

# Activated P2X7 receptor upregulates the expression levels of NALP3 in P388D1 murine macrophage-like cells

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**Abstract.** The aims of the current study were to systematically analyze the regulation of the expression of NALP3 by the P2X7 receptor in P388D1 murine macrophage-like cells, and to investigate the association between the P2X7 receptor and the NALP3 at the molecular level. Cell culture, RNA transfection, adenosine triphosphate (ATP)-induced expression of NALP3, reverse transcription polymerase chain reaction and western blotting were used to explore the association between the P2X7 receptor and NALP3-encoding gene in P388D1 murine macrophage-like cells at the molecular level. The ATP-activated P2X7 receptor can induce the upregulation of NALP3 expression at the gene and protein levels in P388D1 cells. These results demonstrated that the activation of P2X7 increases the expression levels of NALP3 in P388D1 murine macrophage-like cells.

## Introduction

Systemic inflammatory response syndrome (SIRS) is the clinical expression and action of numerous complex intrinsic mediators of the acute phase reaction; SIRS is triggered by events, including infection, trauma, pancreatitis and surgery (1) and may result in multiple organ dysfunction syndrome. Current studies suggest that SIRS is caused by an

imbalance of the pro-inflammatory and anti-inflammatory homeostasis mechanisms within the body. The release of high levels of pro-inflammatory cytokines into the blood, including interleukin-1, interferon- $\gamma$  and phospholipase A2, promotes the production of oxygen free radicals, lipid metabolites and lysosomal enzymes, resulting in a waterfall or cascade effect. While it appears that immune disorders and inflammatory responses cannot be controlled (2,3), regulating the body's inflammatory responses and blocking these pathways in a timely and effective manner is the key to the successful treatment of patients (4,5).

As the NALP3 inflammasome is an important signal receptor and potential therapeutic target in a number of diseases, studies on NALP3 are becoming increasingly important. The NALP3 inflammasome is a macromolecular protein complex, which consists of NALP3, ASC, caspase-1 and CARD-8 (6-9). NALP3 is expressed primarily in the cells with a phagocytic function, for example monocyte-macrophage cell and granulocytes. In addition, certain immune cells express NALP3, including T and B cells. Within the body, NALP3 is primarily distributed in the epithelium of skin, joints, ears, eyes, bladder and ureter (10). When cells are stimulated by external factors, including microbial toxin, peptidoglycan and cathepsin B, intracellular NALP3, ASC, caspase-1 and CARD-8 come together to form a complex protein, the NALP3 inflammasome. Caspase-1 is activated, splicing and activating the pro-inflammatory cytokine interleukin (pro-IL)-1 $\beta$  and pro-IL-18 (11). The NALP3 inflammasome is an important signal receptor that is activated by a number of types of pathogens and danger signals within the body, resulting in an immune response. NALP3 is involved in the development of numerous inflammatory diseases, including inflammatory bowel disease (12), hypersplenism (13), acute pancreatitis associated lung injury (14) and oral inflammatory diseases (15). In addition, certain non-infectious inflammatory diseases have close associations with the NALP3 inflammasome, including the rare autoimmune disease and gout (16).

Based on the above mentioned existing literature, the current study selected the representative P388D1 mouse macrophage cell line as the research focus. Adenosine triphosphate (ATP), an activator of the P2X7 receptor, was used to treat the cells. To elucidate the association between the P2X7 receptor and the NALP3 gene in murine P388D1 macrophage-like cells at

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the molecular level, gene knockout technology was used to manipulate the NALP3 gene and P2X7 receptor. In this study, the role and significance of the NALP3 gene in inflammatory diseases was investigated by observing the activation pathways of NALP3 and its specific expression changes in inflammatory cells.

## Materials and methods

**Main cell solution and reagents.** The murine macrophage-like lymphoma cell line P388D1 was purchased from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China). RPMI-1640 dry medium was obtained from Gibco-BRL (Grand Island, NY, USA). Fetal calf serum (FCS) was purchased from Sijiqing Biological Engineering Materials Co. Ltd (Hangzhou, China). P2X7-small interfering RNA (siRNA) and NALP3-siRNA were purchased from Shanghai GenePharma Co. Ltd (Shanghai, China). Polyclonal rabbit antibody against rat NALP-3 was provided by Abcam Ltd (Hong Kong, China), and horseradish peroxidase (HRP) conjugated goat anti-rabbit antibody was obtained from ZhongShan Goldenbridge Biotechnology (Beijing, China). TRIzol<sup>®</sup> was purchased from Takara Co., Ltd. (Dalian, China).

**Cell culture and transfection.** P388D1 cells were cultured in RPMI-1640 medium, supplemented with 10% (v/v) FCS, 100 U/ml penicillin (Beyotime Institute of Biotechnology, Shanghai, China), and 100 mg/ml streptomycin (Beyotime Institute of Biotechnology). All cells were cultivated at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. The cells were placed into Petri dishes containing six coverslips, onto which cells were plated at a density of ~4x10<sup>5</sup> per coverslip and cultured for 24 h prior to transfection. The medium was refreshed prior to transfection with 4.0 µg isolated P2X7-siRNA and/or NALP3-siRNA. Cells were transfected according to the manufacturer's instructions. Control cells were transfected with an empty vector. Three groups of P388D1 cells were obtained, cells only transfected with NALP3-siRNA, cells that were transfected with P2X7-siRNA and NALP3-siRNA and cells transfected with an empty vector (control).

**ATP-induced expression of NALP-3.** Control, cells transfected with NALP3-siRNA or cells transfected with P2X7-siRNA and NALP3-siRNA were collected from the flask separately. Following counting with a cell counting kit (Beyotime Institute of Biotechnology), ~6x10<sup>6</sup> cells were cultured with added ATP (1 mmol/l; Takara Co., Ltd.) which is the natural agonist of the P2X7 receptor. Meanwhile, a separate parallel control experiment of cells with added adenosine diphosphate (ADP) was performed.

**Reverse transcription quantitative polymerase chain reaction (RT-qPCR).** Total RNA was harvested from the P388D1 cells using TRIzol<sup>®</sup>. The cDNA sequence was produced via reverse transcription under the following conditions: 30°C for 10 min, 42°C for 30 min, 99°C for 5 min and 5°C for 5 min. The resulting cDNA (10 µl) was used to amplify the coding sequence of NALP-3. The sequences of the primers used in the qPCR reactions were as follows: Forward, 5'-CTGTGT

GTGGGACTGAAGCAC-3' and reverse, 5'-GCAGCCCTGCTGTTTCAGCAC-3' for rat NALP-3; forward, 5'-ATCTGGCACCAAACACCTTCTACAATGAGCTGCG-3' and reverse, 5'-CGTCATACTCCTGCTTGCTGATCCACATCTGC-3' for β-actin. PCR was performed in 35 cycles of: 95°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 1 min, terminating at 4°C at the end of the reaction. The transcripts were successfully cloned and sequencing confirmed the target amplification. The PCR products were separated on a 1% agarose gel which contained 0.5 µl/ml ethidium bromide and visualized under ultraviolet light.

**Western blotting.** Control, cells transfected with NALP3-siRNA or cells transfected with P2X7-siRNA and NALP3-siRNA were centrifuged at 1000 x g for 5 min, and the supernatant was pooled. The cells were resuspended in phosphate-buffered saline, counted, and ~6x10<sup>6</sup> cells were aliquoted. These cells were added to 100 µl radioimmunoprecipitation assay buffer (Beyotime Institute of Biotechnology) prior to centrifugation at 12,000 x g at 4°C for 10 min. Following removal of the supernatants, the total protein was mixed with loading buffer (1:1; Takara Co., Ltd.) and heated to 100°C for 5 min prior to loading. Tris-glycine SDS-PAGE gels (concentration, 10%) were used for separation. The total protein was transferred onto a polyvinylidene fluoride (PVDF; OK-kingding Membrane Structure Technology Co., Ltd., Shenzhen, China) membrane with transfer buffer. The PVDF membrane was blocked at 37°C with 5% dry milk in tris-buffered saline for 2 h, and incubated with rabbit anti-rat polyclonal antibody (1:200) at 4°C overnight. Following washing with Tris buffered saline in Tween 20, the membrane was incubated with HRP-conjugated goat anti-rabbit immunoglobulin G (IgG) secondary antibody (1:1,000) at 37°C for 2 h.

**Statistical analysis.** All data are expressed as the mean ( $\bar{x}$ ) ± standard deviation (SD). P<0.05 was considered to indicate a statistically significant difference. All the experiments and statistical analyses were performed four times.

## Results

**Identifying the expression of NALP3 in P388D1 murine macrophage-like cells.** To confirm the expression of NALP3 in P388D1 cells without activating the P2X7 receptor, the expression levels of the NALP3 mRNA were detected by RT-qPCR. The related literature reports the band for NALP3 is at ~252 bp, specifically for the RT-qPCR amplification products of NALP3. β-actin was used as the internal control with a fragment size of ~838 bp. The P388D1 cells were observed to have NALP3 gene expression (Fig. 1A). As the expression of NALP3 mRNA was detected, western blotting was used to detect the expression of the NALP3 protein. The target band was observed on film at 94 kDa. These results demonstrated that the murine P388D1 macrophage-like cells express NALP3 protein (Fig. 1B).

**Identifying the expression of NALP3 in P388D1 murine macrophage-like cells following treatment with ATP.** We know that ATP is the P2X7 receptor agonist (17). To determine whether or not the P2X7 receptor mediates the upregulation

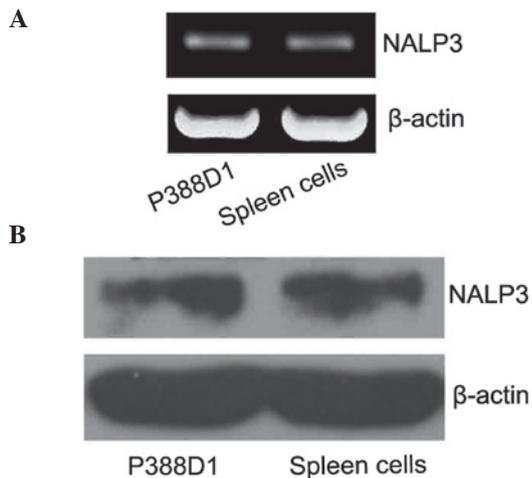


Figure 1. Identification of the expression of NALP3 in the murine P388D1 macrophage-like cells. (A) P388D1 cells exhibited NALP3 gene expression. (B) Western blotting showed that the P388D1 cells expressed the NALP3 protein.

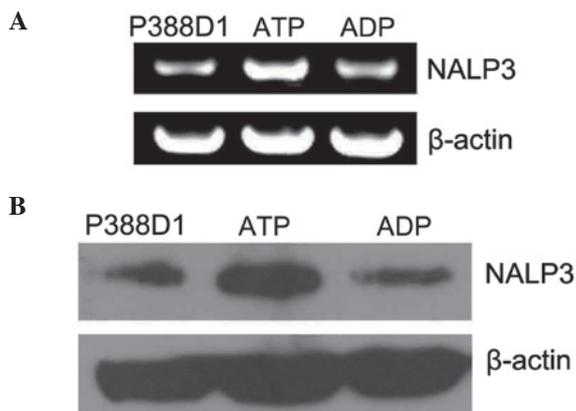


Figure 2. Identification of NALP3 expression in P388D1 cells following treatment with the agonist of the P2X7 receptor, ADP. (A) Expression of NALP3 reverse transcription polymerase chain reaction amplification products showed an increase compared with the normal untreated P388D1 cells. The other group was treated using ADP as a control. (B) Expression of NALP3 protein in P388D1 cells in the group with activation of the P2X7 by ATP for 24 h, using western blotting. The film also showed the expression of the untreated normal P388D1 cells and the control group added with ADP. ADP, adenosine diphosphate; ATP, adenosine triphosphate.

of NALP3 expression, the expression levels of NALP3 were investigated in P388D1 cells in which ATP had activated the P2X7 receptor. Firstly, the expression of NALP3 mRNA in P388D1 cells treated with ATP for 24 h was examined using RT-qPCR, the products of which were separated by 1% agarose gel electrophoresis. This revealed that the expression levels of NALP3 RT-qPCR amplification products (~252 bp) were markedly increased compared with the levels in the normal untreated P388D1 cells (Fig. 2A). The control group was treated with ADP, and the expression level of NALP3 mRNA showed no significant change compared with that of the untreated P388D1 cells ( $P < 0.05$ ,  $\bar{x} \pm SD$ ,  $n = 4$ ) (Fig. 3A). Secondly, the expression levels of NALP3 protein in P388D1 treated with ATP for 24 h were investigated using western blotting (Fig. 2B). The target band was located at 93 KDa on the film and the NALP3 protein

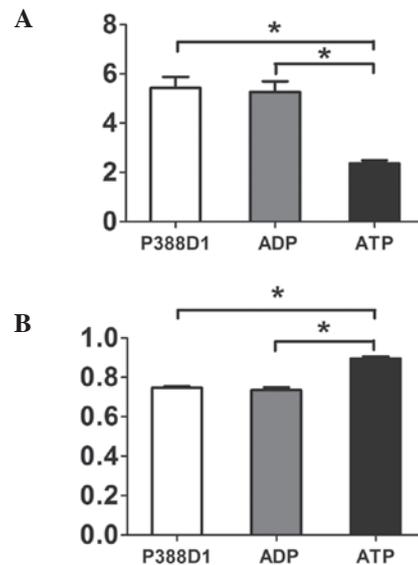


Figure 3. The expression of NALP3 was increased at the gene and protein levels during the activation of the P2X7 by ATP. (A) Semi-quantitative analysis showed that compared with normal untreated P388D1 cells and the control group added with ADP, the expression of NALP3 in the group added with ATP increased markedly at the gene level ( $P < 0.05$ , mean  $\pm$  SD,  $n = 4$ ). (B) A similar phenomenon appeared when the three groups were compared at the protein level. Therefore, the expression of NALP3 was increased at the protein level during the activation of ATP ( $P < 0.05$ , mean  $\pm$  SD,  $n = 4$ ). Due to the different gray values in reverse transcription polymerase chain reaction and western blotting, a high relative value in the histogram above reflects (A) a low level of RNA, while (B) a high level of protein. SD, standard deviation; ADP, adenosine diphosphate; ATP, adenosine triphosphate.

expression levels were found to be increased compared with those of the untreated normal P388D1 cells. The expression level of the NALP3 protein showed no significant change following treatment of cells with ADP compared with those of the normal untreated P388D1 cells ( $P > 0.05$ ,  $\bar{x} \pm SD$ ,  $n = 4$ ) (Fig. 3B). Therefore, the results show that the expression levels of NALP3 increased at the gene and protein levels following the activation of the P2X7 receptor by ATP.

*In P388D1 cells cotransfected with the NALP3-siRNA and P2X7-siRNA plasmids the expression levels of the NALP3 mRNA decreased, while the expression of NALP3 protein remained the same.* Transfection technology was used to elucidate the association between the P2X7 receptor and NALP3 expression levels. Two groups of P388D1 cells were simultaneously transfected with NALP3-siRNA and P2X7-siRNA prior to the addition of ATP. The expression levels of NALP3 mRNA were observed using RT-qPCR (Fig. 4A), but the results showed the expression levels of NALP3 mRNA were reduced in the two groups compared with those of the untreated normal P388D1 cells ( $P < 0.05$ ,  $\bar{x} \pm SD$ ,  $n = 4$ ) (Fig. 5A). The expression levels of NALP3 protein were detected by western blotting. P388D1 cells were transfected with the NALP3-siRNA plasmid prior to ATP treatment (Fig. 4B). There was no significant difference between the expression levels of the NALP3 protein in the transfected cells compared with those of the untreated normal P388D1 cells. In the cells simultaneously transfected with the P2X7-siRNA and NALP3-siRNA prior to ATP treatment, the expression levels of the NALP3 protein showed

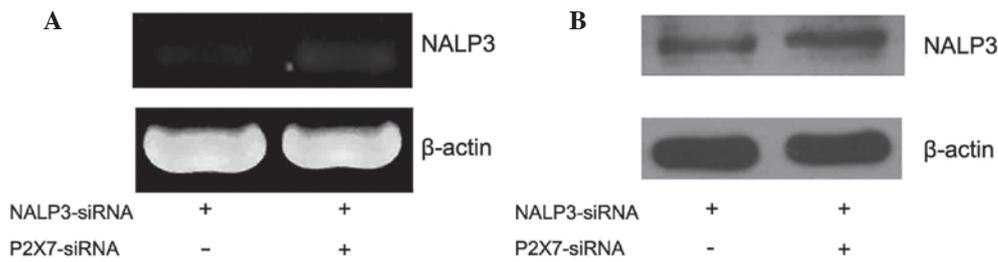


Figure 4. NALP3 expression levels of P388D1 cells following transfection with NALP3-siRNA and/or P2X7-siRNA and treatment with adenosine triphosphate, with  $\beta$ -actin as a reference. (A) NALP3 mRNA expression. (B) Western blot analysis of NALP3 protein expression. siRNA, small interfering RNA.

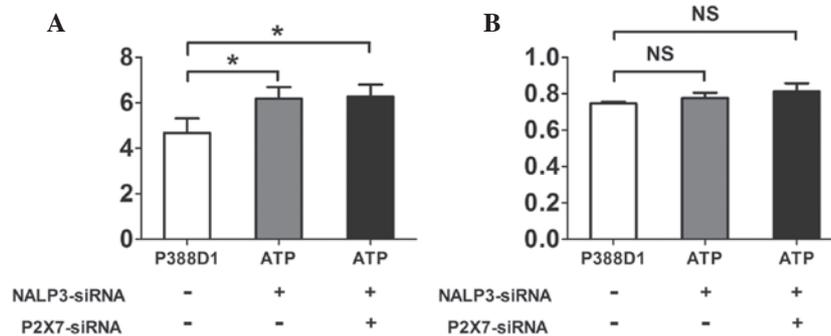


Figure 5. In P388D1 cells cotransfected with NALP3-siRNA and P2X7-siRNA plasmids, the expression of NALP3 mRNA increased, while the expression of NALP3 protein remained the same. (A) In the ATP-treated knockout groups, the expression levels of NALP3 mRNA were reduced compared with the untreated normal P388D1 cells. Data are presented as  $\bar{x} \pm SD$  ( $P < 0.05$ ;  $n = 4$ ). (B) Expression levels of NALP3 protein exhibited no significant difference compared with those of the normal P388D1 cells ( $P > 0.05$ ;  $\bar{x} \pm SD$ ;  $n = 4$ ). Due to the different gray values in the reverse transcription polymerase chain reaction and western blotting gels, a high relative value in the bar chart above reflects (A) a low level of RNA, while (B) a high level of protein. ATP, adenosine triphosphate; siRNA, small interfering RNA; NS, non-significant;  $\bar{x} \pm SD$ , the mean  $\pm$  standard deviation.

no significant difference compared with those of the normal P388D1 cells ( $P > 0.05$ ,  $\bar{x} \pm SD$ ,  $n = 4$ ) (Fig. 5B).

## Discussion

The results of this study demonstrated that when the P2X7 receptor exists on the cell surface, its activation by ATP induces the upregulation of NALP3 expression levels. In other words, the P2X7 receptor and NALP3 gene may have a conduction effect in the inflammatory response. The results of these experiments are consistent with the expected results.

There have been a number of studies undertaken in this area. A previous study determined that ATP activates the P2X7 receptors via pannexin-1, a hemichannel protein in the cell membrane (18). It has also been determined that when ligands recognized by NALP3 enter the cell through the pannexin-1 channel, they activate caspase-1 causing IL-18 and IL-1 $\beta$  to mature and be secreted (19). The aim of the current study was to systematically analyze the regulation of NALP3 expression by the P2X7 receptor in murine P388D1 macrophage-like cells.

To explore the role of the P2X7 receptor in the regulation of NALP3 expression in murine P388D1 macrophage-like cells, the expression levels of NALP3 mRNA and protein were analyzed by RT-qPCR and western blotting. The results of the two techniques demonstrated that the P388D1 cells expressed NALP3 mRNA and protein, without the activation of the P2X7 receptor.

Notably, a previous study indicated that ATP has an important role in the activation of the P2X7 receptor (20). In

order to study the association between the P2X7 receptor and NALP3, an ATP concentration of 1 mM was used as the native agonist of the P2X7 receptor. P388D1 cells were treated with ATP for 24 h, followed by detection of the expression of NALP3 by RT-PCR and western blotting. The expression levels of NALP3 mRNA and protein increased compared with those of the normal P388D1 cells. This result shows that activating the P2X7 receptor induces the upregulation of NALP3 expression at the gene and protein levels. In contrast, when cells were treated with ADP instead of ATP as a control experiment, the detected expression levels of NALP3 showed no significant change in gene and protein expression levels compared with those of the normal untreated P388D cells. The results of a previous study have shown that ATP activates the NALP3 inflammasome by stimulating the P2X7 receptor, which leads to a sudden drop in the levels of K<sup>+</sup> (21). ATP activates the P2X7 receptor and rapidly reduces the opening of K<sup>+</sup> channels. The activated P2X7 receptor recruits pannexin-1 and mediates a gradual increase in the permeability of the transition pore to open gradually (22), which is finally recognized in the plasma by NALP3.

The current study aimed to determine the association between the P2X7 receptor and NALP3 at the molecular level. It has previously been determined that there are a diverse range of signaling molecules that induce the activation of the NALP3 inflammation complex, hence, there are a variety of ways to activate NALP3 (23,24). The P2X7 receptor, activated by ATP, induces the expression of NALP3. Additionally, the formation of pannexin-1, lysosomal damage and the induction of ROS are all able to induce the expression of NALP3.

In the current study, it was revealed that the NALP3 protein has a certain level of expression in normal P388D1 cells. NALP3 expression may be induced by other means, but at this level it did not activate the P2X7 receptor. Gene silencing technology was used to knockout the NALP3 gene, and ATP was added to the cells. The expression levels of NALP3 were reduced in the knockout cells compared with those of the untreated normal P388D1 cells, demonstrating that the transfection had been effective. Secondly, the NALP3 gene and P2X7 receptor were knocked out simultaneously and ATP was added. The expression levels of NALP3 in this group were detected using RT-qPCR and western blotting, and then compared with the expression levels in the normal P388D1 cells. The results showed that the expression level of the knockout cells was reduced at the gene level, but was not significantly different at the protein level compared with the normal untreated P388D1 cells. Hence, while other paths can induce NALP3 expression, the expression of NALP3 protein showed no increase following the removal of the P2X7 receptor. Upon activation of the P2X7 receptor by ATP the expression levels of NALP3 protein increased compared with those of the normal group.

It should be noted that this study detected the expression of NALP3 only, it did not detect the levels of the NALP3 inflammasome. However, NALP3 is a family member of the NALP3 inflammasome, and it has a similar structure and function to the other NALP3 inflammasome family members (6,8,9,25). As the levels of NALP3 were detected in this study, these can be used to infer that the P2X7 receptor may be involved in the activation of the NALP3 inflammasome. However, further studies are required to confirm this hypothesis.

In conclusion, the results of this study demonstrated that the activity of the P2X7 receptor is associated with the expression of NALP3. All the results indicate that the activated P2X7 receptor in murine P388D1 macrophage-like cells mediates the upregulation of NALP3 expression of NALP3, which is closely associated with the activation of the NALP3 inflammasome. The results of this study indicate that in the inflammatory reaction, macrophages that participate in the activation of the P2X7 receptor may have an important role in activating the NALP3 inflammasome.

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### References

- Bone RC, Balk RA, Cerra FB, *et al*: Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 101: 1644-1655, 1992.
- Nyström PO: The systemic inflammatory response syndrome: definitions and aetiology. *J Antimicrob Chemother* 41 Suppl A: 1-7, 1998.
- Zhang XP and Tian H: Pathogenesis of pancreatic encephalopathy in severe acute pancreatitis. *Hepatobiliary Pancreat Dis Int* 6: 134-140, 2007.
- Ono S, Ichikura T and Mochizuki H: The pathogenesis of the systemic inflammatory response syndrome and compensatory anti-inflammatory response syndrome following surgical stress. *Nihon Geka Gakkai Zasshi* 104: 499-505, 2003 (in Japanese).
- Levels JH, Lemaire LC, van den Ende AE, *et al*: Lipid composition and lipopolysaccharide binding capacity of lipoproteins in plasma and lymph of patients with systemic inflammatory response syndrome and multiple organ failure. *Crit Care Med* 31: 1647-1653, 2003.
- Kihlmark M, Rustum C, Eriksson C, *et al*: Correlation between nucleocytoplasmic transport and caspase-3-dependent dismantling of nuclear pores during apoptosis. *Exp cell Res* 293: 346-356, 2004.
- Martinon F, Pétrilli V, Mayor A, *et al*: Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* 440: 237-241, 2006.
- Antonopoulos C, Cumberbatch M, Dearman RJ, *et al*: Functional caspase-1 is required for Langerhans cell migration and optimal contact sensitization in mice. *J Immunol* 166: 3672-3677, 2001.
- Aravind L, Dixit VM and Koonin EV: The domains of death: evolution of the apoptosis machinery. *Trends Biochem Sci* 24: 47-53, 1999.
- Kummer JA, Broekhuizen R, Everett H, *et al*: Inflammasome components NALP 1 and 3 show distinct but separate expression profiles in human tissues suggesting a site-specific role in the inflammatory response. *J Histochem Cytochem* 55: 443-452, 2007.
- Kanneganti TD, Ozören N, Body-Malapel M, *et al*: Bacterial RNA and small antiviral compounds activate caspase-1 through cryopyrin/Nalp3. *Nature* 440: 233-236, 2006.
- Villani AC, Lemire M, Fortin G, *et al*: Common variants in the NLRP3 region contribute to Crohn's disease susceptibility. *Nature Genet* 41: 71-76, 2008.
- Xia Z, Wang G, Wan C, *et al*: Expression of NALP3 in the spleen of mice with portal hypertension. *J Huazhong Univ Sci Technol Med Sci* 30: 170-172, 2010.
- Hartwig W, Werner J, Jimenez RE, *et al*: Trypsin and activation of circulating trypsinogen contribute to pancreatitis-associated lung injury. *Am J Physiol* 277: G1008-G1016, 1999.
- Bostanci N, Emingil G, Saygan B, *et al*: Expression and regulation of the NALP3 inflammasome complex in periodontal diseases. *Clin Exp Immunol* 157: 415-22, 2009.
- Ting JP, Kastner DL and Hoffman HM: Caterpillars, pyrin and hereditary immunological disorders. *Nature Rev Immunol* 6: 183-195, 2006.
- Codocedo JF, Godoy JA, Poblete MI, *et al*: ATP induces NO production in hippocampal neurons by P2X(7) receptor activation independent of glutamate signaling. *PLoS One* 8: e57626, 2013.
- Kanneganti TD, Lamkanfi M, Kim YG, *et al*: Pannexin-1-mediated recognition of bacterial molecules activates the cryopyrin inflammasome independent of Toll-like receptor signaling. *Immunity* 26: 433-443, 2007.
- Willingham SB, Allen IC, Bergstralh DT, *et al*: NLRP3 (NALP3, Cryopyrin) facilitates in vivo caspase-1 activation, necrosis, and HMGB1 release via inflammasome-dependent and-independent pathways. *J Immunol* 183: 2008-2015, 2009.
- Arandjelovic S, McKenney KR, Leming SS, *et al*: ATP induces protein arginine deiminase 2-dependent citrullination in mast cells through the P2X7 purinergic receptor. *J Immunol* 189: 4112-4122, 2012.
- Surprenant A, Rassendren F, Kawashima E, *et al*: The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor (P2X7). *Science* 272: 735-738, 1996.
- Pelegrin P and Surprenant A: Pannexin-1 mediates large pore formation and interleukin-1 $\beta$  release by the ATP-gated P2X7 receptor. *EMBO J* 25: 5071-5082, 2006.
- Duncan JA, Gao X, Huang MT, *et al*: *Neisseria gonorrhoeae* activates the proteinase cathepsin B to mediate the signaling activities of the NLRP3 and ASC-containing inflammasome. *J Immunol* 182: 6460-6469, 2009.
- Qu Y, Ramachandra L, Mohr S, *et al*: P2X7 receptor-stimulated secretion of MHC class II-containing exosomes requires the ASC/NLRP3 inflammasome but is independent of caspase-1. *J Immunol* 182: 5052-5062, 2009.
- Cerwenka A, Baron JL and Lanier LL: Ectopic expression of retinoic acid early inducible-1 gene (RAE-1) permits natural killer cell-mediated rejection of a MHC class I-bearing tumor *in vivo*. *Proc Natl Acad Sci USA* 98: 11521-11526, 2001.