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The nuclear protein Artemis promotes AMPK activation by stabilizing the LKB1–AMPK complex

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ABSTRACT

AMP-activated protein kinase (AMPK) is a hetero-trimeric Ser/Thr kinase composed of a catalytic α subunit and regulatory β and γ subunits; it functions as an energy sensor that controls cellular energy homeostasis. In response to an increased cellular AMP/ATP ratio, AMPK is activated by phosphorylation at Thr172 in the α-subunit by upstream AMPK kinases (AMPKKs), including tumor suppressor liver kinase B1 (LKB1). To elucidate more precise molecular mechanisms of AMPK activation, we performed yeast two-hybrid screening and isolated the complementary DNA (cDNA) encoding the nuclear protein Artemis/DNA cross-link repair 1C (DCLRE1C) as an AMPKα2-binding protein. Artemis was found to co-immunoprecipitate with AMPKα2, and the co-localization of Artemis with AMPKα2 in the nucleus was confirmed by immunofluorescence staining in U2OS cells. Moreover, over-expression of Artemis enhanced the phosphorylation of AMPKα2 and the AMPK substrate acetyl-CoA carboxylase (ACC). Conversely, RNAi-mediated knockdown of Artemis reduced AMPK and ACC phosphorylation. In addition, Artemis markedly increased the physical association between AMPKα2 and LKB1. Taken together, these results suggest that Artemis functions as a positive regulator of AMPK signaling by stabilizing the LKB1–AMPK complex.

1. Introduction

AMP-activated protein kinase (AMPK) is an essential factor for maintaining energy homeostasis following cellular metabolic stress such as exercise, glucose starvation, hypoxia, and ischemia [1]. The activation of AMPK decrease ATP consumption through down-regulation of protein and fat synthesis and increase ATP synthesis through stimulation of glucose uptake and lipid oxidation by phosphorylation of various downstream targets [2]. AMPK is a hetero trimeric Ser/Thr kinase consisting of a catalytic α subunit (α1 or α2) and regulatory β (β1 or β2) and γ subunits (γ1, γ2, or γ3) [1]. Under conditions that increase the AMP/ATP ratio, AMP directly binds to the AMPKα subunit, and AMP binding prevents dephosphorylation of Thr172 in the critical activation loop of the α subunit by protein phosphatase 2A and 2C [2]. The phosphorylation of Thr172, which is required for AMPK activation, is mediated by upstream AMPK kinases (AMPKKs) such as liver kinase B1 (LKB1), calmodulin-dependent protein kinase kinaseβ (CaMKKβ), and TGF-β-activated kinase1 (TAK1) [1]. LKB1, which has been shown to be the dominant AMPKK in many cells [3–5], is a tumor suppressor responsible for Peutz–Jeghers syndrome and sporadic non-small cell lung cancers [6], and it is required for the phosphorylation and activation of AMPK in response to the antidiabetic drug phenformin [5]. However, the precise molecular regulatory mechanisms of AMPK activation have not been completely clarified.

Artemis, also known as DNA cross-link repair 1C (DCLRE1C), is a member of the SNM1 gene family and is characterized by conserved metallo-β-lactamase (MBL) and β-CASP domains [7]. Artemis, which is ubiquitously expressed and is localized in the nucleus [8,9], was originally identified as the product of a causative gene that was deficient in human radiosensitive severe combined immunodeficiency syndrome (RS-SCID) [8] and was shown to function as a nuclease involved in NHEJ-mediated repair of double-strand DNA breaks and V(D)J recombination [10]. In addition, Artemis is involved in cell cycle regulation after DNA damage [11], negative regulation of p53 [12], and ubiquitin-mediated protein degradation [13,14], suggesting that Artemis is a multifunctional protein.

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In this study, in order to elucidate the molecular mechanisms of AMPK activation more precisely, we performed yeast two-hybrid screening and identified Artemis as an AMPKα2-binding protein. Artemis was found to promote LKB1-mediated phosphorylation and activation of AMPK by stabilizing the LKB1–AMPKα2 complex. Our findings suggest that Artemis is a novel positive regulator of the LKB1–AMPK signal.

2. Materials and methods

2.1. Cell culture and transfection

Human osteosarcoma U2OS cells and human embryonic kidney 293 and 293T cells were cultured in Dulbecco's modified Eagle's medium (Wako, Osaka, Japan) supplemented with 10% fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA) at 37 °C in a 5% CO_2 atmosphere. Transfections were performed either by FuGENE HD (Promega, Madison, WI) for plasmid DNA and by HiPerFect (Qiagen, Hilden, Germany) for siRNA, according to the manufacturer's protocol.

2.2. Plasmid construction

Mammalian expression vectors, pCI-neo-3×FLAG and pCI-neo-2×S, were generated by insertion of oligonucleotides encoding three copies of FLAG-tag and two copies of S-tag, respectively, into the pCI-neo mammalian expression vector (Promega). Complementary DNAs (cDNAs) of human AMPKα2, human LKB1 and human Artemis were obtained by reverse transcriptase polymerase chain reaction (RT-PCR) using total RNA extracted from Miapaca-2 cells or 293 cells. The resulting fragment encoding Artemis was digested by restriction enzymes and subcloned into pEGFP-C2 (Clontech, Palo Alto, CA), pCI-neo-2×S or pCI-neo-3×FLAG. Deletion mutants of Artemis were generated by a PCR-based method. The AMPKα2 expression vectors were constructed by inserting the PCR fragment encoding human AMPKα2 into pCI-neo-3×FLAG or pCI-neo-2×S. The constitutively active form of AMPKα2 (AMPKα2 CA), which lacks amino acids 311–552 and Thr172 mutated to Asp, was constructed by a PCR-based method. pGLex-AMPKα2 CA, the yeast two-hybrid bait vector, was constructed by fusing the AMPKα2 CA coding sequence in-frame with the LexA DNA-binding domain encoded in the vector pGLex (provided by Dr. H. Ariga of Hokkaido University). The LKB1 expression vector was constructed by inserting the PCR fragment encoding human LKB1 into pCI-neo-2×S. All constructs were confirmed by DNA sequence analysis.

2.3. Yeast two-hybrid screening

Saccharomyces cerevisiae strain L40 that had been transformed with pGLex-AMPKα2 CA was mated with the Y187 strain carrying the human bone marrow MATCHMAKER cDNA library (Clontech). These mating products were then plated to media lacking tryptophan, leucine, and histidine and supplemented with 5 mM 3-amino-1,2,4-triazole (Sigma, St. Louis, MO). Approximately 1×10^7 colonies were screened for growth in the absence of histidine. Plasmid DNAs derived from positive clones were extracted from yeast cells, and the nucleotide sequences were determined. The interaction between AMPKα2 CA and Artemis in the L40 strain was confirmed by a β-galactosidase assay.

2.4. Immunoprecipitation, affinity pull-down and western blotting

U2OS cells or 293T cells were transiently transfected with the indicated plasmids. Forty-eight hours after transfection, the cells were washed with ice-cold Tris-buffered saline (TBS) and harvested. The cells were lysed with cell lysis buffer containing 20 mM Tris–HCl (pH 7.5), 137 mM NaCl, 0.5% NP-40, 0.5 mM DTT, Complete Protease Inhibitor Cocktail (Roche, Mannheim, Germany), and Phosphatase Inhibitor Cocktail (Sigma). The lysates were incubated on ice for 30 min, and the cell debris was removed by centrifugation at 21,000×*g* for 15 min. The resulting supernatants were incubated with anti-FLAG M2 affinity gel (Sigma) or S-protein agarose (Novagen, Madison, WI) for 2 h at 4 °C, and the precipitates were washed five times with the lysis buffer. For western blotting, whole cell lysates and precipitates were separated by SDS–PAGE and transferred to Immobilon-P membranes (Millipore, Bedford, MA). The membranes were immunoblotted with the following antibodies: anti-S tag (Novagen); anti-FLAG (M2) (Sigma); anti-Artemis (Bethyl, Montgomery, TX); anti-AMPKα, anti-phospho AMPKα (Thr172), anti-AMPKα2, anti-AMPKβ1β2, anti-AMPKγ1, anti-ACC, anti-phospho ACC (Ser79), and anti-β-tublin (Cell Signaling, Danvers, MA); and anti-actin (Millipore). The bound primary antibodies were incubated with horseradish peroxidase-conjugated antibody against mouse or rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) and detected by Immobilon Western HRP Substrate detection reagents (Millipore). Band images were detected using a LAS 4010 system (GE Healthcare Life Sciences, Buckinghamshire, UK).

2.5. RNA interference

Small interfering RNAs (siRNAs) for Artemis and scrambled siRNA were purchased from Ambion (Austin, TX). The siRNA target sequences were as follows: Artemis siRNA #1 5′-GCCUUUAUGCCGGU CUUCCTT-3′ (sense) and Artemis siRNA #2 5′-CCUAGAGCAAC CAC UAAAATT-3′ (sense). U2OS cells and 293 cells at 40–50% confluence were transfected with a 10 nM final concentration of siRNA. Forty-eight hours after transfection, the cells were stimulated with 2 mM of phenformin (Sigma) for 4 h, and the protein of the cells was extracted.

2.6. Quantitative RT-PCR

Total RNA was extracted using the ISOGEN reagent (Nippon Gene, Toyama, Japan). cDNA was synthesized using M-MLV Reverse Transcriptase (Invitrogen) according to the manufacturer's instructions. Real-time RT-PCR was performed as described previously [15]. The relative levels of each transcript were determined using the ddCt method with β-actin as the control. The primers used for PCR were as follows: Artemis, 5′-GAGCTAGAACAGTT CACCGAGAC-3′ and 5′-TGGCAGAGGATCATCAAAGA-3′ [16]; β-actin, 5′-TTGCCGACAGGATGCAGAA-3′, and 5′-GCCGATCCACACGGAG TACT-3′ [15].

2.7. Indirect immunofluorescence

U2OS cells grown on glass cover slips were transfected with the expression vector for green fluorescent protein (GFP)-tagged Artemis with FLAG-tagged AMPKα2 or FLAG-tagged LKB1. Forty-eight hours after transfection, the cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 20 min at 4 °C and reacted with a mouse anti-FLAG (M2) monoclonal antibody. The cells were then reacted with an Alexa Fluor 568-conjugated anti-mouse IgG antibody (Invitrogen) and observed by fluorescence microscopy (BioRevo BZ-9000; Keyence, Osaka, Japan).

3. Results

3.1. Artemis physically associates and co-localizes with AMPKα2

To identify proteins that could be involved in the regulation of AMPK activation, we performed yeast two-hybrid screening with a human bone marrow cDNA library and a constitutively active form of AMPKα2 as the bait. Several AMPKα2-interacting proteins were identified by screening 1×10^7 yeast transformants. DNA sequencing analysis revealed that one of the positive clones that specifically interacted with AMPKα2 was identical to Artemis (Fig. 1A). To determine whether Artemis could associate with AMPKα2 in mammalian cells, we performed immunoprecipitation assays. U2OS cells were transiently transfected with FLAG-tagged Artemis and S-tagged wild-type AMPKα2, and the cell lysates were subjected to immunoprecipitation with the anti-FLAG antibody. The resulting immunoprecipitates were subjected to western blotting with anti-FLAG or anti-S-tag antibody. As shown in Fig. 1B, the AMPKα2 subunit co-precipitated with Artemis. Additionally, the interaction between endogenous Artemis and AMPKα was verified by a co-immunoprecipitation assay with anti-Artemis antibody using U2OS cell extracts (Fig. 1C). We also found that the AMPK activator phenformin did not affect the Artemis–AMPKα interaction. Thus, our data suggest that the AMPKα subunit is associated with Artemis under physiological conditions. Next, to examine the subcellular distributions of Artemis and AMPKα2, we performed indirect immunofluorescence analysis of U2OS cells that had been co-transfected with FLAG-tagged AMPKα2 and GFP-tagged Artemis. Consistent with previous reports [9,17], AMPKα2 was localized in both the cytoplasm and the nucleus and Artemis was exclusively localized in the nucleus (Fig. 1D). The co-localization of AMPKα2 with Artemis was observed in the nucleus. Furthermore, we examined whether Artemis was also able to associate with the AMPK hetero trimer. A pull-down assay with S-protein agarose was performed using cell lysates prepared from 293T cells transfected with S-tagged Artemis. As shown in Fig. 1E, the endogenous β and γ subunits of AMPK were co-purified with S-tagged Artemis, indicating that Artemis can interact with the AMPK hetero trimer. Next, in order to identify the region of Artemis that was responsible for its interaction with AMPKα2, we constructed three deletion mutants of Artemis (Fig. 1F). The immunoprecipitation analysis showed that the AMPKα2-binding site on Artemis was located in the MBL and β-CASP domains.

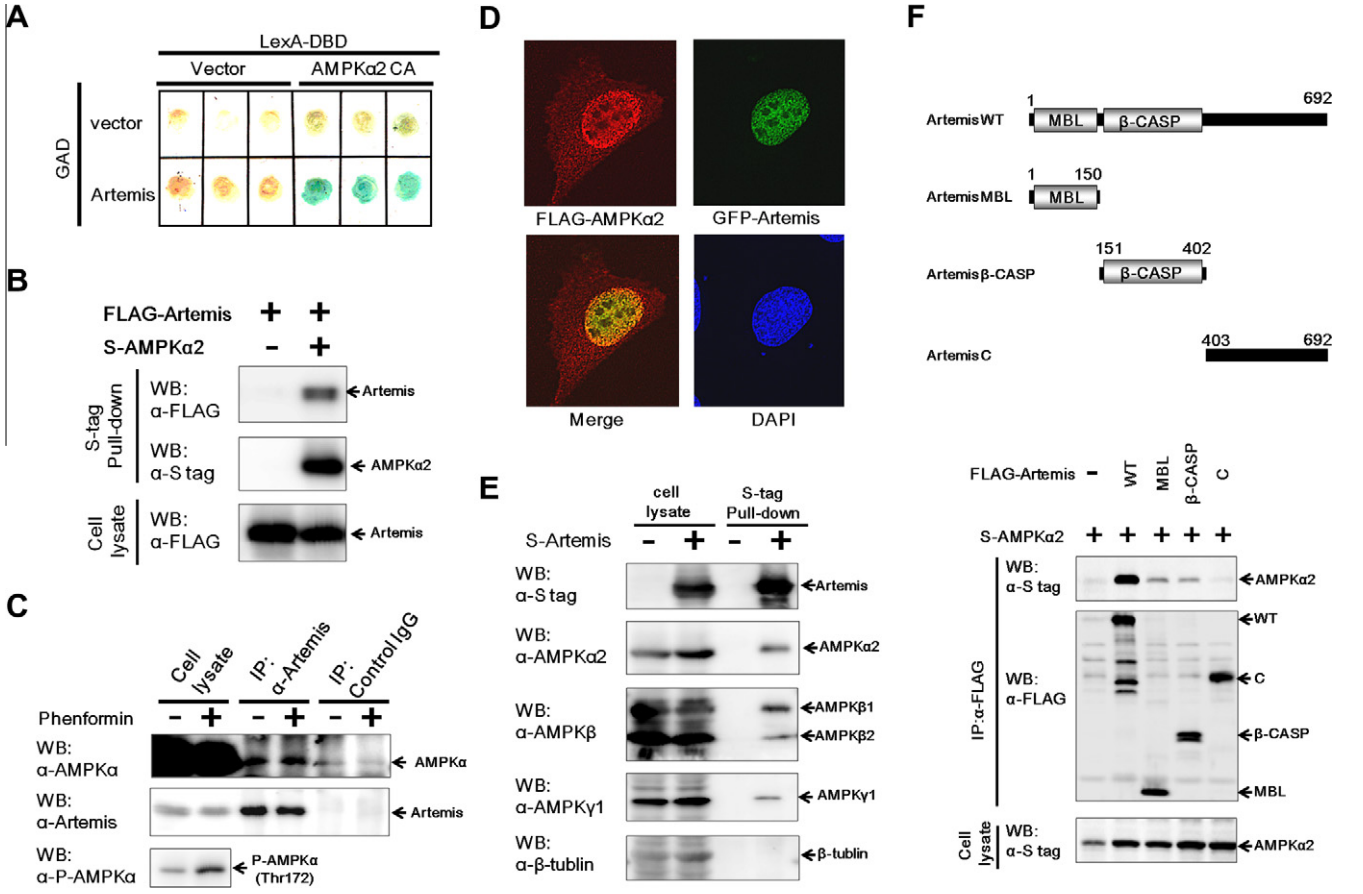

Fig. 1. Interaction between AMPKα2 and Artemis. (A) Yeast L40 cells were transformed with the indicated expression vectors containing the constitutively active form (CA) of AMPKα2 fused to the LexA DNA-binding domain (DBD) and Artemis fused to the GAL4 activation domain (GAD). The β-galactosidase activity of each transformant was measured. (B) U2OS cells were transiently transfected with FLAG-tagged Artemis and S-tagged AMPKα2 (wild-type). The cell lysates were subjected to an S-tag pull-down assay using S-protein agarose, followed by western blotting (WB) with the indicated antibodies. (C) U2OS cells were treated with 2 mM phenformin for 4 h, and the cell lysate were subjected to immunoprecipitation (IP) with anti-Artemis antibody or normal rabbit IgG. The resulting precipitates were subjected to western blotting with anti-AMPKα (pan-anti-AMPKα antibody that recognizes both the AMPKα1 and α2 isoforms) and anti-Artemis antibodies. (D) U2OS cells were co-transfected with FLAG-tagged AMPKα2 and GFP-tagged Artemis. Forty-eight hours after transfection, the cells were fixed, reacted with an anti-FLAG monoclonal antibody, and visualized with an Alexa Fluor 568-conjugated anti-mouse antibody by fluorescence microscopy. Nuclei were detected with 4′,6-diamidino-2-phenylindole (DAPI). (E) 293T cells were transiently transfected with S-tagged Artemis, and the cell lysates were subjected to an S-tag pull-down assay, followed by western blotting with the indicated antibodies. (F) (Upper panel) Schematic representation of the Artemis deletion mutants. The metallo-β-lactamase (MBL) domain and the β-CPSF–Artemis–SNM1–PSO2 (β-CASP) domain are shown. (Lower panel) U2OS cells were transiently transfected with FLAG-tagged deletion mutants of Artemis along with S-tagged AMPKα2 and the cell lysates were subjected to immunoprecipitation with anti-FLAG antibody, followed by western blotting with the indicated antibodies.

3.2. Artemis promotes activation of AMPK

Because Artemis bound to AMPKα2, we next examined whether Artemis was involved in the activation of AMPKα2. To examine the effect of Artemis overexpression on the phosphorylation of AMPKα2 at Thr172, U2OS cells were transiently transfected with S-tagged AMPKα2 and FLAG-tagged Artemis and the cell lysates were subjected to a pull-down assay using S-protein agarose. The resulting precipitates were subjected to western blotting with anti-phospho-AMPKα (Thr172) antibody. As shown in Fig. 2A, the phosphorylation of Thr172 was strongly enhanced by Artemis co-expression. The overexpression of Artemis in 293 cells also enhanced the phosphorylation of endogenous AMPKα at Thr172 in a dose-dependent manner (Fig. 2B). Next, in order to determine whether endogenous Artemis was involved in the regulation of AMPK activation, we performed siRNA-mediated knockdown experiments. Two different siRNAs targeting Artemis were tested for their ability to interfere with Artemis gene expression. Quantitative RT-PCR analysis revealed that two Artemis-specific siRNAs knocked down Artemis gene expression by approximately 70–80% compared with the scrambled siRNA in U2OS cells (Fig. 2C). Using these siRNAs, we examined the effects of knocked-down Artemis on the Thr172 phosphorylation of AMPKα2. U2OS cells were transfected sequentially with scrambled siRNA or two siRNAs targeting Artemis and S-tagged AMPKα2, and then an S-tag pull-down assay was performed. As shown in Fig. 2D, the level of AMPKα2 phosphorylation at Thr172 was clearly decreased by the knockdown of Artemis. We also examined whether phenformin-stimulated AMPK activation was affected by Artemis knockdown. In U2OS and 293 cells, phenformin-stimulated phosphorylation of the AMPKα subunit and the AMPK substrate ACC were also decreased by Artemis knockdown (Fig. 2E). These results indicate that Artemis is a positive regulator of AMPK.

3.3. Artemis stabilizes the LKB1–AMPK complex

Next, we tried to elucidate the molecular mechanism underlying Artemis-promoted AMPK activation. Because LKB1 is the dominant AMPKK in many cells [3–5], we examined the interaction between Artemis and LKB1. The S-tag pull-down assay showed that LKB1 associated with Artemis in U2OS cells (Fig. 3A). Additionally, the indirect immunofluorescence analysis revealed that LKB1 was localized in both the cytoplasm and the nucleus, consistent with a previous report [18], and it co-localized with Artemis only in the

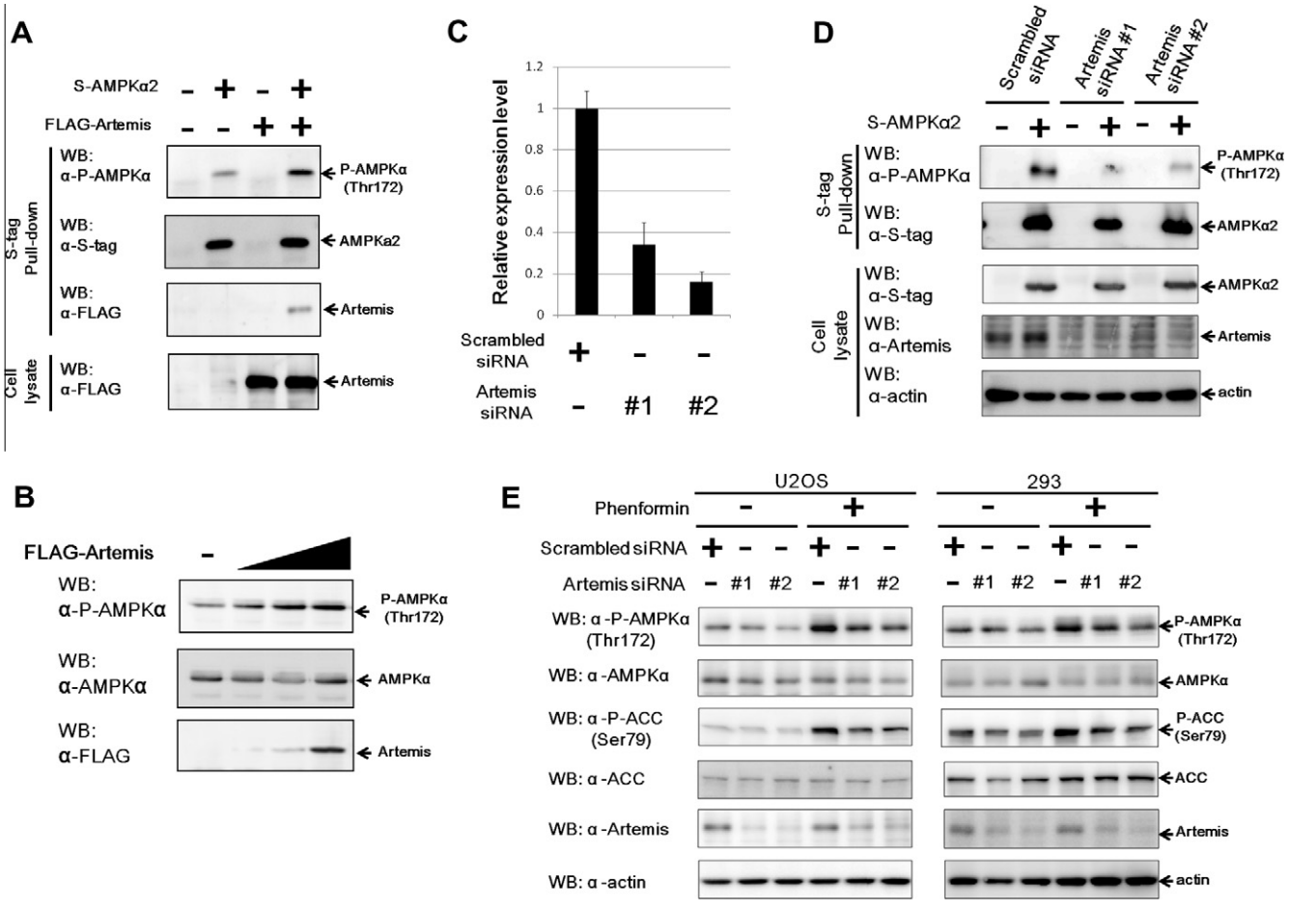

Fig. 2. Artemis promotes AMPK activation. (A) U2OS cells were transiently transfected with FLAG-tagged Artemis and S-tagged AMPKα2 and the cell lysates were subjected to an S-tag pull-down assay, followed by western blotting with the indicated antibodies. (B) 293 cells were transfected with increasing amounts of FLAG-tagged Artemis, and the cell lysates were subjected to western blotting with the indicated antibodies. (C) U2OS cells were transfected with a 10 nM final concentration of scrambled siRNA or two Artemis siRNAs (#1, #2). Forty-eight hours after transfection, RNAs were extracted, and quantitative RT-PCR was performed to the measure knockdown efficiency. Fold-changes in Artemis mRNA levels were calculated with the ddCt method using β-actin as a reference gene. Triplicate experiments were carried out, and the error bars represent standard deviations. (D) U2OS cells were transfected with a 10 nM final concentration of scrambled siRNA or two Artemis siRNAs (#1, #2). Twenty-four hours after siRNA transfection, the cells were transfected with S-tagged AMPKα2 and incubated for an additional 24 h. The cell lysates were subjected to an S-tag pull-down assay, followed by western blotting with the indicated antibodies.(E) U2OS cells and 293 cells were transfected with a 10 nM final concentration of scrambled siRNA or two Artemis siRNAs (#1, #2). Forty-eight hours after transfection, the cells were incubated with 2 mM phenformin for an additional 4 h. The cell lysates were prepared and subjected to western blotting with the indicated antibodies.

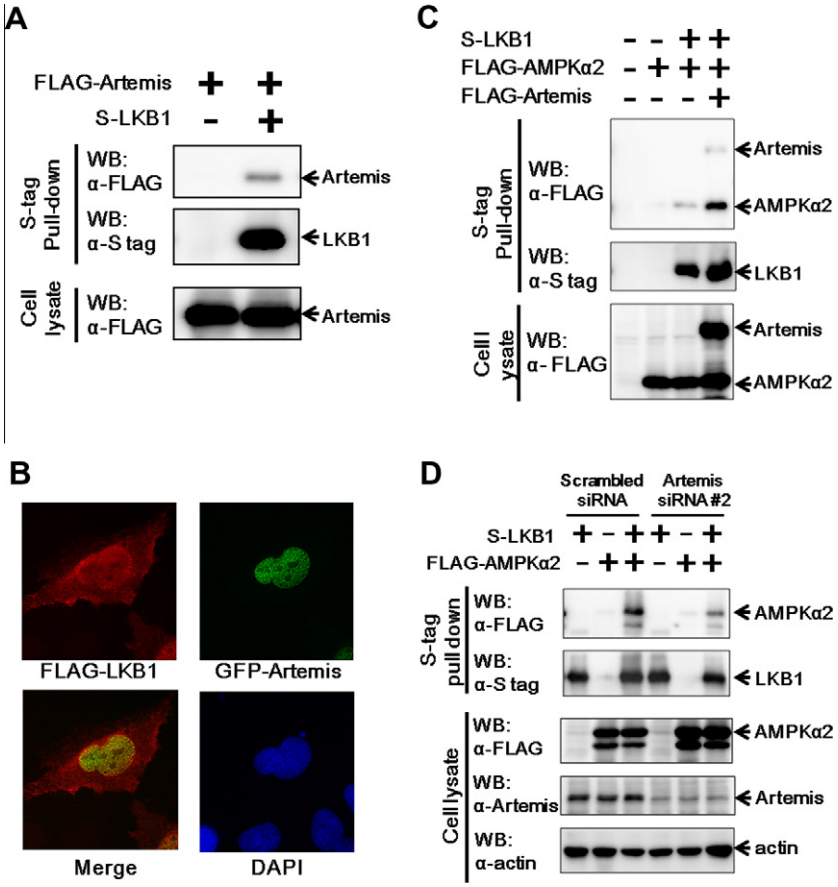

Fig. 3. Artemis stabilizes the LKB1–AMPK complex. (A) U2OS cells were transiently transfected with FLAG-tagged Artemis and S-tagged LKB1 and the cell lysates were subjected to an S-tag pull-down assay, followed by western blotting with the indicated antibodies. (B) U2OS cells were co-transfected with FLAG-tagged LKB1 and GFP-tagged Artemis. The indirect immunofluorescence assay was performed as described in Fig. 1D. (C) U2OS cells were transiently transfected with FLAG-tagged AMPKα2, FLAG-tagged Artemis, and S-tagged LKB1. The cell lysates were subjected to an S-tag pull-down assay, followed by western blotting with the indicated antibodies. (D) U2OS cells were transfected with a 10 nM final concentration of scrambled siRNA or Artemis siRNA #2. Twenty-four hours after siRNA transfection, the cells were transfected with S-tagged LKB1 and FLAG-tagged AMPKα2, and they were incubated for an additional 24 h. The cell lysates were subjected to an S-tag pull-down assay, followed by western blotting with the indicated antibodies.

nucleus of U2OS cells (Fig. 3B). To examine whether the LKB1–AMPKα2 interaction was affected by Artemis overexpression, U2OS cells were transiently transfected with S-tagged LKB1 and FLAG-tagged AMPKα2 together with FLAG-tagged Artemis, and an S-tag pull-down assays was performed. The forced expression of Artemis markedly enhanced the interaction between LKB1 and AMPKα2 (Fig. 3C). To confirm the nvolvement of endogenous Artemis in regulating the LKB1–AMPKα2 interaction, U2OS cells were transfected sequentially with scrambled siRNA or siRNA targeting Artemis and FLAG-tagged AMPKα2 together with S-tagged LKB1, and an S-tag pull-down assay was performed. As shown in Fig. 3D, the association between AMPKα2 and Artemis was markedly decreased in the Artemis-knockdown cells. These results indicate that Artemis stabilizes the LKB1 and AMPKα2 interaction.

4. Discussion

In this study, we identified the nuclear protein Artemis as a novel biding partner of AMPKα2 using a yeast two-hybrid screen. In addition, we found that Artemis promoted AMPK activation by enhancing LKB1-mediated phosphorylation of AMPKα2 through stabilization of the LKB1–AMPKα2 complex.

Artemis has been shown to function as a nuclease involved in NHEJ-mediated repair of double-stranded DNA breaks [7,10]. However, recent reports suggest that Artemis has functions other than serving as a nuclease. Artemis was shown to function as a component of two ubiquitin E3 ligase complexes, SCFFbw7 and Cul4A-DDB1-DDB2 [13,14]. In addition, Artemis was reported to physically associate with the tumor suppressor protein p53 and to inhibit DNA-PKcs-mediated stabilization of p53 [12]. These data suggest that Artemis is involved in multiple biological processes through interactions with different binding partners. The MBL and β-CASP domains that are conserved in the SNM1 family function as interfaces for protein–protein interactions [7]. In this study, we found that Artemis associated with AMPKα2 through the MBL and β-CASP domains and with LKB1 through the MBL domain (Fig. 1F and data not shown). These results indicate that Artemis provides a platform for the formation of the LKB1–AMPKα2 complex, leading to AMPK activation. To determine this possibility, examining whether Artemis directly enhances the interaction between LKB1 and AMPKα2 by forming a ternary complex *in vitro* is required, using purified recombinant proteins.

LKB1-mediated AMPKα-Thr172 phosphorylation is an essential step for the full activation of AMPK [3,5]. However, the precise molecular mechanism of the interaction between LKB1 and AMPKα has not been completely clarified. Post-translational modifications of LKB1 and AMPKα were recently found to be involved in regulating the interaction. In some melanoma cell lines with the oncogenic B-Raf V600E mutation, elevated phosphorylation of LKB1 at Ser325 and Ser428, which is mediated by ERK and RSK, respectively, inhibited the interaction between LKB1 and AMPKα and

led to the down-regulation of AMPK activity [19,20]. Furthermore, Lin et al. recently reported that the acetylation of AMPKα1 mediated by p300 inhibited its association with LKB1 and that the de-acetylation of AMPKα1 mediated by HDAC1 enhanced its physical interaction with LKB1, leading to AMPK activation [21]. Based on these data, we speculate that Artemis promotes the LKB1–AMPK interaction not only by physically recruiting LKB1 and AMPKα2, but also by regulating post-translational modifications of LKB1 or AMPKα. Therefore, it will be interesting to investigate whether the phosphorylation of LKB1 and/or the acetylation of AMPKα are regulated by Artemis.

Indirect immunofluorescence analysis showed that Artemis was exclusively localized in the nucleus, whereas AMPKα2 and LKB1 were localized both in the nucleus and the cytoplasm (Figs. 1D and 3B), suggesting that the interaction of Artemis with LKB1 and AMPKα2 occurred in the nucleus. However, LKB1 has been shown to be allosterically activated in the cytoplasm by association with two co-factors, STRAD (STE20-related adapter) and MO25 (mouse protein 25) [22]. In the presence of STRAD and MO25, the kinase activity of LKB1 increased 10-fold, indicating that the activation of AMPK by LKB1 occurred predominantly in the cytoplasm. Because both AMPK and LKB are known to shuttle between the nucleus and the cytoplasm [23,24], it is speculated that the association between LKB1 and AMPK is promoted by Artemis in the nucleus. Subsequently, the LKB1–AMPK complex would translocates to the cytoplasm, where LKB1 would be activated by its association with STRAD and Mo25, leading to the phosphorylation of AMPKα2 by LKB1. To test this possibility, further analysis is required to examine the effects of Artemis on the level of AMPKα2 phosphorylation in both the nucleus and the cytoplasm by biochemical subcellular fractionation and immunofluorescence.

In conclusion, we have identified Artemis as a novel binding partner of AMPKα2 and demonstrated that Artemis promotes the activation of AMPK. However, the precise molecular mechanism underlying Artemis-mediated stabilization of the LKB1–AMPKα2 complex remains to be elucidated. Additionally, the biological significance of Artemis in the AMPK-mediated regulation of metabolic homeostasis under physiological and pathological conditions is unclear. Because AMPK appears to be a potential therapeutic target for the treatment of obesity, type II diabetes, and cancer [25], elucidation of the role of Artemis in AMPK signaling *in vivo* is important. Further understanding of the role of Artemis in the LKB1–AMPK signal cascade may provide not only critical information about metabolic homeostasis, but also new AMPK-mediated therapeutic approaches to metabolic syndromes and cancer.

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