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Aberrant activation of AMP-activated protein kinase contributes to the abnormal distribution of HuR in amyotrophic lateral sclerosis

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ABSTRACT

Distorted mRNA metabolism contributes to amyotrophic lateral sclerosis (ALS). The human antigen R (HuR) is a major mRNA stabilizer. We report that abnormal localization of HuR was associated with enhanced AMP-activated protein kinase (AMPK) activity in the motor neurons of ALS patients. Activation of AMPK changed the location of HuR in mouse motor neurons and in a motor neuron cell line via phosphorylation of importin-α1. Stimulation of the A_{2A} adenosine receptor normalized the AMPK-evoked redistribution of HuR. This suggests that aberrant activation of AMPK in motor neurons disrupts the normal distribution of HuR, which might imbalance RNA metabolism and contribute to ALS pathogenesis.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease. The major symptoms of ALS patients are muscle weakness and atrophy, which are caused by the degeneration of motor neurons. The clinical presentations of ALS include respiratory insufficiency and voluntary movement impairment [1]. Most patients die after 3–5 years of being diagnosed with ALS. The formation of cytosolic protein aggregates in motor neurons is a hallmark of ALS. In addition, aberrant RNA metabolism because of dysfunction and/or mislocalization of RNA-binding proteins has been implicated in ALS [2]. For instance, mislocalization of TDP-43, a RNA-binding protein that mediates multiple pathways (e.g., gene expression, RNA splicing, microRNA regulation, and mRNA stability [3,4]) is believed to contribute to the pathogenesis of ALS. TDP-43 controls the stabilization of the transcript of the human low-molecular-weight neurofilament (hNFL) by interacting directly with the 3′ untranslated region (3′UTR) of hNFL. In the motor neurons of patients with ALS, cytosolic mislocalization and aggregation of TDP-43 are associated with the downregulation of the hNFL transcript [5]. The human antigen R (HuR) is another important RNA-binding protein that has been implicated in ALS. It was originally identified as a Drosophila embryonic lethal abnormal vision protein that regulates the development of the nervous system in this fly [6]. HuR binds to the adenylate- and uridylate-rich elements (AREs) located in the 3′UTR and stabilizes those transcripts [7]. A wide variety of genes, including those involved in inflammatory processes, cell migration, apoptosis, and cell cycle, are modulated by HuR. Given the importance of RNA stabilization, HuR has been implicated in many human diseases, such as cancers, inflammatory diseases, cardiovascular disorders, and ALS [8,9]. Under normal conditions, HuR is mainly located in the nucleus. Cellular mislocalization of HuR is closely associated with pathogenesis [10,11]. The abundance of the cytoplasmic HuR protein is an indicator of poor prognosis in cancer patients [12,13]. Moreover, HuR is sequestered in intracellular inclusions in motor neurons in mice with ALS, which markedly reduces the binding of HuR to the transcript of the vascular endothelial growth factor and may contribute to cytotoxicity in ALS. The mechanism that regulates the subcellular distribution of HuR remains largely unknown [9].

Abbreviations: $A_{2A}R$, A_{2A} adenosine receptor; AD, Alzheimer's disease; AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; ALS, amyotrophic lateral sclerosis; AMPK, AMP-activated protein kinase; AREs, adenylate- and uridylate-rich elements; ELAV, embryonic lethal abnormal vision; FUS, fused in sarcoma; HD, Huntington's disease; HuR, human antigen R; PD, Parkinson's disease; TDP-43, trans-activation region DNA-binding protein of 43-kDa

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The AMP-activated protein kinase (AMPK) is a key energy sensor that regulates the cellular energy homeostasis [14]. AMPK is a heterotrimeric protein that contains three subunits (α, β, and γ). The activation of AMPK requires the phosphorylation of threonine 172, which is located within the catalytic α subunit and is sensitive to the cellular AMP:ATP ratio [15]. The activity of AMPK is believed to modulate the neuronal susceptibility to stresses in neurodegenerative disorders, including Alzheimer disease, Huntington disease, Parkinson disease, and ALS [16,17]. For example, abnormal activation of AMPK was shown in the spinal cord and primary spinal cord neuronal cultures of a well-characterized ALS mouse model [17]. The functional consequence of such enhanced activity of AMPK is currently unknown. In the present study, we report that elevated AMPK activity was closely correlated with cellular accumulation of HuR in the motor neurons of patients with ALS. Activation of AMPK in a motor neuron cell line (NSC-34) using 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) or a dominant-positive AMPK mutant (AMPK-α1-T172D) caused the subcellular redistribution of HuR via the phosphorylation of importin at Ser105. Suppression of AMPK using a dominant-negative AMPK mutant (AMPK-α1-T172A) or a cAMP-elevating agent normalized the localization of HuR. Collectively, our results suggest that aberrant activation of AMPK in motor neurons triggers the subcellular redistribution of HuR and contributes to the pathogenesis of ALS. Therefore, a therapeutic strategy aimed at the tight control of AMPK activity in motor neurons may be beneficial for ALS patients.

2. Materials and methods

2.1. Human spinal cord sections

Human spinal cord sections were obtained from the NICHD Brain and Tissue Bank for Developmental Disorders (University of Maryland, Baltimore, MD, USA).

2.2. Intrathecal injection

C57BL/6 mice were first anesthetized (100 mg/kg of ketamine plus 7.5 mg/kg of xylazine, intraperitoneal injection), then operated on the space located between L4 and L6, for intrathecal injection. The spinal cord was injected with 5 μL of AICAR (30 μg/μL) or 0.9% saline using a 10 μL Hamilton syringe. Mice were sacrificed 24 h postinjection to collect the L4–L6 region of spinal cord tissues for immunohistochemical staining.

2.3. Cell culture

The NSC-34 cell line used in this study was a kind gift from Dr. Cashman [18]. NSC-34 was maintained in growth medium supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin (Invitrogen GibcoBRL, Carlsbad, CA, USA) in Dulbecco's modified Eagle's medium.

2.4. Constructs

The V5-tagged AMPK mutants (T172A or T172D) were constructed by replacing Thr172 with Ala and Asp, respectively, and were characterized as described previously [19]. The V5-tagged importin-α1 variants (S105A or S105D) were constructed and subcloned into pcDNA3.1 (Invitrogen Life Technologies, Carlsbad, CA, USA) by replacing the Ser105 residue with Ala and Asp, respectively, using standard molecular biology techniques [20].

2.5. Sample preparation and western blotting

NSC34 cells were lysed using RIPA buffer (50 mM Tris–HCl, 0.5% sodium deoxycholate, 1% Triton X-100, and 150 mM NaCl) containing protease and phosphatase inhibitors. The lysates were subjected to western blot analysis, as described previously [19]. The following primary antibodies were used in this study: anti-AMPK-p (1:1,000; Cell Signaling Technology, Danvers, MA, USA), anti-AMPK (1:1,000; GeneTex, Irvine, CA, USA), and anti-α-tubulin (1:10,000; Sigma–Aldrich, St. Louis, MO, USA). The ECL reagents (PerkinElmer, Waltham, MA, USA) were used to detect immunoreactive signals.

2.6. Immunohistochemical and immunocytochemical staining

The analysis of human spinal cord sections (5 μm) was performed using immunohistochemical staining as described previously [19]. In brief, the sections were immunostained at 4 °C for 48 h with the following primary antibodies: anti-HuR (1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and anti-choline acetyltransferase (anti-ChAT; 1:100; Millipore, Billerica, MA, USA). After extensive washing, the sections were incubated for an additional 48 h with an anti-AMPK-p antibody (1:50; Cell Signaling Technology), followed by incubation with the corresponding secondary antibodies at room temperature (RT) for 2 h. The nuclei were stained using DAPI. Images were acquired and analyzed using a confocal microscope (LSM 510; Carl Zeiss, Jena, Germany).

The spinal cord tissues of mice were fixed with 4% paraformaldehyde overnight and hydrated in 0.1 M phosphate buffer solution (PBS) containing 30% sucrose for 36–48 h before paraffin embedding and sectioning (5 μm) were performed using standard protocols. After deparaffinization and rehydration, slices were washed with Na-PBS (81.4 mM Na_2HPO_4, 18.3 mM NaH_2PO_4, and 149.7 mM NaCl; pH = 7.4) and blocked with 3% bovine serum albumin (BSA). All primary antibodies were diluted in Na-PBS containing 1% BSA. The slices were incubated with antibodies for 36–40 h at 4 °C. The primary antibodies used included anti-HuR (1:200; Santa Cruz Biotechnology) and anti-ChAT (1:100, Millipore) antibodies. Incubation with the secondary antibodies (Alexa Fluor 488, Alexa Fluor 568) was carried out at RT for 2 h. Images were acquired using laser confocal microscopy (LSM 780; Carl Zeiss).

NSC-34 cells were fixed with methanol (−20 °C) for 10 min, and then blocked with 3% normal goat serum at RT for 1 h. Cells were then immunostained with the indicated primary antibody at 4 °C for 24 h, washed extensively, and incubated with the corresponding secondary antibody for 1 h. The primary antibodies used in the present study were anti-HuR (1:200; Santa Cruz Biotechnology), and anti-V5 (1:200; GeneTex). The nuclei were stained with DAPI. Images were acquired and processed using a confocal microscope (LSM 510; Carl Zeiss).

2.7. Adenylyl cyclase activity

The preparation of membrane fractions and the adenylyl cyclase (AC) assay were performed as described previously [21]. Briefly, cells were lysed and centrifuged at 50,000×g for 45 min, to collect the P1 membrane fractions, and assayed for the activity of AC in an AC assay buffer (6 mM $MgCl_2$, 0.2 mM EGTA, 100 mM NaCl, 50 mM HEPES, 1 mM ATP, 1 μM GTP, and 0.5 mM 3-isobutyl-1-methylxanthine) for 10 min at 37 °C. Trichloroacetic acid (6%, final concentration) was added to the reaction buffer to terminate the reaction. The amount of cAMP was measured by using a radioimmunoassay as described elsewhere [21].

2.8. Statistics

The data in the figures are presented as means ± S.E.M. of triplicate samples. Each experiment was repeated at least three times. Unless stated otherwise, statistical analyses were performed using

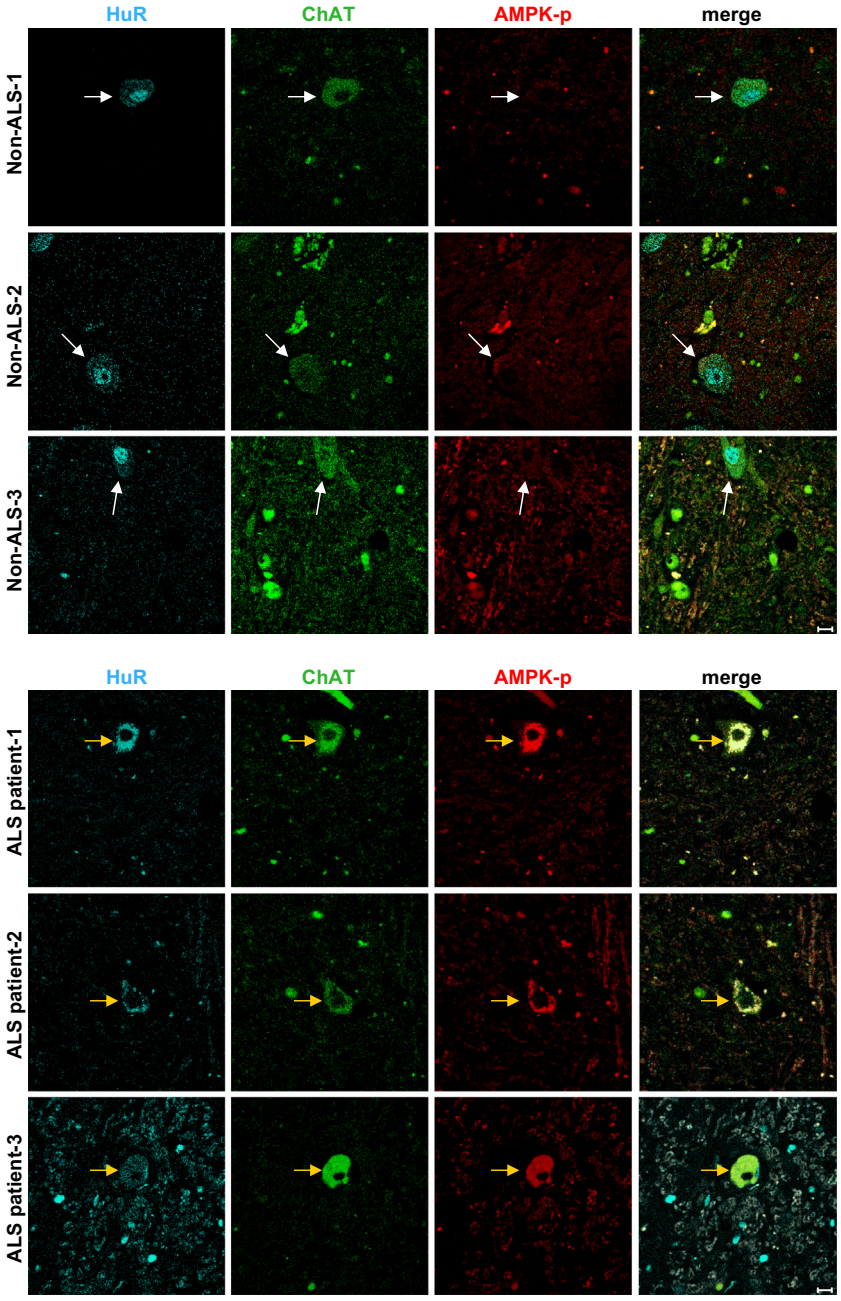

Fig. 1. The cytoplasmic localization of HuR is associated with the abnormal activation of AMPK in the spinal cord of ALS patients. Spinal cord sections from ALS patients and non-ALS controls were immunostained with antibodies against phosphorylated AMPK at Thr172 (AMPK-p, red), a motor neuron marker (ChAT, green), and HuR (light blue). The white arrows marked motor neurons with nuclear localization of HuR, whereas the yellow arrows denote motor neurons with cytosolic localization of HuR and increased expression of AMPK-p. Scale bar, 10 μm.

Table 1
Summary of the demographic data, neuropathology, and experimental results of the human subjects.

Case	Age (yr)	Section area	PMI (hr)	Sex	AMPK activation (in motor neurons)	HuR cytosolic localizatiom (in motor neurons)
ALS-1	73	Spinal cord	10	F	+++	+++
ALS-2	61	Spinal cord	8	M	+++	++
ALS-3	49	Spinal cord	9	M	+++	++
Non-ALS-1	45	Spinal cord	17	M	–	–
Non-ALS-2	42	Spinal cord	4	F	–	–
Non-ALS-3	73	Spinal cord	21	M	–	–

As shown in Fig. 1, human sections were evaluated by immunofluorescence staining of AMPK-p, ChAT, and HuR. Expression of phosphorylated AMPK-α at Thr^{172} (AMPK-p) and of cytosolic HuR (Fig. 1) was detected in the ChAT-positive motor neurons of patients with ALS, but not in those of non-ALS controls. In the spinal cord tissues of each subject, eight to 15 ChAT-positive neurons were scored in eight different sections. +++, 100% of the scored cells were positive; ++, ⩾80% of the scored cells were positive; –, no positive cell was detected. PMI, postmortem interval.

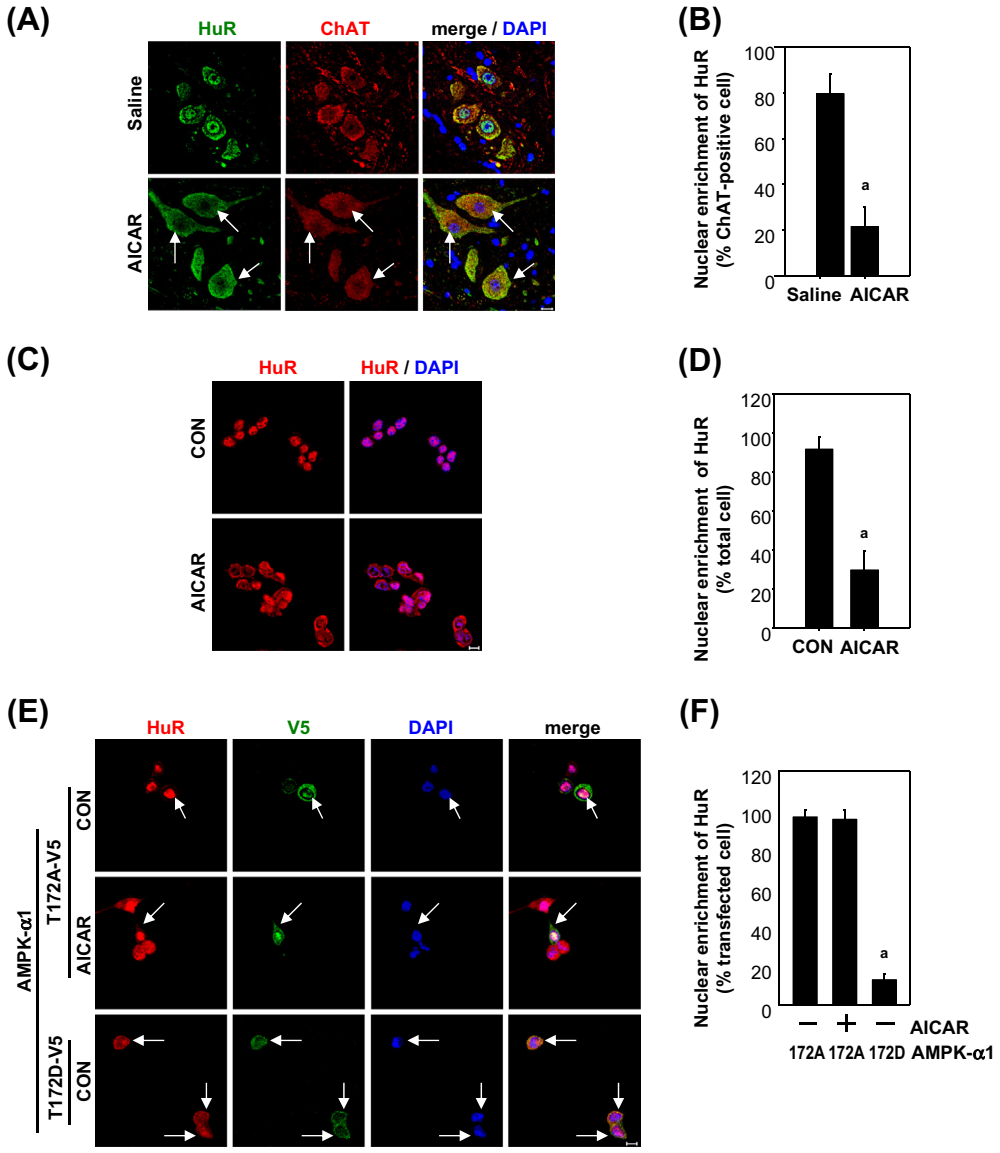

Fig. 2. Activation of AMPK enhances the cytosolic localization of HuR. (A, B) The spinal cord of B6 mice (n = 4–5 per group) was injected with AICAR (30 μg/5 μL/animal) or 0.9% saline (5 μL/animal) for 24 h. The spinal cord tissues were harvested and analyzed using immunohistochemical staining (HuR, green; ChAT, red; nuclei, blue; n = 4–5 in each group). The white arrows indicate motor neurons with cytosolic localization of HuR. For the spinal cord tissue of each mouse, 20–30 ChAT-positive neurons were scored in 3–4 different sections. Quantitation is shown in (B). The data represent the means ± S.E.M. $^{a}P < 0.05$ vs the control. (C and D) NSC34 cells were treated with AICAR (1 mM) for 24 h to activate AMPK. Cells with nuclear enrichment of HuR were quantified using immunofluorescence staining (HuR, red; nuclei, blue) and are shown in (D). The results were quantified as the means ± S.E.M. of three independent experiments. At least 50 cells were scored in each experiment. $^{a}P < 0.05$ vs the control cells. (E) NSC-34 cells were transfected with the indicated AMPK mutant, followed by treatment with AICAR (1 mM, 24 h). The localization of HuR was determined using immunofluorescence staining (HuR, red; AMPKα1-T172A-V5 or AMPKα1-T172D-V5, green; nuclei, blue). The white arrows indicate the transfected cells. The number of transfected cells with nuclear enrichment of HuR was quantified as the means ± S.E.M. in the right panel. $^{a}P < 0.05$ vs cells transfected with AMPK-α1-T172A-V5. Scale bar, 10 μm.

one-way analysis of variance followed by the post hoc Student–Newman–Keuls test. A P-value < 0.05 was considered significant.

3. Results

3.1. Aberrant activation of AMPK evokes the cytoplasmic accumulation of HuR in motor neurons

HuR is a well-characterized mRNA stabilizer and is mainly located in the nucleus. Because increased cytoplasmic accumulation of HuR is associated with diseases [10,11], we first assessed the distribution of HuR in motor neurons. As shown in Fig. 1, HuR existed in the nuclei of the motor neurons of non-ALS (control) subjects, whereas it was detected in the cytoplasm of motor neurons in patients with ALS. Because AMPK controls the cellular localization of HuR in tumor cells and hepatocytes [22,23], next we evaluated the activity of AMPK by measuring the level of phosphorylated AMPK at Thr172 (AMPK-p) using immunohistochemical staining. Motor neurons were identified by the expression of ChAT. The level of AMPK-p in the motor neurons of ALS patients was significantly higher than that observed in normal controls (Figs. 1, S1 and Table 1).

To determine whether activation of AMPK is responsible for the abnormal cellular localization of HuR, we delivered an activator of AMPK (AICAR) into the spinal cord of B6 wild-type mice via intrathecal injection. Consistent with what was observed in human spinal cords, HuR was mainly enriched in the nuclei of ChAT-positive motor neurons, with a low level of HuR detected in the cytoplasm. Activation of AMPK by AICAR enhanced the cytosolic

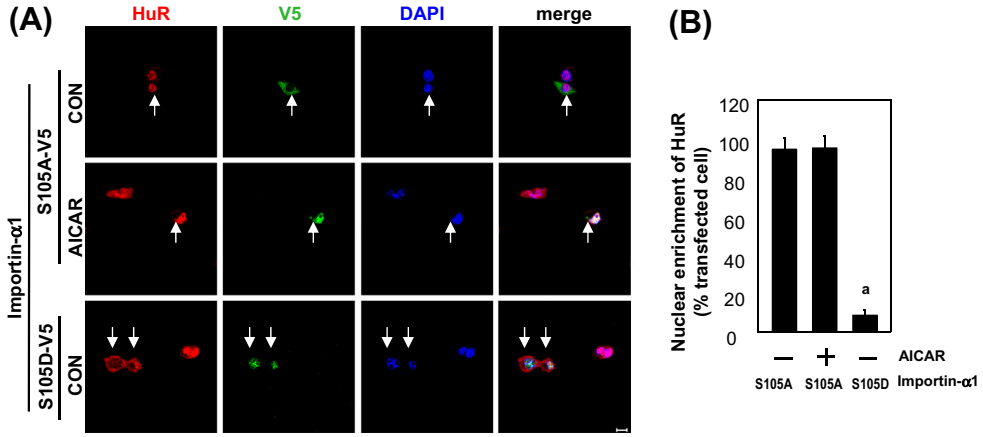

Fig. 3. Importin-α1 functions downstream of AMPK to mediate the cytosolic localization of HuR. NSC34 cells were transfected with the indicated importin-α1 variant for 48 h, followed by treatment with AICAR (1 mM) for an additional 24 h. The localization of HuR was determined using immunofluorescence staining (HuR, red; nuclei, blue; importin-α1-S105A-V5 or importin-α1-S105D-V5, green). The white arrows indicate the transfected cells. The number of cells with nuclear enrichment of HuR was quantified as means ± S.E.M. $^{a}P < 0.05$ vs the importin-α1-S105A group. Scale bar, 10 μm.

expression of HuR (Fig. 2A and B). More than 60% of the ChAT-positive motor neurons expressed no HuR in the nucleus after treatment with AICAR, which suggests that AMPK activation leads to aberrant localization of HuR in motor neurons. Of note, we consistently observed that the AICAR-treated NSC34 cells were slightly larger than control cells. Although the functional impact of this morphological change is currently unknown, previous studies suggest that cellular swelling is a hallmark of necrotic cell death [24,25]. For example, swollen retinal cells were found in SOD1-null mice and such alteration in morphology subsequently leads to necrotic cell death [25]. It would be of great interest to further evaluate whether the AMPK-induced redistribution of HuR would subsequently cause necrotic cell death of motor neurons.

We further evaluated the hypothesis stated above by treating a motor neuron cell line (NSC34) with AICAR. Similar to what was observed in the motor neurons of mice, the activation of AMPK by AICAR enhanced the cytoplasmic accumulation of HuR in NSC-34 cells (Fig. 2C and D), supporting a critical role for AMPK in regulating the distribution of HuR. Such abnormal distribution of HuR is likely to alter the mRNA stability of its target mRNAs, because treatment with AICAR reduced the half-life of the VEGF transcript (which is a downstream target of HuR [9]) in NSC34 cells (Fig. S2B).

The expression of a dominant-positive mutant of AMPK (AMPK-α1-T172D) [19] also evoked cytoplasmic accumulation of HuR in NSC34 cells, as did AICAR. Conversely, the expression of a dominant-negative mutant of AMPK (AMPK-α1-T172A) [19] reversed the AICAR-induced redistribution of HuR (Figs. 2E, F and S2A). Collectively, these findings indicate that activation of AMPK mediates the aberrant cytoplasmic accumulation of HuR in motor neurons.

3.2. Importin-α1 functions downstream of AMPK to mediate the AMPK-evoked cellular accumulation of HuR

Next, we investigated the mechanism that mediates the enhanced redistribution of HuR to the cytoplasm by AMPK. Because the nucleus-to-cytoplasm transport of HuR is mediated by importin-α1, and because AMPK phosphorylates importin-α1 at Ser105 [20], we overexpressed a phosphomimetic mutation of importin-α1 (importin-α1-S105D) that mimics the AMPK-mediated phosphorylated form of importin-α1 in NSC34 cells. As shown in Figs. 3 and S3A, expression of importin-α1-S105D markedly reduced the nuclear enrichment of HuR. Most importantly, the expression of an importin-α1 variant (importin-α1-S105A), which cannot be phosphorylated by AMPK, reduced the effect of AICAR on the abnormal cellular distribution of HuR. Exogenous expression of wild-type importin did not alter the distribution pattern on HuR (Fig. S3B). These findings suggest that phosphorylation of importin-α1 at Ser105 by AMPK mediates the subcellular redistribution of HuR.

3.3. Inhibition of AMPK using an A_{2A} adenosine receptor agonist prevented the cytosolic localization of HuR

We demonstrated previously that the activation of protein kinase A via the stimulation of the A_{2A} adenosine receptor ($A_{2A}R$) inhibits AMPK and has a beneficial effect in Huntington disease [19]. This is of great interest because the activation of $A_{2A}R$ in the spinal cord exerts protective effects [26,27], and the expression of $A_{2A}R$ in spinal cord motor neurons is greatly enhanced by stresses [27]. Consistent with a beneficial effect of $A_{2A}R$ activation, chronic uptake of caffeine, which is an inhibitor of $A_{2A}R$, markedly shortened the life-span of mice with ALS [28]. Therefore, we evaluated whether the activation of $A_{2A}R$ affected the abnormal cellular distribution of HuR in NSC34 cells. As shown in Fig. 4B, NSC34 cells were treated with an $A_{2A}R$ agonist (T1–11, [29]) to stimulate AC activity. The addition of an $A_{2A}R$ antagonist (Sch58261) markedly reduced the T1–11-induced AC activity, which demonstrated that this enhanced AC activity was mediated by $A_{2A}R$. Most importantly, treatment with T1–11 significantly reduced the AICAR-evoked activation of AMPK, as assessed by the level of phosphorylation of AMPK at Thr172 (Fig. 4C and D). Moreover, Sch58261 eliminated the effect of T1–11 on AMPK activation, supporting the contention that T1–11 suppresses AMPK activation via $A_{2A}R$. Consistent with the finding that activation of AMPK is associated with the increase in the cytosolic localization of HuR (Figs. 1 and 2), treatment with T1–11 normalized the AICAR-evoked aberrant cellular localization in an $A_{2A}R$-dependent manner (Fig. 4E and F). Sch58261 or H89 alone did not produce any effect on HuR distribution (Fig. S4). In addition, the effects of T1–11 on AMPK activation and the cytosolic accumulation of HuR were both sensitive to a PKA inhibitor (H89, Fig. 4C–F), which demonstrated that PKA mediates the function of T1–11 in controlling the proper localization of HuR. Therefore, the $A_{2A}R$/PKA pathway might be an important therapeutic target for ALS.

4. Discussion

The modulation of RNA metabolism is one of the key machineries for gene regulation and contributes to the cellular responses to

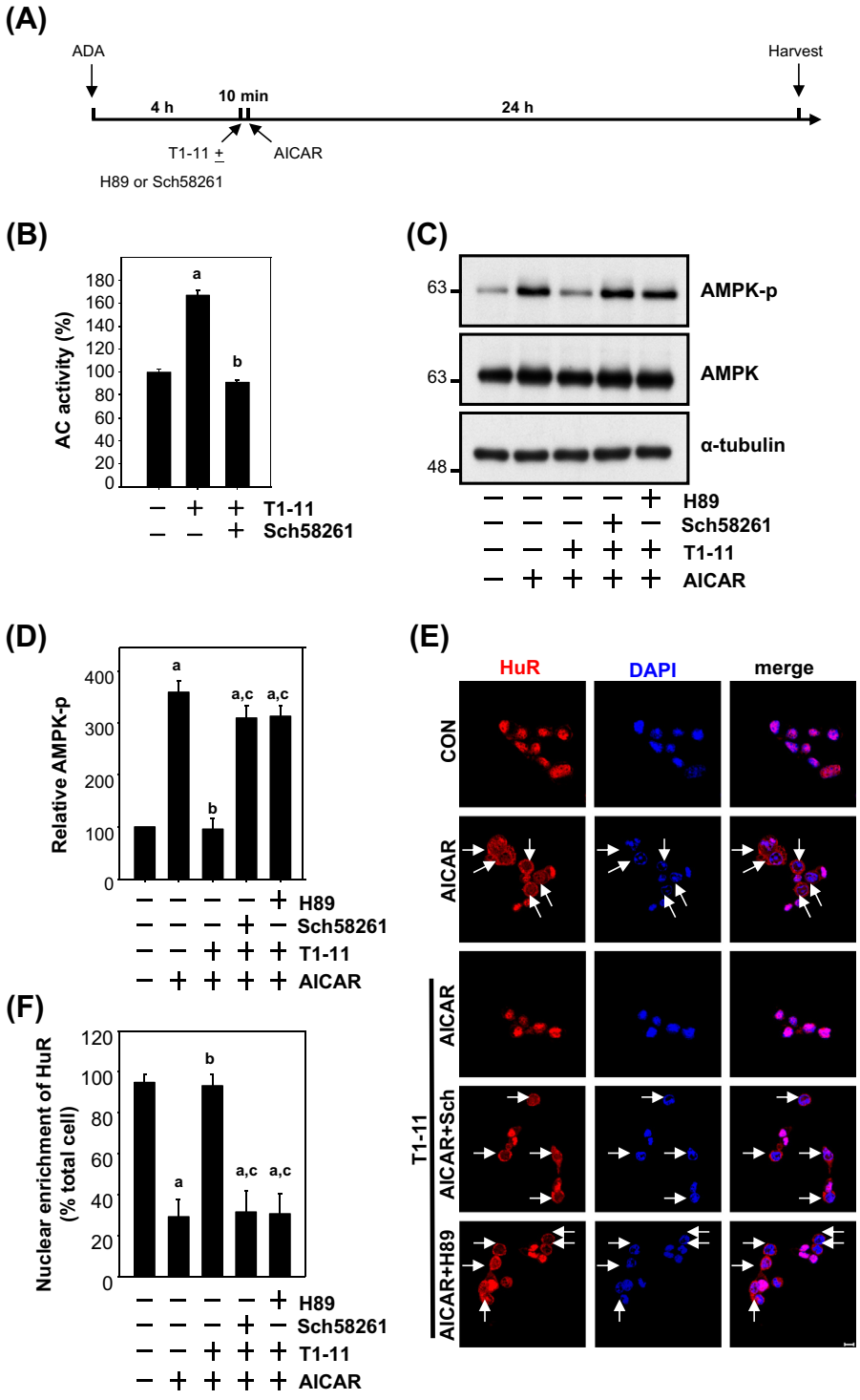

Fig. 4. Activation of $A_{2A}R$ prevents the activation of AMPK and normalizes the cellular distribution of HuR in a PKA-dependent manner. (A) A diagram with the drug pipeline treatment applied to NSC34 cells. (B) NSC34 cells were treated with an $A_{2A}R$ agonist (T1–11, 30 μM) in the absence or presence of an $A_{2A}R$ antagonist (Sch58261, 10 μM) for 20 min at room temperature. Cells were harvested to measure adenylyl cyclase (AC) activity. Data were quantified as means ± S.E.M. $^{a}P < 0.01$ vs the control cells. $^{b}P < 0.01$ vs the T1–11-treated cells. (C–F) NSC34 cells were treated with the indicated drug(s) (AICAR, 1 mM; T1–11, 30 μM; Sch58261, 10 μM; and H89, 10 μM) for 24 h. (C and D) The expression of AMPK-p was determined using western blot analysis. The level of AMPK-p was normalized to that of AMPK, and is presented in (D). The data represent means ± S.E.M. $^{a}P < 0.05$ vs control cells. $^{b}P < 0.05$ vs the AICAR group. $^{c}P < 0.05$ vs the AICAR plus T1–11 group. (E and F) The localization of HuR (red) was determined using immunofluorescence staining. Cells with cytosolic localization of HuR are marked by white arrows (E). The localization of HuR were quantified in (F). At least 50 cells were scored in each experiment. The data are presented as means ± S.E.M. of three independent experiments. $^{a}P < 0.05$ vs the control group. $^{b}P < 0.05$ vs the AICAR group. $^{c}P < 0.05$ vs the AICAR plus T1–11 group. Scale bar, 10 μm.

various external stimuli (including oxidative stress, nutrient deprivation, and UV radiation [30–32]). Abnormal RNA metabolism has been documented in ALS [2]. For example, the alteration in the cellular distribution of several RNA-binding proteins (e.g., TDP-43) may change the stability of the RNAs to which they bind, thus causing abnormal functions that contribute to the pathogenesis of

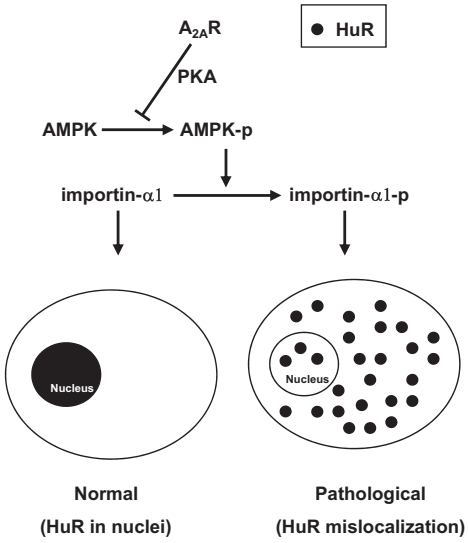

Fig. 5. Schematic representation of the mechanism via which AMPK mediates the cytosolic localization of HuR. In normal conditions, HuR is localized in the nucleus. In pathological conditions, AMPK induces importin-α1 phosphorylation, which leads to the cytosolic localization of HuR. Activation of $A_{2A}R$ using an $A_{2A}R$ agonist reduces AMPK activation and prevents the subcellular localization of HuR.

ALS [5]. We recently demonstrated that AMPK activation causes the mislocalization of TDP-43 in NSC34 cells and mouse spinal motor neurons, and is associated with ALS pathogenesis [33]. TDP-43 has recently been demonstrated to mediate many important RNA-processing functions, including pre-mRNA splicing, microRNA genesis, and gene transcription [3,4,34]. Mislocalization of TDP-43 is expected to cause the loss of nuclear function and alterations in cellular RNA metabolism, which may contribute significantly to the pathology of ALS. In the present study, we demonstrated that activation of AMPK caused the abnormal localization of HuR via phosphorylation of importin-α1. Importantly, AMPK activation also induced an abnormal subcellular distribution of FUS (fused to sarcoma), which is an RNA-binding protein that functions as a component of the hnRNP complex and pre-mRNA splicing (Fig. S5) [34]. Given that importins control the movement of many proteins from the cytoplasm into the nucleus by binding to their nuclear localization signals, our findings support the importance of the AMPK-mediated impairment of importin in ALS, as well as the critical role of RNA metabolism in this disease. Although under AICAR treatment, the distributions of TDP-43 and HuR in NSC34 cells were both altered (Fig. S6), some of the immuno-signals appeared to be overlapped, some were not. It is likely that when the importin-mediated transport is impaired by AMPK activation, abnormal distributions of these importin substrates would occur, likely independently of each other. Further experiments are needed to characterize the potential interaction among these importin substrates.

In addition to the well-characterized TDP-43 and the fused in sarcoma (FUS) protein [35,36], HuR is another key player in RNA metabolism that has been implicated in ALS, as it is sequestered into intracellular inclusions by an ALS-associated mutant superoxide dismutase 1 (SOD1) in the spinal cord of mice expressing mutant SOD1 [9]. Although the AMPK-mediated mislocalization of HuR has been reported in liver cells [22], its role in motor neuron diseases has not been explored. In the present study, we showed for the first time that HuR is aberrantly accumulated in the cytoplasm of motor neurons of patients with ALS (Fig. 1). It should be pointed out that multiple pathogenic pathways occur in ALS, which is a chronic disease that takes years to develop. To validate the causal relationship between the activation of AMPK and the mislocalization of HuR, we confirm the abovementioned finding in mice treated with AICRA intrathecally for 24 h (Fig. 2), suggesting that a short duration of AMPK activation is sufficient to trigger the aberrant distribution of HuR. Using a motor neuron cell line (NSC34), we further demonstrated that such abnormal localization of HuR in motor neurons may be mediated by the activation of AMPK (Figs. 2 and S6). In addition, we demonstrated that importin-α1 acts downstream of AMPK to control the cellular distribution of HuR (Fig. 2), supporting the contention that abnormal nuclear protein import in motor neurons is an important pathogenic pathway in ALS [37]. We also showed that suppression of AMPK by ectopic expression of a dominant variant of AMPK (AMPK-α1-T172A) and a small molecule (T1–11) [29] that activates $A_{2A}R$ effectively normalized the AMPK-evoked cytoplasmic accumulation of HuR (Figs. 2E, 4, and 5). These findings are important, particularly for neurodegenerative diseases, because AMPK is a key energy sensor that can be profoundly regulated in response to various cellular stresses (such as oxidative stress, [38]). Abnormal activation of AMPK has been observed in various neurodegenerative diseases, including Huntington's disease, Alzheimer's disease, and ALS [16,17,19,38,39]. Our study provides much-needed evidence that suggest that the abnormal activation of AMPK in motor neurons imbalances RNA metabolism and contributes to the pathogenesis of ALS.

Despite the tremendous efforts that have been devoted to the development of treatments for ALS, the availability of therapeutic interventions for ALS patients remains very limited. The results of our study suggest that stimulation of PKA is an effective means to normalize the overactivation of AMPK and its pathogenic consequences. Further investigations are needed to assess whether cAMP-elevating reagents (including agonists of $A_{2A}R$) might be used to enhance the sustainability of motor neurons against stresses and to delay the progression of ALS.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2014.12.029.

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