

Panax ginseng Suppresses Endothelin-1-Induced Cardiomyocyte Hypertrophy Through Inhibition of the Local Angiotensin II–AT1 Receptor Signaling Axis

^{1*}Tomohiro Numata, ^{1,2}Hideaki Tagashira, ¹Kaori Sato-Numata, ³Ryo Takayama, ³Aoto Aoyama, ¹Ayako Sakai, ^{1,4}Ha Nam Tran, ⁵Hiroyuki Watanabe

¹Department of Integrative Physiology, Graduate School of Medicine, Akita University, Akita 010-8543, Japan

²Second Department of Pharmacology, School of Pharmaceutical Sciences, Kyushu University of Medical Science, Miyazaki 882-8508, Japan

³School of Medicine, Akita University, Akita 010-8543, Japan

⁴Faculty of Fisheries, University of Agriculture and Forestry, Hue University, Hue 530000, Vietnam

⁵Department of Cardiovascular Medicine, Akita University Graduate School of Medicine, Akita 010-8543, Japan

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Tomohiro Numata: numata@med.akita-u.ac.jp; Hideaki Tagashira: htagashira@phoenix.ac.jp; Kaori Sato-Numata: satokao@med.akita-u.ac.jp; Ryo Takayama: ryo1402.baseball@icloud.com; Aoto Aoyama: aoyama52250210@gmail.com; Ayako Sakai: a.sakai@med.akita-u.ac.jp; Ha Nam Tran: trannamha@huaf.edu.vn; Hiroyuki Watanabe: hirow@doc.med.akita-u.ac.jp

*Corresponding Author

Tomohiro Numata, Ph.D.

Department of Integrative Physiology, Graduate School of Medicine, Akita University

1-1-1 Hondo, Akita 010-8543 Japan

Tel: +81-18-884-6272

E-mail: numata@med.akita-u.ac.jp

Abstract

Panax ginseng (PG) is a traditional herbal medicine widely used for the treatment of cardiovascular disorders in East Asia. Although PG has been reported to attenuate cardiomyocyte hypertrophy, the molecular mechanisms underlying its cardioprotective effects remain incompletely understood. In this study, we investigated whether modulation of the endothelin-1 (ET-1)–angiotensin II (AngII) signaling axis contributes to the anti-hypertrophic action of PG. Neonatal rat ventricular myocytes (NRVMs) were used to evaluate ET-1-induced hypertrophy, and pharmacological experiments using receptor antagonists were performed to examine the signaling pathways involved. Extracellular AngII levels were quantified by ELISA, intracellular Ca^{2+} accumulation was assessed in $\text{AT}_{1\text{A}}\text{R}$ -overexpressing NRVMs, and whole-cell patch-clamp recordings were performed in HEK293T cells co-expressing $\text{AT}_{1\text{A}}\text{R}$ and TRPC6. PG significantly attenuated ET-1-induced cardiomyocyte hypertrophy without altering $\text{ET}_{\text{A}}\text{R}$ or $\text{ET}_{\text{B}}\text{R}$ expression. Pharmacological experiments with the $\text{ET}_{\text{A}}\text{R}$ antagonist BQ-123 suggested that the anti-hypertrophic effect of PG could not be explained solely by direct inhibition of $\text{ET}_{\text{A}}\text{R}$ signaling. PG markedly suppressed ET-1-induced extracellular AngII production, and interaction studies with losartan further implicated the AngII– $\text{AT}_{1\text{A}}\text{R}$ pathway in the anti-hypertrophic action of PG. Moreover, PG inhibited AngII-induced intracellular Ca^{2+} accumulation in $\text{AT}_{1\text{A}}\text{R}$ -overexpressing NRVMs and attenuated AngII-evoked TRPC6-mediated membrane currents without affecting basal channel activity. These findings demonstrate that PG suppresses ET-1-induced cardiomyocyte hypertrophy by inhibiting both ET-1-induced AngII production and downstream $\text{AT}_{1\text{A}}\text{R}$ -dependent signaling, identifying suppression of the AngII– $\text{AT}_{1\text{A}}\text{R}$ signaling axis as a key mechanism underlying its cardioprotective effects.

Keywords: *Panax ginseng*, Cardiomyocyte hypertrophy, Endothelin-1, patch-clamp

Introduction

Cardiac hypertrophy is a major adaptive response to pathological stress and an important precursor to heart failure. Among the neurohumoral factors implicated in pathological cardiac remodeling, endothelin-1 (ET-1) is one of the most potent endogenous stimuli promoting cardiomyocyte hypertrophy (Ito et al., 1991, Ito et al., 1993, Yamazaki et al., 1996, Archer et al., 2017).

Panax ginseng C.A. Meyer has been used for more than 2,000 years in traditional East Asian medicine for the treatment of cardiovascular disorders and general health promotion (World Health, 1999, Yang, 2007, Chinese Pharmacopoeia, 2025). Increasing experimental evidence has demonstrated its cardioprotective effects; however, the molecular targets responsible for these actions remain incompletely understood.

Previous studies, including ours, have demonstrated that PG attenuates ET-1-induced cardiomyocyte hypertrophy, as evidenced by reductions in cardiomyocyte enlargement and the hypertrophic markers ANP and BNP, preserves mitochondrial function by maintaining intracellular Ca^{2+} and reactive oxygen species (ROS) homeostasis, and improves pathological cardiac remodeling in vivo (Tagashira et al., 2025). Additionally, recent research has shown that traditional Kampo medicines containing PG as a principal component, including Moku-boi-to, exert similar cardioprotective and anti-hypertrophic effects (Karmazyn et al., 2011, Liu et al., 2022, Guan et al., 2023, Tagashira et al., 2023, Tagashira et al., 2025). These findings suggest that PG contributes substantially to the cardioprotective actions of ginseng-containing Kampo medicines.

Several molecular mechanisms have been proposed to explain the cardioprotective effects of PG and its constituent ginsenosides. PG suppresses cardiomyocyte hypertrophy through inhibition of the NHE-1/calcineurin–NFAT pathway (Guo et al., 2011), whereas ginsenoside Rg3 attenuates AngII-induced cardiac hypertrophy and fibrosis by modulating the SIRT1/NF- κ B/NLRP3 signaling cascade (Ren et al., 2021). In addition, accumulating evidence indicates that PG functions as a cardiovascular adaptogen by maintaining homeostasis under pathological stress and regulating metabolic pathways, including AMPK and PPAR signaling (Irfan et al., 2020, Zhou et al., 2024). Consistent with these pleiotropic effects, cumulative evidence indicates that specific ginsenoside monomers present in PG can modulate cell membrane receptors and ion channels to stabilize cardiac function (He et al., 2022, Hyun et al., 2022). For instance, ginsenosides have been reported to regulate transmembrane signaling and Ca^{2+} handling under conditions of neurohumoral stress (Kim, 2018). Collectively, these findings indicate that PG exerts anti-hypertrophic effects through multiple cellular processes. However, despite extensive investigation, the primary molecular mechanisms responsible for the anti-

hypertrophic effects of PG remains unknown.

Dysregulation of intracellular Ca^{2+} homeostasis is a key feature of pathological cardiac hypertrophy. In addition to its essential role in excitation–contraction coupling, sustained or spatially altered Ca^{2+} signaling can activate hypertrophic transcriptional programs in cardiomyocytes. A seminal study demonstrated that Ca^{2+} /calmodulin-dependent activation of calcineurin promotes dephosphorylation and nuclear translocation of NFAT, thereby inducing cardiac hypertrophy and fetal gene expression (Molkentin et al., 1998). Ca^{2+} /calmodulin-dependent kinase signaling, particularly CaMKII, also contributes to pathological cardiac remodeling and altered Ca^{2+} handling (Zhang et al., 2003). Importantly, changes in the frequency and spatial pattern of intracellular Ca^{2+} signals can be decoded by the calcineurin/NFAT pathway to induce cardiomyocyte hypertrophy in response to neurohumoral stimuli, including AngII (Colella et al., 2008). ET-1-induced Ca^{2+} signaling has likewise been directly linked to calcineurin/NFAT activation and cardiomyocyte hypertrophy (Higazi et al., 2009). Consistent with the pathological importance of Ca^{2+} dysregulation, abnormal intracellular Ca^{2+} handling has also been shown to contribute directly to the development of the hypertrophic phenotype in human iPSC-derived cardiomyocytes (Lan et al., 2013). Thus, abnormal intracellular Ca^{2+} signaling represents an important mechanistic link between neurohumoral stimulation and pathological cardiomyocyte hypertrophy.

Among the signaling pathways involved in pathological cardiac remodeling, the AngII–AT₁R axis plays a central role in cardiomyocyte hypertrophy and the progression of heart failure (Zhu et al., 2003, Bhullar and Dhalla, 2022). Importantly, accumulating evidence suggests functional cross-talk between ET-1 and AngII signaling pathways, whereby ET-1 stimulates local AngII production and amplifies downstream hypertrophic signaling through autocrine and paracrine mechanisms (Ito et al., 1993, Rosenkranz, 2004). Given that PG consistently suppresses ET-1-induced hypertrophy, we hypothesized that its cardioprotective effects may involve modulation of the ET-1–AngII–AT₁R signaling axis.

Therefore, the present study was designed to elucidate the signaling mechanisms underlying the anti-hypertrophic effects of PG. Using pharmacological, molecular, Ca^{2+} measurements, and electrophysiological approaches, we investigated whether ET-1-induced AngII production contributes to cardiomyocyte hypertrophy and whether suppression of the AngII–AT₁R signaling pathway accounts for the cardioprotective actions of PG. Our findings identify suppression of the AT₁R signaling axis as a key mechanism underlying the anti-hypertrophic effects of PG and provide a mechanistic basis for its cardioprotective actions.

Materials and Methods

Materials

Wistar rats (Japan SLC, Inc., Hamamatsu, Japan), aged 1-3 days after birth, were used to obtain neonatal rat ventricular myocytes (NRVMs) for this experiment. *Panax ginseng* C.A. Meyer root powder (PG; Lot No. 021723), originating from the People's Republic of China, was obtained from Nakaya Hikoju Pharmacy (Ishikawa, Japan). The material was supplied as dried ("Kiboshi") root powder and was used without further processing in our laboratory. Detailed botanical and phytochemical characterization, including representative marker compounds and a reference 3D-HPLC chromatographic profile, is provided in Supplementary Table S1 and Fig. S1. The reference chromatographic profile was provided by Tsumura & Co. and is consistent with the quality specifications of the Japanese Pharmacopoeia. PG was dissolved in dimethyl sulfoxide (DMSO) at a stock concentration of 50 mg/ml and diluted to the desired final concentrations immediately before use. The final concentration of DMSO was kept constant among the experimental groups and did not exceed 0.1%. Endothelin-1 (ET-1), and angiotensin II (AngII) were purchased from the Peptide Institute Inc. (Osaka, Japan). BQ-123 and Losartan potassium were purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan). Rhodamine-conjugated phalloidin for F-actin staining was obtained from Abcam (ab235138; Cambridge, UK). VECTASHIELD mounting medium containing DAPI (Vector Laboratories, Newark, CA, USA) was used for nuclear counterstaining. The Ca^{2+} indicator Fluo-4 AM was obtained from Dojindo Laboratories (Kumamoto, Japan). For heterologous expression experiments, cDNAs encoding rat angiotensin II type 1A receptor ($\text{AT}_{1\text{A}}\text{R}$; GenBank accession no. NM_030985) and human transient receptor potential canonical 6 (TRPC6; GenBank accession no. NM_004621) were cloned and subcloned into pcDNA3.1 (Thermo Fisher Scientific, Waltham, MA, USA) and pIRES2-AcGFP1 (Takara Bio, Shiga, Japan) expression vectors, respectively. The accuracy of all cloned constructs was verified by DNA sequencing. HEK293T cells were kindly provided by the National Institute for Physiological Sciences (Okazaki, Japan) and used for electrophysiological recordings. All other chemicals were of analytical grade and purchased from standard commercial sources.

Isolation and culture of neonatal rat ventricular myocytes

All animal experiments were approved by the Animal Ethics Committee of Akita University, Akita, Japan (approval numbers: a-1-0412 and b-1-0408). The study was performed in strict accordance with institutional guidelines and is reported in compliance with the ARRIVE guidelines 2.0 (pp. 1-2). NRVMs were isolated from 1–3-day-old

neonatal Wistar rats (either sex; total n=85 pups) obtained from Japan SLC, Inc.. Pups were euthanized by rapid decapitation in accordance with institutional guidelines to minimize pain and distress. Isolation was performed using the Pierce Primary Cardiomyocyte Isolation Kit (Thermo Fisher Scientific, Waltham, MA, USA) as previously described (Tagashira et al., 2023, Tagashira et al., 2025). Briefly, the isolated cell suspension was subjected to a 90-min differential pre-plating step on uncoated dishes to minimize non-cardiomyocyte contamination. The non-adherent NRVM-enriched fraction was collected, seeded at experimental densities, and cultured in DMEM supplemented with 10% FBS and penicillin/streptomycin. Cells were maintained for 3–4 days at 37°C with 5% CO₂ before further experiments. The purity of the NRVM cultures was assessed by immunofluorescence staining for cardiac troponin T (cTnT), a cardiomyocyte marker, and platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31), an endothelial cell marker. Cells were stained with an anti-cTnT antibody (1:500; sc-20025, Santa Cruz Biotechnology, Dallas, TX, USA) or an anti-PECAM-1 antibody (1:500; sc-376764, Santa Cruz Biotechnology), and nuclei were counterstained with DAPI. Fluorescence images were acquired using a BZ-X800 fluorescence microscope (Keyence, Osaka, Japan). The percentages of cTnT-positive and PECAM-1-positive cells were determined relative to the total number of DAPI-positive cells. Quantitative analysis showed that $91.8 \pm 2.1\%$ of cells were cTnT-positive and $2.8 \pm 2.1\%$ were PECAM-1-positive (n = 5), confirming the high cardiomyocyte purity of the NRVM cultures used in this study. Representative cTnT/DAPI and PECAM-1/DAPI images are shown in Supplementary Fig. S2. In the experiment of AT₁AR-overexpressing NRVMs, cells were transiently transfected with plasmids encoding AT₁AR using Lipofectamine 2000 (Thermo Fisher Scientific) on the second day after culturing.

Morphological analysis of cardiomyocyte hypertrophy

NRVM hypertrophy was evaluated by measuring cell surface area as previously described (Tagashira et al., 2023, Tagashira et al., 2025). Briefly, cells were fixed with 4% formaldehyde and stained with rhodamine-conjugated phalloidin to visualize F-actin. Fluorescence images were captured randomly using a BZ-X800 microscope (Keyence, Osaka, Japan). The surface areas of at least 100 cells per experimental condition were quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA) and normalized to the control group. Concentration–response curves were fitted using nonlinear regression (variable slope model) in GraphPad Prism 9. IC₅₀ and Hill slope values were calculated from the fitted curves. Unless otherwise indicated, NRVMs were stimulated with 100 nM ET-1 for 48 h.

Pharmacological interaction analysis

To investigate the relationship between PG and receptor-mediated signaling, pharmacological interaction studies were performed using the ET_AR antagonist BQ-123 and the AT₁R antagonist losartan. Concentration–response curves were generated from cardiomyocyte hypertrophy assays in the absence or presence of a fixed concentration of PG (10 µg/mL). The effects of combined treatment were evaluated by directly comparing the experimentally observed concentration–response relationships. These experiments were used to assess whether the inhibitory effect of each receptor antagonist was further modified in the presence of PG.

Measurement of extracellular AngII

To evaluate extracellular AngII production, NRVMs were cultured in serum-free DMEM treated with ET-1 in the presence or absence of PG for 48 h. AngII concentrations in the culture medium were determined using a commercial AngII ELISA kit (ADI-900-204; Enzo Life Sciences, Farmingdale, NY, USA) according to the manufacturer's protocol. Extracellular AngII levels were normalized to the total protein content of the cell lysates.

RNA isolation and PCR analysis

Total RNA was isolated from NRVMs using the NucleoSpin RNA Plus Kit (Takara Bio) and reverse-transcribed using the PrimeScript II 1st Strand cDNA Synthesis Kit (Takara Bio) as previously described (Tagashira et al., 2023, Tagashira et al., 2025). PCR was performed using KOD-Plus-Ver.2 (Toyobo, Osaka, Japan), and the products were visualized on 2% agarose gels with GelRed staining. Quantitative real-time PCR was performed using SYBR Green Real-time PCR Master Mix Plus (Toyobo) on a LightCycler 480 system (Roche Diagnostics, Rotkreuz, Switzerland) following the manufacturer's protocol. Receptor transcripts (AT_{1A}R, AT_{1B}R, ET_AR, and ET_BR) were normalized to β-actin, whereas Agt, Ace, and Ace2 expression levels were normalized to 18S rRNA. Relative gene expression was calculated using the ΔΔCt method. The primer sequences for Agt, Ace, Ace2, and 18S rRNA were adopted from a previous study (Samuel et al., 2012). The specific primer sequences used in this study are summarized in Table 1. The functionality of the AT_{1B}R primer pair was further confirmed in AngII-treated NRVMs, a condition in which *AT_{1B}R* expression was previously demonstrated (Tagashira et al., 2023), by detection of an amplification product of the expected size (345 bp) (Supplementary Fig. S3).

Measurement of intracellular Ca²⁺ in NRVMs

Intracellular Ca²⁺ levels were measured in NRVMs with or without AT₁AR overexpression using Fluo-4 AM as previously described (Tagashira et al., 2023). Briefly, cells cultured in 96-well plates were incubated with 5 μM Fluo-4 AM in HBSS containing 1 mM Ca²⁺ for 30 min at 37°C. After washing, Fluo-4 fluorescence (excitation/emission = 485/538 nm) was quantified using a Fluoroskan Ascent microplate reader (Thermo Fisher Scientific). Thus, Ca²⁺ levels were assessed as population-based fluorescence intensity in each well rather than by fluorescence microscopy or single-cell imaging.

Whole-cell patch-clamp recordings in HEK293T cells

HEK293T cells were cultured in DMEM with 10% FBS and transiently transfected with plasmids encoding AT₁AR and TRPC6 at a 3:1 ratio using Lipofectamine 2000 (Thermo Fisher Scientific). Because TRPC6 was expressed from the pIRES2-AcGFP1 vector whereas the AT₁AR construct did not contain a fluorescent reporter, an excess of the AT₁AR plasmid was used to increase the likelihood that AcGFP-positive TRPC6-expressing cells selected for electrophysiological recording also co-expressed AT₁AR. The use of TRPC6-mediated currents as a functional readout of AngII–AT₁AR signaling was based on previous evidence demonstrating functional coupling of AngII–AT₁AR signaling to DAG-sensitive TRPC3/TRPC6 channels (Onohara et al., 2006). Transfected cells were identified by AcGFP fluorescence and used for electrophysiological recordings 24–48 h post-transfection; empty vector-transfected cells served as controls. Whole-cell membrane currents were recorded at room temperature using an Axopatch 200B amplifier (Molecular Devices, San Jose, CA, USA). Borosilicate glass pipettes (3–5 MΩ) were filled with an intracellular solution containing (in mM): 95 Cs-aspartate, 5 EGTA, 4 MgCl₂, 5 HEPES, and 2 Na₂-ATP (pH 7.2 with CsOH, 300 mOsm/kg with D-mannitol). The extracellular bath solution contained (in mM): 140 NaCl, 5 KCl, 1 MgCl₂, 1 CaCl₂, 10 glucose, and 10 HEPES (pH 7.4 with NaOH, 320 mOsm/kg with D-mannitol). Cells were voltage-clamped at –50 mV, and current–voltage (I–V) relationships were obtained using voltage ramps from –100 to +100 mV. Cells were pretreated with PG for 1 min prior to AngII stimulation. Signals were filtered at 2 kHz, sampled at 10 kHz, and analyzed using pCLAMP (Molecular Devices). Membrane currents were expressed as current density (pA/pF).

Western blot analysis

AT1AR protein expression was examined by Western blot analysis in AT1AR-transfected NRVMs and HEK293T cells. NRVMs were transiently transfected with the AT1AR expression plasmid as described above. HEK293T cells used for the heterologous expression experiments were transfected with the AT1AR expression plasmid. Forty-eight hours after transfection, total protein was extracted from NRVMs or HEK293T cells using RIPA lysis buffer supplemented with protease inhibitors. Protein samples (30 µg) were separated by 10% SDS-PAGE and transferred onto PVDF membranes. After blocking in TBS-T at room temperature for 1 h, the membranes were incubated overnight at 4°C with a rabbit anti-AT1AR (AGTR1) polyclonal antibody (Proteintech, Cat No. 25343-1-AP, dilution 1:1000). After washing with TBS-T, the membranes were incubated with a horseradish peroxidase (HRP)-conjugated donkey anti-rabbit IgG secondary antibody (Amersham, Cat No. NA934, dilution 1:5000) at room temperature for 1 h. Protein bands were visualized using SuperSignal West Dura Extended Duration Substrate (Thermo Fisher Scientific, Cat No. 34075) and captured with a Lumi Vision PRO 400EX imaging system (Aisin Seiki Co., Ltd., Kariya, Japan). To detect the loading control, the membranes were washed with TBS-T and then incubated with Western BLoT Stripping Buffer (Takara Bio, Cat No. T7135A) at room temperature for 30 min with gentle agitation to remove bound antibodies. After washing with TBS-T, the membranes were re-blocked for 1 h and then incubated with a rabbit anti- α -tubulin monoclonal antibody (ABclonal, Cat No. AC049, dilution 1:5000) overnight at 4°C. Subsequent secondary antibody incubation and chemiluminescence detection were performed as described above. Protein bands were visualized using SuperSignal West Dura Extended Duration Substrate (Thermo Fisher Scientific, Cat No. 34075) and captured with an imaging system. Band intensities were quantified using ImageJ software, and AT1AR protein expression was normalized to α -tubulin. Full-length blot images are provided in Supplementary Fig. S4.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 9. Data are expressed as mean $\pm$ standard error of the mean (SEM). For comparisons between two groups, Student's t-test was used after evaluation of variance equality using the F-test. For comparisons among multiple groups, a one-way analysis of variance, followed by Tukey's post hoc test, was used. A value of $P < 0.05$ was considered statistically significant. Goodness of fit for the concentration–inhibition curves was evaluated using the coefficient of determination (R^2). All experiments were repeated at least three times independently unless otherwise stated. Graphs show mean values with SEM. The number

313 of independent experiments, cells, wells, or animals analyzed is indicated in the
314 corresponding figure legends.

Results

PG suppresses ET-1-induced cardiomyocyte hypertrophy in NRVMs

We previously reported that *Panax ginseng* extract (PG) attenuates endothelin-1 (ET-1)-induced cardiomyocyte hypertrophy and mitochondrial dysfunction in neonatal rat ventricular myocytes (NRVMs) and in vivo models (Tagashira et al., 2025). Therefore, before investigating the molecular mechanism underlying the cardioprotective effects of PG, we first confirmed the reproducibility of its anti-hypertrophic effect under identical experimental conditions.

Treatment of NRVMs with 100 nM ET-1 for 48 h resulted in a marked increase in cardiomyocyte size compared with the control group, as shown in Fig. 1A. Consistent with previous findings (Tagashira et al., 2025), PG alone did not significantly alter cardiomyocyte morphology, while PG co-treatment significantly attenuated ET-1-induced cellular enlargement. Quantitative analysis of cell cross-sectional area (CSA) indicated that ET-1 significantly increased CSA relative to the control group (Fig. 1B). In contrast, PG significantly suppressed ET-1-induced hypertrophy, and PG treatment alone did not significantly affect basal cell size. Importantly, in our previous study using the same ET-1-induced NRVM hypertrophy model, PG also significantly suppressed the ET-1-induced upregulation of the established hypertrophic markers ANP and BNP (Tagashira et al., 2025), providing molecular evidence consistent with the morphological changes observed here.

These findings are consistent with previous observations and confirm that PG reproducibly suppresses ET-1-induced cardiomyocyte hypertrophy. Following the successful reproduction of this phenotype, subsequent experiments aimed to identify the molecular mechanism responsible for the anti-hypertrophic action of PG.

Involvement of endothelin receptor signaling in the anti-hypertrophic effect of PG

To investigate whether endothelin receptor signaling contributes to the anti-hypertrophic effects of PG, we first examined the expression of endothelin receptor subtypes in NRVMs. ET-1 stimulation did not significantly alter the mRNA expression levels of either *ET_AR* or *ET_BR*, and PG treatment had no significant effect on receptor expression under basal or ET-1-stimulated conditions (Fig. 2A).

We next evaluated the effect of the ET receptor antagonist BQ-123 (Ihara et al., 1992) on ET-1-induced cardiomyocyte hypertrophy. BQ-123 inhibited ET-1-induced hypertrophy in a concentration-dependent manner, with an IC₅₀ of 91.6 nM and a Hill slope of 0.6 (Fig. 2B), confirming the involvement of endothelin receptor-mediated signaling in this experimental model.

To further examine the relationship between PG and endothelin receptor signaling, the inhibitory effect of BQ-123 was assessed in the presence of PG. As shown in Fig. 2B and C, combined treatment with PG and BQ-123 produced a concentration-dependent inhibition of ET-1-induced cardiomyocyte hypertrophy. The observed response pattern did not suggest that the anti-hypertrophic effect of PG could be explained solely by direct inhibition of ET_AR signaling.

Together with the absence of changes in *ET_AR* or *ET_BR* expression, these findings prompted us to examine whether ET-1 stimulation induces additional extracellular mediators contributing to cardiomyocyte hypertrophy.

PG suppresses ET-1-induced AngII production and local renin–angiotensin system-related gene expression

Because the anti-hypertrophic effects of PG could not be fully explained by alterations in endothelin receptor expression or its direct actions, we next investigated whether ET-1 stimulation induces the production of additional extracellular mediators. In cardiomyocytes, ET-1 and AngII are well known to form a tight reciprocal autocrine/paracrine regulatory network during hypertrophic remodeling (Ito et al., 1993). Based on this established functional link, we focused on AngII as a primary candidate mediator and examined its concentration in the culture supernatant.

As shown in Fig. 3A, ET-1 stimulation markedly increased the concentration of AngII in the culture supernatant compared with the control group. This finding confirms that ET-1 promotes the production and/or release of AngII from cardiomyocytes, establishing a local AngII signaling mechanism in our experimental model. Importantly, pretreatment with PG significantly attenuated this ET-1-induced increase in extracellular AngII levels. To further investigate the mechanism underlying ET-1-induced AngII production, we examined the expression of key components of the local renin–angiotensin system. ET-1 markedly increased the mRNA expression of *Agt*, *Ace*, and *Ace2*, whereas PG significantly attenuated these ET-1-induced increases (Fig. 3B). These findings indicate that ET-1 induces transcriptional activation of multiple components of the local renin–angiotensin system and that PG attenuates this response. These results indicate that PG effectively suppresses not only downstream ET-1 hypertrophic responses but also ET-1-induced activation of the local renin–angiotensin system and the associated increase in AngII production. Given the well-established role of AngII in cardiomyocyte hypertrophy and pathological cardiac remodeling, these data strongly suggest that ET-1-induced AngII production contributes to cardiomyocyte hypertrophy in this model. We therefore next

examined whether direct activation of the AngII–AT₁R signaling pathway contributes to cardiomyocyte hypertrophy and how PG modulates this specific axis.

Involvement of the AngII–AT_{1A}R pathway in ET-1-induced cardiomyocyte hypertrophy

To determine whether the increase in extracellular AngII observed following ET-1 stimulation contributes to cardiomyocyte hypertrophy, we next examined the expression of AngII receptor subtypes and the effects of pharmacological inhibition of the AT₁R signaling. As shown in Fig. 4A, *AT_{1A}R* mRNA was detectable in NRVMs, whereas *AT_{1B}R* transcripts were not detected under any experimental condition despite validation of the AT_{1B}R primer pair (Supplementary Fig. S3). Although AT_{1A}R expression did not differ significantly among groups, these findings indicate that AT_{1A}R is the predominant AngII receptor subtype expressed in this model. We next examined the effects of the AT₁R antagonist losartan. Losartan markedly attenuated ET-1-induced cardiomyocyte hypertrophy (Fig. 4B) and inhibited the hypertrophic response in a concentration-dependent manner, with an IC₅₀ of 151.7 nM and a Hill slope of 0.9 (Fig. 4B). To further investigate the relationship between PG and AT₁R signaling, the inhibitory effect of losartan was evaluated in the presence of PG. As shown in Fig. 4B and C, losartan concentration-dependently suppressed ET-1-induced hypertrophy, whereas the presence of PG did not produce a further enhancement of the maximal inhibitory response under the conditions tested.

These findings, together with the ET-1-induced increase in extracellular AngII and its suppression by PG (Fig. 3), support the involvement of the AngII–AT_{1A}R signaling pathway in the anti-hypertrophic action of PG. Importantly, because ET-1 did not alter AT_{1A}R mRNA expression (Fig. 4A), these results suggest that ET-1 engages this pathway primarily by increasing local AngII availability and thereby activating pre-existing AT_{1A}R, rather than by upregulating AT_{1A}R expression.

PG inhibits AngII-induced Ca²⁺ signaling in AT_{1A}R-overexpressing NRVMs

To determine whether PG modulates AngII–AT_{1A}R signaling, we first characterized the functional impact of PG on AngII-induced cardiomyocyte hypertrophy. Direct application of exogenous AngII (100 nM) induced a hypertrophic response in NRVMs, as evidenced by a significant increase in CSA (Fig. 5A). Notably, pretreatment with PG completely abolished this AngII-induced hypertrophy (Fig. 5A). Importantly, under the same experimental conditions, PG significantly attenuated the AngII-induced decrease in NRVM viability and cell cytotoxicity (Supplementary Fig. S5). In our previous study, PG

alone did not adversely affect cardiomyocyte viability under the experimental conditions examined (Tagashira et al., 2025). Together, these findings argue against nonspecific cytotoxicity as the basis for the anti-hypertrophic effect of PG. Moreover, PG suppressed AngII-induced hypertrophy in a concentration-dependent manner, with an IC_{50} of 10.0 μ g/ml and a Hill coefficient of 2.1 determined from the concentration–inhibition curve (Fig. 5B). Next, to elucidate the precise molecular events immediately downstream of $AT_{1A}R$ activation, we examined AngII-induced intracellular Ca^{2+} accumulation using Fluo-4 fluorescence. Because basal $AT_{1A}R$ expression levels are low in native cardiomyocytes, cDNAs encoding rat $AT_{1A}R$ were transfected into NRVMs. The successful overexpression of $AT_{1A}R$ mRNA in transfected NRVMs was confirmed by both RT-PCR and real-time PCR analyses (Figs. 5C, D; uncropped RT-PCR gel images are provided in Supplementary Fig. S6). Consistent with the increased mRNA expression, Western blot analysis demonstrated a significant increase in $AT_{1A}R$ protein expression in $AT_{1A}R$ -transfected NRVMs compared with non-transfected control cells (Figs. 5E, F), confirming successful $AT_{1A}R$ overexpression at both the mRNA and protein levels. We then examined the effects of AngII and PG on intracellular Ca^{2+} levels in both native and $AT_{1A}R$ -overexpressing NRVMs (Fig. 5G). In native NRVMs without $AT_{1A}R$ overexpression, AngII significantly increased intracellular Ca^{2+} levels, and this increase was significantly attenuated by PG. $AT_{1A}R$ overexpression itself was associated with a modest but significant elevation in basal intracellular Ca^{2+} levels compared with non-transfected control cells. AngII further enhanced intracellular Ca^{2+} accumulation in $AT_{1A}R$ -overexpressing NRVMs, whereas PG significantly suppressed this response. These findings indicate that PG attenuates AngII-induced Ca^{2+} signaling in both native NRVMs and under conditions of enhanced $AT_{1A}R$ expression.

PG inhibits AngII-induced signaling in $AT_{1A}R$ -expressing cells

Before electrophysiological analysis, protein expression from the $AT_{1A}R$ construct used in the present study was confirmed by Western blot analysis in transfected HEK293T cells. $AT_{1A}R$ protein was clearly detected in cells transfected with the $AT_{1A}R$ expression plasmid (Supplementary Fig. S7), confirming that the construct used in the functional experiments produces $AT_{1A}R$ protein. Next, to evaluate the electrophysiological impact of PG on downstream signaling, we examined AngII-induced membrane currents in HEK293T cells co-expressing $AT_{1A}R$ and TRPC6 using whole-cell patch-clamp recordings. TRPC6-mediated currents were used as a functional electrophysiological readout of $AT_{1A}R$ activation. Representative current traces are shown in Figs. 6A and B. Application of AngII induced a marked increase in membrane current amplitude in

AT₁AR/TRPC6-expressing cells (Fig. 6A). In contrast, pretreatment with PG substantially attenuated the AngII-induced current response (Fig. 6B). Current–voltage (I–V) analysis revealed that AngII markedly enhanced both inward and outward currents across the tested voltage range compared with control cells (Fig. 6C). This effect was largely suppressed by PG pretreatment (Fig. 6D). Quantitative analysis demonstrated that AngII significantly increased current densities at both –100 mV and +100 mV, whereas PG markedly reduced these responses (Fig. 6E). To determine whether PG directly affects basal TRPC6 channel activity, membrane currents were examined in the absence of AngII. As shown in Fig. 6F, PG alone had little effect on basal current–voltage relationships. Consistent with these observations, quantitative analysis revealed no significant differences in basal current amplitudes between control and PG-treated cells at either –100 mV or +100 mV (Fig. 6G). Taken together, these findings indicate that PG selectively suppresses AngII-induced AT₁AR-dependent electrophysiological signaling without affecting basal membrane currents. These results support the hypothesis that the anti-hypertrophic effects of PG are mediated, at least in part, through inhibition of the AngII–AT₁AR signaling axis.

Discussion

In the present study, we investigated the molecular mechanism underlying the anti-hypertrophic effects of *Panax ginseng* (PG) in cardiomyocytes. Our major findings demonstrate that: (i) PG reproducibly attenuates ET-1-induced cardiomyocyte hypertrophy (Fig. 1) without altering ET_AR or ET_BR expression (Fig. 2A), (ii) ET-1 stimulation increases extracellular AngII production, which is suppressed by PG (Fig. 3), and pharmacological experiments support the involvement of the AngII–AT₁A_R pathway in the anti-hypertrophic action of PG (Fig. 4); and (iii) PG suppresses acute AT₁A_R-dependent Ca²⁺ signaling and downstream membrane currents (Figs. 5 and 6). Collectively, these findings identify suppression of the AngII–AT₁A_R signaling axis as a previously unrecognized mechanism underlying the cardioprotective effects of PG.

While our previous work demonstrated that PG attenuates ET-1-induced cardiomyocyte hypertrophy and mitochondrial dysfunction in vitro and exerts cardioprotective effects in an isoproterenol-induced in vivo model (Tagashira et al., 2025), the precise molecular mechanism remained unclear. The present study substantially extends these findings by providing a mechanistic explanation: PG modulates a critical upstream neurohumoral pathway rather than merely exerting downstream phenotypic effects.

ET-1 is one of the most potent endogenous inducers of cardiomyocyte hypertrophy and has been implicated in the progression of heart failure and pathological cardiac remodeling (Ito et al., 1991, Yamazaki et al., 1996, Drawnel et al., 2013, Archer et al., 2017). Although endothelin receptor signaling clearly contributed to ET-1-induced hypertrophy in our model, PG did not significantly alter ET_AR or ET_BR expression (Fig. 2A). Moreover, the inhibitory effects observed with PG and the ET_AR antagonist BQ-123 were consistent with PG exerting its anti-hypertrophic action through mechanisms not explained solely by direct modulation of endothelin receptor signaling. These observations prompted us to investigate alternative downstream or parallel mediators linking ET-1 stimulation to cardiomyocyte hypertrophy.

This missing link was resolved by our finding that ET-1 stimulation robustly increased extracellular AngII levels in cultured cardiomyocytes (Fig. 3). Previous pioneering work by Ito et al. (Ito et al., 1993) established that AngII can induce ET-1 production, thereby contributing to hypertrophy via an autocrine/paracrine mechanism. However, the current study uncovers the reverse axis of this neurohumoral relationship, demonstrating that ET-1 reciprocally drives the synthesis and secretion of AngII in a cardiomyocyte-autonomous manner. When integrated with previous evidence that AngII can induce ET-1 production (Ito et al., 1993), our findings support the existence of reciprocal cross-talk between ET-

1 and AngII during cardiac remodeling. Importantly, PG significantly attenuated ET-1-induced AngII accumulation (Fig. 3A), suggesting that disruption of this neurohumoral interaction may contribute to its anti-hypertrophic effect.

The additional analyses provide further insight into the mechanism by which PG suppresses ET-1-induced extracellular AngII accumulation. ET-1 markedly increased the expression of *Agt*, *Ace*, and *Ace2*, whereas PG significantly attenuated these transcriptional responses (Fig. 3B). In particular, several ginsenosides have been shown to directly inhibit ACE enzymatic activity, with protopanaxatriol, protopanaxadiol, and ginsenoside Rh2 exhibiting particularly potent inhibitory effects (Ali et al., 2021). In addition, ginsenoside Rc has been reported to reduce AngII levels while upregulating the ACE2/Ang-(1–7)/Mas axis (Wang et al., 2021). Although these transcriptional changes provide a potential explanation for the reduced AngII accumulation, the present experiments did not assess ACE or ACE2 enzymatic activity or downstream angiotensin peptides. Therefore, the functional contribution of these changes to AngII synthesis and metabolism remains to be established. Further studies examining ACE/ACE2 enzymatic activity and downstream angiotensin peptides will be required to define the precise contribution of these pathways to the PG-mediated reduction in AngII.

This dual neurohumoral connection highlights that the pathogenesis of ET-1-induced hypertrophy is intrinsically intertwined with activation of the local AngII–AT_{1A}R axis. Crucially, our findings elevate our understanding of the cardioprotective scope of PG beyond simple upstream intervention. While PG successfully blunts ET-1-evoked AngII synthesis (Fig. 3), this production-level blockade does not fully account for its overall therapeutic efficacy, as PG retains its robust anti-hypertrophic capacity even against a direct exogenous AngII challenge (Figs. 5A, B). This persistence supports the concept that PG exerts protective effects at two levels: suppression of ET-1-induced AngII production and attenuation of downstream AngII-dependent signaling. This dual-action profile suggests that PG not only reduces mediator availability but also attenuates cellular responsiveness to AngII.

To further investigate this possibility, we used an AT_{1A}R-expressing system to examine acute functional responses to AngII. Our functional and electrophysiological analyses revealed that PG significantly attenuated both AngII-induced intracellular Ca²⁺ elevation (Fig. 5G) and TRPC6-mediated membrane currents (Figs. 6A, B). It should be noted that AT_{1A}R overexpression itself was associated with an elevated basal intracellular Ca²⁺ level. Constitutive activity of AT₁R has been demonstrated in recombinant expression systems, and ligand-independent AT₁R signaling is a recognized property of this receptor (Unal

and Karnik, 2014, Takezako et al., 2017). Thus, the elevated basal Ca^{2+} level observed in $\text{AT}_{1\text{A}}\text{R}$ -overexpressing NRVMs may reflect enhanced basal $\text{AT}_{1\text{A}}\text{R}$ -dependent signaling. Nevertheless, AngII induced a significant further increase in intracellular Ca^{2+} , indicating that the overexpressed receptors remained responsive to ligand stimulation. Accordingly, although the elevated baseline should be considered when interpreting the magnitude of the AngII-induced response, it does not preclude assessment of AngII-evoked Ca^{2+} signaling or its inhibition by PG. Critically, because these inhibitory effects were observed almost immediately (within 1 min) after PG application during acute AngII stimulation (Figs. 6A, B). These rapid inhibitory effects indicate that PG attenuates acute $\text{AT}_{1\text{A}}\text{R}$ -dependent signaling rather than acting solely through distal transcriptional mechanisms. Although TRPC6-mediated currents were used as an electrophysiological readout of $\text{AT}_{1\text{A}}\text{R}$ activation, the fact that PG did not affect basal TRPC6 activity implies that TRPC6 is not the direct target (Figs. 6F, G). Instead, our electrophysiological data provide functional evidence that PG suppresses signaling downstream of $\text{AT}_{1\text{A}}\text{R}$ activation. However, these experiments do not establish whether PG or its constituents directly interact with $\text{AT}_{1\text{A}}\text{R}$ or other receptor-proximal signaling molecules. These results therefore support the conclusion that suppression of the AngII– $\text{AT}_{1\text{A}}\text{R}$ signaling axis represents an important component of the anti-hypertrophic action of PG.

The cardiovascular benefits of PG and its constituent ginsenosides have been widely documented to mitigate oxidative stress, inflammation, and mitochondrial dysfunction in various cardiac models (Kim, 2018, He et al., 2022, Hyun et al., 2022). For instance, specific ginsenosides such as Rg1 and F2 protect against myocardial injury and hypertrophy by mobilizing critical downstream cascades, including AMPK, Nrf2/HO-1, and PI3K/Akt pathways (Qin et al., 2019, Cui et al., 2024). The present findings add a new dimension to these established paradigms by identifying suppression of the ET-1–AngII– $\text{AT}_{1\text{A}}\text{R}$ signaling network as an additional upstream mechanism contributing to the cardioprotective actions of PG.

Several considerations regarding our experimental model should be noted. First, while our electrophysiological and functional analyses demonstrate that PG suppresses $\text{AT}_{1\text{A}}\text{R}$ -dependent signaling, the precise molecular interaction responsible for this effect remains to be determined. Although these findings indicate that PG suppresses $\text{AT}_{1\text{A}}\text{R}$ -dependent signaling, they do not establish a direct physical interaction between PG constituents and $\text{AT}_{1\text{A}}\text{R}$. Whether one or more bioactive constituents of PG directly bind to $\text{AT}_{1\text{A}}\text{R}$ will require further investigation using receptor-binding approaches, such as radioligand or fluorescent competition-binding assays. In addition, because PG is a complex herbal mixture containing numerous bioactive constituents, the specific ginsenoside(s) or

combinations responsible for the observed inhibition of AT₁AR-dependent signaling remain to be identified. Notably, the concentration-dependent inhibition of AngII-induced hypertrophy by PG exhibited a relatively steep Hill slope of 2.1 (Fig. 5B). Although a Hill slope greater than 1 may indicate positive cooperativity in a simple ligand–receptor system, such an interpretation cannot be directly applied to a complex herbal extract such as PG; rather, the observed slope may reflect integrated actions of multiple constituents and/or nonlinear interactions among hypertrophic signaling pathways. The PG preparation used throughout the present study was derived from a single defined lot (Lot No. 021723) of dried (“Kiboshi”) *Panax ginseng* C.A. Meyer root powder, with detailed product and phytochemical information provided in Supplementary Table S1 and Fig. S1. Because ginseng processing can influence its phytochemical composition and pharmacological properties, whether differently processed *P. ginseng* preparations exert comparable effects on the ET-1–AngII–AT₁AR signaling axis remains to be determined. Although the use of a defined lot allowed the same PG preparation to be used consistently across the experiments performed in this study, multiple independent lots were not directly compared; therefore, batch-to-batch reproducibility remains to be established. Identification of these constituent(s), together with validation using multiple independent lots, represents an important direction for future chemical separation and pharmacological studies. Second, the electrophysiological experiments were not designed to determine the contribution of TRPC6 to ET-1-induced cardiomyocyte hypertrophy. TRPC channels, including TRPC1, TRPC3, and TRPC6, have been implicated in pathological cardiac hypertrophy through both receptor-operated and store-operated Ca²⁺ entry pathways (Rubaiy, 2025). Importantly, functional coupling of AT₁R activation to TRPC3/TRPC6 channels through Gq/PLC-dependent signaling has been previously demonstrated, providing a mechanistic basis for the use of TRPC6-mediated currents as a downstream functional readout of AT₁AR activation in our heterologous expression system (Onohara et al., 2006). However, in the present study, TRPC6-mediated currents were employed solely as a functional readout of AT₁AR-dependent signaling in a heterologous expression system. Therefore, the current findings should not be interpreted as evidence that TRPC6 is the principal Ca²⁺ entry pathway mediating AngII-induced Ca²⁺ accumulation or hypertrophic remodeling in NRVMs. Other Ca²⁺ entry pathways, particularly voltage-dependent Ca²⁺ channels such as Cav1.2/L-type Ca²⁺ channels, are important regulators of Ca²⁺ homeostasis in cardiomyocytes and may also contribute to AngII-induced hypertrophic signaling. Indeed, our previous studies in NRVMs have demonstrated the involvement of voltage-dependent Ca²⁺ entry in AngII-induced cardiomyocyte hypertrophy (Tagashira et al., 2024). The present experiments were not designed to

distinguish the relative contributions of TRPC6, Cav1.2, or other Ca^{2+} -handling pathways to the hypertrophic response. Rather, TRPC6-mediated currents were used as an independent functional readout of $\text{AT}_{1\text{A}}\text{R}$ -dependent signaling in the heterologous expression system. Thus, our electrophysiological findings demonstrate that PG suppresses $\text{AT}_{1\text{A}}\text{R}$ -dependent signaling but do not establish TRPC6 as the specific Ca^{2+} channel responsible for the anti-hypertrophic effect of PG in NRVMs. Third, although the current mechanistic exploration was conducted primarily using cultured cardiomyocytes and heterologous expression systems to achieve precise molecular control, the cardioprotective efficacy of PG in vivo was demonstrated in our previous study using an isoproterenol-induced cardiac hypertrophy model (Tagashira et al., 2025). The present study therefore extends these previous observations by providing mechanistic insight into the signaling pathways underlying the cardioprotective effects of PG. It should also be recognized that the cardiomyocyte-level effects identified in the present study do not necessarily predict the systemic hemodynamic actions of PG. Although ginseng has traditionally been considered to exert tonic or pressor effects, its influence on blood pressure is now recognized to be complex and context-dependent, consistent with its proposed adaptogenic and vasomodulatory properties (Irfan et al., 2020). Systemic blood pressure reflects integrated vascular, autonomic, renal, and neurohumoral regulation, whereas the present study specifically addresses the effects of PG on ET-1–AngII– $\text{AT}_{1\text{A}}\text{R}$ signaling in cardiomyocytes. Therefore, suppression of this signaling axis should be interpreted as a cardiomyocyte-level mechanism rather than as evidence for a specific systemic blood pressure response. Taken together, the present findings support a model in which PG interrupts the ET-1–AngII– $\text{AT}_{1\text{A}}\text{R}$ signaling cascade at two complementary levels by suppressing ET-1-induced AngII production and attenuating $\text{AT}_{1\text{A}}\text{R}$ -dependent signaling, thereby preventing intracellular Ca^{2+} accumulation and the development of cardiomyocyte hypertrophy. This proposed mechanism integrating the present findings is summarized in Fig. 7.

In conclusion, the present study demonstrates that PG suppresses ET-1-induced cardiomyocyte hypertrophy through inhibition of the AngII– $\text{AT}_{1\text{A}}\text{R}$ receptor signaling axis. PG attenuated ET-1-induced AngII production (Fig. 3), inhibited AngII-mediated hypertrophic signaling (Figs. 5A, B), and suppressed $\text{AT}_{1\text{A}}\text{R}$ -dependent functional responses (Figs. 5G, 6). These findings identify a novel molecular mechanism underlying the cardioprotective effects of PG and provide new insight into the therapeutic potential of PG for pathological cardiac remodeling and heart failure.

Data availability

The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

Abbreviations

AMPK, adenosine monophosphate-activated protein kinase; AngII, angiotensin II; ANOVA, analysis of variance; AT₁R, angiotensin II type 1 receptor; AT_{1A}R, angiotensin II type 1A receptor; AT_{1B}R, angiotensin II type 1B receptor; CSA, cross-sectional area; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; DNA, deoxyribonucleic acid; ELISA, enzyme-linked immunosorbent assay; ET-1, endothelin-1; ET_A, endothelin type A; ET_AR, endothelin type A receptor; ET_BR, endothelin type B receptor; FBS, fetal bovine serum; HO-1, heme oxygenase-1; I–V, current–voltage; ISO, isoproterenol; LSR, losartan; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa-B; NFAT, nuclear factor of activated T-cells; NHE-1, sodium–hydrogen exchanger 1; NLRP3, NOD-like receptor protein 3; Nrf2, nuclear factor erythroid 2-related factor 2; NRVMs, neonatal rat ventricular myocytes; PCR, polymerase chain reaction; PG, *Panax ginseng*; PI3K, phosphoinositide 3-kinase; ROS, reactive oxygen species; RT-PCR, reverse transcription polymerase chain reaction; SEM, standard error of the mean; SIRT1, sirtuin 1; TRPC6, transient receptor potential canonical 6; Veh, vehicle

Figure Legends

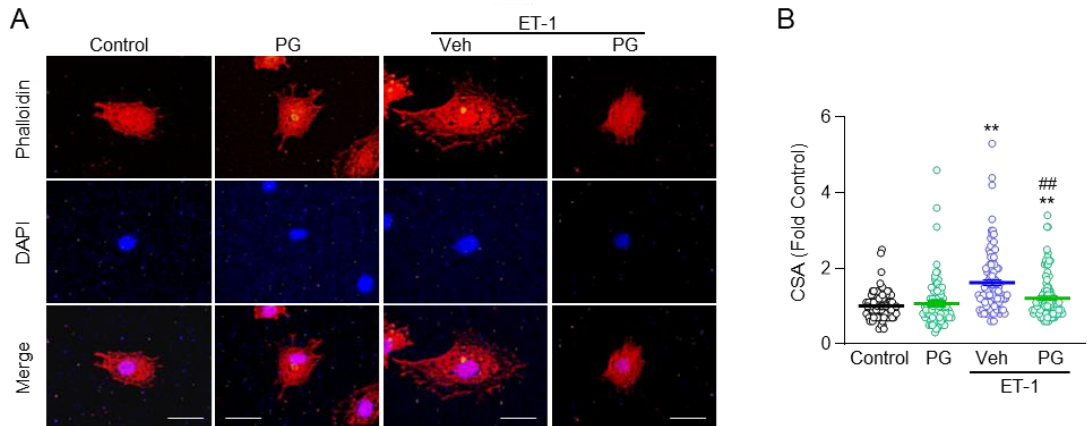


Fig. 1 Inhibitory effect of PG on ET-1-induced cardiomyocyte hypertrophy in NRVMs. (A) Representative fluorescence images of NRVMs stained with rhodamine-conjugated phalloidin (red) to visualize F-actin and counterstained with DAPI (blue) to visualize nuclei. Cells were treated with vehicle alone (Control), PG alone, ET-1 plus vehicle (ET-1 + Veh), or ET-1 plus PG (ET-1 + PG). Scale bar = 50 μ m. (B) Quantification of cell cross-sectional area (CSA) calculated from the rhodamine-conjugated phalloidin-stained images in (A) (n = 103–108 cells/group; n represents the number of individual cells analyzed rather than the number of independent NRVM preparations; cells were sampled from at least five culture dishes). Data were analyzed by one-way ANOVA followed by a Tukey's post hoc test. **P < 0.01 vs. Control; ##P < 0.01 vs. ET-1 + Veh.

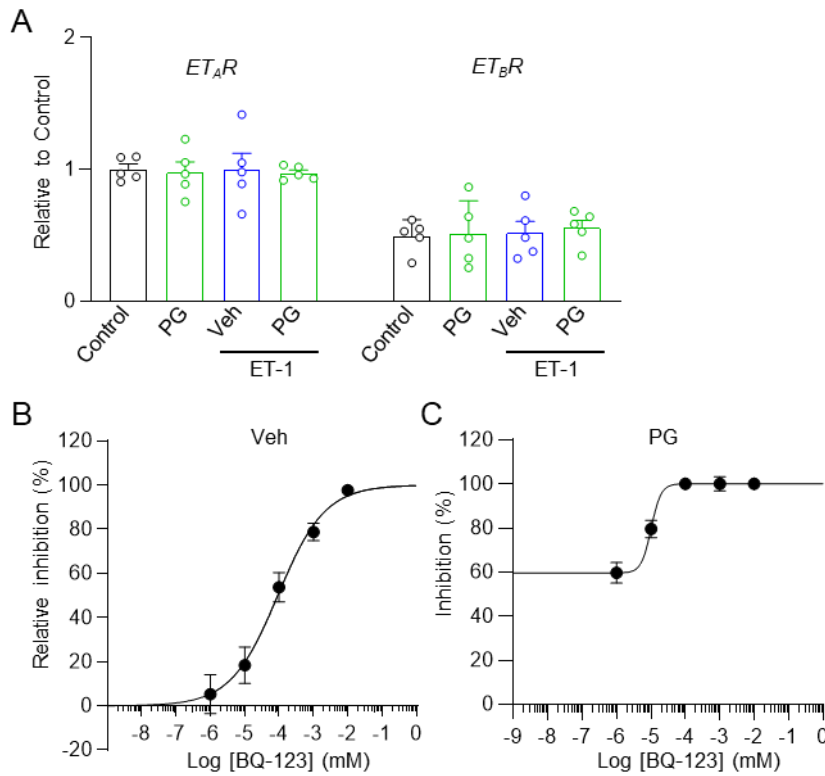


Fig. 2 Effects of PG on endothelin receptor expression and concentration–inhibition curves of BQ-123. (A) Real-time PCR analysis of *ET_AR* and *ET_BR* mRNA expression levels in NRVMs treated with vehicle alone (Control), PG alone, ET-1 plus vehicle (ET-1 + Veh), or ET-1 plus PG (ET-1 + PG). Data are expressed as mean $\pm$ SEM from 5 independent experiments. (B, C) Concentration–inhibition curves of the ET receptor antagonist BQ-123 in the presence of vehicle (B, Veh) or 10 μ g/ml PG (C, PG). The vertical axes indicate relative inhibition (%) or inhibition (%), plotted against the logarithmic concentration of BQ-123 (mM). The goodness of fit for the concentration–inhibition curves in (B) and (C) was $R^2 = 0.9961$ and 0.9909 , respectively. Data points represent mean $\pm$ SEM ($n = 5$).

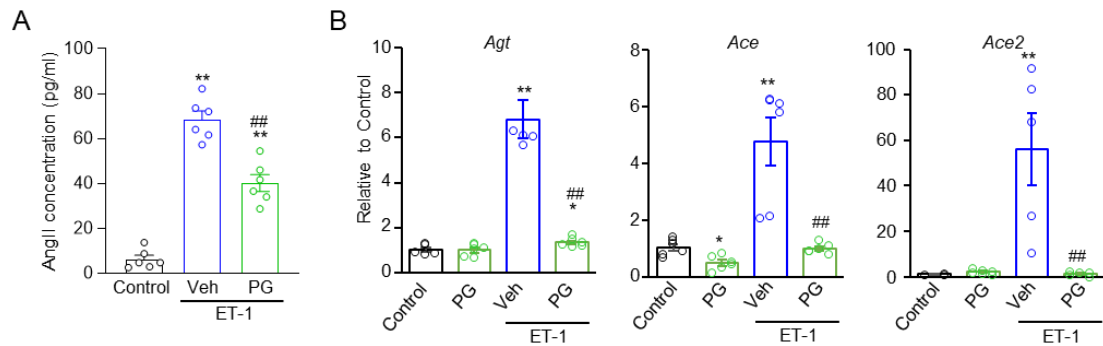


Fig. 3 Effects of PG on ET-1-induced AngII production and expression of local renin-angiotensin system-related genes. (A) Extracellular AngII concentration (pg/ml) in NRVMs treated with vehicle alone (Control), PG alone (PG), ET-1 plus vehicle (ET-1 + Veh), or ET-1 plus PG (ET-1 + PG). (B) Real-time PCR analysis of *Agt*, *Ace*, and *Ace2* mRNA expression in NRVMs under the same experimental conditions. Gene expression levels are expressed relative to the Control group. Data are presented as mean $\pm$ SEM (n = 6). Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. *P < 0.05, **P < 0.01 vs. Control; ##P < 0.01 vs. ET-1 + Veh.

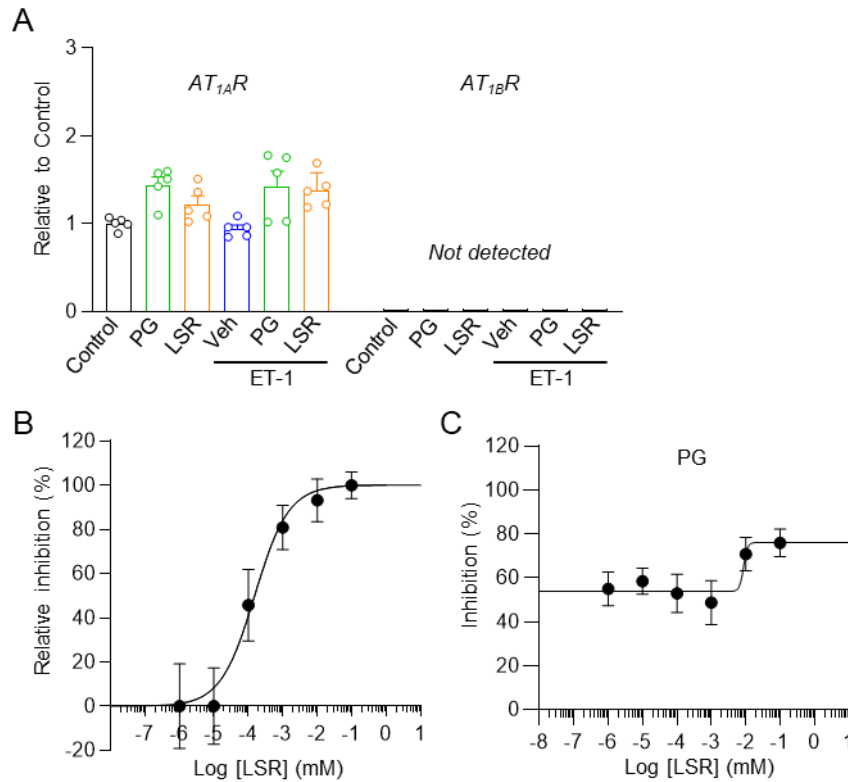


Fig. 4 Effects of PG on angiotensin II receptor mRNA expression and losartan inhibition curves. (A) Real-time PCR analysis of *AT_{1A}R* and *AT_{1B}R* mRNA expression levels in NRVMs treated with vehicle alone (Control), PG alone, losartan alone (LSR), ET-1 plus vehicle (ET-1 + Veh), ET-1 plus PG (ET-1 + PG), or ET-1 plus LSR (ET-1 + LSR). Data are expressed as mean $\pm$ SEM (n = 5). Note that *AT_{1B}R* mRNA was not detected under any conditions. (B, C) Concentration–inhibition curves of the AT₁R antagonist losartan (LSR) against ET-1-induced cardiomyocyte hypertrophy in the presence of vehicle (B, Veh) or 10 μ g/ml PG (C, PG). The vertical axes indicate the percentage of inhibition plotted against the logarithmic concentration of LSR (mM). The goodness of fit for the concentration–inhibition curves in (B) and (C) was $R^2 = 0.9919$ and 0.9903 , respectively. Data points represent mean $\pm$ SEM (n = 5).

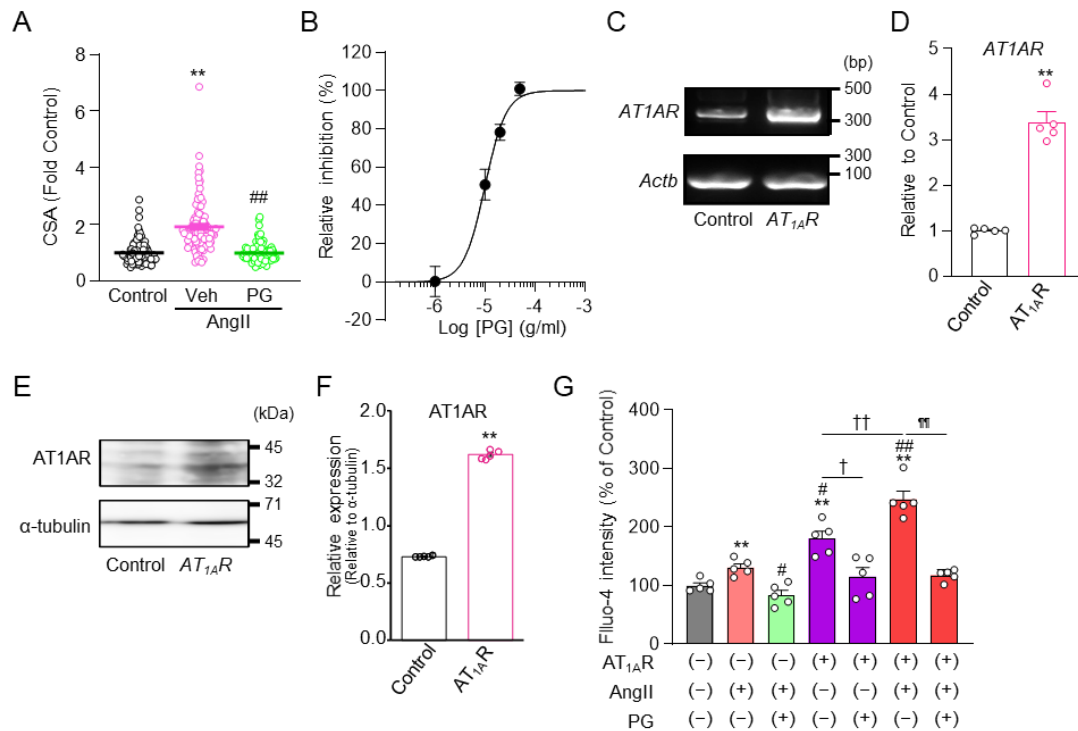
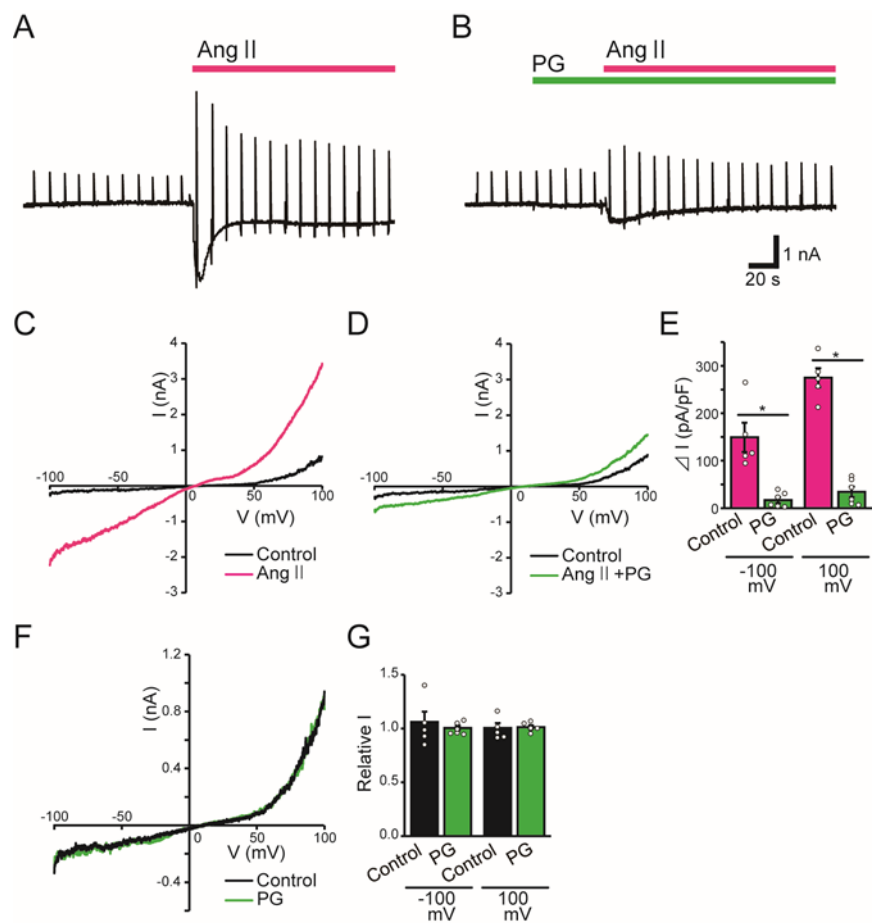


Fig. 5 Effects of PG on AngII-induced hypertrophy and intracellular Ca^{2+} accumulation in $\text{AT}_{1\text{A}}\text{R}$ -overexpressing NRVMs. (A) Quantification of CSA showing the inhibitory effect of PG on AngII-induced cardiomyocyte hypertrophy in NRVMs treated with vehicle alone (Control), AngII plus vehicle (AngII + Veh), or AngII plus PG (AngII + PG). Data are expressed as fold changes relative to the control group ($n = 103$ – 110 cells/group; n represents the number of individual cells analyzed rather than the number of independent NRVM preparations; cells were sampled from at least five culture dishes). $**P < 0.01$ versus Control; $##P < 0.01$ vs. AngII+Veh. (B) Concentration–inhibition curve of PG against AngII-induced cardiomyocyte hypertrophy. The vertical axis indicates relative inhibition (%) plotted against the logarithmic concentration of PG (g/ml) ($n = 102$ – 110 cells/concentration; n represents the number of individual cells analyzed rather than the number of independent NRVM preparations; cells were sampled from at least five culture dishes). The goodness of fit for the concentration–inhibition curve was $R^2 = 0.9954$. (C) Representative agarose gel electrophoresis image of RT-PCR products showing $\text{AT}_{1\text{A}}\text{R}$ (estimated band size: 306 bp) and β -actin (Actb) (estimated band size: 77 bp) mRNA levels in non-transfected control and $\text{AT}_{1\text{A}}\text{R}$ -transfected cells. (D) Real-time PCR analysis confirming the increased expression of $\text{AT}_{1\text{A}}\text{R}$ mRNA in $\text{AT}_{1\text{A}}\text{R}$ -transfected cells compared with non-transfected control cells. Data are expressed as mean $\pm$ SEM ($n = 5$). $*P < 0.01$ vs. Control. (E) Representative Western blot showing AT1AR protein expression in non-transfected control and $\text{AT}_{1\text{A}}\text{R}$ -transfected NRVMs. α -tubulin was used

as a loading control. (F) Quantification of AT₁AR protein expression normalized to α -tubulin. Data are expressed as mean $\pm$ SEM (n = 5). **P < 0.01 vs. Control. (G) Quantitative analysis of intracellular Ca²⁺-dependent Fluo-4 fluorescence measured using a microplate reader and expressed as a percentage of Control. NRVMs without AT₁AR overexpression were treated with AngII in the absence or presence of PG to evaluate the effect of PG on AngII-induced Ca²⁺ accumulation under native conditions. In parallel, AT₁AR-overexpressing NRVMs were treated with AngII and/or PG as indicated. Data are expressed as mean $\pm$ SEM (n = 5). Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. *P < 0.01 vs. AT₁AR(-)/AngII(-)/PG(-); #P < 0.05 and ###P < 0.01 vs. AT₁AR(+)/AngII(-)/PG(-); †P < 0.05 and ††P < 0.01 between the indicated groups; †††P < 0.01 between the indicated groups.

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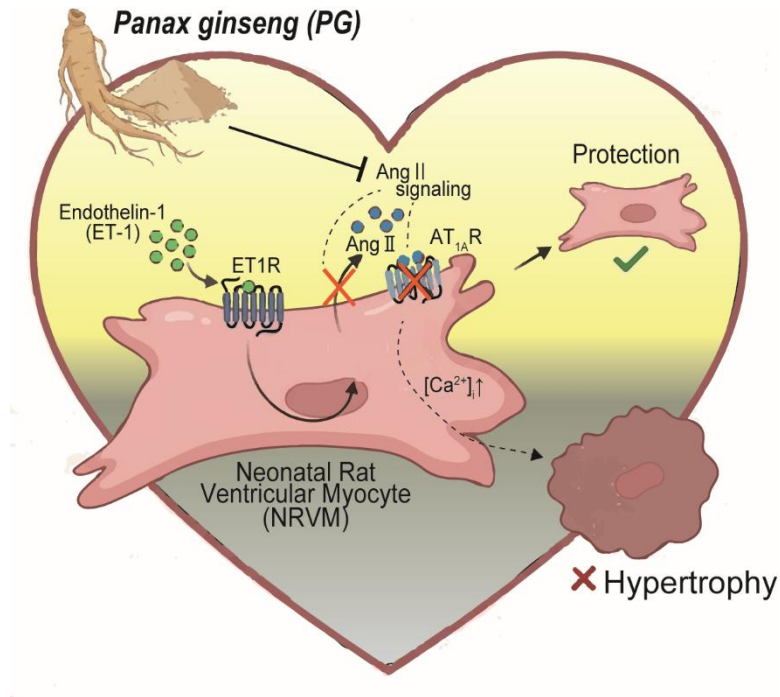


759

760 **Fig. 6** Inhibitory effect of PG on AngII-induced whole-cell currents. (A, B)
761 Representative whole-cell current traces recorded from AT₁AR and TRPC6 expressing
762 HEK293T cells. Upward and downward spikes indicate currents evoked by voltage ramps
763 from -100 to +100 mV. Cells were stimulated with 1 μ M AngII alone (A) or pretreated
764 with 50 μ g/ml PG before AngII stimulation (B). (C, D) Representative current-voltage
765 (I-V) curves obtained during voltage ramps under Control conditions and after AngII
766 stimulation in the absence (C) or presence (D) of PG. (E) Statistical analysis of AngII-
767 induced changes in current density (ΔI (pA/pF)) at -100 and +100 mV in the absence
768 (Control) or presence of PG (n = 5-6). *P < 0.05 between the indicated groups. (F)
769 Representative I-V curves under Control conditions and after treatment with PG alone.
770 (G) Statistical analysis of relative basal current amplitudes at -100 and +100 mV after
771 treatment with PG (n = 5-6). Data are expressed as mean $\pm$ SEM.

772

773



774

775 **Fig. 7** Proposed mechanism underlying the anti-hypertrophic effects of *Panax ginseng*.
 776 ET-1 stimulates cardiomyocyte hypertrophy through activation of the local AngII–AT₁R
 777 signaling pathway. ET-1 promotes extracellular AngII production, leading to AT₁R
 778 activation, intracellular Ca²⁺ accumulation, and downstream hypertrophic signaling. PG
 779 suppresses both ET-1-induced AngII production and AT₁R-dependent signaling, thereby
 780 attenuating Ca²⁺ overload and cardiomyocyte hypertrophy. Red inhibitory lines indicate
 781 the proposed points of pathway modulation by PG based on the findings of the present
 782 study.

783

Table 1 Primer sequences used for RT-PCR and real-time PCR analyses.

Gene (rat)	Forward primer (5'→3')	Reverse primer (5'→3')	Amplicon (bp)
ET _A R	TTCGTCATGGTACCCTTCGA	GATACTCGTTCCATTTCATGG	546
ET _B R	AGCTGGTGCCCTTCATACAGAAGGC	TGCACACCTTTCCGCAAGCACG	919
AT _{1A} R	CGTCATCCATGACTGTAAAATTTTC	GGGCATTACATTGCCAGTGTG	306
AT _{1B} R	CATTATCCGTGACTGTGAAATTG	GCTGCTTAGCCCAAATGGTCC	345
Agt	CCCTGAGCAGTCCGTTTCCT	AAAGTGCAGCGCACCTGAGT	59
Ace	TTTGCTACACAAATGGCACTTGT	CGGGACGTGGCCATTATATT	67
Ace2	TTGAACCAGGATTGGACGAAA	GCCCAGAGCCTACGATTGTAGT	61
Actb (β-actin)	ACTATCGGCAATGAGCGGTTC	ATGCCACAGGATTCCATACCC	77
18S rRNA	GTAGTCGCCGTGCCTACCAT	TCCGGAATCGAACCCTGA	60

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Author information

Authors and Affiliations

Department of Integrative Physiology, Graduate School of Medicine, Akita University, 1-1-1 Hondo, Akita 010-8543, Japan

Tomohiro Numata, Hideaki Tagashira, Kaori Sato-Numata, Ayako Sakai & Ha Nam Tran

Second Department of Pharmacology, School of Pharmaceutical Sciences, Kyushu University of Medical Science, 1714-1 Yoshino-cho, Nobeoka, Miyazaki 882-8508, Japan

Hideaki Tagashira

School of Medicine, Akita University, 1-1-1 Hondo, Akita 010-8543, Japan

Ryo Takayama & Aoto Aoyama

Faculty of Fisheries, University of Agriculture and Forestry, Hue University, 102 Phung Hung Street, Hue 530000, Vietnam

Ha Nam Tran

Department of Cardiovascular Medicine, Graduate School of Medicine, Akita University, 1-1-1 Hondo, Akita 010-8543, Japan

Hiroyuki Watanabe

Contributions

TN: conceptualization, methodology, formal analysis, investigation, visualization, writing – original draft, writing – review & editing, supervision, project administration, funding acquisition. HT: methodology, formal analysis, investigation, visualization, funding acquisition. KSN: investigation, formal analysis, funding acquisition. RT: investigation, formal analysis. AA: investigation, formal analysis. AS: investigation, formal analysis. HNT: investigation, formal analysis. HW: investigation, formal analysis, writing – review & editing. All authors read and approved the final manuscript. The authors declare that all data were generated in-house, that no paper mill was used, and that no AI tool was used for the generation of text or figures.

Corresponding author

Correspondence should be addressed to Tomohiro Numata.

Ethics declarations

Ethics approval

All animal experiments were approved by the Animal Care and Use Committee of Akita University (Approval Nos. a-1-0412 and b-1-0408) and were performed in accordance with the institutional guidelines for the care and use of laboratory animals.

Competing interests

The authors declare no competing interests.

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Use of artificial intelligence tools

The authors did not use generative artificial intelligence tools in the preparation of this manuscript.