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AMPK induces vascular smooth muscle cell senescence via LKB1 dependent pathway

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ABSTRACT

Vascular cells have a limited lifespan with limited cell proliferation and undergo cellular senescence. The functional changes associated with cellular senescence are thought to contribute to age-related vascular disorders. AMP-activated protein kinase (AMPK) has been discussed in terms of beneficial or harmful effects for aging-related diseases. However, the detailed functional mechanisms of AMPK are largely unclear. An aging model was established by stimulating vascular smooth muscle cell (VSMC) with adriamycin. Adriamycin progressively increased the mRNA and protein expressions of AMPK. The phosphorylation levels of LKB1 and acetyl-CoA carboxylase (ACC), the upstream and downstream of AMPK, were dramatically increased by adriamycin stimulation. The expressions of p53 and p21, which contribute to vascular senescence, were also increased. Inhibition of AMPK diminished senescence-associated β-galactosidase (SA-β-gal) staining, and restored VSMC proliferation. Cytosolic translocation of LKB1 by adriamycin could be a mechanism for AMPK activation in senescence. Furthermore, p53 siRNA and p21 siRNA transfection attenuated adriamycin-induced SA-β-gal staining. These results suggest that LKB1 dependent AMPK activation elicits VSMC senescence and p53–p21 pathway is a mediator of LKB1/AMPK-induced senescence.

1. Introduction

Senescence is one of the major risk factor for the development of vascular diseases such as coronary artery disease, hypertension and atherosclerosis [1]. Cellular senescence is accompanied by a specific set of changes in cell function, morphology, and gene expression. Recent studies have indicated that many of the changes in senescent vascular cells are consistent with atherosclerosis [2]. Atherosclerosis is an intrinsically age-related disease, attributed to a proliferative process that selectively affects arterial wall. Atherosclerosis also may be viewed as a form of accelerated vascular aging [3].

Prevention of vascular aging may be important for effective therapy to age-related vascular changes in atherosclerotic degeneration. According to recent report, vascular cell senescence may play a pivotal role in the pathogenesis of atherosclerosis [4]. Cell senescence refers to the process by which cells lose their ability to replicate after a number of cell divisions. Vascular smooth muscle cells (VSMCs) derived from atherosclerotic plaques show a lower rate of cell proliferation in vitro and undergo senescence earlier than normal vessels [5]. However, the molecular mechanisms underlying the increased risk of such conditions that is conferred by aging remained unclear.

A number of studies have demonstrated that senescence is accompanied by an increase in AMP/ATP ratio and AMP-activated protein kinase (AMPK) levels in multiple tissues [6]. AMPK is a crucial cellular energy sensor that controls fatty acid oxidation, myocardial morphology and contractile action [7]. AMPK is a heterotrimeric protein kinase consisting of a catalytic subunit (α) and two regulatory subunits (β and γ). The α subunit contains a kinase domain and exist as both α1 and α2 isoforms, which have different tissue expression patterns. AMPK is activated under conditions that promote the AMP/ATP ratio such as glucose deprivation and muscle contraction [8]. AMPK controls cell metabolism and proliferation in response to low energy levels by phosphorylating a variety of substrates in the cell, including acetyl-CoA carboxylase (ACC), p53, and p21 [9,10]. AMPK activation is also associated with increased activity of an upstream AMPK kinase. LKB1 is one of the upstream kinases of AMPK [11]. The LKB1/AMPK pathway has been implicated in tumor suppression [12].

Recent evidence suggests a role for AMPK signaling in the senescence process. Senescence-associated loss of AMPK activity was reported in skeletal muscle, which may contribute to the interrupted mitochondrial function and intracellular lipid metabolism in advanced age [13,14]. In addition, hypoxic insult was unable to turn on AMPK signaling in hepatic cells in aged rats, indicating a reduced stress tolerance with senescence. However,

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the role of AMPK in VSMC senescence has not been studied despite the high AMPK expression [15] and cardiovascular senescence represents the largest portion of age-related mortality. Since adriamycin has been reported to induce senescence of thymus [16], we therefore tried adriamycin as a chemical inducer of senescence.

In the present study, we investigated whether adriamycin-induced LKB1/AMPK activation can promote VSMC senescence and mechanisms responsible for AMPK-induced VSMC senescence.

2. Materials and methods

2.1. Reagents and antibodies

Adriamycin was supplied by Il-dong Pharmaceuticals Co., Ltd. (Seoul, Korea). Antibodies specifically recognizing AMPK, p-AMPK, p-ACC, p-LKB1, and p53 were purchased from Cell Signaling Technology, Inc. (Beverly, MA, USA). The antibody against p21 was supplied by Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Compound C, an AMPK inhibitor, was provided by Calbiochem (La Jolla, CA, USA). AMPK, LKB1, p53, p21, and control siRNA were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). TO-PRO-3 was obtained from Invitrogen (Carlsbad, CA, USA) for use as a nuclear counterstain.

2.2. Cell culture

Sprague–Dawley rats were anesthetized with pentobarbital (50 mg/kg). VSMCs were isolated from the thoracic aorta. The cells were processed using a 1-mm chop setting in a 10-cm culture dish, and cultured with 50% FBS-Dulbecco's modified eagle's medium (DMEM) with 1% antibiotics in a CO_2 incubator (5% CO_2/95% air, 37 °C). Aortic VSMCs were maintained in DMEM with 10% FBS and 1% antibiotic–antimycotic (penicillin 10,000 U/ml, amphotericin B 25 μg/ml, streptomycin 10,000 μg/ml). We used VSMCs from passages 4 to 8 at 70–90% confluence in 10 cm dishes, and cell growth was arrested by incubation of the cells in serum-free DMEM for 24 h prior to use.

2.3. Senescence-associated β-galactosidase (SA-β-gal) staining

SA-β-gal staining was performed as previously described [6]. Briefly, the VSMCs were seeded in 6-well plates and fixed with 4% formaldehyde for 2–3 min at room temperature. The cells were then washed with PBS and incubated with SA-β-gal staining solution (1 mg/ml of X-gal) (Stratagene, La Jolla, CA, USA), 40 mM citric acid/sodium phosphate buffer, pH 6.0, 5 mM potassium ferricyanide (Sigma, St. Louis, MO, USA), 5 mM potassium ferrocyanide (Sigma, St. Louis, MO, USA), 150 mM NaCl, and 2 mM $MgCl_2$ in 37 °C for 16–17 h to visualize SA β-gal staining.

2.4. Western blot analysis

Whole cell extracts were prepared by lysing the cells in proprep protein extract buffer. The protein concentration was quantified with protein assay reagent from Bio-Rad (Hercules, CA, USA). Equal amounts of protein were mixed with Laemmli sample buffer (Bio-Rad, Hercules, CA, USA) and heated for 5 min at 100 °C before loading. Total protein samples (30 μg) were subjected to 10% SDS–polyacrylamide gel electrophoresis (SDS–PAGE) for 1 h 30 min at 100–120 V. The separated proteins were electrophoretically transferred onto PVDF membranes for 1 h at 100 V using SD semi-dry transfer cell. The membranes were blocked for 1 h at room temperature with 5% non-fat milk in PBS containing 0.05% Tween 20 (PBS-T). The membranes were then incubated overnight with the primary antibodies at a dilution of 1:1000 at 4 °C in PBST. The membranes were then washed with four changes of wash buffer (0.05% Tween 20 in PBS) and incubated for 1 h at room temperature in PBS containing anti-rabbit (Cell Signaling Technology, Inc., Beverly, MA, USA) or anti-mouse Ig G (Sigma, St. Louis, MO, USA) antibodies. Finally, after three more rinses with wash buffer, the membranes were exposed to ECL or ECL plus Western blot analysis detection reagents.

2.5. RT-PCR and real-time PCR analysis

The total RNA was isolated from the cultures using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RT-PCR was performed using 1 μg of total RNA. The total RNA was reverse-transcribed using the Superscript III kit (Invitrogen, Carlsbad, CA, USA). Primers for PCR analysis were synthesized at Bioneer (Daejeon, Korea). Each reaction was carried out as follows: 5 min at 94 °C, 40 cycles of 94 °C for 30 s, 60 °C for 45 s, and 72 °C for 1 min, and 10 min at 72 °C. The primer sequences were as follows: AMPK, forward (F) 5′-TCAGGCACCCTCATATAATC-3′ and reverse (R) 5′-TGACAATAGTCCACACCAGA-3′. AMPK has two isoforms: AMPK α1 and α2. We also determined the mRNA levels of AMPK α1 and α2 in VSMC using real-time PCR. The primers were as follows: AMPK α1, forward (F) 5′-GCAGAGAGATCCAGAACCTG-3′ and reverse (R) 5′-CTCCTTTTCGTCCAACCTTCC-3′ and AMPK α2, forward (F) 5′-GCTGTGGATCGCCAAATTAT-3′ and reverse (R) 5′-GCATCAGCAGAGTGGCAATA-3′. Real-time PCR was performed using the quantitect SYBR green kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Reaction volume was 20 μl and the annealing temperature was 57 °C. Fluorescence was detected using ABI Prism 7700 detection system. Ratios of target gene to GAPDH were calculated and compared in the presence or absence of adriamycin.

2.6. Transfection of siRNA

Transfection of VSMCs with siRNA was performed using Lipofectamine 2000, according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). Aliquots of 1×10^4 cells were plated on 6-well plates the day before transfection and grown to about 70% confluence. The cells were then transfected with 10 μM AMPK, LKB1, p53, and p21 siRNA (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) plus 100 pmol of Lipofectamine for 6 h in Opti-MEM®I reduced serum medium (Invitrogen, Carlsbad, CA, USA). Following an incubation period of 48 h, the protein level was measured using Western blot analysis.

2.7. Cell proliferation assay

VSMC were seeded on 24-well plates at 1×10^4 cells per well in DMEM supplemented with 10% FBS. After different treatments, 50 μl of 1 mg/ml MTT solution was added to each well (0.1 mg/well) and incubated for 4 h. The supernatants were aspirated, and the formazan crystals in each well were solubilized with 200 μl dimethyl sulfoxide. An aliquot of each solution (150 μl) was placed in 96-well plates. Cell proliferation was assessed by measuring the absorbance at 570 nm using a microplate reader. The experiments were repeated 3 times.

2.8. Adenoviral transduction

Adenoviruses expressing the control gene GFP and the dominant-negative isoform of the α1 and α2 subunits of AMPK (AMPK DNα1 and DN α2) were amplified in AD293 cells using standard methodologies. The transductions were carried out in VSMC in serum-free DMEM for 6 h.

2.9. Immunofluorescence analysis

VSMCs were seeded on coverslips in 10 cm dishes, fixed in 4% formaldehyde, and permeabilized with 0.2% Triton X-100. LKB1 primary antibody was used at 1:125 (Cell Signaling Technology, Inc., Beverly, MA, USA) and incubated with cells overnight at 4 °C. Rabbit FITC secondary antibody (Invitrogen, Carlsbad, CA, USA) was used at 1:100 and incubated with cells for 1 h at room temperature. The nuclei were stained with TO-PRO-3 at 1:150. Fixed and immunofluorescently stained cells were imaged using leica confocal microscope (Bannockburn, IL, USA).

2.10. Statistical analysis

All data are represented as the mean ± S.E.M. Differences between data sets were assessed by analysis of variance (ANOVA) followed by Bonferroni's *t*-test. *P* values < 0.05 were considered significant.

3. Results

3.1. AMPK-related protein kinases promoted senescence of VSMC

We examined whether activation of AMPK induced cellular senescence in VSMCs. Adriamycin 500 nM stimulation significantly increased SA-β-gal cell staining, a biomarker of cellular senescence (Fig. 1A). As seen in Fig. 1B, the protein levels of AMPK and AMPK-associated kinase were increased during the adriamycin-induced senescence process. After adriamycin stimulation, mRNA level of AMPK was increased in RT-PCR (Fig. 1C) and AMPK isoforms (AMPK α1 and α2) were also elevated in real-time PCR analysis (Fig. 1D). We tried metformin as an activator of AMPK and investigated relationship between AMPK activation and VSMC senescence. Metformin treatment increased SA-β-gal staining and induced AMPK activation in VSMCs. Metformin-induced AMPK-associated kinase expressions were similar to the changes in adriamycin-induced VSMC senescence (Supplemental Fig. S1). These results showed that activation of AMPK signaling cascades *are relevant to the* senescence in VSMCs.

3.2. Inhibition of AMPK activation prevents VSMC senescence and restores VSMC proliferation

To further demonstrate the interaction of VSMC senescence and AMPK activation, we examined the effects of AMPK inhibitors on senescence. Treatment of compound C, a specific inhibitor of AMPK, reduced the adriamycin-stimulated SA-β-gal-positive cells and promoted proliferation of VSMCs (Fig. 2A and B). The protein levels of p-AMPK, p-ACC, and AMPK were confirmed by Western blotting (Fig. 2C). Genetic inhibition of AMPK with siRNA also diminished the adriamycin-induced SA-β-gal-positive cells and restored proliferation. The protein levels of p-AMPK, p-ACC, and AMPK were confirmed with same protocol (Supplemental Fig. S2). The adriamycin-induced AMPK and ACC phosphorylation in VSMCs was significantly inhibited by pretreatment with compound C or AMPK siRNA. These findings indicated that AMPK plays a role in senescence of VSMC.

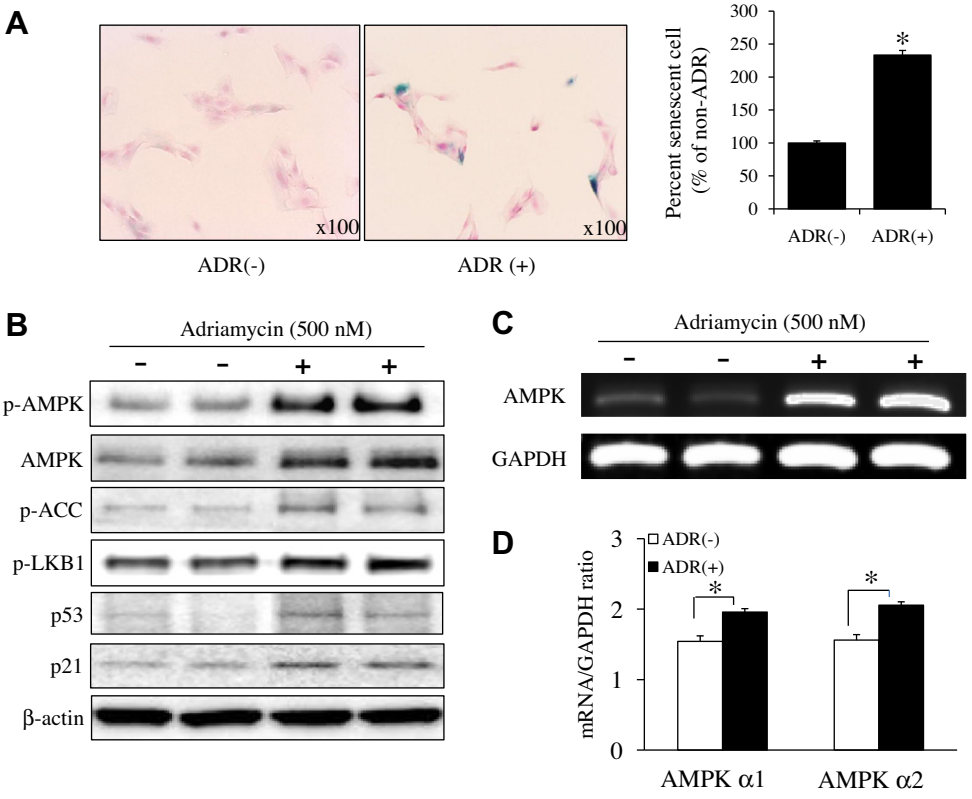

Fig. 1. Adriamycin promoted cell senescence and increased expressions of kinases associated with AMPK signaling pathway. (A) 4 h after treatment with 500 nM adriamycin (ADR), SA-β-gal staining was performed to evaluate the senescent status of the VSMCs. $^{*}p < 0.01$ versus absence of ADR. (B) VSMCs stimulated with 4 h ADR were subjected to Western blotting to determine the level of p-AMPK, p-ACC, p-LKB1, p53, p21, and AMPK proteins. (C) mRNA levels of AMPK increased in VSMCs stimulated with ADR. (D) mRNA levels of AMPK α1 and AMPK α2 in VSMCs were determined using real-time PCR. Representative results from three independent experiments were shown. Values are given as means ± S.E.M ($n = 3$) with $^{*}p < 0.01$ versus absence of ADR.

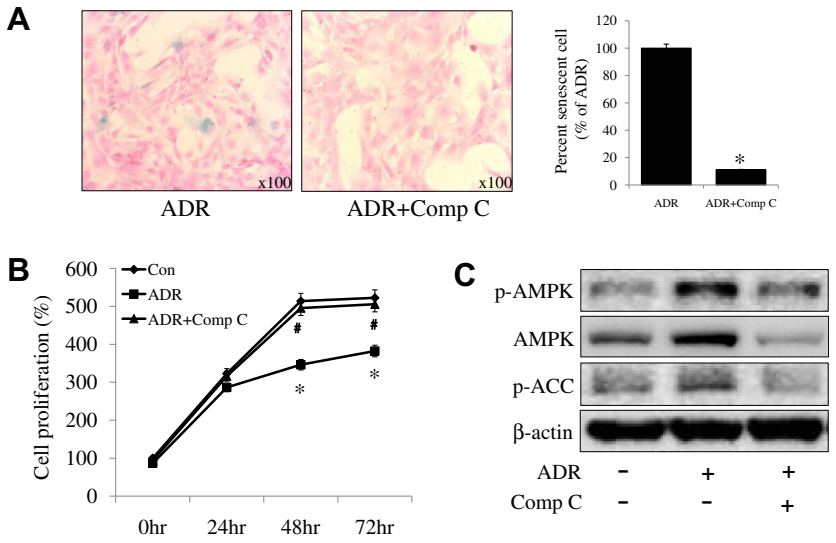

Fig. 2. Pharmacological inhibition of AMPK diminished VSMC senescence and induced VSMC proliferation. (A) VSMCs were treated with compound C (Comp C) 10 μM for 2 h and then stimulated with adriamycin (ADR) 500 nM for 4 h. After incubation, the cells were stained with SA-β-gal. $^{*}p < 0.01$ versus ADR. (B) After ADR treatment, cell proliferation (0, 24, 48, and 72 h) was determined by the MTT assay. Values are given as means ± S.E.M ($n = 6$) with $^{*}p < 0.01$ versus control, $^{\#}p < 0.01$ versus ADR. (C) The levels of p-AMPK, p-ACC, and AMPK proteins were assayed by Western blot. Results were confirmed by three repeated experiments.

3.3. Ad-AMPK-DN prevents VSMC senescence and restores VSMC proliferation

The adenovirus-mediated overexpression system was used to determine whether AMPK could induce senescence in VSMCs. Compared to the GFP controls, VSMCs infected with adenovirus encoding AMPK DNα1 and AMPK DNα2 exhibited remarkably decreased SA-β-gal staining (Fig. 3A). Compared to the GFP control, *expressions of* AMPK DNα1 and AMPK DNα2 promoted the proliferation of VSMCs (Fig. 3B). In Western blotting, transduction of Ad-AMPK DNα1 and Ad-AMPK DNα2 also prevented the increase in phosphorylation of the AMPK and ACC (Fig. 3C). These results confirmed that inhibition of AMPK diminished characteristics of senescent VSMC.

3.4. LKB1 provokes senescence in VSMCs through activation of AMPK

LKB1 is the upstream kinase of AMPK. Under conditions of high cellular energy stress, LKB1 acts as an AMPK activator through an AMP-dependent mechanism [17]. To determine whether LKB1 plays a crucial role in adriamycin-stimulated AMPK activation, we investigated the effect of LKB1 siRNA on VSMCs. LKB1 siRNA reduced the adriamycin-stimulated SA-β-gal positive cells. We also tried LKB1 siRNA to show AMPK is downstream of LKB1 in adriamycin-induced VSMC senescence. In LKB1 siRNA transfected VSMCs, adriamycin failed to activate AMPK (Fig. 4A). Under normal physiological conditions, LKB1 is predominantly localized in the nucleus. In present study, we demonstrated that senescence induced LKB1 translocation from nucleus to cytosol, leading to in-

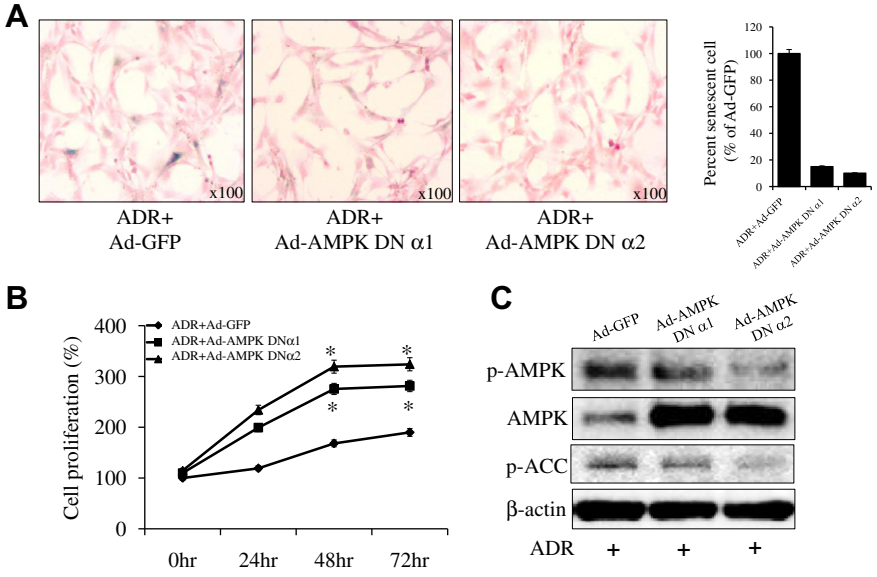

Fig. 3. Dominant-negative AMPK adenovirus abolished VSMC senescence induced by adriamycin. (A) VSMCs were transfected with adenovirus-GFP, AMPK DNα1, and AMPK DNα2 for 6 h and then incubated for 48 h. The infected cells were then treated with adriamycin (ADR) for 4 h and stained with SA-β-gal. $^{*}p < 0.01$ versus Ad-GFP. (B) After ADR treatment, cell proliferation (0, 24, 48, and 72 h) was determined by the MTT assay. Values are given as means ± S.E.M ($n = 6$); $^{*}p < 0.01$ versus Ad-GFP. (C) Inhibition of AMPK by adenoviral overexpression of AMPK DNα1 and α2 attenuated p-AMPK and p-ACC proteins. Results were confirmed by three repeated experiments.

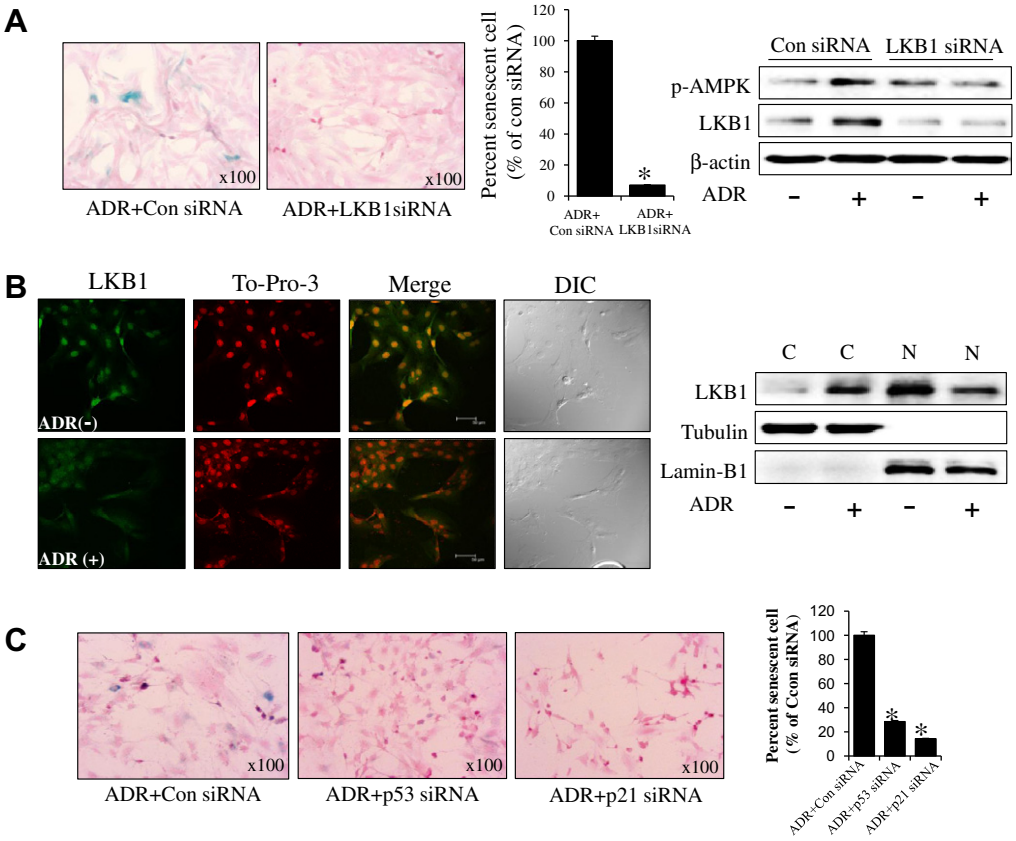

Fig. 4. LKB1 is required for adriamycin-stimulated AMPK activation and p53–p21 signaling in VSMC senescence. (A) VSMCs were transfected with control (Con) siRNA or LKB1 siRNA and then stimulated with adriamycin (ADR) for 4 h. After incubation, the cells were stained with SA-β-gal. VSMCs were subjected to Western blotting to determine the level of p-AMPK and LKB1 proteins. $^{*}p < 0.01$ versus Con siRNA. (B) Confocal microscopy images depict the localization of LKB1 (green) in VSMC treated with or without ADR. The cell nuclei were stained with To-Pro-3 (red), merge of LKB1 and nuclei is shown in yellow. Photomicrographs were taken at the same magnification (×400). ADR-stimulated cytosolic translocation of LKB1 is important for AMPK activation in VSMC senescence. Cytosol (C), Nucleus (N) (C) VSMCs were transfected with Con siRNA, p53 siRNA, and p21 siRNA for 48 h. VSMCs were then stimulated with ADR for 4 h. After incubation, the cells were stained with SA-β-gal. $^{*}p < 0.01$ versus Con siRNA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

creased AMPK activation. Cellular fractionation analysis showed that adriamycin treatment increased nuclear export of LKB1. Fractionation was evaluated by immunoblotting with specific antibodies against tubulin and lamin-B1 (Fig. 4B). Our results demonstrate that enhanced cytosolic localization of LKB1 is the mechanism responsible for adriamycin-stimulated AMPK activation in VSMCs.

Activation of the p53–p21 pathway acts as a major mediator of cellular senescence [18]. Control siRNA transfected VSMC displayed enhanced SA-β-gal-positive cells, and loss of p53 and p21 diminished SA-β-gal positive cells (Fig. 4C). These results indicated that AMPK induced VSMC senescence through LKB1-dependent pathway by promoting the cytosolic localization of LKB1, and the inhibition of p53–p21, downstream of AMPK, decreased SA-β-gal-positive cells.

4. Discussion

Advancing age is a major risk factor for the development of cardiovascular diseases, such as atherosclerosis, hypertension, and stroke. Aging also induces several changes in vascular structure and function, and the risks of aging include increases in arterial intima-media thickness, arterial stiffness, and inflammation [19]. Although cellular senescence is a natural biological process, its role in living organisms is not completely understood. In general, aging can be viewed as an alternative response program to cellular stresses and damages that otherwise may cause programmed cell death [20]. When confronted with metabolic and environmental stressors (e.g. oxidative stress), a self-protective mechanism of aging may be initiated to halt the energy-consuming process of cell proliferation. The duration of cell survival in the non-dividing state after cessation of proliferation is a characteristic of longevity [21]. Mammalian senescence is also triggered by a complex signaling network involving the interactions of multiple proteins [22].

In this study, we elucidated the interplay between LKB1 and AMPK signaling, a well-known stress resistance and longevity-regulating pathways. In recent studies, SIRT1 has been shown to prevent vascular senescence of endothelial cells [23,24]. Moreover, transient activation of LKB1/AMPK signaling protects vascular cells by ensuring the slow consumption of energy stores and maintaining energy homeostasis against stress [25]. LKB1 mediates AMPK activation in response to various cellular stresses and pharmacological agents [26]. Although LKB1/AMPK activation protects cells against energy stress by maintaining energy homeostasis, this pathway can also cause cellular senescence, cell cycle arrest, and apoptosis in eukaryotic systems [27,28]. Activation of LKB1/AMPK contributes to the premature aging of *Zmpste24*$^{-/-}$ mice [29].

In quiescent VSMC, more studies are needed to address the beneficial or adverse effects of LKB1/AMPK signaling in the aging process, the present study examined the role of AMPK-induced VSMC senescence and tried to elucidate the underlying mechanisms.

AMPK and AMPK-associated kinase were increased during the adriamycin-induced senescence process (Fig. 1), this result was similar to the report that showed AMPK activation caused cellular

senescence in a mammalian system [29]. AMPK inhibition by both compound c and AMPK siRNA resulted in profound anti-senescence effect to VSMC and promoted proliferation of VSMCs. AMPK inhibition by adenoviral infection encoding dominant-negative form of AMPK also exhibited an anti-senescence phenotype (Figs. 2 and 3). These results confirmed that AMPK induced senescence in VSMCs and AMPK inhibition could restore proliferation potency that is characteristics of young cell.

We examined whether LKB1 plays a role in adriamycin-induced VSMC senescence. Phosphorylation of LKB1 is significantly up-regulated in adriamycin-induced senescent VSMC (Fig. 1). In contrast, depletion of LKB1 reduced the adriamycin-stimulated SA-β-gal positive cells (Fig. 4A). In response to adriamycin stimulation, LKB1 is translocated from nucleus to cytosol. These results suggested that LKB1 could induce senescence through AMPK activation (Fig. 4B). The LKB1/AMPK downstream signaling mechanisms for inducing VSMC senescence are not clear. Persistent activation of AMPK was reported to accelerated p53-dependent cellular senescence [30]. In the present study, p53 and p21 siRNA transfected VSMCs displayed diminished SA-β-gal cell staining (Fig. 4C).

In summary, these findings suggest that activation of the LKB1/AMPK catabolic pathway may turn a survival strategy into a pro-aging mechanism and contribute to progressive degeneration during cellular senescence. These results support the idea that intervention to modulate AMPK could be a treatment strategy for longevity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.08.071.

References

[1] E.G. Lakatta, D. Levy, Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part I: aging arteries: a "set up" for vascular disease, Circulation 107 (2003) 139–146.
[2] L.T. Cooper, J.P. Cooke, V.J. Dzau, The vasculopathy of aging, J. Gerontol. 49 (1994) 191–196.
[3] A.U. Ferrari, A. Radaelli, M. Centola, Invited review: aging and the cardiovascular system, J. Appl. Physiol. 95 (2003) 2591–2597.
[4] T. Minamino, I. Komuro, Vascular cell senescence: contribution to atherosclerosis, Circ. Res. 100 (2007) 15–26.
[5] C. Matthews, I. Gorenne, S. Scott, N. Figg, P. Kirkpatrick, A. Ritchie, M. Goddard, M. Bennett, Vascular smooth muscle cells undergo telomere-based senescence in human atherosclerosis: effects of telomerase and oxidative stress, Circ. Res. 99 (2006) 156–164.
[6] W. Wang, X. Yang, I. López de Silanes, D. Carling, M. Gorospe, Increased AMP:ATP ratio and AMP-activated protein kinase activity during cellular senescence linked to reduced HuR function, J. Biol. Chem. 278 (2003) 27016–27023.
[7] M. Arad, C.E. Seidman, J.G. Seidman, AMP-activated protein kinase in the heart: role during health and disease, Circ. Res. 100 (2007) 474–488.
[8] D.G. Hardie, D. Carling, The AMP-activated protein kinase–fuel gauge of the mammalian cell?, Eur J. Biochem. 246 (1997) 259–273.
[9] L.A. Witters, B.E. Kemp, Insulin activation of acetyl-CoA carboxylase accompanied by inhibition of the 5′-AMP-activated protein kinase, J. Biol. Chem. 267 (1992) 2864–2867.
[10] H. Motoshima, B.J. Goldstein, M. Igata, E. Araki, AMPK and cell proliferation-AMPK as a therapeutic target for atherosclerosis and cancer, J. Physiol. 574 (2006) 63–71.
[11] Z. Xie, Y. Dong, R. Scholz, D. Neumann, M.H. Zou, Phosphorylation of LKB1 at serine 428 by protein kinase C-zeta is required for metformin-enhanced activation of the AMP-activated protein kinase in endothelial cells, Circulation 117 (2008) 952–962.
[12] L.I. Yoo, D.C. Chung, J. Yuan, LKB1-a master tumor suppressor of the small intestine and beyond, Nat. Rev. Cancer 2 (2002) 529–535.
[13] R.M. Reznick, H. Zong, J. Li, K. Morino, I.K. Moore, H.J. Yu, Z.X. Liu, J. Dong, K.J. Mustard, S.A. Hawley, D. Befroy, M. Pypaert, D.G. Hardie, L.H. Young, G.I. Shulman, Aging-associated reductions in AMP-activated protein kinase activity and mitochondrial biogenesis, Cell Metab. 5 (2007) 151–156.
[14] M.F. McCarty, Chronic activation of AMP-activated kinase as a strategy for slowing aging, Med. Hypotheses 63 (2004) 334–339.
[15] J.Y. Sung, H.C. Choi, Aspirin-induced AMP-activated protein kinase activation regulates the proliferation of vascular smooth muscle cells from spontaneously hypertensive rats, Biochem. Biophys. Res. Commun. 408 (2011) 312–317.
[16] R. Sultana, F. Di Domenico, M. Tseng, J. Cai, T. Noel, R.L. Chelvarajan, W.D. Pierce, C. Cini, S. Bondada, D.K. St. Clair, D.A. Butterfield, Doxorubicin-induced thymus senescence, J. Proteome. Res. 9 (2010) 6232–6241.
[17] K. Sakamoto, A. McCarthy, D. Smith, K.A. Green, D. Grahame Hardie, A. Ashworth, D.R. Alessi, Deficiency of LKB1 in skeletal muscle prevents AMPK activation and glucose uptake during contraction, EMBO J. 24 (2005) 1810–1820.
[18] S.C. Kaul, K. Hasan, R. Wadhwa, CARF regulates p19ARF-p53–p21WAF1 senescence pathway by multiple checkpoints, Ann. NY Acad. Sci. 1067 (2006) 217–219.
[19] G.K. Hansson, Inflammation, atherosclerosis, and coronary artery disease, N. Engl. J. Med. 352 (2005) 1685–1695.
[20] I. Ben-Porath, R.A. Weinberg, When cells get stressed: an integrative view of cellular senescence, J. Clin. Invest. 113 (2004) 8–13.
[21] Y.E. Yegorov, A.V. Zelenin, Duration of senescent cell survival in vitro as a characteristic of organism longevity, an additional to the proliferative potential of fibroblasts, FEBS Lett. 541 (2003) 6–10.
[22] J.F. Passos, C. Simillion, J. Hallinan, A. Wipat, T. von Zglinicki, Cellular senescence: unravelling complexity, Age 31 (2009) 353–363.
[23] H. Ota, M. Eto, M.R. Kano, S. Ogawa, K. Iijima, M. Akishita, Y. Ouchi, Cilostazol inhibits oxidative stress-induced premature senescence via upregulation of Sirt1 in human endothelial cells, Arterioscler. Thromb. Vasc. Biol. 28 (2008) 1634–1639.
[24] I. Mattagajasingh, C.S. Kim, A. Naqvi, T. Yamamori, T.A. Hoffman, S.B. Jung, J. DeRicco, K. Kasuno, K. Irani, SIRT1 promotes endothelium dependent vascular relaxation by activating endothelial nitric oxide synthase, Proc. Natl. Acad. Sci. USA 104 (2007) 14855–14860.
[25] P. Narbonne, R. Roy, *Caenorhabditis elegans* dauers need LKB1/AMPK to ration lipid reserves and ensure long-term survival, Nature 457 (2009) 210–214.
[26] J.E. Kim JE, H.C. Choi, Losartan inhibits vascular smooth muscle cell proliferation through activation of AMP-activated protein kinase, Korean J. Physiol. Pharmacol. 14 (2010) 299–304.
[27] R.J. Shaw, M. Kosmatka, N. Bardeesy, R.L. Hurley, L.A. Witters, R.A. DePinho, L.C. Cantley, The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress, Proc. Natl. Acad. Sci. USA 101 (2004) 3329–3335.
[28] M.B. Chen, W.X. Shen, Y. Yang, X.Y. Wu, J.H. Gu, P.H. Lu, Activation of AMP-activated protein kinase is involved in vincristine-induced cell apoptosis in B16 melanoma cell, J. Cell Physiol. 226 (2011) 1915–1925.
[29] G. Mariño, A.P. Ugalde, N. Salvador-Montoliu, I. Varela, P.M. Quirós, J. Cadiñanos, I. van der Pluijm, J.M. Freije, C. López-Otín, Premature aging in mice activates a systemic metabolic response involving autophagy induction, Hum. Mol. Genet. 17 (2008) 2196–2211.
[30] R.G. Jones, D.R. Plas, S. Kubek, M. Buzzai, J. Mu, Y. Xu, M.J. Birnbaum, C.B. Thompson, AMP-activated protein kinase induces a p53-dependent metabolic checkpoint, Mol. Cell 18 (2005) 283–293.