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Irisin supports integrin-mediated cell adhesion of lymphocytes

Phyoe Kyawe Myint [a], Atsushi Ito, M.D. [a,b], Michael G. Appiah [a], Gideon Obeng [a], Samuel Darkwah, Ph.D. [a], Eiji Kawamoto, M.D., Ph.D. [a,c], Arong Gaowa, Ph.D. [a], Eun Jeong Park, Ph.D. [a], Motomu Shimaoka, M.D., Ph.D. [a,*]

[a] Department of Molecular Pathobiology and Cell Adhesion Biology, Mie University Graduate School of Medicine, Japan
[b] Department of Cardiothoracic and Vascular Surgery, Mie University Graduate School of Medicine, Japan
[c] Department of Emergency and Disaster Medicine, Mie University Graduate School of Medicine, Japan

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ABSTRACT

Irisin, a myokine released from skeletal muscle, has recently been found to act as a ligand for the integrins αVβ5, αVβ1, and α5β1 expressed on mesenchymal cells, thereby playing an important role in the metabolic remodeling of the bone, skeletal muscle and adipose tissues. Although the immune-modulatory effects of irisin in chronic inflammation have been documented, its interactions with lymphocytic integrins have yet to be elucidated. Here, we show that irisin supports the cell adhesion of human and mouse lymphocytes. Cell adhesion assays using a panel of inhibitory antibodies to integrins have shown that irisin-mediated lymphocyte adhesion involves multiple integrins including not only α4β1 and α5β1, but also leukocyte-specific αLβ2 and α4β7. Importantly, mouse lymphocytic TK-1 cells that lack the expression of β1 integrins have exhibited αLβ2- and α4β7-mediated cell adhesion to irisin. Irisin has also been demonstrated to bind to purified recombinant integrin αLβ2 and α4β7 proteins. Thus, irisin represents a novel ligand for integrin αLβ2 and α4β7, capable of supporting lymphocyte cell adhesion independently of β1 integrins. These results suggest that irisin may play an important role in regulating lymphocyte adhesion and migration in the inflamed vasculature.

1. Introduction

Integrins are the largest family of cell-adhesion molecules that mediate adhesive interactions with cognate ligands on opposing cells and in the extracellular matrix (ECM) across a wide range of physiological and pathological processes. There are 18 α integrin subunits and 8 β integrin subunits, assembling at least 24 distinct integrin heterodimers expressed across almost all cell-types in humans and mice [1]. These heterodimeric molecules bind to a distinct array of ligands expressed on cells and/or deposited in the ECM in order to exert biological processes [2]. Depending on the cell types expressing integrins, integrin-mediated cell adhesion plays an important role in various biological phenomenon such as wound healing, host defense, inflammation, thrombosis and cancer metastasis [1,3]. The integrins αLβ2 (a.k.a. lymphocyte function-associated antigen 1, LFA-1) and α4β7 (a.k.a. lymphocyte Peyer's patch adhesion molecules, LPAM-1) represent a subset of integrins exclusively expressed on leukocytes, most prominently on lymphocytes [1,4]. The integrin αLβ2 binds to its major ligand intercellular adhesion molecule-1 (ICAM-1) expressed on endothelial cells. In this way it regulates the systemic re-circulation of lymphocytes though lymph nodes and leukocyte migration to inflamed organs [5]. Integrin α4β7 binds to mucosal addressin cell-adhesion molecule-1 (MAdCAM-1) expressed on gut endothelial cells, thereby governing tissue-specific lymphocyte homing to the intestine [6]. The integrins αLβ2 and α4β7 on lymphocytes cooperatively support adhesive interactions with vascular endothelial cells, thereby regulating lymphocyte extravasation to lymph nodes or inflamed tissues [7].

Irisin is an exercise-inducible peptide proteolytically cleaved from the N-terminal portion of fibronectin type III domain-containing protein 5 (FNDC5) and is secreted into the extracellular space, thereby

Abbreviations: FNDC-5, fibronectin type III domain-containing protein 5; LFA-1, lymphocyte function-associated antigen 1; LPAM-1, lymphocyte Peyer's patch adhesion molecules; ICAM-1, intercellular adhesion molecule-1; MAdCAM-1, mucosal addressin cell adhesion molecule-1; ECM, extracellular matrix; Fc, fragment crystallizable; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; PBMCs, peripheral blood mononuclear cells.

* Corresponding author. Department of Molecular Pathobiology and Cell Adhesion Biology, Mie University Graduate School of Medicine, 2-174 Edobashi, Tsu-city, Mie, 514-8507, Japan.
E-mail address: shimaoka@med.mie-u.ac.jp (M. Shimaoka).

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functioning as a myokine [8]. Irisin has been shown to induce the browning of fat [9], improve metabolic diseases [10,11], and exhibit anti-tumor effects [12]. More recently, irisin has been shown to act as an emerging ligand for a subset of integrins including αVβ5, αVβ1, and α5β1 and plays an important role in metabolic regulation [13]. Irisin secreted from skeletal muscles binds to αVβ5 integrin expressed on osteocytes, thereby inducing the production of sclerostin that causes bone resorption [14]. Irisin also binds to αVβ5 and αVβ1 integrins expressed on adipocyte progenitor cells, thereby mediating *de novo* beige fat biogenesis [15]. Increased concentrations of irisin in the circulation under chronic inflammation conditions such as obesity [16,17] and cancers [18,19] have been reported. Circulating irisin can bind to αVβ5 integrin expressed on endothelial cells, thereby regulating barrier function. Alternatively, increased irisin in the circulation can be deposited on endothelial cells and in the ECM, potentially acting as adhesive substrates for lymphocytes. However, whether the lymphocytic integrins αLβ2 and α4β7 bind to irisin has yet to be demonstrated. Here, we show that irisin binds not only to β1 integrins, but also to αLβ2 and α4β7 integrins, thereby cooperatively supporting lymphocyte adhesion. The results suggest that irisin deposited on inflamed tissues plays an important role in modulating lymphocyte migration.

2. Materials and methods

2.1. Construction of the recombinant human irisin-immunoglobulin Fc portion fusion protein (Irisin-Fc) expression vector

Gene insert-encoding human irisin (ectodomain of FNDC5) was amplified by polymerase chain reaction from a pHLSec2-irisin-his vector with the entire sequence of irisin using the KOD FX Neo reagents (TOYOBO, Osaka, Japan) and digested with restriction enzymes, HindIII and BamHI. pHLSec2-irisin-his was a gift from Harold Erickson (Addgene plasmid # 122729) [20]. A forward primer with an engineered HindIII restriction enzyme site (5′GTTTAAACTTAAGCTTGCCA CCATGGGCATCCTT3′) and a reverse primer with an engineered BamHI site (5′GAGTTTTGTCGGATCCCTCCTTCATGGTCACCTC3′) were used. An expression plasmid vector encoding irisin-Fc protein was constructed by sub-cloning the digested insert into the Fc vector plasmid (Addgene plasmid # 8636) at the in-frame site to the gene encoding the Fc receptor of human immunoglobulin gamma-1 (IgG-1) using a Gibson Assembly.

2.2. Expression and purification of Irisin-Fc fusion proteins

Expression of irisin-Fc protein was induced by the transfection of irisin-Fc plasmid into FreeStyle 293-F cells (Thermo Fisher Scientific Japan, Tokyo, Japan) using Lipofectamine 2000 reagents (Invitrogen, Tokyo, Japan). An Fc plasmid without sub-cloning was used as a control plasmid to produce control Fc protein. The transfectants were cultured in serum-free Opti-MEM (Gibco, Invitrogen, Tokyo, Japan) and the supernatant was collected after seven days. Irisin-Fc or control Fc protein secreted in the supernatant were purified using the Protein A-affinity Amicon Pro purification system (Millipore-Japan, Tokyo, Japan). The purity of Irisin-Fc and control Fc proteins was determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequent staining with Coomassie Brilliant Blue dye (EzStain Aqua, ATTO, Osaka, Japan). One microgram of each protein, determined by the bicinchoninic acid (BCA) method, was reduced and denatured with 2-mercaptoethanol at 95 °C for 5 min. Western blot analysis using goat anti-human IgG (Fc specific)-antibody conjugated with horseradish peroxidase (Sigma-Aldrich) incubated at 4 °C overnight was performed to detect the Fc portion of proteins. Signal development of blots was conducted using an ImmunoStar®LD luminescence kit (Waka, Osaka, Japan) and images were analyzed with a ImageQuant LAS 4000mini (GE Healthcare, Tokyo, Japan).

2.3. Cell culture

Jurkat and TK1 cells were obtained from ATCC (Manassas, VA, USA) and cultured according to the manufacturer's instructions. Briefly, cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Nacalai Tesque, Kyoto, Japan) supplemented with 10% fetal bovine serum (FBS) (Equitech-Bio, Kerrville, TX), penicillin (100 U/mL)/ streptomycin (100 μg/mL) (Nacalai Tesque, Kyoto, Japan) in an atmosphere of 5% CO_2 at 37 °C until they reached approximately 80% confluency for further procedures.

2.4. Preparation of peripheral blood mononuclear cells (PBMCs)

Isolation of PBMCs was carried out as previously described [21]. Blood samples were collected from healthy volunteers in citrate-treated tubes after obtaining informed consent for the procedure. Blood samples were subjected to a Percoll Plus (GE Healthcare) density gradient centrifugation at 1000 ×*g* for 20 min. Subsequently, the supernatant containing PBMCs was collected and centrifuged at 1200 rpm for 5 min. Finally, PBMCs were suspended in HEPES-buffered saline (HBS). The Institutional Review Board of Mie University School of Medicine approved the study protocol (approval number: 3026).

2.5. Cell-adhesion assay

A cell-adhesion assay using 96-well V-bottom plates was performed as previously reported [22]. Briefly, Irisin-Fc and control Fc protein were prepared in 10 μg/ml phosphate-buffered saline (PBS) and a 100-μl aliquot of protein preparation was dispensed in each well. Ten μg/ml Bovine serum albumin (BSA) was used as a negative control, while 1 μg/ml human recombinant ICAM-1-Fc chimera protein (R&D systems, Minneapolis, MN, USA), 1 μg/ml human recombinant MAdCAM-1 Fc chimera protein (R&D systems, Minneapolis, MN, USA), 1 μg/ml mouse recombinant ICAM-1-Fc chimera protein (BioLegend, San Diego, CA, USA), or 1 μg/ml fibronectin (Sigma-Aldrich, Tokyo, Japan) was included as a positive control. Plates were incubated at 4 °C overnight and twice washed with PBS. The plates were then blocked by 1% BSA in HBS at 37 °C for 1 h. A one-hundred microliter aliquot of HBS containing 2 x 10^4 PBMCs or Jurkat cells labelled with 2′, 7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF, Life Technologies, Eugene, OR, USA), either in the presence of 2 mM ethylenediaminetetraacetic acid (EDTA) or 5 mM $MgCl_2$ and 1 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), was dispensed in each well. Samples in the presence of EDTA were used for determining background adhesion, as integrin-mediated interactions require divalent cations such as Mg^{2+}, and $MgCl_2$ plus EGTA are known to induce activated ligand-competent integrins [22]. For antibody inhibition experiments, BCECF-labeled cells were incubated with 0.5 μg/ml of blocking monoclonal antibodies (mAb) to integrins or control IgG at 37 °C for 10 min before dispensing into each well. The plates were subsequently incubated at room temperature for 5 min and then centrifuged at 800 rpm unless otherwise indicated for 5 min using a swinging bucket rotor (EX-125, TOMY SEIKO, Osaka, Japan). Accumulated non-adhered cells at the nadir of the V-bottom wells were detected by a 2030 ARVO X-2 Multilabel Reader (PerkinElmer Japan, Kanagawa, Japan). The binding percentage of the cells to the V-bottom plate was calculated as previously described [22].

2.6. Flow cytometry

Flow cytometry analysis was used to detect the cell-surface expression of different integrin subunits in TK1 cells, Jurkat cells and PBMCs. Cells (2 x 10^5) were twice washed with flow-cytometry staining buffer (FACS buffer) containing phosphate buffer saline supplemented with 2% FBS, 0.05% NaN_3 and 2 mM EDTA. They were then subjected to probing with fluorescence-conjugated primary antibodies, and incubated on ice

for 1 h. Subsequently, excess antibodies were washed with FACS buffer. Finally, cells were resuspended in 250 μl of FACS buffer before being analyzed with a BD Accuri C6 plus flow cytometer (BD Biosciences, San Jose, CA).

2.7. Reagents and antibodies

The PE-conjugated monoclonal antibodies (mAbs) against human-integrin α1, α2, α3, α4, α5, α6, α8, α9, αE, β2, β5, β7, the APC-conjugated anti-integrin αM, αX mAb, the FITC-conjugated anti-human-integrin β3, β1, αL and their respective isotype IgG mAbs were purchased from BioLegend (San Diego, CA, USA). Anti-human-integrin β1 mAb (P5D2) was purchased from R&D Systems (Minneapolis, MN, USA) and the anti-human integirn α5 mAb (P1D6) was obtained from Abcam (Cambridge, MA, USA). The anti-human integrin α4 mAb (P4C2), the anti-human integirn αL mAb (TS1/22), the anti-human integrin β2 mAb (TS1/18), and the mouse IgG1 isotype control mAb (MOPC-21) were purchased from BioLegend (San Diego, CA, USA). The conjugated mAbs against mouse integrins and their respective isotype control mAbs were obtained from BioLegend (San Diego, CA, USA). Anti-mouse-integrin β2 mAb (M18/2) was purchased from eBioscience (San Diego, CA, USA), while anti-mouse integrin α4 mAb (PS/2) was from Southern Biotech (Birmingham, USA). The αL mAb (H155-78), the β7 mAb (FIB27) and isotype control antibody were purchased from BioLegend (San Diego, CA, USA).

2.8. Enzyme-linked immunosorbent assay (ELISA)

The interactions of irisin with integrins αLβ2 and α4β7 were studied by an ELISA-type experiment using purified recombinant integrin αLβ2 and α4β7 proteins as previously described [23]. Briefly, recombinant human integrin α4β7 (Cat: 5397-A3, R&D Systems), recombinant human integrin αLβ2 (Cat: 3868-AV, R&D Systems), or recombinant mouse integrin αLβ2 (Cat: 7825-AB, R&D Systems) to the concentration of 3 μg/ml was immobilized to wells of a 96-well transparent microtiter plates and incubated overnight at 4 °C. Bovine serum albumin at 3 μg/ml in HEPES buffer saline (HBS) was included as a negative control. Integrin- or BSA-coated wells were washed 3 times with HBS and blocked with a blocking buffer (1% BSA in HBS) for 1 h at room temperature. After washing wells 3 times with HBS, 100-μl aliquot of HBS containing 2 or 10 μg/ml irisin and 1 mM $MgCl_2$ was dispensed to each well. Fc protein (2 μg/ml) was used as a negative control. Recombinant human MAdCAM-1-Fc protein (2 μg/ml) (Cat: 6056-MC, R&D Systems), recombinant human ICAM-1-Fc protein (2 μg/ml) (Cat: 720-IC, R&D Systems), and recombinant mouse ICAM-1-Fc protein (2 μg/ml) (Cat: 553004, BioLegend) were used as positive controls for human integrin α4β7, human integrin αLβ2, and mouse integrin αLβ2, respectively. After incubating for 3 h at room temperature, the plate was washed three times with HBS. Then, 100-μl aliquot of HBS containing horseradish peroxidase-conjugated goat anti-human immunoglobulin G (Fc specific)-antibody (Sigma-Aldrich) diluted at 1:3000 was added and incubated at 4 °C for 1 h. After washing wells three times with HBS, 100μL/well substrate solution (BD OptEIA; BD Biosciences, Franklin Lakes, NJ, USA) was added and reaction allowed to proceed for 20 min before being stopped by the addition of 100μL/well stop solution (BD OptEIA; BD Biosciences, Franklin Lakes, NJ, USA). Absorbance at OD450 was measured with the 680XR microplate reader (Bio RAD).

2.9. Statistical analysis

Statistical analysis was performed using SPSS software v24.0 (IBM Crop, Armonk, NY, USA). All data were presented as the mean ± S.E.M (standard error of mean). Comparisons between two groups was done using the Student's *t*-test (independence, two-tailed), while those involving more than two groups were carried out using one-way ANOVA (analysis of variance), followed by Tukey post-hoc test. A probability (p) value < 0.05 was regarded as statistically significant.

3. Results

3.1. Irisin supports cell adhesion of lymphocytic jurkat cells in a concentration-dependent manner

Irisin protein was recombinantly expressed as a Fc-fusion protein in a mammalian cell-line. Irisin-Fc-fusion protein and Fc protein, as a reference, secreted to the culture supernatant were affinity-purified to homogeneity using a protein A column, as was evident by a major single band appearing in the SDS-polyacrylamide gel electrophoresis (PAGE) under reducing conditions followed by Coomassie Brilliant Blue (CBB) staining (Fig. 1A). Irisin-Fc and Fc proteins migrated at 60 kDa and 36 kDa, respectively, displaying a larger molecular weight than that estimated by the deduced amino acid sequences due to glycosylation (Fig. 1A). The intactness of the fusion protein was confirmed by the presence of the Fc portion of both proteins detected by Western Blot analysis probed with goat anti-human-Fc IgG conjugated with horseradish-peroxidase (data not shown).

To investigate the adhesive interaction of irisin with human lymphocytic Jurkat cells, we carried out a cell-adhesion assay in which either irisin-Fc protein or control Fc protein was immobilized onto the bottom of 96-well plates. ICAM-1-Fc and fibronectin were included as positive controls (Fig. 2A). The cell-adhesion assay involving Jurkat cells using different concentrations of ligands exhibited concentration-dependent, sigmoidal-like binding curves to the immobilized ligands. Jurkat cells binding to ICAM-1 and fibronectin reached the plateau at 2 μg/ml, with approximately 50% maximal binding at 1 μg/ml (Fig. 1B). In contrast, Jurkat cells binding to irisin plateaued at 10 μg/ml with 50% maximal binding at 6 μg/ml (Fig. 1B). As we did not measure the site densities of ICAM-1, fibronectin, or irisin immobilized on plastic, we were unable to accurately compare the binding strengths of each interaction.

3.2. Integrin αLβ2 supports Jurkat and PBMC adhesion to irisin by cooperating with integrins α4β1 and α5β1

While Jurkat cells highly express the integrins α4β1, α5β1, and αLβ2, they express only low levels of αV integrin (Fig. 3A). To study which integrins support cell adhesion to irisin, we tested a panel of inhibitory antibodies to integrins using cell-adhesion assays (Fig. 4A). The antibody to the β1 integrin subunit that blocks both α4β1 and α5β1 integrins inhibited cell adhesion of Jurkat cells to irisin. The antibody to the α4 subunit that blocks α4β1 integrin, as well as the antibody to the α5 subunit that blocks α5β1 integrin, both partially inhibited cell adhesion of Jurkat cells to irisin. Of note, the antibody to the αL subunit and that to the β2 subunit, both of which block αLβ2 integrin, partially inhibited the adhesion of Jurkat cells to irisin. These results suggest that α4β1, α5β1, and αLβ2 cooperatively support Jurkat cell adhesion to irisin.

We have extended our investigations to human primary cells using peripheral blood mononuclear cells (PBMC) that predominantly express α4β1, α5β1, and αLβ2 (Figs. 2B and 3B). Using PBMCs, we have obtained similar results to those using Jurkat cells, thereby confirming that multiple integrins -namely, α4β1, α5β1, and αLβ2 - cooperatively support cell adhesion to irisin (Fig. 4B).

3.3. Mouse lymphocyte TK-1 cells exhibit αLβ2 and α4β7 integrin-mediated cell adhesion to irisin

As the amino acid sequences of human and mouse irisin are identical, we expanded our focus to examine the ability of irisin to support the cell adhesion of mouse lymphocyte TK-1 cells. TK-1 cells express only the integrins αLβ2 and α4β7, but lack β1, β3, and β5 integrins (Figs. 2C and 3C). Cell-adhesion assays in the presence or absence of a panel of inhibitory antibodies have demonstrated robust cell adhesion to ICAM-1

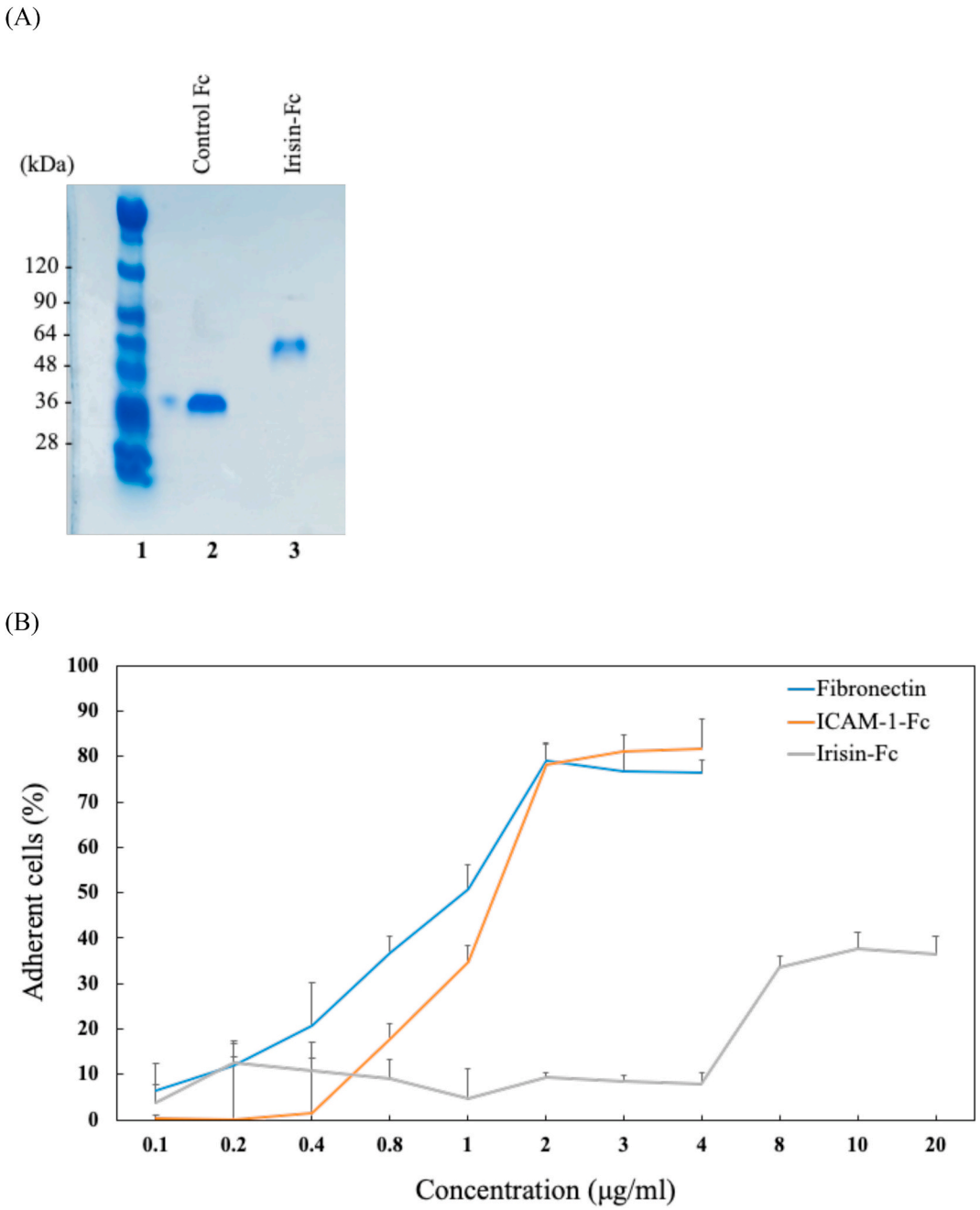

Fig. 1. Cell adhesion of lymphocytic Jurkat cells to irisin, in comparison to ICAM-1, and fibronectin (A) A representative SDS-PAGE image of recombinant irisin-Fc fusion protein; lane 1 protein molecular weight maker; lane 2, control-Fc protein; lane 3, irisin-Fc protein. (B) Jurkat cells showed concentration-dependent binding to fibronectin (FN, blue), inter-cellular adhesion molecule-1 (ICAM-1, red), and irisin (gray). Data are expressed as the mean ± SEM of three independent experiments carried out in triplicate. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and MAdCAM-1, which serve as reference ligands for αLβ2 and α4β7, respectively. Furthermore, we have shown that irisin supports cell adhesion of TK-1 cells in an αLβ2 and α4β7 integrin-dependent manner independently of β1, β3, and β5 integrins (Fig. 4C).

3.4. Binding of irisin to purified recombinant integrin αLβ2 and α4β7 proteins

To confirm the specific interactions of irisin with integrin αLβ2 and α4β7, we have performed ELISA-type experiments using purified recombinant integrin proteins. We used commercially available recombinant integrin proteins of mouse αLβ2 (Fig. 5A), human αLβ2 (Fig. 5B), and human α4β7 (Fig. 5C), but not mouse α4β7 that is currently unavailable. ICAM-1- and MAdCAM-1-Fc have shown good specific binding to αLβ2 and α4β7, respectively, thereby validating the experimental setting (Fig. 5A–C). The ELISA-type experiments have demonstrated that irisin-Fc but not control Fc bound well to human and mouse αLβ2 and human α4β7. These results have substantiated binding of irisin with integrin αLβ2 and α4β7.

4. Discussion

Integrins αVβ5, αVβ1, and α5β1 on the cell surface of osteocytes and adipocytes have been shown to bind to irisin, thereby playing important roles in cellular metabolic regulation [14,15]. However, the interactions of irisin with other integrins have yet to be elucidated. As irisin levels in

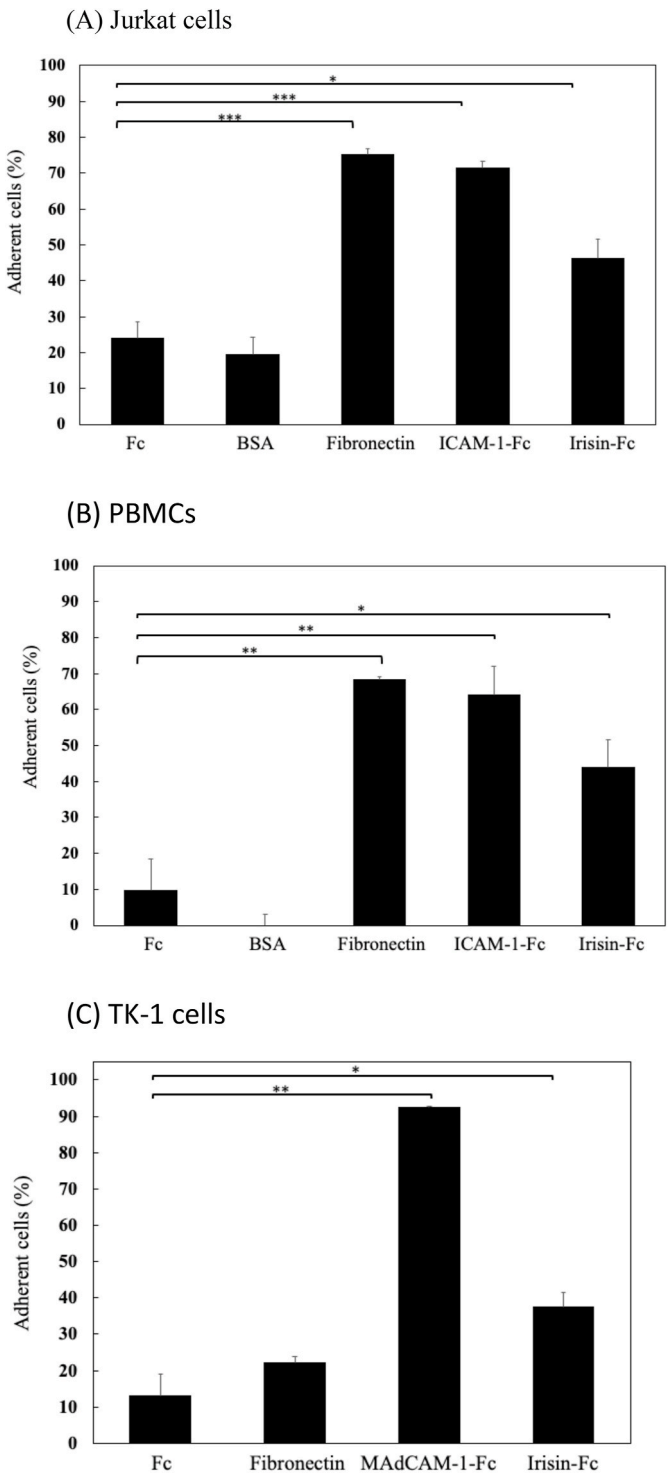

Fig. 2. Cell adhesion of human and mouse lymphocytes to irisin (A) Jurkat cells (B) PBMCs, and (C) TK1 cells. Data are expressed as the mean ± SEM of three independent experiments carried out in triplicate. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with Fc.

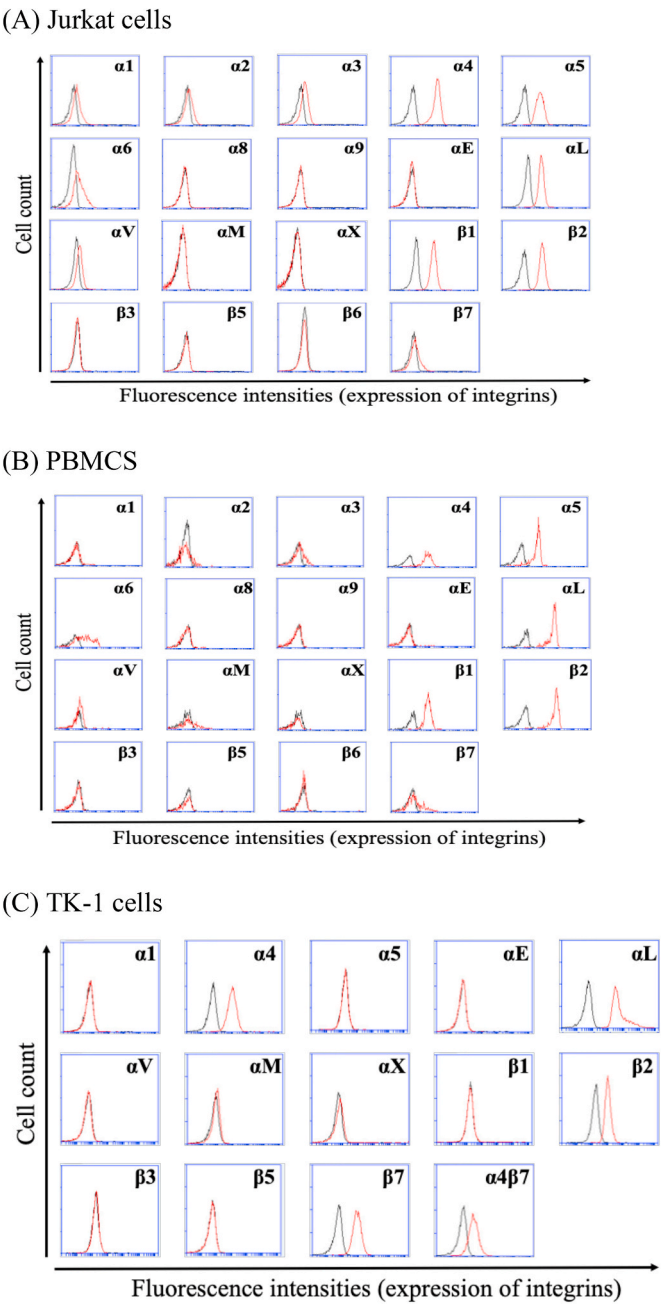

Fig. 3. Immunofluorescent cytometry analysis of integrin expression. Representative FACS histograms of flow cytometry analysis illustrating the expression of different integrin subunits on (A) Jurkat cells, (B) PBMCs, and (C) TK1 cells. The background staining with isotype control antibodies is shown as black lines, whereas specific monoclonal antibody staining is shown as red lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

plasma increase during inflammation [24], potentially being deposited on the surface of endothelial cells as well as in the interstitial space in inflamed tissue, determining whether irisin also acts as a ligand for such lymphocyte integrins as αLβ2 and α4β7 is of great interest. In this study, using cell- and purified protein-based assays, we have shown that irisin serves as a ligand for αLβ2 and α4β7 integrins, thereby supporting cell adhesion of human and mouse lymphocytes. The interactions of αLβ2 and α4β7 integrins with irisin could play additional roles that supplement the well-established αLβ2-ICAM-1 and α4β7-MAdCAM-1 interactions, potentially fine-tuning leukocyte adhesion and migration at inflamed tissues.

Our results are of great significance, as growing numbers of studies have shown that irisin interacts with leukocytes such as monocytes/macrophages that express several integrins including αLβ2, thereby modulating their cellular functions. Irisin increases the phagocytic activity of macrophages while reducing their respiratory burst activities [25]. Irisin treatment inhibits lipo-polysaccharide (LPS)-induced inflammatory cytokine production in RAW 264.7 cells and peritoneal

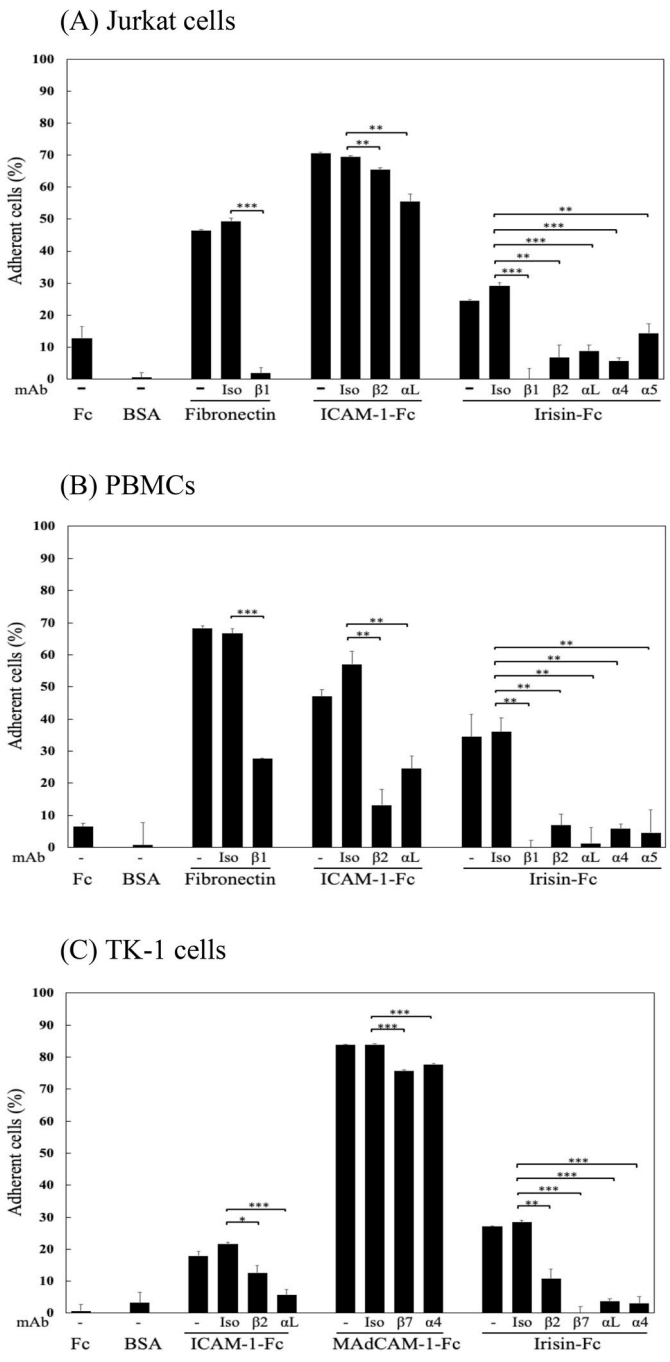

Fig. 4. Specific integrins involved in lymphocyte adhesion to irisin studied using a panel of inhibitory antibodies. Binding of (A) Jurkat cells and (B) PBMCs to Fc, BSA, fibronectin, ICAM-1-Fc and irisin-Fc in the presence or absence of blocking anibodies (C) Binding of TK1 cells to Fc, BSA, ICAM-1-Fc, MAdCAM-1-Fc and irisin-Fc in the presence or absence of blocking antibodies. (A–C) Data are expressed as the mean ± SEM of three independent experiments carried out in triplicate. *p < 0.05, **p < 0.01, ***p < 0.001 compared with an isotype control in each group.

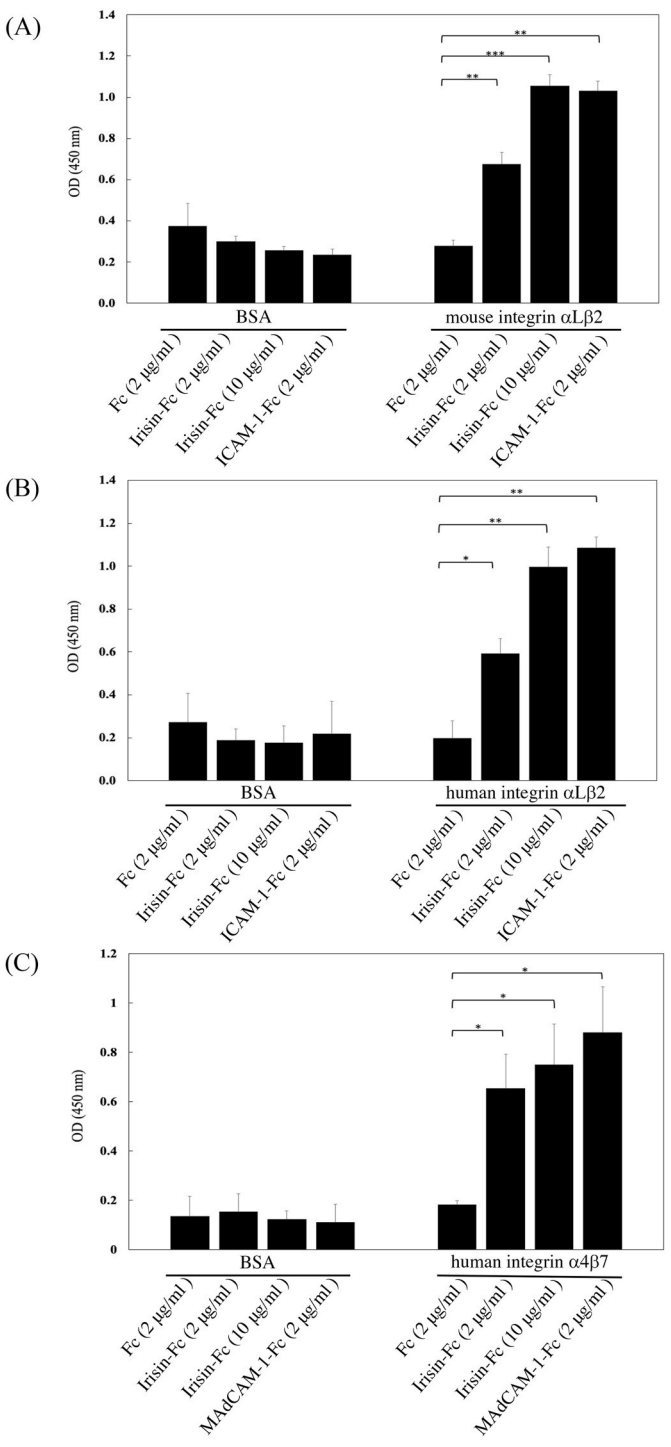

Fig. 5. Purified protein based-ELISA type experiments to study the interaction of irisin with integrin αLβ2 and α4β7. Binding of irisin- or control-Fc to immobilized proteins such as; mouse αLβ2 (A), human αLβ2 (B), human α4β7 (C), and BSA as a control (A–C); was examined. (A–C) Data are expressed as the mean ± SEM of three independent experiments carried out in triplicate. *p < 0.05, **p < 0.01, ***p < 0.001 compared with control Fc in each group.

macrophages [10]. Another study has also shown that pre-treatment with irisin lowers LPS-induced cytokine production in macrophages via the downregulation of TLR4 and MyD88 protein levels [26]. The *in vivo* study showed that administration of recombinant irisin alleviated inflammation in an experimental colitis model, suggesting that irisin has anti-inflammatory properties [27]. Irisin's anti-inflammatory effects could be mediated either by directly interfering with the adhesive functions of leukocyte integrins and/or by transmitting anti-inflammatory signals through integrins. Previous studies demonstrated that irisin elicited αVβ5-mediated signaling in osteocytes and adipocytes, leading to the activation of focal adhesion kinase (FAK) [14,15]; however, the expression of αVβ5 integrin in leukocytes is usually minimal. Thus, future investigations are required to study how irisin activates signaling cascades though leukocyte integrins including αLβ2 and α4β7, both of which have been shown to transduce signaling

through FAK upon ligation [28,29].

Our results show that the adhesive interactions of lymphocytes with irisin involve multiple integrins such as α4β1, α5β1, αLβ2 and α4β7. Of note, irisin binds to multiple integrins as do so some ECM integrin ligands such as fibronectin and collagens. Computational docking simulations of the irisin-αVβ5 interaction suggest the presence of a surface exposed RGD-like XXD integrin-binding motif [14] within the irisin molecule. Integrin α5β1 recognizes the RGD motif, whereas α4β1, αLβ2 and α4β7 do not. Thus, it is possible that irisin could contain more than one integrin-binding site. Compared to the well-established interactions of αLβ2-ICAM-1, α4β7-MAdCAM-1, and of α4β1/α5β1-finbronectin, integrin-irisin interactions seem to be of lower affinity and avidity, although a strictly quantitative analysis involving measurements of ligand densities has yet to be performed. The ability of irisin to bind to multiple integrins simultaneously could compensate for its low affinity and avidity, thereby facilitating its putative role in supporting lymphocyte adhesion and migration in inflammation.

In conclusion, we have demonstrated that irisin serves as a novel ligand for integrin αLβ2 and α4β7, thereby supporting lymphocyte adhesion. We are just beginning the exploration of the biological roles played by irisin in the regulation of leukocyte migration in inflammation. As increasing evidence has pointed to the important roles played by irisin in metabolic regulation, further investigations of irisin biology may lead to the realization that irisin is at the focal point of metabolic regulation of leukocyte migration, which is an emerging research topic [30,31].

Author statement

Phyoe Kyawe Myint: Conceptualization, Data Curation, Investigation, Writing - Original Draft, Formal analysis. Atsushi Ito: Validation, Investigation. Michael G. Appiah: Validation, Investigation. Gideon Obeng: Validation, Investigation. Samuel Darkwah Ph.D: Validation, Investigation. Eiji Kawamoto MD, Ph.D.: Project administration, Funding acquisition. Arong Gaowa Ph.D: Project administration, Funding acquisition. Eun Jeong Park Ph.D: Conceptualization, Supervision, Writing - Review & Editing, Project administration, Funding acquisition, Formal analysis. Motomu Shimaoka MD, Ph.D: Conceptualization, Formal analysis, Supervision, Writing - Review & Editing, Project administration, Funding acquisition

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Declaration of competing interest

The authors declare that they have no conflicts of interests.

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