Biochemical and Biophysical Research Communications 423 (2012) 270–275

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

SIRT1 inhibits proliferation of pancreatic cancer cells expressing pancreatic adenocarcinoma up-regulated factor (PAUF), a novel oncogene, by suppression of β-catenin

Il-Rae Cho [a], Sang Seok Koh [b,c], Waraporn Malilas [a], Ratakorn Srisuttee [a], Jeong Moon [a], Young-Whan Choi [d], Yoshiyuki Horio [e], Sangtaek Oh [f], Young-Hwa Chung [a,*]

[a] WCU, Department of Cogno-Mechatronics Engineering, Pusan National University, Busan 609-735, Republic of Korea
[b] Immunotherapy Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-333, Republic of Korea
[c] Department of Functional Genomics, University of Science and Technology, Daejeon 305-333, Republic of Korea
[d] Department of Horticultural Bioscience, Pusan National University, Miryang 627-706, Republic of Korea
[e] Department of Pharmacology, Sapporo Medical University, Sapporo 060-8556, Japan
[f] Department of Advanced Fermentation Fusion Science and Technology, Kookmin University, Seoul 136-702, Republic of Korea

ARTICLE INFO

Article history:
Received 6 May 2012
Available online 26 May 2012

Keywords:
SIRT1
β-Catenin
PAUF
Pancreatic cancer

ABSTRACT

Because we found in a recent study that pancreatic adenocarcinoma up-regulated factor (PAUF), a novel oncogene, induces a rapid proliferation of pancreatic cells by up-regulation of β-catenin, we postulated that β-catenin might be a target molecule for pancreatic cancer treatment. We thus speculated whether SIRT1, known to target β-catenin in a colon cancer model, suppresses β-catenin in those pancreatic cancer cells that express PAUF (Panc-PAUF). We further evaluated whether such suppression would lead to inhibition of the proliferation of these cells. The ectopic expression of either SIRT1 or resveratrol (an activator of SIRT1) suppressed levels of β-catenin protein and its transcriptional activity in Panc-PAUF cells. Conversely, suppression of SIRT1 expression by siRNA enhanced β-catenin expression and transcriptional activity. SIRT1 mutant analysis showed that nuclear localization of SIRT1 is not required for reduction of β-catenin. Treatment with MG132, a proteasomal inhibitor, restored β-catenin protein levels, suggesting that SIRT1-mediated degradation of β-catenin requires proteasomal activity. It was reported that inhibition of GSK-3β or Siah-1 stabilizes β-catenin in colon cancer cells, but suppression of GSK-3β or Siah-1 using siRNA in the presence of resveratrol instead diminished β-catenin protein levels in Panc-PAUF cells. This suggests that GSK-3β and Siah-1 are not involved in SIRT1-mediated degradation of β-catenin in the cells. Finally, activation of SIRT1 inhibited the proliferation of Panc-PAUF cells by down-regulation of cyclin-D1, a target molecule of β-catenin. These results suggest that SIRT1 activation may be a therapeutic strategy for treatment of pancreatic cancer cells that express PAUF via the down-regulation of β-catenin.

1. Introduction

Pancreatic cancer has an extremely poor prognosis, with a 5-year survival rate of less than 5% [1,2]. The only potential curative treatment for pancreatic cancer is surgery, but only 10–20% of patients are candidates for surgery at the time of presentation. To overcome obstacles such as late diagnosis, the aggressive nature of the tumor, and resistance to existing therapeutic regimens [3], novel tumor-specific biomarkers and genetic pathways that may lead to potential diagnostic and therapeutic targets are desperately needed. During a search for early biomarkers of pancreatic cancer, we discovered that a novel secretory protein, pancreatic adenocarcinoma up-regulated factor (PAUF), is highly expressed in pancreatic cancer tissues. The introduction of PAUF into Chinese hamster ovary (CHO) cells causes tumor formation in xenografted nude mice [4]. We later demonstrated that PAUF expression enhances migration and invasion of pancreatic cancer cells by up-regulating CXCR4, which eventually facilitates the metastasis of pancreatic cancer [5]. We further reported that PAUF contributes to oncogenesis of pancreatic cells via the up-regulation of β-catenin [6].

β-Catenin is a crucial effect molecule in the canonical Wnt signaling pathway involved in cell proliferation, differentiation and oncogenesis [7]. Constitutive activation of the β-catenin pathway has been found in 90% of clinical colorectal cancer tissues. In addition, this pathway is aberrantly activated in many other cancers such as prostate, breast, ovary, and skin cancers. The β-catenin signal is initiated by the interaction of Wnts (Wnt1, 3a, and 8) with

* Corresponding author. Fax: +82 55 350 5653.
E-mail address: younghc@pusan.ac.kr (Y.-H. Chung).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.05.107

the Frizzled receptor and low-density lipoprotein receptor-related protein (LRP) 5/6 co-receptors [8]. The signal is then transduced through the disheveled protein to negatively regulate glycogen synthase kinase-3β (GSK-3β), resulting in the accumulation of cytoplasmic β-catenin. The β-catenin is then translocated into the nucleus, where it forms a complex with the T cell factor/lymphocyte enhancer factor (TCF/LEF) families of transcription factors. These complexes activate the expression of β-catenin-responsive genes, such as cyclin-D1, c-Jun, c-Myc and peroxisome proliferator-activated receptor-β [9–12]. In quiescent cells, β-catenin is maintained in the cytoplasm at low levels. This is facilitated by its interaction with scaffolding proteins, adenomatous polyposis coli (APC) protein, axin, and protein kinases, such as casein kinase-1α (CK-1α) and GSK-3β. CK-1α and GSK-3β phosphorylate β-catenin on Ser-45 and Ser-33/37/Thr-41 sites, respectively, leading to its ubiquitination and proteasomal degradation [13,14]. Alternatively, in the Siah-1-dependent pathway, Siah-1 interacts with the carboxyl terminus of APC, recruits the ubiquitination complex, and promotes the degradation of β-catenin through an F-box protein in the E3 ubiquitin ligase complex [15].

SIRT1 is highly conserved deacetylase that is reported to regulate the life span of organisms in response to caloric restriction [16,17]. However, role of SIRT1 in regulating the worm and fly lifespan has recently become controversial [18]. SIRT1 can deacetylate not only histones [19], but also non-histone proteins including NF-κB p65 [20], p53 [21], Ku70 [22] and Foxo3a [23]. Moreover, the role of SIRT1 in tumor formation is also controversial. The overexpression of SIRT1 in chemo-resistant tumor cells and clinical tissues has been frequently detected [24]. Some studies have indicated that SIRT1 can promote cancer cell growth by blocking cellular senescence via direct deacetylation of p53 [21], FOXO [23], and E2F1 [25]. In contrast, other studies have indicated that ectopic expression resulting in elevated SIRT1 levels reduced cell proliferation and tumor formation in a colon cancer model [26]. Additionally, the activation of SIRT1 by resveratrol also limited cell growth and reduced tumor formation in a breast cancer model [27].

In this study, we explored a therapeutic strategy for the treatment of pancreatic cancer cells that stably express PAUF. We report that SIRT1 inhibits the proliferation of pancreatic cancer cells through suppression of β-catenin.

2. Materials and methods

2.1. Cell cultures and transfection

Panc-1 cells that stably expressed vector (Panc-Vec) and PAUF (Panc-PAUF) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 1% penicillin, 1% streptomycin, and G418 (500 μg/ml). Panc-Vec and Panc-PAUF cells were plated at 5×10^5 cells per 60-mm-diameter plate 24 h before transfection. The cells were then transfected with 0.5–4 μg of DNA using lipofectamine 2000 (Invitrogen, Carlsbad, CA).

2.2. Reagents and antibodies

MG132, cycloheximide, and resveratrol were purchased from Calbiochem (San Diego, CA). For immunoblotting, anti-GSK-3β, anti-cyclin-D1, anti-GFP, and β-actin antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-phospho-GSK-3(Ser-9), and anti-β-catenin antibodies were acquired from Cell Signaling Biotechnology (Danvers, MA). Anti-SIRT1 and anti-Siah1 antibodies were obtained from Abcam (Cambridge, MA) and Cosmobio (Tokyo, Japan), respectively.

2.3. Western blotting assay

Cells were harvested and lysed with lysis buffer (0.15 M NaCl, 1% Nonidet P-40, 50 mM Tris [pH7.5]) containing 0.1 mM Na_2VO_3, 1 mM NaF, and protease inhibitors (Sigma–Aldrich, St. Louis, MO). For immunoblotting, proteins from whole-cell lysates were resolved by 10% or 12% SDS–PAGE and transferred to nitrocellulose membranes. Primary antibodies were used at 1:1000 or 1:2000 dilutions, and secondary HRP-conjugated antibodies (Santa Cruz) were analyzed at 1:2000 dilution in 5% nonfat dry milk. After final washing, nitrocellulose membranes were exposed using chemiluminescence assays (GE-Amersham, Piscataway, NJ).

2.4. Luciferase reporter assay

HEK 293 T cells were transfected with TOP Flash-vectors or FOP Flash-vectors. To normalize transfection efficiency, a pGK-βgal vector that expresses β-galactosidase from a phosphoglucokinase promoter was included in the transfection mixture. At 48 h post-transfection, cells were washed with cold PBS and lysed in lysis solution (25 mM Tris [pH7.8], 2 mM EDTA, 2 mM DTT, 10% glycerol, and 1% Triton-X100). Luciferase activity was measured with a luminometer using a luciferase kit (Promega, Madison, WI).

2.5. siRNA transfection

Cells were trypsinized and incubated overnight to achieve 60–70% confluency before siRNA transfection. SIRT1 siRNA (100 nM, 5′-ACUUUGCUGUAACCCUGUA (dTdT)-3′ as sense and 5′-UACAGGGUUACAGCAAAGU (dTdT)-3′ as anti-sense), GSK-3β siRNA (200 nM, 5′-GACACUAAAGUGAUUGGAA (dTdT)-3′ as sense and 5′-UUCCAAUCACUUUAGUGUC (dTdT)-3′ as anti-sense), Siah1 siRNA (200 nM, 5′-CAGCAUAAGUCCAUUACAA (dTdT)-3′ as sense and 5′-UUGUAAUGGACUUAUGCUG (dTdT)-3′ as anti-sense), or negative control siRNA was mixed with Lipofectamine 2000 (Invitrogen). All siRNA was purchased from Bioneer (Daejeon, Korea). The cells were incubated with the transfection mixture for 6 h and then rinsed with DMEM containing 10% serum. The cells were incubated for an additional 42 h before harvest.

2.6. Statistical analysis

Data are presented as a means ± standard deviation. Student's *t* test was used to compare groups, and *p*-values <0.05 were considered significant.

3. Results

3.1. SIRT1 inhibits protein levels of β-catenin and its transcriptional activity

Because we found in a recent study that PAUF enhances the expression of β-catenin, leading to a rapid proliferation of Panc-1 pancreatic cancer cells [6], we postulated that β-catenin might be a target molecule for cancer treatment of Panc-1 cells that stably express PAUF (Panc-PAUF). Meanwhile, although a line of evidence has shown that ectopic induction of SIRT1 suppresses β-catenin-driven colon cancer in a mouse model [26], the detailed mechanism by which SIRT1 suppresses β-catenin remains to be illustrated. We thus examined whether SIRT1 inhibits the expression of β-catenin in Panc-PAUF cells. To directly test this question, we first introduced SIRT1 and examined the levels of β-catenin protein in Panc-Vec and Panc-PAUF cells. As shown in Fig. 1A, β-catenin expression was significantly diminished by SIRT1 in both cell lines. In addition, we introduced a Top-Flash luciferase

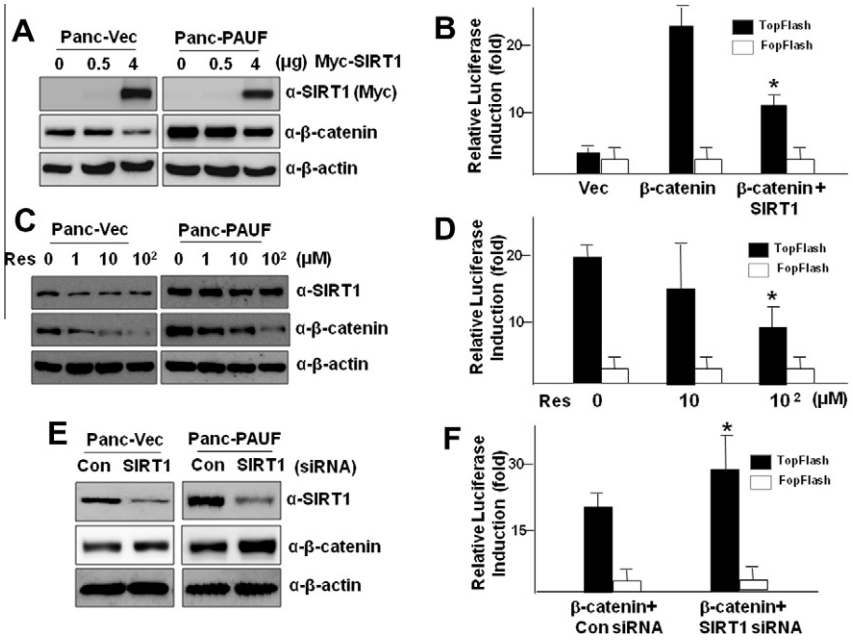

Fig. 1. Expression and activation of SIRT1 reduced levels of β-catenin protein and its transcriptional activity. (A) After Panc-Vec and Panc-PAUF cells were transfected with Myc-tagged SIRT1 vector, cell lysates were prepared 48 h post-transfection. Proteins were separated by 10% SDS–polyacrylamide gel electrophoresis (PAGE) and the expression of β-catenin was examined by immunoblotting using anti-β-catenin antibody. (B) HEK 293 T cells were transfected with Flag-tagged β-catenin (1 μg) or Flag-tagged β-catenin (1 μg) plus Myc-tagged SIRT1 vector (1 μg) in the presence of Top-Flash (1 μg) or Fop-Flash luciferase reporter vector (1 μg). Transfection efficiency was normalized with the β-galactosidase reporter vector, pGK-β-gal. The results shown are the average of triplicate wells. Error bars indicate standard deviation. (*; β-catenin vs. β-catenin plus SIRT1; $p < 0.01$). (C) Panc-Vec and Panc-PAUF cells treated with resveratrol (Res; 1, 10, 100 μM) and cell lysates were prepared 24 h post-treatment. The expression of β-catenin was examined by immunoblotting using anti-β-catenin antibody. (D) HEK293 T cells were transfected with Flag-tagged β-catenin vector in the presence of Top-Flash or Fop-Flash luciferase reporter vector and were treated with resveratrol for 24 h. Luciferase activity was measured as described in (B) (*; Res 0 vs. 10^2; $p < 0.01$). (E, F) Panc-Vec and Panc-PAUF cells were transfected with SIRT1 siRNA (100 nM) for 48 h. The expression of β-catenin and transcriptional activity of β-catenin were evaluated as described in Fig. 1D. (*; control siRNA vs. SIRT1 siRNA; $p < 0.01$).

reporter vector, controlled by a β-catenin/TCF-4 complex, to examine whether the decreased β-catenin levels caused by SIRT1 lead to functional suppression and binding to the β-catenin/TCF-binding element in the nucleus. As shown in Fig. 1B, SIRT1 transfection reduced β-catenin-mediated transcriptional activity ($p < 0.01$), but SIRT1 transfection together with Fop-Flash vector carrying a mutant TCF binding site failed to diminish β-catenin-mediated transcription activity. Moreover, we treated Panc-PAUF cancer cells with resveratrol, a polyphenol compound that is a SIRT1 deacetylase activator. Resveratrol treatment reduced β-catenin protein levels and β-catenin transcriptional activity in a dose-dependent manner (Fig. 1C, D). To further explore whether SIRT1 directly regulates levels of β-catenin, we suppressed SIRT1 protein levels with siRNA and examined the levels of β-catenin protein and β-catenin transcriptional activity ($p < 0.01$). As shown in Fig. 1E, suppression of SIRT1 protein with siRNA dramatically enhanced the expression of β-catenin. The effect of SIRT1 suppression on the up-regulation of β-catenin expression is more dramatic in Panc-PAUF cells than in Panc-Vec cells. In addition, we examined β-catenin transcriptional activity using the Top-Flash luciferase reporter vector. As seen in Fig. 1F, SIRT1 siRNA treatment conversely enhanced β-catenin-mediated transcriptional activity as compared to control siRNA treatment ($p < 0.01$). These results suggest that levels of SIRT1 protein are directly involved in regulation of the level of β-catenin protein in the cell.

3.2. Nuclear localization of SIRT1 is not required for the decrease of β-catenin expression

Because SIRT1 can be localized in either the nucleus or the cytosol, depending on the cell context and developmental stage of cell [28], we speculated whether nuclear localization of SIRT1 is necessary for the suppression of β-catenin expression in Panc-PAUF cells. To answer this question, we introduced SIRT1 localization mutants such as GFP-conjugated nuclear export sequence (NES) mutant SIRT1 (localizes in the nucleus; GFP-NES-SIRT1) and GFP-conjugated nuclear localization sequence (NLS) mutant (mainly localizes in the cytosol; GFP-NLS-SIRT1), as described elsewhere [28], into Panc-PAUF cells. As control, we used GFP-conjugated wild type (WT) SIRT1 (GFP-WT-SIRT1) as a positive control and GFP as a negative control. As shown in Fig. 2A, GFP-conjugated WT and NES mutant SIRT1 were mainly localized in the nucleus while GFP-conjugated NLS mutant SIRT1 was detected in the cytosol. Transfection of Panc-PAUF cells with GFP-WT-SIRT1, GFP-NLS-SIRT1, and GFP-NES-SIRT1 vectors reduced β-catenin levels to a similar degree (Fig. 2B). However, GFP alone did not decrease β-catenin protein levels. This result suggests that nuclear localization of SIRT1 is not necessary for down-regulation of β-catenin.

3.3. SIRT1-mediated degradation of β-catenin is independent of GSK-3β and Siah-1 but is dependent of proteasome

GSK-3β is involved in phosphorylation of β-catenin, leading to degradation of β-catenin [16,17]. Siah-1, E3 ubiquitin ligase is also associated with degradation of β-catenin independent of GSK-3β [15] We thus explored whether GSK-3β or Siah-1 participates in SIRT1-mediated degradation of β-catenin. To test this question, we investigated whether suppression of GSK-3β or Siah-1 in Panc-PAUF cells protects β-catenin degradation from SIRT1 activity. Panc-PAUF cells had similar levels of GSK-3β and Siah1 proteins as Panc-Vec cells (Fig. 3A). However, Panc-PAUF cells had more phosphorylation of GSK-3β (an inactive form) than Panc-Vec cells, indicating that Panc-PAUF cells has an intracellular environment conducive to the stabilization of β-catenin. When

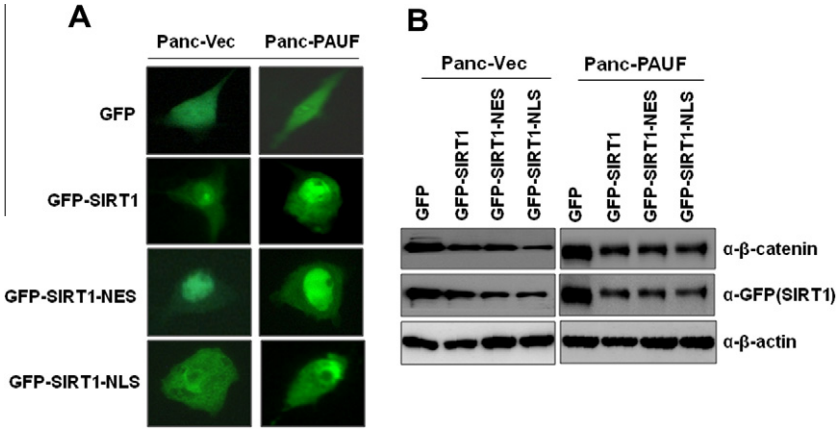

Fig. 2. Nuclear localization of SIRT1 is not necessary for SIRT1-mediated degradation of β-catenin. (A and B) Panc-Vec and Panc-PAUF cells were transfected with GFP-conjugated NES-SIRT1 mutant (3 μg), GFP-NLS-SIRT1 mutant vector (3 μg), GFP-WT SIRT1 (3 μg) as a positive control, and GFP (3 μg) as a negative control for 48 h. The level β-catenin protein was examined with anti-β-catenin antibodies and the level of GFP protein was used to control for transfection.

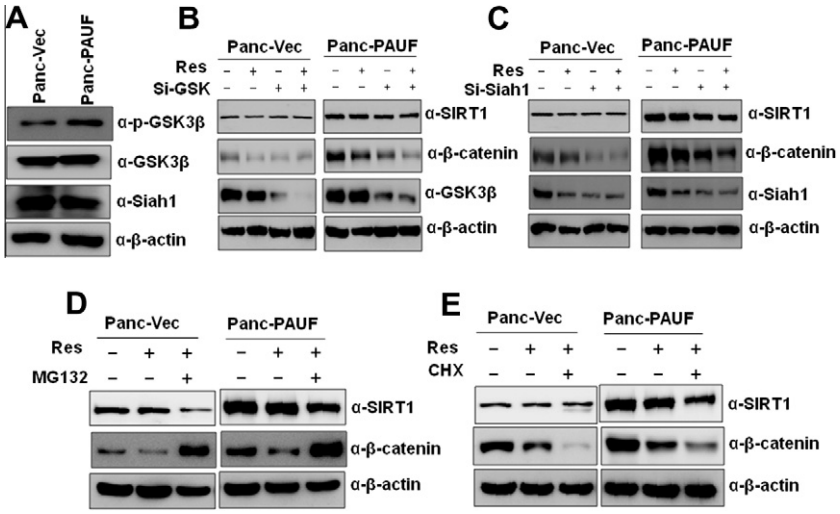

Fig. 3. SIRT1-mediated degradation of β-catenin is not required for GSK-3β and Siah-1 but for proteosome. (A) Endogenous GSK-3β, phospho-GSK3β and Siah-1 protein levels were examined in Panc-Vec and Panc-PAUF cells. Each protein level was examined by immunoblotting using the corresponding antibodies. (B and C) Panc-Vec and Panc-PAUF cells were treated with GSK-3β siRNA (Si-GSK; 200 nM) or Siah-1 siRNA (Si-Siah; 200 nM) for 48 h and then prepared as cell lysates. After confirming suppression of GSK-3β or Siah-1, β-catenin protein levels were evaluated with anti-β-catenin antibodies. (D and E) Panc-Vec and Panc-PAUF cells were treated with resveratrol for 24 h. The cells were subsequently treated with MG132 (10 μM) or cycloheximide (CHX; 10 μM) for additional 8 h and the cell lysates were prepared for immunoblotting. The expression of β-catenin was examined using anti-β-catenin antibody.

Panc-Vec and Panc-PAUF cells were transfected with GSK-3β siRNA alone, the resulting suppression of GSK-3β levels mildly reduced β-catenin expression (Fig. 3B). Moreover, combined treatment with resveratrol and GSK-3β siRNA significantly reduced β-catenin levels (Fig. 3B). Siah-1 siRNA treatment slightly reduced β-catenin expression as observed in GSK-3β siRNA treatment (Fig. 3C). Combined treatment with resveratrol and Siah-1 siRNA significantly suppressed β-catenin protein levels (Fig. 3C). These results indicate that suppression of GSK-3β and Siah-1 expression does not prevent SIRT1–mediated degradation of β-catenin in Panc-PAUF cells, considering that inhibition of GSK-3β or Siah-1 stabilizes β-catenin protein levels in colon cancer cells [29,30].

Although it is well known that the phosphorylation of β-catenin takes place before ubiquitination by GSK-3β action and subsequent degradation occurs through the proteasome system [13,14], we speculated whether SIRT1 activation could reduce β-catenin protein levels through the proteasome-mediated degradation. To answer this question, Panc-Vec and Panc-PAUF cells were treated with resveratrol in the presence or absence of MG132, a proteasome inhibitor. We found that MG132 treatment completely blocked SIRT1-induced β-catenin degradation, leading to more β-catenin than in untreated Panc-Vec and Panc-PAUF cells (Fig. 3D). These results suggest that β-catenin degradation caused by SIRT1 activation occurs via proteasomes. Next, we speculated whether SIRT1-induced β-catenin degradation requires a new protein synthesis. To test this, Panc-Vec and Panc-PAUF cells were treated with cycloheximide in the presence of resveratrol. As shown in Fig. 3E, the inhibition of new protein synthesis with cycloheximide further enhanced the degradation of β-catenin in Panc-Vec and Panc-PAUF cells treated with resveratrol as compared to Panc-Vec and Panc-PAUF cells treated with resveratrol alone. This result implies that β-catenin degradation induced by SIRT1 does not require a new protein synthesis at least, although the mechanism of cycloheximide-induced acceleration of SIRT1-induced β-catenin degradation remains unclear.

3.4. SIRT1 activation inhibits proliferation of Panc-PAUF cells

Finally, we speculated whether SIRT1 activation can inhibit proliferation of Panc-PAUF cells. If our hypothesis is true, SIRT1

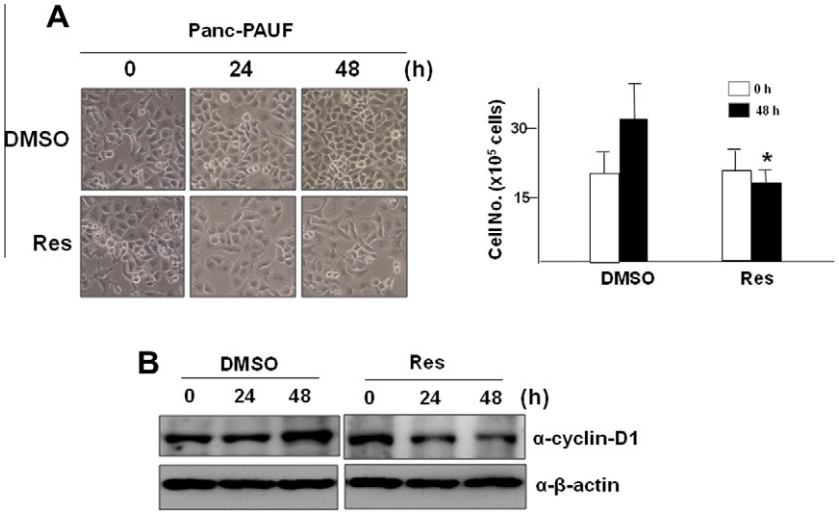

Fig. 4. Activation of SIRT1 inhibits proliferation of Panc-PAUF cells through down-regulation of β-catenin-cyclin-D1 signaling. (A) Panc-PAUF cells were treated with or without resveratrol (50 μM) and cell proliferation was observed under light microscopy for 48 h. Cell proliferation was also evaluated using Trypan blue exclusion. (*; DMSO treatment at 48 h vs. Res treatment at 48 h; $p < 0.01$). (B) Cell lysates from Panc-PAUF cells treated with DMSO or resveratrol were prepared for the examination of cyclin-D1 protein levels. Cyclin-D1 protein levels were evaluated with immunoblotting using anti-cyclin-D1 antibody.

activation may be a useful regimen for the treatment of pancreatic cancer. To answer this question, we treated Panc-PAUF cells with resveratrol. As shown in Fig. 4A, resveratrol treatment inhibited cell proliferation after 24 h of exposure. The number of viable mock-treated Panc-PAUF cells was approximately 2 times that of resveratrol-treated Panc-PAUF cells 48 h post-treatment (Fig. 4A) ($p < 0.01$). After Panc-PAUF cells were treated with resveratrol or DMSO as a control, cell lysates were prepared at 24 and 48 h after the treatment. The expression of β-catenin was examined by immunoblotting using anti-β-catenin antibody. We further examined the expression of β-catenin target gene such as cyclin-D1 because elevated expression of cyclin-D1 is preferentially detected in proliferating and tumorigenic cells. As seen in Fig. 4B, the expression of cyclin-D1 was lower in resveratrol-treated cells than in DMSO-treated cells at 24 and 48 h after the treatment. These results suggest that SIRT1 activation can be a potential therapeutic approach for treatment of Panc-PAUF cells.

4. Discussion

In a previous study, we outlined a molecular mechanism by which PAUF contributes to the development of pancreatic cancer by up-regulation of β-catenin, leading to the rapid proliferation of pancreatic cells [6]. We found that PAUF-induced phosphorylation of β-catenin follows a different phosphorylation pattern than that achieved by treatment with Wnt3a or Bt_2-cAMP (a cAMP analogue; Protein kinase A activator). Because Akt-GSK-3β signaling is used by PAUF, Wnt3a, and Bt2-cAMP, the hyper-phosphorylation of Ser-33/37 and Thr-41 is thought to lead to the ubiquitination and proteasomal degradation of β-catenin. However, while Wnt3a and Bt2-cAMP treatment reduced β-catenin phosphorylation at Ser-33/37 and Thr-41, PAUF-induced β-catenin hyper-phosphorylation of these sites actually stabilized β-catenin levels [6]. Based on these results, we imagined that PAUF may also recruit or activate a molecule that is downstream of GSK-3β, thereby interrupting the ubiquitin E3 ligase-mediated degradation of β-catenin that would otherwise occur. However, we observed that neither GSK-3β nor Siah-1 siRNA treatment blocked degradation of β-catenin during the activation of SIRT1. This indicates that SIRT1-mediated degradation of β-catenin takes place independently of GSK-3β or Siah-1. We believe that SIRT1 may block the actions of the signaling molecules that are provoked by PAUF. Further studies are therefore needed in order to illustrate the molecular mechanism by which SIRT1 degrades β-catenin in Panc-PAUF cells.

Several studies have suggested that SIRT1 plays a role as a tumor suppressor. One study found that mouse embryonic fibroblasts derived from SIRT1-null mice are prone to spontaneous immortalization [31]. SIRT1 also has been shown to inhibit androgen receptor-dependent cell proliferation of prostate tumor cells [32]. Recent studies have reported that SIRT1 overexpression inhibits the growth of colon cancer cells, depending on β-catenin activity. The same study also showed that SIRT1 sequesters β-catenin, thereby interrupting translocation of β-catenin to the nucleus [26]. However, while that study found that overexpression of SIRT1 did not induce β-catenin degradation, we observed that SIRT1 overexpression induced degradation of β-catenin. This differential outcome may result from the use of different cancer cells in each study (colon vs. pancreas). We also found that cytoplasmic localization of SIRT1 was still able to suppress β-catenin protein levels in Panc-PAUF cells, as compared to wild type SIRT1 that was localized in the nucleus. We are currently investigated how the different localizations of SIRT1 both result in the down-regulation of β-catenin.

Acknowledgment

This study was supported by a two-year grant by Pusan National University Research Grant.

References

[1] P. Ghaneh, C. Tudur-Smith, J.P. Neoptolemos, Comment on "adjuvant therapy in pancreatic cancer: a critical appraisal", Drugs 67 (2007) 2487–2490 [Discussion 2491–2483].
[2] A. Maitra, R.H. Hruban, Pancreatic cancer, Annu. Rev. Pathol. 3 (2008) 157–188.
[3] R.H. Hawes, Q. Xiong, I. Waxman, K.J. Chang, D.B. Evans, J.L. Abbruzzese, A multispecialty approach to the diagnosis and management of pancreatic cancer, Am. J. Gastroenterol. 95 (2000) 17–31.
[4] S.A. Kim, Y. Lee, D.E. Jung, K.H. Park, J.Y. Park, J. Gang, S.B. Jeon, E.C. Park, Y.G. Kim, B. Lee, Q. Liu, W. Zeng, S. Yeramilli, S. Lee, S.S. Koh, S.Y. Song, Pancreatic adenocarcinoma up-regulated factor (PAUF), a novel up-regulated secretory protein in pancreatic ductal adenocarcinoma, Cancer Sci. 100 (2009) 828–836.
[5] Y. Lee, S.J. Kim, H.D. Park, E.H. Park, S.M. Huang, S.B. Jeon, J.M. Kim, D.S. Lim, S.S. Koh, PAUF functions in the metastasis of human pancreatic cancer cells and upregulates CXCR4 expression, Oncogene 29 (2010) 56–67.

[6] I.R. Cho, S.S. Koh, H.J. Min, S.J. Kim, Y. Lee, E.H. Park, S. Ratakorn, B.H. Jhun, S. Oh, R.N. Johnston, Y.H. Chung, Pancreatic adenocarcinoma up-regulated factor (PAUF) enhances the expression of beta-catenin, leading to a rapid proliferation of pancreatic cells, Exp. Mol. Med. 43 (2011) 82–90.
[7] P. Polakis, Wnt signaling and cancer, Genes Dev. 14 (2000) 1837–1851.
[8] R.H. Giles, J.H. van Es, H. Clevers, Caught up in a Wnt storm: Wnt signaling in cancer, Biochim. Biophys. Acta 1653 (2003) 1–24.
[9] O. Tetsu, F. McCormick, Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells, Nature 398 (1999) 422–426.
[10] T.C. He, A.B. Sparks, C. Rago, H. Hermeking, L. Zawel, L.T. da Costa, P.J. Morin, B. Vogelstein, K.W. Kinzler, Identification of c-MYC as a target of the APC pathway, Science 281 (1998) 1509–1512.
[11] T.C. He, T.A. Chan, B. Vogelstein, K.W. Kinzler, PPARdelta is an APC-regulated target of nonsteroidal anti-inflammatory drugs, Cell 99 (1999) 335–345.
[12] A. Saadeddin, R. Babaei-Jadidi, B. Spencer-Dene, A.S. Nateri, The links between transcription, beta-catenin/JNK signaling, and carcinogenesis, Mol. Cancer Res. 7 (2009) 1189–1196.
[13] B. Rubinfeld, I. Albert, E. Porfiri, C. Fiol, S. Munemitsu, P. Polakis, Binding of GSK3beta to the APC-beta-catenin complex and regulation of complex assembly, Science 272 (1996) 1023–1026.
[14] H. Aberle, A. Bauer, J. Stappert, A. Kispert, R. Kemler, Beta-catenin is a target for the ubiquitin-proteasome pathway, EMBO J. 16 (1997) 3797–3804.
[15] J. Liu, J. Stevens, C.A. Rote, H.J. Yost, Y. Hu, K.L. Neufeld, R.L. White, N. Matsunami, Siah-1 mediates a novel beta-catenin degradation pathway linking p53 to the adenomatous polyposis coli protein, Mol. Cell 7 (2001) 927–936.
[16] C.L. Brooks, W. Gu, How does SIRT1 affect metabolism, senescence and cancer?, Nat Rev. Cancer 9 (2009) 123–128.
[17] S.J. Lin, P.A. Defossez, L. Guarente, Requirement of NAD and SIR2 for life-span extension by calorie restriction in Saccharomyces cerevisiae, Science 289 (2000) 2126–2128.
[18] C. Burnett, S. Valentini, F. Cabreiro, M. Goss, M. Somogyvari, M.D. Piper, M. Hoddinott, G.L. Sutphin, V. Leko, J.J. McElwee, R.P. Vazquez-Manrique, A.M. Orfila, D. Ackerman, C. Au, G. Vinti, M. Riesen, K. Howard, C. Neri, A. Bedalov, M. Kaeberlein, C. Soti, L. Partridge, D. Gems, Absence of effects of Sir2 overexpression on lifespan in C. elegans and Drosophila, Nature 477 (2011) 482–485.
[19] A. Vaquero, M. Scher, D. Lee, H. Erdjument-Bromage, P. Tempst, D. Reinberg, Human SirT1 interacts with histone H1 and promotes formation of facultative heterochromatin, Mol. Cell 16 (2004) 93–105.
[20] F. Yeung, J.E. Hoberg, C.S. Ramsey, M.D. Keller, D.R. Jones, R.A. Frye, M.W. Mayo, Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase, Embo J. 23 (2004) 2369–2380.
[21] J. Luo, A.Y. Nikolaev, S. Imai, D. Chen, F. Su, A. Shiloh, L. Guarente, W. Gu, Negative control of p53 by Sir2alpha promotes cell survival under stress, Cell 107 (2001) 137–148.
[22] H.Y. Cohen, C. Miller, K.J. Bitterman, N.R. Wall, B. Hekking, B. Kessler, K.T. Howitz, M. Gorospe, R. de Cabo, D.A. Sinclair, Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase, Science 305 (2004) 390–392.
[23] A. Brunet, L.B. Sweeney, J.F. Sturgill, K.F. Chua, P.L. Greer, Y. Lin, H. Tran, S.E. Ross, R. Mostoslavsky, H.Y. Cohen, L.S. Hu, H.L. Cheng, M.P. Jedrychowski, S.P. Gygi, D.A. Sinclair, F.W. Alt, M.E. Greenberg, Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase, Science 303 (2004) 2011–2015.
[24] F. Chu, P.M. Chou, X. Zheng, B.L. Mirkin, A. Rebbaa, Control of multidrug resistance gene mdr1 and cancer resistance to chemotherapy by the longevity gene sirt1, Cancer Res. 65 (2005) 10183–10187.
[25] Y. Yang, W. Fu, J. Chen, N. Olashaw, X. Zhang, S.V. Nicosia, K. Bhalla, W. Bai, SIRT1 sumoylation regulates its deacetylase activity and cellular response to genotoxic stress, Nat. Cell Biol. 9 (2007) 1253–1262.
[26] R. Firestein, G. Blander, S. Michan, P. Oberdoerffer, S. Ogino, J. Campbell, A. Bhimavarapu, S. Luikenhuis, R. de Cabo, C. Fuchs, W.C. Hahn, L.P. Guarente, D.A. Sinclair, The SIRT1 deacetylase suppresses intestinal tumorigenesis and colon cancer growth, PLoS One 3 (2008) e2020.
[27] R.H. Wang, K. Sengupta, C. Li, H.S. Kim, L. Cao, C. Xiao, S. Kim, X. Xu, Y. Zheng, B. Chilton, R. Jia, Z.M. Zheng, E. Appella, X.W. Wang, T. Ried, C.X. Deng, Impaired DNA damage response, genome instability, and tumorigenesis in SIRT1 mutant mice, Cancer Cell 14 (2008) 312–323.
[28] M. Tanno, J. Sakamoto, T. Miura, K. Shimamoto, Y. Horio, Nucleocytoplasmic shuttling of the NAD+-dependent histone deacetylase SIRT1, J. Biol. Chem. 282 (2007) 6823–6832.
[29] H. Choi, J. Gwak, M. Cho, M.J. Ryu, J.H. Lee, S.K. Kim, Y.H. Kim, G.W. Lee, M.Y. Yun, N.M. Cuong, J.G. Shin, G.Y. Song, S. Oh, Murrayafoline A attenuates the Wnt/beta-catenin pathway by promoting the degradation of intracellular beta-catenin proteins, Biochem. Biophys. Res. Commun. 391 (2010) 915–920.
[30] S. Park, J. Gwak, M. Cho, T. Song, J. Won, D.E. Kim, J.G. Shin, S. Oh, Hexachlorophene inhibits Wnt/beta-catenin pathway by promoting Siah-mediated beta-catenin degradation, Mol. Pharmacol. 70 (2006) 960–966.
[31] K.F. Chua, R. Mostoslavsky, D.B. Lombard, W.W. Pang, S. Saito, S. Franco, D. Kaushal, H.L. Cheng, M.R. Fischer, N. Stokes, M.M. Murphy, E. Appella, F.W. Alt, Mammalian SIRT1 limits replicative life span in response to chronic genotoxic stress, Cell Metab. 2 (2005) 67–76.
[32] M. Fu, M. Liu, A.A. Sauve, X. Jiao, X. Zhang, X. Wu, M.J. Powell, T. Yang, W. Gu, M.L. Avantaggiati, N. Pattabiraman, T.G. Pestell, F. Wang, A.A. Quong, C. Wang, R.G. Pestell, Hormonal control of androgen receptor function through SIRT1, Mol. Cell Biol. 26 (2006) 8122–8135.