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Aquatide Activation of SIRT1 Reduces Cellular Senescence through a SIRT1-FOXO1-Autophagy Axis

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Abstract

Ultraviolet (UV) irradiation is a relevant environment factor to induce cellular senescence and photoaging. Both autophagy- and silent information regulator T1 (SIRT1)-dependent pathways are critical cellular processes of not only maintaining normal cellular functions, but also protecting cellular senescence in skin exposed to UV irradiation. In the present studies, we investigated whether modulation of autophagy induction using a novel synthetic SIRT1 activator, heptasodium hexacarboxymethyl dipeptide-12 (named as Aquatide), suppresses the UVB irradiation-induced skin aging. Treatment with Aquatide directly activates SIRT1 and stimulates autophagy induction in cultured human dermal fibroblasts. Next, we found that Aquatide-mediated activation of SIRT1 increases autophagy induction via deacetylation of forkhead box class O (FOXO) 1. Finally, UVB irradiation-induced cellular senescence measured by SA-β-gal staining was significantly decreased in cells treated with Aquatide in parallel to occurring SIRT1 activation-dependent autophagy. Together, Aquatide modulates autophagy through SIRT1 activation, contributing to suppression of skin aging caused by UV irradiation.

Key Words: Cutaneous cellular senescence, UV irradiation, Aquatide, SIRT1, Autophagy

INTRODUCTION

Autophagy is an evolutionarily conserved intracellular process of delivering cytosolic compartments into the lysosome for degradation (Mizushima *et al*., 2008). While autophagy process is induced by cellular stresses, such as nutrient starvation, oxidative stress, and pathogenic infection, two main protein kinase complexes, the unc-51-like kinase complex along with mammalian target of rapamycin complex 1; and the class III phosphatidylinositol 3-kinase complex, are responsible for recruitment of proteins required for autophagy in the phagophore assembly site to initiate phagophore formation (Boya *et al*., 2013; Lapaquette *et al*., 2015). Phagophore then elongates to form a vesicular structure, termed as autophagosome (Boya *et al*., 2013; Lapaquette *et al*., 2015). Subsequently, matured autophagosome fuses with lysosome, leading to the formation of a single membrane autolysosome (Boya *et al*., 2013; Lapaquette *et al*., 2015). Physiological

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levels of autophagy induction are required for normal cellular functions, *i.e.*, i) the elimination of aged, damaged organelles, and aggregated, excessive cellular proteins as well as pathogens (Mizushima *et al*., 2008; Boya *et al*., 2013); ii) regulation of apoptosis, differentiation, inflammation, and immunity in multiple cells/tissues, including skin (Nagar, 2017). Whereas, dysfunction of autophagy mechanism is known to influence the pathogenesis of diverse human disorders, including skin aging (Rubinsztein *et al*., 2011; Nagar, 2017).

SIRT1, the human homologue to sir2, is a NAD-dependent class III histone deacetylase, while is known as 'longevity protein' because its important role in extension of life-span and reduction of aging and aged-related diseases (Donmez and Guarente, 2010; Haigis and Sinclair, 2010). SIRT1 has a wide range of target substrates, *e.g.*, histone and non-histone proteins, including a transcriptional factor forkhead box class O (FOXO) 1, which has been reported to be tightly associated with autophagy induction (Huang and Tindall, 2007; Ng and Tang, 2013). Because prior studies demonstrated that both autophagy and SIRT1 are implicated as a key signaling process/molecule to modulate aging and age-related disorders (Ng and Tang, 2013), activation of autophagy and/or SIRT1 using chemicals is a considerable strategy to delay aging and to reduce age-associated diseases.

Skin continuously expose to deleterious external environments, such as ultraviolet (UV) irradiation, oxidative stress, mechanical stress, and pathogens, which threaten normal cutaneous functions (Denda *et al*., 2000; Sanches Silveira and Myaki Pedroso, 2014). In particular, UV irradiation is the most common environment factor to cause cellular senescence and skin aging (Holleran *et al*., 1997; Sanches Silveira and Myaki Pedroso, 2014). Prior studies have demonstrated that skin aging could be delayed by modulations of age-related cellular processes/proteins, such as autophagy and SIRT1 (Donmez and Guarente, 2010; Rubinsztein *et al*., 2011). Resveratrol is a naturally occurring polyphenol that activates both SIRT1 and autophagy pathways (Morselli *et al*., 2010, 2011). We recently synthesized heptasodium hexacarboxymethyl dipeptide-12 (Aquatide) containing the structures of resveratrol and pyrrolidone carboxylic acid (PCA), a natural moisturizing factor (NMF), which improves the overall skin integrity. We here demonstrated that Aquatide activates SIRT1, which then stimulates autophagy induction through deacetylation of FOXO1, leading to attenuation of UV-irradiation-mediated increase in cellular senescence and skin aging. These results indicate that SIRT1-autophagy axis is a key, considerable determinant in the regulation of skin aging.

MATERIALS AND METHODS

Synthesis and purification of aquatide

Synthesis of heptasodium hexacarboxymethyl dipeptide-12 (Aquatide) was performed using a standard Fmoc-based solid-phase peptide synthetic strategy, as described previously (Wang, 1973). Briefly, the process of Aquatide synthesis was initiated by loading the fluorenylmethyloxycarbonyl (Fmoc)-protected amino acid to 2-chlorotrityl chloride resin, followed by reaction with a solution of Fmoc-Lys(Dde)-OH (N_{α}-Fmoc-N_{ε}-Dde-L-lysine,N_{α}-Fmoc-N_{ε}-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]-L-lysine) (21.3 g, 2 equivalents) and N, N-Diisopropylethylamine (29.9 ml, 8 equivalents) in order to synthesize Fmoc-Lys(Dde)-O-2-chlorotrityl-resin. Subsequently, Fmoc was removed by treating a solution of 20% piperidine in dimethylformamide, and Fmoc-Lys(Fmoc)-OH was coupled to yield Fmoc-Lys(Fmoc)-Lys(Dde)-O-2-chlorotrityl resin. After removal of both Fmoc and Dde on the N-terminus of Fmoc-Lys(Fmoc)-Lys(Dde)-O-2-chlorotrityl resin, the peptide was alkylated using tert-butyl bromoacetate, an alkylating agent, to form H-Lys(H)-Lys(H)-O-2-chlorotrityl resin. Finally, the peptide was cleavage from 2-chlorotrityl-resin and side-chain protein groups using a cleavage solution (trifluoroacetic acid: triisopropylsilane: water=95: 2.5: 2.5, *v/v/v*), and the peptide was then carboxylated using bromoacetic acid to achieve the final form, which is heptasodium hexacarboxymethyl dipeptide-12 (Aquatide). Aquatide was purified by semi-preparative reversed-phase HPLC purification system (SPD-20A, Shimadzu corporation, Kyoto, Japan) with a column of BDS Hypersil C18 (4.6×250 mm, 130 Å, 5 μm) and Alliance HPLC 2695 separations module equipped with 2489 UV/Vis detector (Waters, Milford, MA, USA). In addition, LC-MS (SQD2, Waters) analysis was performed to measure the molecular weight of Aquatide, as described previously (Akritopoulou-Zanze *et al*., 2004).

Cell culture

Cultured human dermal fibroblasts isolated from neonatal foreskins were grown in M106 fibroblast growth medium containing low serum growth supplement (Thermo Fisher Scientific, Waltham, MA, USA) under the Institutional Review Board-approval protocols (University of California San Francisco, CA, USA; Incospharm corporation, Daejeon, Korea). Cell viability and cytotoxicity were determined using MTT assay kit in accordance with the manufacturer's instructions.

Three-dimensional organotypic skin culture and immunohistochemistry

Three-dimensional (3D) organotypic skin cultures with an air-tissue interface were prepared using human epidermal keratinocytes and dermal fibroblasts, as described previously (Sun *et al*., 2015). Briefly, gels comprised a 1:1 mixture of Matrigel (Becton Dickinson, San Jose, CA, USA) and type I collagen (Upstate, St. Charles, MO, USA) containing 4×10^6 cells/mL of fibroblasts, to which 3×10^5 keratinocytes were added. After establishment of 3D skin model, Aquatide was treated to the medium, and incubated for 24 h. The gels were bisected, fixed in formal saline, and processed to paraffin. Sections (10 μm) were stained with anti-LC3-II antibody (abcam, Cambridge, MA, USA), as described previously (Park *et al*., 2013).

Western blot analysis: Western blot analysis was performed as described previously (Park *et al*., 2016). Briefly, Cell lysates, prepared in radioimmunoprecipitation assay buffer, were resolved by electrophoresis on 4-12% Bis-Tris protein Gel (Life Technologies, Carlsbad, CA, USA). Resultant bands were blotted onto polyvinylidene difluoride membranes, probed with appropriate antibodies, and detected using enhanced chemiluminescence (Thermo Fisher Scientific). The intensity of bands was measured with a LAS-3000 (Fuji Film, Tokyo, Japan).

Immunofluorescence: Immunofluorescence were performed as described previously (Park *et al*., 2013). Cells were treated with Aquatide or vehicle for 24 h. LC3-II distribution was assessed using anti-LC3-II (abcam) and anti-rabbit IgG

conjugated with fluorescein isothiocyanate (Life Technologies). Cells were counterstained with the nuclear marker DAPI (Vector Laboratories, Burlingame, CA, USA) and images were viewed under a fluorescence microscope (Carl Zeiss, Thornwood, NY, USA).

Deacetylase activity of SIRT1 and SIRT2: The SIRT1 and SIRT2 deacetylase activities were measured using a SIRT1 Fluorescent Activity Assay Kit or a SIRT2 Fluorescent Activity Assay Kit (Enzo Life Science, Plymouth Meeting, PA, USA), which are designed to measure the lysyl deacetylase activity of the recombinant human SIRT1 and SIRT2, according to the manufacturer's instructions.

SIRT1-Aquatide binding assay: SIRT1 binding to Aquatide was assessed by enzyme linked immunosorbent assay (ELISA)-based binding assay. Recombinant Sirt1 (0.1 μM) in PBS was immobilized overnight in 96-well microtiter plate at 4°C. The wells were incubated with 300 ul of blocking solution (Sigma-Aldrich, St. Louis, MO, USA) for 1 h at room temperature. The plates were washed three times with PBS with 0.05% Tween 20 (PBST), and biotin-labeled Aquatide in PBST was added over a range of concentrations. 1 h after incubation at 37°C, plate was washed with PBST to remove unbound Aquatide, and further incubated with HRP conjugated avidin (Sigma-Aldrich) in PBST for 1 h at 37°C. The wells were washed five times with PBS-T and added with 200 ul of TMB solution (Thermo Fisher Scientific) as chromogenic substrate. Levels of binding were assessed by absorbance at 450 nm.

DNA transfection and immunoprecipitation: Cells were transfected at 70-80% confluency with vectors for Flag-FOXO1, HA-SIRT1, and myc-p300 (pcDNA3.1) in a 0.5:1:3 ratio by calcium phosphate (Life Technologies), as described previously (Lim *et al*., 2015). Briefly, total transfected DNA for each sample was normalized by adding empty vector DNA. 24 h after transfection, cells were harvested in immunoprecipitation lysis buffer containing protease/phosphatase inhibitors (Thermo Fisher Scientific). After clarification, lysates were immunoprecipitated with anti-Flag (M2) conjugated-agarose (Sigma-Aldrich), washed 5 times with lysis buffer and eluted with Flag peptide (100 μg/ml). Samples were boiled in Laemmli buffer for SDS-PAGE and performed Western blotting with antibodies against Flag (M2, Sigma-Aldrich), and acetyl lysine (Cell signaling, Danvers, MA, USA).

Transmission electron microscopy: Cells were fixed immediately in 2% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 h at 4°C. Following three washes in phosphate buffer, the tissues were post-fixed with 1% osmium tetroxide on ice for 2 h and washed three times, all in phosphate buffer. The cells were then embedded in pure Epon 812 mixture after dehydration in ethanol and following infiltration in a mixture of propylene oxide and epon. Polymerization was conducted with pure resin at 70°C for 24 h. Sections were obtained with a model MT-X ultramicrotome (RMC, Tucson, AZ, USA) and then collected on 100 mesh copper grids. After staining with 2% uranyl acetate and lead citrate, the sections were visualized by cryogenic Transmission electron microscopy (Cryo-TEM) (JEM-1400 Plus, at 120kV) and Bio-HVEM (JEM -1000BEF, at 1000kV) (JEOL, Tokyo, Japan).

UV irradiation and β-Galactosidase Histochemical Staining: Cells pretreated with Ex-527 (1 μM) and Aquatide (50 μM) for 16 h were exposed to a high dose of UVB (50 mJ/cm^{-2}), as described previously (Uchida *et al*., 2010), followed by further incubation for 72 h. To determine cellular

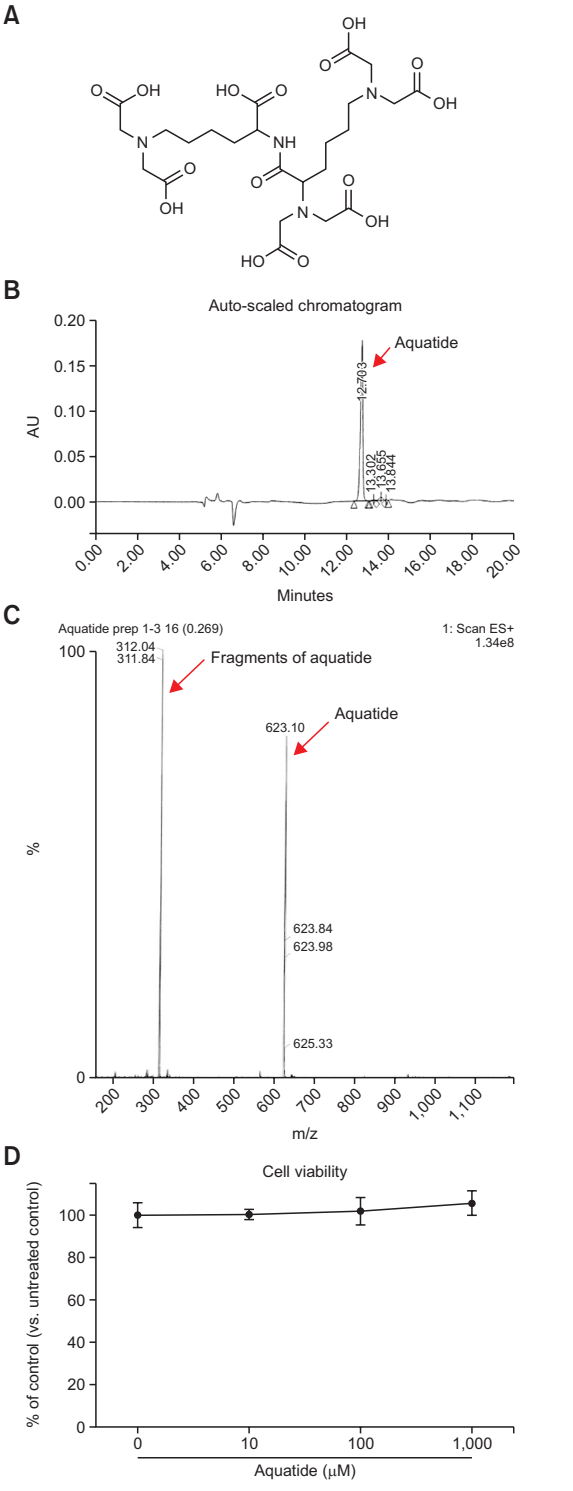

Fig. 1. Synthesis of Aquatide and cell viability in response to Aquatide treatment. Aquatide was synthesized using a standard Fmoc-based solid-phase peptide synthetic protocol. The chemical structure of Aquatide (A). The purity of Aquatide was advanced by the purification process by semipreparative reversed-phase HPLC system (B), and the expected molecular weight was measured by LC-MS analysis (C). Cultured human dermal fibroblasts were incubated with the indicated concentration of Aquatide for 24 h. Cell toxicity was determined by MTT assay (D). Similar results were obtained when the experiment was repeated (in triplicate) using different cell preparations.

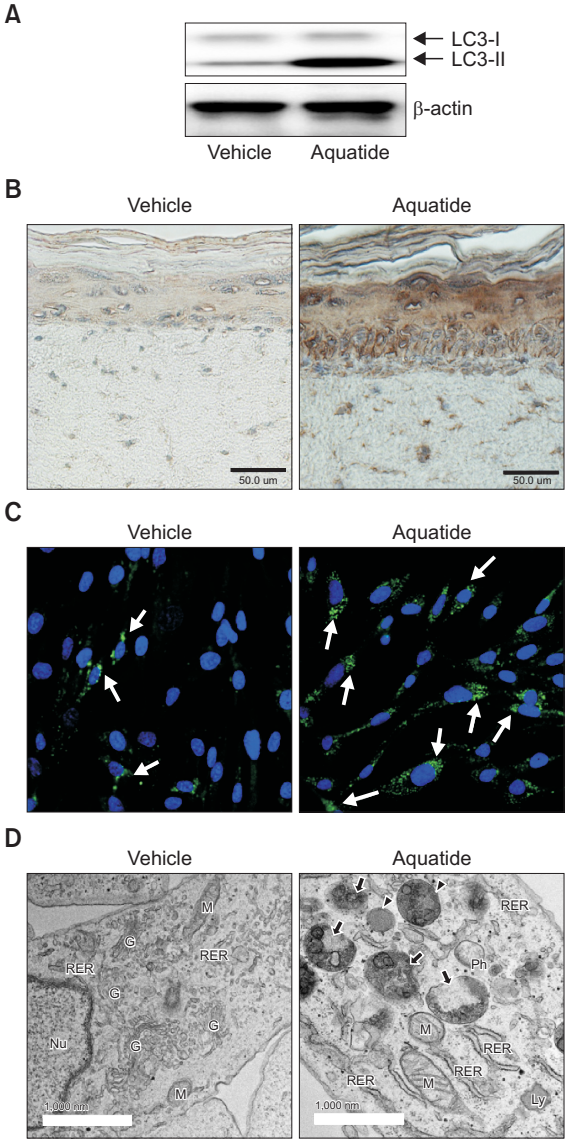

Fig. 2. Aquatide stimulates autophagy induction. Cells or 3D organotypic skin cultures were treated with Aquatide (100 μM) for 24 h. LC3-II protein levels were determined by Western blotting (A), Immunohistochemistry (B), and Immunofluorescence (C). Autophagy compartments were visualized by a TEM. Similar results were obtained when the experiment was repeated (more than twice) using different cell preparations. Green staining corresponds to LC3-II staining. Arrows and arrow heads indicate autolysosome, autophargosome, respectively. Ph: phargophore, M: mitochondria, RER: rough endoplasmic reticulum, G: golgi apparatus, Ly: lysosome.

senescence, cells were fixed in 4% formaldehyde and performed the β-galactosidase staining assay using senescence β-galactosidase staining kit (Cell signaling) according to the manufacturer's instructions. Images were taken with an inverted microscope (CK-41, Olympus, Tokyo, Japan). The number of positive SA-β-galactosidase cells stained with blue-green and the total number of cells were counted under microscope in parallel.

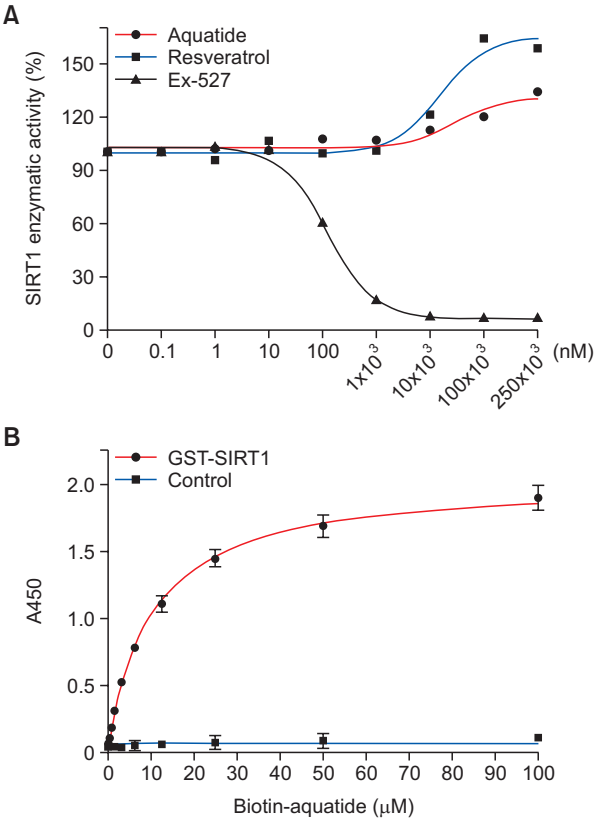

Fig. 3. Aquatide is a specific SIRT1 activator. SIRT1 activities in response to either Aquatide or resveratrol, a known SIRT1 activator, were assessed using a Fluor de lys fluorescent assay system (A). Ex-527 (1 μM), a SIRT1 inhibitor, was employed as a negative control. SIRT1 binding to Aquatide was measured by enzyme linked immunosorbent assay (ELISA)-based binding assay using recombinant SIRT1 and biotin-labeled Aquatide. Levels of binding were assessed by absorbance at 450 nm using plate reader (B).

Statistical analyses

All experiments were repeated at least three times. For each experiment, results from triplicate samples were expressed as the mean ± standard deviation (SD). Significance between groups was determined with unpaired Student *t* test. The *p*-values were set at <0.01.

RESULTS

Aquatide stimulates autophagy induction in cultured human fibroblasts

Aquatide was synthesized according to the standard protocol of Fmoc-based solid-phase peptide synthesis (Fig. 1A-1C). The purity and expected molecular weight of synthesized Aquatide were 96.19% and 622.58, respectively. Because the level of light chain (LC)3-II conversion from LC3-I is a well-known biomarker to determine the classical autophagy induction/pathway (Lapaquette *et al*., 2015), we first measured the levels of LC3-II conversion from LC3-I in fibroblasts treated with Aquatide. Western blot and immunohistochemistry/immunofluorescence analyses revealed that a significant increase in LC3-II protein levels in cells after incubation with Aquatide

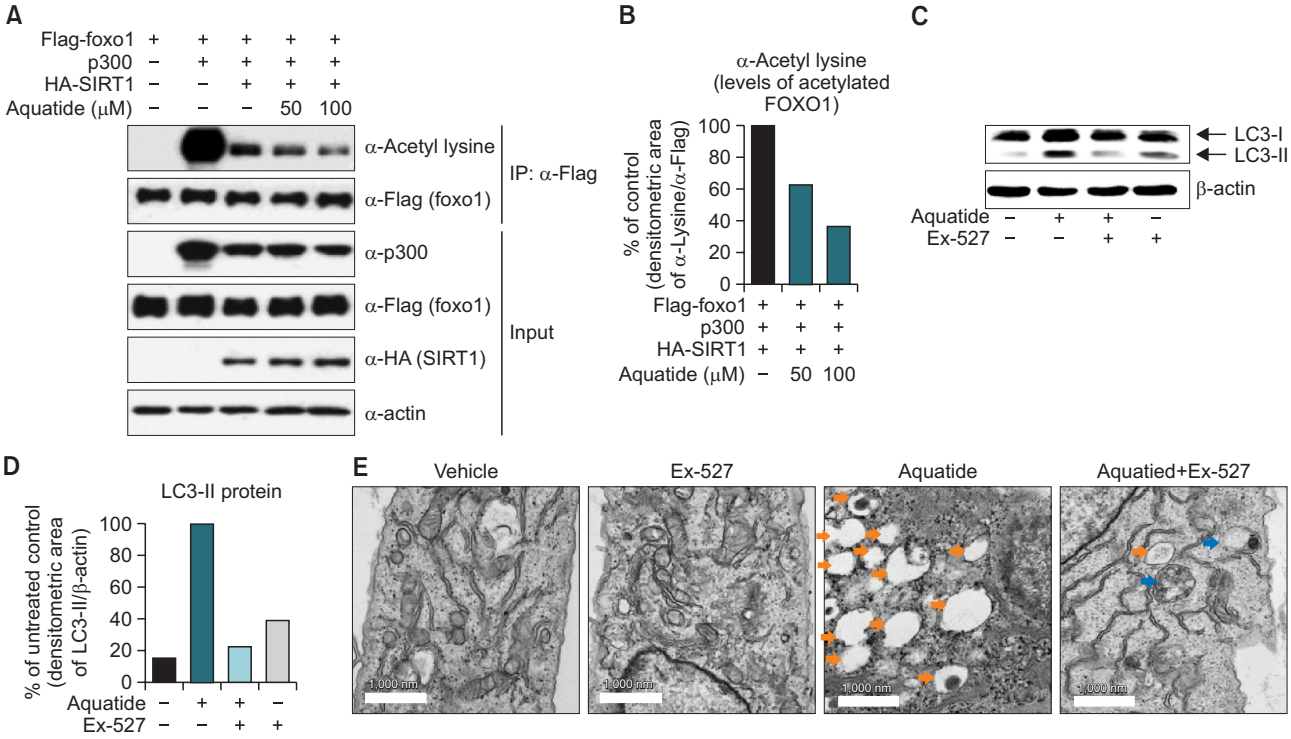

Fig. 4. Aquatide activation of SIRT1 accounts for autophagy induction. Cells overexpressed with vectors for Flag-FOXO1, HA-SIRT1, and myc-p300 in a 0.5:1:3 ratio were treated with Aquatide for 24 h. Cell lysates were immunoprecipitated with anti-Flag and immunoblotted with indicated antibodies (A). The intensity of acetylated FOXO1 detected by Acetyl Lysine antibody from experiment of (A) was quantified and the integrated areas were normalized to the corresponding value of Flag (B). Cells pretreated with Ex-527 (1 μM) were treated with Aquatide (100 μM) for 24 h. LC3-II protein levels were determined by Western blotting (C). The intensity of LC3-II from experiment of (C) was quantified and the integrated areas were normalized to the corresponding value of β-actin (D). Autophagy compartments were visualized by a TEM (E). Similar results were obtained when the experiment was repeated (more than twice) using different cell preparations. Orange- or blue-colored arrows indicate autolysosome and autophagosome, respectively. Scale bar= 1000 nm.

(Fig. 2A-2C). We next studied autophagy by assessing the formation of increased autophagic compartments, *e.g.*, autophagosome and autolysosome, using electron microscopy (TEM) (Fig. 2D). Autophagic components were evident in cells treated with Aquatide. These results indicated that Aquatide is an inducer of autophagy. Since Aquatide at concentration ≤1000 μM did not affect significantly cell viability (Fig. 1D), we employed Aquatide at concentrations of 50-100 μM in subsequent studies.

Aquatide activates SIRT1, but not SIRT2

We next determined whether Aquatide activates human sir2 homologs, SIRT1 and SIRT2, by assessing the lysyl deacetylase activity of the recombinant human SIRT1 and SIRT2, using fluor de lys-SIRT1/SIRT2 fluorometric assay kits, which are employed for screening candidate inhibitors or activators of the enzyme (Sakai *et al*., 2015; Zhang *et al*., 2016) . Both resveratrol and Aquatide significantly activates SIRT1, albeit resveratrol shows more potent activation compared with Aquatide (Fig. 3A). In contrast to SIRT1, no SIRT2 activation was found in response to Aquatide treamtent (Supplementary Fig. 1), suggesting that Aquaitde is a specific activator for SIRT1. Because prior studies revealed that resveratrol activates SIRT1 by its direct binding to SIRT1 protein (Borra *et al*., 2005), we next investigated whether Aquatide binds to SIRT1. Binding assay revealed that Aquatide bound to SIRT1 similar to resveratrol, while the binding affinity was lower than resveratrol (Fig. 3B). These results suggest that Aquatide activates SIRT1 by its direct binding to SIRT1.

Aquatide-mediated activation of SIRT1 stimulates autophagy induction via deacetylation of FOXO1

Prior studies demonstrated that SIRT1, which is a NAD^+-dependent histone deacetylase, modulates cellular function by deacetylation of target substrates, transcriptional factors such as including forkhead box class O (FOXO) 1 (Huang and Tindall, 2007; Ng and Tang, 2013; Qiu *et al*., 2015). FOXO1 activated by SIRT1-mediated deacetylation induces autophagy (Huang and Tindall, 2007; Ng and Tang, 2013). We next examined whether Aquaitde-induced activation of SIRT1 alters acetylation levels of FOXO1. Immunoprecipitation studies showed that acetylation of FOXO1 detected by anti-acetyl lysine antibody was dramatically increased in cells co-transfected with recombinant FOXO1 and an acetylase, p300 (Fig. 4A). Increased acetylation of FOXO1 by p300 was significantly attenuated by overexpression of recombinant SIRT1 (Fig. 4A, 4B). Moreover, SIRT1-mediated decrease in acetylation levels of FOXO1 was further declined in immunoprecipitated fractions following Aquatide treatment (Fig. 4A, 4B). These results indicated that Aqautide-activated SIRT1 decreases acetylation levels of FOXO1. We next addressed whether Aquaitde-induced activation of SIRT1 is required for autophagy induc-

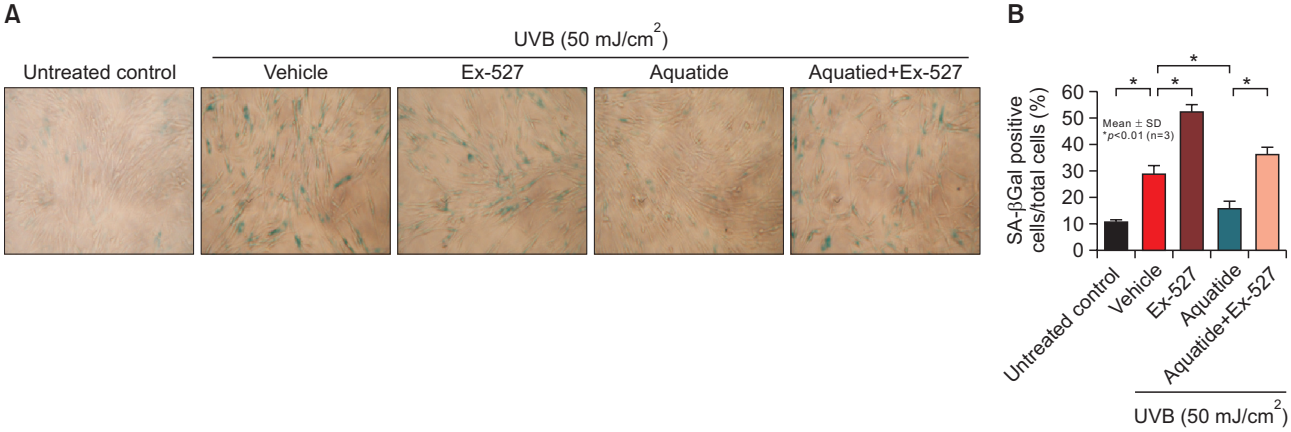

Fig. 5. Aquatide reduces cellular senescence by SIRT1-mediated induction of autophagy. Cultured human dermal fibroblasts pretreated with Ex-527 (1 μM), an inhibitor of SIRT1, and Aquatide (50 μM) were exposed to UVB irradiation (50 mJ/cm^{-2}), followed by further incubation for 72 h. Cellular senescence was determined by β-galactosidase staining assay (A). The number of positive β-galactosidase cells stained with blue-green and the total number of cells were counted under microscope (B). Similar results were obtained when the experiment was repeated (in triplicate) using different cell preparations. *p<0.01 (n=3).

tion. We first confirmed that Ex-527, an inhibitor of SIRT1, does not alter the levels of autophagy induction measured by LC3-II conversion (Fig. 4C, 4D) and the formation of autophagic compartments (Fig. 4E). Western blot and EM analyses revealed that co-incubation of cells with Aquatide and Ex-527 significantly attenuated induction of autophagy (Fig. 4C-4E). Together, these results indicate that Aquatide induces autophagy via SIRT1-FOXO1 axis.

Aquatide-induced autophagy reduces skin aging through a SIRT1-dependent mechanism

UVB irradiation crosses the epidermis and reaches the upper dermis, in which fibroblasts are a major cell type, contributing to the generation of oxidative stress in dermal fibroblasts, resulting in induction of cellular senescence and photo aging (Rosette and Karin, 1996; Rittie and Fisher, 2002). Therefore, we next investigated whether Aquatide reduces cellular senescence in UVB irradiation-exposed human dermal fibroblasts, a model of UV irradiation-induced skin photo-aging, by assessing SA-β-gal levels that are a marker of the cell senescence (Lee *et al*., 2006). SA-β-gal staining assay revealed that SA-β-gal positive cells were elevated in human dermal fibroblast following UV irradiation, while UV irradiation-induced senescence was significantly decreased in cell incubated with Aquatide (Fig. 5). We next elucidated whether SIRT1 activation is responsible for the Aquatide-mediated decreases in cellular senescence. Inhibition of SIRT1 by a pharmacological inhibitor, Ex-527, significantly reduced the Aquatide-mediated decreases in SA-β-gal positive cells, while increased number of SA-β-gal positive cells were found in cell treated with inhibitor alone, compared to vehicle control (UV irradiation alone) (Fig. 5), suggesting that Aquatide suppresses UVB-induced cell senescence through SIRT1-dependent induction of autophagy.

DISCUSSION

Autophagy pathway is stimulated in response to certain conditions, *e.g.*, nutrient depletion, oxidative stress, and microbial infection, in multiple cells/tissues, including skin (Rubinsztein *et al*., 2011; Nagar, 2017). Basal levels of autophagy induction are required for the maintenance of cellular homeostasis through elimination of the aged, damaged, or unnecessary organelles and dysfunctional proteins. Exogenous perturbations such as UV irradiation, oxidative stress, chemicals and mechanical stress accelerate skin aging (Rubinsztein *et al*., 2011; Sanches Silveira and Myaki Pedroso, 2014; Nagar, 2017). Oxidative stress derived from endogenous metabolic activity also stimulates aging (Sanches Silveira and Myaki Pedroso, 2014). Autophagy is a pathway to reduce aging process through removing substances that stimulate/cause aging, while a decrease in autophagy induction has been reported to accelerate skin aging (Rubinsztein *et al*., 2011). Prolonged UV exposure-mediated skin aging is characterized as photo aging (Sanches Silveira and Myaki Pedroso, 2014; Rinnerthaler *et al*., 2015). Both natural- and photo-aging further develops skin aging (Sanches Silveira and Myaki Pedroso, 2014; Rinnerthaler *et al*., 2015). Aged skin shows the loss of elasticity due to changes in extracellular matrix structure and composition, which are caused by increased production and/or activity of matrix metalloproteases, leading to decrease in production of pro-collagen, collagen, and elastin (Cherng *et al*., 2012; Liebel *et al*., 2012). Recent studies have been demonstrated that autophagy levels in aged human dermal fibroblasts are significantly lower than those of young fibroblasts (Rubinsztein *et al*., 2011). We here showed that Aquatide-mediated increases in autophagy significantly suppresses UV-irradiation-induced cellular senescence, suggesting that Aquatide could be a chemical to delay skin aging.

SIRT1 that activates FOXO1 known as longevity gene is also implicated in the development of skin aging (Huang and Tindall, 2007; Ng and Tang, 2013), *i.e.*, SIRT1 activation→deacetylation of FOXO1→increased Rab7 expression→stimulate autophagy induction (Qiu *et al*., 2015). A modulation of SIRT1 pathways is a strategy to suppress cellular senescence and skin aging. Our present study demonstrated that a newly designed chemical (Aquatide) that is based on structures of both resveratrol (SIRT1/Autophagy activators) and pyrrolidone carboxylic acid (recognized as NMF). We found that similar to resveratrol (Borra *et al*., 2005), Aquatide acti-

vates SIRT1 through its binding to SIRT1, leading to stimulate autophagy induction. Pharmacological inhibition of SIRT1 activation diminished Aquatide-mediated suppression of UV-induced cell senescence (see Fig. 5). Thus, SIRT1 activation serves a mechanism of Aquatide-mediated anti-aging activity. It is noted that although SIRT1 activation is lower than resveratrol, Aquatide has additional molecular feature that is NMF functions due to a mimic of pyrrolidone carboxylic acid with seven carboxyl residues in the structure (Bonte, 2011), *i.e.*, 1) enhances moisturizing activity, contributing to improve skin barrier function; 2) reduces skin aging, chemical damages, physical insult or pathological conditions.

Taken together, our present studies illuminate that Aquatide activation of SIRT1 stimulates autophagy induction, leading to contribute the protective mechanism against UVB-irradiation-induced cellular senescence and skin aging. These results further suggest that pharmacological stimulation of either SIRT1 and/or autophagy pathway could represent therapeutic approaches to prevent skin aging caused by UV irradiation.

CONFLICT OF INTEREST

There are no conflict of interest.

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