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Functional Expression of Toll-Like Receptor 2 in the Trigeminal Ganglion Neurons

삼차신경절 신경세포에서의 TLR2의 기능적 발현

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ABSTRACT

Functional Expression of Toll-Like Receptor 2 in the Trigeminal Ganglion Neurons

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Pattern recognition receptors (PRRs), such as Toll-Like Receptors (TLRs) expressed by immune cells, and play an important role in innate immunity in response to pathogens which enter into the host. PPRs in immune cells recognize pathogen-associated molecular patterns (PAMPs) in pathogens, which activate intracellular signaling to release inflammatory cytokines. Recently, it has been reported that PRRs are also expressed on sensory neurons, and thus can directly recognize pathogens and modulate pain and inflammation. However, little is known about whether and what PRRs work in the trigeminal

ganglia (TG).

This study was aimed to determine various PRRs expressed in the TG by examining the molecular and functional expression of PRRs through RT-PCR, real-time PCR, and Fura-2 based Ca^{2+} imaging in the TG.

PRRs were used such as NOD1 (Nucleotide Binding Oligomerization Domain Containing 1), NOD2, P2X7 (P2X purinoceptor 7), CD14 (Cluster of Differentiation 14), NLRP3 (NOD-, LRR- and Pyrin Domain-containing Protein 3), RAGE (Receptor for Advanced Glycation Endproducts), TLR1-7, TLR9, and these mRNAs were expressed overall in TG and dorsal root ganglia (DRG). Quantitative real-time PCR analysis confirmed that Toll-like receptor 2 (TLR2) was significantly higher in the TG. By using Ca^{2+} imaging, we confirmed that the intracellular Ca^{2+} responses of the TG neurons were observed with two agonists of TLR2 (Pam2CSK4, Pam3CSK4).

When we further examined changes of TLR2, MyD88, TNF-α and IL-1β mRNA expression in the TG after tooth pulp exposure, TLR2, MyD88, TNF-α and IL-1β mRNA expression levels were increased in the ipsilateral TG compared to the contralateral TG. Also, the expression level of TLR2 was confirmed by single cell RT-PCR in dental primary afferent (DPA) neurons.

Therefore, these results revealed that the molecular and functional expression of PRRs in the TG, and we suggest that TLR2 may play a functional role in tooth pain.

Key Words: Pattern recognition receptors, Pathogen-associated molecular patterns, Trigeminal ganglia, Toll-like receptor 2, Pulp-exposure model

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ABBREVIATIONS

PRRs	Pattern Recognition Receptors
PAMPs	Pathogen-associated molecular patterns
CD14	Cluster of Differentiation 14
NLRP3	NOD-, LRR- and Pyrin domain-containing protein 3
P2X7	P2X purinoceptor 7
NOD	Nucleotide Binding Oligomerization Domain Containing
RAGE	Receptor for Advanced Glycation Endproducts
TLR	Toll-Like Receptors
GAPDH	Glyceraldehyde 3-Phosphate Dehydrogenase
DPA	Dental Primary Afferent neurons
qPCR	Quantitative real-time Polymerase Chain Reaction
TG	Trigeminal Ganglia
DRG	Dorsal Root Ganglia

1. INTRODUCTION

The immune system acts against pathogens such as Gram-positive and Gram-negative bacteria and parasites, and plays a major role in protecting the host against diseases. It has both physical and chemical defenses that create an environment whereby microorganisms find it difficult to survive and cause an inflammatory reaction [15, 16]. The innate immune system, which is the first to respond comprises largely of macrophages, dendritic cells and neutrophils, which have Pattern Recognition Receptors (PRRs). PRRs recognize specific structural motifs found on microorganisms, and are known as pathogen-associated molecular patterns (PAMPs), which generate an immune response by inducing intracellular signal transduction pathways and the release of inflammatory cytokines [15, 17].

The interaction between pathogens and immune cells has been well documented, but studies in recent years have reported that sensory neurons also have PRRs that regulate pain and inflammation [18, 19]. In other words, pain signals are transmitted not only by activating sensory neurons through the interaction between pathogens and immune cells, but also through a direct interaction between pathogens and sensory neurons [20]. However, PRR studies in sensory neurons are a little known, additionally there have not been much studies conducted in the trigeminal ganglia (TG). Therefore, we investigated the molecular and functional expression of PRRs in the TG.

In the present study, we confirmed the expression of PRRs in the TG using reverse transcription-polymerase chain reaction (RT-PCR),

and carried out a quantitative analysis by quantitative real-time PCR (qPCR).

From the results, we confirmed that Toll-like receptor 2 (TLR2) was significantly expressed in the TG. Amongst the TLR family members, TLR2 is a Gram-positive bacterial receptor and recognizes PAMPs such as lipoteichoic acid and di- and tri-acylated lipopeptides [8]. TLR2 forms a heterodimer with TLR1 or TLR6 depending on the stimulating ligand [12]. Using the TLR2 agonists Pam2CSK4 and Pam3CSK4, which target TLR2/6 and TLR2/1 heterodimers each, we examined the functional expression of TLR2 in the TG neurons through Ca^{2+} imaging technique. In addition, after tooth pulp exposure, changes in the expression of TLR2 was determined via qPCR and TLR2 mRNA levels in dental primary afferent (DPA) neurons are confirmed through single cell RT-PCR.

2. MATERIALS AND METHODS

2.1. Animals

All surgical and experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Seoul National University. Male C57BL/6 mice of 5~7 weeks old were used for the experiments and maintained with standard lab chow (pellet diet) and water ad libitum. All experiments were performed in accordance with relevant guidelines and regulations that were confirmed by IACUC.

2.2. Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNA was isolated from mouse TG with Direct-zol RNA Miniprep (Zymo Research). Then, 0.5 - 1 μg of the total RNA was used for cDNA synthesis by the QuantiTect Reverse Transcription Kit (Qiagen) and agarose gel electrophoresis was performed. The primers used are listed in the Table 1 and the mRNA of NOD1 and ACTB is obtained from primerbank. GAPDH was used as a control.

2.3. Real-Time PCR assay

Both TGs were rapidly harvested and the total RNA was transcribed to cDNA as described in the RT-PCR Method. Real-time PCR was performed with a StepOnePlus Real-Time PCR System (Applied Biosystems, USA) using SYBR Green mix solution. The primer pairs are indicated in the Table 1. Reaction mixtures were denatured for 2 minutes at 50°C and 10 minutes at 95°C and subjected to 40 cycles for 15 seconds at 95°C and annealing for 1 minutes at 60°C. Gene expression was normalized using the △△CT method with ACTB as the reference gene. $P<0.05$ was considered to indicate significant differences.

2.4. Retrograde Labeling of DPA Neurons

Dental primary afferent (DPA) neurons were retrograde-labeled with a fluorescent dye [23, 24]. The anesthesia was induced with pentobarbital (50 to 80 mg/kg, intraperitoneal). The anesthetized animal was drilled of the left and right maxillary first molars under microscope. The dentin and tooth pulp were exposed with a low-speed dental drill and a round bur and filled in DiI (1,1′-dioctadecyl-3,3,3′3′-tetramethylindocarbo-cyanine perchlorate). Sealing material was resin-based and it occluded molar holes to prevent DiI leaking. Two weeks after DiI application, mice were used for scRT-PCR.

2.5. Single-Cell Reverse Transcription PCR

Under a fluorescence microscope, isolectin B4-FITC positivity in DiI-labeled DPA neurons was examined. Neurons were collected randomly in size and put into a PCR tube containing reverse transcription agents. Complementary DNA (cDNA) synthesis was carried out after the sampling and cDNAs were used for nested PCR amplifications.

2.6. TG cultures and calcium imaging

TGs from mice (5-7 weeks) were dissected into neurobasal medium (Life Technologies), dissociated in 1 mg/ml collagenase A (Cat# 10 103 578 001, Roche) and 2.4 U/ml dispase II (Cat# 04 942 078 001, Roche) diluted in filtered and autoclaved HBSS for 60 min at 37°C in 5.0% CO2. After trituration with glass Pasteur pipettes, TG cells were centrifuged over a 15% BSA, plated on PDL-coated cell culture dishes in B27 supplemented neurobasal-A medium.

TG neurons were used for Ca^{2+} imaging 1 day after plating. Fura-2-based ratiometric Ca^{2+} imaging was performed to measure intracellular Ca^{2+} change as previously described [14]. The TG cells were grown to 70% confluence in coverslips and loaded with 2 μM Fura-2 (Invitrogen) for 1 hour at 37°C in 5.0% CO2. The coverslips were mounted onto the microscope (Nikon Eclipse Ti) and washed with Extracellular Solution (140mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$, 10mM HEPES, and 10mM Glucose; 290 to 310 mOsm, pH 7.3± 0.1 adjusted with NaOH) and imaged at room temperature. 340/380 ratiometric images were processed and intracellular free Ca^{2+} concentration was measured with MetaFluor software (v 7.8.13.0;

Molecular Devices). Culture bath solution was applied to the TG neurons and TLR2 agonists were flowed onto neurons followed by washout after application. In all experiments, 1 μg/ml Pam2CSK4 or Pam3CSK4 (Invivogen), 1 μM Capsaicin (Tocris), and 50 mM KCl (Sigma) were applied. TLR2 agonists-positive neurons that displayed calcium transients with ≥8% of the maximal amplitude by 50 mM KCl, were used for the analysis.

2.7. Chemicals

All TLR2 agonists were purchased from Invivogen; Pam2CSK4 (Cat# tlrl-pm2s-1), Pam3CSK4 (Cat# tlrl-pms). All compounds are added 1 ml of endotoxin-free water (provided) and vortex to homogenize for preparation of stock solution. Drugs were diluted to their final concentration with the bath solution for Ca^{2+} imaging. Capsaicin stock solution (Sigma-Aldrich) was made in ethanol and stored at -20°C.

2.8. Data Analysis and Statistics

Data in figures are presented as mean ± SEM. All statistical analyses were performed using Prism software (version 5; GraphPad). Statistical significance was achieved by *t*-test. Differences with P values <0.05 were regarded as the criterion for statistical significance.

Table 1. List of Primers Used

Target Gene (Amplicon)	Sequence (5′->3′)	Target Gene (Amplicon)	Sequence (5′->3′)
TLR1 (106)	TCAAGTGTGCAGCT GATTGCT TGCTAACGTGCCGA AGAGATC	TLR2 (305)	TCTCTGGGCAGTCT TGAACA TCGCGGATCGACTT TAGACT
TLR2 (118)	AGACACTGGGGGTA ACATCG AGAGAAGTCAGCCC AGCAAA	TLR3 (108)	TCACGCAGTTCAGC AAGCTAC CCTCTTCGCAAACA GAGTGCA
TLR4 (125)	ATGGGAGGACAATC CTCTGGT AGGTCCAAGTTGCC GTTTCTT	TLR7 (132)	ACTTGCCTCCTGAG GTTTTTGA CGCTGAGGTCCAAA ATTTCCAG
TLR5 (130)	GCAGGATCATGGCA TGTCAAGT ATCTGGGTGAGGTT ACAGCCTT	TLR9 (135)	ATTCTCTTCATGGA CGGGAACC AGGCACCTTTGTGA GGTTGTTA
TLR6 (128)	ACACAATCGGTTGC AAAACACC GGAAAGTCAGCTTC GTCAGGA	TRPV1 (115)	GCGACCATCCCTCA AGAGTT ATACTCCTTGCGAT GGCTGA
TRPV1 (293)	CATCTTCACCACGG CTGCTTA AGAACACCATGGAA GCCACAT		

Target Gene (Amplicon)	Sequence (5′->3′)	Target Gene (Amplicon)	Sequence (5′->3′)
CD14 (127)	TTGGCCCAGTCAGC TAAACTC AGGGCTAGGGTTCC TATCCAG	RAGE (124)	GAGAGCCTGGGAGT AGTAGACC CATTCAGCTCTGCA CGTTCCT
NOD1 (132)	TCATCTGGTCACCA ACATTCGA TGGATCTTTCGGAC CTTGTCAA	NOD2 (103)	TGGACACAGTCTGG AACAAGG CAGGACCCATACAG TTCAAAGG
NLRP3 (142)	GGTCAGGATCTCGC ATTGGTT TCCCCAGCTTCTGC AAGTTAC	P2X7 (142)	GTTCCTACCCCCAA TGTGTCC CTTGCAGACTTTTC CCAAGCC
GAPDH (268)	GTTCCTACCCCCAA TGTGTCC ACCTGTTGCTGTAG CCGTATC	GAPDH (127)	TTGTGATGGGTGT GAACCAC CCTTCCACAATGCC AAAGTTG
TNF-α (115)	GCCTCTTCTCATTC CTGCTTG CTGATGAGAGGGAG GCCATT	MyD88 (166)	CAAAGGAACTGGGA GGCATCA CGGTCGGACACACA CAACTTA
IL-1β (122)	GGCTGCTTCCAAAC CTTTGAC AGCTTCTCCACAGC CACAAT	ACTB (154)	GGCTGTATTCCCCT CCATCG CCAGTTGGTAACAA TGCCATGT

3. RESULTS

3.1. The expression of Pattern-recognition receptors (PRRs) in TG and DRG through RT-PCR

We investigated PRRs that were representative of membrane-bound and cytoplasmic PRRs. A number of PRRs have been identified, such as the Toll-like receptors (TLRs); Nucleotide-binding domain leucine-rich repeat containing (NLRs); P2X purinoceptor 7, that has a role in activating the NLRP3 inflammasome and increases inflammatory mediators [1]; CD14 (Cluster of differentiation 14) that detects bacterial lipopolysaccharide (LPS) and other molecular patterns such as lipoteichoic acid [2]; and RAGE (Receptor for advanced glycation endproducts) who's ligand is high mobility group box 1 (HMGB1) and results in pro-inflammatory gene activation, and showed high mRNA expression levels in inflamed dental pulp compared to healthy dental pulp [3].

Fourteen candidate genes were selected for this study. We first determined the expression of the PRRs in the TG using whole tissue RT-PCR. With these receptors, mRNA was detected in the TG **(Fig. 1)**. Spleen tissue was used as a positive control **(Fig. 1A)** and mRNA for the PRRs was compared between TG and DRG **(Fig. 1B)**. Most of the PRRs described above are found in the TG.

3.2. The expression of Pattern-recognition receptors (PRRs) in TG and DRG through real-time PCR

For quantitative analysis of the selected target genes, we then examined the mRNA expression levels using qPCR **(Fig. 2A-B)**. Compared with DRG, the mRNA expression levels of P2X7 and almost all TLRs were higher in the TG, but there were no significant changes observed in these groups. Interestingly, in the TG, TLR2 was significantly increased when compared to DRG ($p < 0.05$). There were no differences in the expression levels of CD14, NLRP3, NOD1, NOD2 and RAGE between TG and DRG.

3.3. Toll-like receptor 2 agonists-induced calcium transient in mouse trigeminal ganglion neurons

Fura-2-loaded Ca^{2+} imaging was examined to see whether Ca^{2+} responses were induced in the TG neurons to confirm their functional expression. Since TLR2 signaling is Ca^{2+} dependent [4], we examined whether TLR2 agonists activate TLR2 in the TG neurons. TG neurons were exposed to 100 ng/ml and 1 μg/ml of TLR2 specific agonists; Pam2CSK4 (P2C), Pam3CSK4 (P3C), 1nM Capsaicin (CAP) and 50mM KCl. P2C and P3C elicited Ca^{2+} transients in a subset of the TG neurons **(Fig. 3B)**. Amongst the KCl-responding TG neurons, 28.6% responded to 100 ng/ml of P2C (n = 4 of 14); 45.1% responded to 1 μg/ml of P2C (n = 23 of 51), and 63.2% responded to 1 μg/ml of P3C (n = 12 of 19). TLR2 KO mice were used to confirm whether the generated Ca^{2+} transients were due to TLR2 activation. There were no neurons responsive to 1 μg/ml of P2C and P3C of the

KCl responding neurons in TLR2 KO mice **(Fig. 3B).** TLR2 agonist-responding neurons were seen in almost small-sized TG neurons and the percentage of TLR2+TRPV1+ responsive neurons were: 1 μg/ml P2C+CAP+ (17.6%, *n* = 9 of 51) and 1 μg/ml P3C+CAP+ neurons (31.6%, *n* = 6 of 19) **(Fig. 3-C).**

3.4. The expression changes of Toll-like receptor 2 twenty-four hours after tooth pulp exposure through real-time PCR

Tooth enamel is the outermost surface and the hardest part of the tooth, and the dental pulp, which exists inside the tooth is a place with nerves and blood vessels. It serves to provide nutrients to the dentin and allows the dentin to sense signals, so it detects irritation and protects the teeth through a pain response [21]. Once the tooth enamel is eroded, dentin is exposed to the oral environment and becomes degraded, predominantly by Gram-positive bacteria, including *Streptococcus mutans*, *Streptococcus sobrinus* and *Lactobacillus acidophilus* that primarily regulate dentin caries lesions [5].

In this study, one side of the maxillary first molar was drilled and the tooth dentin was exposed. Since the infiltration of inflammatory cells occurs within 24 hours [6], we conducted an experiment at 1 day post injury, assuming that inflammatory conditions caused by bacteria had occurred [7].

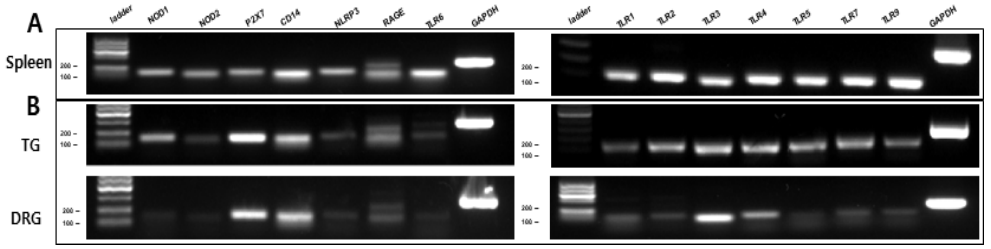
The expression levels of TLR2, MyD88, TNF-α and IL-1β were analyzed by the detection of the relevant mRNA in total TG. As seen in Figure 4, the result that was induced by tooth injury was found in the ipsilateral TG, and the ipsilateral TG was nearly twice as high as

the contralateral TG **(Fig. 4A-D)**. We found that TLR2, MyD88, TNF-α and IL-1β in the right TG was significantly up-regulated following pulp exposure ($P < 0.05$).

3.5. Molecular expression of TLR2 in DPA Neurons through scRT-PCR

We then examined TLR2 mRNA expression in DiI-labeled DPA neurons by scRT-PCR **(Fig. 5A)**. TLR2 mRNA was detected in 12.5% (n = 5/40) of DPA neurons, and 40% (n = 2 of 5) of TLR2 positive-neurons co-expressed TRPV1 mRNA **(Fig. 5B)**.

Figure 1.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Spleen
B
TG
DRG
ladder
NOD1
NOD2
P2X7
CD14
NLRP3
RAGE
TLR6
GAPDH
ladder
TLR1
TLR2
TLR3
TLR4
TLR5
TLR7
TLR9
GAPDH
200
100

Figure 1. The expression of Pattern recognition receptors (PRRs) in TG and DRG through RT-PCR

(A) The representative gels showing RT-PCR products from mouse spleen as a positive control.

(B) The representative gels showing RT-PCR products from TG and DRG. Predicted sizes for selected markers are NOD1 (132bp), NOD2 (103bp), P2X7 (142bp), CD14 (127bp), NLRP3 (142bp), RAGE (124bp), TLR1 (106bp), TLR2 (118bp), TLR3 (108bp), TLR4 (125bp), TLR5 (130bp), TLR6 (103bp), TLR7 (132bp), TLR9 (135bp), GAPDH (268bp).

Figure 2.

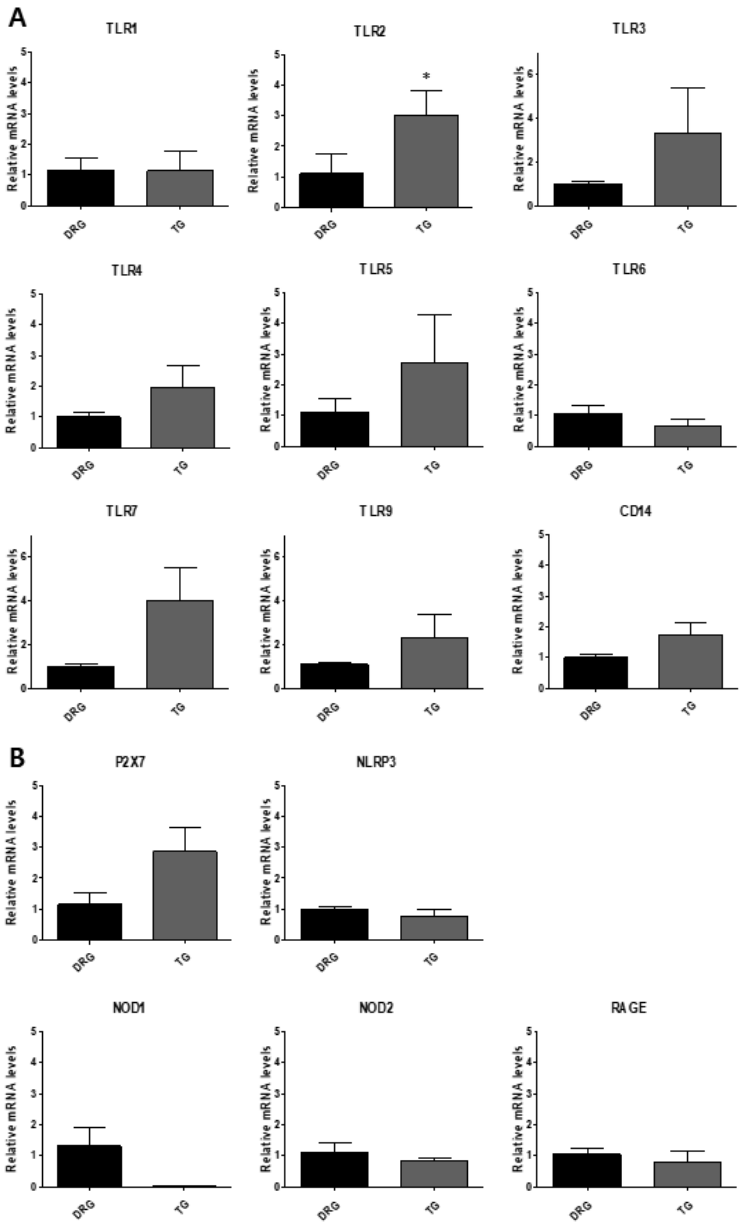
A
TLR1
TLR2
TLR3
TLR4
TLR5
TLR6
TLR7
TLR9
CD14
B
P2X7
NLRP3
NOD1
NOD2
RAGE
Relative mRNA levels
DRG
TG
*

Figure 2. The expression of Pattern recognition receptors (PRRs) mRNA by real-time PCR analysis

(A) The results of real-time PCR are shown in naïve mice and compared with Toll-like receptors 1-7, 9 and CD14 mRNA expression in TG and DRG. (n = 3 animals per each group).

(B) The results of real-time PCR are shown in naïve mice and compared with P2X7, NOD1, NOD2, NLRP3 and RAGE mRNA expression in TG and DRG. (n = 3 animals per each group).

Figure 3.

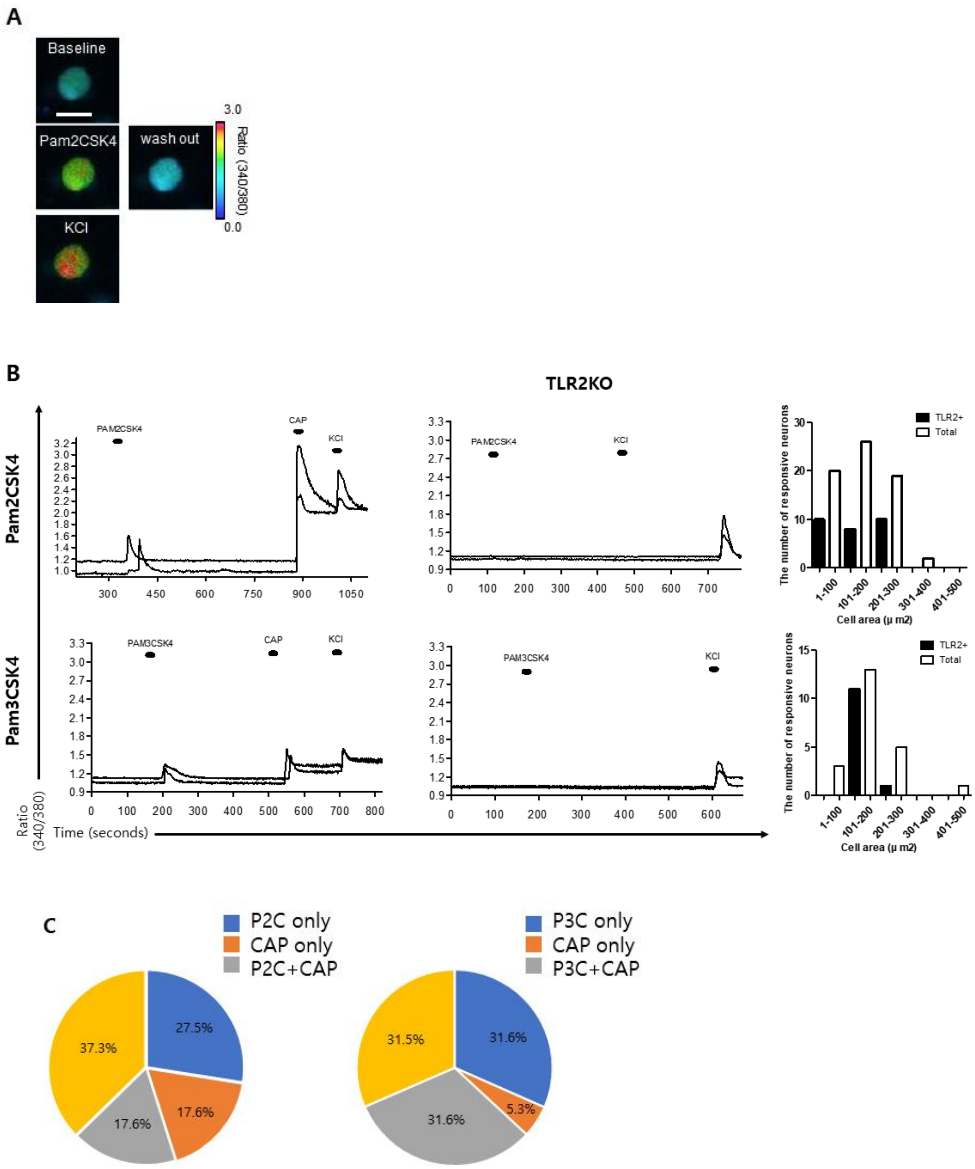
A
Baseline
Pam2CSK4
wash out
KCl
3.0
Ratio (340/380)
0.0
B
TLR2KO
Pam2CSK4
Pam3CSK4
PAM2CSK4
CAP
KCl
PAM3CSK4
Ratio (340/380)
Time (seconds)
TLR2+
Total
The number of responsive neurons
Cell area (μ m2)
1-100
101-200
201-300
301-400
401-500
C
P2C only
CAP only
P2C+CAP
27.5%
17.6%
17.6%
37.3%
P3C only
CAP only
P3C+CAP
31.6%
5.3%
31.6%
31.5%

Figure 3. Effects of Pam2CSK4 and Pam3CSK4 on intracellular calcium transients in TG neurons

(A) Representative cell image of pseudocolor ratiometric $[Ca^{2+}]_i$ images in TG neurons. Scale bars: 30 μm.

(B) Representative traces and percentage of TG responsive neurons after 100 ng/ml (28.6%, n = 4 of 14) Pam2CSK4, 1 μg/ml (45.1%, *n* = 23 of 51) Pam2CSK4 and 1 μg/ml (63.2%, n = 12 of 19) Pam3CSK4 respectively in naïve mouse. No responsive neurons to 1 μg/ml Pam2CSK4 and Pam3CKS4 in TLR2 KO mice. Size distribution of Pam2CSK4 responsive neurons (1 to 101 μm2, n = 6; 101 to 200 μm2, n = 7; 201 to 300 μm2, n = 9; 301 to 400 μm2, n = 1) and Pam3CSK4 responsive neurons (1 to 101 μm2, n = 1; 101 to 200 μm2, n = 8; 201 to 300 μm2, n = 3). n = 2 mice.

(C) Pie graph representation: percentage of 1 μg/ml P2C+CAP+ (17.6%, *n* = 9 of 51) and 1 μg/ml P3C+CAP+ neurons (31.6%, *n* = 6 of 19).

Figure 4.

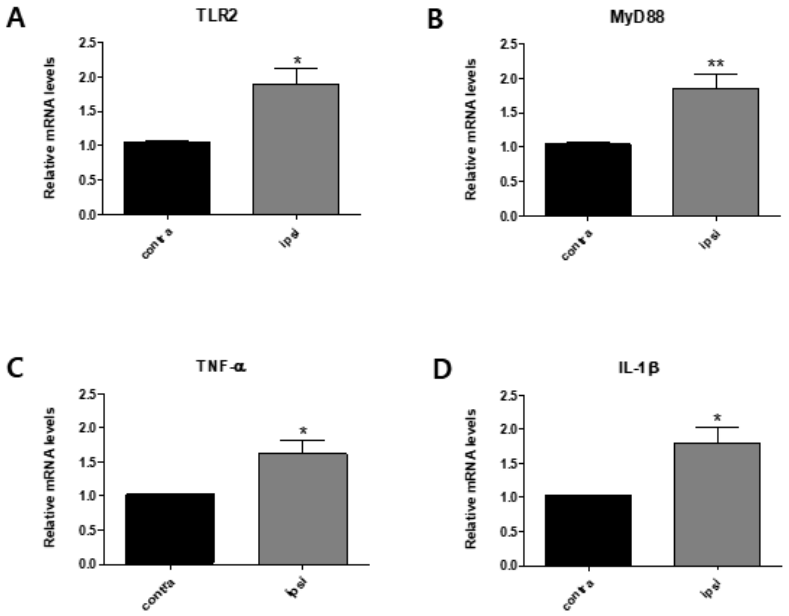
A
TLR2
Relative mRNA levels
2.5
2.0
1.5
1.0
0.5
0.0
*
contra
ipsi
B
MyD88
Relative mRNA levels
**
contra
ipsi
C
TNF-α
Relative mRNA levels
*
contra
ipsi
D
IL-1β
Relative mRNA levels
*
contra
ipsi

Figure 4. The mRNA expression of TLR2, MyD88, TNF-α *and* IL-1 β in the TG after tooth pulp exposure.

(A-D) The relative fold change of TLR2, MyD88, TNF-α and IL-1β expression in the TG ipsilateral (right) to the exposured pulp after 24h. The expression levels of these genes were normalized to the Ct values of ACTB. The graphs show relative expression values that calculated using the ΔΔCt method.

Figure 5.

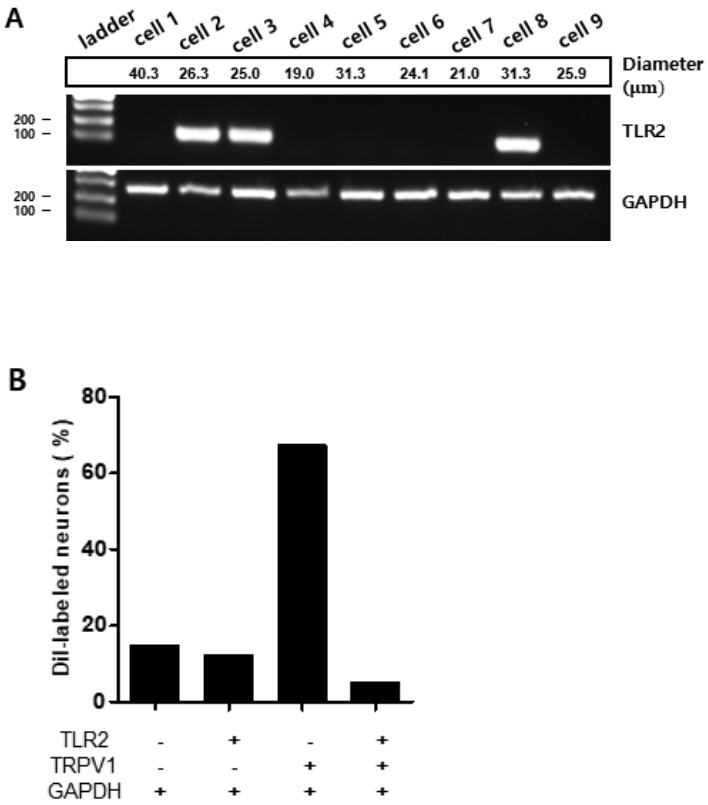
A
ladder
cell 1
cell 2
cell 3
cell 4
cell 5
cell 6
cell 7
cell 8
cell 9
40.3
26.3
25.0
19.0
31.3
24.1
21.0
31.3
25.9
Diameter (μm)
200
100
TLR2
200
100
GAPDH
B
Dil-labeled neurons (%)
80
60
40
20
0
TLR2 - + - +
TRPV1 - - + +
GAPDH + + + +

Figure 5. Molecular expression of TLR2 mRNA in dental primary afferent (DPA) neurons by single-cell reverse transcription polymerase chain reaction (scRT-PCR).

(A) scRT-PCR of TLR2 mRNA in DPA neurons. Predicted sizes are 118 bp for TLR2, 115 bp TRPV1 and 268 bp for GAPDH.

(B) Expression of collected DPA neurons showed TLR2+ only (12.5%, n = 5), TRPV1+ only (67.5%, n = 27), TLR2+TRPV1+ (5%, n = 2), and TLR2-TRPV1- (15%, n = 6). n = 40 neurons from 11 mice.

4. DISCUSSION

In this study, we aimed to determine whether PRRs were molecularly and functionally expressed in the TG, and focused more on TLR2, which produced a significant finding. In Ca^{2+} imaging, TG neurons responded to TLR2 agonists and P2C, P3C - induced Ca^{2+} transients were generated. In the dental pulp exposure model, an increase in the expression level of TLR2, MyD88, TNF-α and IL-1β was observed. Therefore, our results suggest that TLR2 in the TG is functionally expressed, thus minimally contributing to tooth pain.

One of the main causes of tooth pain is tooth decay, and the bacteria that cause tooth decay are known to be Gram-positive bacteria [5]. TLR2, identified as a result of PRR screening in the TG tissue, plays a role as a receptor for Gram-positive bacteria. TLR2 recognizes PAMPs of Gram-positive bacteria and causes inflammation, and the release of inflammatory cytokines, chemokines, etc. [21]. A recent study revealed that TLR2 expressed in immune cells was expressed in primary sensory neurons, such as TG and DRG [8], and the expression of TLR2 in DPA neurons was confirmed through scRT-PCR. Therefore, we suggest the possibility of inflammation and tooth pain from the interaction of bacteria and PRRs expressed in DPA neurons.

The model used in the current study was the pulp exposure model, which destroys the dentin and minimizes pulp inflammation [9]. It has been found that it was possible to increase bacterial invasion and cause inflammation because there was easy access from the surrounding environment in the mouth [10]. Since mice are smaller

than rats, it was not easy to proceed with the process of exposing the pulp using a dental drill, so an experiment was conducted with one maxillary molar in the mouse model. Since inflammation occurs within 24 hours after pulp exposure [10, 11], we confirmed the expression of TLR2, MyD88, TNF-α and IL-1β in the TG after 24 hours. Tooth decay is caused by the reaction of bacteria distributed in the mouth and sugar from ingested food, so an additional experiment was conducted to allow sufficient time for the bacteria in the mouth to enter into the exposed pulp. After a week, two-thirds of the teeth were visually confirmed to turn brown, although this was not seen in some mice. We concluded that the experimental effect, which tooth decay occurs naturally would be difficult to see.

TLR2 acts as a TLR6/TLR2 and TLR1/TLR2 heterodimer [12]. As TLR2 is a Gram-positive receptor, we used TLR2 agonists-based on lipoproteins, pathogens associated molecular patterns of Gram-positive bacteria. Ca^{2+} imaging was conducted with Pam2CSK4, an agonist of TLR6/TLR2 and Pam3CSK4, an agonist of TLR1/TLR2. The Pam3CSK4 responding neurons were 18% higher than Pam2CSK4 in the TG neurons. We analyzed capsaicin sensitive neurons to determine the correlation between TLR2 and TRPV1 in the TG neurons [8, 13]. The results showed that TLR2+capsaicin+ sensitive neurons had an similar percentage, with TLR2 only positive neurons. These results suggest the possibility that the TG neurons responding to TLR2 agonists, express TRPV1 and were more likely to act as a TLR1/2 heterodimer. However, the subpopulation which the TLR2-induced calcium transient still needs to be investigated.

In conclusion, our study identified the molecular expression of various PRRs in TG and DRG, and showed that TLR2 was

functionally expressed in the TG neurons. Identification of the function of TLR2 in tooth pain needs to be investigated in future studies.

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국문초록

삼차신경절에서의 패턴인식수용체 스크리닝

외부에서 병원균이 들어왔을 경우, 면역세포에 발현되어있는 톨-유사수용체 (Toll-Like Receptors, TLRs)와 같은 패턴인식수용체 (Pattern Recognition Receptors, PRRs)가 선천 면역에 중요한 역할을 한다. 이러한 표면수용체들을 통해 면역세포들은 병원체 연관 분자패턴 (pathogen-associated molecular patterns, PAMPs)을 인식하여 세포 내 신호전달을 이루고 염증성 사이토카인의 분비를 유도하여 면역 방어 체계를 활성화하는 역할을 한다. 최근 들어, 패턴인식수용체는 감각신경세포에서도 발현되어 있어 병원균을 직접 인식하여 통증과 염증을 조절할 수 있다는 것이 밝혀졌다. 하지만 감각신경세포에서 연구된 패턴인식수용체는 일부분이며 특히 삼차신경절 (Trigeminal Ganglia)에서 연구가 많이 이루어지지 않았다.

본 연구에서는 삼차신경절에서 어떤 종류의 패턴인식수용체가 발현되어 있는지를 규명하고자 하였다. 그리고 이를 규명하기 위하여 RT-PCR, real-time PCR 및 Fura-2를 기반으로 한 칼슘이미징 기법을 통해 패턴인식수용체의 분자 및 기능적 발현을 조사하였다.

삼차신경절과 척수후근신경절에서 패턴인식수용체인 NOD1 (Nucleotide Binding Oligomerization Domain Containing 1), NOD2, P2X7 (P2X purinoceptor 7), CD14 (Cluster of Differentiation 14), NLRP3 (NOD-, LRR- and Pyrin Domain-containing Protein 3), RAGE (Receptor for Advanced Glycation Endproducts), TLR1-7, TLR9 mRNA가 전반적으

로 발현되어 있음을 확인하였다. 또한 real-time PCR을 통해 정량적 분석을 한 결과, TLR2가 삼차신경절에서 척수후근신경절에 비해 상대적으로 유의미하게 높게 발현되어 있었다. 이를 바탕으로 TLR2의 기능적 발현을 조사하기 위해 진행한 칼슘이미징에서 TLR2의 두 가지 작용제 (Pam2CSK4, Pam3CSK4) 를 처리했을 때 약물에 의해서 세포 내 칼슘 농도가 증가하는 것을 확인하였다.

추가적으로 치수노출모델을 우측상악구치부 위치에 유발하고 삼차신경절에서 TLR2, MyD88, TNF-α, 그리고 IL-1β mRNA 발현을 확인했을 때, 치수를 노출하지 않은 반대측 삼차신경절에 비해 치수를 노출한 삼차신경절에서 유의미하게 발현량이 증가한 것을 확인하였다. 또한 dental primary afferent (DPA) neurons에서의 TLR2 mRNA 발현량은 single cell RT-PCR 기법을 통해 확인하였다.

따라서 이상의 연구결과를 통하여 삼차신경절에서 패턴인식수용체의 분자 및 기능적 발현을 확인하고, 더 나아가 치수노출모델에 적용하여 TLR2가 치아 통증에 있어 기능적 역할을 할 수 있음을 제시하였고 실제로 TLR2를 통한 통증전달이 일어나는지 규명하기 위한 후속연구가 필요하다.

주요어 : 패턴인식수용체, 병원체 연관 분자패턴, 삼차신경절, TLR2, 치수노출모델

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