Abstract. Sepsis is characterized by systemic inflammatory responses. In the present study, the role of deleted in liver cancer 1 (DILC), interleukin (IL)-6, signal transducer and activator of transcription 3 (STAT3), and Toll-like receptor 4 (TLR4) in the pathogenesis of sepsis was investigated. Reverse transcription-quantitative polymerase chain reaction analysis and western blotting were performed to evaluate the effects of lipopolysaccharide (LPS) on the expression of DILC, IL-6, STAT3, and TLR4, in addition to the effects of DILC and IL-6 on the synthesis of tumor necrosis factor (TNF-α), chemokine ligand 5 (CCL5), E-selectin and C-X-C motif chemokine receptor 1 (CXCR1). In addition, the regulatory association between DILC, IL-6, STAT3 and TLR4 was investigated. LPS reduced the expression level of DILC, and enhanced the expression of IL-6, STAT3 and TLR4. DILC directly and negatively regulated the synthesis of IL-6, as demonstrated by the markedly decreased luciferase activity in cells transfected with a wild-type DILC plasmid. On the other hand, compared with the scramble control, DILC and IL-6 small interfering (si)RNAs significantly suppressed the expression of IL-6, STAT3 and TLR4. In addition, DILC siRNA enhanced the expression of IL-6, STAT3 and TLR4, whereas the expression levels of TNF-α, CCL5, E-selectin and CXCR1 in LPS-treated THP-1 cells were downregulated following transfection with DILC and IL-6 siRNAs. In patients with sepsis, DILC expression was inhibited, although the expression levels of IL-6, STAT3 and TLR4 were upregulated. Therefore, DILC may mediate the crosstalk between the cascades of IL-6/STAT3 and TNF-α signaling, indicating that DILC may act as a prognostic biomarker of sepsis, and may serve as a potential therapeutic target for the treatment of sepsis.

Introduction

Sepsis is one of the most frequent causes of mortality among hospitalized patients. The mortality rate of sepsis is similar to that of myocardial infarction (1). In North America, the number of hospitalized patients (per 100,000 patients) suffering from septicemia or sepsis increased by 70% between 2001 (2) and 2008 (3), and the incidence of severe postoperative sepsis has increased by three times (from 0.3 to 0.9%) (2,3). In addition, the incidence of sepsis is particularly high among older people and tends to increase with advancing age (1). In North America, ~90% of the population are unfamiliar with the medical term ‘sepsis’, whereas the majority of those who understand this term do not know that sepsis is the biggest contributor to mortality (4).

Among the Toll-like receptors (TLRs), TLR4 serves an important role in a variety of inflammatory disorders, including asthma, sepsis and chronic obstructive pulmonary disease. Therefore, the TLR4 signaling pathway may be very important in the regulation of these disorders. It was reported that TAK-242, a new small-molecule cyclohexene derivative, may selectively suppress TLR4 signaling and may lead to beneficial outcomes in a mouse model of endotoxins (5). TLR4 acts as a receptor for a variety of endogenous ligands, including heat shock proteins, hyaluronic acid, fibrinogen and high mobility group box 1, and the interaction between endogenous ligands and TLR4 is involved in a number of human diseases, including sepsis.

Interleukin (IL)-6 is a multifunctional cytokine released by a variety of cells; however, the level of IL-6 in healthy populations is undetectable (6). In trauma patients, an early increase in IL-6 may be associated with the magnitude of trauma, the severity of proinflammatory reactions and the incidence of complications (sepsis, multiple organ failure and mortality) (7,8). Similarly, IL-10 is a major anti-inflammatory factor and its expression levels are undetectable within healthy individuals. The expression level of IL-10 is increased in
patients with sepsis, and correlates with the magnitude of sepsis and the likelihood of mortality (9). The ratio of IL-6/IL-10 has long been used to evaluate the immune system in intensive care unit patients, as such a ratio may reveal the balance between proinflammatory and anti-inflammatory responses (systemic inflammatory syndrome and compensatory anti-inflammatory response) (10). A reduction in the IL-6/IL-10 ratio may affect patient prognosis and the occurrence of postoperative sepsis (11).

As transcripts of >200 bp in length, long noncoding (lnc) RNAs do not demonstrate any significant protein-coding capability (12). At present, an increasing amount of data has demonstrated that lncRNAs participate in a variety of pathological and physiological processes, and hence may impact various cellular functions (13). It has been recognized that nuclear factor (NF)-κB serves as a bridge between cancer and inflammation (14). A recent study demonstrated the critical role of NF-κB in the maintenance and spread of cancer stem cells (CSCs) (15). In addition, it has been reported that Inc-deleted in liver cancer 1 (DILC) may induce the interaction between the autocrine IL-6/signal transducer and activator of transcription 3 (STAT3) cascade and tumor necrosis factor (TNF)-α/NF-κB signaling in liver CSCs (LCSCs), thus linking liver inflammation to the dissemination of LCSCs (16). It has been demonstrated that the expression of IL-6 may be inhibited by DILC, whereas the expression of STAT3, a downstream component of the IL-6 signaling pathway, may be enhanced by IL-6 stimulation (16). Furthermore, IL-6 signaling has been demonstrated to modulate TLR4-dependent inflammatory responses via STAT3 (17). In the present study, the role of DILC, IL-6, STAT3 and TLR4 in the pathogenesis of sepsis was investigated.

Materials and methods

Sample collection. A total of 36 patients, including 18 patients with sepsis and 18 healthy participants, were involved in the present study. The mean age of all subjects was 56 years old (42-78 years), and the ratio of female to male subjects was 1.0:1.5. Peripheral blood samples from 18 participants diagnosed with sepsis and 18 people free of any health problems, were collected using Lymphocyte Separation Medium (Human) (Applygen Technologies Inc., Beijing, China), and stored at -80°C. Written informed consent was obtained from all subjects. All data processing and sample collection were approved by the Ethics Committee of Guangdong Academy of Medical Sciences. The present study was performed according to the Declaration of Helsinki.

Peripheral blood mononuclear cell (PBMC) isolation. PBMCs were maintained at -80°C until use. A 70-μm strainer (Falcon; Corning, Inc., Corning, NY, USA) was utilized for cellpassaging to yield single cell suspensions, according to the manufacturer's protocol. Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% fetal bovine serum (FBS, Thermo Fisher Scientific, Inc.), 100 mg/ml streptomycin sulfate and 100 U/ml penicillin sodium was utilized to culture the cells into 48-well plates with 5% CO₂ at 37°C. Growth medium was changed every 2 days until the cells were passaged, then transferred to a new dish; at 80% confluence, the PMBCs were passaged again, and cells at passage 2-3 were utilized for analysis.

Cell culture and transfection. DMEM (Gibco; Thermo Fisher Scientific, Inc.) containing 10% FBS (Thermo Fisher Scientific, Inc.), 100 mg/ml streptomycin sulfate and 100 U/ml penicillin sodium was utilized to maintain THP-1 cells under a humidified atmosphere with 5% CO₂ at 37°C. DILC mimic (5′-AAAT ATCGTACAAGCTATGGCGTGTAACGGGGAAGCAGGG ATCGGCACATTACCGCGGTAGCCCTGAGCGCCGCGGCGGTAGCGCAAAAATTTGTTGTTAAGGAGCG CGCCGCGAAATCGGCGCACAATTTTCTTTGGCGCAC GGTGATGGACAAAGATGATGTTCTCTGGT-3') and its negative control were synthesized by Guangzhou RiboBio Co., Ltd. (Guangzhou, China). When the cells reached 80% confluence, Lipofectamine® 2000 (Thermo Fisher Scientific, Inc.) was used to transfect the DILC mimic or NC into THP-1 cells according to the manufacturer's protocol. The time interval between the completion of transfection and the subsequent experiments was 4 h. The culture cells were also treated with LPS (1 mg/ml) for 30 min. A total of three independent experiments were carried out.

RNA isolation and reverse transcription - quantitative polymerase chain reaction (RT-qPCR) analysis. TRizol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) was used to extract total RNA from THP-1 cells and PBMCs, according to the manufacturer's protocol. A TaqMan micro (mi)RNA reverse transcription kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used to reverse transcribe the RNA to DNA (cDNA) with designed primers including primers for DILC (forward: 5′-ATGGCAAAAGATGTCGGTTCAATTG-3'; reverse: 5′-TCGTTCTTCGTCATCTTCG-3'), IL-6 (forward: 5′-ATGAACTCC TTCCACCAAGC-3'; reverse: 5′-CTCATATTTCGCCGAG AGGCCCTCAAGCTGACTG-3'), STAT3 (forward: 5′-ACT CCATGCTGAAAA-3'; reverse: 5′-CAGTGTCGACCAGG AGAGA-3'), and TLR4 (forward: 5′-AGCCACCTCTCT ACCTTTAATTTGA-3'; reverse: 5′-GCCGTGTTGAAATA GGTTAGAAAG-3') synthesized by Applied Biosystems (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. The Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used to perform RT-qPCR, with a 20-μl mixture containing 10 μl SYBR advantage qPCR Premix (Clontech Laboratories, Inc., Mountainview, CA, USA), 1 μl cDNA, 7 μl H₂O and 1 μl each forward and reverse primer. The temperature conditions were: 95°C for 3 min, followed by 30 cycles of 94°C for 40 sec, 56°C for 35 sec and final extension at 72°C for 60 sec. The relative expression levels of DILC, IL-6, STAT3 and TLR4 were quantified using the standard curve method. U6 (primer sequence forward: 5′-ATTGCATATCATATGCT ACCGTA-3'; reverse: 5′-AGGCAGATTAAGTGAGCTG-3') and NAPDH (primer sequence forward: 5′-CCGAGGATTTT CAGACATCC-3'; reverse: 5′-TGGATGATAGCGCAAG GCT-3') were utilized internal control for mRNAs, respectively. Relative expression levels of DILC, IL-6, STAT3 and TLR4 were presented using the 2^ΔΔCq method (18). All reactions were run in triplicate.
Luciferase assay (promoter). A luciferase assay was performed to examine whether DILC alters the transcriptional ability of the promoter of IL-6. qPCR was performed according to the protocol described above to amplify the full promoter segment from DNA templates collected from PBMCs. IL-6 promoter-luciferase reporter plasmids containing either a wild type (WT) or mutant (MUT) DILC binding site were generated accordingly. Sequencing was performed to confirm the reporters with WT or MUT DILC binding sites. Endonuclease (New England BioLabs, Inc., Ipswich, MA, USA) was used to digest the pcDNA3-control vector and amplified fragments. Subsequently the qPCR products were inserted into the pcDNA3-control vector (Ambion; Thermo Fisher Scientific, Inc.). THP-1 cells were seeded into 96-well plates at a final concentration of 3x10^4 cells/ml, and transfected with 50 ng pcDNA3-DILC (WT or MUT) via electroporation (Lonza Group Ltd., Basel, Switzerland). A dual-luciferase reporter assay system (Promega Corporation, Madison, WI, USA) was used to determine the firefly and Renilla luciferase activity 48 h post-transfection. All tests were repeated in triplicate.

Western blot analysis. Ice-cold PBS was used to wash the THP-1 cells twice, and radioimmunoprecipitation buffer (Pierce; Thermo Fisher Scientific, Inc.) was used to lyse cells and tissue sample to isolate protein for 30 min on ice, according to the manufacturer's protocols. The lysates were subjected to centrifugation for 20 min at 12,000 x g at 4°C. A bicinechonic acid protein assay kit (Boster Biological Technology, Pleasanton, CA, USA) was used to quantify proteins. SDS-PAGE on a 10% gel was used to separate 30-µg protein samples, and electroblotting was employed to transfer the target protein onto a polyvinylidene difluoride (PerkinElmer, Inc., Waltham, MA, USA) membrane for 90 min. To block the membrane, 5% non-fat milk was applied to the membrane to avoid nonspecific binding for 2 h at room temperature. The primary antibody anti-IL-6 (1:5,000; cat. no. sc-130326; Santa Cruz Biotechnology Inc., Dallas, TX, USA), primary antibody anti-STAT3 (1:5,000; cat. no. sc-8019; Santa Cruz Biotechnology, Inc.), primary antibody anti-TLR4 (1:5,000; cat. no. sc-52962; Santa Cruz Biotechnology Inc.) and anti-β-actin (1:10,000; cat. no. sc-47778, Santa Cruz Biotechnology Inc.) were used to incubate the membrane at 4°C for 12 h. Horseradish peroxidase (HRP)-labeled anti-mouse goat secondary antibody was used to treat the membrane at 37°C for 1 h (1:3,000; cat. no. 7074; Cell Signaling Technology, Inc.). A chemiluminescence detection system (Pierce; Thermo Fisher Scientific, Inc.) was used to determine the protein bands, and the Bio-Rad Chemi-DocXRS (Bio-Rad Laboratories, Inc., Hercules, CA, US) was utilized to visualize the bands of the target proteins. Each test was performed in triplicate.

ELISA analysis. A total of 50 mM carbonate-bicinechonic buffer supplemented with mouse polyclonal immunoglobulin G (IgG) anti-TNF-α (cat. no. 6945; Cell Signaling Technology, Inc.)/CCL5 (cat. no. 2988; Cell Signaling Technology, Inc.) /E-selectin (cat. no. 14-0627-82; Thermo Fisher Scientific, Inc., Waltham, MA, USA)/CXCR1 antibodies (1:1,500; cat. no. MA1-201; Thermo Fisher Scientific, Inc.) were used to treat THP-1 cells on 96-well plates at a final concentration of 3x10^4 cells/ml, and maintained at 4°C for 12 h. PBS supplemented with 4% bovine serum albumin (BSA, LI-COR Biosciences, Lincoln, NE, USA) was used to treat for another 60 min and PBS with 0.1% Tween-20 was used to wash the plates. A total of 50 µl purified TNF-α/CCL5/E-selectin/CXCR1 antibodies and clinical samples were distributed into 96 wells, incubated for 120 min at 37°C, and washed and cultured for 60 min at 37°C in 100 µl PBS, containing 1% BSA and rabbit monoclonal IgG anti-TNF-α (cat. no. 6945; Cell Signaling Technology, Inc.)/CCL5 (cat. no. 2988; Cell Signaling Technology, Inc.)/E-selectin (cat. no. 14-0627-82; Thermo Fisher Scientific, Inc.)/CXCR1 (cat. no. MA1-201; Thermo Fisher Scientific, Inc.) at a dilution of 1:1,500. HRP-conjugated secondary antibodies (1:3,000; cat. no. 7074; Cell Signaling Technology, Inc.) and 1% BSA (LI-COR Biosciences) were used to maintain the plate. A microplate reader was used to determine the HRP activity with the use of O-phenylenediamine based on the absorbance at 492 nm, and H₂O₂ served as the enzyme substrates. All tests were performed in triplicate.

Statistical analysis. SPSS 13.0 software (SPSS, Inc. Chicago, IL, USA) was used to perform all statistical analyses. All results are presented as the mean ± standard deviation. Unpaired t-tests and one-way analysis of variance followed by Mann-Whitney U test were used to analyze the significance comparisons among more than two groups. P<0.05 was considered to indicate a statistically significant difference.

**Results**

Lipopolysaccharide (LPS) alters the production of inflammatory mediators. LPS, a widely recognized stimulator of inflammation, was used in the present study to treat THP-1 cells. RT-qPCR was performed to determine whether LPS affected the expression of relevant inflammatory cytokines. In particular, the expression levels of DILC, IL-6, STAT3 and TLR4 in the cells treated with or without LPS was measured. As presented in Fig. 1A, the expression of DILC reached a stable state at 4 h following treatment with LPS; however, the levels of DILC expression were decreased in LPS-treated THP-1 cells compared with control cells. Conversely, the expression levels of IL-6 (Fig. 1B), STAT3 (Fig. 1C) and TLR4 (Fig. 1D) were increased in LPS-treated cells. Consistent with the observation that treatment with LPS upregulated the protein expression levels of STAT3 (19), these results suggested that LPS inhibited the expression of DILC and enhanced the expression of IL-6, STAT3 and TLR4.

Lnc-DILC binds to the promoter of IL-6 and suppresses the transcription of IL-6. As presented in Fig. 2A, a putative complementary binding site of Inc-DILC was located within the promoter region of IL-6. To further verify the regulatory association between Inc-DILC and the IL-6 promoter, an oligo mimic (oligoribonucleotide) and its mutated control (oligo mut), in addition to an oligodeoxynucleotide (ODN) decay and its mutated control (ODN mut), were synthesized based on the sequence of the binding locus (Fig. 2B). Subsequently, the luciferase activity of IL-6 was detected. As presented in Fig. 2C, the luciferase activity in cells transfected with the WT DILC vectors was markedly decreased compared with the control cells, whereas the IL-6 luciferase activity in cells transfected
with the mutant DILC vectors remained unchanged, indicating that lnc-DILC bound to the promoter of IL-6 and suppressed its production.

**Lnc-DILC alters the expression of IL-6, STAT3 and TLR4 in LPS-treated THP-1 cells.** To further understand whether DILC affects the productions of inflammatory cytokines involved in sepsis, LPS-treated THP-1 cells were transfected with WT or MUT DILC vectors, and the mRNA expression level of IL-6, STAT3 and TLR4 in these cells was measured using RT-qPCR and western blot analyses. Transfection with WT DILC significantly reduced the mRNA levels of IL-6 and TLR4, whereas transfection with mutant DILC did not alter the mRNA expression levels of IL-6 and TLR4 (Fig. 3). In addition, it was observed that the WT and MUT DILC vectors demonstrated a minimal effect on the mRNA expression levels of STAT3 (Fig. 3). Furthermore, the protein expression levels of IL-6, STAT3, phosphorylated (p)-STAT3 and TLR4 in these cells was measured and it was observed that transfection with WT DILC reduced the protein expression levels of IL-6, STAT3, p-STAT3 and TLR4, whereas transfection with MUT DILC did not alter the protein expression levels of these genes (Fig. 3).

**Lnc-DILC alters the levels of TNF-α, CCL5, E-selectin and CXCR1 by regulating the expression of IL-6.** To investigate the effects of lnc-DILC on the expression of TNF-α, CCL5, E-selectin and CXCR1, THP-1 cells were stimulated with LPS and transfected with the WT or MUT DILC vectors in conjunction with DILC and IL-6 siRNAs. Subsequently, the expression levels of TNF-α, CCL5, E-selectin and CXCR1 within these cells was determined using RT-qPCR and western blot analyses. As presented in Fig. 5, the levels of TNF-α (Fig. 5A), CCL5 (Fig. 5B), E-selectin (Fig. 5C) and CXCR1 (Fig. 5D) in the cells transfected with the WT DILC and IL-6 siRNA were significantly reduced, whereas the levels of these cytokines were significantly increased following transfection DILC siRNA.

**Expression of sepsis-associated genes varies between septic and healthy groups.** In the present study, 36 subjects, including 18 patients with sepsis and 18 healthy control subjects, were recruited and the expression levels of DILC, IL-6, STAT3, TLR4, TNF-α, CCL5, E-selectin and CXCR1 in these subjects were measured. As presented in Fig. 6, the sepsis group exhibited decreased levels of DILC, and increased levels of IL-6 and TLR4, compared with the healthy group; however, the levels of STAT3 between the two groups exhibited no significant difference. Additionally, the levels of TNF-α (Fig. 7A), CCL5 (Fig. 7B), E-selectin (Fig. 7C) and CXCR1 (Fig. 7D) were higher in the sepsis group compared with the control group.

**Discussion**

The interaction between lnc-DILC and the promoter region of IL-6 was observed in the present study. In addition, the effects of lnc-DILC overexpression and lnc-DILC knockdown, which may affect LCSC expansion, STAT3 activation and IL-6 transcription, were eliminated by the ODN decoy and the oligo mimic of lnc-DILC, respectively, thus suggesting that the interaction between lnc-DILC and the promoter of IL-6 exerted an inhibitory effect on LCSC expansion by suppressing the IL-6/STAT3 axis (15). During the expansion of LCSCs, it
has been suggested that lnc-DILC serves as a bridge linking NF-κB signaling to the autocrine IL-6/STAT3 cascade (15). It has additionally been demonstrated that lnc-DILC may inhibit the expansion of LCSCs by suppressing the IL-6/STAT3 signaling pathway. Additionally, the elevated expression levels of IL-6 have been revealed to induce the expansion of CSCs by stimulating the expression of STAT3 in hepatocellular carcinoma, and colon, lung, breast and prostate cancers (20‑23). In addition, a study demonstrated the ability of STAT3 to enhance the expression of IL-6 in cancer cells (24). For example, the mRNA levels of IL-6 increased in the tumor tissues of gp130 mutant mice under abnormal stimulation with STAT3 (25). Ogura et al (26) reported that the expression levels of IL-6 and other cytokines may be lowered by suppressing the activity of STAT3 in fibroblasts and macrophages (26). In addition, a study suggested that treatment with STAT3-siRNA may

Figure 2. Lnc-DILC binds to the promoter of IL-6 and suppresses the transcription of IL-6. (A) Sequence and location of the postulated lnc-DILC binding locus in the IL-6 promoter. (B) Sequences of RNA oligoribonucleotides and DNA decoy (oligodeoxyonucleotides) used in the present study. (C) Luciferase activity in cells was markedly decreased following transfection with vectors containing wild-type DILC. *P<0.05. DILC, deleted in liver cancer 1; IL, interleukin; lnc, long non-coding; mut, mutant.

Figure 3. Plasmids containing DILC were transfected into cells treated with LPS. (A) IL-6 mRNA was under-expressed in cells transfected with DILC. (B) STAT3 mRNA expression levels were much lower in cell transfected with DILC. (C) TLR4 mRNA expression levels were decreased in cells transfected with DILC. DILC, deleted in liver cancer 1; IL, interleukin; mut, mutant; p, phosphorylated; STAT3, signal transducer and activator of transcription 3; TLR4, Toll-like receptor 4.
reduce the mRNA expression levels of vascular endothelial growth factor, IL-10 and IL-6 in human melanoma cells (27).

As a proinflammatory cytokine expressed by fibroblasts, endothelial cells and monocytes, IL-6 may activate T and
Figure 6. Expression of sepsis-associated genes including DILC, IL-6 mRNA, STAT3 and TLR4 vary between septic and healthy groups. (A) DILC was under-expressed in subjects with sepsis. (B) IL-6 mRNA was highly expressed in subjects with sepsis. (C) STAT3 expression levels in subjects with sepsis were not significantly different. (D) Subjects with sepsis exhibited higher expression levels of TLR4. *P<0.05. DILC, deleted in liver cancer 1; IL, interleukin; STAT3, signal transducer and activator of transcription 3; Toll-like receptor 4.

Figure 7. Expression of sepsis-associated genes including TNF-α, CCL5, E-selection mRNA, and CXCR1 vary between septic and healthy groups. (A) TNF-α was highly expressed in subjects with sepsis. (B) CCL5 expression levels in subjects with sepsis were increased. (C) E-selectin mRNA was highly expressed in subjects with sepsis. (D) Subjects with sepsis exhibited a higher level of CXCR1. *P<0.05. CCL5, chemokine ligand 5; CXCR1, C-X-C motif chemokine receptor 1; TNF-α, tumor necrosis factor-α.
B lymphocytes, and induce fever (28). A previous report demonstrated that IL-6 may serve a critical role in inflammatory responses against the invasion of microbes (29). For example, a previous study indicated that a high concentration of IL-6 was associated with higher mortality and risk of severe sepsis (30), whereas the critical role of the IL-6 signaling pathway in systemic inflammation has been observed within IL-6-deficient mice (31).

As pattern recognition receptors, TLRs serve important roles in the mediation of innate immunity so as to provide protection against endotoxins (32). It has been demonstrated that LPS-induced TLR4 activation may trigger the phosphorylation of STAT3 primarily via myeloid differentiation primary response gene MyD88 (MyD88), an adaptor protein of TLR4. In addition, the suppression of p38, a downstream target of the TLR4 signaling, may lead to a decreased level of p-STAT3 at the tyrosine site, while p38 suppression and TLR4 deficiency may inhibit stress-induced DNA-binding activity and the phosphorylation of STAT3. Collectively, these data suggested a connection between STAT3 and TLR4/p38 in chronic stress (33), and it appears that TLR4/p38/IL-10 activates STAT3 following chronic stress (33). Previous studies have revealed that the expression levels of TLR4 are increased in the monocytes of patients with sepsis and healthy subjects challenged with LPS (34,35). In addition, the stimulation of TLR4 may lead to the expression of anti- and proinflammatory factors by activating two distinct signaling pathways (36). In particular, TLR4 is a unique member of the TLRs due to its ability to mediate the Toll-interleukin receptor (TIR)-domain-containing-adapter-inducing interferon-β (TRIF) and MyD88-dependent pathways (37). Through TNF receptor-associated factor-6, interferon (IFN)-β and the TRIF-dependent signaling pathway are involved in the mediation of NF-κB activation (38). In addition, IFN-β has been reported to be involved in the regulation of late-stage hyper-inflammation in sepsis (39).

LPS is secreted by gram-negative bacteria and is a pathogen-associated molecular pattern. Once recognized by TLR4, LPS is able to activate a signaling cascade and lead to the upregulated expression of certain cytokines that are associated with the onset and progression of sepsis (40). The majority of sepsis cases (>50%) are triggered by gram-negative bacteria that are primarily recognized by TLR4 (1). For example, Tsujimoto et al (41) demonstrated that the serum levels of TLR4 protein increased markedly in patients with sepsis upon pathogen infection (41), and it has been suggested that the protein expression levels of TIR domain-containing adaptor protein and TLR4, rather than gene polymorphisms, may be associated with the severity of sepsis (42). LPS increased STAT3 mRNA expression, whereas DILC or IL-6 did not, suggesting that LPS did not increase STAT3 mRNA expression via the DILC/IL-6 signaling pathway (43).

In conclusion, the association between IncRNA DILC and sepsis was investigated; to the best of our knowledge, the present study was the first to demonstrate that DILC directly inhibited the expression of IL-6, which subsequently modulated TLR4-dependent inflammatory responses via STAT3. In addition, TLR4 may act as an important mediator of inflammatory responses in sepsis. Therefore, it was hypothesized in the present study that Inc-DILC may act as a potential prognostic marker of sepsis, and may additionally be considered to be a potential therapeutic target for the treatment of sepsis.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors’ contributions

WH planned the study, collected and analyzed the data, and prepared the manuscript; LH planned the study, collected and analyzed the data; MW collected, analyzed and interpreted the data, and prepared the manuscript; MF collected the literature and prepared the manuscript; and, HZ planned the study, organized the funding and prepared the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All data processing and sample collection were approved by the Ethics Committee of Guangdong Academy of Medical Sciences. The present study was performed according to the Declaration of Helsinki.

Patient consent for publication

Written informed consent was obtained from all patients.

Competing interests

The authors declare that they have no competing interests.

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