Biochemical and Biophysical Research Communications 388 (2009) 543–548

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

LRRC19, a novel member of the leucine-rich repeat protein family, activates NF-κB and induces expression of proinflammatory cytokines

Limin Chai [a], Lingyun Dai [a], Yongzhe Che [b], Jie Xu [a], Guojing Liu [b], Zhujun Zhang [a,*], Rongcun Yang [a,c,*]

[a] Department of Immunology, Nankai University School of Medicine, Nankai University, PR China
[b] Department of Anatomy, Nankai University School of Medicine, Nankai University, PR China
[c] Key Laboratory of Bioactive Materials, Ministry of Education, Nankai University, PR China

ARTICLE INFO

Article history:
Received 18 July 2009
Available online 11 August 2009

Keywords:
LRRC19
NF-κB
Leucine-rich repeat protein
Toll-like receptor ligands

ABSTRACT

We have identified a new functional transmembrane receptor, LRRC19 (leucine-rich repeat containing 19), that belongs to the LRR protein family. LRRC19's central core has four analogous LRR repeating modules in a juxtaposed array and a casein kinase (CK2) phosphorylation site in the cytoplasmic domain. LRRC19 mRNA was found in the kidney, spleen and intestine of adult mice using both RT-PCR and *in situ* hybridization. LRRC19 does not contain a cytoplasmic Toll/IL-1 receptor (TIR) domain but was able to activate NF-κB and induce production of proinflammatory cytokines. LRRC19 shares a close evolutionary relationship with multiple Toll-like receptors (TLRs), especially TLR3. Importantly, the TLR3 ligand, as well as other TLR ligands, significantly promoted the expression of proinflammatory cytokines and the activation of NF-κB by LRRC19. Thus, LRRC19 may play an important role in inducing innate immune responses in certain tissues such as the kidney.

Introduction

Leucine-rich repeats (LRRs) are widespread structural motifs of 20–30 amino acids with a characteristic repetitive sequence pattern rich in leucine residues. Proteins containing leucine-rich repeats (LRRs) form a continually expanding family that includes intracellular, extracellular and cell-surface members to allow detection of a diverse set of antigens [1]. LRR proteins, both intracellular and extracellular, have well-characterized functions in the innate immune system that are similar in diverse organisms from plants to mammals [2]. Dolan et al. [3] have recently catalogued all the extracellular LRR (eLRR) proteins in worms, flies, mice and humans, including a total of 369 proteins, among them LRR_Ig/Fn3, LRR_Tollkin, LRR_other, and LRR_only . These proteins are reported to participate in many biologically important processes with such varied functions as adhesion and invasion of host cells by pathogenic bacteria [4], disease resistance and pathogen recognition in plants [5], immune response [6,7], cell adhesion, and signaling [8] and extracellular matrix assembly [9], etc. However, the function and expression patterns of these unusual proteins have not yet been fully elucidated.

The field of innate immunity has largely been fueled by the molecular identification of critical receptors and signaling pathways involved in pathogen recognition. Five families of PRRs (pattern-recognition receptors), TLRs (Toll-like receptors), NLRs (nucleotide binding and oligomerization domain-like receptors), RLRs (retinoic acid-inducible gene-1-like receptors) and CLRs (C-type lectin receptors) have been identified as important components of innate immunity, participating in the sensory system for host defense against the invasion of infectious agents [10]. Because the receptors in the Toll receptor superfamily are similar in their cytoplasmic domains, they share some signaling components, leading to the activation of NF-κB and production of inflammatory cytokines. These proteins are characterized by a common molecular architecture primed for protein–protein interactions [11,12]. The structure of these proteins also shows similarities, forming a curved shape with a parallel beta-sheet on the concave side and helical elements primarily on the convex side [11,12].

In this study, we identify a novel functional transmembrane receptor LRRC19 (leucine-rich repeat containing 19) that may be involved in innate immune responses. LRRC19 is a member of the LRR_only group [3], having a close evolutionary relationship with Toll-like receptors (TLRs). Although LRRC19 contains no cytoplasmic Toll/IL-1 receptor (TIR) domain, it can activate NF-κB and induce the expression of proinflammatory cytokines with or without the stimulus of Toll-like receptor ligands. This protein appears to be specifically expressed in the kidney, spleen and intestine. Given the fact that this transmembrane receptor is recognized by

* Corresponding authors. Address: Department of Immunology, Nankai University, School of Medicine, Weijin Road 94#, Nankai District, Tianjin 300071, PR China. Fax: +86 22 23502554.
E-mail addresses: zhang_zhujun@nankai.edu.cn (Z. Zhang), ryang@nankai.edu.cn (R. Yang).

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.08.043

multiple Toll-like receptor ligands, LRRC19 may play an important role in mediating innate immune responses for tissues such as kidney by functioning as a transmembrane receptor.

Materials and methods

Cell culture. HEK293T and RAW264.7 cell lines were purchased from ATCC and maintained in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal bovine serum (Sigma), 100 U/ml penicillin and 100 μg/ml streptomycin in a 5% CO_2 atmosphere at 37 °C.

Molecular cloning of mouse and human LRRC19 cDNA as well as LRRC19Δ. The cDNAs containing the coding sequence of LRRC19 from mouse kidney and biopsy tissue of human kidney were isolated by RT-PCR. The primers for mouse LRRC19 were as follows: forward primer, 5′-ATGAAAGTCACACGCTTCATGTTTTGGC-3′ and reverse primer, 5′-CTTTTCTTCATGTACCTCATTGATATCT-3′. The primers for human LRRC19 (HLRRC19) were as follows: forward primer, 5′-ATGAAAGTCACAGGCATCACAATCC-3′ and reverse primer, 5′-ATTTTCTTCACATAATTCATGGATA-3′. The primers for LRRC19Δ (LRRC19 absent of extracellular region) were as follows: forward primer, 5′-ATGAAATGCCCAATATGGTAC-3′ and reverse primer, 5′-ATTTTCTTCACATAATTCATGGATA-3′. The PCR products were then ligated into pcDNA3.1-V5 vector (Invitrogen) and sequenced at Invitrogen Company. The DNA sequence was 100% identical with the one deposited in the public source (NCBI reference Sequence No. NM_175305.4 or NM_022901).

RT-PCR analysis. To investigate the expression of LRRC19 in different tissues of adult mice, total RNAs were extracted from kidney, spleen, intestine, liver, and heart. Mouse LRRC19 cDNA was amplified by RT-PCR, using the primers described above. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control. The primers for GAPDH were as follows: forward primer, 5′-GTGGCAAAGTGGAGATTGTTG-3′ and reverse primer, 5′-CAGTCTTCTGGGTGGCAGTGAT-3′. The PCR conditions were as follows: 95 °C for 5 min, 35 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s. PCR products were visualized on 1.0% (wt/vol) agarose gel.

Transfection and reporter assays. PcDNA3.1-V5-LRRC19 (mouse or human) and pNF-κB-SEAP (secreted alkaline phosphatase) (BD, Clontech) were co-transfected HEK293T cells with Lipofectamine 2000 (Invitrogen) in 24-well plates for SEAP assay. The pcDNA3.1 empty vector was used as a negative control. After 24 h, the cells were stimulated using 2 μg/ml peptidoglycan (PGN), 1 μg/ml synthetic bacterial lipoprotein (PAM3CSK4), 10 μg/ml poly I: C, 100 ng/ml lipopolysaccharide (LPS), 10 ng/ml flagellin or 3 μg/ml bacterial DNA (B.DNA). The supernatants were collected and analyzed by chemiluminescence secreted alkaline phosphatase assay according to the manufacturer's protocol (BD Clontech).

Immunofluorescence. RAW264.7 cells were transfected with pcDNA3.1-V5-LRRC19 (mouse), rinsed twice with PBS and then fixed for 5 min in 4% paraformaldehyde at 4 °C. Fixed cells were blocked in 10% goat serum in PBS for 10 min at room temperature and then incubated with anti-V5-FITC antibody. Images were collected by confocal microscopy (Fluoview FV300, Olympus Optical Corp.).

In situ hybridization. Tissues of mice were formaldehyde-fixed and paraffin-embedded according to standard procedures. Briefly, 5-μm sections were deparaffinized, rehydrated through a graded series of ethanol, and washed with water treated with 0.1% DEPC three times for 5 min. *In situ* hybridization of LRRC19 was done according to the manufacturer's protocol (TBD sci). Staining was completed with the Diaminobenzidine Staining Kit. The probes (probe 1: 5′-AGCTGTGGTGCATCGAAGAGGCGTATC-3′, probe 2: 5′-TGATAGCAATGAAGATAAGCAGTGAAGTT-3′, probe 3: 5′-CAGGCATGGTGGTTGTAACTCAGCAG-3′) were designed based on the published genomic sequence of mouse LRRC19 mRNA (GenBank NM.022901) and labeled with Digoxin (DIG). These probes recognized sequences conserved in the mouse LRRC19 mRNA sequence. The sense probes were used as negative controls.

ELISA. The culture supernatants were harvested after stimulation. A commercial sandwich enzyme-linked immunosorbent assay (ELISA) kit was used for quantitation of IL-8 (Pierce Endogen). Cytokine levels were quantified from two to three titrations with standard curves and expressed as the number of picograms per milliliter.

Results

LRRC19 gene and protein structure

Using either SMART or Pfam with several transmembrane-prediction and motif-detection programs, we identified a novel transmembrane LRRC19 protein. Consistent with the prediction by Dolan et al. [3], LRRC19 contains four leucine-rich repeats, which were numbered sequentially and arranged in tandem array in the extracellular domain as shown in Fig. 1B. An open reading frame of 1095 bp encoded a protein of 364 amino acids. The first N-terminal 20 amino acids constitute a cleavable signal peptide as predicted by signal P and SP-HMM [13,14]. Thus, the mature protein has 344 aa and a calculated molecular mass of 41.48 kDa. Notably, LRRC19 has a putative protein kinase casein kinase2 (CK2) phosphorylation site (Fig. 1B). A single-span transmembrane domain was defined by Tmpred [15] between A265 and I285. LRRC 19 was shown to be a membrane protein by ectopic expression in RAW264.7 cells after transfection with the murine LRRC19 expression plasmid pcDNA3.1-V5-LRRC19 (Fig. 1C). In addition, similar to other members of the LRR family, the entire LRRC19 cDNA has multiple exons separated by 1.6-kb, 4.9-kb, 2.3-kb, 1.1-kb or 650-bp introns respectively (not shown), supporting replication slippage and exon shuffling mechanisms in LRR protein evolution [16,17]. LRRC19 is highly conserved across species (Fig. S1), suggesting that it mediates an important function [18].

Expression of LRRC19 gene in different tissues and organs

Next we searched the tissue distribution of LRRC19 based on the expressed sequence tags (EST) data base sequences. According to computational information, LRRC19 mRNA was expressed in the kidney (EST Sequence No. BY369353.1), spleen (EST Sequence No. AI178908.1) and intestine (EST Sequence No. CK382141.1) (Fig. 2A). To show the expression pattern of LRRC19, we first detected the levels of LRRC19 mRNA in different tissues and organs from C57BL6 and BALB/c mice. As shown in Fig. 2B, LRRC19 was strongly expressed in the kidney, suggesting that LRRC19 might play a specific role in this organ. However, in the spleen, a tissue that expresses abundant levels of other TLRs, LRRC19 was also expressed, although at a lower level, implying a possible role for this receptor in the spleen. Additionally, LRRC19 could be detected in intestinal tissue. The control band, amplified using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) specific primers, did not reveal differences in these tissues.

Using LRRC19 mRNA specific probes and *in situ* hybridization, we further examined the distribution and localization of LRRC19 mRNA in adult mouse tissues of C57BL/6 and BALB/C mice. As shown in Fig. 2C, LRRC19 mRNA was mainly expressed in renal tubular epithelial cells in the kidney of C57BL/6. The splenic cords and the germinal center of the spleen also stained positively (Fig. 2C and not shown). In addition, LRRC19 mRNA specific probe activities were also observed in the small intestine lamina propria and epithelium mucosae cells, as seen in Fig. 2C. Control incuba-

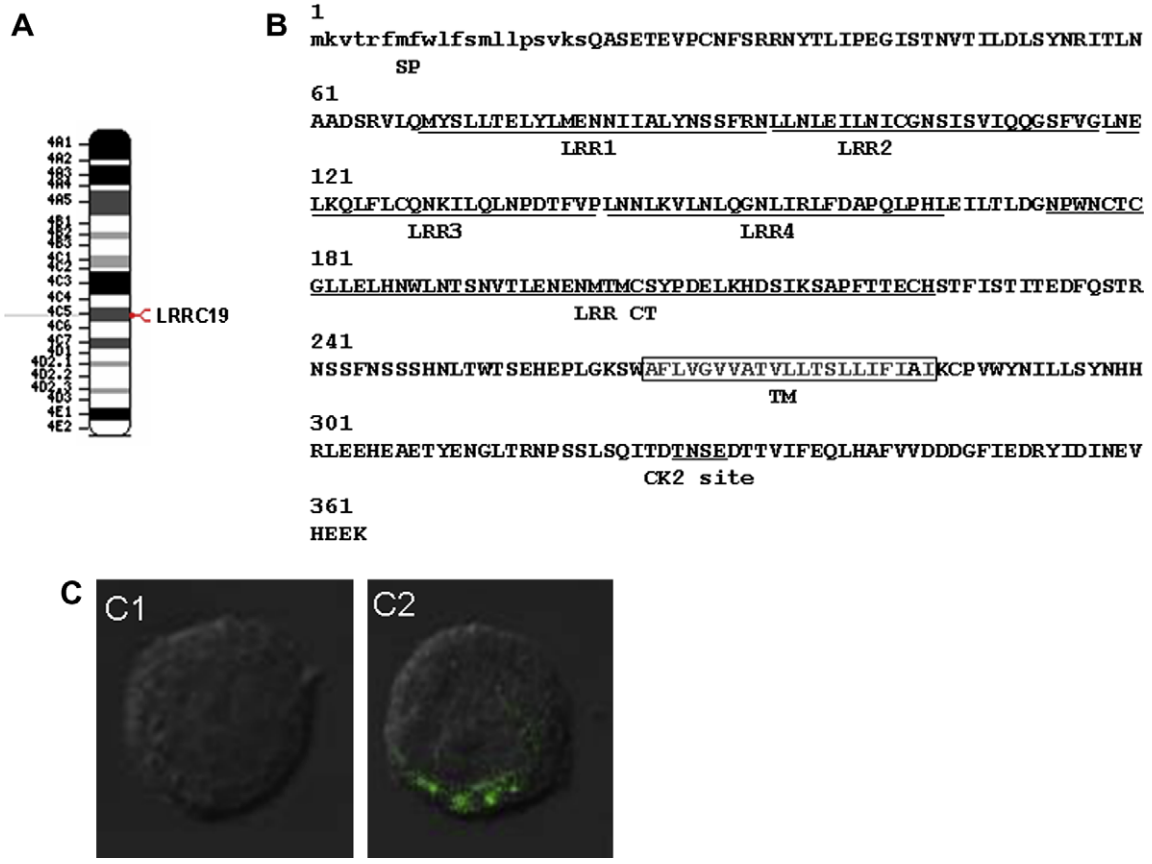

Fig. 1. Leucine-rich repeat containing (LRRC19) gene and protein structure. (A) The ideogram of mouse chromosome 4 with the LRRC 19 gene C5 (94303344–94316835 bp) position. (B) A schematic diagram of LRRC19 protein. The signal peptide is in lowercase. LRRs are numbered sequentially from 1 to 4 as indicated and underlined. The transmembrane domain is outlined by paled letters. The putative protein kinase CK2 phosphorylation site is also marked with underlined letters. SP, signal peptide; LRRs, leucine-rich repeats; LRR CT, LRR C-terminal domain; and TM, transmembrane domain. (C) Demonstration of LRRC19 as a transmembrane protein receptor. Raw264.7 cells were transfected with pcDNA3.1-V5-LRRC19. Cells were stained by FITC labeled anti-V5 antibody. Images were collected by confocal microscopy (400×) (C2). Image collected by bright-light was used as control (C1).

tions with the LRRC19 sense probe were negative (not shown). However, we failed to detect LRRC19 in heart and lung, which were not significantly stained. Similar results were found in BALB/C mice. Thus, our results suggest that LRRC19 is expressed in the kidney, spleen, and intestine.

Relationship of LRRC19 with leucine-rich repeat protein superfamily members

To understand the relationship of LRRC19 to the members of the leucine-rich repeat protein superfamily, a phylogenetic tree analysis of LRRC19 together with the known LRR members was performed using the ClustalW and ClustalX programs [3] (Fig. 3A). The mouse protein sequences used in this study were obtained from the public database deposited as NCBI reference sequences. As presented in the phenogram (Fig. 3A and [19]), Class-I, II, III, IV, and V extracellular matrix and TLRs occupy different branches of the phylogenetic tree. LRRC19 has the closest distance to Toll-like receptor 3 (Fig. 3A and Fig. S2). The sequence of LRRC19 is also more closely related to that of Toll-like receptors 4, 11, and 12 (Fig. 3A and Fig. S2). Tertiary structure analysis showed a higher similarity of LRRC19 with Toll-like receptors 3, 4 and 11 than with TLR6 (Fig. 3B). However, the cytoplasmic domain of LRRC19 has no apparent homology to TLRs, which have an intracytoplasmic signaling domain TIR, highly conserved across the TLRs as well as in the receptors for IL-1 and IL-18, suggesting that LRRC19 was a member of the LRR superfamily independently of TLRs. In addition, as shown in Fig. 3B, LRRC19 is similar to recent structural studies on LRR proteins, supporting the characteristic horseshoe structure of LRR proteins [20,21].

Ectopic LRRC19 activates NF-κB and induces expression of proinflammatory cytokines

Although LRRC19 lacks the intracytoplasmic signaling domain TIR, it has a CK2 (casein kinase2) phosphorylation site (Fig. 1B) that is involved in the induction of NF-κB via a mechanism dependent on phosphorylation of p65 at both Ser536 and Ser276 sites [22]. We therefore hypothesized that LRRC19 may be involved in the activation of NF-κB. To analyze the functional potential of LRRC19, HEK293T cells that lacked the expression of endogenous LRRC19 were used to investigate whether LRRC19 could activate NF-κB. Prior work has shown that ectopic expression of Toll-like receptors in HEK293T cells permits reconstitution of signaling induced by their ligands [23,24]. Our results show that LRRC19 caused the activation of signal transduction, as detected by NF-κB activity (Fig. 4B), even in the absence of TLR ligands. To eliminate the effect of the extracellular portion of LRRC19, we deleted the LRRC19 extracellular region (LRRC19Δ) and then tested the effect on NF-κB activity. As shown in Fig. 4, transfection of LRRC19Δ also activated NF-κB. Thus, our results suggest a new mechanism of signaling, independent of the intracytoplasmic domain TIR, by which LRRC19 can activate NF-κB, a transcription factor required for proinflammatory signaling.

A

Tissue Name	Transcripts per million	Gene EST/Total EST
Kidney	346	43/124081
Spleen	31	3/95037
Intestine	34	3/85879
Lung	0	0/99429
Heart	0	0/53750

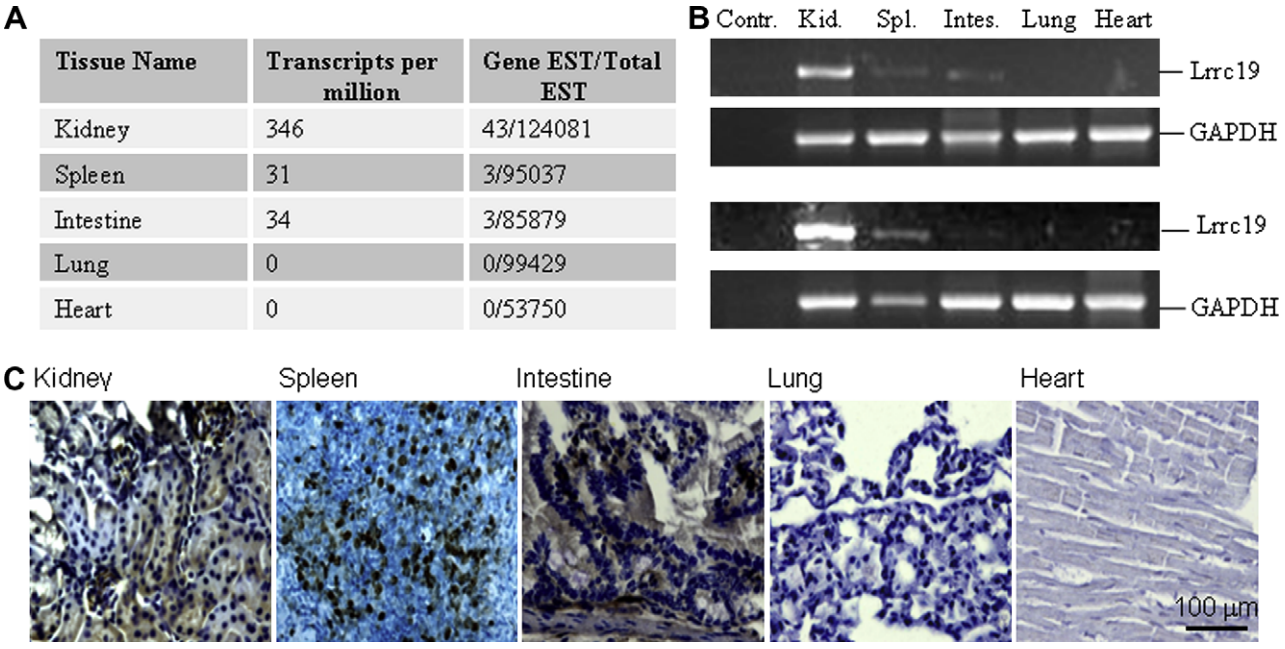

Fig. 2. Tissue distribution of LRRC19. (A) LRRC19 tissue distribution based on the expressed sequence tags (EST) data base sequences. According to computational information, LRRC19 mRNA was expressed in kidney, spleen and intestine but not in heart and liver. (B) RT-PCR analysis to determine the expression pattern of LRRC19 mRNA in organs of C57BL/6 (upper panel) and BALB/c (lower panel) mice. LRRC19 is predominantly expressed in the kidney, with lower levels of expression in spleen and intestine. GAPDH probe was used as a control for DNA loading. The PCR products were analyzed on 1.0% TBE–agarose gel and photographed under UV light. (C) Localization of LRRC19 mRNA in tissues of C57BL/6 mice by *in situ* hybridization. Expression of LRRC19 is detected by incubation with the anti-sense probe of LRRC19 in kidney, spleen and intestine but not in lung and heart. The specimens were observed and photographed under a light microscope (Leica, DM RXA2) at magnification of 400×.

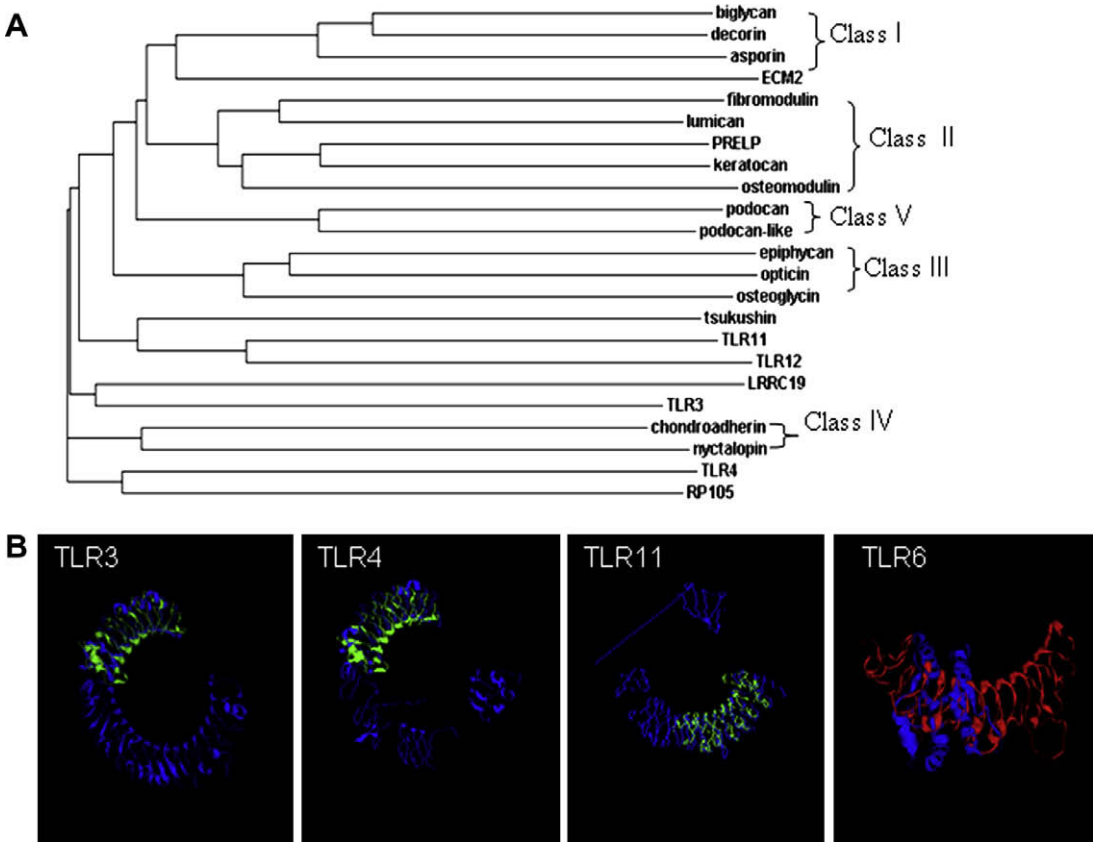

Fig. 3. (A) Phylogenetic tree analysis of LRRC19 together with other mouse LRR members. The LRRC19 is closely related to that of Toll-like receptor 3, 4, 11, and 12. Notably, TLR3, 4, 11, 12, tsukushin and RP105 are surface receptors. (B) Comparison of the tertiary structure of mouse TLR3, TLR4, TLR6 or TLR11 to the predicted tertiary structure of LRRC19 (composed using PyMOL Molecular Graphics System, http://www.pymol.org). The tertiary structure of LRRC19 (green structure in TLR3, TLR4, TLR11 and red structure in TLR6) was closer to TLR3, TLR4 and TLR11 than to TLR6 (blue structure in TLR3, TLR4, TLR11 and TLR6). (For interpretation of the references in colour in this figure legend, the reader is referred to the web version of this article.)

LRRC19 can be recognized by different kinds of Toll-like receptor ligands

Given the homology of LRRC19 to TLRs, we tested the cellular function of LRRC19 in response to different kinds of TLR ligands, such as peptidoglycan (PGN), synthetic bacterial lipoprotein (PAM3CSK4), poly I:C and lipopolysaccharide (LPS), flagellin (FAL) and bacterial DNA (B. DNA) by examining the activity of NF-κB and the expression of the chemokine IL-8. As expected, all of these TLR ligands effectively promote NF-κB activation by LRRC19 (Fig. 4B). Conversely, human LRRC19 lacking the extracellular region (LRRC19□) did not significantly upregulate the activity of NF-κB in response to different TLR ligands. Interestingly, LRRC19-transfected HEK293T cells also produce higher levels of IL-8 upon exposure to these TLR ligands (Fig. 4C), thus confirming our data showing that LRRC19 can be recognized by multiple TLRs.

Discussion

The LRR proteins are known to participate in many biologically important processes, such as cell adhesion and signaling [9,25,26], adhesion and invasion of host cells by pathogenic bacteria [4,5], disease resistance and pathogen recognition in plants [27–29] and the immune response [1,6,7,30,31]. Here, we have reported the identification and characterization of a new member of the LRR family, LRRC19. LRRC19 can activate NF-κB and induce the production of proinflammatory cytokines. Importantly, this protein can be recognized by most TLR ligands to promote NF-κB activity and induce expression of the IL-8 cytokine, suggesting that LRRC19 may be involved in innate immune responses.

TLRs are characterized structurally by an extracellular leucine-rich repeat (LRR) domain, a conserved pattern of juxtamembrane cysteine residues, and an intracytoplasmic signaling domain Toll/IL-1 resistance (TIR) that is highly conserved across the TLRs as well as in the receptors for IL-1 and IL-18 [32]. Similar to TLRs, LRRC19 has a conserved extracellular LRR domain and a TLR-like pattern of juxtamembrane cysteines. Unlike the TLRs, however, LRRC19 lacks a TIR domain, but contains 79 intracytoplasmic amino acids and has a protein kinase CK2 phosphorylation site in the cytoplasmic domain, which was found to be involved in the signal transduction pathway mediated by CK2. CK2 is a highly conserved tetrameric serine/threonine kinase present in all eukaryotic organisms. CK2 has been postulated to control the function of many proteins through changes in phosphorylation that affect protein stability, protein–protein interactions, and subcellular localization, thus regulating many fundamental cellular properties [33,34]. CK2 is indeed involved in the synergistic induction of NF-κB via a mechanism dependent on phosphorylation of p65 at both Ser536 and Ser276 sites. Toll-like receptor ligands could therefore activate the NF-κB transcription factor through a protein kinase CK2 phosphorylation site in the LRRC19 cytoplasmic domain.

We demonstrate that LRRC19 is predominantly expressed in the kidney, spleen and intestine. Renal tubular cells play an important regulatory role in immune-mediated diseases, with an active participation in the response to local inflammation. The spleen, including T cells, B cells and plasma cells, macrophages and dendritic cells, is an important organ in regulating different immune responses. The intestinal epithelial cell barrier also plays an important role in maintaining mucosal immune homeostasis. Our findings showing that LRRC19 responds to multiple natural ligands such as LPS, PGN, etc., and is expressed in the kidney, spleen, and intestine suggest that LRRC19 may be involved in the response to certain pathogenic microorganisms that cause infection.

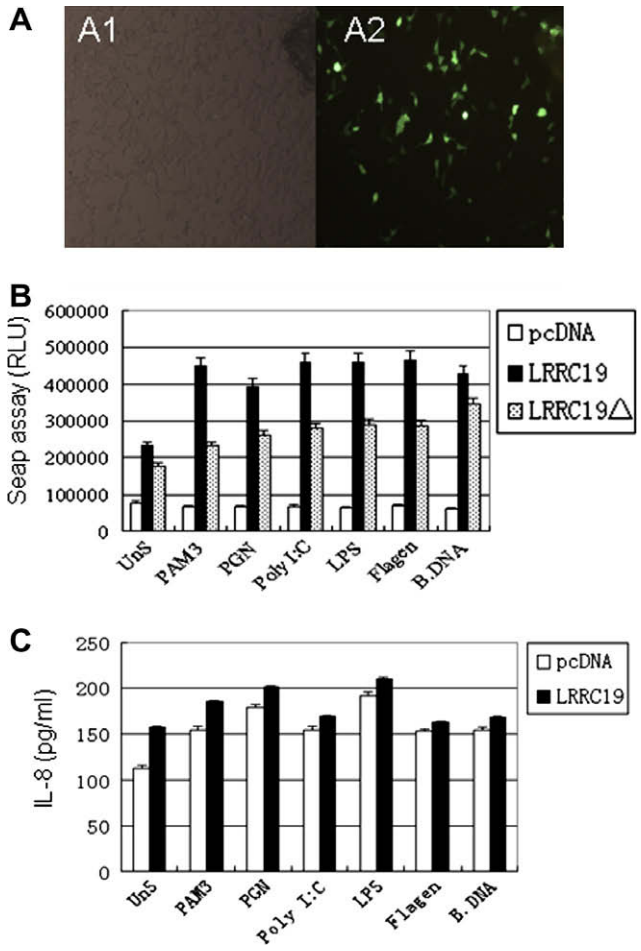

Fig. 4. LRRC19 induces activation of NF-κB and endogenous cytokine production. (A) Transfection of 293T cells with LRRC19 expression plasmids. 293T cells were transfected with pcDNA3.1-V5-LRRC19. After 24 h, cells were incubated with anti-V5-FITC antibody for 1.5 h at room temperature. Images were collected by confocal microscopy (400×) (A2). Image collected by bright-light was used as control (A1). (B) Multiple Toll-like receptor ligands activated NF-B through LRRC 19 receptors. 293T cells were co-transfected with pNF-κB-SEAP (secreted alkaline phosphatase) and the expression plasmids of LRRC19 or LRRC19Δ by Lipofectamine 2000 (Invitrogen). Alkaline phosphatase activity in the supernatants was measured following treatment with PAM3CSK4 (PAM3), PGN, LPS, polyI:C, flagellin, bacterium DNA (B. DNA), or untreated (Uns) cells. The empty expression vector (pcDNA) was used as control. NF-B induced alkaline phosphatase activities in the supernatant were measured by the chemiluminescence secreted alkaline phosphatase assay according to the manufacturer's protocol. RLU, relative light units. (C) TLR ligands induced the release of IL-8 by LRRC19 receptors. 293T cells were transfected with the expression plasmids of LRRC19, and then stimulated by different TLR ligands according to the protocol described in materials and methods. IL-8 secretion was detected by ELISA kit according to the manufacturer's protocol (Jingmei Corp.).

Disclosures

The authors have no financial conflict of interest.

Acknowledgments

This research was supported by NSFC Grant 30771967, 30872315; The Ministry of Science and Technology grants 06C26211200695, 2008AA02Z129 and 2006AA020502, and Tianjin Municipal Science and Technology Commission grants 07JCZDJC03300 and 06ZHCXSH04800.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.08.043.

References

[1] A.P. West, A.A. Koblansky, S. Ghosh, Recognition and signaling by toll-like receptors, Annu. Rev. Cell Dev. Biol. 22 (2006) 409–437.
[2] J. Bella, K.L. Hindle, P.A. McEwan, S.C. Lovell, The leucine-rich repeat structure, Cell. Mol. Life Sci. 65 (2008) 2307–2333.
[3] J. Dolan, K. Walshe, S. Alsbury, K. Hokamp, S. O'Keeffe, T. Okafuji, S.F. Miller, G. Tear, K.J. Mitchell, The extracellular leucine-rich repeat superfamily: a comparative survey and analysis of evolutionary relationships and expression patterns, BMC Genomics 8 (2007) 320.
[4] H.H. Niemann, W.D. Schubert, D.W. Heinz, Adhesins and invasins of pathogenic bacteria: a structural view, Microbes Infect. 6 (2004) 101–112.
[5] H. Bierne, C. Sabet, N. Personnic, P. Cossart, Internalins: a complex family of leucine-rich repeat-containing proteins in Listeria monocytogenes, Microbes Infect. 9 (2007) 1156–1166.
[6] . I.B. Rogozin, L.M. Iyer, L. Liang, G.V. Glazko, V.G. Liston, Y.I. Pavlov, L. Aravind, Z. Pancer, Evolution and diversification of lamprey antigen receptors: evidence for involvement of an AID-APOBEC familycytosine deaminase, Nat. Immunol. 8 (2007) 647–656.
[7] N.J. Gay, M. Gangloff, Structure and function of Toll receptors and their ligands, Annu. Rev. Biochem. 76 (2007) 141–165.
[8] J. Bellaa, K.L. Hindlea, S.C. Lovella, The leucine-rich repeat structure, Cell. Mol. Life Sci. 10 (2008) 1–27.
[9] E. Hohenester, S. Hussain, J.A. Howitt, Interaction of the guidance molecule Slit with cellular receptors, Biochem. Soc. Trans. 34 (2006) 418–421.
[10] E.M. Pålsson-McDermott, L.A. O'Neill, Building an immune system from nine domains, Biochem. Soc. Trans. 35 (2007) 1437–1444.
[11] S.G. Buchanan, N.J. Gay, Structural and functional diversity in the leucine-rich repeat family of proteins, Prog. Biophys. Mol. Biol. 65 (1996) 1–44.
[12] B. Kobe, A.V. Kajava, The leucine-rich repeat as a protein recognition motif, Curr. Opin. Struct. Biol. 11 (2001) 725–732.
[13] S. Barash, W. Wang, Y. Shi, Human secretory signal peptide description by hidden Markov model and generation of a strong artificial signal peptide for secreted protein expression, Biochem. Biophys. Res. Commun. 294 (2002) 835–842.
[14] S.R. Eddy, Profile hidden Markov models, Bioinformatics 14 (1998) 755–763.
[15] B. Persson, P. Argos, Prediction of transmembrane segments in proteins utilising multiple sequence alignments, J. Mol. Biol. 237 (1994) 182–192.
[16] W. Wang, Y. Yang, L. Li, Y. Shai, Synleurin, a novel leucine-rich repeat protein that increases the intensity of pleiotropic cytokine responses, Biochem. Biophys. Res. Commun. 305 (2003) 981–988.
[17] E.D. Loh, S.R. Broussard, L.F. Kolakowski, Molecular characterization of a novel glycoprotein hormone G-protein-coupled receptor, Biochem. Biophys. Res. Commun. 282 (2001) 757–764.
[18] R. Roubin, S. Pizette, V. Ollendorff, J. Planche, D. Birnbaum, O. Delapeyriere, Structure and developmental expression of mouse Garp, a gene encoding a new leucine-rich repeat-containing protein, Int. J. Dev. Biol. 40 (1996) 545–555.
[19] Y. Mochida, D. Parisuthiman, M. Kaku, J. Hanai, V.P. Sukhatme, M. Yamauchi, Nephrocan, a novel member of the small leucine-rich repeat protein family, is an inhibitor of transforming growth factor-β signaling, J. Biol. Chem. 281 (2006) 36044–36051.
[20] I.T. Weber, R.W. Harrison, R.V. Iozzo, Model structure of decorin and implications for collagen fibrillogenesis, J. Biol. Chem. 271 (1996) 31767–31770.
[21] J.E. Scott, Proteodermatan and proteokeratan sulfate (decorin, lumican/fibromodulin) proteins are horseshoe shaped. Implications for their interactions with collagen, Biochemistry 35 (1996) 8795–8799.
[22] S.M. Kweon, B. Wang, D. Rixter, J.H. Lim, T. Koga, H. Ishinaga, L.F. Chen, H. Jono, H. Xu, J.D. Li, Synergistic activation of NF-kappaB by nontypeable H. influenzae and S. pneumoniae is mediated by CK2, IKK beta-I kappa B alpha, and p38 MAPK, Biochem. Biophys. Res. Commun. 351 (2006) 368–375.
[23] E. Latz, A. Visintin, E. Lien, K.A. Fitzgerald, K.A. Fitzgerald, B.G. Monks, E.A. Kurt-Jones, D.T. Golenbock, T. Espevik, Lipopolysaccharide rapidly traffics to and from the Golgi apparatus with the toll-like receptor 4-MD-2-CD14 complex in a process that is distinct from the initiation of signal transduction, J. Biol. Chem. 277 (2002) 47834–47843.
[24] E.A. Kurt-Jones, L. Popova, L. Kwinn, L.M. Haynes, L.P. Jones, R.A. Tripp, E.E. Walsh, M.W. Freeman, D.T. Golenbock, L.J. Anderson, R.W. Finberg, Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus, Nat. Immunol. 1 (2000) 398–401.
[25] H. Kresse, E. Schönherr, Proteoglycans of the extracellular matrix and growth control, J. Cell. Physiol. 189 (2001) 266–274.
[26] A. Matilla, M. Radrizzani, The Anp32 family of proteins containing leucine-rich repeats, Cerebellum 4 (2005) 7–18.
[27] B.J. DeYoung, R.W. Innes, Plant NBS-LRR proteins in pathogen sensing and host defense, Nat. Immunol. 7 (2006) 1243–1249.
[28] L. McHale, X. Tan, P. Koehl, R.W. Michelmore, Plant NBS-LRR proteins: adaptable guards, Genome Biol. 7 (2006) 212.
[29] C.A. Ryan, A. Huffaker, Y. Yamaguchi, New insights into innate immunity in *Arabidopsis*, Cell. Microbiol. 9 (2007) 1902–1908.
[30] F. Martinon, J. Tschopp, NLRs join TLRs as innate sensors of pathogens, Trends Immunol. 26 (2005) 447–454.
[31] Z. Pancer, M.D. Cooper, The evolution of adaptive immunity, Annu. Rev. Immunol. 24 (2006) 497–518.
[32] K. Takeda, T. Kaisho, S. Akira, Toll-like receptors, Annu. Rev. Immunol. 21 (2003) 335–376.
[33] J.D. Shaul, A. Farina, T. Huxford, The human IKKbeta subunit kinase domain displays CK2-like phosphorylation specificity, Biochem. Biophys. Res. Commun. 374 (2008) 592–597.
[34] R.O. Escárcega, S. Fuentes-Alexandro, M. García-Carrasco, A. Gatica, A. Zamora, The transcription factor nuclear factor-kappa B and cancer, Clin. Oncol. 19 (2007) 154–161.