Yonsei Medical Journal
Vol. 45, No. 2, pp. 359 - 361, 2004

Brief Communication

Cloning of TLR3 Isoform

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Toll-like receptor (TLR) 3 is a member of the TLR family that confers innate immunity by recognizing viral pathogens. Herein, we report that the TLR3 isoform is expressed on human primary cells and cell lines. This isoform has 2,520 bp cDNAs compared to the 2,712 bp of full cDNA, is produced by deletion of an intron-like sequence within exon 4 and is co-expressed with wild type TLR3 in primary human astrocytes and glioblastoma cell lines. This finding suggests the TLR3 isoform in astrocytes may have a different immunological role for binding ligands during the immune response in brain.

Key Words: Human TLR3, isoform

Toll-like receptors (TLRs) have been shown to play important roles in the recognition of bacterial components. Until now, ten TLR members have been reported in humans.[1] Among these ten TLRs, TLR4 mediates LPS signaling and TLR2 is an essential receptor for other bacterial components: lipoprotein, peptidoglycan and lipoteichoic acid.1 It has been reported that TLR6 forms a heterodimer with TLR2, which mediates the responsiveness to PGNs and zymosan, but not lipoproteins.[2] TLR5[3] and TLR9[4] have recently been shown to mediate signals from flagella and bacterial DNA, respectively. However, TLR3 is known to mediate signaling by dsRNA,[5] which suggests that TLR3 may be responsible for viral infection, whereas other TLRs are mainly responsible for bacterial products.

Among human cells, immature dendritic cells[6] and intestinal epithelial cells[7] have been reported to express TLR3. Its expression in other blood cells, including monocytes, granulocytes, NK cells, T cells and B cells and macrophages, has not been detected at the transcriptional level.[8] Cloning of TLR3 cDNA has showed a 3,029 bp (904 aa ORF) product composed of 5 exons.[9]

Recently, TLR3 cDNA was cloned in our laboratory from LN215 cells, a human glioblastoma cell line, and deletion of part of the exon 4 of this isoform was identified. The alternative splicing in exon 4 may influence the ligand binding, as this exon translates the extracellular regions. Therefore, sequences of TLR3 cDNA was compared between primary astrocytes and astrocytoma cell lines (LN215 and A172).

LN215 and A172, glioblastoma cell lines, were cultured in 10% FCS (Gibco BRL, Grand Island, NY)-DMEM (Gibco BRL), containing 1% nonessential amino acids (Sigma, St. Louis, MO, USA). The LN215 cell line was kindly provided by Dr. E. G. Van Meir (Department of Neurosurgery, Laboratory of Tumor Biology and Genetics, Lausanne, Switzerland). Fetal astrocytes were isolated from a 25-week-old human fetus. Astrocytes were cultured in 10% FCS (Gibco BRL, Grand Island, NY, USA)-DMEM (Gibco BRL, Grand Island, NY, USA), containing 1% nonessential amino acids (Sigma, St. Louis, MO, USA).

Total RNA was isolated using an RNeasy kit (Qiagen, Santa Claris, CA, USA). The level of TLR3 mRNA was measured by RT-PCR. Total RNA, 1 μg, was used to synthesize cDNA with 0.1

Received March 31, 2004
Accepted April 12, 2004

This work was supported by research grant from KOSEF (RO1-2002-000-00210-0 (2002)).

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O.D. of random hexamer (Pharmacia, Uppsala, Sweden) and 200 U of M-MLV reverse transcriptase (Gibco BRL, Grand Island, NY, USA). The TLR3 primers used were; forward: 5′-GAT CTG TCT CAT AAT GGC TTG TCA-3′ (456 - 479) and reverse: 5′-GTT TAT CAA TCC TGT GAA CAT AT-3′ (1,140 - 1,117). The PCR conditions were as follows: denaturation at 94℃ for 30s, annealing at 52℃ for 30s and extension at 72℃ for 30s. The PCR buffer contained 10 mM Tris-HCl (pH 10), 2.0 mM $MgCl_2$ and 50 mM KCl with 1.25 U of Taq polymerase (Takara, Tokyo, Japan). After 30 PCR cycles, an additional extension at 72℃ for 10 min was performed. Amplification of 30 RT-PCR cycles belonged to the log phase. GAPDH was used as the internal control. Representative results of the RT-PCR assays, which were performed three times, are shown in results.

For western blotting, cells were lysed by sonicating three times for 10 seconds. Lysates were centrifuged for 10 min at 12,000 rpm, and the soluble supernatant fraction used for the western blot analysis. For gel electrophoresis, 100 μg of each protein was loaded onto an 8% PAGE. Proteins were blotted onto a nitrocellulose membrane (Amersham Pharmacia Biotech., Piscataway, NJ) and incubated with 5 μg/mL anti-human rabbit TLR3 polyclonal antibodies that were produced in our laboratory. After three washes in PBS containing 0.5% Tween 20, the membranes were incubated with secondary peroxidase- conjugated goat anti-rabbit IgG (Jackson Immuno Research, Baltimore, MD) for 2 h, washed three times in PBS containing 0.1% Tween 20, and developed on an ECL system (Amersham Pharmacia Biotech, Piscataway, NJ, U.S.A.).

The isoform of TLR3 was cloned from cDNA of human astrocytes. Fig. 1 illustrates the schematic structures of the wild type TLR3 mRNA and the spliced form. The TLR3 isoform mRNA showed splicing of the mRNA that contained the splice donor and acceptor sites in exon 4. The isoform lacking a 192 bp fragment was produced by alternative splicing within exon 4. RT-PCR using nested primers was performed, and the isoform observed in the primary astrocytes and astrocy-

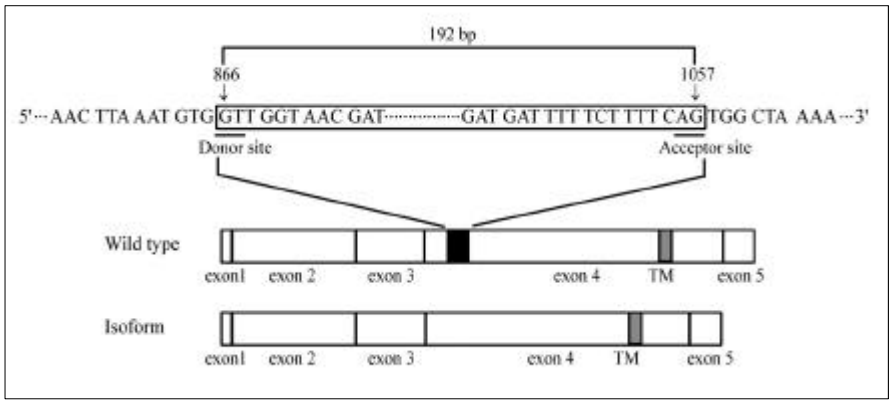

Fig. 1. Schematic diagram of the TLR3 isoform showing cDNA. The splice donor and acceptor sites reside within exon 4. The isoform lacking 192 bp is produced by alternative splicing within exon 4.

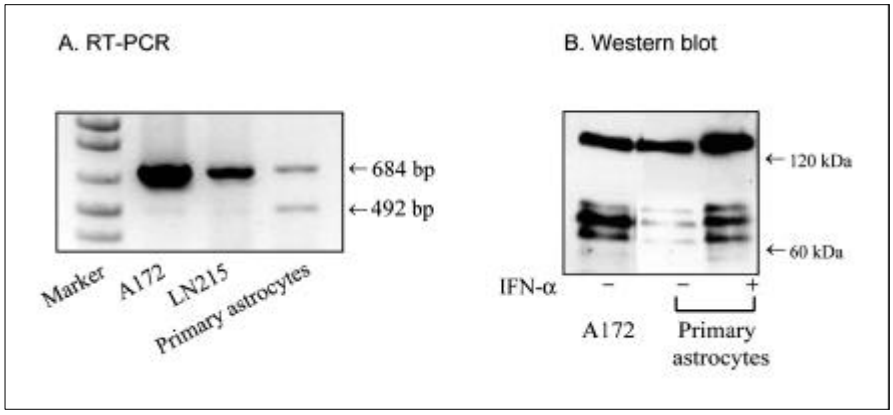

Fig. 2. Expression of the TLR3 isoform in human astrocytoma cell lines and primary astrocytes. RT-PCR was performed with TLR3 nested primers. Western blot was carried out with polyclonal rabbit antiserum.

toma cell lines, LN215 and A172 (Fig. 2A), respectively. Western blotting suggested the isoform was expressed at the protein level (Fig. 2B). The major band (app. 120 kDa) was the wild type TLR3, with the smaller bands (above 60 kDa) potentially being the isoform. The smaller isoform was induced more after treatment with IFN-α (Fig. 2B). The functional significance of this isoform was not clarified in the study. A recent paper suggested the putative ligand binding site in TLRs might reside in leucine rich repeats (LRRs).[10] In TLR3, LRRs with inserts after position 15 exist as 969-1,065, 1,140-1,221, 1,443-1,518 and 1,593-1,686 bp fragments. The identified isoform of TLR3 has a 866-1,057 bp deletion. Therefore, a partial deletion was found in the first LRR with inserts after position 15 in TLR3. A further functional study, such as a ligand binding assay, will confirm the biological activity of the isoform.

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