation, RP: Reporter gene assay, qP: Quantitative polymerase chain reaction, Bi: Biotin-seq.

Target miRNA	miRNA confidency	Methods	*Target miRNA*	miRNA confidency	Methods
hsa-let-7a-5p	High	IP	*hsa-miR-302a-3p*	High	IP
hsa-let-7d-5p	High	IP	*hsa-miR-30a-5p*	High	IP
hsa-let-7e-5p	High	IP	*hsa-miR-30d-5p*	High	IP
hsa-miR-103a-3p	High	IP	*hsa-miR-30e-3p*	High	IP
hsa-miR-106a-5p	High	IP	*hsa-miR-30e-5p*	High	IP
hsa-miR-1271–5p	High	IP	*hsa-miR-320a-3p*	High	IP
hsa-miR-1287–5p	High	IP	*hsa-miR-320b*	Low	IP
hsa-miR-130 b-3p	High	IP	*hsa-miR-320c*	Low	IP
hsa-miR-135 b-3p	High	IP	*hsa-miR-34a-5p*	High	IP,Bi
hsa-miR-148a-3p	High	IP	*hsa-miR-3679-5p*	Low	IP
hsa-miR-15a-3p	High	IP	*hsa-miR-384*	Low	qP, RP
hsa-miR-15 b-5p	High	IP	*hsa-miR-423-3p*	High	IP
hsa-miR-181a-5p	High	IP	*hsa-miR-423-5p*	High	IP
hsa-miR-181 b-5p	High	IP	*hsa-miR-424-5p*	High	IP
hsa-miR-182–5p	High	IP	*hsa-miR-454-3p*	High	IP
hsa-miR-183–5p	High	IP	*hsa-miR-484*	Low	IP
hsa-miR-186–5p	High	qP,RP	*hsa-miR-500a-3p*	High	IP
hsa-miR-18a-5p	High	IP	*hsa-miR-503-5p*	High	IP
hsa-miR-18 b-5p	High	IP	*hsa-miR-548w*	Low	IP
hsa-miR-193 b-3p	High	IP	*hsa-miR-641*	Low	qP, RP
hsa-miR-195–5p	High	IP	*hsa-miR-651-5p*	High	IP
hsa-miR-196a-5p	High	IP	*hsa-miR-7-5p*	High	IP
hsa-miR-19a-3p	High	IP	*hsa-miR-708-5p*	High	IP
hsa-miR-19 b-3p	High	IP	*hsa-miR-92a-3p*	High	IP
hsa-miR-21–5p	High	IP	*hsa-miR-92b-3p*	High	IP
hsa-miR-22–5p	High	IP	*hsa-miR-942-5p*	High	IP
hsa-miR-25–3p	High	IP	*hsa-miR-29a-3p*	High	IP
hsa-miR-26a-5p	High	IP	*hsa-miR-29b-3p*	High	IP
hsa-miR-27a-3p	High	IP	*hsa-miR-29c-3p*	High	IP
hsa-miR-28–5p	High	IP			

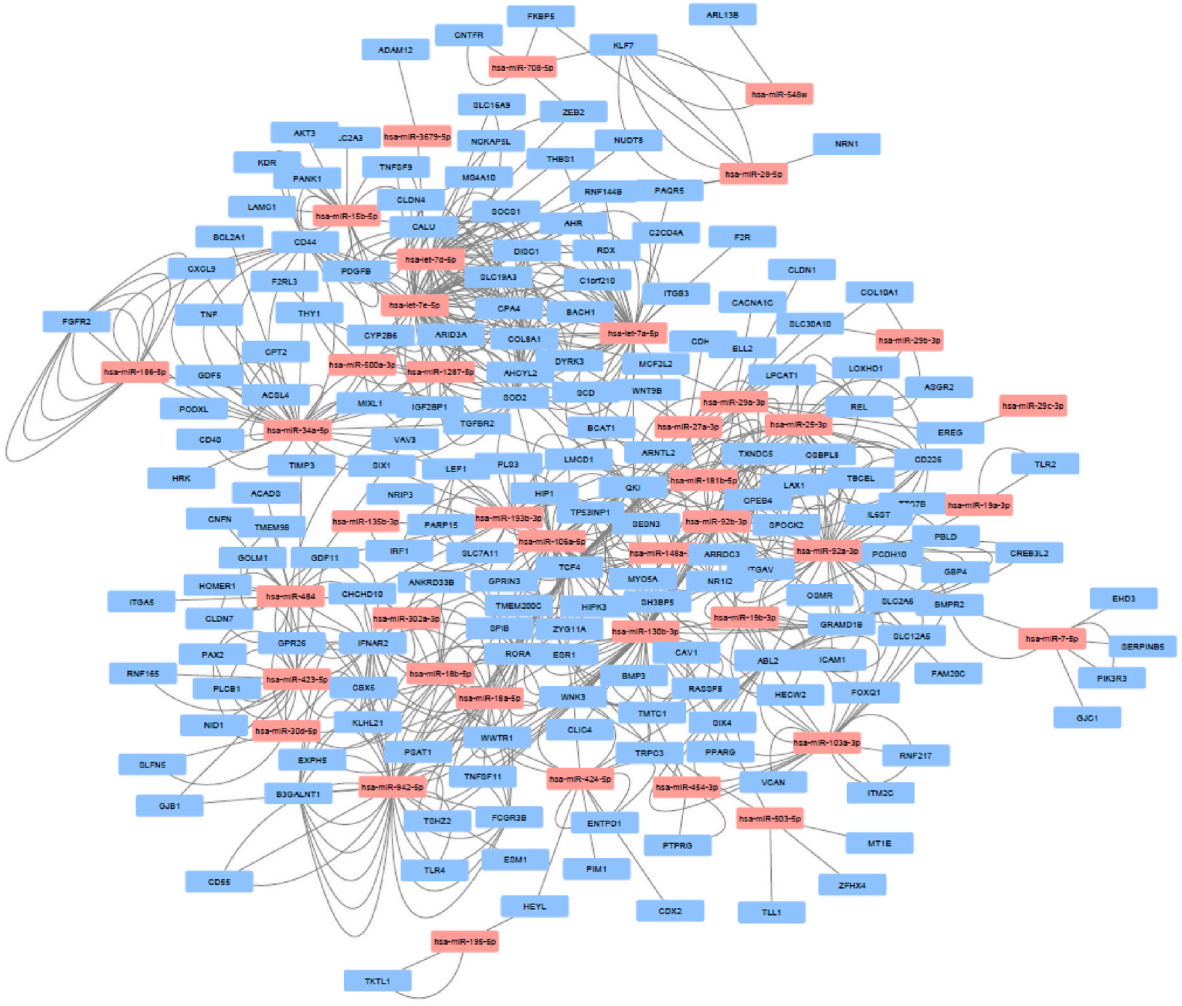

Fig. 3. Gene-miRNA network between IBD DEGs (Blue) and CRNDE's target miRNAs (Red).

database with 553 edges (average node degree: 15.3) and average local clustering coefficient 0.67 (Fig. 4). These proteins revealed the central proteins of the network such as U2 Small Nuclear RNA Auxiliary Factor 2 (U2AF2), serine and arginine rich splicing factor 1 (SRSF1), ILF3 interleukin enhancer binding factor 3 (ILF3), DEAD-box helicase family member (DDX3X), ELAV Like RNA Binding Protein 1 (ELAVL1), TIA1 Cytotoxic Granule Associated RNA Binding Protein Like 1 (TIAL1) and heterogeneous nuclear ribonuclear proteins (HNRNPs) family (Table 3).

mRNA Splicing (HSA-72165), Insulin-like Growth Factor-2 mRNA Binding Proteins (IGF2BPs/IMPs/VICKZs) bind RNA (HSA-428359), competing endogenous RNAs (ceRNAs) regulate PTEN translation (HSA-8948700), Post-transcriptional silencing by small RNAs (HSA-426496), FGFR2 alternative splicing (HSA-6803529), regulation of MECP2 expression and activity (HSA-9022692) and MAPK6/MAPK4 signaling (HSA-5687128) were some of the enriched pathways for targeting proteins in the Reactome database.

3.4. CRNDE has positive regression with NF-kB pathway genes

RNA-seq data analysis in section 3.1 revealed that 11 NF-kB pathway genes are significantly up regulated in IBD patients. Furthermore, Regression analysis with the ggpubr package in R program revealed that most of these up-regualted genes in the NF-kB pathway have positive regression with CRNDE expression in IBD patients (Fig. 5). LY96 ($R = 0.8$), VCAM1 ($R = 61$), CD40 ($R = 0.61$), PLAU ($R = 0.73$), ICAM1 ($R = 0.62$), PTGS2 ($R = 0.62$), IL1B ($R = 0.62$), IL1R1 ($R = 0.61$) and IL8 ($R = 0.68$) had regression score >0.5 and p value < 0.05. Positive regression for TNF and TNFSF11 could not be statistically proven (p-value >0.05).

3.5. CRNDE is upregulated after inflammation induction in the HT-29 cell line

For validating our bioinformatics analysis results, colonic model cell lines, including the HT-29 and Caco-2, were inflamed. Inflammation induction was checked by *RELA* (NF-kB P65 subunit) expression and then, *CRNDE* expression was evaluated using Real-time PCR. Based on Real-time PCR results, *CRNDE* is significantly upregulated in inflamed HT-29 cells compared to control cells (Relative expression 3.35, standard error 0.055, and p-value 0.014). However, slight upregulation of the CRNDE in inflamed Caco-2 cells could not be statistically proven (Relative expression 1.17, standard error 0.03, and p-value 0.111) (Fig. 6).

3.6. CRNDE is upregulated in IBD patients' colonic tissue samples

We measured the expression of *CRNDE* in colonic biopsies and PBMC samples of 18 UC patients and 18 matched controls (Table 4). Based on Real-time PCR results, *CRNDE* is significantly upregulated in UC patients' colonic biopsies compared to controls by a mean factor of 2.091 (standard error = 0.942–4.947) and P value 0.0001. Hence upregulation of *CRNDE* in PBMC samples couldn't be statistically proven (mean factor = 1.695, standard error = 0.451–10.339, P value = 0.32) (Fig. 7).

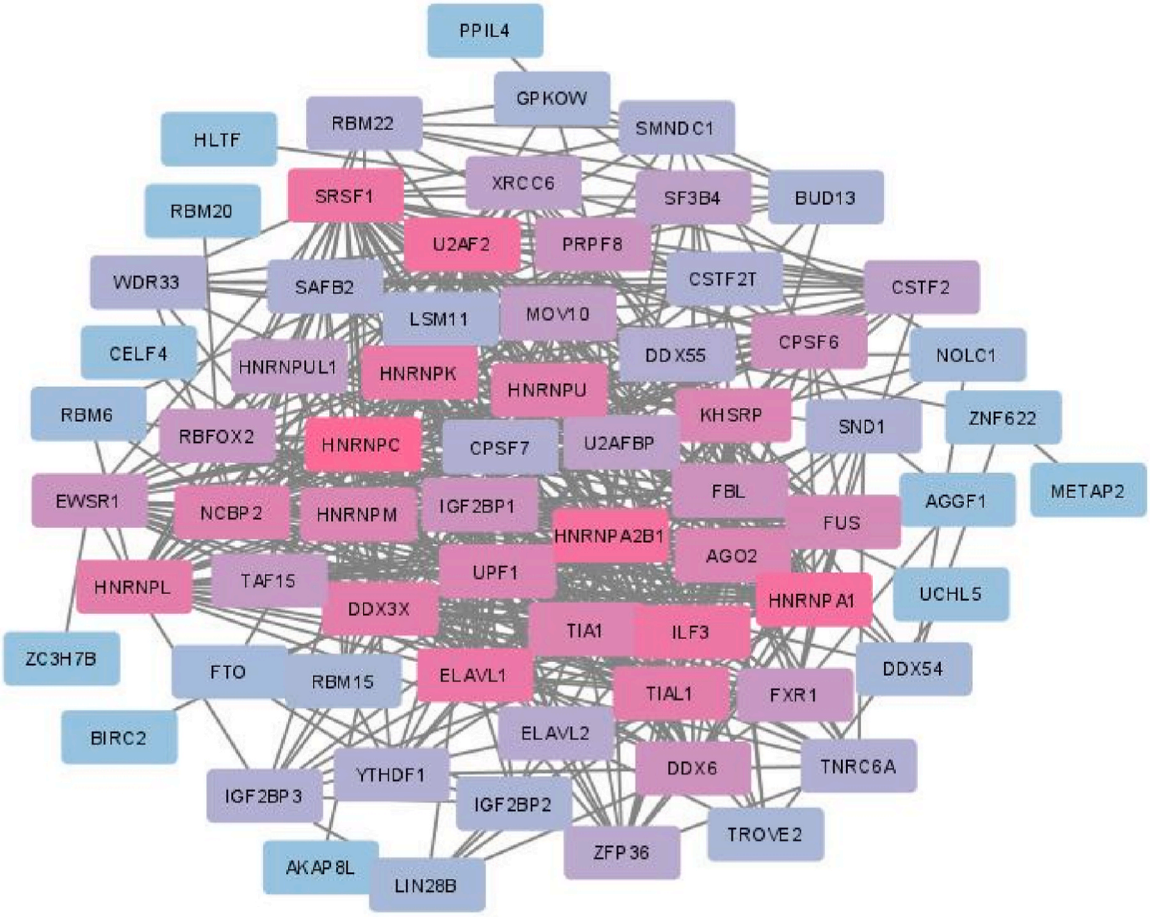

Fig. 4. Protein-protein interaction network for CRNDE's target proteins (string-db.org). visualized by the Cytoscape tool. Nodes are colored by gradient based on their degree number. High-degree nodes pink, medium-degree nodes purple and low-degree nodes are displayed by blue color.

Table 3
Central proteins from PPI network for CRNDE's target proteins. Proteins with degree ≥30 and highest Betweenness and closeness centrality and neighborhood connectivity were selected.

Protein name	Degree	Betweenness centrality	Neighborhood connectivity	Closeness centrality
HNRNPC	41	0.07433149126616731	22.463414634146343	0.7010309278350515
HNRNPA2B1	38	0.06067403635014974	22.763157894736842	0.6732673267326732
HNRNPA1	38	0.04825232097310949	23.605263157894736	0.6799999999999999
U2AF2	37	0.07731824820966438	22.216216216216218	0.6601941747572816
SRSF1	35	0.03570837961330831	23.542857142857144	0.6415094339622641
ILF3	35	0.031940767757445775	24.514285714285716	0.6476190476190475
ELAVL1	34	0.03636423501333448	23.764705882352942	0.6355140186915889
HNRNPK	33	0.018721294157474776	25.424242424242426	0.6355140186915889
TIAL1	31	0.034428057412145815	25.225806451612904	0.6126126126126126
DDX3X	31	0.0794198676128049	24.225806451612904	0.6238532110091742
HNRNPL	31	0.02010829749361029	25.06451612903226	0.6181818181818182
HNRNPU	30	0.03459259576859074	25.0	0.6238532110091742

4. Discussion

The lncRNA CRNDE (colorectal neoplasia differentially expressed) plays an important role in regulating pathways such as proliferation, apoptosis, and metastasis. It is overexpressed in various cancers and is thought to influence response to chemotherapy drugs and radiotherapy [19,30]. Microarray analysis on UC patients showed that *CRNDE* is upregulated (log fold change: 4.69) in UC colonic tissues compared to controls [31]. Therefore, we hypothesize that *CRNDE* up-regulation in UC patients may exacerbate inflammation by activating and promoting the NF-κB pathway. In our study, we found that *CRNDE* is significantly upregulated in the colonic tissue of patients with UC, as confirmed by RNA sequencing and Real-time PCR data. Also, the inflamed HT-29 cell line showed an up-regulation in *CRNDE* expression compared to untreated cells. By analyzing lncRNA-miRNA and protein-protein interactions, we have discovered a potential method of modulating the inflammatory response and the NF-kB pathway by CRNDE lncRNA.

CRNDE is reported to be associated with inflammatory pathways. A study on astrocytes revealed that overexpression of *CRNDE* increased the expression of key factors in the toll-like receptor signaling pathway and triggered downstream transcription factors such as nuclear factor kappa B and numerous cytokines [24]. Based on our bioinformatics study, inflammation pathways such as Toll-like receptor signaling pathway, Cytokine-cytokine receptor interaction, NF-κB signaling

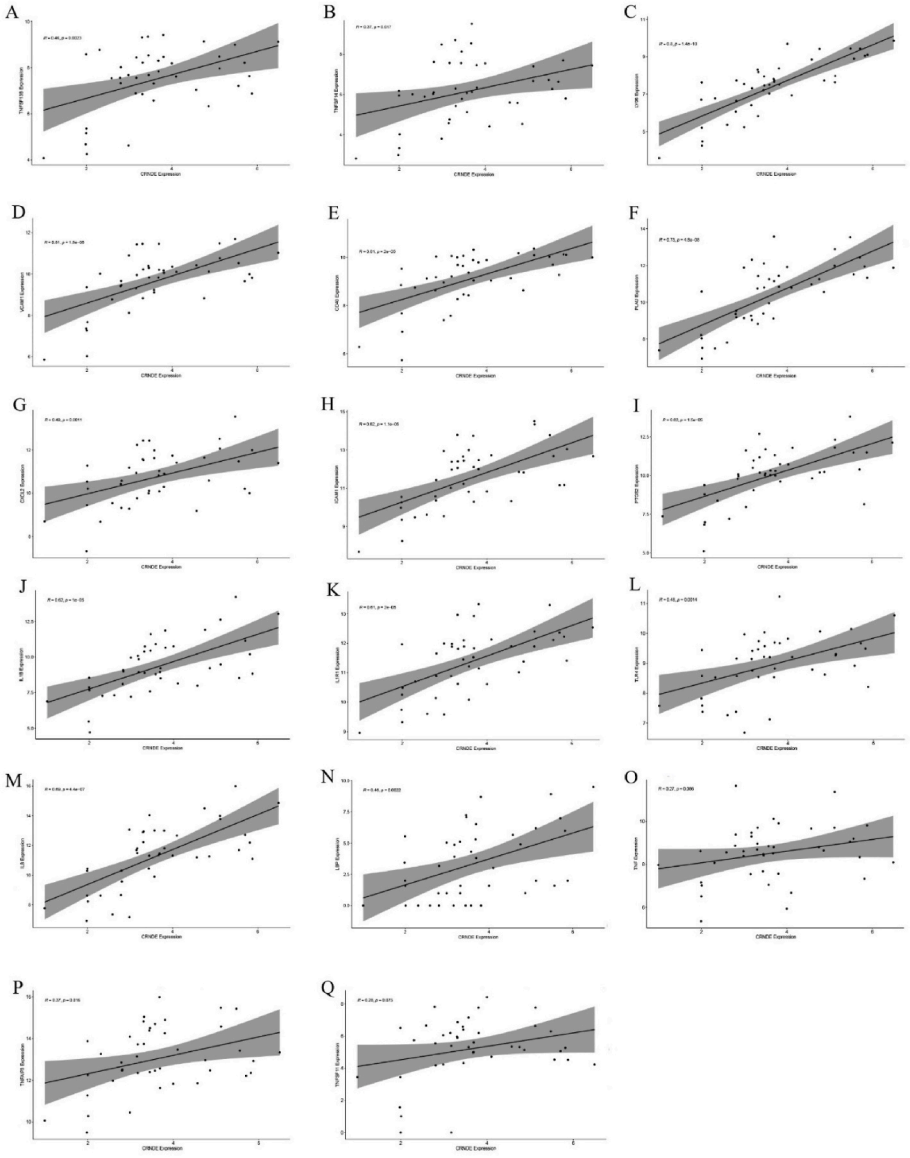

Fig. 5. Regression analysis results between CRNDE expression and differentially expressed NF-kB pathway genes. A: TNFSF13B (R = 0.46, P = 0.0023), B: TNFSF14 (R = 0.34, P = 0.017), C: LY96 (R = 0.8, P = 1.4e-10), D: VCAM1 (R = 0.61, P = 1.5e-05), E: CD40 (R = 0.61, P = 2e-05), F: PLAU (R = 0.73, P = 4.8e-08), G: CXCL2 (R = 0.49, P = 0.0011), H: ICAM1 (R = 0.62, P = 1.1 e−05), I= PTGS2 (R = 0.62, P = 1.5e-05), J: IL1B (R = 0.62, P = 1e-05), K:IL1R1 (R = 0.61, P = 2e-05), L: TLR4 (R = 0.48, P = 0.0014), M: IL8 (R = 0.69, P = 4.4e-07), N: LBP (R = 0.46, P = 0.0022), O:TNF (R = 0.27, R = 0.086), P:TNFAIP3 (R = 0.37,P = 0.016), Q: TNFSF11 (R = 0.28, P = 0.075).

pathway, and TNF signaling pathway; proliferation and tumorgenesis pathways such as PI3K-Akt signaling pathway, Ras signaling pathway and Proteoglycans in cancer; and also differentiation pathways such as Hippo signaling pathway and JAK-STAT signaling pathway are regulated by CRNDE. Meanwhile, *CRNDE* expression had a positive correlation with most of the NF-kB pathway genes. Likewise, Dong Zhu-ge et al. reported that LPS exposure increased *CRNDE* expression in WI-38 cells and activated the NF-κB and JAK/STAT pathways. CRNDE also induced FOXM1 expression [32]. Another study on patients with sepsis revealed that TLR4 expression was upregulated with the rise of CRNDE expression ex vivo. Tests also uncovered that CRNDE could induce the expression of inflammatory biomarkers, such as TNF-α, IL-1β, NF-kB, and IL-6 [33]. Knockout of *CRNDE* changes PI3K/AKT signaling function, which controls cell proliferation, migration, and invasion [34]. Other studies revealed that PI3K/AKT disregulation leads to gut barrier disruption and develops a leaky gut [35]. Furthermore, bioinformatics studies indicate that CRNDE is significantly involved in the MAPK signaling pathway, apoptosis, and Wnt signaling pathway [36]. It was recently cleared that in patients with IBD, Hippo, Notch, and Wnt signaling pathways are dysregulated, resulting in depletion or excess of a cell lineage in the intestine and promoting gut barrier disruption and chronic inflammation [37]. According to this evidence, CRNDE may play a crucial role in triggering inflammation and proliferation, and tumorigenesis of IBD. Based on the regression analysis between *CRNDE* expression and NF-kB pathway genes, it's revealed that by increasing the expression of *CRNDE*, inflammatory mediators also increased their expression, and this overexpression could lead to severe or chronic inflammation in the gut.

We employed bioinformatics tools to determine the potential pathways and mechanisms of CRNDE in UC pathogenesis. To aid us in this endeavor, we have turned to DIANA-tools, a web server that provides advanced utilities and databases for investigating miRNA function. The primary objective of this reference repository is to analyze experimentally supported miRNA-gene interactions and interpret their functions

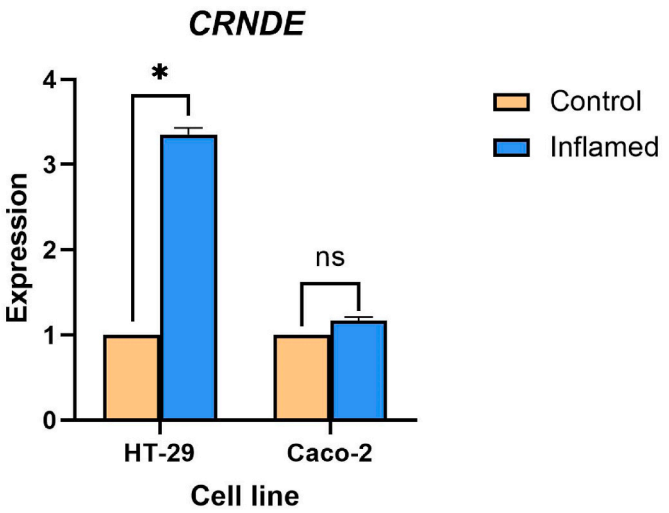

Fig. 6. Relative expression of CRNDE in colon model cell lines. CRNDE is significantly upregulated in inflamed HT-29 cells compare to control cells (Relative expression 3.35, standard error 0.055, and p value 0.014). However, slightly up-regulation of the CRNDE in inflamed Caco-2 cells could not be proven statistically (Relative expression 1.17, standard error 0.03, and p value 0.111).

Table 4
Patients characteristics in each sampling group.

	UC	Controls
Age	38.3 ± 12.3	57.2 ± 10.1
Sex	Male: 11	Male: 10
	Female: 7	Female: 8
Sampling site of colon	Ascending: 1	Ascending: 2
	Transverse: 3	Transverse: 3
	Descending: 4	Descending: 3
	Rectum/Sigmoid: 9	Rectum/Sigmoid: 9
Family history of cancer	Yes: 7	Yes: 3
	No: 11	No: 15

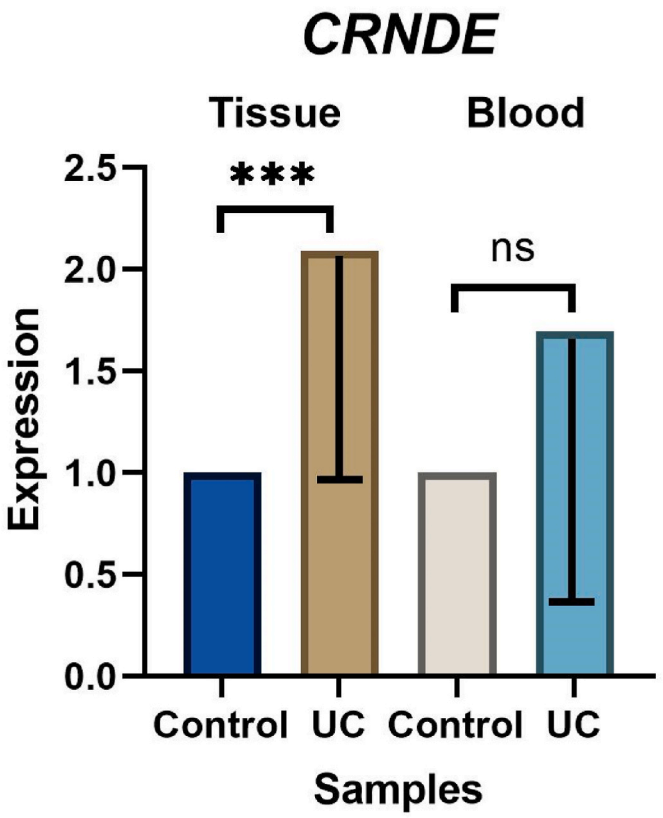

Fig. 7. CRNDE Relative expression in patients' samples. Based on Relative Expression results in tissue and blood samples, CRNDE is significantly up regulated in UC patients' colonic biopsies compare to controls by mean factor of 2.091 (standard error = 0.942–4.947) and P value 0.0001. However Its increased expression in blood samples couldn't confirmed statistically (mean factor = 1.695, standard error = 0.451–10.339, P value = 0.32).

using the available tools [38]. Furthermore, miRWalk provides a comprehensive perspective on the genetic networks of miRNA-gene pathways and distinct experimentally validated data [39]. Based on the experimental data obtained from these tools, we can predict lncRNA-miRNA and mRNA-miRNA interactions with a reasonable accuracy.

Our study confirms these results and highlights the role of CRNDE in controlling various signaling pathways by sponging related miRNAs as Fan Yang et al. used a mouse model of IBD and HT-29, LOVO, and Caco-2 cell lines to investigate the mechanism of interaction between microRNA-495 (miR-495) and CRNDE. Results showed that CRNDE promoted DSS-induced colonic epithelial cell apoptosis via suppressing miR-495 and indicating CRNDE as a novel target for treating IBD [40]. MiRNAs let-7a and let-7d were predicted as targets for CRNDE in this study and were recently reported to be associated with activating the NF-kB pathway [41]. Another reported miRNA in this study, hsa-miR-106a-5p, was previously investigated for its role in regulating IL-10 and its association with autoimmune diseases such as Grave's disease and Multiple sclerosis [42,43]. MiR-30a-5p and miR-30d were other predicted miRNAs whose potential in promoting epithelial to mesenchymal transition in inflammatory stress has been demonstrated recently [44].

NPInter database established on experimentally determined functional interactions between noncoding RNAs and protein-related biomacromolecules (PRMs) (proteins, mRNAs, or genomic DNAs) [45] were employed to detect CRNDE-protein interactions such ILF3, heterogeneous nuclear ribonuclear proteins (hnRNPs) family, and DDX3X in this study. ILF3 is an RNA-binding protein known for participating in cellular antiviral responses and innate immunity. ILF3 promotes angiogenesis through cytokine-inducible mRNA stabilization of pro-angiogenic transcripts and is upregulated in many cancers such as gastric and colorectal cancers and correlates with poor prognosis [46–48]. On the other hand, hnRNPK acted downstream of the TNFα-TNFR2 signaling pathway, directly interacting with and stabilizing YAP on the target gene and regulating the expression of YAP target genes [49]. In general, HnRNPs have shown the ability to modulate inflammatory mediator expression by affecting mRNA stability of COX-2, TNFα, IL-1B, and iNOS, and their overexpression could result in many cancers [50]. Another RNA-binding protein, DDX3X, is associated with tumorigenesis, tumor progression, inflammation, innate immunity, and cellular stress response. It is also reported to activate NLRP3 inflammasomes in response to viral infections too [51,52]. Although all these proteins are associated with inflammatory pathways and immunity, more studies must focus on their role in IBD pathogenesis.

LncRNAs have revolutionized the way we understand gene regulation and have led to the development of advanced diagnostic and therapeutic methods for various diseases. Therefore, it is necessary to analyze gene expression, and associated pathways, and monitor the functional roles of genes to understand their impact on disease progression [53]. Various studies have investigated the clinical significance of lncRNAs in inflammatory bowel diseases. For instance, a study conducted by Marianna Lucafò et al. demonstrated that the GAS5 lncRNA could regulate the activity of matrix metalloproteinases (MMPs) and cause tissue damage. Additionally, the study found that the expression of GAS5 lncRNA was reduced in inflamed sites of the colon in patients with IBD [54]. On the other hand, the expression of IFNG-AS1 lncRNA helps

to maintain a balance between the production of inflammatory and anti-inflammatory cytokines in T-cells. Its expression is elevated in the intestinal mucosa of patients with actively inflamed IBD [55]. Our study focuses on the expression of *CRNDE* in an inflammatory condition *in vitro*. We examined the expression of *CRNDE* in the Caco-2 and HT-29 cell lines before and after inflammation induction to determine if inflammation could elevate its expression in colon cell line models. Our results indicated that *CRNDE* expression was indeed elevated in these models, and we confirmed these findings through expression analysis on colonic samples from patients with UC. Based on this evidence, we can conclude that CRNDE may serve as a potential inflammatory marker in colonic samples of UC patients with active inflammation. However, we also found that CRNDE promote the NF-κB pathway and modulate inflammatory responses by sponging anti-inflammatory miRNAs and interacting with transcription proteins which suggest that the lncRNA CRNDE might have significant implications for managing and understanding ulcerative colitis. CRNDE is reported as a biomarker for colorectal cancer and is highly associated with oncogenic markers and previous studies mentioned CRNDE as a valuable lncRNA marker for diagnosis and therapy of solid and hematological malignancies [19]. Therefore, *CRNDE* over-expression in UC colonic samples can be a potential risk factor for developing colorectal cancer in inflamed areas of the colon in the future too.

5. Conclusion

Recently, lncRNAs have become a hot topic in molecular research and their potential for controlling gene expression directly and indirectly highlighted their role in the pathogenesis of various complex diseases and cancers. This study predicted some miRNA and protein targets of CRNDE lncRNA associated with the pathogenesis of IBD. These results are based on bioinformatics analysis and must be confirmed in future studies. CRNDE is a known lncRNA for promoting cancer in various studies and is presented as a cancer biomarker. Its association with proliferation and inflammation pathways indicates that it could be a marker for promoting inflammation in IBD patients too. Due to the cancer-promoting and inflammation induction potential of CRNDE, it could be a suitable candidate for further studies and a possible therapeutic target in controlling and treating IBD.

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6. Declaration

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

7. Ethical approve

Sample collections and experimental protocols were approved by the Isfahan University of Medical Science Ethical Committee (IR.MUI.MED.REC.1399.1143). All patients fully read and signed the informed consent.

9. Author's contribution

NK: Lab work, Bioinformatics and statistical analysis, writing. MF: Lab work, article proofreading. NK and MK: Study design. SS and HAA: Patients recruitment and sample collection. MK, MZ, and SBG: Study supervision and article revisions. All authors read and approved the final manuscript.

8. Data availability statement

The RNA-seq dataset analyzed in this study is available on the GEO repository (www.ncbi.nlm.nih.gov/geo) with the accession number GSE83687.

CRediT authorship contribution statement

Nesa Kazemifard: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Maryam Farmani:** Writing – review & editing, Data curation. **Shaghayegh Baradaran Ghavami:** Supervision, Resources. **Mohammad Kazemi:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Shabnam Shahrokh:** Data curation. **Hamid Asadzadeh Aghdaei:** Resources, Funding acquisition. **Mohammadreza Zali:** Supervision, Resources.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

11 Abbreviations

IBD	Inflammatory bowel disease
NF-κB	nuclear factor κB
UC	ulcerative colitis
CD	Crohn's disease
lncRNAs	Long non-coding RNAs
LPS	lipopolysaccharide
TNFα	tumor necrosis factor α
IL	Interleukin
CRNDE	colorectal neoplasia differentially expressed
Caco-2	Cancer coli-2
DEGs	differentially expressed genes
PCA	principal component analysis
logFC	log Fold Change
KEGG	Kyoto encyclopedia of genes and genomes
DMEM	Dulbecoo's modified eagles medium
FBS	fetal bovine serum
ETEC	enterotoxigenic *E. coli*
MOI	Multiplicity of Infection
PCR	Polymerase chain reaction
PBMC	Peripheral Blood Mononuclear Cell

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