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Expression Analysis of Long Noncoding RNA-MALAT1 and Interleukin-6 in Inflammatory Bowel Disease Patients

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ABSTRACT

Inflammatory bowel disease (IBD) manifests as chronic inflammation within the gastrointestinal tract. The study focuses on a long noncoding RNA (lncRNA) known as Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1). MALAT1's misregulation has been linked with various autoimmune diseases and regulates proinflammatory cytokines. The role of IL6 in immune-triggered conditions, including IBD, is another focal point. In this research, the expression of MALAT1 and IL6 in IBD patients was meticulously analyzed to uncover potential interactions.

The study involved 33 IBD patients (13 with Crohn's disease and 20 with ulcerative colitis) and 20 healthy counterparts. Quantitative real-time polymerase chain reaction determined the MALAT1 and IL6 gene expression levels. The competitive endogenous RNA (ceRNA) regulatory network was constructed using several tools, including LncRRIsearch and Cytoscape. A deep dive into the Inflammatory Bowel Disease database was undertaken to understand IL6's role in IBD. Drugs potentially targeting these genes were also pinpointed using DGIdb.

Results indicated a notable elevation in the expression levels of MALAT1 and IL6 in IBD patients versus healthy controls. MALAT1 and IL6 did not show a direct linear correlation, but IL6

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could serve as MALAT1's target. Analyses unveiled interactions between MALAT1 and IL6, regulated by hsa-miR-202-3p, hsa-miR-1-3p, and has-miR-9-5p. IL6's pivotal role in IBD-associated inflammation, likely interacting with other cytokines, was accentuated. Moreover, potential drugs like CILOBRADINE for MALAT1 and SILTUXIMAB for IL6 were identified.

This research underscored MALAT1 and IL6's potential value as targets in diagnosis and treatment for IBD patients.

Keywords: Crohn disease; Gene regulatory networks; Inflammatory bowel diseases; Interleukin-6; MALAT1 long non-coding RNA, human; Ulcerative colitis

INTRODUCTION

Inflammatory bowel disease (IBD) is a group of chronic inflammatory disorders of the gastrointestinal tract with two major phenotypes that vary in pathophysiology, symptoms, and management, namely ulcerative colitis (UC) and Crohn's disease (CD).[1,2] CD is more common in young people and usually features a transmural inflammatory pattern, potentially affecting every part of the gastrointestinal tract from the mouth to the anus. In contrast, UC primarily affects the mucosal and submucosal layers of the colon.[3] IBD is caused by a convergence of inappropriate immune responses driven by genetic susceptibility as well as environmental and microbial factors.[4]

Noncoding RNAs are transcriptional and translational gene regulators, the most prominent of which are microRNAs (miRNAs) and long noncoding RNAs (lncRNAs).[5] Recently, the crucial roles of noncoding RNAs have been investigated in several diseases, including rheumatoid arthritis,[6] psoriasis,[7] osteoarthritis,[8] asthma,[9] and IBD.[5,10] Several noncoding genome regions have been identified as IBD risk loci through genome-wide association studies.[11] However, the precise mechanisms by which lncRNAs contribute to disease development remain elusive. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is a fairly novel lncRNA located on chromosome 11.q.13.[12] Dysregulation of *MALAT1* expression has been reported in many cancers as well as autoimmune diseases, where *MALAT1* can act as a regulator of inflammation.[13-17] *MALAT1* seems to have a dual effect on inflammation. First, *MALAT1* induces the production of proinflammatory cytokines such as interleukin (IL)-6 by downregulating zinc finger protein 36, thereby increasing the severity of food allergies.[14] Moreover, the knockdown of *MALAT1* attenuated the inflammatory response in an acute lung injury model.[18] Conversely, *MALAT1* downregulates *IL6* and tumor necrosis factor-alpha (*TNF-α*) through binding to nuclear factor kappa-light-chain-enhancer of activated B cells (*NF-κB*) subunits.[19] *IL6* is a cytokine that regulates inflammation, and its inappropriate production is associated with numerous malignancies and immune-mediated diseases like IBD.[20]

Our understanding of the role of lncRNAs, especially *MALAT1*, and their mechanism of action still needs to be completed. Furthermore, the interaction of *MALAT1* and *IL6* in IBD has yet to be thoroughly examined. In this study, we evaluated the expression of *MALAT1* and *IL6* in IBD patients' tissue samples and investigated the possible competing endogenous RNA (ceRNA) network and potential drugs using bioinformatics analysis.

MATERIALS AND METHODS

Subjects

This case-control study assessed 33 IBD patients (13 with CD and 20 with UC) and 20 healthy controls. All study volunteers were referred to the Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Inclusion criteria for this study included the age of 15 years or older with a confirmed diagnosis of CD or UC who were currently under medical management for their condition at our institution. Meanwhile, patients with other gastrointestinal disorders, a history of gastrointestinal surgery, a history of malignancy or autoimmune diseases other than IBD, and those who had taken antibiotics within the last three months were excluded from further investigation. All participants had Iranian ethnicity and provided written informed consent for the present study prior to the sampling procedure.

RNA Isolation and Quantitative Real-time PCR

The intestinal tissue samples were provided during diagnostic colonoscopy, frozen immediately in liquid

nitrogen, and stored at −80°C. RNA was purified from the biopsy samples using the RNeasy Mini Kit (50) (Qiagen, Germany) according to the kit protocol. A Nanodrop ND-1000 spectrophotometer quantified the RNA concentration (Nanodrop Technologies), and its quality was assessed based on A260/A280 and A260/A230 ratios. The Revert Aid RT Reverse Transcription Kit (Thermo Fisher Scientific, USA) synthesized cDNA from 1 μg of total RNA. *MALAT1* and *IL6* expression were evaluated using a polymerase chain reaction (PCR) cycler (Rotor-Gene Q MDx; Qiagen GmbH). cDNA fragments were used as templates to amplify the *MALAT1* and *IL6* genes using BioFACT 2X Real-Time PCR Master Mix (Biofact, Korea) according to the manufacturer's protocol. The experimental protocol was performed as follows: Thermocycling conditions consisted of an initial activation step for 15 minutes at 95°C, 35 cycles at 94°C for 30 seconds, and 60°C for 35 seconds, with melting curve analysis. The primer sequences of the qPCR are listed in Supplementary Table. The *B2M* gene was used as an endogenous control gene.

Competing Endogenous RNA (ceRNA) Network Construction

By constructing ceRNA networks, we were able to predict MALAT1 and IL6 interaction. The first approach was to identify the lncRNA-mRNA interactions based on physicochemical forces. To do this, we employed the LncRRIsearch tool (http://rtools.cbrc.jp/LncRRIsearch/)[21] to predict the interaction energy. Based on the proposed methods in the LNCRRIsearch tool and the RIblast algorithm,[22] threshold interaction energy was set to −12 kcal/mol here. Hence, we reported the transcripts of *MALAT1*, which had a minimum free energy of less than −12 kcal/mol, as candidate transcripts that may interact with the *IL6* gene. Finding the lncRNA-RNA interaction based on their networks was the goal of the second approach. To provide it, a number of bioinformatics tools were utilized. The RNAInter V4.0 database (http://www.rnainter.org/)[23] was used to identify the most valid RNAs, including miRNAs, lncRNAs, mRNAs, and transcription factors related to *IL6* and *MALAT1*. We selected the compounds with a confidence greater than 0.40 out of 1. The NPInter V4.0 (http://bigdata.ibp.ac.cn/npinter4)[24] was used to detect RNAs, DNAs, and proteins interacting with *MALAT1* and *IL6* independently. We obtained the entire experimentally confirmed RNA interaction database and sorted it by species. Only interactions related to *IL6* or *MALAT1* in homo sapiens were considered. Subsequently, hTFtargets (http://bioinfo.life.hust.edu.cn),[25] a bioinformatics prediction website, was used to predict the targets of transcription factors and identify potential binding sites between the transcription factor and the lncRNA. We chose the transcription factors that target *IL6* or *MALAT1* in the human colon. We integrated prior steps to shed light on the probable mechanism of overexpression of *MALAT1* and *IL6* and their association. We generated a tab-delimited file including the source node and target node for *MALAT1* and *IL6* interactions. Finally, we utilized CytoScape to construct the network and uncover a potential mechanism for the *MALAT1* and *IL6* relationship.[29]

Exploring the Role of *IL6* in the Development of IBD

We employed the Inflammatory Bowel Disease Database to investigate the involvement of *IL6* in various biological processes, biological actions, cellular components, and pathways, as well as its interaction with genes associated with IBD. The Inflammatory Bowel Disease Database (https://www.cbrc.kaust.edu.sa/ibd/index.php?p=home) is a web-based resource developed by the Computational Bioscience Research Center (CBRC) based on text-mining algorithms that extract relevant information from the scientific literature. The database provides a comprehensive, up-to-date collection of gene expression data and related metadata, including information on the experimental conditions and clinical characteristics of the IBD patients from whom the samples were collected.

Drug-Gene Interaction (DGI)

In the present investigation, we utilized the Drug Gene Interaction Database (DGIdb) to identify potential drug-gene interactions involving *IL6* and *MALAT1*. DGIdb (https://www.dgidb.org/) is a database that provides a comprehensive resource for information on drug-gene interactions. It is a web-based tool that integrates data from various sources, including drug-gene interaction databases, pharmacological databases, and gene annotation databases, to create a unified resource for exploring drug-gene interactions.[26] Finally, the gene-drug network was visualized via Cytoscape (Version 3.9.1).

Statistical Analysis

A relative expression software tool (REST) was used for comparison and statistical analysis of relative expression results of real-time PCR. The association between lncRNA *MALAT1* and *IL6* gene expression was assessed via linear regression. The receiver operating characteristics (ROC) curve was constructed to describe diagnostic specificity. Statistical analysis was performed using IBM SPSS version 21, and data was plotted with GraphPad Prism (version 8; GraphPad Software, Inc.). The significance was determined using paired t-test, in which $p<0.05$ was considered significant.

RESULTS

General Statistical Information

A total of 33 patients with IBD and 20 healthy individuals were included in the survey. The patients had a mean age of 33.40±2.440, while the controls had a mean age of 55±12.732. The patients' mean body mass index (BMI) was 22.62±0.87, whereas, for the controls, it was 25.68±4.26. In terms of gender distribution, there were 16 (46.9%) males and 17 (53.1%) females among the IBD patients. There were 9 (45%) males and 11 (55%) females for the healthy controls. Among the IBD patients, 20 (60.6%) were diagnosed with UC, and 13 (39.4%) had CD (Table 1).

Expression of *MALAT1* in IBD Tissue Samples

The differences in the expression levels of *MALAT1* in the biopsy sample between IBD patients and normal controls were found to be statistically significant ($p<0.001$; 95% CI, 0.006–16.018) (Figure 1). Real-time PCR data analysis of the expression levels of the *MALAT1* gene showed a significant difference between UC samples compared to controls ($p<0.0054$; 95% CI, 0.002–29.761). We also found a considerable difference in *MALAT1* expression level between CD samples and controls ($p<0.001$; 95% CI, 0.000–27.753). There was no significant difference between the samples from CD patients compared with those from the UC patients.

Table 1. Demographic data of study population

Demographic categories	Ulcerative colitis	Crohn disease	Healthy controls
Gender			
Female	10	7	11
Male	10	6	9
Current age	33.40 ± 2.440		55±12.732
Age at diagnosis			
<16	2	0	-
17–40	14	9	-
>40	2	2	-
Unknown	2	2	-
BMI	22.62 ± 0.87		25.68 ± 4.26
Smoking or alcohol consumption			
No	20	13	20
Yes	0	0	0

BMI: body mass index

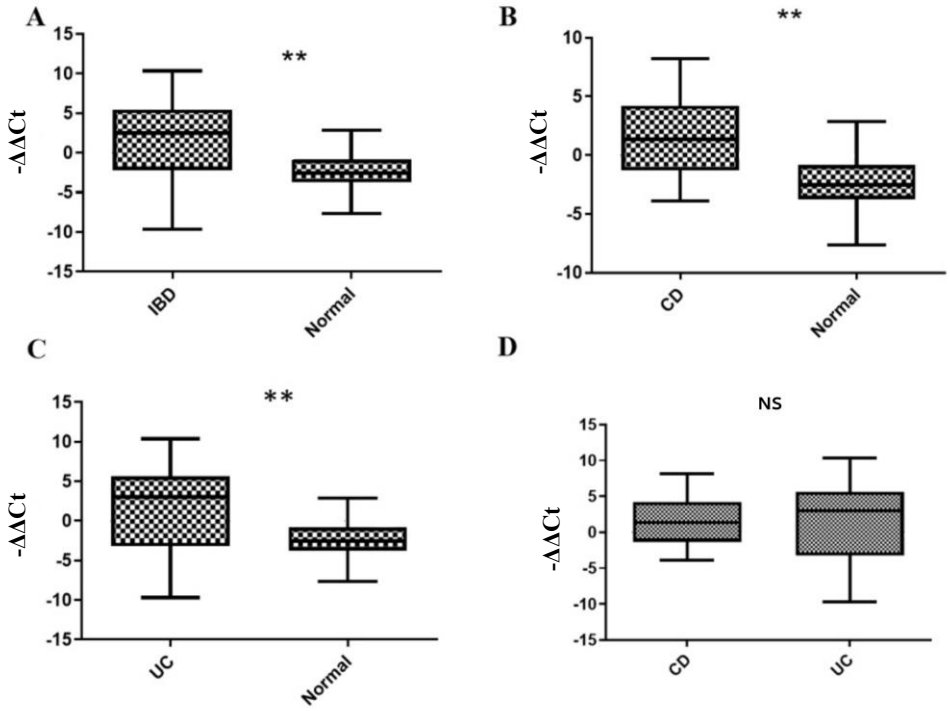

Figure 1. Real-time quantitative PCR (qRT-PCR) was used to evaluate the gene expression levels. qRT-PCR analysis of the lncRNA expression levels of *MALAT1*. (A) The *MALAT1* expression levels in inflammatory bowel disease (IBD) tissues compared to healthy tissues, (B) Crohn's disease (CD) patients vs. healthy controls, (C) Ulcerative colitis (UC) patients vs. healthy controls, and (D) CD compared with UC patients. NS: Non-significant, **: p<0.05 **: p<0.01.*

Expression of *IL6* in the IBD Tissue Samples

The differences in the expression levels of *IL6* in the biopsy sample between patients with IBD and normal controls were statistically significant (p<0.001; 95% CI, 0.128–45.096; Figure 2). Real-time PCR data analysis of the *IL6* gene showed a significant difference between UC samples compared to controls (p<0.001; 95% CI, 0.047–52.425), with significant differences also found between CD samples compared to controls (p<0.001; 95% CI, 0.001–3.880). Again, there was no significant difference between the samples from CD patients compared with those from UC patients.

Correlation between the expression of *MALAT1* and *IL6* in IBD Patients

A linear regression analysis was performed to evaluate the correlation of expression levels between the *MALAT1* and *IL6* genes. No significant association was observed between the levels of *MALAT1* and the *IL6* gene in tissues of patients with IBD (R<0.001; p<0.8942). No significant association was observed between the levels of *MALAT1* and *IL6* genes in tissues from patients with UC (R<0.01; p<0.09685). In addition, no significant association was observed between the levels of *MALAT1* and *IL6* genes in tissues from patients with CD (R=0.01581, p=0.6823).

Characteristics of *MALAT1* and *IL6* Expressions as Diagnostic Biomarkers for IBD

The ROC curves and the area under the ROC curves were analyzed on 33 patients and 20 control subjects to examine the features of *MALAT1* and *IL6* expressions as potential diagnostic biomarkers for IBD. The ROC curve analysis for *MALAT1* showed an area under the curve of 0.7820 (p<0.001; 95% CI, 0.6574–0.9066;), and the ROC curve analysis for *MALAT1* showed an area under the curve of 0.8727 (p<0.00195; 95% CI, 0.7743–0.9710;) (Figure 3 and Table 2).

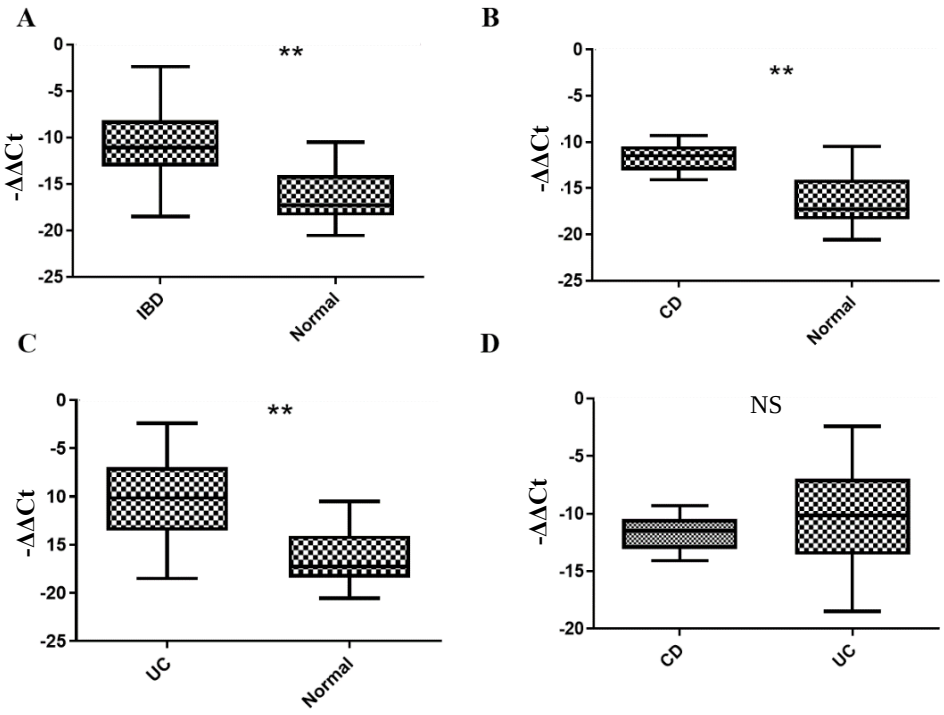

Figure 2. Real-time quantitative PCR (qRT-PCR) was used to evaluate the gene expression levels. qRT-PCR analysis of the mRNA's expression levels of *IL6* at different tissues. (A) The *IL6* expression in inflammatory bowel diseases (IBD) patients and normal tissue. (B) The *IL6* expression in Crohn's disease (CD) patients and normal tissue. (C) The *IL6* expression in Ulcerative colitis (UC) patients and normal tissue. (D) The *IL6* expression in CD compared with UC patients. NS: Non-significant, **: p<0.05 **: p<0.01.*

Table 2. Evaluating the diagnostic potential of MALAT1 and IL6 in distinguishing between inflammatory bowel disease patients and healthy control tissues

Variable	*MALAT1*	*IL6*
Cutoff	7.993	8.370
Specificity (%)	90%	85%
Sensitivity (%)	65.63%	78.13%
Area	0.7820	0.8727
%95 CI	0.6574–0.9066	0.7743–0.9710

MALAT1: metastasis associated in lung adenocarcinomatranscript 1; IL6: Interleukin 6; CI: Confidence interval

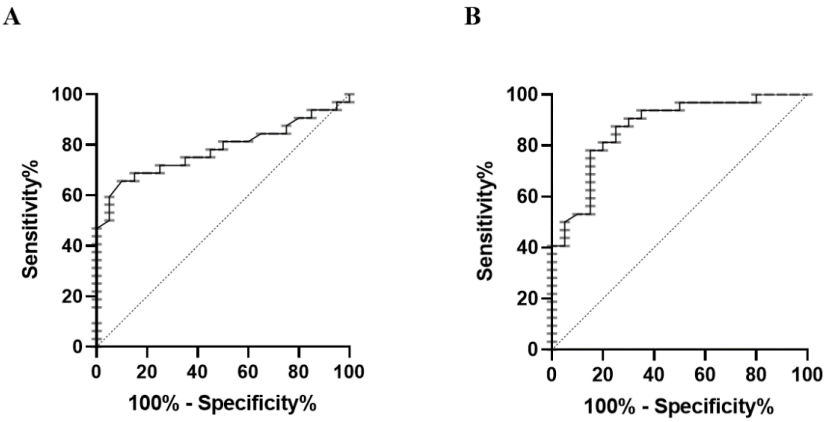

Figure 3. ROC curves of normalized (A) *MALAT1* and (B) *IL6* gene expressions to evaluate sensitivity and specificity. ROC, receiver-operating characteristic; *MALAT1*, metastasis-associated lung adenocarcinoma transcript 1; *IL6*, Interleukin 6.

MALAT1 and *IL6* Networks

Several bioinformatics tools were used in this investigation. LncRRIsearch database predicted 3 Physicochemical interactions between *MALAT1* transcripts and *IL6* by RIblast, including *MALAT1-201*, *MALAT1-202*, *MALAT1-204*, and *MALAT1-213* (Table 3). Additionally, the miRNA-based network, which included *hsa-miR-202-3p*, *hsa-miR-1-3p*, *hsa-miR-9-5p*, and CCAAT enhancer binding protein beta *(CEBPB)* transcription factor, was implemented using the Cytoscape software (Figure 4).

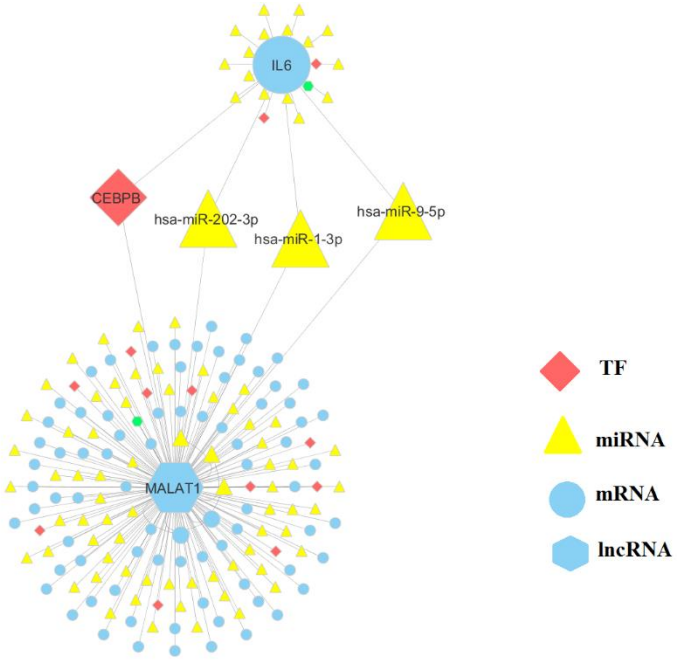

Figure 4. The gene regulatory network based on miRNAs and transcription factors between metastasis associated in lung adenocarcinoma transcript 1 (MALAT1) and IL6 was implemented using Cytoscape software (An open-source platform designed for the visualization of intricate networks and integrating these with any type of attribute data).

Table 3. Interaction of transcripts of *MALAT1* with *IL6* based on LncRRIsearch

Ensemble ID	Name	Sum of interaction energies	Predicted local basepairing interactions (n)
ENST00000508832(MALAT1-002)	*MALAT1-201*	−12.19 kcal/mol	1
ENST00000534336(MALAT1-001)	*MALAT1-202*	−76.57 kcal/mol	6
ENST00000610481(MALAT1-008)	*MALAT1-204*	−13.41 kcal/mol	1
ENST00000618227(MALAT1-016)	*MALAT1-213*	−12.49 kcal/mol	1

MALAT1: metastasis associated in lung adenocarcinomatranscript 1; IL6: Interleukin 6

Investigating the Involvement of IL6 in IBD

IBD is characterized by dysregulated inflammation and cytokine production. In this context, we identified several genes such as *IL10*, *IL17A*, *IL4*, *NFKB1*, *TNF*, and *CXCL8* that have been identified as being associated with *IL6* and IBD. Figure 5 depicts the biological process of the inflammatory response, highlighting the potential relevance of IL6 to the pathogenesis of IBD.

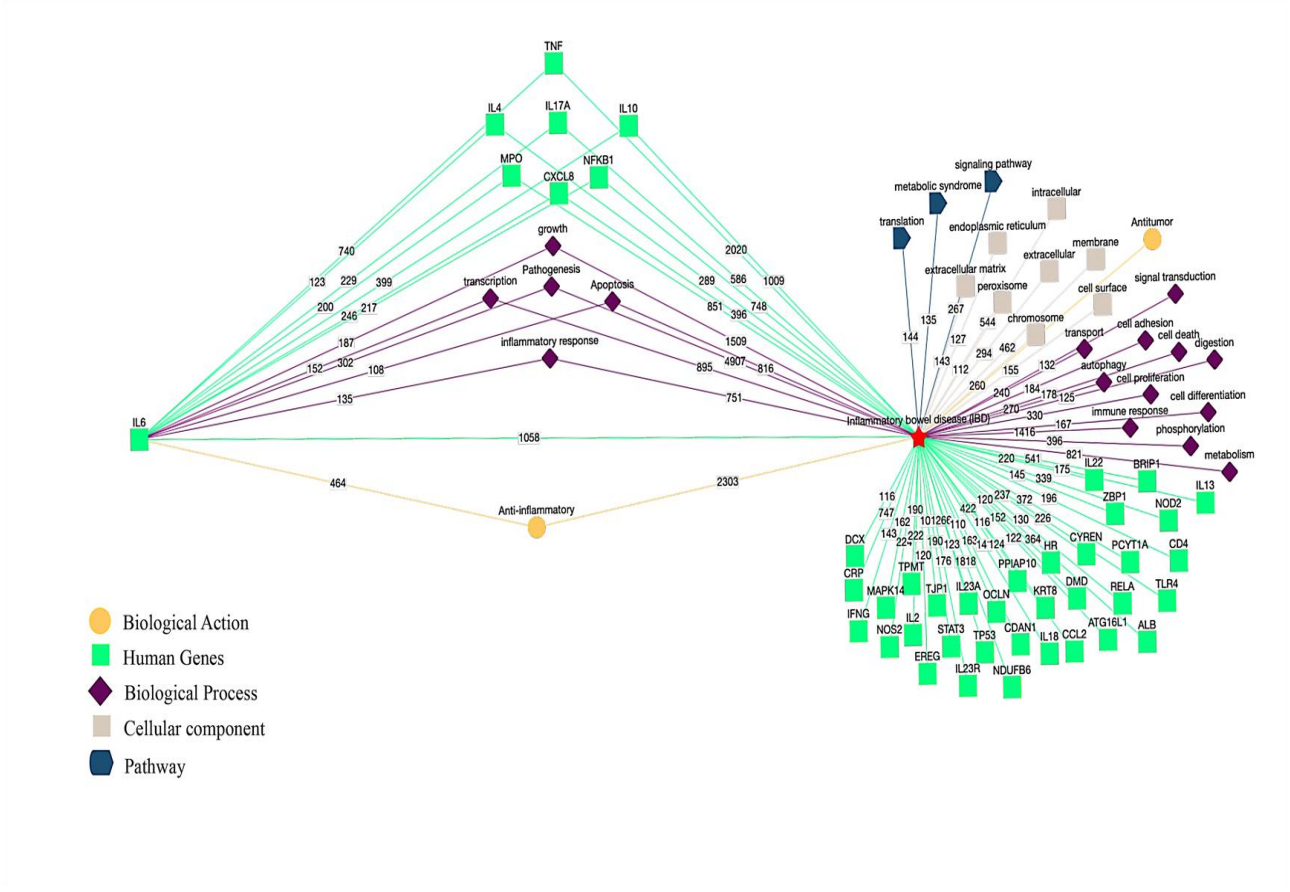

Figure 5. Involvement of the *IL6* gene in biological action, biological process, cellular component, and genes correlated with IBD using the "Inflammatory Bowel Disease" database. The numbers displayed on the edges indicate the total number of studies that have investigated the association between the variables connected by the edge (The "Inflammatory Bowel Disease" Database was used to construct this network.).

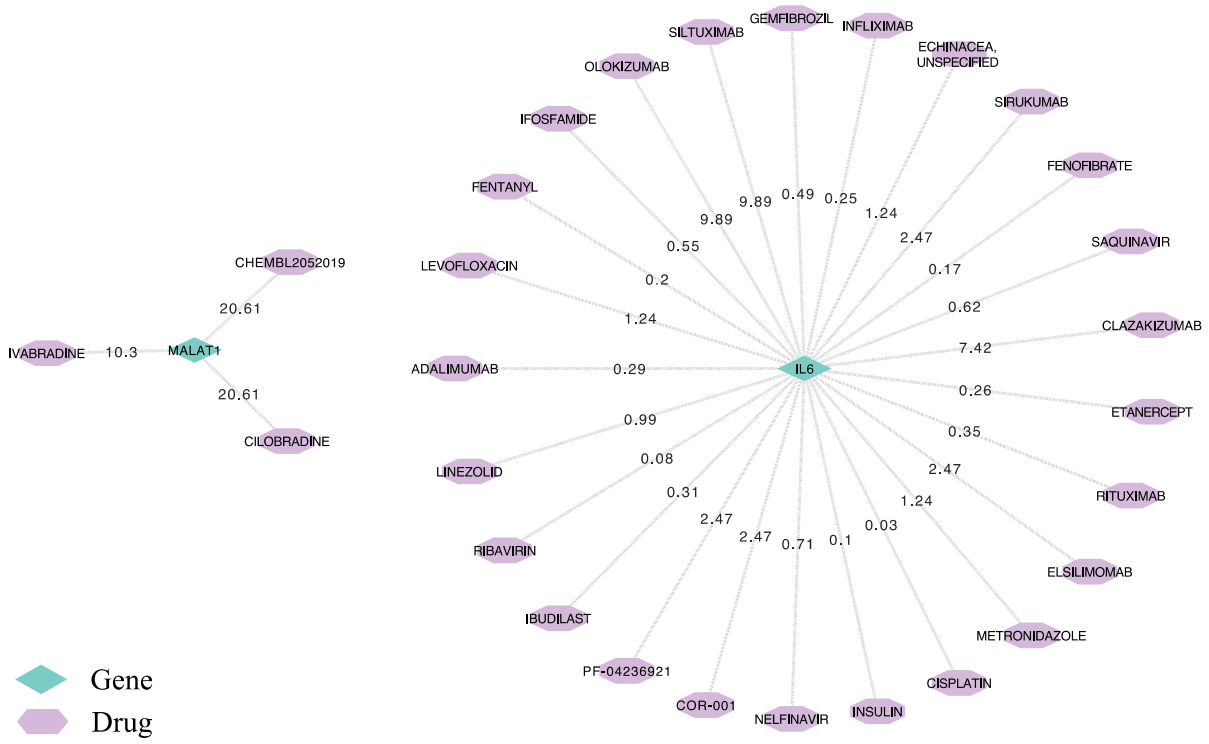

Figure 6. Gene-drug network construction based on the Drug-gene interaction database (DGIdb) using the Cytoscape software. The numbers on the edges indicate interaction scores.

Drug-gene Interaction

To determine which drugs, interact with the *MALAT1* or *IL6* genes, we utilized DGIdb and found that CILOBRADINE and CHEMBL2052019 had the highest interaction score (20.61) with the *MALAT1* gene, while SILTUXIMAB and OLOKIZUMAB had the highest interaction score (9.89) with *IL6* (Supplementary Table and Figure 6).

DISCUSSION

UC and CD are common presentations of IBD. The heterogeneity and lack of specific diagnostic tests for the disease are significant problems, and a better understanding of molecular pathogenesis may improve patient management.[28] In the present study, we analyzed the expression of *MALAT1* and *IL6* as well as their ceRNA networks in IBD patients. Finally, we investigated the potential drugs for these genes based on bioinformatics databases.

Evidence suggests that lncRNAs have a crucial role in regulating the immune system.[29] *MALAT1* is a nuclear-retained RNA that regulates transcriptional and post-transcriptional genes and is involved in various disease pathogenesis, such as cancer, diabetes, and IBD.[17,30] Our study showed that *MALAT1* expression increased in IBD patients compared to healthy controls, and the same results were also observed in both CD and UC patients. In the same vein, a higher expression level of *MALAT1* and antisense noncoding RNA at the *INK4* locus (*ANRIL*) was reported in UC patients' mucosal tissue and plasma samples.[31] Another study showed increased expression of *MALAT1* in an in vitro colitis model. The researchers concluded that the upregulation of *MALAT1* induces apoptosis and inflammation.[27] We further tested whether *MALAT1* expression differed in CD and UC samples and observed no statistically significant difference between the two groups. Although CD and UC are different phenotypes of IBD, we postulated that the function of *MALAT1* in both is likely identical.

MALAT1 overexpression can alter inflammatory responses and dysregulate the production of inflammatory cytokines, including *TNF-α* and *IL6*. *IL6*,

a crucial cytokine in the acute-phase response, is involved in the pathogenesis of several inflammatory disorders such as IBD.[32] We demonstrated that the tissue level of *IL6* was significantly increased in IBD patients. Numerous studies have revealed that serum *IL6* significantly rises in IBD patients, particularly in the active stage of the disease.[33] Parallel to our findings, Li et al. discovered that patients with active UC disease had considerably higher levels of *IL6* than patients with inactive UC disease and controls.[34]

Diagnosis and management of IBD have advanced considerably in the past decades.[35,36] In particular, the advent of biomarkers has improved the tools for early diagnosis. Our study explored the diagnostic ability of *MALAT1* and *IL6* in IBD, suggesting that they may be possible biomarkers for detecting this disease with specificities of 90% and 85% and sensitivities of 65.63% and 78.13%, respectively. Jones et al. also investigated the relationship between IBD activity and some biomarkers, including high-sensitivity CRP, *IL6*, fecal calprotectin, and lactoferrin. They demonstrated that higher high-sensitivity CRP and *IL6* levels were associated with more severe CD activity.[37] Up to this point, *MALAT1* has been mentioned as a diagnostic marker for various malignancies.[38] However, the usefulness of lncRNA as a diagnostic marker for IBD has yet to be well investigated.

It is worth mentioning that while our study revealed elevated levels of both *MALAT1* and *IL6* in both CD and UC patients, their correlation was not statistically significant. Other studies have reported controversial results. Through analyzing the relationship between the severity of normal-tension glaucoma and the *MALAT1* haplotype, Jinliang Yue et al. found that *MALAT1* boosts *IL6* production by downregulating *miR-1* expression.[30] On the other hand, the overexpression of *MALAT1* reduces traumatic brain injury-induced edema by blocking the *AQP4* and *NF-κB/IL6* pathways.[39] This disparity in findings may be attributable to differences in tissue types, races, sample sizes, methodologies, and in vitro or in vivo environments.

In order to elucidate the possible physicochemical-based interactions between mRNA and lncRNAs, we employed the LncRRIsearch web server. The analysis revealed that *IL6* protein-coding RNA interacts with three *MALAT1* transcripts. Furthermore, the investigation of ceRNA networks led us to discover three regulatory pathways of *MALAT1* and *IL6*, including *MALAT1/hsa-miR-202-3p/IL6*, *MALAT1/hsa-miR-1-3p/IL6*, and *MALAT1/ has-miR-9-5p /IL6*. By competing with endogenous RNA (ceRNA), lncRNA acts as a natural miRNA sponge. This reduces the inhibitory impact of miRNA on target genes, allowing lncRNA to upregulate the expression of target genes.[40] The gene encoding *miRNA-202-3p* is located on chromosome 10q26.3, which has recently been studied regarding some diseases such as gastric cancer, colorectal cancer, scleroderma, and lymphomagenesis.[41] Additionally, several studies have highlighted this miRNA's anti-inflammatory role.[42] Sun et al. found that *miRNA-202-3p* attenuates the inflammatory response by decreasing *TNF-α*, *IL1β*, *IL6*, and *IL8* levels in rat serum after spinal cord injury modeling.[41] On the other hand, a study asserted that *MALAT1* promotes osteosarcoma lung metastasis by sponging *miR-202*.[43] It has been shown that *miR-9* plays a role in the *NF-kB* signaling pathway, and the absence of *miR-9* leads to an increase in the expression of cytokines promoting inflammation.[44] Further, the downregulation of *TUG1* (taurine-up-regulated gene 1) lncRNA suppresses inflammation by inhibiting *miR-9-5p* in multiple sclerosis. Furthermore, it was shown that *miR-9-5p* ameliorates MS by suppressing *TNF-α*, *IFN- γ*, *IL6*, and *IL17*.[45] A study demonstrated that upregulated *MALAT1* decreased the expression of *miR-9* in in-vitro and in-vivo models of osteoarthritis.[46] For the first time, the function of *miR-1-3p* was investigated in cardiac cells, indicating its role in regulating cardiac morphogenesis and cell cycle.[47] In addition, *miR-1-3p* expression was decreased in various cancers, including lung cancer,[48] and colorectal cancer.[49] Li et al, examined the impact of lipopolysaccharide stimulation on the activation and proliferation of normal human astrocytes. Consequently, *miR-1-3p* decreased *CCL2*, *IL6*, and *TNF-α* by inhibiting the *H19* lncRNA.[50] Meanwhile, in human breast cancer cells, *miR-1* levels declined as upregulated *MALAT1* was competitively bound with *miR-1*, which led to migration and invasion.[51] In earlier research, it was discovered that the CEBPB transcription factor was 150 kb downstream of the IBD risk locus. Furthermore, analysis of transcription start-sites in biopsies of IBD identifies *CEBPB* as possibly implicated in the disease.[52]

Further, we found that *IL6* plays a pivotal role in the inflammatory responses of IBD in conjunction with other cytokines. Consistent with our results, Wang et al. have demonstrated that *IL6* promotes *NF-κB* activation in the Caco2-BBE cell line, as a epithelial intestinal model.[53] Meanwhile, Kontoyiannis et al. have shown

that excessive *TNF-α* can compromise the epithelial integrity of intestinal cells, leading to apoptosis and diminished barrier function. Targeting *TNF-α* with anti-TNF therapies has proven effective in improving clinical scores, promoting mucosal healing, and extending relapse-free periods in numerous patients.[54] In another study, *IL10*, *IL10RA*, and *IL10RB* gene mutations have been linked to IBD. In mice, deficiency of *IL10* or its receptor has been shown to result in spontaneous colitis.[55,56]

In summary, this study demonstrates significant roles for *MALAT1* and *IL6* in the pathogenesis of IBD, suggesting that these genes may have potential value as targets in the diagnosis and treatment. In addition to investigating the feedback controls between the lncRNA *MALAT1* and *IL6*, we suggest exploring the possible association of *MALAT1* with other inflammatory cytokines in a larger cohort of IBD patients. This can provide a more comprehensive understanding of the role of *MALAT1* in regulating inflammation in IBD. Furthermore, given the potential regulatory role of *MALAT1* in ceRNA networks, it would be valuable to evaluate the efficacy of ceRNA-targeting drugs in modulating the *MALAT1*-associated ceRNA network and inflammation in IBD patients in future studies. Such investigations can shed light on the development of targeted therapies for IBD and improve patient outcomes.

STATEMENT OF ETHICS

The study was conducted according to the Declaration of Helsinki guidelines and approved by the Ethics Committee of the Research Institute for Gastroenterology and Liver Diseases at Shaheed Beheshti University of Medical Sciences, Tehran, Iran (ethics code: IR.SBMU.RIGLD.REC.1395.120). All patients provided written informed consent before the study.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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