Evaluation of Punicalagin as a multi-targeted therapeutic agent against endometrial cancer

ZEESHAN AHMAD BHUTTA[1,2*], DOHEE AHN[1*], HONG KYU LEE[3] and KYUNG-CHUL CHOI[1]

[1]Laboratory of Biochemistry and Immunology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungcheongbuk 28644, Republic of Korea; [2]State Key Laboratory of Molecular Vaccinology and Molecular, Diagnostics and Center for Molecular Imaging and Translational Medicine, School of Public Health, Xiamen University, Xiamen, Fujian 361002, P.R. China; [3]Department of Companion Animal Health, College of Biomedical Science and Health, Inje University, Gyeongsangnam 50834, Republic of Korea

Received December 22, 2025; Accepted July 1, 2026

DOI: 10.3892/ijo.2026.5922

Abstract. Endometrial cancer (EC) has become an increasing clinical concern as the incidence is rising, and treatment options available for advanced disease or recurrent disease are limited. In the present study, the anticancer potential of punicalagin (PCG), a natural ellagitannin polyphenol that comes from pomegranate, was characterized using both *in vitro* and *in vivo* models in EC. Two EC cell lines (Ishikawa and SNU-539) treated with increasing doses of PCG showed dose-dependent inhibition of cell proliferation and demonstrated a decrease in colony formation. PCG inhibited Transwell migration and an increase in E-cadherin expression, indicating an inhibition of epithelial-mesenchymal transition. Further experimental work characterized the mechanisms by which PCG acted and revealed that it decreased the mitochondrial membrane potential and subsequently increased levels of reactive oxygen species, which led to apoptosis as shown by increased BAX expression and Hoechst/PI staining. In addition, PCG showed signs of autophagy, especially in the Ishikawa cells, as indicated by increased levels of LC3-IIB and the formation of autophagic vacuoles. *In vivo* studies using a xenograft mouse model showed that treatment with PCG significantly reduced tumor volume and weight, whereas body weight was not significantly affected, thus highlighting strong anticancer efficacy coupled with very low toxicity. Overall, the present study highlights PCG as a promising natural compound with multitarget anticancer activity against EC and warrants further preclinical and clinical research as a potential treatment option.

Correspondence to: Professor Kyung-Chul Choi, Laboratory of Biochemistry and Immunology, College of Veterinary Medicine, Chungbuk National University, 1 Chungdae, Seowon, Cheongju, Chungcheongbuk 28644, Republic of Korea
E-mail: kchoi@cbu.ac.kr

Dr Hong Kyu Lee, Department of Companion Animal Health, College of Biomedical Science and Health, Inje University, 197 Inje, Gimhae, Gyeongsangnam 50834, Republic of Korea
E-mail: hklee83@inje.ac.kr

*Contributed equally

Key words: endometrial cancer, carcinoma, apoptosis, autophagy, punicalagin

Introduction

Endometrial cancer (EC) is a major global health issue, with ~420,368 new cases diagnosed globally as of 2022 (1,2). The American Cancer Society reports ~68,270 new cases of uterine cancer will be diagnosed in 2026, resulting in ~14,450 expected deaths due to uterine cancers (3). The incidence of EC has increased by more than 1% per year since the mid-2000s, particularly among non-White racial and ethnic women (4,5).

The etiology of EC involves a complicated spectrum of molecular biology, including oncogenic mutations in tumor suppressor genes (PTEN, TP53 and ARID1A), activation of oncogenes (KRAS, CTNNB1 and HER2/neu), defects in DNA mismatch repair pathways, aberrations in hormonal signaling, epigenetic changes and dysregulated angiogenesis (6). The Cancer Genome Atlas (TCGA) identifies four molecular subtypes of ECs characterized by POLE ultra-mutated, microsatellite instability (MSI) hyper-mutated, high copy number with TP53 mutations, and low copy number with microsatellite stability (7). While the four core TCGA molecular subtypes provide an objective framework, contemporary clinical deployment faces active debates regarding pragmatic risk stratification surrogate systems (such as ProMisE) and how to classify ambiguous overlap patterns or heterogeneous mutations within individual tumors.

Treatment options for EC include surgery, radiation therapy, chemotherapy and recently approved immunotherapy combining Pembrolizumab with Lenvatinib. However, advanced and recurrent ECs pose a unique challenge with limited available therapies to improve patient outcomes (8-10). The clinical need for improved outcomes has led to renewed interest in using natural compounds as possible agents to

improve patient care, particularly those with safety when used in humans and multiple mechanisms of action (11,12).

Despite advancements in surgical procedures, chemotherapy and immunotherapy, advanced and recurrent ECs remain highly challenging due to treatment resistance and systemic toxicity. This clinical bottleneck has fueled scientific interest in natural bioactive compounds, such as polyphenols and flavonoids, as safer alternatives or adjuncts to standard therapies. Natural products possess diverse chemical structures capable of simultaneously modulating multiple oncogenic pathways, inducing apoptosis, reversing drug resistance, and suppressing metastatic potential with low systemic toxicity, making them highly attractive for modern oncological research (10,12-15).

Punicalagin (PCG) is a notable polyphenolic compound categorized as a hydrolysable ellagitannin (Fig. 1), and it can be found in considerable amounts in several parts of the pomegranate (*Punica granatum L.*), such as peel, seeds and juice (16). PCG is available in both α and β anomeric forms and is characterized by its relatively large water-soluble molecular structure with gallagyl and hexahydroxydiphenoyl moieties esterified to a glucose unit. Hydrolyzing PCG easily produces ellagic acid and other metabolites, which are considered to drive its biological properties (17,18). PCG is associated with several medicinal properties, making it a compound of significant scientific interest, particularly in cancer treatment. As a strong antioxidant, it can scavenge free radicals and chelate pro-oxidant metal ions, thereby safeguarding cells against the damaging effects of oxidative stress. In addition to being a potent antioxidant, PCG has strong anti-inflammatory, antimicrobial and antiviral properties that contribute to its protective effects across multiple pathological conditions. Regarding cancer, PCG exhibits particularly strong anticancer activity across a variety of cancers, including gynecological, gastrointestinal and breast cancers (13,19-23).

Epithelial-mesenchymal transition (EMT) is a process of great importance in the initiation, progression and dissemination of EC. During EMT, epithelial tumor cells lose typical cell-to-cell adhesion and polarity, adopting a more migratory and invasive mesenchymal phenotype. EMT is also associated with increased potential for metastases, worse prognosis, and responses to conventional treatment in EC (20). Autophagy, an intricate process of lysosomal degradation, has the potential to both promote and inhibit endometrial carcinogenesis. Autophagy can support tumor cell survival under metabolic stress, whereas dysregulated autophagy can facilitate cellular senescence or apoptosis, and in some cases may prevent tumor initiation or progression (24). Apoptosis, or programmed cell death, is a critical mechanism that removes damaged or abnormal cells from tissues, but numerous endometrial tumors gain the ability to suppress apoptosis, which contributes to uncontrolled cell proliferation and tumor growth and delays therapeutic responses (25).

There is now compelling data that natural products may modulate these cross-cutting biological processes in the context of cancer (12). Natural products (for example, flavonoids, polyphenols and terpenoids) are from dietary sources and medicinal plants and have been shown to target signaling pathways associated with cell adhesion and motility in order to inhibit EMT, reverse drug resistance, and increase autophagic flux, as well as restore apoptosis in cancer cells through the intrinsic and extrinsic pathways (12,15). The more complex structural chemistry of natural products could be an advantage in addressing the complexity and heterogeneity of EC biology. Also, natural products are typically associated with lower systemic toxicity, making them more attractive agents for incorporation into adjunct or alternative therapeutic outlooks. Therefore, the intent of the present study was to investigate the use of PCG in the modulation of EMT, autophagy and apoptosis in EC. The present study examined safer yet efficacious methods for patients with EC suffering from this common cancer.

Materials and methods

Reagents and chemicals. A stock solution of the 80 mM PCG was maintained in the lab by dissolving it in dimethyl sulfoxide (DMSO). PCG was purchased from ChemCruz (Santa Cruz Biotechnology, Inc.).

Cell culture and media. EC cell line, Ishikawa, was acquired from the American Type Culture Collection. DMEM (Welgene, Inc.) supplemented with fetal bovine serum (FBS) (R&D Systems, Inc.) (10%), antibiotics (1%), and HEPES (1%) was used to maintain the cells' proliferation throughout the experiments. Moreover, another endometrial cell line, SNU-539, was obtained from the Korean Cell Line Bank. The cells were cultured in RPMI-1640 medium (R&D Systems, Inc.) supplemented with FBS (10%), antibiotics (1%) and HEPES (1%) for maintenance and cell proliferation. Furthermore, these cells were provided with a humid environment containing 5% CO_2 at 37˚C during the trial period. On reaching the confluence of 70-80% (after every 3 days), the cells were sub-cultured using the 1% Trypsin-EDTA (Invitrogen; Thermo Fisher Scientific, Inc.) for 3 min.

Cell viability assay and IC_{50} determination. EC cells were subjected to the water-soluble tetrazolium salt (WST)-8 assay to check the viability of cells at various doses at different time intervals after treating the cells with PCG. Briefly, a total of $3x10^3$ cells were seeded in a 96-well plate for 24 h. After that, the cells were treated with different doses of PCG (0, 0.78125, 1.5625, 3.125, 6.25, 12.5, 25, 50, 100 and 200 μM) for a total time duration of 24 and 48 h. Later on, the cells' viability was determined by Quanti-MAXTM WST-8 Cell Viability Assay Kit (BIOMAX) as per the manufacturer's guidelines and the absorbance at 450 nm by employing the Synergy NEO 2 multimode plate reader (BioTek; Agilent Technologies, Inc.). The normalized cell viability data from independent experiments were fitted to a non-linear regression model using an online pharmacological dose-response calculator (PharmCalculator; https://pharmcalculator.github.io/IC_{50}-triplicate-normolized/). Specifically, a four-parameter logistic curve (a sigmoidal dose-response with variable slope) was used to model the relationship between the log-transformed concentration of PCG and the percentage of cell viability. IC_{50} values and their corresponding SD were generated directly from the fitted regression curves.

Colony formation assay. The cells were seeded in the 6-well plates at a density of 200 cells per well and incubated for 24 h

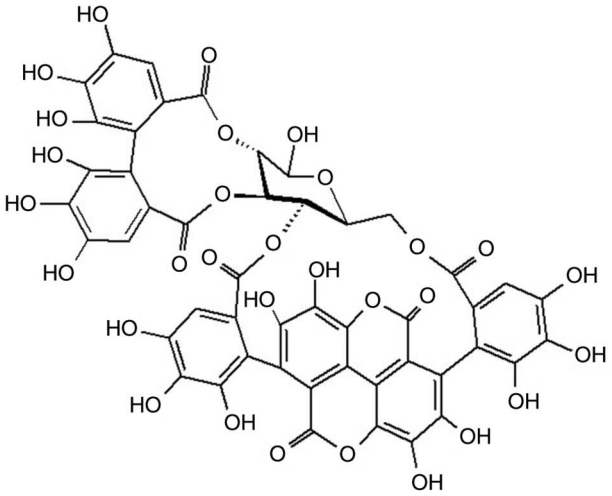


Figure 1. Chemical structure of Punicalagin.

at 37˚C prior to treating the cells with PCG. After treatment, the cells were further incubated for 48 h at 37˚C. After 48 h, the fresh media were maintained in the cell culture wells for 14 days. Later, the cells were fixed using 100% methanol (for 20 min) and stained with a 0.5% crystal violet solution (for 15 min). Lastly, the wells were washed using the DPBS. Colonies containing more than 50 cells were considered a single colony and were automatically quantified using ImageJ software (version 1.54f; National Institutes of Health).

Transwell migration assay. The 24-well Transwell chamber (8.0-μm pore membrane) was used in the present study. A total of ~4x10^3 cells per well were seeded in the upper chamber in 250 μl of serum-free medium, and 750 μl of serum-free medium was added to the lower chamber. After 48 h incubation at 37˚C, the cells in the upper chamber were removed with cotton swabs and fixed and stained with 4% paraformaldehyde and a 0.1% crystal violet solution. The cells penetrated through the membrane and were detected using a fluorescence microscope.

JC-10 assay. EC cells were seeded in the 96-well black plate at a density of 4x10^3 cells per well for 24 h prior to treatment with PCG. After treating the cells with PCG, the plates were incubated again for 48 h. Later, the cells were treated with 5 μM of JC-10 (AdipoGen Life Sciences) dissolved in DMSO and incubated for 30 min. After that, the plates were quickly transferred to LionheartTM FX Automated Microscope (BioTek; Agilent Technologies, Inc.) to detect the distribution in mitochondrial membrane potential (MMP).

DCFH-DA assay. The DCFH-DA assay was also used in the present study to detect the formation of ROS species in the Ishikawa and SNU-539 cells. Briefly, the cells were seeded at 4x10^3 cells per well in a 96-well black plate for 24 h, and then for 48 h after treating the cells with different doses of PCG. Later, the cells were treated with DCFH-DA (5 μM) and Hoechst33342 (Invitrogen; Thermo Fisher Scientific, Inc.) (2 μg/ml) for 30 min and detected the formation of ROS using the LionheartTM FX Automated Microscope (BioTek; Agilent Technologies, Inc.).

Hoechst 33342/PI double staining. Hoechst 33342/PI double staining assay was used in the present study to detect the apoptosis in the Ishikawa and SNU-539 cells. Briefly, the cells were seeded at 4x10^3 cells per well in a 96-well black plate for 24 h, and then for 48 h after treating the cells with different doses of PCG. Later on, the cells were treated with propidium iodide (20 μg/ml) and Hoechst33342 (5 μg/ml) for 30 min and detected the formation of ROS using the LionheartTM FX Automated Microscope (BioTek; Agilent Technologies, Inc).

Acridine orange (AO) staining. To detect the presence of acidic vesicular organelles, indicative of autophagy, AO staining was used. Briefly, after the initial incubation of 24 h, the cells were treated with various doses of PCG for 48 h. Cells were provided with 5% CO_2 and 37˚C during the incubation. After that, the cells were treated with AO staining (1 μg/ml) for 20 min. Lastly, the images were captured using the LionheartTM FX Automated Microscope after 2 washes with DPBS. Images captured were further analyzed using the Gen5 v 3.14.03 software (BioTek; Agilent Technologies, Inc).

Western blot analysis. For the western blot analysis, the Ishikawa and SNU-539 cells were treated with 50 and 100 μM PCG for 24 h. After this, the cells were scraped using a cell scraper, centrifuged at 300 x g for 5 min. The cells were then mixed with PRO-PREP™ Protein Extraction Solution (C/T) (Intron Biotechnology, Inc.) and kept at -20˚C for 20 min. Lysates were subsequently centrifuged at 13,000 x g for 5 min, and the supernatant containing the extracted protein was collected. Protein concentration was quantified using the BCA protein assay. A total of 30 μg of protein per lane was separated by SDS-PAGE (10%) and transferred to PVDF membranes. Membranes were blocked with 5% BSA (MilliporeSigma) and incubated with the primary antibodies listed in Table I (overnight at 4˚C), followed by the appropriate secondary antibody [1:3,000 dilution for 1 h at room temperature; Goat Anti-Rabbit IgG (H+L)-HRP Conjugate; cat. no. 1706515; Bio-Rad Laboratories, Inc.). Where multiple targets were probed on the same membrane, membranes were stripped according to the manufacturer's instructions and reprobed for the additional targets (BAX, E-cadherin and LC3). For LC3, the LC3-I band was below the limit of detection under the present experimental conditions, and only the LC3-II band was reliably resolved. As the LC3-I band was absent, an LC3-II/LC3-I ratio could not be calculated; autophagy was therefore quantified by normalizing LC3-II band intensity directly to the GAPDH loading control. GAPDH served as the internal loading control, and all densitometric band intensities were normalized against GAPDH. Protein bands were detected using Cytiva Amersham ECL Start Western Blotting Detection Reagent and imaged on a LuminoGraph II system (ATTO Corporation). Band quantification was performed using ImageJ software (version 1.54f; National Institutes of Health).

In vivo study. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Chungbuk National University (approval no. CBNUA-24-00 075-01; Cheongju, Korea) and conducted in accordance with institutional guidelines for the care and use of laboratory animals. A total of 12 female BALB/c nude mice (5 weeks old)

Table I. List of antibodies used in western blot analysis.

Protein	Supplier	Identifier	Source/Description	Dilution
BAX	Cell Signaling Technology, Inc.	5023	Rabbit mAb	1:1,000
E-cadherin	Cell Signaling Technology, Inc.	3195	Rabbit mAb	1:1,000
LC3A/B	Cell Signaling Technology, Inc.	12741	Rabbit mAb	1:1,000
GAPDH	Cell Signaling Technology, Inc.	4970	Rabbit mAb	1:1,000

were obtained from KOATECH Ltd. and cared for in specific pathogen-free conditions at a controlled temperature of 22±2˚C, a relative humidity of 50±10%, and a 12-h light/dark cycle. The animals had *ad libitum* access to a standard sterile pellet diet and water. At the start of the experiment, the 12 mice had an initial mean body weight of 24.1±1.7 g. To establish the Ishikawa cell xenograft model, $5x10^6$ Ishikawa cells resuspended in 100 μl of phosphate-buffered saline (PBS) were subcutaneously implanted into the right flank of each mouse. After the average tumor diameter reached ~5 mm, mice were divided into two groups (n=6 per group) randomly [using a simple randomization protocol (computer-generated random numbers)]: i) PCG-treated group, which received PCG at 20 mg/kg of body weight via the intraperitoneal route, ii) PBS-treated control group, which received PBS by the intraperitoneal route. The growth of the tumor would be measured every two days. On day 14, post-treatment, all mice were euthanized, and tumor tissues were collected. Animals were euthanized by cervical dislocation using a dedicated instrument. Death was confirmed by cessation of respiration and heartbeat and the absence of the pedal withdrawal reflex prior to tumor excision. The tumor growth was measured every two days using a digital caliper, and tumor volume (V) was calculated using the standard formula: V=(Length x width2)/2.

Statistical analysis. All the experiments were repeated at least 3 times for the consistency of the results, and the data obtained were analyzed using the GraphPad Prism 10.4.1 (GraphPad Software Inc.; Dotmatics). Two-way ANOVA and post hoc Dunnett's multiple comparison test were used to analyze the data obtained, and the results are shown as the mean ± standard deviations or standard errors of the means. $P<0.05$ is deemed statistically significant when compared with the negative control. For *in vivo* tumor growth curves, a two-way repeated-measures ANOVA was used. For all *in vitro* assays, cells treated with 0.1% (v/v) DMSO served as the negative vehicle controls. The final concentration of DMSO in all experimental culture media was strictly maintained at ≤0.1% (v/v) to eliminate solvent-induced cytotoxicity.

Results

Anti-proliferative effects of PCG on EC cells. The EC cells, Ishikawa and SNU-539, were treated with various doses of PCG (0, 0.78125, 1.5625, 3.125, 6.25, 12.5, 25, 50, 100 and 200 μM) for 24 and 48 h. The viability of cancer cells was evaluated using the water-soluble tetrazolium salt (WST) assay. PCG reduced the growth of EC cells in a dose and time-dependent manner (Fig. 2A-D). With the increase in time, the effectiveness of PCG at lower doses increased. These results indicate the antiproliferative activity of PCG against EC cells. Treating the SNU-539 cells for 24 h resulted in an estimated IC_{50} of 37.621±5.714 μM, demonstrating the high initial susceptibility of this cell line to the compound, whereas extending the incubation period to 48 h yielded an estimated IC_{50} of 43.149±5.876 μM. Moreover, at 24 h post-treatment, Ishikawa cells exhibited a relatively resistant phenotype, with an estimated IC_{50} of 80.429±22.680 μM, whereas prolonged exposure to 48 h significantly enhanced the compound's cytotoxic efficacy, resulting in a reduced IC_{50} of 49.193±7.024 μM.

Inhibitory effects of PCG on the colony forming ability of EC cells. The Ishikawa and SNU-539 cells were treated with PCG doses ranging from 6.25 to 100 μM for 48 h, and then the cells were cultured for another 14 days. There was a dose-dependent decrease in the number of colonies of both cell lines. The efficacy of PCG on Ishikawa cells (Fig. 3A) was more prominent when compared with the SNU-539 cells (Fig. 3B). Higher doses of PCG (25, 50 and 100 μM) showed a more significant effect in Ishikawa and SNU-539 cells when compared with lower doses of PCG. This experiment indicated the inhibitory effect of PCG on the colony-forming ability of EC cells. Notably, while short-term cell viability assays indicated an initial metabolic transition beginning at 25 μM (Fig. 2), the long-term colony formation assay displayed a more drastic phenotypic suppression at 50 and 100 μM across both cell lines (Fig. 3). This variation in sensitivity between the two assays is attributed to the drastically lower initial cell seeding density used during clonogenic tracking, which renders individual cells more susceptible to persistent chemical stress over a 14-day window compared with short-term, high-density cell cultures.

Inhibitory effect of PCG on the migratory ability of EC cells. To investigate whether PCG reduces the migration ability of *EC* cells, a Transwell migration assay was performed to assess the ability of cells to migrate through the Transwell membrane after PCG treatment. The results of the Transwell migration assay revealed that the PCG (25, 50 and 100) was able to successfully inhibit the Transwell migration ability of both Ishikawa and SNU-539 cell lines (Fig. 4A and B). The effects of PCG in inhibiting Transwell migration were equally improved in both Ishikawa and SNU-539 cell lines (26). Furthermore, the results were further analyzed using the western blot assay. It was found that PCG increased the expression of E-cadherin in Ishikawa cells (Fig. 4C). In summary, PCG was found to inhibit the migratory ability of EC cells.

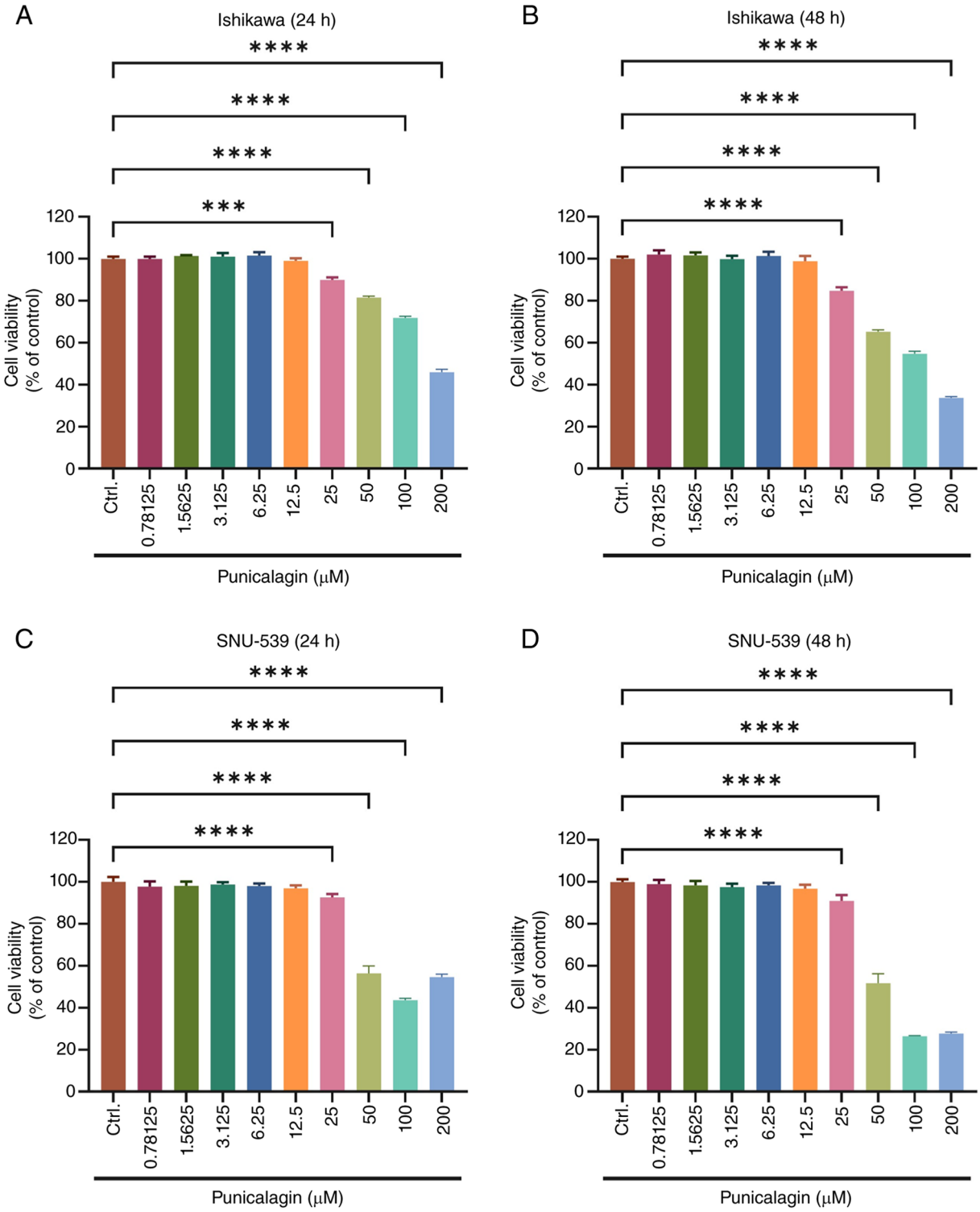


Figure 2. PCG dose-dependently decreases the proliferation of EC cells. The EC cell lines, Ishikawa and SNU-539, were treated with PCG (from 0.78125 to 200 μM) for 24 and 48 h. Cell proliferation was detected by the WST assay. (A-D) PCG caused a decrease in the proliferation of (A and B) Ishikawa cells and (C and D) SNU-539 cells. Following the statistical analysis, the data in the graphs have been derived from at least three repeated experiments and shown as the mean ± SD. Compared with control, ***P≤0.001 and ****P≤0.0001. PCG, punicalagin; EC, endometrial cancer.

PCG disrupts mitochondrial function and increases reactive oxygen species (ROS) levels in EC cells. The importance of MMP in the mechanism of cell death of cancer cells is well established. A JC-10 assay was conducted to detect MMP changes following PCG treatment of Ishikawa and SNU-539 cell lines for a period of 48 h. The PCG treatment decreased the MMP of the cancer cells, which was consistent for both Ishikawa (Fig. 5A) and SNU-539 cells (Fig. 5B). Moreover, ROS are significantly involved in the anticancer action of numerous cancer drugs. It has been identified that elevated ROS can initiate apoptosis, necroptosis, ferroptosis, autophagy, and other mechanisms of cell death in cancer

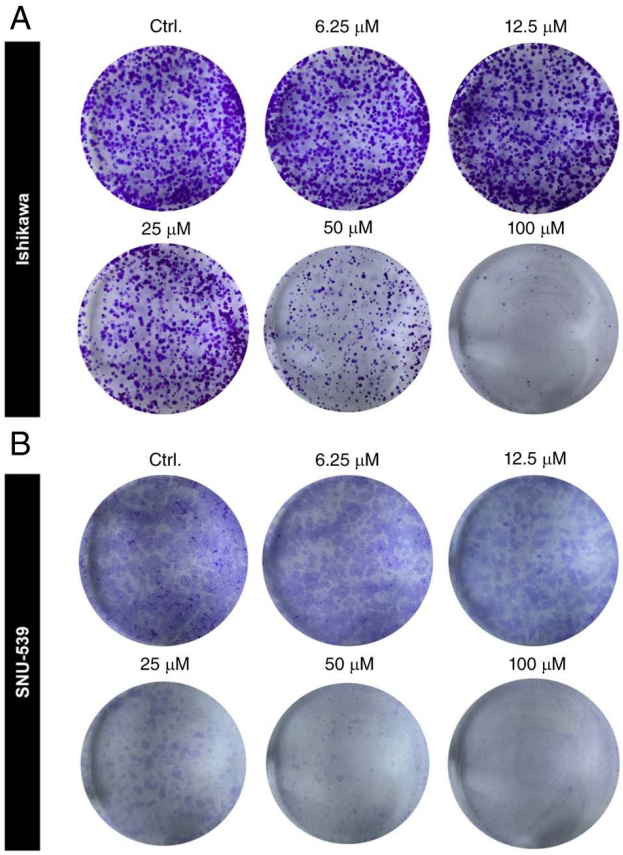


Figure 3. PCG suppresses the colony formation ability of EC cells. The EC cell lines, Ishikawa and SNU-539, were treated with PCG (from 6.25 to 100 μM) for 48 h and then incubated for 14 days. (A and B) PCG causes a decrease in the colony formation ability of the (A) Ishikawa and (B) SNU-539 cells. The inhibitory effect was more prominent in Ishikawa as compared with SNU-539 cells. PCG, punicalagin; EC, endometrial cancer.

cells (27). As expected, ROS levels in both EC cell lines increased after treatment with PCG (Fig. 6A and B). These results indicated that the antitumor effects of PCG in EC may be due to the disruption of mitochondrial function and an increase in ROS.

Proapoptotic effects of PCG on EC cells. After confirmation of the antiproliferative effect of PCG on EC cells, the mechanism of cell death was investigated. Apoptosis was identified and quantified using the Hoechst 33342/PI double staining. It was found that treatment with PCG caused dose-dependent apoptosis of the Ishikawa cells (Fig. 7A), while only a high dose of 100 μM in SNU-539 cells (Fig. 7B). The apoptotic rate was more dose-dependent in Ishikawa cells than in the SNU-539 cells. Moreover, a western blot analysis revealed increased levels of the proapoptotic BCL2-associated X, apoptosis regulator (BAX) protein (Fig. 7C). These results indicate that PCG prevents proliferation by triggering apoptosis in EC cells.

Effect of PCG on autophagy in EC cells. AO staining was utilized to identify if PCG treatment resulted in autophagy. The formation of red vesicles after AO staining was detected by fluorescence microscopy. Treatment with PCG resulted in autophagy in the Ishikawa cells (Fig. 8A). However, no such statistically significant effect was seen with SNU-539 cells (Fig. 8B). Furthermore, the western blot assay also revealed a conversion of LC3I to LC3II in Ishikawa cells after 24 h of treatment, indicating that PCG is able to trigger autophagy to inhibit the proliferation of Ishikawa cells (Fig. 8C). In a nutshell, PCG can cause autophagy marker accumulation in the Ishikawa cells, but not in SNU-539 cells.

In vivo efficacy of PCG in xenograft mice model. Following the successful establishment of the xenograft model employing Ishikawa cells, the tumor-bearing mice were subjected to treatment using a control group (Control) and a drug-treated group (20 mg/kg, once every two days) for 14 days (Fig. 9A), and the outcome revealed a significant attenuation of tumor volume and significant reduction of tumor weight after PCG treatment with elongating time (Fig. 9C-E). Data from both the *in vitro* and *in vivo* studies confirmed a significant inhibition of proliferative behavior by PCG in EC.

Discussion

The current study presents extensive evidence pertaining to the anticancer effects of PCG against EC cells, establishing its merit as a promising natural treatment via several overlapping mechanisms of action. It was demonstrated that PCG produces dose-dependent cytotoxicity in both Ishikawa and SNU-539 EC cell lines, while also affecting several pivotal cellular processes involved in cancer progression, such as cell proliferation, migration, mitochondrial functionality, and programmed cell death. This research extends the established anticancer applications of PCG in various malignancies to the first observations in EC, thereby providing a useful basis for clinical translational potential. The diverse mechanisms executed by PCG give it the unique ability to address primary tumor growth and metastatic potential, rendering this natural compound a potentially useful therapy against EC, particularly considering the currently limited treatment options available for patients with advanced and recurrent disease.

The results presented in the current study are consistent with previous peer-reviewed literature on PCG's anticancer properties, while also providing new and valuable perspectives provided specifically to EC studies. The dose-dependent growth inhibition of the Ishikawa and SNU-539 EC cell lines (0.78125-200 μM) is consistent with concentration ranges utilized in other cancer studies where PCG has shown to be cytotoxic, including up to 200 μM in cervical cancer (CC) (21); IC_{50} values between 100-200 μM in gastric cancer (GC) (20), and between 50-75 μM in lung cancer A549 cells (9). The similar and consistent concentration-response relationships across cell lines of different cancer types suggest that PCG has a similar mechanism of action with antiproliferative effect regardless of cancer type under investigation. Crucially, these findings should be interpreted within the modern TCGA molecular classification framework of EC. The Ishikawa cell line represents a well-differentiated, estrogen receptor-positive endometrioid adenocarcinoma, which typically correlates with the copy-number low or MSI subtypes. Conversely, the SNU-539 cell line represents a more aggressive, poorly differentiated phenotype. By demonstrating that PCG exerts strong anti-proliferative effects across both models, the present data

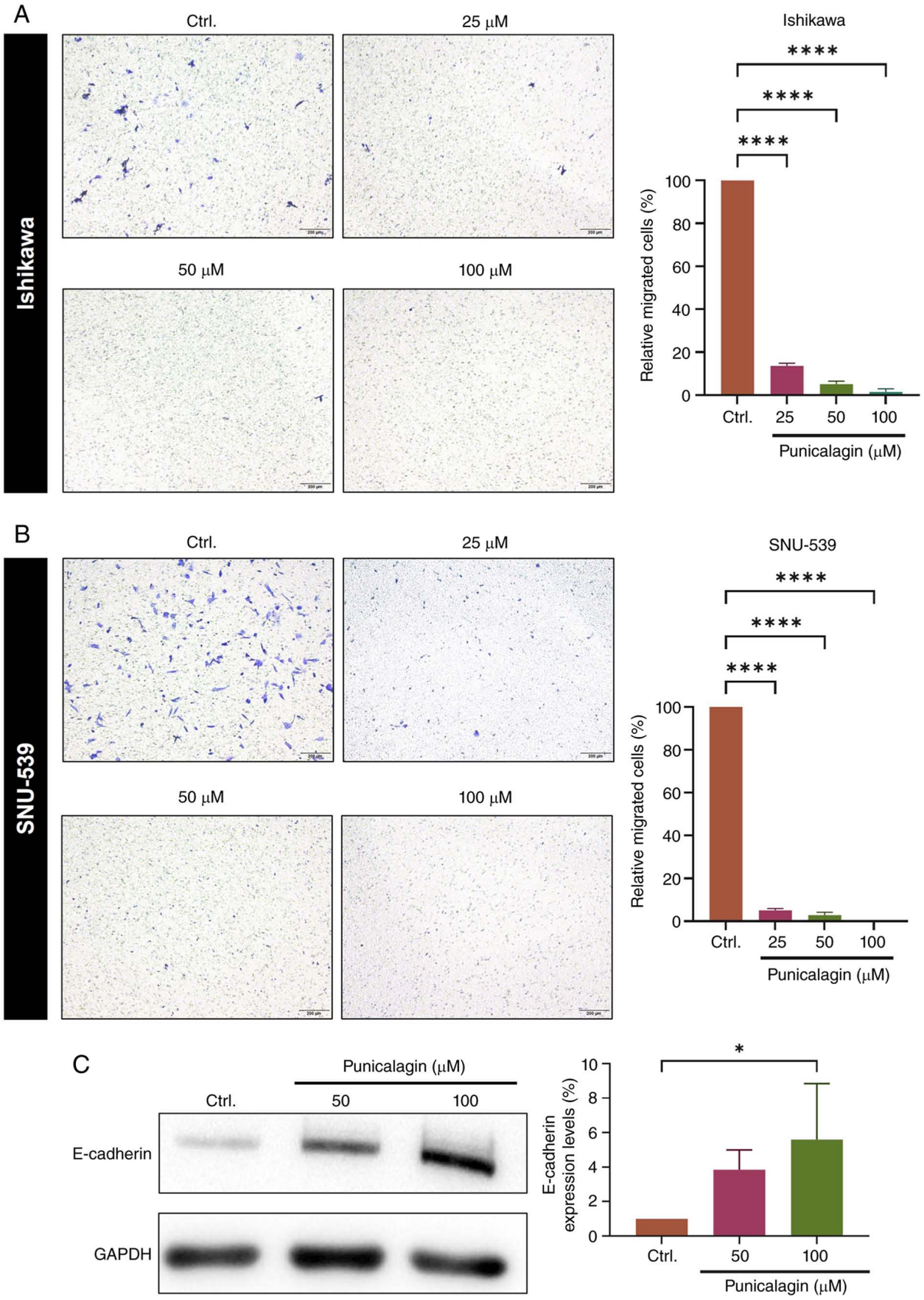


Figure 4. PCG suppresses the Transwell migration ability of EC cells. To assess the Transwell migration ability of PCG on the EC cells, the Ishikawa and SNU-539 were subjected to 48 h to PCG at various doses (25, 50, and 100 μM). (A and B) PCG treatment resulted in the decreased Transwell migration ability of the (A) Ishikawa and (B) SNU-539 cells. (C) Western blot assay revealed an increase in E-cadherin in Ishikawa cancer cells. Data were obtained after statistical analysis using at least three repeated experiments, presented in graphs as the mean ± SD. Compared with control, *P≤0.05 and ****P≤0.0001. PCG, punicalagin; EC, endometrial cancer.

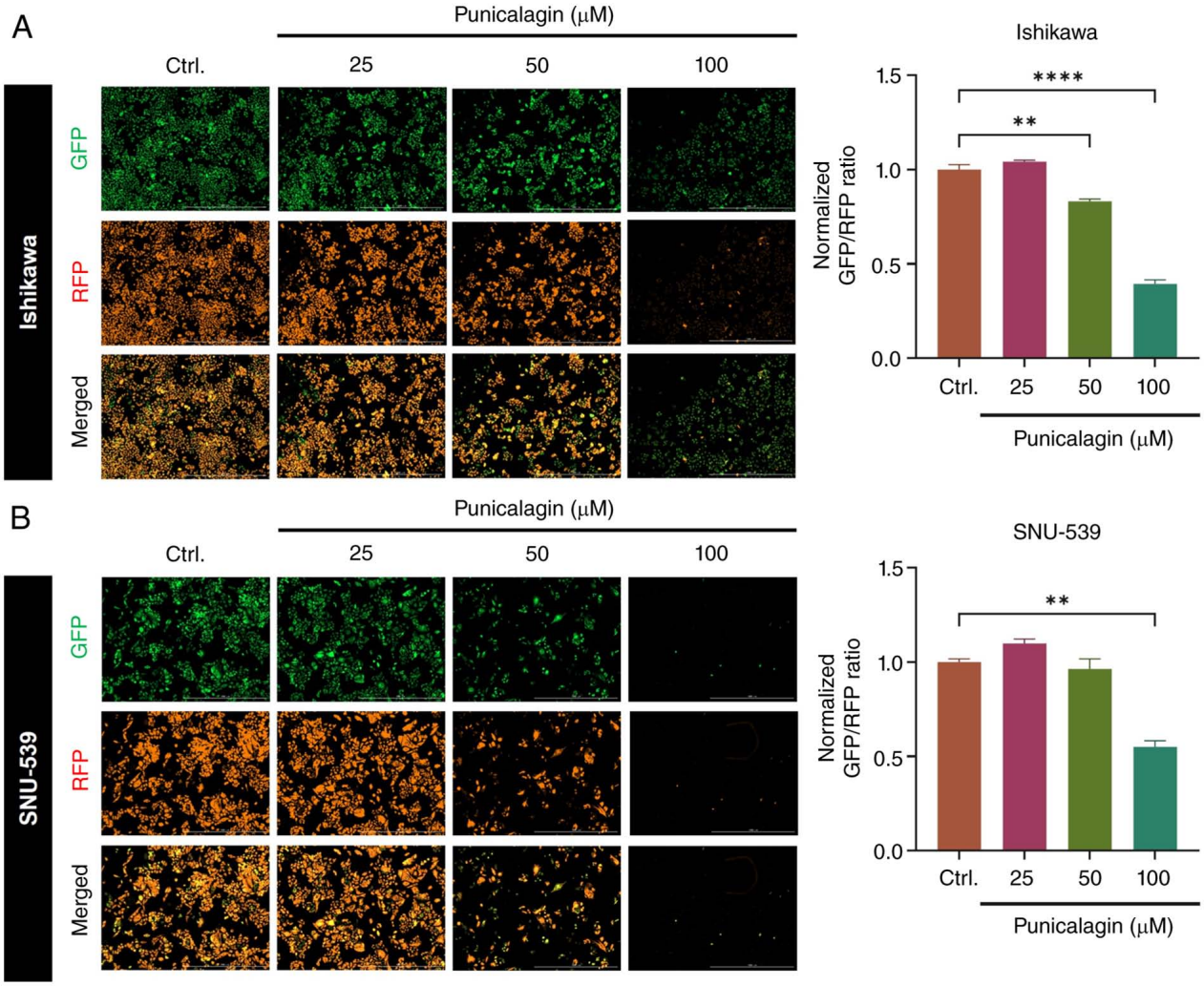


Figure 5. PCG disrupts the MMP in endometrial cancer cells. (A and B) Representative images for the analysis of MMP through JC-10 assay in (A) Ishikawa cells and (B) SNU-539 cells. All cells were seeded at a density of $4x10^3$ per well in cell culture dishes and treated with (0.1% DMSO) and PCG (25, 50 and 100 μM). Data were obtained after statistical analysis using at least three repeated experiments, presented in graphs as the mean ± SD. Compared with control, **P≤0.01 and ****P≤0.0001. PCG, punicalagin; MMP, mitochondrial membrane potential.

suggest therapeutic potential that spans across distinct molecular and histopathological backgrounds. However, because EC is highly heterogeneous, it is recognized that the present findings using these two specific models cannot be broadly generalized to all subtypes, such as POLE-ultra-mutated or p53-aberrant serous carcinomas, which require dedicated evaluation in future studies.

The inhibition of capacity for colony formation observed in the present experiments in both EC -derived cell lines, especially in Ishikawa cells, is consistent with previous research conducted with PCG in other gynecological cancers that demonstrate similar patterns of differential sensitivities across varying cancer types (20,28). It has been previously found that PCG demonstrated variable responses across isolated cellular epigenetics and molecular characteristics, and genetic alterations between a cell line in a cancer type (29). The additional finding that Ishikawa cells had a significantly greater response than SNU-539 in each of the assays is consistent with the personalized cancer care paradigm, which is based on differences in tumor heterogeneity.

The indication that PCG suppressed migratory activity using a Transwell assay and increased E-cadherin expression is an important contribution to understanding PCG's anti-metastatic mechanisms in EC. Because multiple other types of cancers have reported similar findings demonstrating PCG's ability to inhibit EMT through the upregulation of E-cadherin and downregulation of N-cadherin in breast cancer (30), the reduction of MMP-2 and MMP-9 in CC (21), and the suppression of invasion through modulation of similar MMPs in GC (20), this conclusion is backed by strong evidence from the literature. The upregulation of E-cadherin, specifically, represents the first direct evidence of PCG's anti-EMT effects in EC, bringing to bear well-established mechanisms to this specific malignancy. It is noted, however, that evaluating E-cadherin expression alongside a Transwell migration assay provides an initial look at cellular motility rather than a complete validation of EMT reversal or matrix invasion. Documenting a full EMT blockade requires evaluating comprehensive mesenchymal markers (such as N-cadherin, Vimentin, Snail, or Slug) alongside Matrigel-coated Transwell invasion assays to confirm disrupted extracellular matrix

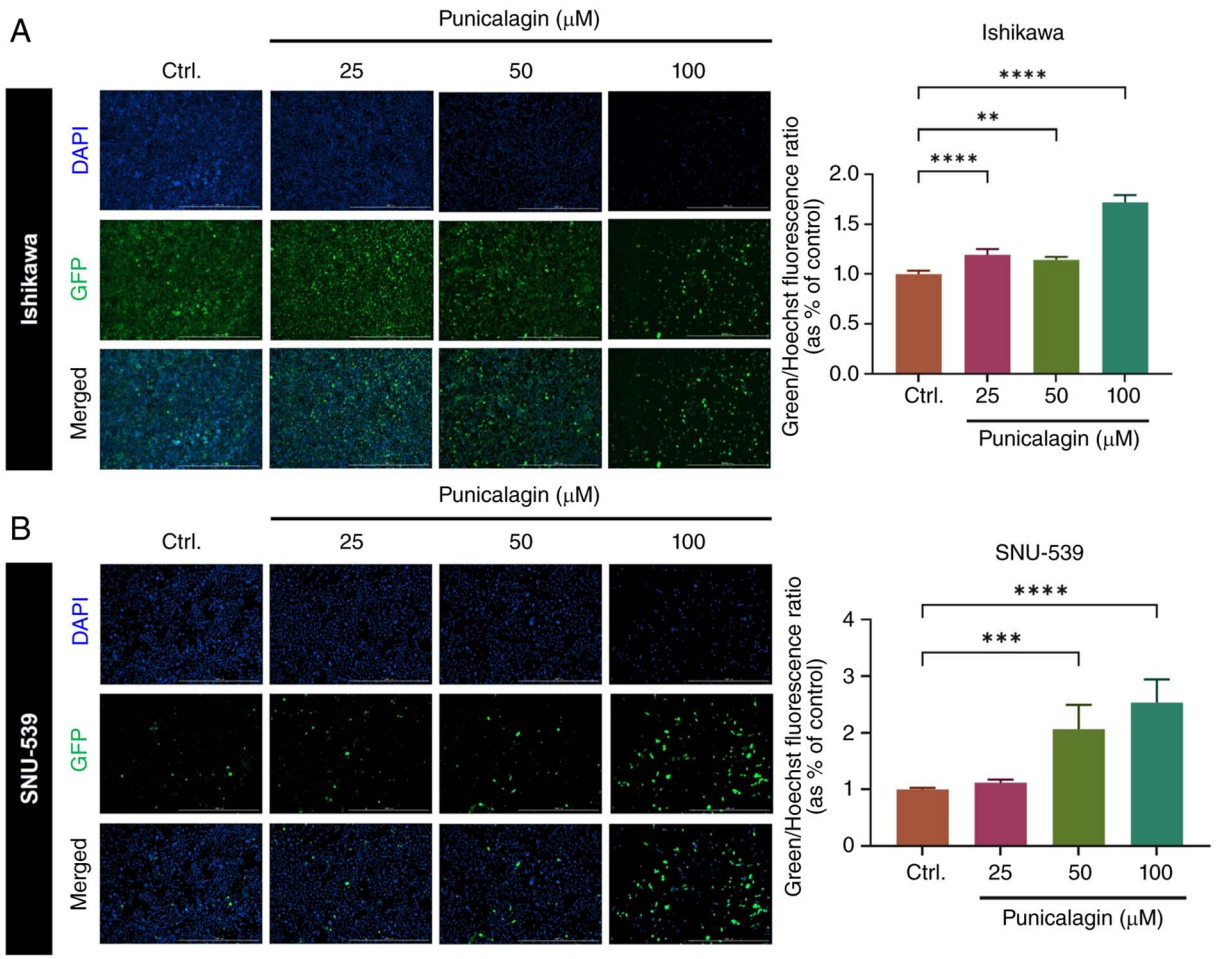


Figure 6. PCG causes an increase in ROS production in EC cells. For the quantification of the ROS, the EC cells were subjected to PCG for 48 h before employing the DCFH-DA assay. (A and B) There was an increase in the ROS generation in (A) Ishikawa and (B) SNU-539 cells. Data were obtained after statistical analysis using at least three repeated experiments, presented in graphs as the mean ± SD. Compared with control, $^{**}P\leq0.01$, $^{***}P\leq0.001$ and $^{****}P\leq0.0001$. PCG, punicalagin; ROS, reactive oxygen species; EC, endometrial cancer.

degradation. Future studies are required to overcome this limitation of the present study.

The mitochondrial impairment associated with ROS generation after treatment with PCG is consistent with mechanisms described in other types of cancers. Researchers have shown PCG can abolish MMP in lung cancer A549 cells, with an increase in mitochondrial ROS production (31); cause dysfunction of mitochondria with increased superoxide radicals in colon cancer Caco-2 cells (32); cause mitochondrial dysfunction with resulting apoptosis in glioblastoma U87MG cells (33); and disrupt mitochondrial function with increased production of ROS in leukemic cells (19). The consistent nature of the mitochondrial-targeting effects observed in different cancer types indicates a mechanistic basis by which PCG induces death in cancer cells due to increasing oxidative stress and disrupting energy metabolism. Crucially, this intracellular accumulation of ROS acts as a centralized upstream mechanism that bridges the various biological phenomena observed in the present study, highlighting an important pharmacological phenomenon known as the polyphenol pro-oxidant paradox. Although PCG is widely recognized as a classic antioxidant capable of scavenging free radicals in normal tissues, its role characteristically shifts toward a pro-oxidant state inside malignant cells. This cancer-specific toxicity occurs because the intracellular microenvironment of tumor cells features higher baseline metabolic stress, altered pH, and elevated levels of transition metal ions (such as copper and iron) that can catalyze the autoxidation of polyphenolic structures, generating high concentrations of superoxide anions. On one hand, this excessive ROS directly targets the mitochondrial membrane, causing a loss of MMP, which facilitates the leakage of pro-apoptotic factors into the cytosol and triggers the intrinsic apoptosis cascade, as substantiated by the present finding of elevated pro-apoptotic BAX expression. On the other hand, this severe oxidative and metabolic stress simultaneously prompts the cell to activate autophagy as an initial stress or death response, explaining the pronounced conversion of LC3-I to LC3-II observed predominantly in the highly responsive Ishikawa cells. Taken together, the observations of ROS accumulation, loss of MMP, BAX upregulation, and LC3-II conversion are consistent with an integrated ROS-mitochondria axis in which early oxidative stress acts as a proximal trigger for downstream mitochondrial damage. It is noted that the causal directionality of this axis is inferred from the convergence of these endpoints and from mechanisms established in other malignancies, rather than from direct rescue experiments in the models of the present study. This interconnected ROS-mitochondria axis branches

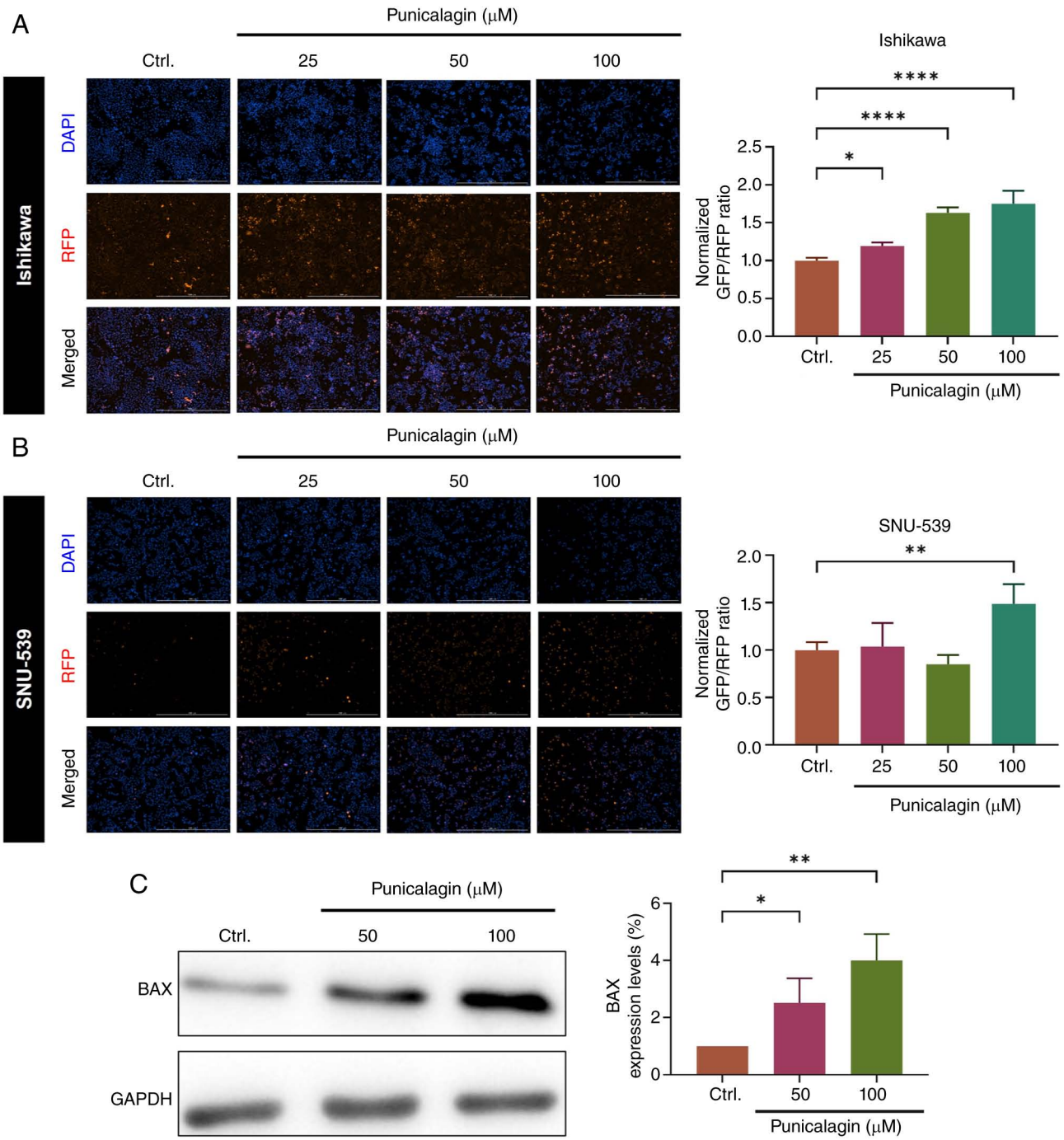


Figure 7. PCG causes apoptosis in endometrial cancer cells. (A and B) Representative images for the analysis of Hoechst 33342/PI double staining in (A) Ishikawa and (B) SNU-539 cells. All cells were seeded at a density of $4x10^3$ per well in cell culture dishes and treated with (0.1% DMSO) and PCG (25, 50 and 100 μM). A dose dependent increase in apoptotic cells was noted in the Ishikawa cells, while only at high concentration (100 μM) in SNU-539 cells. (C) Representative image of the BAX expression in the Ishikawa cells. Data were obtained after statistical analysis using at least three repeated experiments, presented in graphs as the mean ± SD. Compared with control, *P≤0.05, **P≤0.01 and ****P≤0.0001. PCG, punicalagin.

out to drive both apoptosis and autophagy depending on the genetic background and tolerance thresholds of the specific EC cell line, explaining the elegant multi-targeted potency of PCG and providing a more unified, in-depth perspective on its therapeutic mechanism against endometrial malignancies.

A primary limitation of the present study is that the proposed ROS-mitochondria axis, while supported by measurements of intracellular ROS, MMP, BAX and LC3-II, was not subjected to direct functional interrogation. Specifically, antioxidant rescue experiments (for example, N-acetylcysteine co-treatment) were not performed to confirm that the apoptotic and autophagic phenotypes are ROS-dependent. Caspase-3/9 activation was not examined to confirm execution of the intrinsic apoptotic cascade, neither true autophagic flux was validated via p62/SQSTM1 turnover or co-treatment with late-stage lysosomal inhibitors (chloroquine or bafilomycin A1). These experiments represent the essential next step to formally establish causality within the axis and are a priority for future investigation.

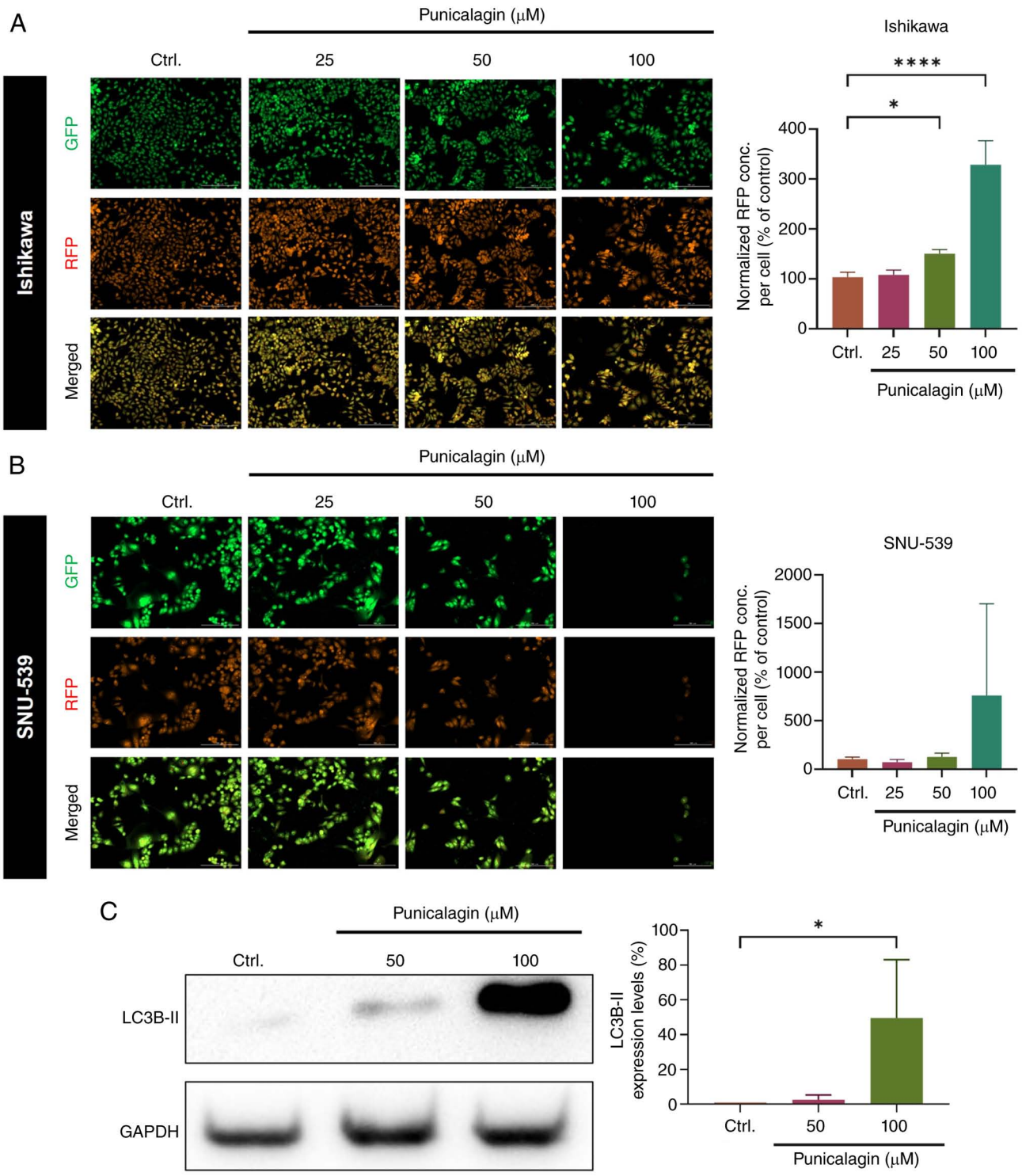
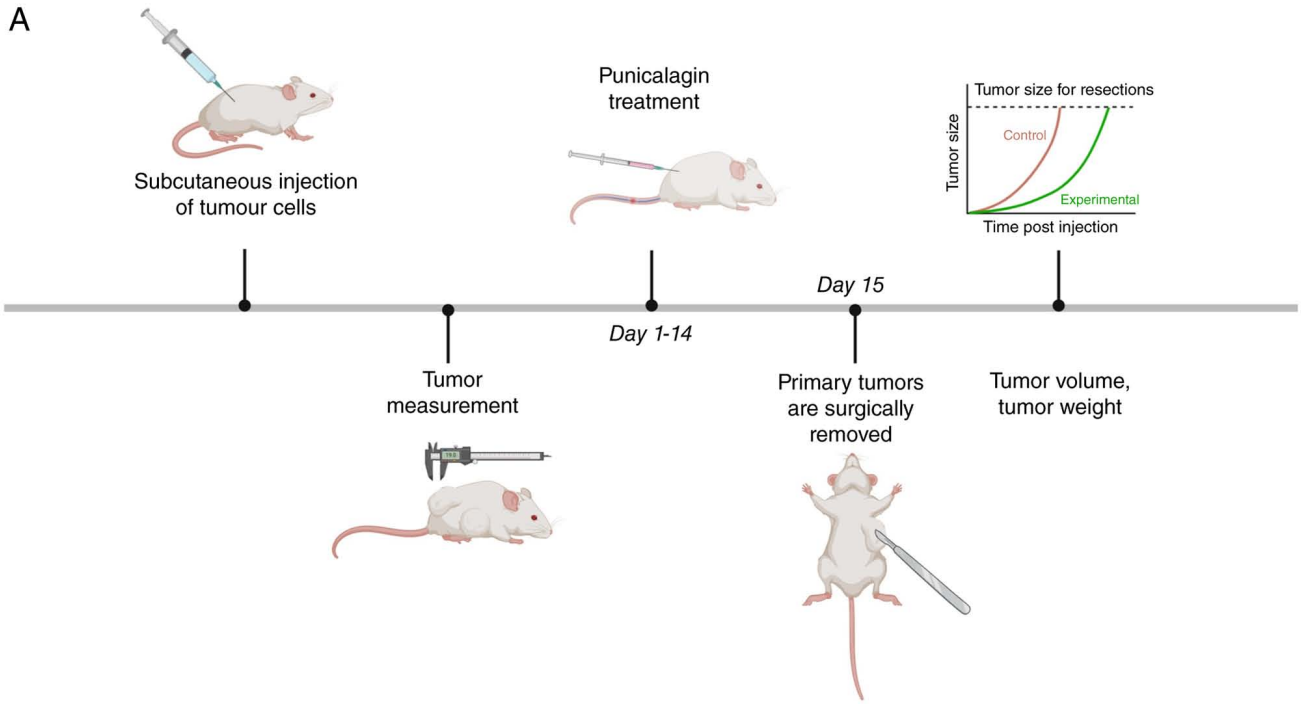
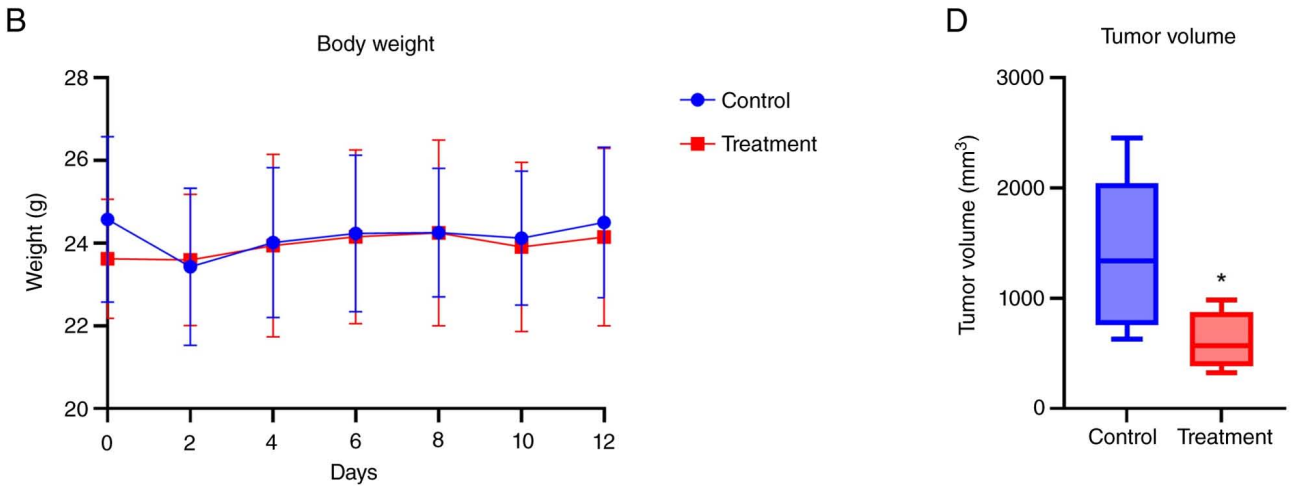


Figure 8. PCG causes autophagy in endometrial cancer cells. (A and B) Representative images for the analysis of autophagy through Acridine Orange staining in (A) Ishikawa and (B) SNU-539 cells. All cells were seeded at a density of $4x10^3$ per well in cell culture dishes and treated with (0.1% DMSO) and PCG (25, 50 and 100 μM). PCG caused autophagy in the Ishikawa cell line. The staining result was statistically insignificant in SNU-539 cells. (C) Representative image of the LC3-IIB expression in the Ishikawa cells. Data were obtained after statistical analysis using at least three repeated experiments, presented in graphs as the mean ± SD. Compared with control, $^{*}P\leq0.05$ and $^{****}P\leq0.0001$. PCG, punicalagin.

The apoptotic impacts observed through Hoechst-PI staining, as well as the upregulation of BAX, represent PCG's most consistently reported and well-researched mechanism of action. There is a plethora of literature that supports these findings, occurring as a result of PCG upregulating Bax and downregulating Bcl-2, while also activating caspases 3 and 9 in CC cells (21), promoting elevated activated caspase-3 levels in GC (20), further promoting increases in caspase-3 activity and cleaved caspase-3 levels in colon cancer (34), as well as employing multiple caspase upregulation involving Bax/Bcl-2 ratios in leukemic cells (19). The difference in sensitivity exhibited between Ishikawa and SNU-539 cells, in which





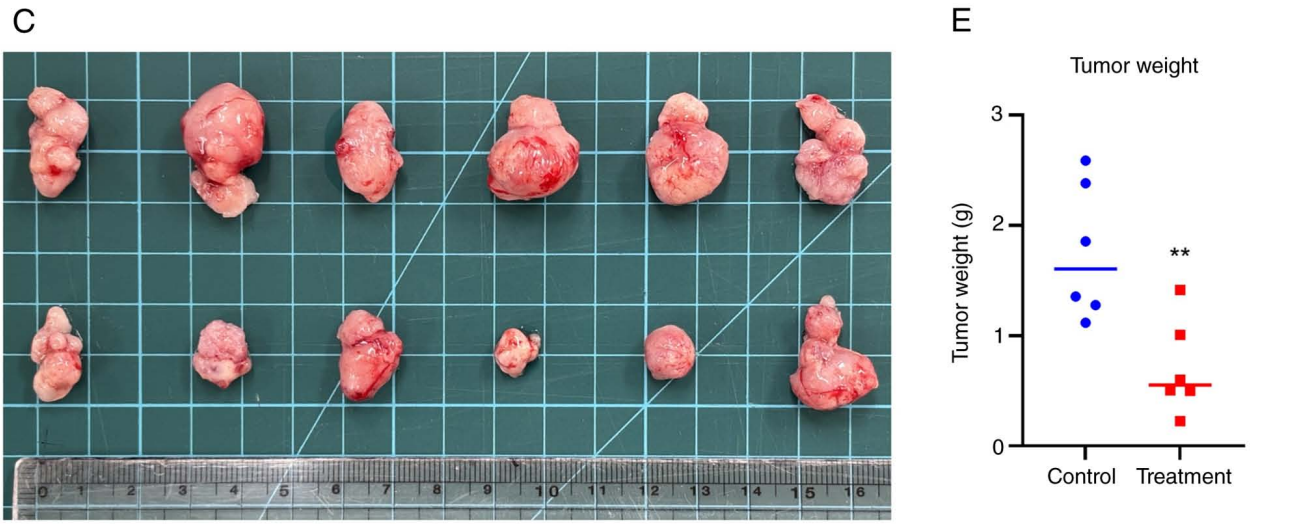


Figure 9. Punicalagin suppresses the tumor growth in xenograft Ishikawa tumors. The rates of tumor growth in xenograft mice were measured with an electric caliper after every 2 days. (A) Visual representation of the *in vivo* study plan. (B) There was no significant difference in the body weight of the control and treatment mice. (C) Representative images of the tumor growth. The upper line of the tumors represents the control group, while the lower line of tumors represents the treatment group. There was a marked difference in tumor growth. Furthermore, there was a significant reduction in (D) tumor volume and (E) tumor weight in xenograft mice. Data were presented in graphs as the mean ± SD Compared with control, $^{*}P \leq 0.05$ and $^{**}P \leq 0.01$.

SNU-539 cells required higher concentrations of PCG for statistically significant evidence of apoptosis, is well established with PCG research, which is well-contextualized by the cells and the genetic background that are utilized in discovery work.

The autophagy induction and demonstration through AO staining and upregulation of LC3-II in EC cells, and particularly Ishikawa cells, establish autophagy-inducing mechanisms in this malignancy for the first time to the best of our knowledge. The existing literature has established PCG's ability to induce autophagy through an increase in LC3-II conversion and beclin-1 upregulation in papillary thyroid carcinoma (35), LC3-II increase and AMPK-p27 signaling activation in glioblastoma (33), and has also been shown to promote autophagic degradation of HPV oncoproteins in CC (36), and induce autophagy through mTOR downregulation in leukemia (19). The pattern of induction of autophagy, dependent on the cell line, as observed in the degree of autophagy induction in Ishikawa cells compared with the other cell lines, also aligns with the available literature demonstrating that induction of autophagy by PCG is dependent on cellular context and genetic background. However, it is recognized that the current evaluation relies primarily on AO vesicular staining and LC3-II protein upregulation, which cannot definitively distinguish between highly active autophagic clearance and a block in late-stage lysosomal fusion. Evaluating true autophagic flux via p62/SQSTM1 degradation curves or functional co-treatments with late-stage lysosomal inhibitors such as chloroquine or bafilomycin A1 remains a critical limitation.

The demonstrated *in vivo* efficacy in xenograft mice, with substantial reductions in tumor volume and weight, provides important translational support for the anticancer activity of PCG. Prior work has developed a nano-formulated PCG that demonstrated similar *in vivo* efficacy in GC xenograft models (37). With respect to tolerability, no significant body-weight loss was observed in treated animals under the tested conditions over the 14-day study period. It is emphasized, however, that body weight alone is an insufficient indicator of systemic safety, and therefore conclusions regarding low systemic toxicity cannot be drawn. A comprehensive toxicity evaluation, including serum biochemical markers (such as alanine aminotransferase, aspartate aminotransferase and creatinine) and histological examination of major organs (such as liver and kidney), was not performed in the present study. Such an assessment is required to formally characterize the systemic safety profile and therapeutic index of PCG and is a priority for future investigation. It is noted that the existing toxicology literature reports favorable tolerability for PCG at comparable doses in rodents (36), although this does not substitute for direct safety evaluation within the current experimental system.

In conclusion, the present study shows strong anticancer activity from PCG against EC via numerous synergistic mechanisms, with regard to growth inhibition, apoptosis stimulation, autophagy induction, mitochondrial dysfunction and migration ability inhibition. The varied responses observed between Ishikawa and SNU-539 emphasized the importance of cellular context to treatment efficacy and potential biomarker development in the future to stratify patients. The successful advancement from *in vitro* evidence to significant tumor suppression in a xenograft mouse model, with no significant body-weight loss observed under the tested conditions, provides encouraging evidence for the potential therapeutic activity of PCG. Specifically, this compound is uniquely positioned to address a critical unmet clinical need in patient populations suffering from advanced, recurrent, or hormone-independent endometrioid endometrial malignancies that have grown resistant to standard cytotoxic regimes. In a clinical setting, the favorable tolerability profile reported for PCG, pending confirmation through comprehensive toxicological evaluation, would position it as a candidate either as an adjunctive partner to sensitize tumors to low-dose standard chemotherapies (such as platinum-based agents), thereby mitigating systemic side effects, or as a long-term maintenance therapy to suppress metastatic recurrence in post-operative patients. Further research will be required to derive dosing regimens, establish predictive biomarkers for PCG's anticancer effects, and examine combination strategies with conventional therapies, therefore further establishing PCG as a natural compound worthy of clinical investigation. Given the rising incidence of EC globally and the urgent need for safer, more effective therapeutic alternatives, PCG represents a valuable addition to the armamentarium against this increasingly prevalent malignancy, offering hope for improved patient outcomes with reduced treatment-related toxicity.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Regional Innovation System and Education program through the Chungbuk Regional Innovation System and Education Center; the Ministry of Education and the Chungcheongbuk of Republic of Korea (grant no. 2025-RISE-11-014-03); and the Sejong Fellowship through the NRF funded by the Ministry of Science and ICT (grant no. RS-2025-00557567). In addition, the present study was also supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT; RS-2026-25486352).

Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Authors' contributions

KCC conceptualized and supervised the study, and performed project administration. ZAB, DA and HKL developed methodology, validated data and conducted formal analysis. ZAB and DA conducted investigation, wrote the original draft, and visualized data. ZAB and KCC curated data; HKL and KCC wrote, reviewed and edited the manuscript, and acquired funding. ZAB and DA confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

All animal experiments were approved by the Institutional Animal Care and Use Committee of Chungbuk National

University (approval no. CBNUA-24-00075-01; Cheongju, Korea) and conducted in accordance with institutional guidelines for the care and use of laboratory animals.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Hwang WY, Kim JH, Noh JJ, Baek MH, Choi MC, Lee YJ, Lee M, Suh DH, Kim YB and Kim DY: Clinical practice guidelines for uterine corpus cancer: An update to the Korean society of gynecologic oncology guidelines. J Gynecol Oncol 36: e71, 2025.
2. Siegel RL, Miller KD, Fuchs HE and Jemal A: Cancer statistics, 2022. CA Cancer J Clin 72: 7-33, 2022.
3. Siegel RL, Kratzer TB, Wagle NS, Sung H and Jemal A: Cancer statistics, 2026. CA Cancer J Clin 76: e70043, 2026.
4. Gao S, Wang J, Li Z, Wang T and Wang J: Global trends in incidence and mortality rates of endometrial cancer among individuals aged 55 years and above from 1990 to 2021: An analysis of the global burden of disease. Int J Womens Health 17: 651-662, 2025.
5. Siegel RL, Kratzer TB, Giaquinto AN, Sung H and Jemal A: Cancer statistics, 2025. CA Cancer J Clin 75: 10-45, 2025.
6. Ribeiro-Santos P, Martins Vieira C, Viana Veloso GG, Vieira Giannecchini G, Parenza Arenhardt M, Müller Gomes L, Zanuncio P, Silva Brandão F and Nogueira-Rodrigues A: Tailoring endometrial cancer treatment based on molecular pathology: Current status and possible impacts on systemic and local treatment. Int J Mol Sci 25: 7742, 2024.
7. Bostan IS, Mihaila M, Roman V, Radu N, Neagu MT, Bostan M and Mehedintu C: Landscape of endometrial cancer: Molecular mechanisms, biomarkers, and target therapy. Cancers (Basel) 16: 2027, 2024.
8. Tuninetti V, Farolfi A, Rognone C, Montanari D, De Giorgi U and Valabrega G: Treatment strategies for advanced endometrial cancer according to molecular classification. Int J Mol Sci 25: 11448, 2024.
9. Baker-Rand H and Kitson SJ: Recent advances in endometrial cancer prevention, early diagnosis and treatment. Cancers (Basel) 16: 1028, 2024.
10. Singla RK, Behzad S, Khan J, Tsagkaris C, Gautam RK, Goyal R, Chopra H and Shen B: Natural kinase inhibitors for the treatment and management of endometrial/uterine cancer: Preclinical to clinical studies. Front Pharmacol 13: 801733, 2022.
11. Deng R, Zong GF, Wang X, Yue BJ, Cheng P, Tao RZ, Li X, Wei ZH and Lu Y: Promises of natural products as clinical applications for cancer. Biochim Biophys Acta Rev Cancer 1880: 189241, 2025.
12. Bhutta ZA and Choi KC: Phytochemicals as novel therapeutics for triple-negative breast cancer: A comprehensive review of current knowledge. Phytother Res 39: 364-396, 2025.
13. Jang JY, Kim D, Im E and Kim ND: Therapeutic potential of pomegranate extract for women's reproductive health and breast cancer. Life (Basel) 14: 1264, 2024.
14. Dehelean CA, Marcovici I, Soica C, Mioc M, Coricovac D, Iurciuc S, Cretu OM and Pinzaru I: Plant-derived anticancer compounds as new perspectives in drug discovery and alternative therapy. Molecules 26: 1109, 2021.
15. Kangra K, Kakkar S, Mittal V, Kumar V, Aggarwal N, Chopra H, Malik T and Garg V: Incredible use of plant-derived bioactives as anticancer agents. RSC Adv 15: 1721-1746, 2025.
16. Bhutta ZA, Go RE and Choi KC: Effect of punicalagin on the autophagic cell death in triple-negative breast cancer cells. Toxicol Res 40: 585-598, 2024.
17. Berdowska I, Matusiewicz M and Fecka I: Punicalagin in cancer prevention-via signaling pathways targeting. Nutrients 13: 2733, 2021.
18. Zhang H, Wang M, Yu G, Pu J, Tian K, Tang X, Du Y, Wu H, Hu J, Luo X, *et al*: Comparative analysis of the phenolic contents and antioxidant activities of different parts of two pomegranate (*Punica granatum* L.) cultivars: 'Tunisia' and 'Qingpi'. Front Plant Sci 14: 1265018, 2023.
19. Subkorn P, Norkaew C, Deesrisak K and Tanyong D: Punicalagin, a pomegranate compound, induces apoptosis and autophagy in acute leukemia. PeerJ 9: e12303, 2021.
20. Sun DP, Uen YH, Kang NW, Chang CC, Tian YF, Fang CL and Lin KY: Punicalagin restricts growth, promotes apoptosis, and reduces invasion in human gastric cancer cells. Dose Response 22: 15593258241264954, 2024.
21. Tang J, Li B, Hong S, Liu C, Min J, Hu M, Li Y, Liu Y and Hong L: Punicalagin suppresses the proliferation and invasion of cervical cancer cells through inhibition of the beta-catenin pathway. Mol Med Rep 16: 1439-1444, 2017.
22. Huang M, Wu K, Zeng S, Liu W, Cui T, Chen Z, Lin L, Chen D and Ouyang H: Punicalagin inhibited inflammation and migration of fibroblast-like synoviocytes through NF-κB pathway in the experimental study of rheumatoid arthritis. J Inflamm Res 14: 1901-1913, 2021.
23. Hussein L, Gouda M and Buttar HS: Pomegranate, its components, and modern deliverable formulations as potential botanicals in the prevention and treatment of various cancers. Curr Drug Deliv 18: 1391-1405, 2021.
24. Jalali P, Shahmoradi A, Samii A, Mazloomnejad R, Hatamnejad MR, Saeed A, Namdar A and Salehi Z: The role of autophagy in cancer: from molecular mechanism to therapeutic window. Front Immunol 16: 1528230, 2025.
25. Su Z, Yang Z, Xu Y, Chen Y and Yu Q: Apoptosis, autophagy, necroptosis, and cancer metastasis. Mol Cancer 14: 48, 2015.
26. Hallas-Potts A, Dawson JC and Herrington CS: Ovarian cancer cell lines derived from non-serous carcinomas migrate and invade more aggressively than those derived from high-grade serous carcinomas. Sci Rep 9: 5515, 2019.
27. An X, Yu W, Liu J, Tang D, Yang L and Chen X: Oxidative cell death in cancer: mechanisms and therapeutic opportunities. Cell Death Dis 15: 556, 2024.
28. Huang T, Zhang X and Wang H: Punicalagin inhibited proliferation, invasion and angiogenesis of osteosarcoma through suppression of NF-κB signaling. Mol Med Rep 22: 2386-2394, 2020.
29. Devor EJ, Gonzalez-Bosquet J, Thiel KW and Leslie KK: Genomic characterization of five commonly used endometrial cancer cell lines. Int J Oncol 57: 1348-1357, 2020.
30. Pan L, Duan Y, Ma F and Lou L: Punicalagin inhibits the viability, migration, invasion, and EMT by regulating GOLPH3 in breast cancer cells. J Recept Signal Transduct Res 40: 173-180, 2020.
31. Berköz M and Krośniak M: Punicalagin induces apoptosis in A549 cell line through mitochondria-mediated pathway. Gen Physiol Biophys 39: 557-567, 2020.
32. Omar U, Aloqbi A, Yousr M and Howell NK: Effect of punicalagin on human colon cancer caco-2 cells. Malays J Nutr 22: 125-136, 2016.
33. Wang SG, Huang MH, Li JH, Lai FI, Lee HM and Hsu YN: Punicalagin induces apoptotic and autophagic cell death in human U87MG glioma cells. Acta Pharmacol Sin 34: 1411-1419, 2013.
34. Sun DP, Huang HY, Chou CL, Cheng LC, Wang WC, Tian YF, Fang CL and Lin KY: Punicalagin is cytotoxic to human colon cancer cells by modulating cell proliferation, apoptosis, and invasion. Hum Exp Toxicol 42: 9603271231213979, 2023.
35. Cheng X, Gao Y, Yao X, Yu H, Bao J, Guan H, Sun Y and Zhang L: Punicalagin induces apoptosis-independent autophagic cell death in human papillary thyroid carcinoma BCPAP cells. RSC Adv 6: 68485-68493, 2016.
36. Xie X, Hu L, Liu L, Wang J, Liu Y, Ma L, Sun G, Li C, Aisa HA and Meng S: Punicalagin promotes autophagic degradation of human papillomavirus E6 and E7 proteins in cervical cancer through the ROS-JNK-BCL2 pathway. Transl Oncol 19: 101388, 2022.
37. Chen XX, Khyeam S, Zhang ZJ and Zhang KY: Granatin B and punicalagin from Chinese herbal medicine pomegranate peels elicit reactive oxygen species-mediated apoptosis and cell cycle arrest in colorectal cancer cells. Phytomedicine 97: 153923, 2022.