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ORIGINAL RESEARCH

Ellagic Acid Mediates the Delay of Dermal Fibroblast Senescence via CSNK2A1

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Objective: This research seeks to explore the impact of Ellagic Acid (EA) on the aging process of human dermal fibroblasts Hs68 cells and to uncover the mechanisms involved.

Methods: Senescence was induced in Hs68 cells with H_2O_2, followed by treatment with EA and CSNK2A1 inhibitor (Silmitasertib). Bioinformatics identified EA's downstream targets. Cell viability was assessed by MTT assays, and senescence markers (γH2AX, p16, p19, p53), CSNK2A1, Nrf2, and NF-κB p65 were analyzed by Western blot. Inflammatory cytokines (IL-6, TNF-α, IL-1β) and oxidative stress markers (SOD, MDA, GSH/GSSG) were measured. ROS levels were assessed by fluorescence staining, senescence by SA-β-gal staining, cell cycle by flow cytometry and apoptosis by TUNEL assay.

Results: Senescent cells showed increased γH2AX, p16, p19, and p53 expression, with reduced viability. EA inhibited senescence in a dose-dependent manner, with cytotoxicity at 60 μM. EA upregulated CSNK2A1, decreased β-galactosidase activity, restored cell viability and cycle progression, and reduced apoptosis. EA alleviated oxidative stress by enhancing Nrf2 expression, reducing ROS and MDA, and increasing SOD and GSH/GSSG. Silmitasertib negated these effects. EA also reduced IL-6, TNF-α, and IL-1β, inhibiting NF-κB p65, with anti-inflammatory effects mediated by CSNK2A1.

Conclusion: EA delays dermal fibroblast senescence by modulating CSNK2A1, mitigating oxidative stress and inflammation, and may serve as a potential therapeutic for aging and age-related diseases.

Keywords: ellagic acid, CSNK2A1, cellular aging, oxidative stress, inflammatory response

Introduction

Cellular senescence is an inevitable physiological process linked to organismal aging, marked by an irreversible halt in cell proliferation after limited divisions.[1] It plays a vital role in tissue repair but is associated with age-related diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders.[2–4] Senescent cells lose proliferative capacity and exhibit metabolic abnormalities, with increased expression of markers like p16, p21, p53, and γH2AX, indicating significant intracellular and extracellular changes.[5] Oxidative stress and inflammation are major contributors to senescence;[6,7] as the antioxidant defense system weakens with age, elevated reactive oxygen species (ROS) levels lead to DNA, protein, and lipid damage, triggering cellular dysfunction and senescence.[8] Additionally, chronic inflammation through the senescence-associated secretory phenotype (SASP) accelerates tissue and organ aging.[9] These factors form the complex biological basis of cellular senescence.

Recent advances in aging research have highlighted natural compounds with potential anti-senescence effects.[10] Ellagic acid (EA), a polyphenol abundant in fruits and nuts such as pomegranates and walnuts, is noted for its antioxidant, anti-inflammatory, and anticancer properties.[11] Studies show EA can mitigate cellular damage and delay aging by neutralizing ROS and modulating inflammatory responses.[12,13] However, while EA's protective effects against oxidative stress-induced senescence are known, the precise molecular mechanisms remain unclear. Given EA's properties, investigating its regulatory mechanisms in senescent cells is a crucial scientific inquiry.

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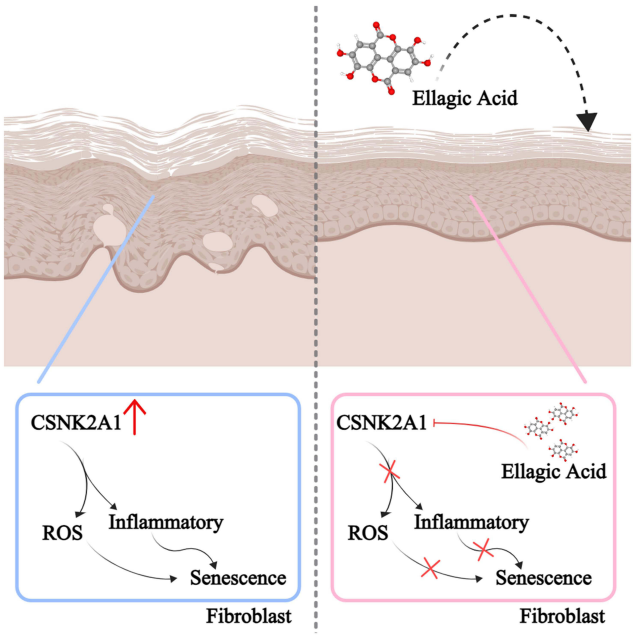
Graphical Abstract



To explore EA's mechanisms of action, we analyzed its interactions with potential target proteins using the STITCH database, identifying Casein Kinase 2 Alpha 1 (CSNK2A1) as a strongly interacting target. CSNK2A1, a conserved serine/threonine protein kinase, is integral to cell cycle regulation, DNA repair, apoptosis, and transcriptional control.[14,15] It serves as a central regulator in intracellular signaling pathways and its dysregulation is implicated in cellular senescence and neurodegenerative diseases.[16,17] Additionally, CSNK2A1 mediates cellular responses to stress, influencing cell adaptation to external stimuli.[18] Therefore, we hypothesize that CSNK2A1 may be a potential target for EA in inhibiting cellular senescence.

This study examines EA's effects on hydrogen peroxide (H_2O_2)-induced senescence in Hs68 cells, focusing on CSNK2A1-mediated mechanisms. Using an in vitro model, we assessed EA's impact on senescence, oxidative stress, and inflammation. Our findings reveal EA's potential as an anti-senescence agent, offering insights for age-related disease interventions and anti-aging therapies with natural compounds.

Methods

Reagents and Instruments

Reagents and instruments used in this study are listed in Table 1 and Table 2 respectively.

Cell Culture and Treatment

Human dermal fibroblast cell line Hs68 (B164789) was procured from Mingzhoubio (Ningbo, China) and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin in a 37°C, 5% CO2 incubator. To establish a senescence model, cells were exposed to H_2O_2 (0, 100, 250, 500 nM) for 2 hours.[19] Following oxidative stress induction, cells were treated with EA (0–100 μM) for 24 hours and/or with Silmitasertib (25 μM) for 48 hours.[20] Both EA and Silmitasertib were dissolved in dimethyl sulfoxide (DMSO), and an equal amount of DMSO was used as a negative control (NC). The study did not implement any type of blind procedure and the overall flow chart of the method is shown in Figure S1.

Table 1 Reagent Information

Reagent	Catalog	Company	Location
FBS	A5670701	Thermo Fisher Scientific	MA, USA
Penicillin-Streptomycin (10,000 U/mL)	15140122	Thermo Fisher Scientific	MA, USA
DMEM	11965092	Thermo Fisher Scientific	MA, USA
H_2O_2	88597	Sigma-Aldrich	St. Louis, MO, USA
Ellagic Acid (EA)	HY-B0183	MedChemExpress	New Jersey, USA
Silmitasertib	HY-50855	MedChemExpress	New Jersey, USA
DMSO	D2650	Sigma-Aldrich	St. Louis, MO, USA
MTT Solution	HY-15924	MedChemExpress	New Jersey, USA
PBS	AM9624	Thermo Fisher Scientific	MA, USA
SOD Assay Kit	19160	Sigma-Aldrich	St. Louis, MO, USA
MDA Assay Kit	ab238537	Abcam	Cambridge, UK
GSH/GSSG Ratio Kit	ab138881	Abcam	Cambridge, UK
IL-6 ELISA Kit	EZIL6	Sigma-Aldrich	MO, USA
TNF-α ELISA Kit	EZHTNFA-150K	Sigma-Aldrich	MO, USA
IL-1β ELISA Kit	ab214025	Abcam	Cambridge, UK
RIPA Lysis Buffer	ab170197	Abcam	Cambridge, UK
BCA Protein Assay Kit	P0010	Beyotime	Shanghai, China
PVDF Membrane	ab133411	Abcam	Cambridge, UK
BSA	V900933	Sigma-Aldrich	MO, USA
CSNK2A1 (1:500)	ab76040	Abcam	Cambridge, UK
Nrf2 (1:200)	ab62352	Abcam	Cambridge, UK
NF-κB p65 (1:1000)	ab288751	Abcam	Cambridge, UK
GAPDH (1:10000)	ab181602	Abcam	Cambridge, UK
HRP-Conjugated Secondary Antibody (1:1000)	ab6721	Abcam	Cambridge, UK
TBST Solution	ZS405-3	Zomanbio	Beijing, China
DCFH-DA	HY-D0940	MedChemExpress	New Jersey, USA
Paraformaldehyde (4%)	MA0192	Meilune	Dalian, China
Immunostaining Permeabilization (Triton X-100)	P0096	Beyotime	Shanghai, China
Goat Serum	C0265	Beyotime	Shanghai, China
DAPI	D9542	Sigma-Aldrich	St. Louis, MO, USA
Anti-Fluorescence Quenching Mounting Medium	F4680	Sigma-Aldrich	MO, USA
RNase	EN0531	Thermo Fisher Scientific	MA, USA
Propidium Iodide	HY-D0815	MedChemExpress	New Jersey, USA
TUNEL Cell Apoptosis Detection Kit	C1086	Beyotime	Shanghai, China
TBST Solution	ZS405-3	Zomanbio	Beijing, China
DCFH-DA	HY-D0940	MedChemExpress	New Jersey, USA
Paraformaldehyde (4%)	MA0192	Meilune	Dalian, China
Immunostaining Permeabilization (Triton X-100)	P0096	Beyotime	Shanghai, China
Goat Serum	C0265	Beyotime	Shanghai, China
DAPI	D9542	Sigma-Aldrich	St. Louis, MO, USA
Anti-Fluorescence Quenching Mounting Medium	F4680	Sigma-Aldrich	MO, USA
RNase	EN0531	Thermo Fisher Scientific	MA, USA
Propidium Iodide	HY-D0815	MedChemExpress	New Jersey, USA
TUNEL Cell Apoptosis Detection Kit	C1086	Beyotime	Shanghai, China

3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyl Tetrazolium Bromide (MTT)

Hs68 cells were seeded into 96-well plates (1×10^4 cells/well). Upon reaching 70% confluence, a total of 10 μL of MTT solution [5 mg/mL prepared in phosphate buffer saline (PBS)] was added to each well and incubated with cells at 37°C for 4 hours. After removing the medium, 100 μL of DMSO was added to solubilize formazan crystals. Plates were gently

Table 2 Instrument/Software

Instrument/Software	Model/Catalog	Company	Location
ELISA Reader	1410101	Thermo Fisher Scientific	MA, USA
Fluorescence Microscope	DM2500	Leica	Wetzlar, Germany
Chemiluminescence Imaging System	BeyoECL Star (A38554)	Thermo Fisher Scientific	MA, USA
Image Analysis Software	ImageJ V1.8.0.112	NIH	Madison, WI, USA
Flow Cytometer	A24858	Thermo Fisher Scientific	MA, USA
Flow Cytometry Software	FlowJo v10.8.2	BD	Ashland, Oregon, USA
Graphing Software	GraphPad Prism 9.0	Dotmatics	Boston, MA, USA

agitated for 10 minutes. Absorbance was measured at 560 nm using an enzyme-linked immunosorbent assay (ELISA) reader.

Biochemical Analysis

Cell culture supernatants were collected to determine levels of superoxide dismutase (SOD), malondialdehyde (MDA), glutathione (reduced/oxidized) (GSH/GSSG) ratio, Interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNF-α), and Interleukin-1 beta (IL-1β) in the samples using respective commercial assay kits following the manufacturers' instructions. Absorbance was measured using a microplate reader.

Western Blotting (WB)

Total proteins were extracted from Hs68 cells using radioimmunoprecipitation assay lysis buffer, and protein concentration was quantified using the bicinchoninic acid (BCA) Protein Assay Kit. Proteins were separated by SDS-PAGE and transferred onto polyvinylidene fluoride membranes. Membranes were blocked with 10% goat serum solution and incubated overnight at 4°C with primary antibodies against CSNK2A1, Nrf2, nuclear factor kappa B (NF-κB) p65, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Following this, membranes were incubated with HRP-conjugated secondary antibodies at room temperature for 2 hours. Bands were visualized using BeyoECL Star chemiluminescence substrate and quantified using ImageJ software.

Detection of ROS

Cells were incubated with 10 μM 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) at room temperature in the dark for 30 minutes. After washing, cells were mounted with anti-fade mounting medium and visualized under a fluorescence microscope. Fluorescence intensity was quantified using ImageJ software.

Senescence-Associated β-Galactosidase (SA-β-Gal) Staining

Cells were fixed with 0.6 mL of fixative buffer for 15 minutes at room temperature and then incubated with SA-β-gal staining solution at 37°C overnight. After rinsing with PBS, stained cells were observed under a microscope. The percentage of SA-β-Gal positive cells (blue-green) was calculated based on total cell count.

Flow Cytometry

Cells were resuspended in PBS with RNase A (100 μg/mL) and incubated at 37°C for 30 minutes. Next, propidium iodide (PI; 50 μg/mL) staining solution was incubated with cells in the dark at room temperature for 30 minutes. PI staining fluorescence signals were analyzed using a flow cytometer, and cell cycle distribution was determined with FlowJo software.

Terminal Deoxynucleotidyl Transferase dUTP Nick-End Labeling (TUNEL)

Cells were fixed with 4% paraformaldehyde for 30 minutes, permeabilized with 0.1% Triton X-100 in PBS for 2 minutes on ice, and incubated with 50 μL TUNEL solution at 37°C in the dark for 60 minutes. Cells were counterstained with

DAPI working solution (1 μg/mL) and mounted with anti-fade mounting medium. Apoptotic nuclei were visualized under a fluorescence microscope. The apoptosis rate was quantified using ImageJ software.

Statistical Analysis

Statistical analysis was conducted using GraphPad Prism 9 software, with data presented as mean ± standard deviation (SD). A *t*-test was used for comparisons between two groups. For comparisons involving three or more groups, one-way or two-way analysis of variance (ANOVA) was applied, followed by Tukey's post hoc test. A difference was deemed significant if $p < 0.05$.

Results

EA Effectively Inhibits the Aging of Hs68 Cells

To explore the impact of EA on aging cells, we initially assessed the expression of senescence-associated proteins and observed alterations in cell viability. H_2O_2 is commonly used to induce cellular aging; thus, we established an in vitro aging model of Hs68 cells using this method. After induction with H_2O_2 (100 nM, 250 nM, 500 nM), the expression of p16, p19, p53, and γH2AX proteins increased (Figure 1A), and cell viability decreased (Figure 1B), indicating successful establishment of the aging model, with the 250 nM concentration showing the most effective induction of aging cells. We then evaluated the effects of EA (1–100 μM). MTT assay results indicated that EA reduced cellular aging in

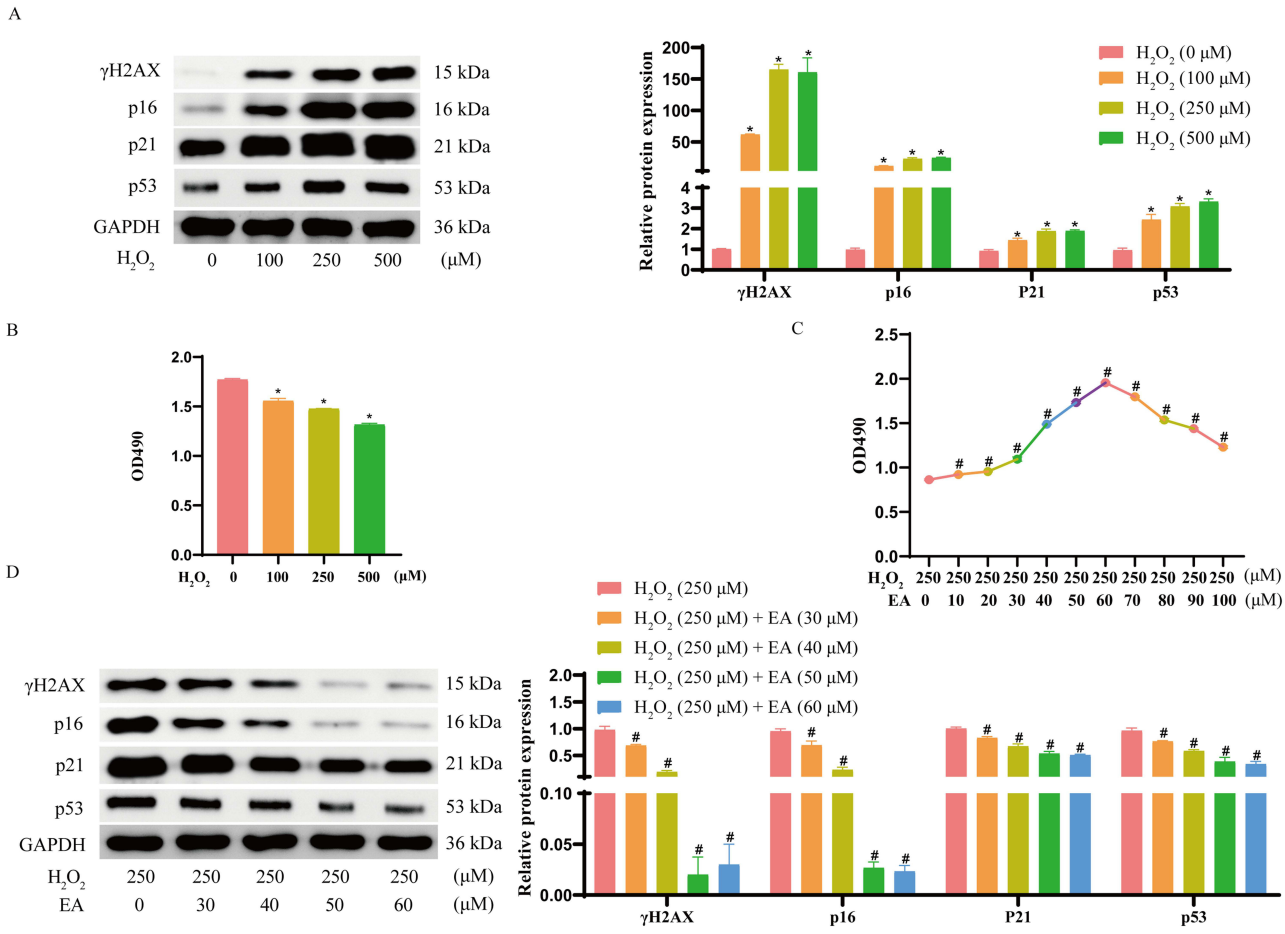


Figure 1 Ellagic acid effectively inhibits senescence in Hs68 cells. Cells were treated with H_2O_2. (**A**) Western blot analysis of p16, p21, p53, and γH2AX protein expression. (**B**) MTT assay for cell viability. Cells were treated with H_2O_2 and Ellagic acid. (**C**) MTT assay for cell viability. (**D**) Western blot analysis of p16, p21, p53, and γH2AX protein expression. *represents $p < 0.05$ versus the H_2O_2 (0 μM) group; # represents $p < 0.05$ versus the H_2O_2 (250 μM) group.

Abbreviations: γH2AX, Phosphorylated H2A Histone Family Member X; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; H_2O_2, Hydrogen Peroxide; GAPDH, Glyceraldehyde-3-Phosphate Dehydrogenase.

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a concentration-dependent fashion, with cell toxicity becoming evident at concentrations of 60 μM and higher (Figure 1C). Western blot results demonstrated that EA significantly inhibited the expression of senescence-associated proteins in the aging Hs68 model. These data suggest that EA effectively inhibits the aging of Hs68 cells (Figure 1D).

EA Significantly Promotes the Expression of CSNK2A1 Protein, Which is Downregulated Due to Aging

To elucidate the mechanisms by which EA inhibits cellular aging, we first explored downstream target factors of EA through bioinformatics analysis. Using the STITCH database (http://stitch.embl.de/cgi/input.pl?UserId=5d5hKidRT7HO&sessionId=YA0fPIZobXLl), we identified that EA has a strong binding affinity with CSNK2A1 (Figure S2). Furthermore, CSNK2A1 dysregulation is closely associated with cellular senescence.[16] Subsequently, we assessed the expression of CSNK2A1 protein by Western blot. The results showed that CSNK2A1 is significantly downregulated in aging cells (Figure 2A), while EA (30, 40, 50, 60 μM) significantly enhances the expression of CSNK2A1 in aging cells, with the 50 μM concentration showing the most pronounced effect (Figure 2B). These data suggest that EA may inhibit cellular senescence in a dose-dependent manner by promoting the expression of CSNK2A1.

EA Mediates the Delay of Cellular Aging by Regulating CSNK2A1

In H_2O_2-treated cells, we observed increased β-galactosidase activity (Figure 3A), cell cycle arrest in the G1 phase (Figure 3B), decreased cell viability (Figure 3C), and elevated apoptosis (Figure 3D), all markers of cellular aging. Treatment with EA significantly reduced β-galactosidase activity, restored normal cell cycle progression and viability, and inhibited apoptosis in senescent Hs68 cells (Figure 3A–D). Furthermore, the addition of Silmitasertib, an established

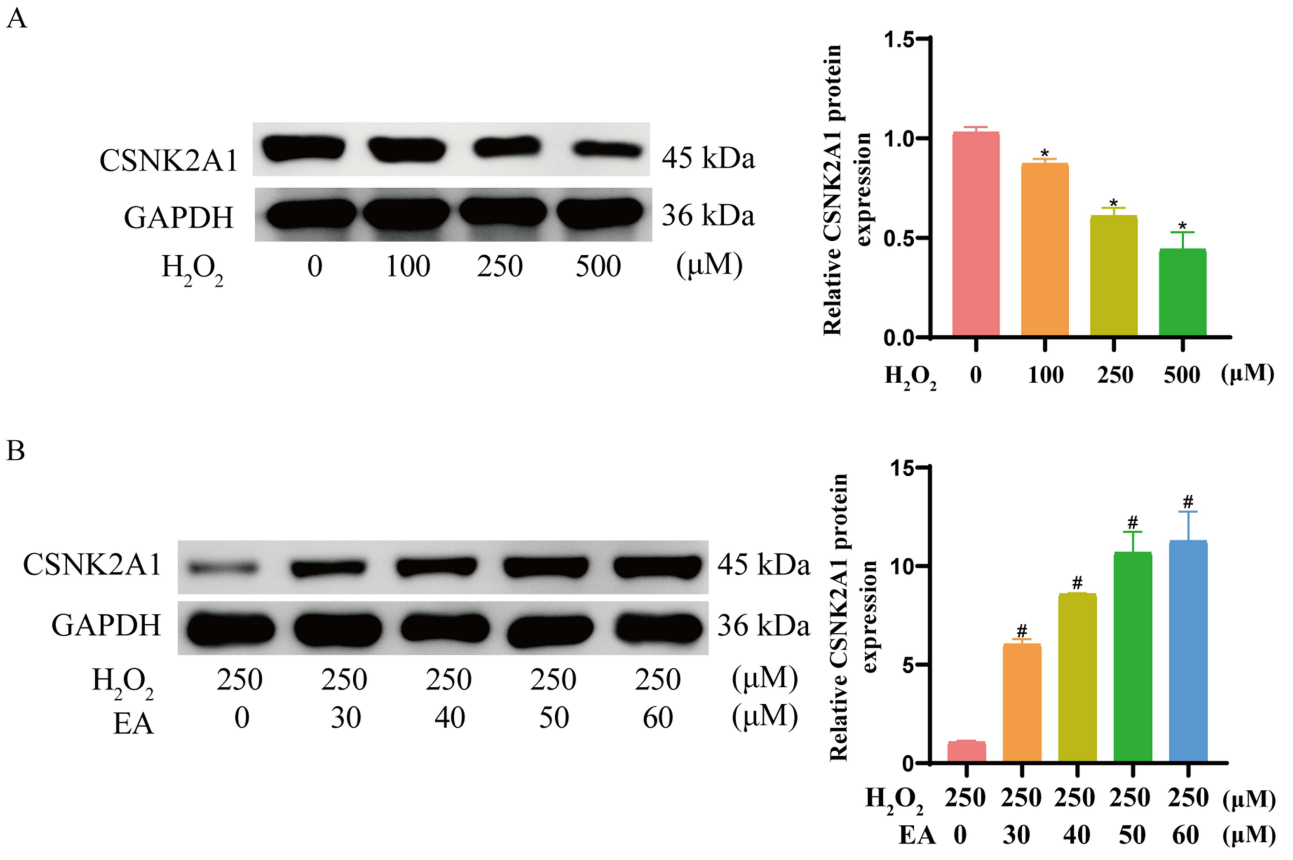

Figure 2 Ellagic acid upregulates CSNK2A1 protein expression. (**A** and **B**) Western blot analysis of CSNK2A1 expression levels. *represents $p < 0.05$ versus the H_2O_2 (0 μM) group; #represents $p < 0.05$ versus the H_2O_2 (250 μM) group.
Abbreviations: CSNK2A1, Casein Kinase II Subunit Alpha; H_2O_2, Hydrogen Peroxide; GAPDH, Glyceraldehyde-3-Phosphate Dehydrogenase.

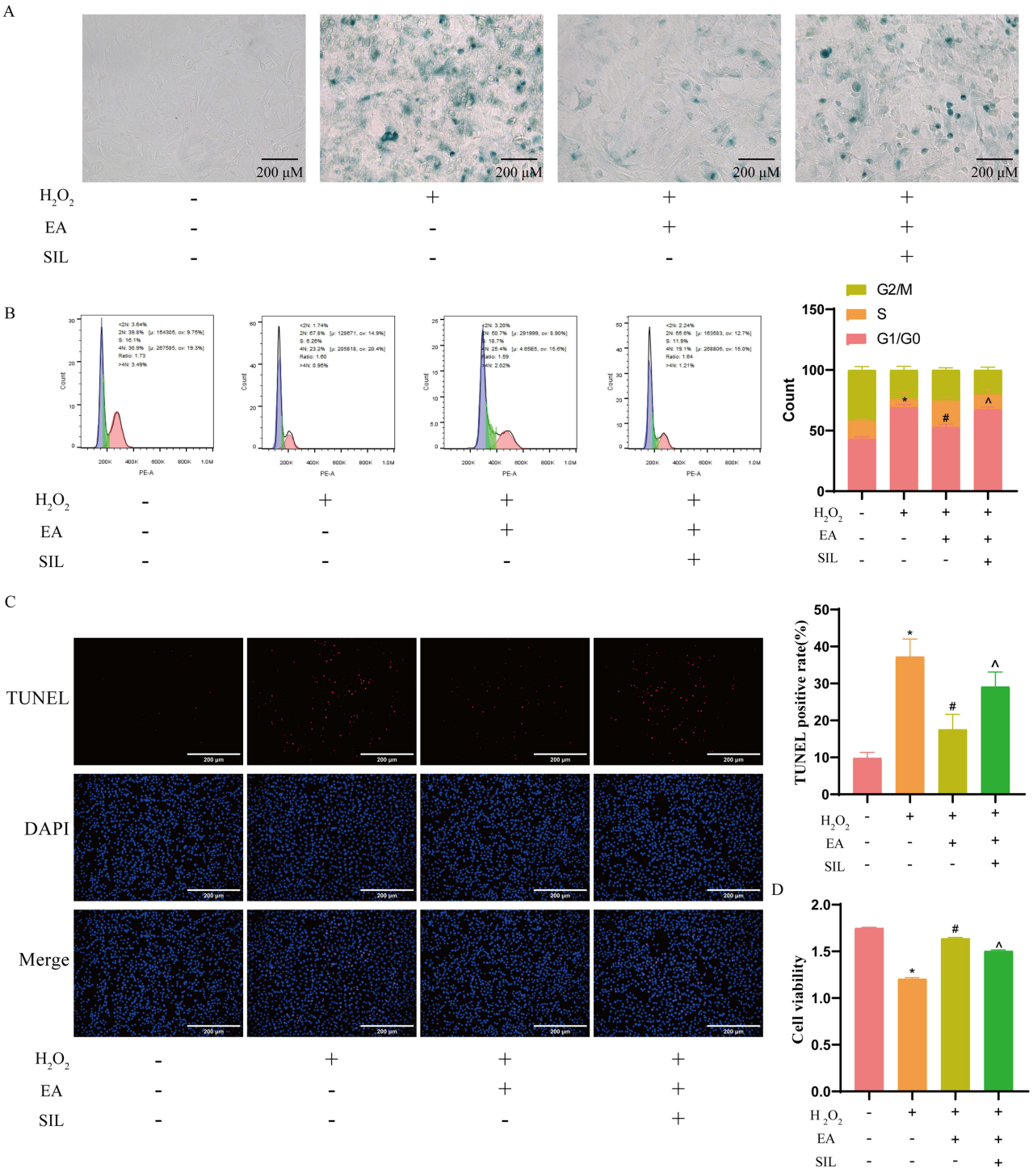

Figure 3 Ellagic acid mediates CSNK2A1 to delay cellular senescence. (**A**) SA-β-gal staining to detect senescence-associated β-galactosidase activity. (**B**) Flow cytometry analysis of cell cycle. (**C**) TUNEL assay for apoptosis detection. (**D**) MTT assay for cell viability. *represents $p < 0.05$ versus the untreated group; #represents $p < 0.05$ versus the H_2O_2 group; ^represents $p < 0.05$ versus the H_2O_2 + EA group.

Abbreviations: SIL, Silmitasertib; H_2O_2, Hydrogen Peroxide; CSNK2A1, Casein Kinase II Subunit Alpha; TUNEL, Terminal Deoxynucleotidyl Transferase dUTP Nick-End Labeling; GAPDH, Glyceraldehyde-3-Phosphate Dehydrogenase.

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CSNK2A1 inhibitor, nullified EA's anti-aging effects, indicating that EA delays cellular aging, at least in part, through the upregulation of CSNK2A1 (Figure 3A–D).

EA-Mediated CSNK2A1 Alleviates Oxidative Stress in Aging Cells

Oxidative stress is a key driver of cellular aging, and in H_2O_2-treated cells, we observed elevated ROS levels (Figure 4A), decreased SOD activity, increased MDA levels, a lowered GSH/GSSG ratio (Figure 4B), and a marked reduction in Nrf2 expression (Figure 4C)—indicating heightened oxidative stress. EA treatment significantly reduced

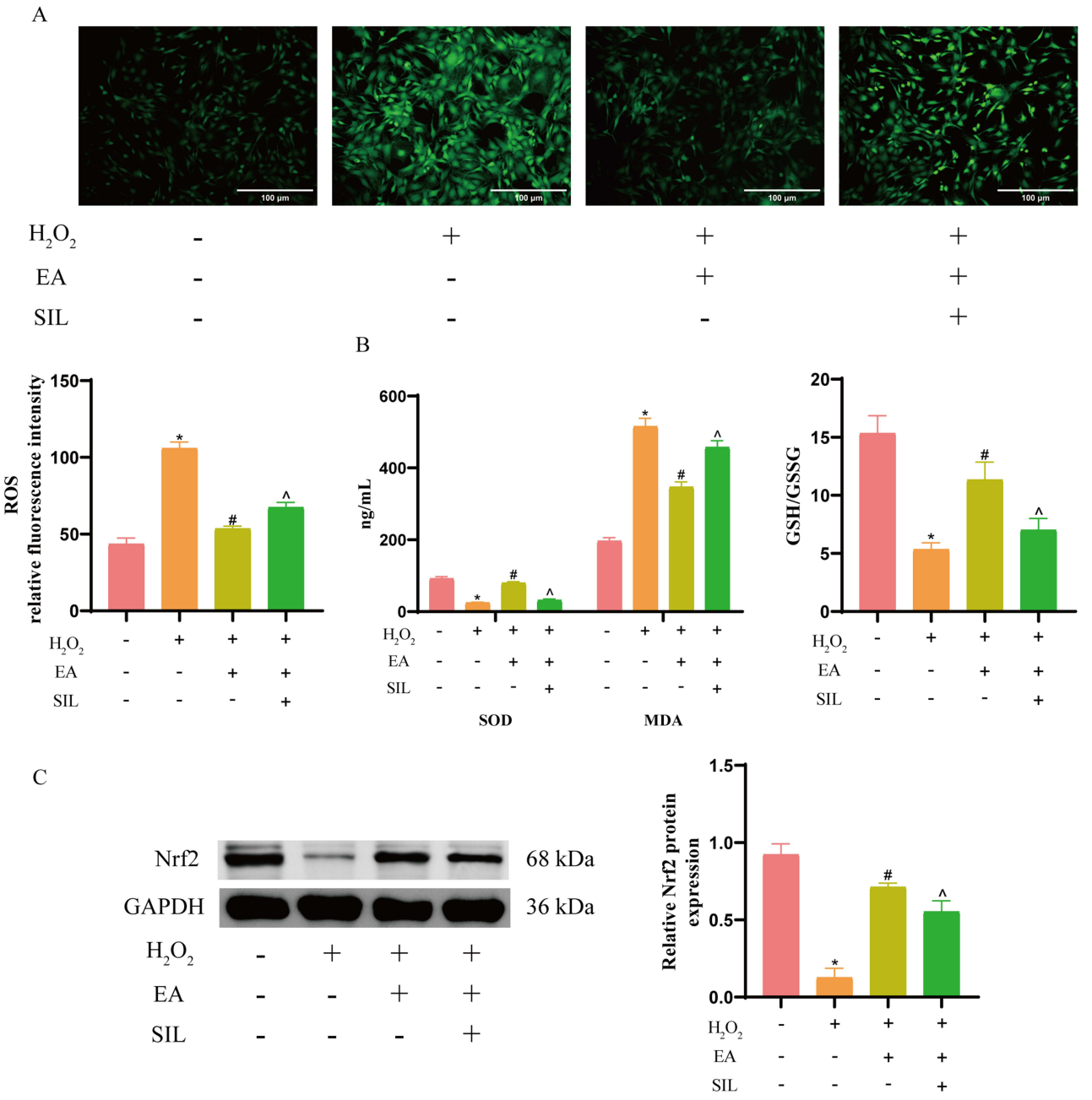

Figure 4 Ellagic acid mediates CSNK2A1 to alleviate oxidative stress in senescent cells. (**A**) Fluorescence staining to detect ROS levels. (**B**) ELISA assay to measure antioxidant enzyme SOD, oxidative stress marker MDA, and GSH/GSSG ratio. (**C**) Western blot analysis of Nrf2 levels. *represents $p < 0.05$ versus the untreated group; #represents $p < 0.05$ versus the H_2O_2 group; ^represents $p < 0.05$ versus the H_2O_2 + EA group.

Abbreviations: SIL, Silmitasertib; H_2O_2, Hydrogen Peroxide; CSNK2A1, Casein Kinase II Subunit Alpha; SOD, Superoxide Dismutase; MDA, Malondialdehyde; GSH/GSSG, Glutathione (Reduced/Oxidized); Nrf2, Nuclear Factor Erythroid 2-Related Factor 2; GAPDH, Glyceraldehyde-3-Phosphate Dehydrogenase.

ROS and MDA levels, and raised both SOD activity, the GSH/GSSG ratio increased Nrf2 expression, in aging cells (Figure 4A–C). Furthermore, using Silmitasertib abolished these antioxidant effects, suggesting that EA mitigates oxidative stress in aging cells by upregulating CSNK2A1 (Figure 4A–C).

EA-Mediated CSNK2A1 Alleviates Inflammation in Aging Cells

Inflammatory responses are a hallmark of aging cells. When assessing inflammatory markers, we discovered that in H_2O_2 -treated cells, the levels of inflammatory factors such as IL-6, TNF-α, and IL-1β were significantly elevated. However, EA notably lowered the levels of inflammatory factors in aging cells. Additionally, Silmitasertib negated the anti-inflammatory effects of EA on aging cells (Figure 5A). NF-κB is a multifunctional transcription factor, and its pathway activation is strongly linked to inflammation, with NF-κB p65 acting as a marker for this activation.[21] We found that in aging cells, NF-κB p65 expression was increased, while EA treatment significantly decreased NF-κB p65 levels in these cells (Figure 5B). These data suggest that EA alleviates inflammation in aging cells to some extent by promoting CSNK2A1 expression and inhibiting NF-κB pathway activation.

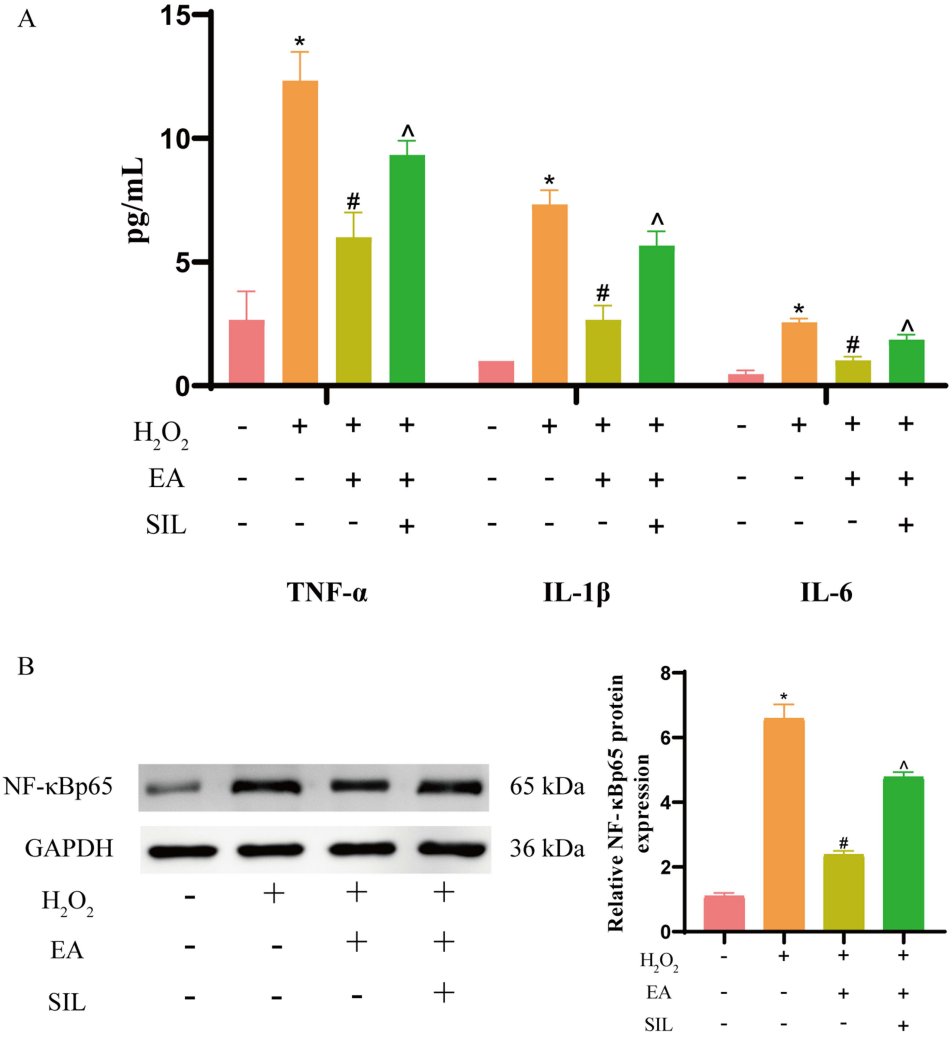

Figure 5 Ellagic acid mediates CSNK2A1 to mitigate inflammatory response in senescent cells. (**A**) ELISA assay to detect inflammatory factors (IL-6, TNF-α, IL-1β). (**B**) Western blot analysis of NF-κB p65 protein levels. *represents $p < 0.05$ versus the untreated group; #represents $p < 0.05$ versus the H_2O_2 group; ^represents $p < 0.05$ versus the H_2O_2 + EA group.

Abbreviations: SIL, Silmitasertib; H_2O_2, Hydrogen Peroxide; CSNK2A1, Casein Kinase II Subunit Alpha; IL-6, Interleukin-6; ELISA, Enzyme-Linked Immunosorbent Assay; TNF-α, Tumor Necrosis Factor-Alpha; IL-1β, Interleukin-1 Beta; GAPDH, Glyceraldehyde-3-Phosphate Dehydrogenase.

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Discussion

In this study, we established an Hs68 cell aging model to investigate the effects of EA on aging cells and its underlying mechanisms. We found that EA effectively inhibits H2O2-induced cellular aging. Interestingly, we observed that CSNK2A1, a protein with strong interaction with EA, is significantly downregulated in aging cells. EA can promote the expression of this protein in aging cells and influence oxidative stress and inflammation, thereby effectively inhibiting cellular aging.

Human skin aging is a gradual process driven by the accumulation of ROS, leading to fibroblast dysfunction and wrinkle development.[22] Senescent cells exhibit reduced activity and loss of division, along with increased expression of senescence markers such as p16, p21, p53, and γH2AX.[5] p16 and p21 are important cell cycle inhibitors that can promote cell cycle arrest or apoptosis.[23] p53, known as the "guardian of the genome", is an upstream factor of p21 and plays a crucial role in the cell's response to DNA damage and other stress responses.[24] γH2AX is commonly used as a marker of DNA damage and is an important phenomenon in the cellular aging process.[25] H_2O_2 is a common inducer of cellular aging models and can effectively simulate aging mechanisms associated with oxidative stress.[26] In this study, H_2O_2 was effectively employed to create an in vitro aging model in Hs68 cells, which was indicated by decreased cell viability and heightened levels of senescence markers such as p16, p21, p53, and γH2AX. Previous research has shown that phenolic compounds like EA can counteract aging by neutralizing free radicals and exhibiting both antioxidant and anti-inflammatory effects.[27] In this study, EA treatment notably reduced the levels of p16, p21, p53, and γH2AX in the aging cell model. These findings reinforce the anti-aging properties of EA. While its anti-aging benefits have been documented, the specific molecular mechanisms behind these effects are still not fully understood.

To clarify how EA produces its anti-aging effects, we began by searching the STITCH database, which led us to identify CSNK2A1 as a protein that interacts strongly with EA. We then investigated CSNK2A1 as a potential downstream target of EA. CSNK2A1 is a serine/threonine protein kinase essential for various cellular functions, including proliferation, transcription, apoptosis, DNA repair, and cell cycle control.[28,29] Numerous studies have indicated that CSNK2A1 is downregulated in aging cells. Research by Li et al found that downregulation of CSNK2A1 induces cellular aging,[30] while Song et al. It has been shown that reducing CSNK2A1 levels triggers the expression of factors associated with the senescence-associated secretory phenotype.[31] Consistent with these findings, our study also observed a significant downregulation of CSNK2A1 in aging cells. Interestingly, EA was found to significantly inhibit the downregulation of CSNK2A1 in aging cells. However, whether EA acts on aging cells through this target remains unclear. Silmitasertib (CX-4945) is a targeted inhibitor of CSNK2A1. Further investigation revealed that EA significantly reduced β-galactosidase activity, restored cell cycle and cell viability, and decreased apoptosis in aging cells. However, the use of Silmitasertib abolished the anti-aging effects of EA on aging cells. This further confirms that EA inhibits cellular aging through CSNK2A1. Nevertheless, this study was limited to a single cell line and lacked in vivo experiments. Future research should consider using multiple cell lines and further validate the effects of EA in vivo models.

Oxidative stress is a crucial factor driving cellular aging, with Nrf2 serving as an important regulator of oxidative stress.[32] Xu et al found that VDR mitigates osteoblast aging by activating the Nrf2 pathway.[33] EA, a polyphenolic compound with antioxidant potential, has been reported to have therapeutic potential in various diseases, including myocardial injury, renal damage, and Parkinson's disease.[34,35] CSNK2A1, the alpha subunit of CSNK2, has been demonstrated to activate Nrf2 by promoting the autophagic degradation of Keap1 and stimulating AMPK.[36] However, whether EA alleviates oxidative stress in aging cells through CSNK2A1 remains unclear. In this study, H2O2-treated aging cells exhibited downregulation of Nrf2 protein expression, a significant increase in ROS levels, reduced SOD activity, and elevated oxidative stress markers MDA. EA notably counteracted these effects by reinstating Nrf2 expression, lowering ROS and MDA levels, and enhancing SOD activity. These results indicate that EA mitigates cellular aging through mechanisms related to oxidative stress. Notably, when using the CSNK2A1-targeting inhibitor Silmitasertib, the antioxidant effects of EA were partially reversed, further supporting the role of CSNK2A1 in alleviating oxidative stress in aging cells induced by EA.

Additionally, inflammatory responses also play a critical role in cellular aging.[7] We observed that in H2O2-induced senescent cells, the expression of pro-inflammatory factors such as IL-6, TNF-α, and IL-1β was significantly elevated, while treatment with EA led to a marked reduction in these inflammatory factors. Furthermore, the use of Silmitasertib partially inhibited the anti-inflammatory effects of EA, suggesting that CSNK2A1 may exert its anti-aging effects by regulating inflammatory responses. The NF-κB signaling pathway is a key regulatory mechanism in inflammation.[37] Our results indicate that EA, by upregulating CSNK2A1, inhibits the activation of the NF-κB pathway, thereby mitigating the inflammatory response in senescent cells.

However, one limitation of this study is the exclusive use of a single cell line, which may not fully capture the complexity and heterogeneity of the biological processes involved. Additionally, the absence of in vivo validation restricts the generalizability of the findings to physiological conditions. Future studies involving multiple cell lines and animal models are warranted to confirm and extend these results.

Conclusion

In summary, EA effectively boosts CSNK2A1 expression in senescent cells, mitigates oxidative stress and inflammation, and consequently slows down the aging of dermal fibroblasts. As such, EA holds potential as a promising candidate for clinical treatment of aging and age-related diseases in the future.

Data Sharing Statement

The data used and analyzed during the current study are available from the corresponding author.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors state that they have no financial or commercial ties to other entities that could be seen as a conflict of interest in the research.

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