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RSK2-induced stress tolerance enhances cell survival signals mediated by inhibition of GSK3β activity

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ABSTRACT

Our previous studies demonstrated that RSK2 plays a key role in cell proliferation and transformation induced by tumor promoters such as epidermal growth factor (EGF) in mouse and human skin cells. However, no direct evidence has been found regarding the relationship of RSK2 and cell survival. In this study, we found that RSK2 interacted and phosphorylated GSK3β at Ser9. Notably, GSK3β phosphorylation at Ser9 was suppressed in RSK2$^{-/-}$ MEFs compared with RSK2$^{+/+}$ MEFs by stimulation of EGF and calcium ionophore A23187, a cellular calcium stressor. In proliferation, we found that RSK2 deficiency suppressed cell proliferation compared with RSK2$^{+/+}$ MEFs. In contrast, GSK3β$^{-/-}$ MEFs induced the cell proliferation compared with GSK3β$^{+/+}$ MEFs. Importantly, RSK2$^{-/-}$ MEFs were induced severe cellular morphology change by A23187 and enhanced G1/G0 and sub-G1 accumulation of the cell cycle phase compared with RSK2$^{+/+}$ MEFs. The sub-G1 induction in RSK2$^{-/-}$ MEFs by A23187 was correlated with increase of cytochrome *c* release, caspase-3 cleavage and apoptotic DNA fragmentation compared with RSK2$^{+/+}$ MEFs. Notably, return back of RSK2 into RSK2$^{-/-}$ MEFs restored A23187-induced morphological change, and decreased apoptosis, apoptotic DNA fragmentation and caspase-3 induction compared with RSK2$^{-/-}$/mock MEFs. Taken together, our results demonstrated that RSK2 plays an important role in stress-tolerance and cell survival, resulting in cell proliferation and cancer development.

1. Introduction

The p90-kDa ribosomal S6 kinases (p90^{RSKs}) are a family of serine/threonine kinases including RSK1, RSK2, RSK3, RSK4, MSK1 and MSK2 [1], and regulated their activities responding to many growth factors, peptide hormones, neurotransmitters [2,3] and environmental stresses such as ultraviolet (UV) light [4] through the mitogen-activated protein (MAP) kinases, key regulators of cell proliferation and oncogenesis [5,6]. Activated RSK2 is translocated to the nucleus and phosphorylates diverse nuclear proteins, including c-Fos, ELK-1, histones, cyclic AMP responsive element binding protein (CREB) protein [7–11], activating transcription factor 4 (ATF4) [12], p53 [13], NFAT3 [14] and ATF1 [15]. Because RSK2 enzyme has broad substrate spectrum, it might be able to interact with diverse substrates that regulates cell proliferation, differentiation, transformation and stress resistance, depending on the specific context.

GSK3 that is present in mammals in two isoforms, α and β, is an ubiquitous serine/threonine kinase [16] and identified as an enzyme to phosphorylate glycogen synthase, resulting in inhibition of glycogen synthesis [17]. The general regulatory mechanism is the phosphorylation of GSK3β at Ser9 or GSK3α at Ser21, which inhibits activity, by through phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway [18]. In contrast, GSK3 enzyme activity is induced by intramolecular autophosphorylation of Tyr216 in GSK3β and Tyr279 in GSK3α, resulting in enhancement of the stability of the enzymes [19]. Because dysregulation of GSK3 activity is associated with variety of human diseases such as mood disorder, neurodegenerative disease, cancer, inflammatory reaction and cardiovascular disease and cellular apoptosis, GSK3 must be tightly regulated [20].

The intrinsic apoptosis signaling pathway can be induced by numerous stimuli, which cause cell damage including DNA damage, oxidative stress and endoplasmic reticulum (ER) stress. The intrinsic apoptosis cascade causes the destruction of mitochondria mediated by enhancement of GSK3 activity [20]. When cells are stimulated with apoptotic stimuli such as DNA damage or ER stress, GSK3β activity is increased in mitochondria [21,22]. Moreover, the activation of GSK3β in nucleus induces p53-dependent expression of Bax in response DNA damage [23,24] and inhibits CREB, which blocks the CREB-mediated expression of the

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anti-apoptotic protein Bcl-2 [25]. Interestingly, our previous publications indicated that RSK2 phosphorylates and inactivates proapoptotic protein BAD, resulting in induction of cell survival [26], and RSK2 up-regulates the transcription of anti-apoptotic Bcl-2 through CREB phosphorylation and activation [27]. On account of apoptosis is presumed to have a protective function against carcinogenesis and cell survival, these effects suggest that the function of RSK2 might have correlation in enhancing tumorigenesis and cell survival. These possibilities have been provided by our researches that RSK2 phosphorylates and activates various transcription factors and epigenetic factors involved in cell proliferation and transformation [12–15]. However, molecular mechanisms on the RSK2-mediated cell survival have not been clearly understood.

In this study, we found that GSK3β was a binding partner and a substrate of RSK2. RSK2 phosphorylated GSK3β at Ser9, resulted in the enhancing of cell survival by the induction of calcium stress tolerance.

2. Materials and methods

2.1. Reagents and antibodies

Chemical reagents, including Tris, NaCl, and SDS for molecular biology and buffer preparation were purchased from Sigma–Aldrich (St. Louis, MO, USA). Cell culture medium and other supplements were purchased from Life Science Technologies, Inc. (Rockville, MD, USA). Antibodies for Western blot analysis were purchased from Cell Signaling Technology, Inc. (Beverly, MA, USA), Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA), or Millipore Korea Co., Ltd, (Gangnam-gu, Seoul, Korea).

2.2. Cell culture and transfection

293T, GSK3β$^{+/+}$ and GSK3β$^{-/-}$, and RSK2$^{+/+}$ and RSK2$^{-/-}$ mouse embryonic fibroblasts (MEFs) were cultured with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics at 37 °C in a 5% CO_2 incubator. The cells were split at 90% confluent and medium was changed every other day. To stimulate calcium in RSK2$^{+/+}$ and RSK2$^{-/-}$ MEFs, the cells (1×10^6) was seeded, cultured for overnight at 37 °C in a 5% CO_2 incubator, starved for 16–24 h and then treated indicated dose of A23187.

2.3. Mammalian two-hybrid assay

293T cells (2.0×10^4) were seeded into 48-well plates and incubated with 10% FBS-DMEM for 18 h before transfection. The DNAs, pACT-GSK3β, pBIND-kinases and pG5-luciferase, were combined in the same molar ratio and the total amount of DNA was not more than 100 ng per well. The transfection was performed using jetPEI following the manufacturer's recommended protocols. The cells were disrupted by addition of cell lysis buffer directly into each well of the 48-well plate and then aliquots of 20 μl were added to each well of a 96-well luminescence plate. The luciferase activity was measured automatically by computer program (PerkinElmer, Waltham, MA, USA). The relative luciferase activity was calculated and normalized based on the pG5-luciferase basal control. For assessment of transfection efficiency and protein amount, the luciferase assay, *renilla* luciferase activity assay or Lowry protein assay were used.

2.4. MTS assay

To estimate proliferation, GSK3β$^{+/+}$, GSK3β$^{-/-}$, RSK2$^{+/+}$ and RSK2$^{-/-}$ MEFs (1×10^3 cells/well) were seeded into 96-well plates. After culturing for 2 h, 20 μl of the CellTiter 96® Aqueous One Solution (Promega, Madison, WI) were added to each well and cells were then incubated for 1 h at 37 °C and 5% CO_2. To stop the reaction, 25 μl of a 10% SDS solution were added and absorbance was measured at 492 and 690 nm using xMark microplate spectrophotometer (Bio-RAD, Philadelphia, PA, USA). The MTS assay was conducted at 24 h intervals for 96 h.

2.5. Western blotting

Samples containing equal amounts of protein were resolved by the appropriate percentage SDS-PAGE and then transferred onto PVDF (polyvinylidene difluoride) membranes. The membranes were incubated in blocking buffer and then probed with specific primary antibodies against appropriate target proteins. The Western blots were visualized using an enhanced chemiluminescence detection system (ECL, Amersham Biosciences Corp., Piscataway, NJ. USA).

2.6. In vitro kinase assay

A GST-GSK3β protein was used as a substrate for an *in vitro* kinase assay with active RSK2 (Millipore Korea Co., Ltd, Gangnam-gu, Seoul, Korea)). Reactions were carried out at 30 °C for 30 min in a mixture containing 50 μM unlabeled ATP and 10 μCi [γ-^{32}P] ATP, and then were stopped by adding 6× SDS sample buffer. ^{32}p-labeled GSK3β was visualized by autoradiography. The input was confirmed using an identical experimental set by Western blotting using specific antibodies as indicated. To examine the phosphorylation site conformation of GSK3β by RSK2, GST-GSK3β and active RSK2 were mixed with cold ATP alone and conducted at 30 °C for 30 min. The GSK3β phosphorylation at Ser9 by active RSK2 was visualized Western blotting using specific primary and HRP-conjugated secondary antibodies as indicated.

2.7. Cell cycle and apoptosis analysis

RSK2$^{+/+}$ or RSK2$^{-/-}$ cells (2×10^5) were seeded into 60-mm dishes and cultured for 16 h at 37 °C in a 5% CO_2 incubator. The cell was starved 24 h with 0.1% FBS-DMEM and then stimulated with indicated dose of A23187 for 12 h. For the cell cycle analysis, the cells were trypsinized, fixed with methanol, stained with propidium iodide (PI; Sigma–Aldrich, St. Louis, MO, USA). For the apoptosis analysis, the cells were harvested, washed with cold PBS and the directly stained with PI and annexin V (Life Science Technologies, Inc, Rockville, MD, USA). The cells were analyzed for cell cycle phase, sub-G1 populations or apoptosis using FACS Calibur flow cytometer (BD Bioscience, San Jose, CA, USA) as described by Ahmad et al. [28].

2.8. DNA fragmentation assay

RSK2$^{+/+}$ and RSK2$^{-/-}$ MEFs, the cells (1×10^6) was seeded and cultured to 90% confluent at 37 °C in a 5% CO_2 incubator. The cells were stimulated with indicated dose of A23187 for 24 h and harvested. The cells were suspended with 500 μl of cell lysis buffer [10 mM Tris–HCl (pH 8.0), 100 mM EDTA (pH 8.0), 0.5% SDS] and treated with 100 μg/ml of proteinase K at 55 °C for 4 h. The total genomic DNA was isolated by phenol/chloroform extraction and ethanol precipitation, and RNA was eliminated by adding RNase A. Total 5 μg of genomic DNA was electrophoresed onto 1.2%

agarose gel and DNA fragmentations were visualized by ethidium bromide staining and photographed.

3. Results and discussion

3.1. RSK2 deficiency enhances A23187-induced apoptosis

Activated RSK2-mediated pro-apoptotic BAD phosphorylation at serine 112 inactivates BAD and induces cell survival [26]. Our previous results demonstrated that UVB stimulation induces sub-G1 accumulation in RSK2$^{-/-}$ MEFs compared with RSK2$^{+/+}$ MEFs [4]. A recent report indicated that UV-induced apoptosis signaling was also enhanced by intracellular calcium influx [29], Based on these results, we hypothesized that RSK2 might a survival factor when cells are stimulated with A23187. To examine the hypothesis, we stimulated RSK2$^{+/+}$ or RSK2$^{-/-}$ MEFs with A23187. We found that attached RSK2$^{-/-}$ MEFs in culture vessels were dramatically increased the G1 cell cycle phase and decreased S cell cycle phase compared with attached RSK2$^{+/+}$ MEFs by A23187 in dose dependent manner (Fig. 1A). RSK2 deficiency induced morphological changes with cytoplasmic shrinkage, fragmentation and detachment (Supplementary Fig. 1A). Notably, RSK2 deficiency increased sub-G1 cell cycle phase to about 75% in the total cell population compared with about 20% of sub-G1 cell cycle phase in RSK2$^{+/+}$ MEFs (Fig. 1B and Supplementary Fig. 1B). Importantly, we found that RSK2$^{-/-}$ MEFs showed dramatic induction of cytosolic cytochrome *c* and reduction of membrane cytochrome *c* by

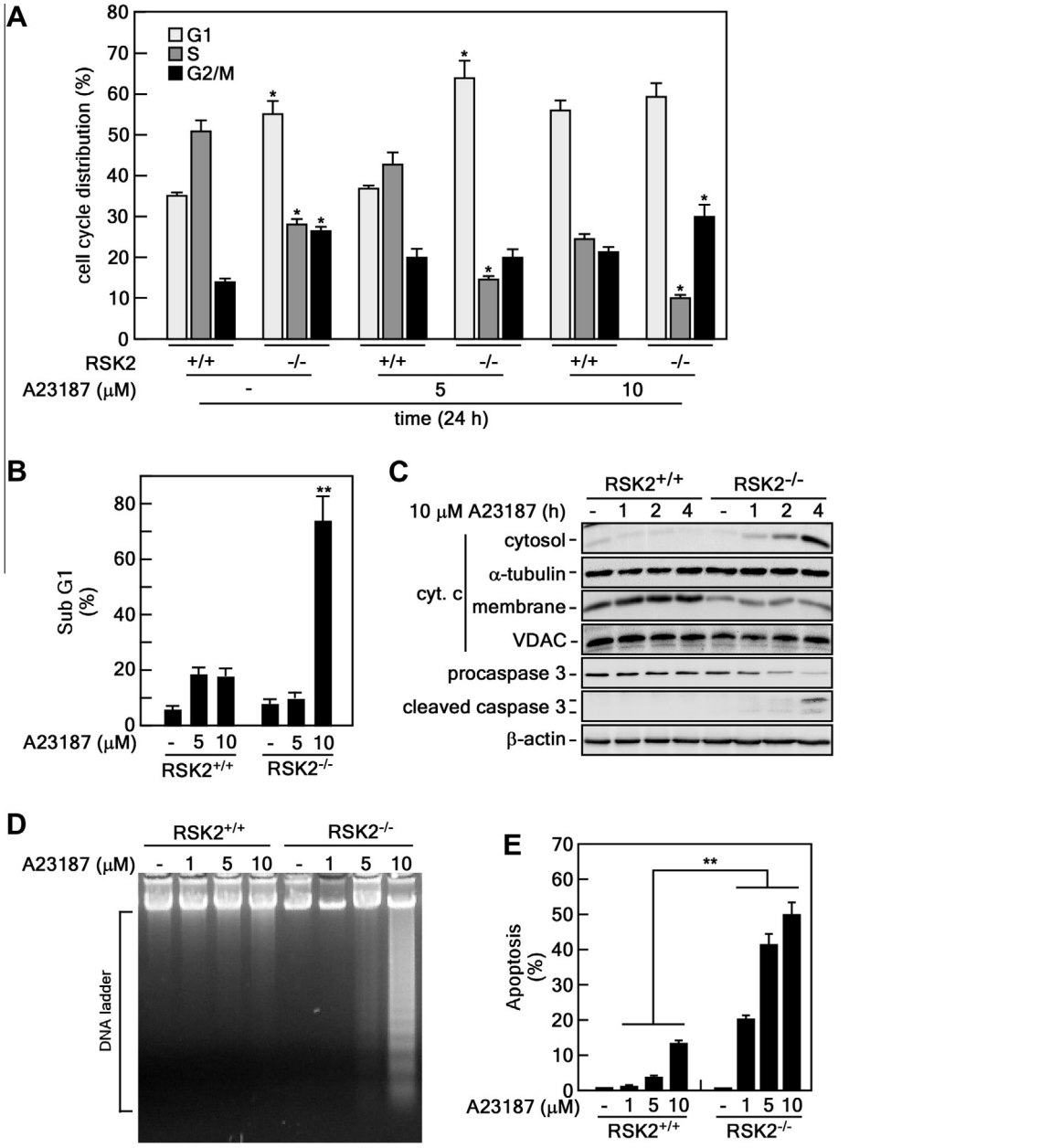

Fig. 1. RSK2 deficiency enhances A23187-induced apoptosis. (A–B) RSK2 deficiency induces accumulation of G1/G0 cell cycle phase by A23187. RSK2$^{+/+}$ and RSK2$^{-/-}$ MEFs were analyzed cell cycle distribution and Sub-G1 by treatment of A23187 as described in "Materials and methods" ($^{*}p < 0.01$ and $^{**}p < 0.001$). (C) RSK2$^{-/-}$ MEFs enhance cytochrome *c* release and caspase-3 cleavage by A23187. (D) RSK2 deficiency enhances DNA fragmentation induced by A23187. RSK2$^{+/+}$ and RSK2$^{-/-}$ MEFs were treated with A23187 and DNA fragmentation was visualized as described in "Materials and methods". (E) RSK2 deficiency induces apoptosis by A23187. RSK2$^{+/+}$ and RSK2$^{-/-}$ MEFs were treated with A23187 and analyzed apoptosis by PI/annecxin V staining using FACS Calibur flow cytometer.

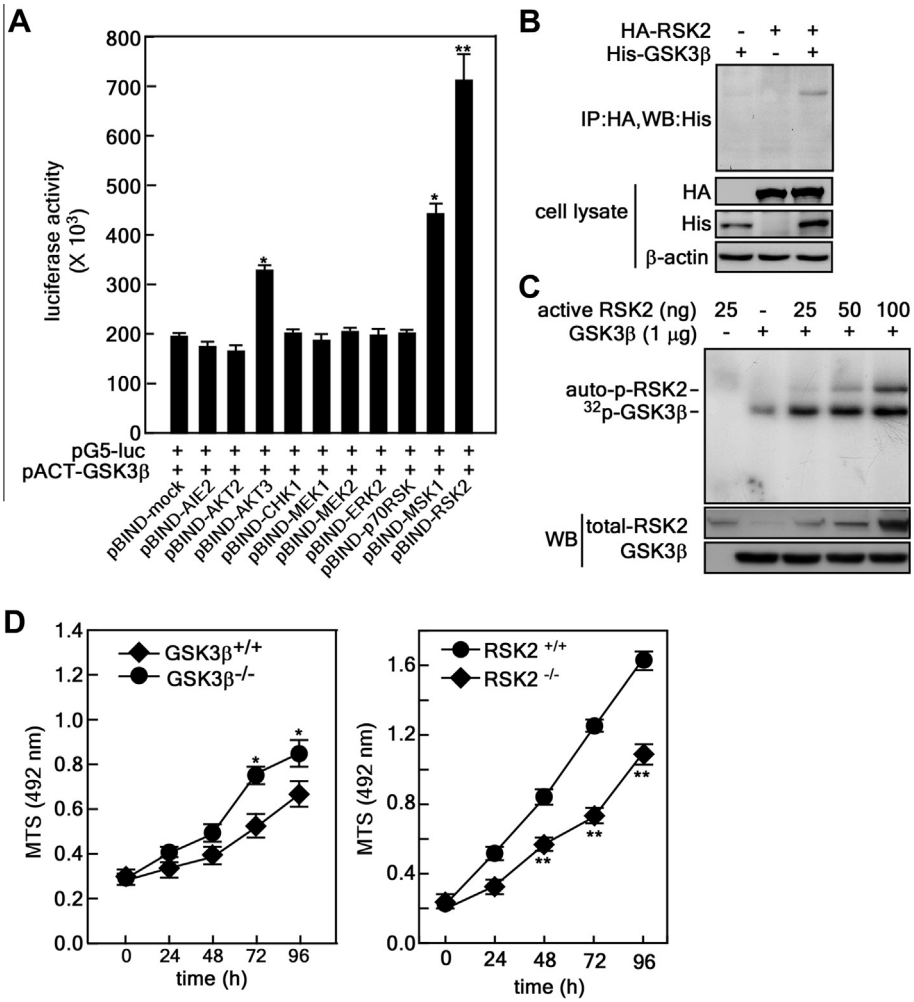

Fig. 2. GSK3β is a binding partner and substrate of RSK2. (A) Identification of protein–protein interaction in *ex vivo* by mammalian two-hybrid assay. The pBIND-AKT2 and -AKT3 were used as positive controls (*$p < 0.05$; **$p < 0.005$). (B) Immunoprecipitation of RSK2 with GSK3β. The expression vectors were transfected into 293 cells as indicated and protein binding was analyzed by co-immunoprecipitation (IP) and Western blotting using HA and His antibodies as indicated. (C) *In vitro* kinase. Active RSK2 and GSK3β purified from Sf1 insect cells were combined with 10 μCi of [γ-^{32}p] ATP and *in vitro* kinase assay was conducted as described in "Materials and methods". (D) Cell proliferation analysis of GSK3β$^{+/+}$, GSK3β$^{-/-}$, RSK2$^{+/+}$ and RSK2$^{-/-}$ MEFs. The GSK3β$^{+/+}$, GSK3β$^{-/-}$, RSK2$^{+/+}$ or RSK2$^{-/-}$ MEFs (1×10^3) were seeded into 96-well culture plates and cell proliferation was measured for 96 h in 24 h intervals by MTS assay (*$p < 0.05$; **$p < 0.005$).

A23187 compared with RSK2$^{+/+}$ MEFs (Fig. 1C). The cytochrome *c* release into cytosol by A23187 induced cleavage of caspase-3, a critical modulating factor inducing cell apoptosis, and reduced procaspase-3 in RSK2$^{-/-}$ MEFs (Fig. 1C). In contrast, RSK2$^{+/+}$ MEFs showed marginal reduction of procaspase-3 by A23187 (Fig. 1C), indicated that RSK2 might inhibit upstream caspases of caspase-3 or activate calcium pumps from cytosol toward ER inside or outside of cells. As expected, we observed extensive DNA fragmentation in RSK2$^{-/-}$ MEFs by A23187 treatment compared with RSK2$^{+/+}$ MEFs (Fig. 1D). Moreover, the quantification analysis of apoptotic cell population demonstrated that RSK2$^{-/-}$ MEFs were significantly increased the apoptosis by A23187 compared with RSK2$^{+/+}$ MEFs (Fig. 1E). Taken together, RSK2 plays an important role in stress-tolerance and cell survival induced by calcium stress.

3.2. GSK3β is a binding partner and substrate of RSK2

MAP kinase including ERKs, p38 kinases and JNKs, and AKT/GSK3β signaling pathways are activated by diverse extracellular stimulations in a short time including growth factors, cytokines and environmental stresses such as UVB stimulation, and regulate cell proliferation, transformation and stress resistance, depending on the specific context [14,30]. To examine the signaling pathway to involve the GSK3β-mediated stress tolerance induced by calcium, we conducted mammalian two-hybrid assay to identify the novel binding partners of GSK3β. We found that GSK3β interacted strongly with RSK2 (Fig. 2A). GSK3β and RSK2 binding was stronger than that of GSK3β and AKT3 (Fig. 2A), which is a well-known upstream kinase of GSK3β at Ser9 when cells are stimulated with growth factors such as insulin [31]. To confirm whether GSK3β and RSK2 were interact each other or not, we ectopically expressed RSK2 and GSK3β in 293T cells and then verified GSK3β and RSK2 interaction by immunoprecipitation. We found that GSK3β interacted with RSK2 (Fig. 2B). Although previous publications indicated that RSK was an upstream kinase of GSK3β [32], no direct evidence has been provided *in vitro*. To examine this hypothesis, we conducted *in vitro* kinase assay using purified GST-GSK3β and active RSK2, which are commercially available, and [γ-^{32}p]ATP radioisotope, and found that GSK3β was phosphorylated by RSK2 (Fig. 2C). Notably, we confirmed that the proliferation of GSK3β$^{-/-}$ and RSK2$^{+/+}$ MEFs was faster than those of GSK3β$^{+/+}$ and RSK2$^{-/-}$, respectively (Fig. 2D, left and right graphs). Taken together, these

A

kinase	position	aa sequences	score	cutoff	score/ cutoff
RSK2 c.seq.*		RxRxxS			
p70S6K	9	SGRPRTTSFAESCK	8.75	4.25	2.06
RSK	9	SGRPRTTSFAESCK	4.45	0.90	4.97
RSK	426	GQTNNAASASASNS	1.68	0.90	1.88
RSK	428	TNNAASASASNST*	2.42	0.90	2.71
RSK	430	NAASASASNST***	2.40	0.90	2.68
RSK	432	ASASASNST*****	2.82	0.90	3.15
RSK	433	SASASNST******	1.58	0.90	1.76

* RSK2 c.seq. indicates RSK2 recognition consensus amino acid sequences

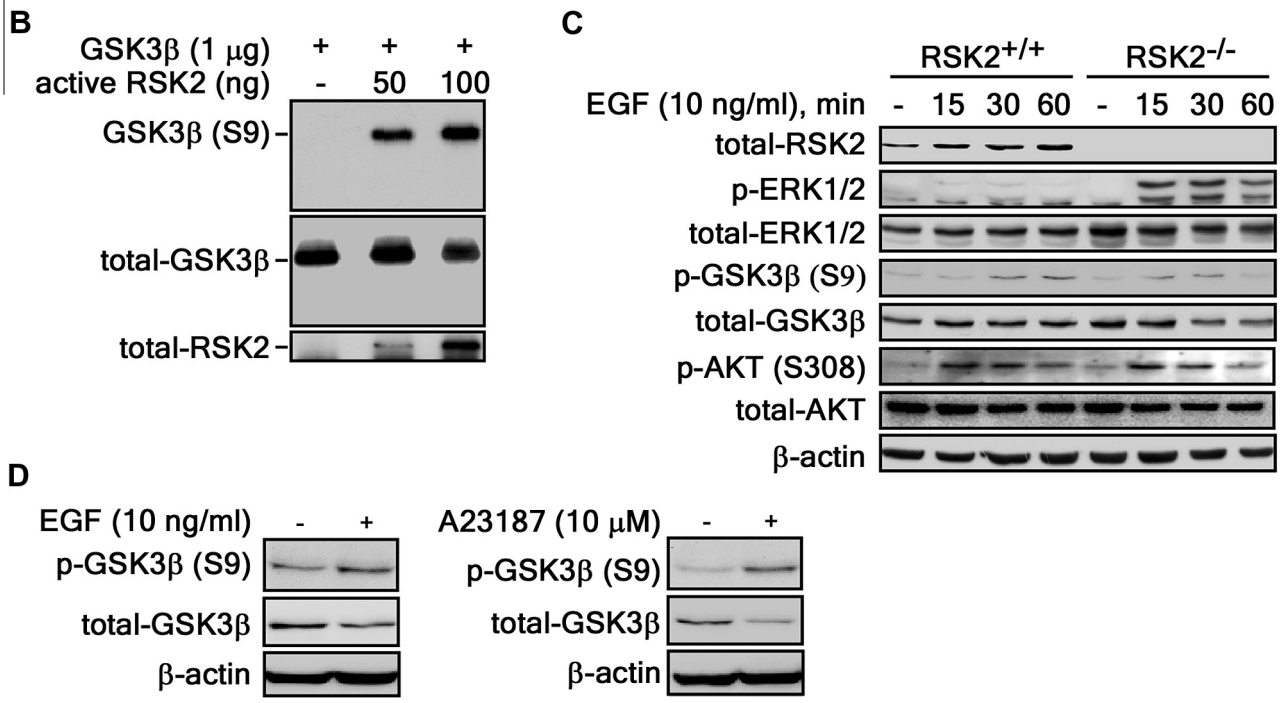

Fig. 3. GSK3β Ser9 is a target phosphorylation amino acid by RSK2. (A) Putative phospho-amino acid(s) of GSK3β that could be phosphorylated by RSK were identified using the Group-based Prediction System (v2.1). (B) RSK2 phosphorylates GSK3β at Ser9. *In vitro* kinase assay using the GSK3β protein and active RSK2 was conducted as described in "Materials and methods". (C) RSK2 deficiency attenuates EGF-induced GSK3β phosphorylation at Ser9. Indicated protein levels were analyzed by Western blotting by stimulation of EGF in RSK2$^{+/+}$ and RSK2$^{-/-}$ MEFs. (D) The phosphorylation of GSK3β at Ser9 was compared by EGF or A23187 stimulation in RSK2$^{+/+}$ MEFs as indicated.

results demonstrated that GSK3β is a direct substrate of RSK2 and RSK2/GSK3β signaling axis might play an important role in cell proliferation.

3.3. GSK3β Ser9 is a target phosphorylation amino acid by RSK2

Previous our report demonstrates that RSK2-mediated phosphorylation consensus amino acid sequences is RxRxxS/T [33]. To search whether GSK3β contained RSK2 target amino acid or not, we downloaded human GSK3β amino acid from human protein reference databases (http://www.hprd.org) and surveyed RSK2 target amino acid consensus sequences. We found that GSK3β Ser9 contained RxRxxS/T RSK2-mediated phosphorylation target consensus sequences (Supplementary Fig. 2A). None of other known phosphorylation sites of GSK3β were matched with RSK2 target consensus amino acid motif (Supplementary Fig. 2A). To verify whether GSK3β Ser9 was a putative RSK2 target or not, we searched kinase-specific phosphorylation sites using Group-based prediction system (GPS v2.1) [34]. We found that GSK3β Ser9 showed the highest score/cutoff values about 4.97 among predicted phosphorylation sites by RSK family members (Fig. 3A). Furthermore, RSK showed the highest score/cutoff values about among the predicted putative kinases to phosphorylate GSK3β at Ser9 compared with about 3.13 of the AKT2, which is a well-known GSK3β (Supplementary Fig. 2B). To confirm the phosphorylation amino acid of GSK3β by RSK2, we conducted *in vitro* kinase assay with active RSK2 and GST-GSK3β, and confirmed the phosphorylation of GSK3β at Ser9 by Western blotting. Notably, phosphorylation of GSK3β at Ser9 was increased by RSK2 in a dose dependent manner (Fig. 3B), indicated that RSK2 is a GSK3β kinase. We further confirmed that EGF-induced GSK3β phosphorylation at Ser9 in RSK2$^{+/+}$ MEFs was suppressed in RSK2$^{-/-}$ MEFs (Fig. 3C).

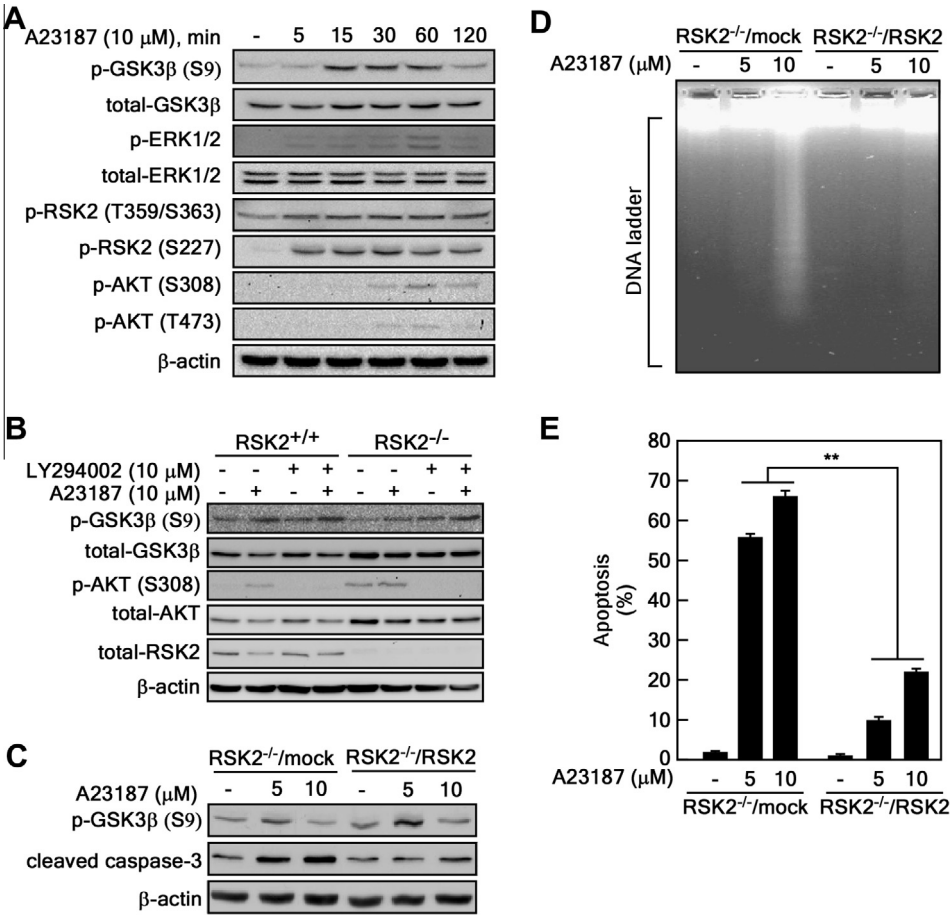

Fig. 4. Adding back RSK2 to RSK2$^{-/-}$ MEFs restores stress tolerance. (A) A23187-induced phospho-signaling protein profiles. RSK2$^{+/+}$ MEFs were stimulated with A23187 and then indicated protein levels were analyzed by Western blotting. (B) RSK2 deficiency attenuates A23187-induced GSK3β phosphorylation at Ser9. The GSK3β phosphorylation at Ser9 induced by A23187 treatment was compared in RSK2$^{+/+}$ and RSK2$^{-/-}$ MEFs by combination of A23187 and LY294005 as indicated. (C) RSK2 reintroduction into RSK2$^{-/-}$ MEFs induces GSK3β phosphorylation at Ser9 and reduces caspase-3 cleavage by A23187 treatment. (D) Re-introduction of RSK2 into RSK2$^{-/-}$ MEFs restores A23187-induced DNA fragmentation. (E) Adding back RSK2 to RSK2$^{-/-}$ MEFs suppresses A23187-induced apoptosis.

However, the GSK3β phosphorylation induced by EGF did not totally disappeared in RSK2$^{-/-}$ MEFs (Fig. 3C), indicated that EGF-induced AKT might involve in GSK3β phosphorylation at Ser9. In addition, we found that GSK3β phosphorylation at Ser9 was similarly induced by EGF or A23187 treatment and total protein levels of GSK3β were suppressed by EGF or A23187 treatment (Fig. 3D). These results demonstrated that GSK3β was a direct substrate of the RSK2 and phosphorylated by RSK2 at Ser9.

3.4. Adding back RSK2 to RSK2$^{-/-}$ MEFs restores stress tolerance

Our previous studies indicate that RSK2 inhibits BAD activity [26] and enhances Bcl-2 gene expression [27]. Because apoptosis have a protective function against carcinogenesis, we examined and confirmed that sub-G1 population was increased in RSK2$^{-/-}$ MEFs compared with RSK2$^{+/+}$ MEFs when the cells were exposured with UVB (4 kJ/m^2) [4]. A recent report indicated that UV-induced apoptosis signaling was also enhanced by intracellular calcium influx [29]. Furthermore, our results demonstrated that RSK2$^{-/-}$ MEFs induced apoptosis by A23187 treatment (Fig. 1). Thus, we examined the A23187-induced phosphorylation protein profiles in RSK2$^{+/+}$ MEFs and found that GSK3β phosphorylation at Ser9 was followed by the phosphorylation of RSK2 at Ser227 (Fig. 4A). Importantly, the phosphorylation of AKT at Ser308 and Thr473 was showed later than GSK3β phosphorylation at Ser9 (Fig. 4A). Moreover, GSK3β phosphorylation at Ser9 induced by A23187 did not suppressed by co-treatment of A23187 and LY294002, a specific inhibitor of PI3K, in RSK2$^{+/+}$ MEFs (Fig. 4B). These results demonstrated that GSK3β Ser9 phosphorylation signaling induced by A23187 is mediated through ERKs/RSK2 signaling pathway, not through the PI3K/AKT signaling pathway. Notably, RSK2$^{-/-}$ MEFs showed different phosphorylation patterns of GSK3β at Ser9 compared with RSK2$^{+/+}$ MEFs by A23187, LY294002 or A23187/LY294002 treatment (Fig. 4B), indicated that other signaling pathway to phosphorylate GSK3β at Ser9 might exist except RSK2 and AKT. Additionally, inhibitory effect of LY294002 on the PI3K was confirmed by inhibition of AKT phosphorylation at Ser308 by co-treatment with A23187 and LY294002 (Fig. 4B). We further confirmed that adding back of RSK2 into RSK2$^{-/-}$ MEFs (RSK2$^{-/-}$/RSK2) [15] showed the increase of GSK3β phosphorylation at Ser9 (Fig. 4C). In contrast with GSK3β phosphorylation, cleaved caspase-3 content was suppressed in RSK2$^{-/-}$/RSK2 MEFs compared with RSK2$^{-/-}$/mock MEFs (Fig. 4C). Notably, the increased DNA fragmentation induced by A23187 in RSK2$^{-/-}$/mock MEFs was suppressed in RSK2$^{-/-}$/RSK2 MEFs compared with RSK2$^{-/-}$/mock MEFs (Fig. 4D). Notably, the apoptotic population was significantly reduced in RSK2$^{-/-}$/RSK2 MEFs compared with RSK2$^{-/-}$/mock MEFs by A23187 treatment (Fig. 4E). These results suggested that RSK2 might play an important role in stress tolerance and cell survival.

Our previous results demonstrated that RSK2-mediated BAD phosphorylation suppressed apoptosis, resulting in the increase

of cell survival [26]. Moreover, we have demonstrated that RSK2 plays an important role in cell proliferation, transformation and cancer development in human [4,35,36]. Generally, cells transformed to cancer cells gains resistance from the cell death induced by environmental stresses and chemotherapeutic compounds [37]. Although molecular mechanisms are necessary to elucidate RSK2/GSK3β signaling pathway in stress tolerance and cell survival in future, our study demonstrates that a regulation mechanism of RSK2-mediated GSK3β activity might give a context to enhance cell survival, resulting in acquirement of abilities to increase cell proliferation and anti-therapeutic resistance in human cancer.

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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.bbrc.2013.09.042.

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