Research Square

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The proinflammatory role of guanylate binding protein 5 in inflammatory bowel diseases

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Abstract

Background: NLRP3 inflammasome is implicated in the pathogenesis of inflammatory bowel diseases (IBD). Since guanylate binding protein 5 (GBP5) induces the NLRP3 inflammasome activity, we aim to investigate the potential role of GBP5 in IBD pathogenesis.

Results: The expression of GBP5, NLRP3 inflammasome and related cytokines and chemokines were examined in two cohorts of IBD patients and healthy controls, by microarray transcriptome analysis and quantitative real time PCR. Cellular localization of GBP5 in colonic biopsies were examined by immunohistochemistry and immunofluorescence with confocal microscopy. For functional studies, *GBP5* was induced by interferon γ or silenced by siRNA or CRISPR/CAS9 technique, and inflammatory activities were evaluated at mRNA and protein levels. We found that expression of *GBP5* was elevated in colonic mucosa in two geographically and culturally distinct IBD cohorts. In colonic tissues of IBD patients, *GBP5* expression was mainly confined to immune cells and the levels of *GBP5* expression were correlated with those of the inflammatory cytokines and chemokines. In cultured T and macrophage cells, expression of proinflammatory cytokines and chemokines were increased when *GBP5* was induced, while *GBP5* deficiency leads to decreased expression of proinflammatory mediators including gasdermin D, caspase 1, cytokines and chemokines.

Conclusions: GBP5 is required in the expression of many pro-inflammatory cytokines and chemokines in intestinal immune cells. In addition, GBP5 may up-regulate inflammatory reactions through an inflammasome mediated mechanism. Since GBP5 plays a pro-inflammatory role at the early steps of the inflammatory cascades of IBD pathogenesis, and is implicated in IBD patients of distinct genetic and environmental backgrounds, targeting GBP5 could be an effective strategy for the management of IBD.

Background

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic and relapsing inflammatory diseases mainly affecting the intestines. The pathogenesis of IBD is not known, but generally believed to be driven by abnormalities in genetics, environment, gut microbiota and immunity(1).

NLRP3 (NOD-like receptor family pyrin domain containing 3) inflammasome comprises the inflammasome sensor NLRP3, the adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD domain) and the effector caspase 1. Activation of NLRP3 inflammasome leads to the production of active form of proinflammatory cytokines IL1β and IL18. Different groups have reported that colonic mucosa of IBD patients exhibited higher levels of caspase 1(2), IL1β(2–5), and IL18(6), demonstrating an association of elevated NLRP3 inflammasome activity with IBD. Animal studies provided a chain of evidence in support of a key role for NLRP3 inflammasome in colitis. NLRP3 gene knockout mice are protected from dextran sodium sulfate (DSS)(7) or 2,4,6-trinitrobenzene sulfonic acid (TNBS)(8) induced colitis compared to wild type animals. Similar protection was also observed with

pharmacological inhibition of caspase 1 with pralnacasan(7) or with caspase 1 knockout mice(9, 10). Along this line, inhibition of IL1β or its receptor suppressed experimental colitis(11, 12). Importantly, IL-1 receptor blockade using anakinra resulted in a rapid and sustained improvement in patients with colitis(13).

Further support for a causal role of NLRP3 inflammasome in IBD pathogenesis came from studies on CARD8, an inhibitor for NLRP3. Single nucleotide polymorphism (SNP) studies found that a loss-of-function mutation in CARD8 is associated with IBD(14, 15). Without the inhibitory CARD8 activity, the un-checked NLRP3 inflammasome activity leads to elevated IL1β, and consequently intestinal inflammation. Patients with CARD8 loss-of-function mutation specifically responded to IL1β blockers, but not to anti-TNFα(16), demonstrating the role of CARD8-NLRP3-IL1β axis in IBD.

Guanylate binding protein 5 (GBP5), a member of the GBP family, is a GTPase highly inducible by interferon(17). GBP5, but not other members of the GBP family, binds NLRP3 to promote ASC assembly, thus positively regulates NLRP3 inflammasome, as demonstrated in *GBP5* knock-out mice and cell culture models(18, 19). Consistent with its effect on inflammasome assembly, GBP5 was reported to stimulate NF-κB signaling, induce the expression of interferon (IFN) and other proinflammatory factors, and inhibit the replication of influenza A virus in a cell culture model(20). Therefore, we hypothesize that GBP5 plays a key role in IBD through regulating NLRP3 inflammasome activity. Here, we show that GBP5 is highly elevated in the colonic mucosa of IBD patients, and that GBP5 is required in the stimulated secretion of proinflammatory mediators in cell culture models.

Results

Elevated GBP5 expression in the colonic mucosa of IBD patients

We first examined *GBP5* mRNA expression in patients with IBD using a published transcriptome dataset generated with colonic biopsies from European patients. Demographic and clinical characteristics of these patients were described previously(21). Elevated *GBP5* expression was observed in both CD and UC patients compared to healthy controls, with fold changes of 8.6 and 8.1, respectively (Fig. 1A). To confirm these findings, a validation cohort including healthy controls, CD and UC patients from south China was enrolled (Supplementary Table S1) and *GBP5* mRNA levels in colonic mucosa were evaluated by quantitative real-time PCR (qRT-PCR). Similarly, elevated *GBP5* expression was observed in CD and UC patients compared to healthy controls, with fold changes of 13.1 and 3.8, respectively (Fig. 1B).

To examine the GBP5 expression at protein level, inflamed and adjacent non-inflamed colonic tissue from the same CD patients underwent colon resection were subjected to immunohistochemistry (IHC) staining. With all the samples examined, GBP5 staining was more intense in the inflamed tissue than in the non-inflamed tissue (Fig. 1C and D). Interestingly, GBP5 positive cells were mostly found in the lamina propria, with a few exceptions located at the luminal or glandular epithelium (Fig. 1C).

Immune cell specific GBP5 expression in the colon of CD patients

To better understand the tissue distribution of GBP5, IHC staining of GBP5 was examined at the entire depth of the inflamed and non-inflamed colonic tissues of CD patients underwent colon resection. In the inflamed colon, GBP5 positive cells were densely populated in mucosa, mostly lamina propria (Fig. 2A, I). Besides, GBP5 positive cells were frequently observed in other layers of the colonic tissue including submucosa, circular muscle, longitudinal muscle, serosa, and mesentery (Fig. 2A, II-VI). Less GBP5 positive cells were observed in colonic layers of the non-inflamed tissue from the same patient (Fig. 2B). It is noteworthy that GBP5 was not detected in muscle cell (Fig. 2A, III, IV) or endothelial cell, but detected in the blood cells (Fig. 2A, VI). Another outstanding observation was the enrichment of GBP5 positive cells in Peyer's patch (mucosa, Fig. 2A, VII) and lymph node (submucosa, Fig. 2A, VIII). The elevated expression of GBP5 across the colonic layers of CD patients is in line with the fact that CD is usually inflicted by transmural inflammation.

To determine the cellular distribution of GBP5, inflamed colon tissues from CD patients were subjected to immunofluorescence staining for GBP5 and immune cell marker proteins. Confocal microscopy showed that, most of the CD3 positive cells, most of the CD40 positive cells, and the majority of CD68 positive cells expressed GBP5 (Fig. 2C). CD3, CD40 and CD68 are marker proteins for T lymphocyte, antigen presenting cells (dendritic cells, macrophages and B cells)(22) and macrophages, respectively. Thus the above results demonstrated immune cell specific expression of GBP5.

Transcriptome analysis reveals association between GBP5 and inflammatory reaction pathways

To identify potential links between *GBP5* and IBD pathogenesis, we performed hierarchical clustering of GBP5 with 102 available cytokine and chemokine genes with the transcriptome dataset generated from colonic mucosa of IBD patients (GSE16879)(21). The clustering result showed that *GBP5* shared a similar expression pattern with genes coding for proinflammatory cytokines and chemokines including *IL1B* and *IL6*, and they were highly elevated in most of the CD and UC patients compared to the healthy controls (Fig. 3A). Interestingly, the anti-inflammatory cytokine *IL10* exhibited a similar expression pattern as *GBP5*. The elevated *IL10* expression in IBD was observed previously and was thought to reflect a futile effort of patient immune system to control the excessive inflammatory reaction(23). Next, we performed Pearson correlation analyses between every two genes based on the transcriptome data (Fig. 3B). With a threshold of correlation coefficient greater than 0.6, 486 genes were correlated with *GBP5*. In comparison, 226, 123, and 112 genes were correlated with *GBP1*, *GBP2* and *GBP4*, respectively. Apparently, at the transcription level, *GBP5* had the largest impact on IBD among all *GBP* family genes. We then performed Gene Ontology (GO) enrichment analysis with all 486 genes correlated with *GBP5*. Top "Biological Process", "cellular compartment" and "molecular function" are listed in Fig. 3C. The top "Biological Process", including "leukocyte migration", "response to molecule of bacterial origin", "response to lipopolysaccharide" and "cellular response to molecule of bacterial origin" are related to inflammatory

responses to bacterial infection. In addition, the top “cellular compartment” and the top “molecular function” such as “collagen-containing extracellular matrix” and “extracellular matrix structural constituent”, are closely related to lymphocyte migration and infiltration. These results are consistent with previous reports that GBP5 plays a critical role in host defense against bacterial pathogens(24, 25).

GBP5 deficiency down-regulates pro-inflammatory chemokines and cytokines in cultured cells

For further understanding of the association between *GBP5* and inflammatory processes, we performed *GBP5* siRNA knockdown in Jurkat cells to determine the impact of *GBP5* on chemokine and cytokine secretion. Diminished GBP5 expression in Western blot analysis indicated efficient knockdown of *GBP5* gene in Jurkat cells (Supplementary Fig. S1A). The cell culture supernatants were then subjected to Luminex chemokine and cytokine assay. Compared to cells treated with control RNA, upon stimulation with IFNγ and lipopolysaccharide (LPS), *GBP5* siRNA treated cells exhibited decreased levels of CCL2, CCL8, CCL13, CCL25, CXCL10, CXCL11, CXCL12, CXCL16, CX3CL1, IL-1β, IL-10 and MIF in the cell culture supernatant (Supplementary Fig. S1B). In addition, CCL19, CCL20, CCL22, CXCL2 and CXCL13 were not detected in the supernatant after *GBP5* knockdown, but detected in the supernatant of the cells treated with control RNA (Supplementary Fig. S1B). *GBP5* knockdown also caused a trend of decreased levels in some proinflammatory chemokines and cytokines, including CCL1, CCL11, CCL15, CCL21, CCL26, CCL27, CXCL1, CXCL5, IL-16 and TNF-α, but statistical significance was not achieved (Supplementary Fig. S1B). CCL7, CCL17, CCL23, CXCL6, CXCL9, GM-CSF, IL-2, IL-4 and IL-6 were not detected in any of the samples (Supplementary Fig. S1B).

For a precise evaluation of *GBP5* impact on chemokine and cytokine secretion, *GBP5* gene was removed from THP-1 cell by CRISPR/CAS9 method and confirmed by Western blot showing no GBP5 expression in $GBP5^{-/-}$ THP-1 cells (Fig. 4A). Global mRNA expression of the $GBP5^{-/-}$ THP-1 cells were assessed by RNA sequencing. As expected, *GBP5* mRNA was reduced in $GBP5^{-/-}$ THP-1 cells compared to the wildtype controls, with or without induction by IFNγ plus LPS (Fig. 4B, C). Surprisingly, *GBP5* deficiency greatly reduced the mRNA expressions of many inflammation and immune related genes, including (1) IFNγ response genes such as *AIM2, CASP1, GSDMD*, and *IL1B*, and (2) IFNγ non-response genes such as *NLRP3, CASP11* and *IL18* (Fig. 4B).

Comparing the $GBP5^{-/-}$ with wildtype THP-1 cells, many differentially expressed genes, including 2298 genes up-regulated and 2813 genes down-regulated in $GBP5^{-/-}$ cells, were identified (Fig. 4C). The mRNA expression for most proinflammatory cytokines and chemokines were reduced or undetected in $GBP5^{-/-}$ cells (Supplementary Fig. S2). We then performed GO enrichment analysis with the list of genes down-regulated in $GBP5^{-/-}$ cells. The identified top “Biological Process”, “cellular compartment” and “molecular function” are related to inflammatory signaling, immune cell migration, neutrophil activation, secretion of cytokines and chemokines, and other immune and inflammatory events (Fig. 4D).

Using a different approach for functional analysis, gene set enrichment analysis (GSEA) identified a similarly broad suppression of immune and inflammatory functions in *GBP5*$^{-/-}$ THP-1 cells, including antigen processing, chemokine signaling, cytokine signaling, leukocyte migration and NK cell cytotoxicity (Fig. 4E). Compared to GO enrichment analysis that uses partial information of the biological pathways (in our case, the down-regulated genes), GSEA considers all available information of relevant genes for functional analysis. Thus both methods identified immune and inflammatory pathways as down-regulated pathways in *GBP5*$^{-/-}$ THP-1 cells.

Next, we examined the altered immune and inflammatory pathways at proteins level. Compared to the wildtype controls, THP-1 cells with *GBP5* deficiency exhibited decreased levels of CCL2, CCL8, CCL13, CCL21, CCL25, CCL26, CXCL5, CXCL11, CXCL12, CX3CL1, IL1β, IL-10 and IL16 in the cell culture supernatant (Fig. 5). In addition, CCL1, CCL3, CCL7, CCL11, CCL15, CCL19, CCL20, CCL22, CCL23, CCL24, CCL27, CXCL1, CXCL2, CXCL6, CXCL9, CXCL10, CXCL13, CXCL16, IL-2, IL-4, IL-6, CXCL8, TNF-α and GM-CSF were not detected in the supernatant of *GBP5* knockout cells, but detected in the WT controls (Fig. 5). CCL17 was again not detected in any of the samples (Fig. 5). Therefore, *GBP5* deficiency led to decreased secretion for most of the inflammatory mediators analyzed.

Given the close relation among GBP5, inflammasomes and IL1β, we examined the intracellular protein expression of some related molecules. Western blots showed that *GBP5* deficiency greatly decreased the expression of gasdermin D, caspase 1 and pro-IL1β (Fig. 6). Since the active form for gasdermin D or caspase 1 was not observed in any of the samples, the decreased production of IL1β is not likely due to impaired proteolytic activation by inflammasomes in *GBP5* deficiency. Rather, the transcription and expression analysis indicate that the decreased IL1β production is part of the consequence of broad inhibition of inflammatory gene expression in *GBP5* deficiency.

Discussion

Here, we show for the first time the elevated expression of *GBP5* in colonic mucosa of patients with IBD, and that *GBP5* is required for the stimulated secretion of inflammatory cytokines and chemokines, including IL1β, from T lymphocytes and macrophages. In colonic tissues of IBD patients, the expression of *GBP5* was mainly confined to immune cells and the levels of *GBP5* expression were correlated with those of the inflammatory markers. Importantly, expression of proinflammatory cytokines and chemokines were increased when *GBP5* was induced, while *GBP5* deficiency leads to decreased production of the proinflammatory mediators in T and macrophage cells. Thus, the specific role of GBP5 in IBD pathogenesis is to facilitate the stimulated production of inflammatory mediators in intestinal immune cells. Our observation of highly elevated *GBP5* expression at the inflamed colonic mucosa in two geographically and culturally distinct IBD cohorts indicates that GBP5 plays a common and important role in the early steps of IBD pathogenesis, and therefore, is a potential therapeutic target for the management of IBD patients of various genetic and environmental backgrounds.

Our results suggest that GBP5 may promote inflammation through two mechanisms. Firstly, gene knockout studies indicated that GBP5 is required for the stimulated expression of many inflammatory cytokines such as IL1β, and is required for the expression of many IFNγ non-response cytokines such as IL18. Studies at both mRNA and protein levels indicated that GBP5 had a large impact on the expression of these proinflammatory cytokines and chemokines. Once IL1β is induced, in turn, IL1β may stimulate the production of other pro-inflammatory cytokines and chemokines including IL6, IL8, CCL2, CCL5, CXCL1, CXCL2, CXCL3, CXCL6 and IFNγ(26, 27). The outstanding knowledge gap is how the cytoplasmic, inflammasome interacting GBP5 regulates gene expression? Secondly, since GBP5 promotes NLRP3 inflammasome activity(18), and NLRP3 inflammasome is required for the excessive inflammatory reactions in IBD (reviewed in (28)), it is expected that NLRP3 inflammasome may mediate the pro-inflammatory effect of GBP5. This is supported by our observation that the expression of caspase 1, gasdermin D and IL1β was elevated when *GBP5* is induced, and was reduced when *GBP5* was down-regulated by siRNA or gene knockout. The puzzle here is the observation that the active forms of caspase 1 and gasdermin D were not observed in IFNγ primed THP-1 cells. For both mechanisms, future efforts are needed to unravel the puzzles and to join the knowledge gaps.

One major challenge for the interpretation of our data came from a few studies that reported protective role for NLRP3 inflammasome in mouse models of colitis, that is, mice lacking NLRP3, ASC or caspase 1 were more susceptible for colitis in DSS and azoxymethane (AOM) models(29–33). The discrepancies regarding the role of NLRP3 inflammasome in colitis may be explained by environmental factors including microbiota(8) and diet(34). In addition, Zaki et al. argued that the protective role of NLRP3 inflammasome is due to its beneficial effect on epithelial barrier function via induction of IL18 in epithelial cells(30). However, this argument does not reconcile with the facts that little NLRP3 inflammasome activity presents in the intestinal epithelial cells and that IL18 production by these cells is independent of NLRP3 inflammasome(35).

Besides the conventional NLRP3 inflammasome mediated mechanism, a caspase 11 dependent pathway may relay the inflammatory signal from GBP5. Mice and cell culture studies suggested that, upon detection of cytoplasmic LPS, GBP proteins encoded on mouse chromosome 3 activate caspase 11 dependent cell autonomous immune responses(25). This is consistent with current knowledge that, structural and functional alterations in the gut microbiota, especially in gram-negative bacteria(36), and their cell wall component LPS(37), are implicated in the pathogenesis of IBD. In support of a causal role for bacteria and LPS in IBD, we identified the following pathways as the top features of the GBP5 associated colonic mucosal transcriptome in IBD: “response to molecule of bacterial origin”, “response to lipopolysaccharide” and “cellular response to molecule of bacterial origin”. On the other hand, it is noteworthy that virus infection is implicated in IBD pathogenesis(38), and GBP5 has been identified as an IFN induced virus restriction factor, that interferes with virus assembly(39), or through induction of innate immune mediators(20). Further study is required to clarify potential collaborative roles of caspase 11 and GBP5 in IBD pathogenesis, and whether viral or bacterial infection trigger elevated GBP5 expression in the intestines of IBD.

Conclusions

In summary, GBP5 is highly expressed in the colonic immune cells of IBD patients. Induction of GBP5 is required for the stimulated production of proinflammatory cytokines and chemokines, while GBP5 deficiency decreases the expression of the proinflammatory mediators. Since GBP5 plays a pro-inflammatory role at the early steps of the inflammatory cascades of IBD pathogenesis, and is implicated in IBD patients of distinct genetic and environmental backgrounds, targeting GBP5 could be an effective strategy for the management of IBD.

Materials And Methods

Human samples

Colonic pinch biopsies were obtained from patients with IBD at the Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou, China. Healthy control colonic biopsy samples were from patients suspected of intestinal diseases but diagnosed normal according to biopsy. Colon resections were obtained from patients with IBD underwent colectomy. Non-inflamed control specimens were obtained 5 cm away from the inflamed lesion. Written informed consents were obtained from all donors. This study was approved by the Institutional Review Board of the Sixth Affiliated Hospital of Sun Yat-sen University.

RNA extraction, complementary DNA (cDNA) synthesis, and quantitative real-time PCR (qRT-PCR)

Total RNA from cultured cells was isolated using TRIZol (Invitrogen, USA). For RNA isolation from clinical samples, colonic tissues were disrupted with lysis beads (Luka, China) before RNA isolation with AllPrep® DNA/RNA Micro Kits (Qiagen, USA). Using total RNA as template, cDNA was synthesized with Fast Reverse Transcription kits (ES Science, China). For gene expression analysis, qRT-PCR was performed with FastStart Essential DNA Green Master (Roche, UK) on LightCycler® 96 (Roche, UK). Gene expression levels were normalized with β-actin gene as the reference gene according to the $2^{-\Delta\Delta CT}$ method. The sequences of the qRT-PCR primers are: GBP5, forward: 5'-CCTGATGATGAGCTAGAGCCTG-3', and reverse: 5'-GCACCAGGTTCTTTAGACGAGA; β-actin, forward: 5'-TTGTTACAGGAAGTCCCTTGCC-3', and reverse: 5'-ATGCTATCACCTCCCCTGTGTG-3'.

Transcriptome analysis

Global transcriptome analysis of the colonic tissue from IBD patients used the published microarray dataset generated from the colonic mucosa of IBD patients and healthy controls (GSE16879, http://www.ncbi.nlm.nih.gov/geo/)(21). Data were normalized by MAS5 method before further analysis. Unsupervised hierarchical clustering and Gene Ontology (GO) enrichment was performed with R (3.6.3).

For transcriptome analysis of the wildtype and *GBP5* knockout THP-1 cells, total RNA from parental THP-1 cells and *GBP5* knockout THP-1 clone B2, with and without stimulation (IFNγ and LPS) for 16 hours, respectively, was isolated as described above. The RNA preparation was qualified with an Agilent 2100

bioanalyzer (Thermo Fisher Scientific, USA). Oligo(dT)-attached magnetic beads were used to purified mRNA, which served as templates for cDNA synthesis. Libraries were constructed with the cDNA preparations at BGI-Shenzhen, China, amplified with phi29 to make DNA nanoball (DNB) which had more than 300 copies of one molecular, and sequenced on a BGIseq500 platform (BGI-Shenzhen, China). The sequencing data was filtered with SOAPnuke (v1.5.2) to remove sequences of adapters, to remove reads with low-quality base percentage (base quality less than or equal to 5) higher than 20%, and to remove reads whose unknown base ('N' base) percentage is higher than 5%. The clean reads were mapped to the reference genome using HISAT2 (v2.0.4). After that, Ericscript (v0.5.5) and rMATS (V3.2.5) were used to identify fusion genes and differentially spliced genes (DSGs), respectively. Bowtie2 (v2.2.5) was applied to align the clean reads to a human mRNA database built by BGI (ShenZhen, China), and the expression level of gene was calculated by RSEM (v1.2.12). The heatmap was drawn by pheatmap (v1.0.8) according to the gene expression levels. Differential expression analysis was performed using the DESeq2(v1.4.5) with Q value ≤ 0.05. GO (http://www.geneontology.org/) and KEGG (https://www.kegg.jp/) enrichment analysis of differentially expressed genes was performed by Phyper (https://en.wikipedia.org/wiki/Hypergeometric_distribution) based on Hypergeometric test. The statistical significance for multiple tests were adjusted by Bonferroni method. Transcriptiome data for wildtype and *GBP5* knockout THP-1 cells are available at: https://www.biosino.org/node/, accession ID: OEP002938.

Immunohistochemistry

Formalin-fixed paraffin-embedded intestine tissues from inflamed and non-inflamed sites of IBD patients were sectioned and collected onto glass slides. Antigen retrieval was performed in 10mM sodium citrate (pH6.0) at 100℃ for 15 minutes. GBP5 was stained with PV-6000 immunohistochemistry (IHC) kit (ZSGB-BIO, China) according to the protocol of the manufacturer, with the GBP5 antibody (Catalogue #: 67798) from Cell Signaling (USA). The images were collected with Slide Scanning System SQS-1000 (TEKSQRAY, China). Quantitation of the GBP5 signals was performed with ImageJ with IHC-Toolbox plugins.

Immunofluorescence

Tissue section was prepared as in IHC method. Primay antibodies include anti-GBP5 (Cat. #: 67798, Cell Signaling, USA), anti-CD3 (Cat. #: 60181-1-Ig, Proteintech, China), anti-CD40 (Cat. #: ab280207, Abcam, USA) and anti-CD68 (Cat. #: ARG10514, Arigo, China). Secondary antibodies include Alexa Fluor647 or 488 conjugated goat anti-rabbit or goat anti-mouse antibodies (Invitrogen, USA). Cell nuclei were stained with 4',6-Diamidine-2'-phenylindole dihydrochloride (DAPI, Sigma-Aldrich, USA). Slides were visualized with a TCS-SP8 confocal microscope (Leica, Germany).

Cell culture and gene knockdown with siRNA

Human cell lines Jurkat, THP-1 and 293T cells were purchased from American Type Culture Collection (ATCC, USA). Cells were cultured in RPMI 1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum (Gibco, USA) maintained at 37℃ and 5% CO2. To knockdown GBP5 in Jurkat cells, a pool of two different siRNAs (1, 5'-GCCATAATCTCTTCATTCA-3'; and 2, 5'-GCTCGGCTTTACTTAAGGA-3') were used.

Scrambled sequence of the siRNA was used as control. RNA was synthesized at Ribo-Bio (China) and provided lyophilized. RNA was reconstituted with nuclease-free water to reach a final concentration of 20μM, before transfection using Lipofectamine RNAiMAX (Life technologies, USA) following the manufacturer's instructions. At 48 hours post transfection, cells were stimulated with human interferon γ (IFNγ, 25μg/ml, Novoprotein, China) and Lipopolysaccharides from Escherichia coli O55:B5 (LPS, 500ng/ml, Sigma-Aldrich, USA) for 16 hours before harvest.

Western blot

Cells were lysed in RIPA (Radio-Immunoprecipitation Assay) buffer (P0013B, Beyotime, China) supplemented with protease inhibitors (Cat. #: HY-K0011, MedChemExpress, China) and phosphatase inhibitors (Cat. #: HY-K0021 and HY-K0022, MedChemExpress, China). Lysates were mixed with loading buffer containing SDS and DTT, and heated at 95℃ for 5 min. Proteins of interest were probed with the following antibodies: anti-GBP5 antibody as described above, anti-GSDMD (Cat. #: 97558, Cell Signaling, USA), anti-caspase 1 (Cat. #: 3866, Cell Signaling, USA), anti-IL-1β (Cat. #: 12703, Cell Signaling, USA), and anti-GAPDH (Cat. #: 60004-1-Ig, Proteintech, China). HRP conjugated secondary antibodies anti-rabbit IgG and anti-mouse IgG were from Proteintech, China.

GBP5 Knockout cell line

Two all-in-one sgRNA plasmids (HCP260159-CG04-3-10-a and HCP260159-CG04-3-10-b) for human *GBP5* were purchased from GeneCopoeia (China). In addition to DNA elements required for the generation of recombinant lentivirus, these plasmids carry Cas9 sequence and one of the following sgRNAs targeting the exon 3 of *GBP5*: gRNA1, 5'-AGTTCTCGATGAGGCACATG-3'; gRNA2, 5'-CATTACGCAACCTGTAGTTG-3'. Recombinant lentivirus was produced by transfecting 293T cells with the two sgRNA plasmids together with lentiviral packaging plasmids psPAX2 and pMD2. *GBP5* knockout THP-1 cells were generated by infecting THP-1 cells with the recombinant lentiviruses followed by G418 selection. After obtaining single cell colonies from limiting dilution, three THP-1 clones (A1, B1, B2) harboring all-allelic deletion of the 80-bp in exon 3 of GBP5 locus were identifiedby PCR and validated by DNA sequencing of the PCR products. The primers used for PCR screening are: forward, 5'-AGTAGTATGTCCCCAGGTTC-3', and reverse, 5'- AAGACCAGCTGTAGCCTAAA – 3'.

Luminex-based assays

The cell culture supernatant was collected after IFNγ + LPS stimulation. Multiple cytokines and chemokines were examined with Luminex liquid suspension chip at Wayen Biotechnologies Shanghai, Inc. (China). The Bio-Plex Pro Human Chemokine Panel 40-Plex kit (Cat. #: 171AK99MR2, Bio-rad, USA) was used following the manufacturer's instructions.

Statistical analysis

Data was analyzed by either one-way ANOVA and Dunn's test or two tailed Student's t-test using GraphPad Prism (v7.0). All *in vitro* experiments were performed in triplicate. $P < 0.05$ was considered to be statistically significant.

Abbreviations

AOM, azoxymethane;

ASC, apoptosis-associated speck-like protein containing a CARD domain;

CD, Crohn's disease;

DSS, dextran sodium sulfate;

GBP, guanylate binding protein;

GSEA, gene set enrichment analysis;

IBD, inflammatory bowel diseases;

IFN, interferon;

IHC, immunohistochemistry;

LPS, lipopolysaccharide;

NLRP3, NOD-like receptor family pyrin domain containing 3;

SNP, single nucleotide polymorphism;

TNBS, 2,4,6-trinitrobenzene sulfonic acid;

UC, ulcerative colitis.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of the Sixth Affiliated Hospital of Sun Yat-sen University (No. 2019ZSLYEC-186). Written informed consents were obtained from all donors.

Consent for publication

Not Applicable.

Availability of data and materials

Transcriptome data for patients with inflammatory bowel diseases and healthy control subjects (GSE16879) are available at: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE16879.

Transcriptiome data for wildtype and GBP5 knockout THP-1 cells are available at: https://www.biosino.org/node/, accession ID: OEP002938.

Competing interests

All authors declare no competing interest.

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Authors' contributions

LZ, JK, and RZ conceived and designed this study. XL, YL, SC, YH, YZ, Wenxia W, and JK collected patient samples and clinical data. YL, SC, YH, and SG performed experiments. YL, SG, XL, LZ, JK, and RZ analyzed data. YL and LZ prepared the manuscript. All authors critically revised the manuscript. All authors had access to the study data and had reviewed and approved the final manuscript.

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Not Applicable.

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Figures

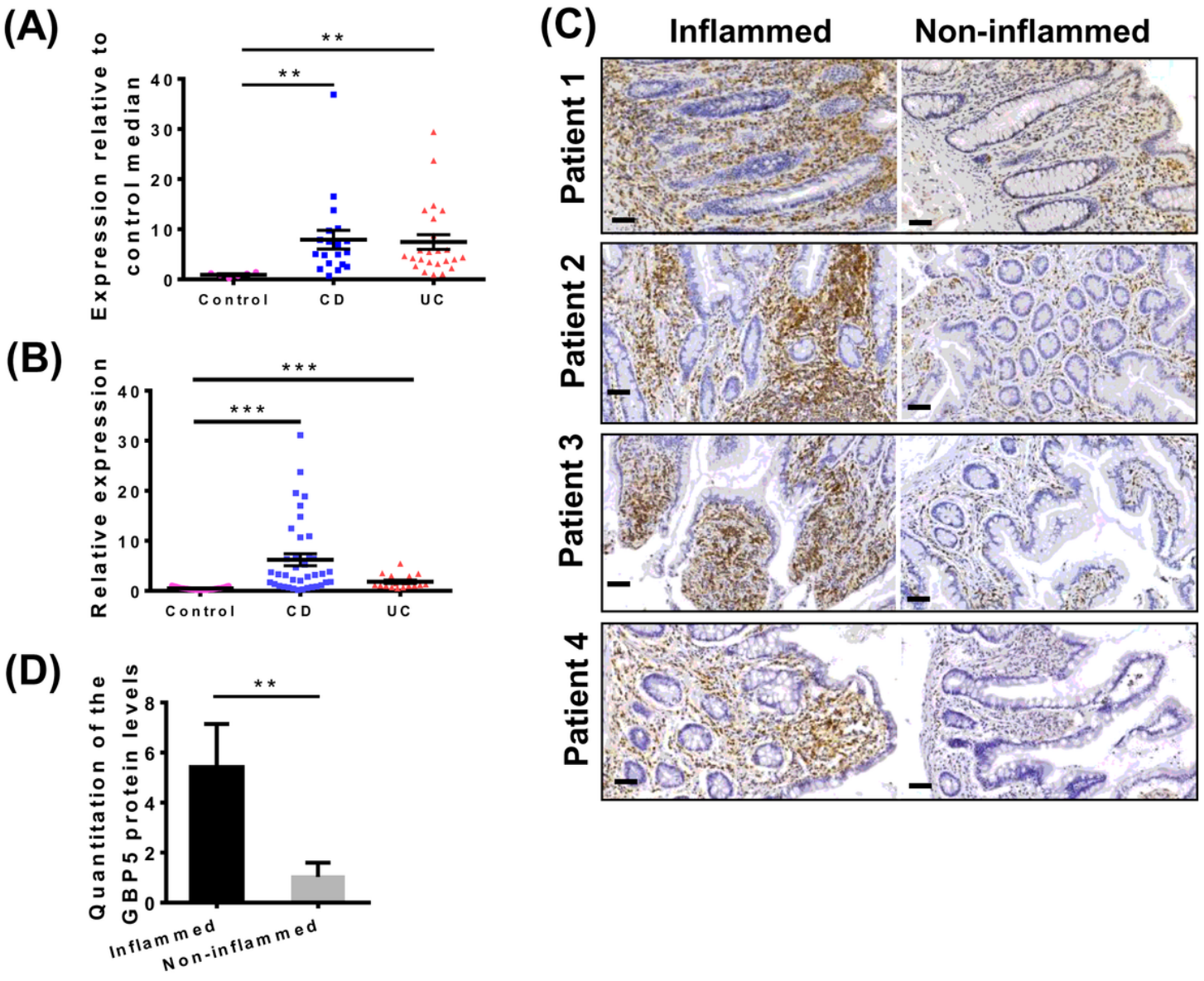

Figure 1

GBP5 is highly expressed in the inflamed intestinal tissue of IBD patients. (A) Messenger RNA expression of GBP5 in the colonic mucosa of healthy controls (*n*=6), patients with Crohn's disease (CD, *n*=19) and ulcerative colitis (UC, *n*=24). Data are from a microarray dataset generated from a European cohort. **, *P*<0.01, Dunn's multiple comparison test. (B) Quantitative RT-PCR analysis of GBP5 mRNA in colonic mucosa from a Chinese cohort including healthy controls (*n*=35), patients with CD (*n*=38) and UC (*n*=17). ***, *P*<0.001, Dunn's multiple comparison test. (C) Immunohistochemical staining of GBP5 in colonic mucosa from 4 representative patients with CD. Images of inflamed and non-inflammed sites from the same patient are compared side by side. Bar=50μm. (D) Quantitation of the GBP5 staining in the inflammed and non-inflammed sites in (C). **, P<0.01, paired student t-test.

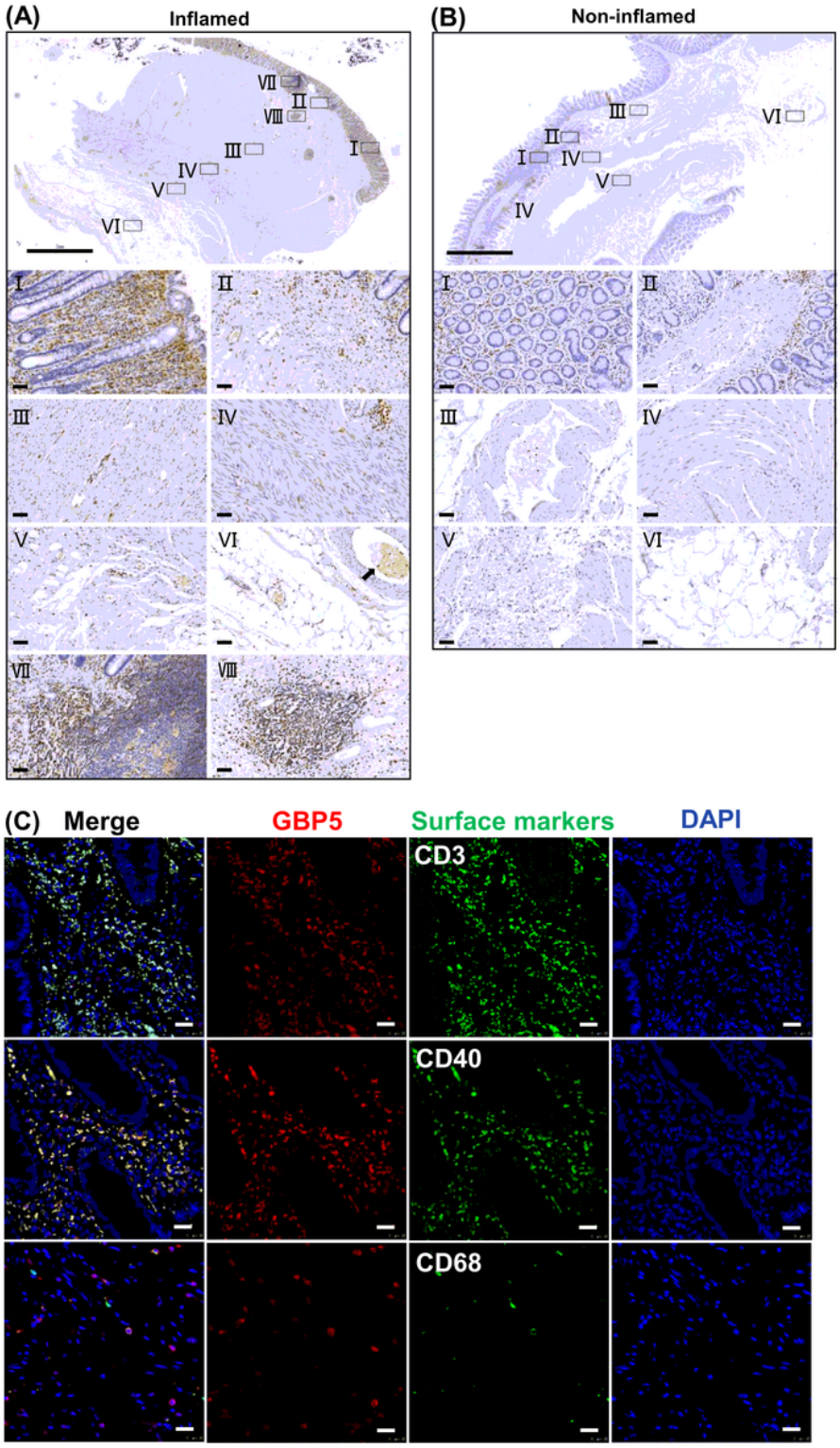

Figure 2

Immune cell specific GBP5 expression in patients with CD. (A) Immunohistochemical staining of a colonic biopsy section from a representative CD patient with an anti-GBP5 antibody: an inflamed area. The top image shows a panoramic view of the section. Bar = 2mm. Details are shown for boxed areas representing different anatomic structures. Ⅰ, mucosa; Ⅱ, muscularis mucosa and submucosa; Ⅲ, Circular muscle; Ⅳ, Longitudinal muscle; Ⅴ, Serosa; Ⅵ, Mesentery, arrow indicates a blood vessel; Ⅶ, Peyer's patch in

mucosa; □, Lymph node in submucosa. Bar = 50μm. (B) Immunohistochemical staining of a colonic biopsy section from a representative CD patient with an anti-GBP5 antibody: a non-inflamed area. The top image shows a panoramic view of the section. Bar = 2mm. Details are shown for boxed areas representing different anatomic structures. □, mucosa; □, lamina propria, muscularis mucosa and submucosa; □, a blood vessel in submucosae layer; □, circular muscle and longitudinal muscle; □, serosa; □, mesentery. Bar = 50μm. (C) Immunofluorescence staining of inflamed colon tissue from a representative CD patient with antibodies against GBP5, CD3, CD40 and CD68. Bar = 50μm.

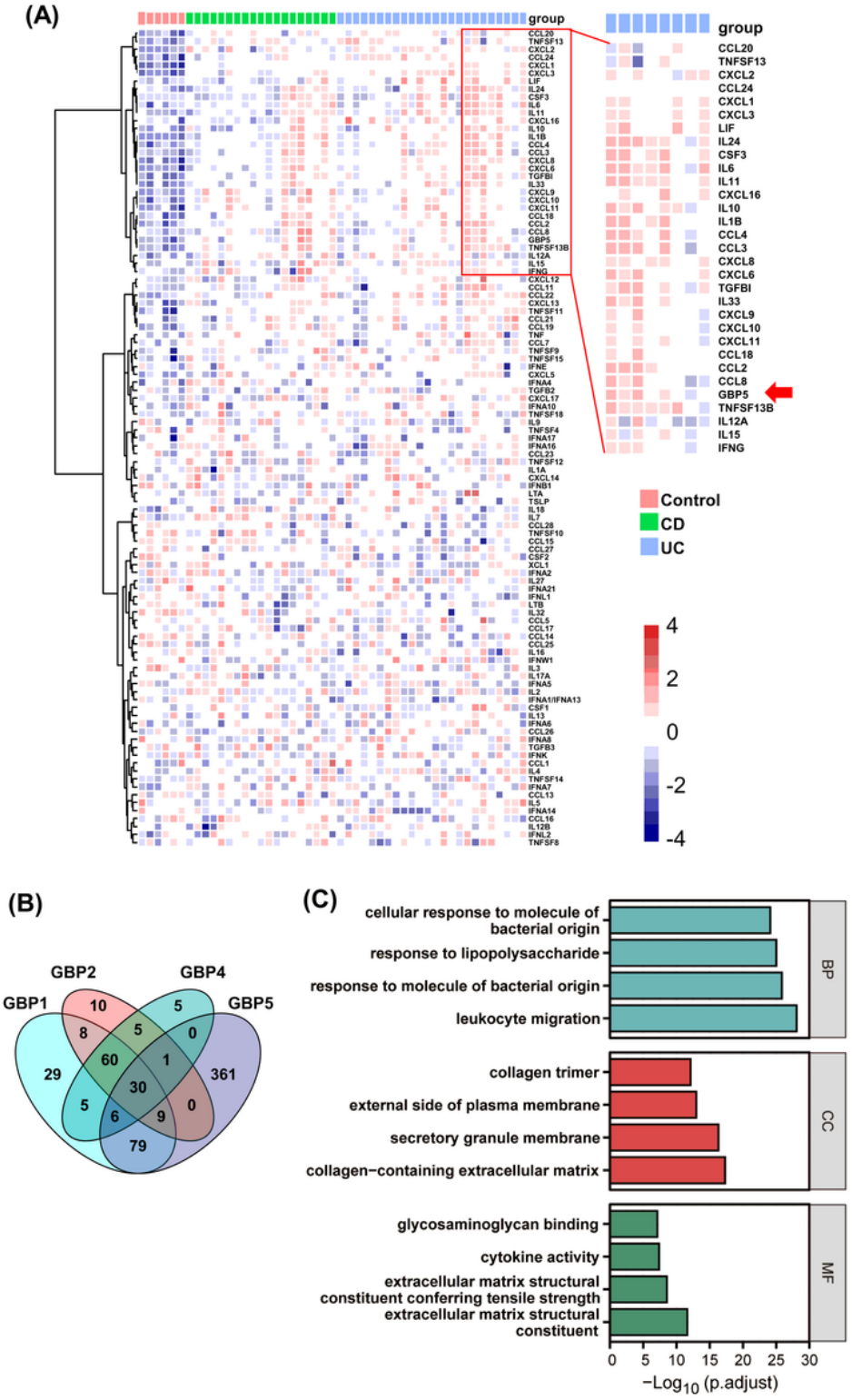

Figure 3

GBP5 is implicated in inflammatory processes in IBD according to transcriptome analysis. (A) Heatmap for mRNA expression levels of GBP5 (arrow), available cytokines and chemokines based on transcriptome data generated from colonic mucosal biopsies of healthy controls (n=6), patients with Crohn's disease (CD, n=19) and ulcerative colitis (UC, n=24). Unsupervised hierarchical clustering of *GBP5*, cytokine and chemokine genes was performed. Genes in the red box are more closely clustered with GBP5. (B) Venn plot of the number of genes associated with GBP1, GBP2, GBP4 and GBP5, based on their mRNA expression levels. Associations with a Pearson's correlation coefficient no less than 0.6 are counted. (C) Gene Ontology (GO) analysis of GBP5 associated genes. The cut-off value for the input gene list is Pearson's correlation coefficient no less than 0.6. BP, biological process; CC, cellular compartment; MF, molecular function. All samples from GSE16879 are included (n=90).

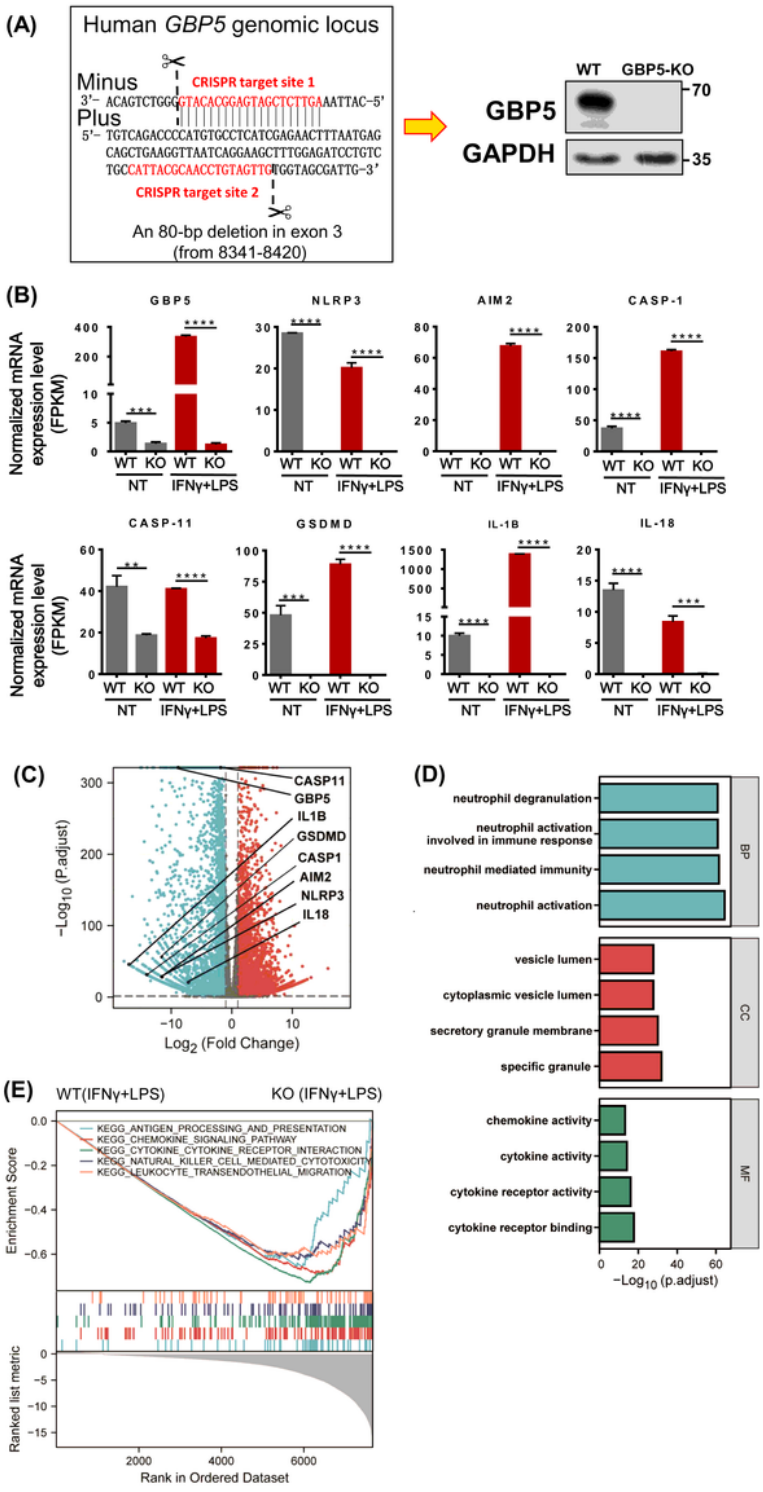

Figure 4

GBP5 Knockout down-regulated the expression of proinflammatory mediator genes in THP-1 cells. The transcriptomes of wildtype and *GBP5*$^{-/-}$ THP-1 cells, with or without stimulation (IFNγ and lipolopolysaccharide (LPS)), respectively, were analyzed by RNAseq technique. N=3 for each treatment group. (A) Generation of *GBP5* knockout (*GBP5*$^{-/-}$) THP-1 cell line by CRISPR/Cas9-mediated genome editing. The two CRISPR target sites are highlighted in red. Loss of GBP5 was confirmed by Western blot.

WT, wildtype control. (B) Messenger RNA expression of *GBP5* and related genes in wildtype and *GBP5*$^{-/-}$ THP-1 cells (clone B2), with or without stimulation (IFNγ and LPS), respectively. FPKM, fragments per kilobase million. **, P<0.01; ***, P<0.001; ****, P<0.0001; unpaired student t-test. (C) Differential gene expression between wildtype and *GBP5*$^{-/-}$ THP-1 cells: volcano plot. Vertical and horizontal dashed lines indicate the cut-off values for differentially expressed genes:|Log_2 (Fold Change)| >1 and p.adjust <0.05. (D) GO (Gene Ontology) analysis of down-regulated genes in *GBP5* knockout cells. The cut-off values for selecting differentially expressed genes for the input gene list: Log_2(Fold Change) <-2, and p. adjust <0.05. BP, biological process; CC, cellular compartment; MF, molecular function. (E) Gene set enrichment analysis (GSEA) of *GBP5*$^{-/-}$ THP-1 cells (clone B2) transcriptome compared to wildtype THP-1 cells. Upper panel: the enrichment score curves of the top KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways exhibiting decreased expression in *GBP5*$^{-/-}$ THP-1 cells: immune related pathways. Middle panel: distribution of the genes related to the pathways indicated in the upper panel. The genes were ranked according to their differential expression between wildtype and *GBP5*$^{-/-}$ THP-1 cells. Genes of higher rank (left) exhibit relatively higher expression in wildtype THP-1 cells. Lower panel: Graphical representation of the correlations of the gene expression levels with the phenotypes: wildtype or *GBP5* knockout. Genes on the right are more negatively correlated with *GBP5* deficiency.

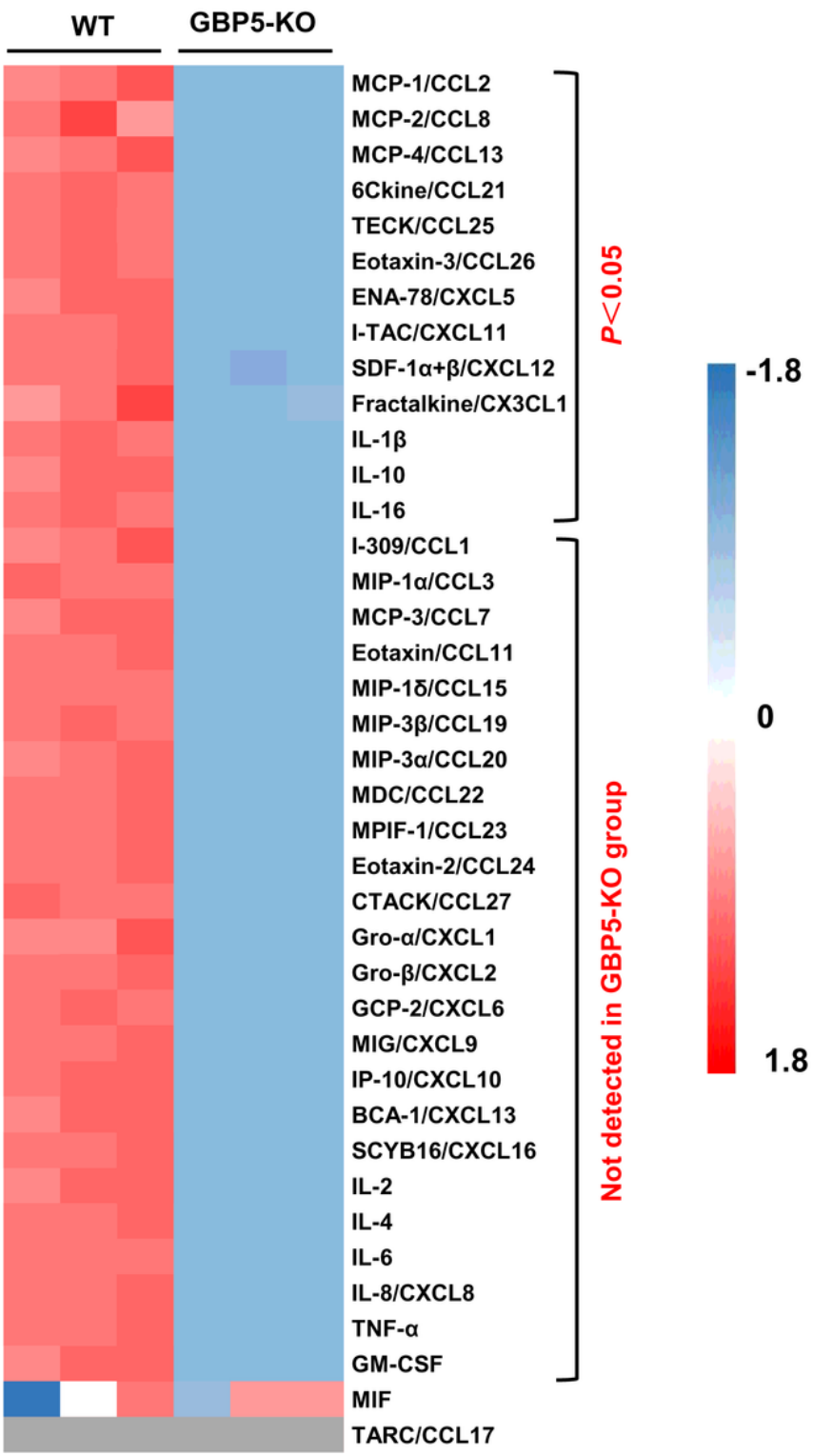

Figure 5

Decreased chemokine and cytokine secretion in *GBP5* knockout THP-1 cells. The protein levels of chemokines and cytokines in the cell culture supernatant of wildtype (WT) and *GBP5* knockout (KO) THP-1 cells are plotted. Cells were primed with IFNγ and LPS before sample collection. The concentrations of cytokines and chemokines were determined by Luminex liquid suspension chip. Data were normalized as (x-mean)/SD. Gray blocks indicate no detection. *P* values were from Student *t*-tests.

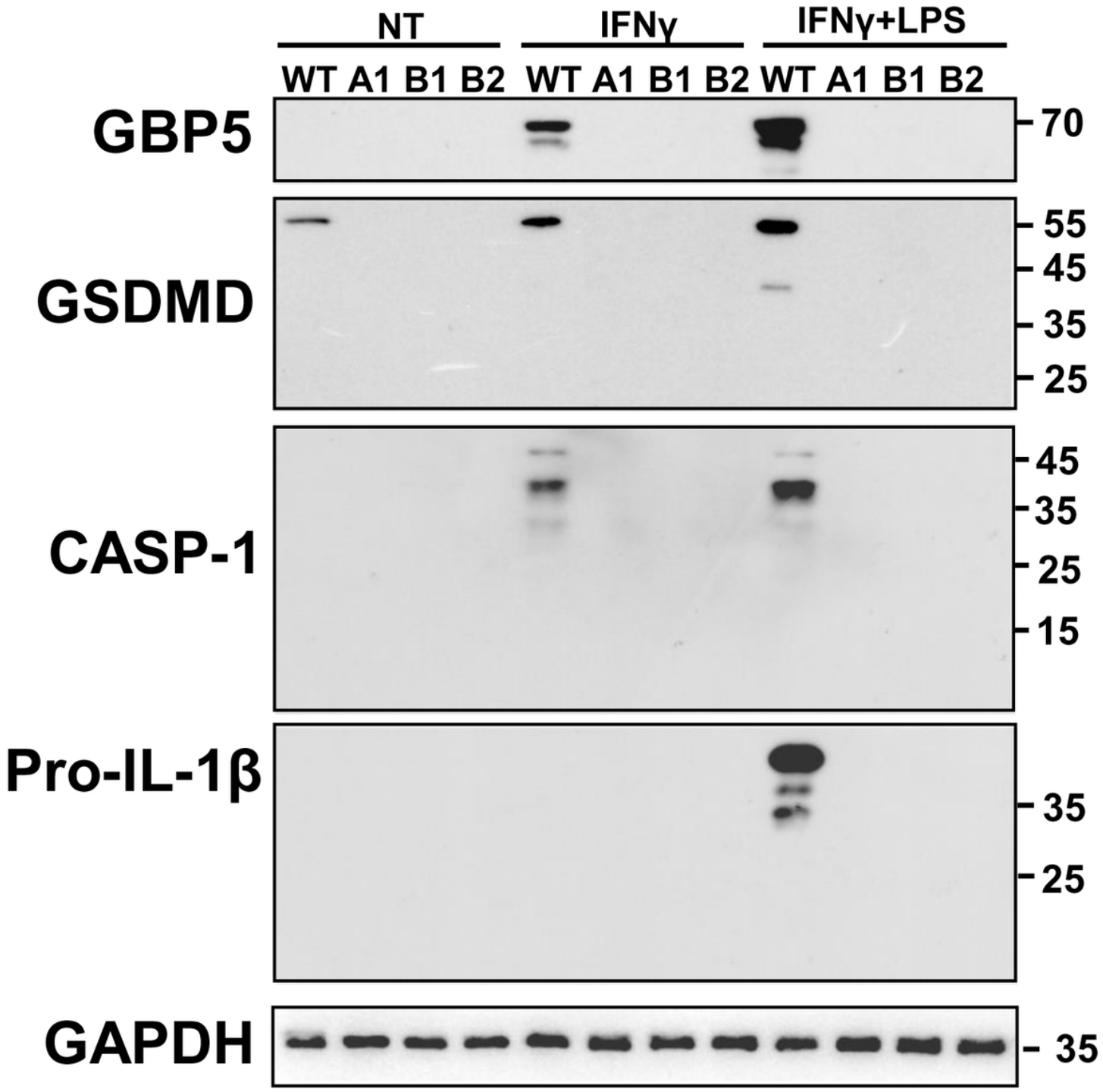

Figure 6

Diminished expression of pyroptosis related proteins in *GBP5* knockout THP-1 cells. were subjected to Wildtype THP-1 cells and *GBP5* knockout THP-1 cell clones (A1, B1, and B2) were left untreated (NT), treated with IFNγ (25ng/ml) only, or treated with IFNγ (25ng/ml) plus LPS (500ng/ml) for 16 hours, before Western blot analysis with antibodies against GBP5, gasdermin D (GSDMD), caspase 1 (CASP-1), pro-IL1β and GAPDH, respectively.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GBP5TableS1.docx
- GBP5figureS1.tif
- GBP5figureS2.tif