Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

C-terminal tagging enhances the detection sensitivity of interleukin receptor type 1

Ayuko Moriyama [1, 2], Saya Imaoka [1, 2], Tsuyoshi Sasagawa [3, 4], Machi Hosaka [3, 5], Isao Kato [1, 6], Hiroki Tamura [7, 8], Rie Takeuchi [1, 2], Mariko Tsunoda [2, 9], Masatake Asano [2, 9*]

[1] *Division of Applied Oral Science, Nihon University Graduate School of Dentistry, Tokyo, Japan*
[2] *Department of Pathology, Nihon University School of Dentistry, Tokyo, Japan*
[3] *Division of Oral Structural Functional Science, Nihon University Graduate School of Dentistry, Tokyo, Japan*
[4] *Department of Orthodontology, Nihon University School of Dentistry, Tokyo, Japan*
[5] *Department of Oral Surgery, Nihon University School of Dentistry, Tokyo, Japan*
[6] *Department of Partial Denture Prosthodontics, Nihon University School of Dentistry, Tokyo, Japan*
[7] *Division of Oral Health Sciences, Nihon University Graduate School of Dentistry, Tokyo, Japan*
[8] *Department of Pedodontics, Nihon University School of Dentistry, Tokyo, Japan*
[9] *Division of Immunology and Pathobiology, Dental Research Center, Nihon UniversitySchool of Dentistry, Tokyo, Japan*

Article Info

Article history:

Received: January 18, 2024
Accepted: October 07, 2024
Published: October 31, 2024

Use your device to scan and read the article online

Abstract

Substances released outside of the cells during cell necrosis are collectively called danger-associated molecular patterns (DAMPS) or alarmins. A pro-inflammatory cytokine, interleukin-1α (IL-1α) is known as a typical alarmin. IL-1α transmits signals by binding to IL-1 receptor 1 (IL-1R1), type I protein, expressed on the cell membrane of target cells, but detection of IL-1R1 at the protein and mRNA levels is difficult. Although the reasons are not elucidated, we attempted to add the HiBiT-tag to the N-terminus (N'-R1) or C-terminus (C'-R1) of IL-1R1 to examine whether the detection sensitivity can be augmented. Increase in detection sensitivity will allow the investigation of its function and subcellular localization much further. Using uterine cervical cancer-derived HeLa cells and its derivative CR-R1-4 cells lacking IL-1R1, C'-R1 was demonstrated to significantly increase the detection sensitivity of IL-1R1. Furthermore, the signal transduction function of neither N'-R1 nor C'-R1 was affected. Immunofluorescence cell staining revealed that wild-type IL-1R1 is mainly localized in the nucleus, whereas C'-R1 is localized both in the nucleus and the cytoplasm. The above results showed that adding a tag to the C-terminus of IL-1R1 increases detection sensitivity while maintaining its function. In the future, we would like to further investigate the relationship between changes in the intracellular localization of C'-R1 and increases in detection sensitivity.

Keywords: IL-1R1, Localization, HeLa, Alarmin, HiBiT.

1. Introduction

Substances released outside cells during cell necrosis are collectively called danger-associated molecular patterns (DAMPS) or alarmins [1]. Interleukin-1α (IL-1α) is a typical alarmin, which is produced in cells as a precursor (pIL-1α) and then cleaved by various enzymes to form an N-terminal propiece IL-1α (ppIL-1α) and mature IL-1α (mIL-1α) on the C-terminal side. Of these three molecular species, pIL-1α and mIL-1α are secreted extracellularly and transmit signals by binding to type 1 IL-1 receptor (IL-1R1) expressed on the cell membrane of target cells [2, 3]. IL-1R1 has three immunoglobulin-like domains in the extracellular region and a toll/IL-1 receptor (TIR) domain in the cytoplasm [3]. When pIL-1α or mIL-1α binds to IL-1R1, it recruits IL-1R3, and as a result, the TIR domains of both receptors associate, thereby transmitting a signal. Based on this, the TIR domain is considered to be extremely important for signal transduction.

The prototype of the TIR domain is the insect protein Toll [4, 5]. Toll is responsible for the innate immunity of adult flies and regulates the expression of the Cecropin gene [6]. Since the discovery of Toll, a series of mammalian Tolls have been identified [7] Among them, MyD88 encompassing an N-terminal death domain and a C-terminal TIR domain [4, 8], plays am intrinsic role in IL-1/IL-1R1 signaling [8, 9].

On the other hand, IL-1R2 is considered a decoy receptor because it does not have a TIR domain in the cytoplasm [10, 11] and cannot transmit a signal even if the ligand IL-1α binds to it. Therefore, IL-1R1 is an essential molecule for IL-1α signal transduction. However, IL-1R1 is expressed in extremely small numbers on the cell surface, making it difficult to detect using methods such as flow cytometry [12]. Furthermore, detecting IL-1R1 by

* Corresponding author.
E-mail address: asano.masatake@nihon-u.ac.jp (Masatake Asano).
Doi: http://dx.doi.org/10.14715/cmb/2024.70.10.7

western blot using the commercially available antibodies and IL-1R1-transfected cell lysate was also extremely difficult in our hands. Therefore, we wondered if it would be possible to increase the detection sensitivity of IL-1R1 and enable more detailed analysis of its function and subcellular localization by adding a tag to IL-1R1. In this study, we aimed to improve detection sensitivity by attaching a HiBiT-tag, a peptide tag of 11 amino acids (GTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGC), developed by Promega (WI, USA), to IL-1R1, and also investigated its localization and function.

2. Materials and Methods

2. 1. Cell Culture and ELISA

Human uterine cervical cancer -derived HeLa cells were obtained from the Health Science Research Resource Bank (Osaka, Japan). Cells were grown at 37°C in Dulbecco's Modified Eagle's Medium (DMEM, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal calf serum (FCS, Serana Europe GmbH, Brandenburg, Germany), 50 µg/ml streptomycin, and 50 U/ml penicillin (Sigma-Aldrich, St. Louis, MO, USA) in a 5% CO_2 incubator. Additionally, a cell line lacking IL-1R1 (CR-R1-4 cells [13]) was established using the CRISP/Cas9 system and cultured under the same conditions.

Cell reactivity to IL-1β was determined by culturing the cells (5×10^4 cells / 24 wells) in the presence or absence of recombinant IL-1β (10 ng/ml, PeproTech, Cranbury, NJ, USA) for 3 h. After incubation, the culture supernatant was collected and centrifuged (14,000 × g, 1 min) to remove the cell debris. The supernatants were transferred to new tubes and then subjected to IL-8 measurement with IL-8 ELISA Kit (R&D Systems, Minneapolis, MN, USA).

2. 2. Plasmid construction and transfection

IL-1R1 full-length cDNA was purchased from the Institute of Physical and Chemical Research (Saitama, Japan). Using this as a template, the full length was amplified by PCR, and inserted into the EcoRI site of the pMKIT-neo vector using the Gibson Assembly system (pMKIT-R1, New England Biolabs, Ipswich, MA, USA). Furthermore, a HiBiT- tag was inserted directly below the N-terminal signal peptide using the quick change site-directed mutagenesis method (Agilent Technologies, Santa Clara, CA, USA) (Figure 1A). This vector was designated as the N'-R1 vector. On the other hand, a plasmid with a HiBiT tag inserted just above the stop codon at the C-terminus of IL-1R1 was used in the experiment as C'-R1 (Figure 1A). For functional analysis of IL-1R1, the transmembrane region of pMKIT-R1 vector was deleted using the quick change site-directed mutagenesis method (Agilent Technologies, Santa Clara, CA, USA). These vectors were designated as ΔTM-R1 and used for transfection experiment (Figure 1B).

Transfection was performed using Lipofectamin 3000 (0.75 µl) (Thermo Fisher Scientific, Waltham, MA, USA). Briefly, each plasmid (250 ng) and Plus reagent (0.75 µl) were dissolved in OPTI-MEM (25 µl, GE Healthcare, Chicago, IL, USA) in one tube. In the other tube, Plus 3000 reagent (Thermo Fisher Scientific, Waltham, MA, USA) was mixed with OPTI-MEM (25 µl). Both solutions were mixed and reacted for 15 min at room temperature, then added to the culture medium and incubated at 37°C for 18 h.

2. 3. Fluorescent immunostaining

HeLa cells or CR-R1-4 cells (5×10^4 cells / well) [13] were seeded on a cover slip (10 mm in diameter: Matsunami Glass, Osaka, Japan) in a 24-well plate. After culturing at 37°C for 18 h, transfection was conducted as described above. After transfection, the cells were washed with phosphate-buffered saline (PBS, Takara Bio, Shiga, Japan) warmed to 37°C and fixed with 2% paraformaldehyde (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). Permeabilization was performed with 1% triton X-100 (Nacalai Tesque, Kyoto Japan) / PBS for 10 min at room temperature. After washing with PBS, the coverslip was incubated with 1% bovine serum albumin-containing PBS (1% BSA-PBS) for 15 min at room temperature. After blocking, the cells were incubated with mouse anti-human IL-1R1 (1/3,000, Abcam, Cambridge, CB2 0AX, UK) or mouse anti-HiBiT antibody (1/1,000, Promega, Madison, WI, USA) for 18 h. After washing with PBS, the coverlslips were further incubated with FITC-conjugated goat anti-mouse IgG antibody (Proteintech, Rosemont, IL, USA) for 1 h in the dark. After further washing with PBS, coverslips were mounted on a glass slide using DAPI-Fluoromount-G (Southern Biotech, Birmingham, AL, USA). An all-in-one fluorescence microscope (All-in-one Fluorescence Microscope BZ-X810, Keyence, Osaka, Japan) was used for observation and image capture.

2. 4. Western blotting (WB)

CR-R1-4 cells were transfected with pMKIT-R1, N'-R1, C'-R1 or pMKIT-neo. For WB, the cells were lysed with 100 µl of cell lysis solution (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5% Triton X-100). The samples were collected and centrifuged (14,000 × g, 3 min at 4°C). The supernatants were transferred to the new tubes and total protein concentration was measured using the Bio-Rad protein assay kit (Bio-Rad, California, USA). Fifty ng of protein was electrophoresed using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to Immobilon-P (Sigma-Aldrich, Tokyo,

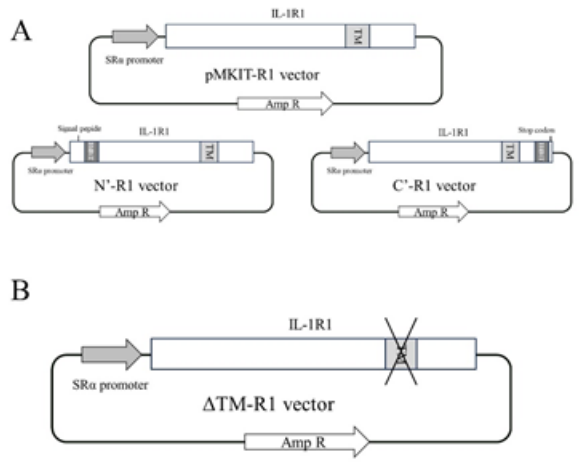

Fig. 1. Expression vector used in the experiment. IL-1R1 was inserted into the EcoRI site of the pMKIT-neo vector (pMKIT-R1) using the Gibson Assembly method. Using pMKIT-R1 as a template, a HiBiT-tag was inserted directly below the N- terminal signal peptide by the quick change site-directed mutagenesis method (N'-R1 vector). Similarly, a HiBiT-tag was inserted just above the C-terminal stop codon (C'-R1). The transmembrane region of IL-1R1 was deleted by site-directed mutagenesis and designated as ΔTM-R1 vector.

Japan). WB was performed according to the previously reported method [14]. Briefly, the membrane was incubated with 1% BSA-PBST (0.5% Tween 20 containing 1% BSA-PBS) for 1 h at room temperature. After blocking, the membrane was further incubated with the primary antibody. As the primary antibody, mouse anti-HiBiT antibody (Promega, Madison, WI, USA) was diluted (1/1,000) with 1% BSA-PBST and incubated for 1 h at room temperature. A horseradish peroxidase (HRP)-labeled goat anti-mouse IgG (H+L) antibody (Jackson ImmunoResearch, West Grove, PA, USA; 1/5,000) was used as the secondary antibody, and the reaction was performed at room temperature for 1 h. GAPDH was used as an endogenous control. Rabbit anti-human GAPDH antibody (1/10,000, Proteintech, Rosemont, IL, USA) was used as the primary antibody, and HRP-labeled goat anti-rabbit IgG (H+L) antibody (Jackson ImmunoResearch, West Grove, PA, USA; 1/6,000) was used as the secondary antibody.

For the analysis of intracellular localization, the cytoplasmic and nuclear extracts were purified using NE-PER™ Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific, Waltham, MA, USA). CR-R1-4 cells were transfected with pMKIT-R1, C'-R1. Total protein concentration was estimated as described above and 50 ng of protein was subjected to WB. Lamin B was used as a nuclear endogenous control. Rabbit anti-human Lamin B antibody (Proteintech, Rosemont, IL, USA; 1/1,000) was used as the primary antibody, and HRP-labeled goat anti-rabbit IgG (H+L) antibody (Jackson ImmunoResearch, West Grove, PA, USA; 1/6,000) was used as the secondary antibody. GAPDH was used as an endogenous control. Mouse anti-human GAPDH antibody (1/10,000, Santa Cruz Biotechnology, Heidelberg, Germany) was used as the primary antibody, and HRP-labeled goat anti-mouse IgG (H+L) antibody (Jackson ImmunoResearch, West Grove, PA, USA; 1/6,000) was used as the third antibody. The bands were detected using a Clarity Western ECL Substrate (Bio-Rad, California, USA). The data were incorporated and analyzed with iBright imaging system (Thermo Fisher Scientific, Waltham, MA, USA).

2. 5. Statistics

Statistically significant differences were determined by two-tailed Student's t-test and Tukey's test. A p-value <0.05 was considered significant. All data were plotted as mean ± standard deviation (SD).

3. Results

3. 1. Importance of cell membrane-spanning region

The reactivity of HeLa cells to IL-1β was examined. In the presence of IL-1β, HeLa cells were induced to secrete 2962.1 ± 281.3 pg/mL of IL-8 to the culture supernatant. In contrast, in the absence of IL-1β, IL-8 secretion was less than 5 pg/mL (Figure 2A). This indicated that IL-1R1 was functionally expressed in HeLa cells. Next, the IL-1β reactivity of the IL-1R1-deficient cell line CR-R1-4 cells [13] was examined. Irrespective of the existence of IL-1β, CR-R1-4 cells did not secrete IL-8 (Figure 2B). When the IL-1R1 was forced expressed in CR-R1-4 cells, significant IL-8 secretion (2882.2 ± 830.4 pg/mL) was observed in response to IL-1β (Figure 2C) compared to pMKIT-neo vector transfectant (control, 808.8 ± 99.8 pg/mL).

Next, we examined the importance of transmembrane region of IL-1R1 for IL-1β reactivity. CR-R1-4 cells were transfected with ΔTM-R1 and stimulated with or without IL-1β. As shown in Figure 2C, reactivity to IL-1β was completely abolished indicating the indispensable role of transmembrane region for IL-1β reactivity.

3. 2. Detection of IL-1R1

Next, we attempted to detect IL-1R1 at the protein level using a cell lysate of IL-1R1 transfectant in CR-R1-4 cells. In WB, a slight band around 75 kDa could be detected in the transfectant, and no IL-1R1 band could be detected in the control (Figure 3A). We further attempted to detect IL-1R1 with commercially available IL-1R1 ELISA kit. However, IL-1R1 was not detected (data not shown). Based on these data, we attempted to increase detection sensitivity of IL-1R1 by adding the HiBiT-tag.

The results of WB were shown in Figure 3B. The clear 75 kDa band was only detected in C'-R1 transfectant. However, the band could be detected neither'in N'-R1 nor pMKIT-R1 transfectant. These results indicated that IL-1R1 detection sensitivity was augmented only when HiBiT-tag was added to C-terminus of IL-1R1.

3. 3. Functional comparison between N- or C-terminus tagging

Next, we investigated changes in receptor function between N- and C-terminus HiBiT-tagging on IL-1R1. When CR-R1-4 cells was transfected with pMKIT-neo,

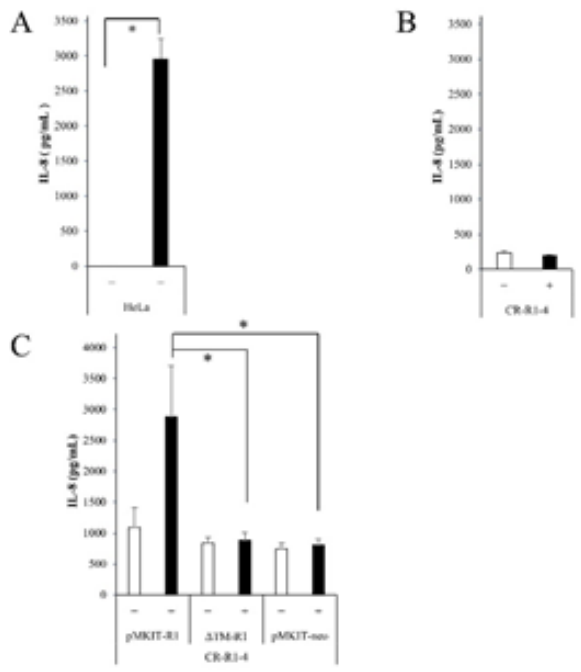

Fig. 2. IL-1β reactivity of HeLa cells. (A) HeLa cells (5 × 10^4 cells/24 wells) were cultured for 3 h in the presence (+) or absence (-) of recombinant IL-1β (10 ng/mL), and the IL-8 concentration in the culture supernatant was measured by ELISA. Each data was expressed as mean ± SD *P<0.05 (n = 3). (B) CR-R1-4 cells (5 × 10^4 cells/24 wells) were cultured for 3 h in the presence (+) or absence (-) of recombinant IL-1β (10 ng/mL), and IL-8 in the culture supernatant was measured by ELISA. (n = 3). © CR-R1-4 cells were transfected with pMKIT-R1, ΔTM-R1, and pMKIT-neo vectors. The transfectants were cultured for 3 h in the presence (+) or absence (-) of recombinant IL-1β (10 ng/mL). The IL-8 concentration in the culture supernatants was measured by ELISA. Each data was expressed as mean ± SD *P<0.05 (n=3).

the cells did not respond to IL-1β (IL-8 in secretion; IL-1β (-) 600.4 ± 49.1 pg/mL vs IL-1β (+) 653.6 ± 49.4 pg/mL). In contrast, pMKIT-R1 (1528.7 ± 113.4 pg/m'), N'-R1 (1695.4 ± 67.2 pg/mL), 'nd C'-R1 transfectants (1381.1 ± 9.9 pg/mL) showed significant IL-1β response (Figure 3C). The results indicated that neither N- nor C-terminus tagging affected IL-1R1 function. Based on these results, we focused on 'he C'-R1 transfectant for further study.

3. 4. Subcellular localization of C'-R1

To examine the C-terminus HiBiT-tagging on the subcellular localization of IL-1R1, C'-R1 and pMKIT-R1 were transfected to CR-R1-4 cells. The nuclear or cytoplasmic extracts were obtained and subjected to WB. In pMKIT-R1 transfectant, IL-1R1 was detected as a faint 75 kDa band in nuclear extract (Figure 4 left upper row) but not in the cytoplasmic extract. In contrast, with the C'-R1 transfectant, IL-1R1 reactivity was significantly augmented. IL-1R1 was detected in both nuclear and cytoplasmic extracts, however, the band in the cytoplasmic extract was extremely stronger than that of nuclear extract (Figure 4, right upper row). The results suggested that adding HiBiT-tag to the C-terminus of IL-1R1 may augment the detection sensitivity of IL-1R1.

We next examined the subcellular localization of IL-1R1 with immunofluorescence cell staining.

When the pMKIT-R1 transfectant was observed, significantly weak fluorescence was mainly detected in the cell nuclei (Figure 5 upper row). In the C'-R1 transfectant, however, fluorescence could be observed not only in the nucleus but also in the cytoplasm (Figure 5, lower row). This suggested that the localization of IL-1R1 may be changed by adding an HiBiT-tag to the C-terminus.

4. Discussion

It has already been shown that HeLa cells enhance IL-8 production in response to IL-1 stimulation [15], and we obtained similar results in this study. This indicated that IL-1R1 and IL-1R3, which are essential for IL-1 signal transduction, are expressed in HeLa cells while maintaining their functions [2]. Therefore, we attempted to detect the expression of endogenous IL-1R1 in HeLa cells using several types of commercially available antibodies. Although IL-1R1 has been reported to be detected in various organs of mice and rats by immunohistochemical techniques [16-18], we observed extremely weak fluorescence only in nuclei of IL-1R1-transfected, but non-transfected, HeLa cells by immunofluorescent staining. However, it could not be detected by WB or ELISA using whole-cell lysate. Our results are in consistent with the previous reports. Although mouse pre-B lymphoblast cells (70Z/3) can transduce IL-1 signal, detection of IL-1R1 at the protein and mRNA levels [19] using conventional methods is extremely difficult. The difficulty might be due to the fact that only about 20 IL-1R1 molecules on the cell surface is sufficient to transduce the IL-1-binding signal [12]. These facts prompted us to examine if it would be possible to increase the detection sensitivity by adding a tag to IL-1R1. For this purpose, we added HiBiT-tag to either N- or C-termini of IL-1R1. The results revealed that only the C-terminus tagging improves the detection sensitivity of IL-1R1. Liu et al. [19] created special genetically modified mice and searched for differences in IL-1R1 function among cell types in the brain. In consistent with our data,

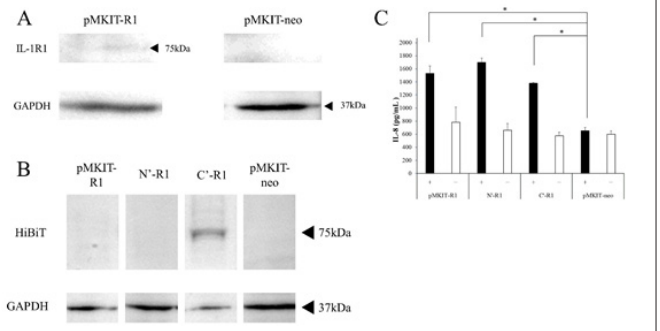

Fig. 3. Detection of IL-1R1 in CR-R1-4 cells. (A) pMKIT-R1 (left) or pMKIT-neo (right) were transfected into CR-R1-4 cells. The cell lysates were subjected to WB. GAPDH was used as an endogenous control. The representative data are shown. (n = 5). (B) CR-R1-4 cells were transfected with pMKIT-R1 (lane 1), N'-R1(lane 2), C'-R1 (lane 3) or pMKIT-neo (lane 4), and the cell lysates were subjected to WB with anti-HiBiT antibody. GAPDH was used as an endogenous control. The representative data are shown. (n = 3). (C) CR-R1-4 cells (5×10^4 cells/24 wells) were transfected with pMKIT-R1, N'-R1, C'-R1, or pMKIT-neo, and cultured in the presence or absence of recombinant IL-1β (10 ng /mL) for 3 h. The culture supernatant was harvested and subjected to IL-8 ELISA. Each data was expressed as mean ± SD *p<0.05 (n = 3).

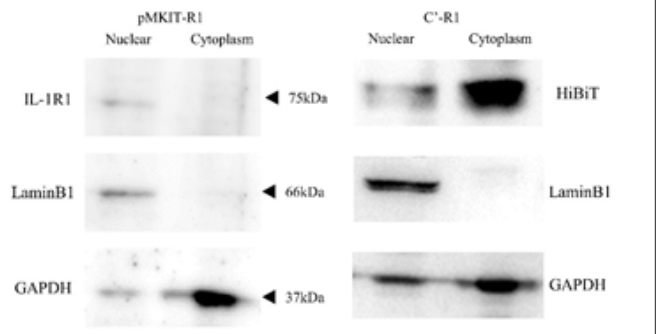

Fig. 4. Comparison of subcellular localization between wild-type IL-1R1 and C'-R1. Cytoplasmic and nuclear fractions were extracted from pMKIT-R1 (left) or C'-R1-transfected CR-R1-4 cells (right) and subjected to WB using anti-IL-1R1 or anti-HiBiT antibodies. GAPDH and LaminB1 were used as internal controls. The representative data are shown. (n = 3).

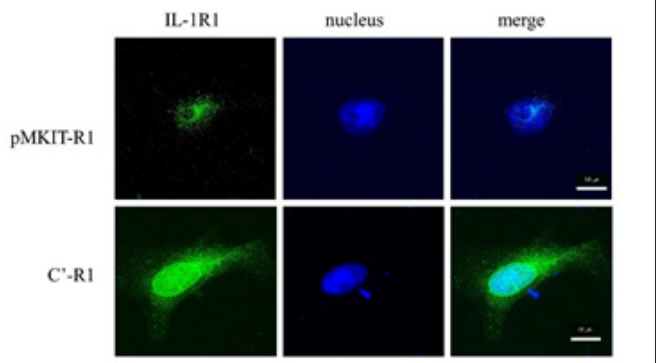

Fig. 4. Comparison of subcellular localization between wild-type IL-1R1 and C'-R1. Cytoplasmic and nuclear fractions were extracted from pMKIT-R1 (left) or C'-R1-transfected CR-R1-4 cells (right) and subjected to WB using anti-IL-1R1 or anti-HiBiT antibodies. GAPDH and LaminB1 were used as internal controls. The representative data are shown. (n = 3).

only C-terminus tagging resulted in the successful IL-1R1 detection. Over expression of C'-R1 to CR-R1-4 cells recovered the reactivity to IL-1β indicating the functional integrity of C'-R1 molecule. Unexpected result was the changes in intracellular localization of C'-R1. IL-1R1 is localized primarily in the nucleus, but C'-R1 moves to the cytoplasmic space.

What is the relationship between changes in the intracellular localization of IL-1R1 and improved detection sensitivity? The intracellular region of IL-1R1 encompasses the TIR domain [20], which is composed of three important regions, box-1, 2 and 3. Both box-1 and 2 are reported to be indispensable for the IL-1R1 signaling [21]. Box-3 is speculated to be important for the adaptor molecule interaction [22]. The change in intracellular localization might be due to the conformational change of IL-1R1 without affecting the signaling function.

Additionally, the fact that signal transduction was not inhibited by adding HiBiT-tag to the C-terminus indicates that it does not significantly affect the association with adapter molecules such as MyD88. IL-1R1 undergoes intramembrane proteolysis (RIP) by presenilin-dependent γ-secretase [23], and the extracellular region is released outside the cell. As a result, plasma membrane and cytoplasmic region, termed the C-terminal domain (CTD), is generated and this CTD has been suggested to be involved in intracellular and nuclear signaling [24]. The increased detection sensitivity of C'-R1 in this study may be due to inhibition of RIP by the HiBiT-tag. This possibility is only confirmed by examining whether CTD-mediated signaling is affected in C'-R1 transfectant or not.

In this study, we were also unable to detect endogenous IL-1R1 mRNA in HeLa. This may be due to mRNA stability [25, 26]. Although there are no reports regarding the stability of IL-1R1 mRNA, it has been reported that the RNA-binding protein tristetraprolin (TTP) is involved in the stability of the ligand IL-1β mRNA [27]. We also investigated the involvement of the intracellular proteolytic system [28] and confirmed that pretreatment with MG132, a proteasome inhibitor, did not affect the detection sensitivity of IL-1R1. (data not shown). IL-1R1 turnover is reported to takes approximately 11 h [29], and this time frame is considered to be sufficient for IL-1R1 detection at the protein level. The CR-R-1-4 cells used to confirm the intracellular localization of IL-1R1 were cells in which IL-1R1 was knocked out using the CRISP/Cas9 system [13]. A higher amount of IL-8 was secreted by CR-R1-4 cells compared to parental HeLa cells. Although the biological function of IL-1R1 nuclear localization is not clarified, this may suggest the involvement of IL-1R1 in gene transcription activity, and we would like to investigate this in the future, as well as why it is difficult to detect IL-1R1 biochemically.

As mentioned above, IL-1R1 was mainly localized in the nucleus in HeLa cell. It has been reported that IL-1 bound to IL-1R1 on the cell surface is internalized and translocated to the nucleus [29-31]. Some of these reports suggest that nuclear localization of IL-1R1 is dependent on a nuclear localization signal present in the N-terminal region of IL-1α, since the nuclear localization of IL-1R1 is not confirmed in the absence of IL-1α [32]. However, in patients with dermatomyositis, IL-1R1 is expressed in the nucleus of skeletal muscle cells [33], and Kuno et al. [34] also found that IL-1R1 has an amino acid sequence extremely similar to NLS at its C-terminus. Using the cell fractionation experiment, nuclear IL-1R1 was partially translocated to the cytoplasm by adding HiBiT-tag to the C-terminus. This suggested the possibility that the above sequence functions as an NLS, and that its function was inhibited by the addition of the HiBiT-tag. The meaning of IL-1R1 nuclear localization and its function should be clarified in future research.

Conflict of interests
The author has no conflicts with any step of the article preparation.

Consent for publications
The author read and approved the final manuscript for publication.

Ethics approval and consent to participate
No human or animals were used in the present research.

Informed consent
The authors declare that no patients were used in this study.

Availability of data and material
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions
Ayuko Moriyama and Masatake Asano conceived the study and designed the experiments. Ayuko Moriyama, Saya Imaoka, Tsuyoshi Sasagawa, Machi Hosaka, Isao Kato, Hiroki Tamura, Rie Takeuchi, Mariko Tsunoda contributed to the data collection. Ayuko Moriyama and Masatake Asano performed the data analysis and interpreted the results. Ayuko Moriyama and Masatake Asano wrote the manuscript. All authors have read and approved the manuscript.

Funding
This work was supported by a Grant-in-aid for Scientific Research (C) (2020-2022), a Grant-in-aid for Young Scientists (2019-2021) and (22K17027). The Research grant from Scholarship Fund for Young/Women Researchers and Uemura Yasuo Haruko Fund; a grant from the Dental Research Center, Nihon University School of Dentistry.

References

1. Oppenheim JJ, Yang D. (2005) Alarmins: chemotactic activators of immune responses. Curr Opin Immunol 17(4):359-365. doi: 10.1016/j.coi.2005.06.002
2. Di Paolo NC, Shayakhmetov DM. (2016) Interleukin 1α and the inflammatory process. Nature Immunol 17(8):906-913. doi: 10.1038/ni.3503
3. Boraschi D, Italiani P, Weil S, Martin M U. (2018) The family of the interleukin-1 receptors. Immunol Rev 281(1):197-232. doi: 10.1111/imr.12606
4. Mitcham J L, Parnet P, Bonnert T P, Garka K E, Gerhart M J, Slack J L, et al. (1996) T1/ST2 Signaling Establishes It as a Member of an Expanding Interleukin-1 Receptor Family. J Biol Chem 271(10):5777–5783. doi: 10.1074/jbc.271.10.5777
5. Ravizza T, Vezzani A. (2006) Status epilepticus induces time-dependent neuronal and astrocytic expression of interleukin-1

receptor type I in the rat limbic system. Neuroscience 137(1):301-308. doi: 10.1016/j.neuroscience.2005.07.063

6. Lemaitre B, Nicolas E, Michaut L, Reichhart J M, Hoffmann J A. (1996) The dorsoventral regulatory gene cassette spätzle/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell 86(6), 973–983. doi: 10.1016/s0092-8674(00)80172-5
7. Rock F L, Hardiman G, Timans J C, Kastelein R A, Bazan J F. (1998) A family of human receptors structurally related to Drosophila Toll. Proc Natl Acad Sci U S A 95(2):588–593. doi: 10.1073/pnas.95.2.588
8. Wesche H, Henzel W J, Shillinglaw W, Li S, Cao Z. (2013) Pillars article: MyD88: an adapter that recruits IRAK to the IL-1 receptor complex. Immunity. 1997. 7: 837-847. J Immnunol 190(1):5-15. PMID: 23264669
9. Medzhitov R, Preston-Hurlburt P, Kopp E, Stadlen A, Chen C Q, Ghosh S, et al. (1998) MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. Mol Cell 2(2):253–258. doi: 10.1016/s1097-2765(00)80136-7
10. Curtis B M, Gallis B, Overell R W, McMahan C J, DeRoos P, Ireland R, et al. (1989) T-cell interleukin 1 receptor cDNA expressed in Chinese hamster ovary cells regulates functional responses to interleukin 1. Proc Natl Acad Sci USA 86(9):3045-3049. doi: 10.1073/pnas.86.9.3045
11. Muñoz E, Zubiaga A M, Sims J E, Huber B T. (1991) IL-1 signal transduction pathways. I. Two functional IL-1 receptors are expressed in T cells. J Immunol 146(1):136-143. PMID: 1845804
12. Sims J E, Gayle M A, Slack J L, Alderson M R, Bird T A, Giri J G, et al. (1993) Interleukin 1 signaling occurs exclusively via the type I receptor. Proc Natl Acad Sci USA 90(13):6155-6159. doi: 10.1073/pnas.90.13.6155
13. Hanayama M, Ishiyama Y, Sadamura M, Moriyama A, Imaoka S, Tsunoda M, et al. (2022) Establishment of interleukin-1 receptor type 1 knockout Hela strain by CRISP/Cas9 system. J Biomed Sci Res 4(2) :161. doi: 10.36266/JBSR/161
14. Omagari D, Mikami Y, Suguro H, Sunagawa K, Asano M, Sanuki E, et al. (2009) Poly I:C-induced expression of intercellular adhesion molecule-1 in intestinal epithelial cells. Clin Exp Immunol 156(2):294-302. doi: 10.1111/j.1365-2249.2009.03892.x
15. Yang H T, Cohen P, Rousseau S. (2008) IL-1 beta-stimulated activation of ERK1/2 and p38alpha MAPK mediates the transcriptional up-regulation of IL-6, IL-8 and GRO-alpha in HeLa cells. Cell Signal 20(2):375-380. doi: 10.1016/j.cellsig.2007.10.025
16. Konsman J P, Vigues S, Mackerlova L, Bristow A, Blomqvist A. (2004) Rat brain vascular distribution of interleukin-1 type-1 receptor immunoreactivity: relationship to patterns of inducible cyclooxygenase expression by peripheral inflammatory stimuli. J Comp Neurol 472(1):113-129. doi: 10.1002/cne.20052
17. Matsuwaki T, Eskilsson A, Kugelberg U, Jönsson J, Blomqvist A. (2014) Interleukin-1 beta induced activation of the hypothalamus-pituitary-adrenal axis is dependent on interleukin-1 receptors on non-hematopoietic cells. Brain Behav Immun 40:166-173. doi: 10.1016/j.bbi.2014.03.015
18. Ravizza T, Vezzani A. (2006) Status epilepticus induces time-dependent neuronal and astrocytic expression of interleukin-1 receptor type I in the rat limbic system. Neuroscience 137(1):301-308. doi: 10.1016/j.neuroscience.2005.07.063
19. Liu X, Yamashita T, Chen Q, Belevych N, Mckim D B, Tarr A J, et al. (2005) Interleukin 1 type 1 receptor restore: a genetic mouse model for studying interleukin 1 receptor-mediated effects in specific cell types. J Neurosci 35(7):2860-2870. doi: 10.1016/j.neuroscience.2005.07.063
20. Gay N J, Keith F J. (1991) Drosophila Toll and IL-1 receptor. Nature 351(6325):355-356. doi: 10.1038/351355b0
21. Slack J L, Schooley K, Bonnert T P, Mitcham J L, Qwarnstrom E E, Sims J E, et al. (2000) Identification of two major sites in the type I interleukin-1 receptor cytoplasmic region responsible for coupling to pro-inflammatory signaling pathways. J Biol Chem 275(7):4670-4678. doi: 10.1074/jbc.275.7.4670
22. Radons J, Falk W, Dove S. (2015) Identification of critical regions within the TIR domain of IL-1 receptor type I. Int J Biochem Cell Biol 68:15-20. doi: 10.1016/j.biocel.2015.08.009
23. Elzinga B M, Twomey Ciara, Powell J C, Harte F, McCarthy J V. (2009) Interleukin-1 receptor type 1 is a substrate for gamma-secretase-dependent regulated intramembrane proteolysis. J Biol Chem 284(3):1394-1409. doi: 10.1074/jbc.M803108200.
24. Hébert S S, Serneels L, Tolia A, Craessaerts K, Derks C, Filippov M A, Müller U, Strooper B D. (2006) Regulated intramembrane proteolysis of amyloid precursor protein and regulation of expression of putative target genes. EMBO Rep 7(7):739-745. doi: 10.1038/sj.embor.7400704
25. Wells M L, Parera L, Blackshear P J. (2017) An ancient family of RNA-binding proteins: Still important! Trends Biochem Sci 42(4):285-296. doi: 10.1016/j.tibs.2016.12.003
26. Sung H B, Yoon K K. (2020) The emerging role of RNA modifications in the regulation of mRNA stability. Exp Mol Med 52(3):400-408. doi: 10.1038/s12276-020-0407-z
27. Sneezum L, Eislmayr K, Dworak H, Sedlyarov V, Heron A L, Ebner F, et al. (2020) Context-dpendent IL-1 mRNA-destabilization by TTP prevents dysregulation of immune homeostasis under steady state conditions. Front Immunol 11:1398. doi: 10.3389/fimmu.2020.01398
28. Yanan L, Shujing L, Huijian W. (2022) Ubiquitination-proteasome system (UPS) and autophagy two main protein degradation machineries in response to cell stress. Cells 11(5):851. doi: 10.3390/cells11050851
29. Mizel S B, Kilian P L, Lewis J C, Paganelli K A, Chizzonite R A. (1987) The interleukin 1 receptor. Dynamics of interleukin 1 binding and internalization in T cells and fibroblasts. J Immunol 138(9):2906-2912. doi: https://doi.org/10.4049/jimmunol.138.9.2906
30. Qwarnstrom E E, Page R C, Gillis S, Dower S K. (1988) Binding, internalization, and intracellular localization of interleukin-1 beta in human diploid fibroblasts. J Biol Chem 263(17):8261-8269. doi: https://doi.org/10.1016/S0021-9258(18)68472-8
31. Grenfell S, Smithers N, Miller K, Solari R. (1989) Receptor-mediated endocytosis and nuclear transport of human interleukin l alpha. Biochem J 264(3):813-822. doi: 10.1042/bj2640813
32. Curtis B M, Widmer M B, deRoos P, Qwarnstrom E E. (1990) IL-1 and its receptor are translocated to the nucleus. J Immunol 144(4):1295-1303. doi: https://doi.org/10.4049/jimmunol.144.4.1295
33. Grundtman C, Salomonsson S, Dorph C, Bruton J, Andersson U, Lundberg I E. (2007) Immunolocalization of interleukin-1 receptors in the sarcolemma and nuclei of skeletal muscle in patients with idiopathic inflammatory myopathies. Arthritis Rheum 56(2):674-687. doi: 10.1002/art.22388
34. Kuno K, Okamoto S, Hirose K, Murakami S, Matsushima K. (1993) Structure and Function of the Intracellular Portion of the Mouse Interleukin 1 Receptor (Type I). J Biol Chem 268(18):13510-13518. doi: https://doi.org/10.1016/S0021-9258(19)38679-X