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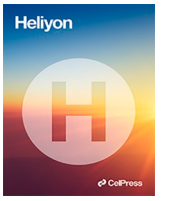

Research article

Melatonin alleviates aging-related heart failure through melatonin receptor 1A/B knockout in mice

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ABSTRACT

Age-related cardiovascular diseases continue to be important issues that contribute to the societal burden. Unveiling the molecular mechanisms underlying age-related cardiovascular diseases provides novel opportunities to delay aging and facilitate early disease diagnosis and treatment. This study utilized knockout mice lacking melatonin receptors type 1A (MT1) and 1B (MT2). Ultrasonography, pathological staining, and transcriptomics were used to investigate the role of MT1/2 in the hearts of aging mice. Knockout of both receptors decreased ejection fraction and exacerbated fibrosis, inflammation, oxidative stress, and apoptosis levels in aging mice. Our findings indicated that the cardiac function of MT1 knockout mice was more severely affected than that of MT2 knockout mice. Additionally, we observed that intraperitoneal administration of melatonin (20 mg/kg/day for 90 days) ameliorated abnormal cardiac function in aging mice. However, the absence of MT1/2 resulted in the inability of melatonin to improve cardiac function. Our study, utilizing an aging polymerase chain reaction assay and cell experiments, revealed that melatonin receptors potentially influence cardiac function in aging mice through their effects on leukocyte differentiation antigen 14 (CD14) expression. Consequently, melatonin receptors, particularly MT1, are key contributors to cardiac aging, and therapeutic interventions targeting this receptor are promising for delaying the progression of cardiac aging.

1. Introduction

Cardiovascular disease (CVD) is a primary health concern in aging populations. Aging is a major risk factor for CVD (heart failure [HF]) and plays an important role in its occurrence and development [1]. Approximately 1 % of the population over 50 years and 8.4 % over 75 is affected by HF. Cardiac aging is marked by cardiomyocyte enlargement, interstitial fibrosis, infiltration of inflammatory cells, and ongoing myocardial rigidity [2–5]. The pathogenesis of cardiac aging includes inflammatory response, oxidative stress, autophagy disorders, metabolic changes, impaired calcium regulation, activation of neuroendocrine pathways, and accumulation of senescent cells. The stimulation of pathological factors such as oxidative stress and inflammation can cause stress senescence of cardiomyocytes [6]. Studying cardiac aging and its mechanisms in older adults, investigating key factors in the process, and identifying

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novel targets for clinical interventions are essential.

Melatonin is a pleiotropic neurohormone and the most important hormone involved in the circadian rhythm [7]. Age also affects melatonin levels, with peak levels higher in neonates and declining in older adults [8]. Numerous studies have shown that melatonin has anti-aging properties [9,10]. It has a wide range of biological effects in biological rhythm, reproductive system, immune system, digestive system, central nervous system, antioxidant and anti-tumor, and has been used in hypnosis, immune regulation, anti-aging treatment [11]. In recent years, researchers have found that Melatonin has anti-inflammatory, antioxidant, blood pressure and lipid lowering effects, while Melatonin and its receptors play important roles in CVD pathogenesis, and notably, the mechanisms by which they exert their pharmacological effects are becoming more well-understood [12,13]. Melatonin receptor type l (MT1) and melatonin receptor type 2 (MT2) are the primary melatonin receptors in the human body, belonging to the A family of G-protein-coupled receptors. MT1 is encoded by the *MTNR1A* gene and has 350 amino acids. *MTNR1B* encodes MT2 and has 362 amino acids [14]. MT1 and MT2 are highly homologous, with an overall sequence similarity of 55 %, and a sequence similarity in the transmembrane region reaching 70 % [15,16]. MT1 and MT2 are most densely distributed in the human nervous system, including the hypothalamus, retina, cerebral cortex, hippocampus, midbrain, and pituitary gland. MT1 and MT2 are widely distributed in other human body organs and tissues and play important physiological roles. Although MT1 and MT2 are activated by melatonin to exert their functions, they exhibit considerable differences in their specific distributions in the human body. The higher the expression of MT1 in breast cancer cells, the stronger the sensitivity of tumor cells to melatonin [17]. Additionally, melatonin participates in the MT2-mediated RZR/RORα pathway, leading to the inhibition of colon cancer cell proliferation [18]. The melatonin receptor agonist, ramelteon, induces MT2 activation and reactive oxygen species (ROS) release for cardioprotection [19]. Melatonin membrane receptor 2 activation is a key determinant for melatonin-mediated cardioprotection in cardiac ischemia-reperfusion injury [20,21].

Animal aging has been used to explore mechanical models of cardiac aging. Rats and mice are equivalent to 85-year-old humans at 24 months [22]. However, in this study, the MT1 and MT2 knockout mice barely survived for 24 months. The survival rate was approximately 50 % at 12 months. Therefore, we selected 12-month-old mice as an aging model. Macroscopic changes in the hearts of aged mice included adventitial fat deposition, aortic valve calcification, atrial hypertrophy/dilatation, and left ventricular wall thickening. DNA double-strand breaks trigger a DNA damage response, which activates the recruitment of ataxia-telangiectasia mutated (ATM)- and ataxia telangiectasia and Rad3 (ATR)-related kinases to the site of damage and leads to Ser139 phosphorylation of histone γH2AX, which in turn activates the p53/p21 and p16/Rb signaling pathways [23,24]. Research indicates that mice with p53 overexpression have a reduced lifespan and exhibit premature aging characteristics [25]. In mice and humans, p16 expression increases with age [26].

The involvement of melatonin and its receptors in heart failure (HF) treatment has been previously investigated [27]. Administering melatonin before and after ischemia has resulted in equal levels of cardio-protection in ischemia-reperfusion-injured hearts. Its beneficial effects on cardiac arrhythmias and cardiac mitochondrial function and dynamics depend on the activation of MT2. However, aging models have not confirmed whether MT1 and MT2 play a role in HF. Thus, in this study, knockout mice were utilized to examine the significance of MT1 and MT2 in aging and to elucid the potential mechanisms through which melatonin safeguards the aging mouse heart via these receptors.

2. Materials and methods

2.1. Animals and treatment

MT1 and MT2 KO C57BL/6 male mice were purchased from the Cyagen Biosciences (Suzhou, China). Litter control and knockout mice were fed for 12 months (23–25 g). Young C57BL/6 male mice were selected litter control at 8 weeks of age (23–25 g), and purchased from Liaoning Changsheng biotechnology co., Ltd. All mice were maintained under specific pathogen-free, environmentally controlled (Temperature: 20–25 °C; Humidity: 50 ± 5 %) barrier conditions in individually ventilated cages, fed a standard rodent laboratory diet and given free access to water and food. Mice were then randomly divided into four groups: Young group (n = 6), Aging group (n = 6), MT1 group (n = 6) and MT2 group (n = 6). Nine months old mice and MT KO mice were injected Melatonin (selleck, S1204, 20 mg/kg/day) via intraperitoneal for 90 days. Mice were then randomly divided into six groups: Aging group (n = 6), Aging + melatonin group (n = 6), MT1 group (n = 6), MT1 + melatonin group (n = 6), MT2 group (n = 6) and MT2 + melatonin group (n = 6). All procedures were approved by the Animal Care and Use Committee of Dalian Medical University (AEE21079). These experiments were conducted according to established animal welfare guidelines. We performed the procedures in accord with the Guidelines. Animal handling, care, killing, and other procedures involving animals were conducted in compliance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

2.2. Echocardiography

After removing chest hair using a hair removal cream, the mice were anesthetized by inhalation of 1.5 % isoflurane (Ezvet) and their chests were coated with an ultrasound coupling gel. Mice were anesthetized and echocardiograms were performed using Vevo2100 high resolution imaging system (Visualsonic, LTD.), and then LV ejection fraction (EF%) and LV fractional shortening (FS%) were calculated [28].

2.3. Histopathology

All mice were euthanized after being anesthetized by injection of tribromoethanol (T48402, sigma-aldrich, 20 mL/kg, i.p.) following echocardiography. Normal saline was drawn into a 1 mL syringe. A puncture was then made in the left ventricle with the injection needle, which was left in place. Normal saline was slowly injected for perfusion to eliminate residual blood in the heart. Finally, the heart was removed with scissors. The heart was collected and stored at −80 °C for further experiments or fixed with 4 % paraformaldehyde for histological analysis. Heart tissues were fixed by 4 % paraformaldehyde. The fixed heart tissues were dehydrated with a series of ethanol solutions: 85 % ethanol for 40 min, followed by 90 % ethanol for another 40 min, then immersed in two changes of anhydrous ethanol for each change lasting for 40 min, subsequently treated with two changes of xylene each lasting for 40 min, further processed in liquid paraffin for 1 h to prepare paraffin blocks, and the paraffin blocks were prepared using a tissue embedding mold. The heart tissues were cut into 5 μm sections. The paraffin sections of heart tissue were dewaxed in the first xylene for 10 min, the second xylene for 10 min, the first anhydrous ethanol for 5 min, the second anhydrous ethanol for 5 min, 95 % ethanol for 5 min, and 80 % ethanol for 5 min. Then rinse under running water for 10 min. Hematoxylin-eosin(H&E) staining and Masson's trichrome staining were performed with a hematoxylin-eosin staining kit (Solarbio, G1120) and a Masson's trichrome stain kit (Solarbio, G1340). The dyeing method was carried out according to the manufacturer's instructions.

2.4. Immunohistochemical

For immunohistochemistry staining, the paraffin sections of heart tissue were dewaxed in the first xylene for 10 min, the second xylene for 10 min, the first anhydrous ethanol for 5 min, the second anhydrous ethanol for 5 min, 95 % ethanol for 5 min, and 80 % ethanol for 5 min. Then washed 3 times with PBS. After the sections were performed antigen retrieval, the tissue sections were treated with 3 % H2O2 for 10 min and blocked with goat serum for 1 h at room temperature. Then, the sections were incubated with α-SMA (1:500, ARG66381, arigo), iNOS (1:200, ARG56509, arigo) and P16 (1:50, ARG41366, arigo) at 4 °C overnight. After incubation with secondary antibody, the DAB staining solution was added to each section. Then, the nucleus was stained with hematoxylin staining solution. Finally, neutral balsam was used and observed by light microscope (OLYMPUS, BX53) [29].

2.5. Immunofluorescent analysis

For immunofluorescent staining, the paraffin sections of heart tissue were dewaxed in the first xylene for 10 min, the second xylene for 10 min, the first anhydrous ethanol for 5 min, the second anhydrous ethanol for 5 min, 95 % ethanol for 5 min, and 80 % ethanol for 5 min. Then washed 3 times with PBS. After the sections were performed antigen retrieval,The sections were incubated with enhanced immunostaining permeabilization solution for 3 min and blocked with 5 % BSA in room temperature for 1 h. Then, the sections were incubated with primary antibodies against Collagen III (1:150, 22734-1-AP, Proteintech) and γ-H2AX (1:150, ab81299, abcam) overnight at 4 °C. After washed 3 times with PBS, incubation with secondary antibody. And the sections were washed 3 times with ddH2O, using antifade mounting medium with DAPI to seal the sections. The sections were observed by fluorescence microscope (OLYMPUS, BX53).

2.6. Wheat germ agglutinin (WGA)

After the sections were dewaxed in the first xylene for 10 min, the second xylene for 10 min, the first anhydrous ethanol for 5 min, the second anhydrous ethanol for 5 min, 95 % ethanol for 5 min, and 80 % ethanol for 5 min. Then washed 3 times with PBS. WGA staining reagent (2 ng/ml, Sigma, Green; FL-1021, VECTOR, Red) was used for 1h at room temperature. After wash 3 times with ddH2O, using antifade mounting medium seal the sections. And the cardiomyocyte of surface area was measured with ImageJ [30].

2.7. PCR array and data analysis

Total RNAs were extracted from Heart. the cDNA was subjected to PCR Array analysis (Aging PCR Array plate, Wcgene Biotech, China). We defined genes with p values < 0.05 and Foldchange>1.5 or <0.666 as significantly different genes. The Heatmap and volcano plot are made through Xiantao website (https://www.xiantao.love/).

2.8. Western blot analysis

Heart tissues and cells were lysed in RIPA Lysis Buffer added with PMSF, phosphatase inhibitor and protease inhibitor. The protein concentration was determined by BCA Protein Assay Kit. Then the protein samples (30 μg/lane) were separated by 10 % or 12.5 % SDS-polyacrylamide gels and transferred to polyvinylidene fluoride membranes (PVDF). The membranes were blocked with 5 % skimmed milk at room temperature for 2h, and incubated with each primary antibody against p-AKT (1:1000, 66444-1-Ig, Proteintech), AKT (1:1000, 60203-2-Ig, Proteintech), p-ERK1/2 (1:500, 4370, CST), ERK1/2 (1:1000, 4695, CST), Collagen III (1:1000, 22734-1-AP, Proteintech), α-SMA (1:1000, A17910, ABclonal), NLRP3 (1:1000, PA5-88709, inventrigen), p-SMAD2/3 (1:1000, 8828, CST), SMAD2/3 (1:1000, 5678, CST), TGF-β(1:1000, CST), NOX2 (1:1000, 19013-1-AP, Proteintech), NOX4 (1:1000, 14347-1-AP, Proteintech), p53 (1:1000, 60283-2-lg, Proteintech), CD14 (1:1000, A5737, ABclonal), Mrpl43 (1:1000, A17793, ABclonal), Zmpste24 (1:1000, A18593, ABclonal), and β-actin (1:4000, T0022, Affinity Biosciences)overnight at 4 °C. Then incubated with a secondary

antibody for 1 h at room temperature. After washing the membranes with TBST, using ECL luminescence solution to observe the protein bands.

2.9. RNA isolation and quantitative real-time PCR

The heart tissue and cells were fully lysed with 1 ml TRIzol, then 0.2 ml chloroform was added, then stratified, centrifuged at 4 °C at 13300g for 15 min after full shock, the upper transparent liquid was collected into a new RNA-free EP tube, the same volume of isopropyl alcohol was added, mixed and placed at −20 °C for 20 min. Centrifuge 13300*g* for 15 min, Discard the supernatant and add 75 % ethanol prepared with 1 ml DEPC water and anhydrous ethanol, reverse mix, centrifuge 13300*g* at 4 °C for 15 min; After centrifugation, discard the supernatant, precipitate in the fume hood and blow until transparent, add an appropriate amount of DEPC water to dissolve RNA. After the concentration was determined, 1 μg RNA was reverse-transcribed into cDNA using reverse transcription kit. Real-time quantitative PCR was performed on Applied Biosystems 7500 Fast (ABI, USA) to detect the expression levels of related genes using the universal blue qPCR SYBR Green Master Mix. The relative expression of mRNA was calculated by $2^{-\Delta\Delta Ct}$. The primers sequences were shown in Table 1.

2.10. TDT-mediated dUTP nick end-labeling (TUNEL) staining

After the sections were dewaxed in the first xylene for 10 min, the second xylene for 10 min, the first anhydrous ethanol for 5 min, the second anhydrous ethanol for 5 min, 95 % ethanol for 5 min, and 80 % ethanol for 5 min. Then washed 3 times with PBS, the sections were incubated with DNase-free protease K (20 μg/ml) and washed 3 times with PBS. Thereafter, endogenous peroxidase blocking buffer was added to each section. Next, the sections were incubated with biotin labeling solution for 1h at 37 °C, and then incubated with labeled reaction termination solution. After washing 3 times with PBS, streptavidin-HRP working solution was used to incubate. After DAB staining, the nucleus was stained with hematoxylin staining solution. Finally, neutral balsam was used and apoptotic cells were observed by light microscope (OLYMPUS, BX53) [31].

2.11. Dihydroethidium (DHE)

DHE (sigma) was used to evaluate the production of superoxide anion in the heart sections. After the sections were dewaxed in the first xylene for 10 min, the second xylene for 10 min, the first anhydrous ethanol for 5 min, the second anhydrous ethanol for 5 min, 95 % ethanol for 5 min, and 80 % ethanol for 5 min. Then washed 3 times with PBS, DHE solution (1:600) was incubated for 10 min at room temperature. After wash 3 times with ddH2O, using antifade mounting medium seal the sections. And the sections were observed

Table 1
The primer sequence for qRT-PCR.

Gene		Primer
Mouse *nppa*	Forward	5' - GTGCGGTGTCCAACACAGAT - 3′
	Reverse	5' - TCCAATCCTGTCAATCCTACCC - 3′
Mouse *nppb*	Forward	5' - AGTCCTTCGGTCTCAAGGCA - 3′
	Reverse	5' - CCGATCCGGTCTATCTTGTGC - 3′
Mouse *Acta2*	Forward	5' - CCCAGACATCAGGGAGTAATGG - 3′
	Reverse	5' - TCTATCGGATACTTCAGCGTCA - 3′
Mouse *Col1a1*	Forward	5' - TAAGGGTCCCCAATGGTGAGA - 3′
	Reverse	5' - GGGTCCCTCGACTCCTACAT - 3′
Mouse *Col3a1*	Forward	5' - CTGTAACATGGAAACTGGGGAAA - 3′
	Reverse	5' - CCATAGCTGAACTGAAAACCACC - 3′
Mouse *Il-1β*	Forward	5' - CTCGACTCAGTGCGACTTCTC - 3′
	Reverse	5' - CGAATGTTCATCCGCCAATTCAG - 3′
Mouse *Il-6*	Forward	5' - CTGCAAGAGACTTCCATCCAG - 3′
	Reverse	5' - AGTGGTATAGACAGGTCTGTTGG - 3′
Mouse *Tgfβ1*	Forward	5' - CCACCTGCAAGACCATCGAC - 3′
	Reverse	5' - CTGGCGAGCCTTAGTTTGGAC - 3′
Mouse *Cybb*	Forward	5' - CCTCTACCAAAACCATTCGGAG - 3′
	Reverse	5' - CTGTCCACGTACAATTCGTTCA - 3′
Mouse *Nox4*	Forward	5' - CCTTTTACCTATGTGCCGGAC - 3′
	Reverse	5' - CATGTGATGTGTAGAGTCTTGCT - 3′
Mouse *Actb*	Forward	5' - GTGACGTTGACATCCGTAAAGA - 3′
	Reverse	5' - GCCGGACTCATCGTACTCC - 3′
Rat *CD14*	Forward	5' - GCCCAATTCAGGATTGTCAGAC - 3′
	Reverse	5' - GTTATACGCCTCCGACTGGG - 3′
Rat *Mrpl43*	Forward	5' - TTCCATTGCCCACGTCTACC - 3′
	Reverse	5' - TGCAAGGAGGCCACATGAAT - 3′
Rat *Zepste24*	Forward	5' - CCTGCGGAGAAGCGAATCTT - 3′
	Reverse	5' - TCTGGTGGTACATGAGTCGT - 3′
Rat *Actb*	Forward	5' - CTATGAGGGTTACGCGCTCC - 3′
	Reverse	5' - ATGTCACGCACGATTTCCCT - 3′

with a fluorescence microscope (OLYMPUS, BX53) [32].

2.12. Cell culture and treatment

H9C2 cells were obtained from the Procell, and were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10 % fetal bovine serum and 1 % penicillin-streptomycin, at 37 °C, with 5 % CO_2, and treated after reaching a confluence of ~80 %.H9C2 cells were pretreated with melatonin (50 μM) and luzindole (10 μM) for 1 h and then stimulated with D-gal (10 g/L) for 24 h. Cells were then processed for cytochemical staining of β-gal.

2.13. SA-β-gal staining

To estimate cell senescence, staining for SA-β-gal was performed with a Senescence β-Galactosidase Staining Kit (Solarbio, G1580), following the manufacturer's protocols. Briefly, AC16 cells were cultured in six-well plate. The cells were washed with PBS after different treatments. Add 1 ml β-Gal Fixative to each pore and fix for 15 min at room temperature, and wash cells 3 times. We added 1 ml Dyeing Working Solution to each hole, and incubated overnight at 37 °C. After wash cells 3 times with PBS, the cells were observed and images were taken with an inverted microscope (OLYMPUS, IX73).

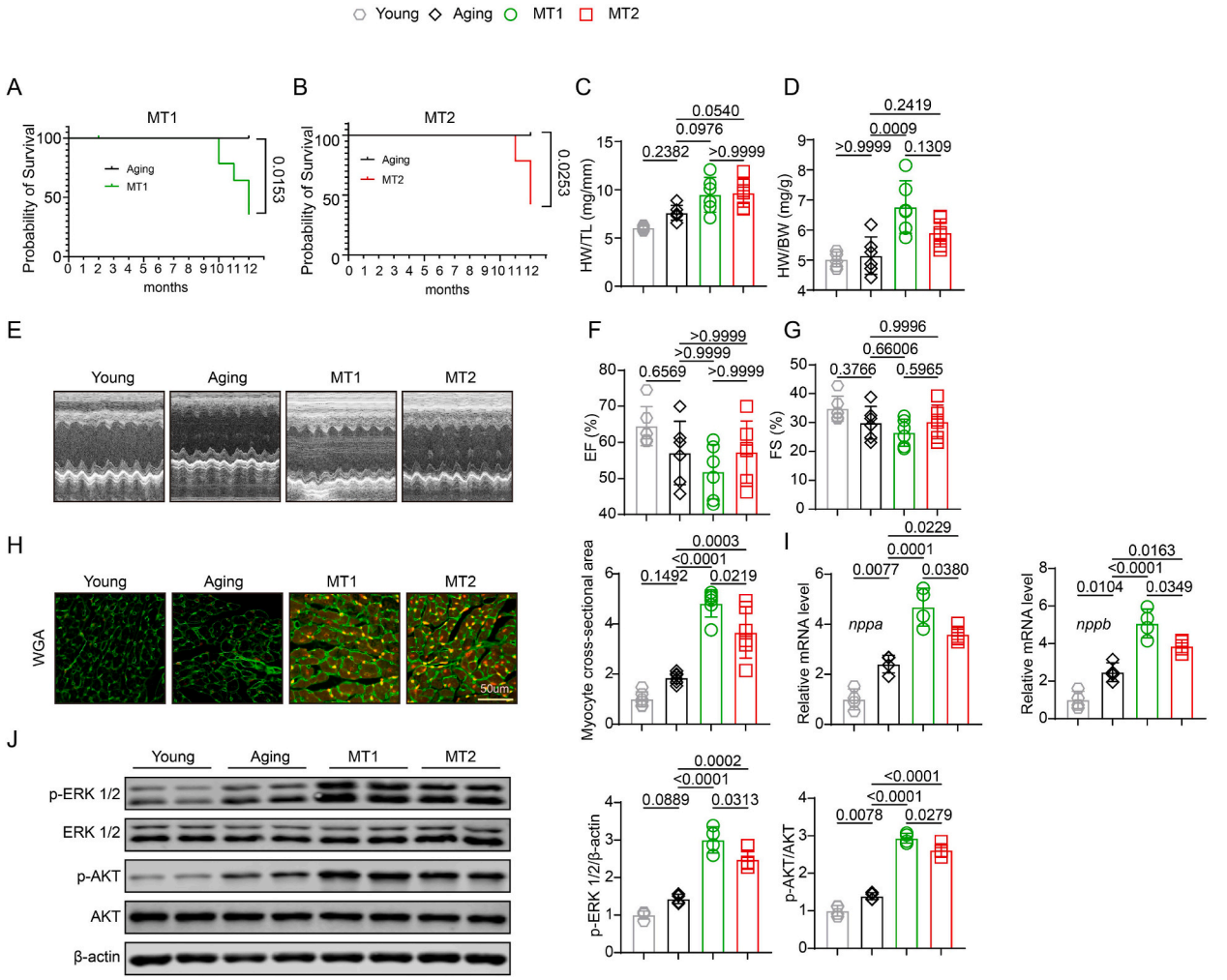

Fig. 1. Deletion of MT1 and MT2 inhibits the protective effect of melatonin on cardiac function in aging mice. (A) Survival curves of mice in MT1 group and aging group; (B) Survival curves of mice in MT2 group and aging group; (C) the ratios of heart weight to tibia length (HW/TL); (D) the ratios of heart weight to body weight (HW/BW); (E) M-mode echocardiography; (F) quantification of EF%; (G) quantification of FS%; (H) Heart cross-sections were stained with WGA; (I) The mRNA expression of *Nppa* and *Nppb* in each group; (J) The expression of p-ERK 1/2, ERK 1/2, p-AKT, AKT and β-actin in each group.

2.14. Statistics

All experimental results are expressed as mean ± SD. One-way ANOVA or two-way ANVOAs were performed for comparisons among three or four groups (correct test by Bonferroni). $P < 0.05$ were considered statistically significant.

3. Results

Deletion of MT1 and MT2 inhibits the protective effect of melatonin on cardiac function in aging mice.

To investigate the impact of melatonin receptor knockout on the hearts of aging mice, MT1/2 knockout mice and wild-type mice were fed for 12 months. The survival curve indicated that the young and aging mice did not die, whereas the mortality rate of MT1 knockout mice was approximately 60.0 %, and that of MT2 knockout mice was 64.3 % (Fig. 1A and B). Cardiac hypertrophy is an crucial risk factor for myocardial aging. We found no significant difference in the myocardial weight to tibia (HW/TL) ratio between aging and young mice. Compared with aging mice, the HW/TL ratio of MT1/2 knockout mice was not significantly different (Fig. 1C). However, compared with the control group, the HW/body weight (BW) of MT1/2 knockout mice was significantly different. We found no statistically significant difference in the HW/BW ratio between aging and young mice. Compared with aging mice, the HW/BW of MT1 knockout mice was significantly different; however, the HW/BW of MT2 knockout mice was not significantly different (Fig. 1D). Subsequently, an echocardiography was performed. There was no statistical difference in% EF and% FS between the four groups. The EF% and FS% values of MT1 and MT2 knockout mice significantly differed from those of the young mice (Fig. 1E–G). The size of cardiomyocytes in each group of mice was measured using wheat germ agglutinin (WGA) staining. The results showed that the cardiomyocytes were larger in aged mice than in young mice. Compared with the cardiomyocytes of aged mice, those of the MT1/2 knockout group were significantly larger. Moreover, the cardiomyocytes in the MT1 knockout group were significantly larger than those in the MT2 knockout group (Fig. 1H). We also tested myocardial-hypertrophy-related indicator genes, and compared them with young mice; the mRNA expression of *Nppa* and *Nppb* in aging mice were significantly increased, while compared with aging mice, the mRNA expression of *Nppa* and *Nppb* was significantly increased in the hearts of MT1/2 knockout mice (Fig. 1I). We also tested myocardial hypertrophy related indicator proteins. The results showed that compared with young mice, the expression of p-ERK 1/2 and p-AKT in aging mice was significantly increased. In contrast, compared with aging mice, the expression of p-ERK 1/2 and p-AKT was significantly increased in the hearts of MT1/2 knockout mice (Fig. 1J).

3.1. Deletion of MT1 and MT2 promotes myocardial inflammation and fibrosis in aging mice

Inflammatory aging is a low-grade chronic inflammatory state that occurs during the aging process. We found that aging mice had more inflammatory aggregates than young mice. Melatonin inhibited cardiac inflammation in aging mice, and MT1/2 knockout mice exhibited more nuclear aggregates than the aging group using H&E staining (Fig. 2A). We also examined inducible nitric oxide synthase (iNOS) expression. The results showed that iNOS expression was significantly higher in the aging group than in the young group. Compared with the aging group, the expression of iNOS in MT1/2 knockout mice was significantly increased. iNOS expression in MT1 knockout mice was significantly higher than in the MT2 knockout group (Fig. 2A). Masson's experiment showed that fibrosis levels were significantly higher in aged mice than in control mice. Fibrosis levels were significantly increased in the MT1/2 knockout mice. The degree of fibrosis was significantly increased in MT1 knockout mice than in MT2 knockout mice (Fig. 2B). We investigated collagen III expression in the hearts of mice from each group using immunofluorescence staining. The results showed that Collagen III expression was significantly higher in aged mice than in young mice. Compared with aging mice, Collagen III expression levels were significantly increased in MT1/2 knockout mice. Compared with MT2 knockout mice, the collagen III expression level was significantly increased in MT1 knockout mice (Fig. 2C). Smooth muscle actin (SMA) expression was measured using immunohistochemistry. SMA expression was significantly higher in the aging group than in the young group. Compared with the aging group, SMA expression in MT1/2 knockout mice was significantly increased. SMA expression in MT1 knockout mice was significantly higher than in the MT2 knockout group (Fig. 2D). We also tested the expression of inflammation- and fibrosis-related indicator genes. The results showed that compared with young mice, α-SMA, tgfb1, Col1, Col3, interleukin (IL) 1b, and IL-6 mRNA expression were significantly increased in aging mice, and compared with aging mice, the mRNA expression of α-SMA, tgfb1, Col1, Col3, il1b, and il6 were significantly increased in the hearts of MT1/2 knockout mice (Fig. 2E). We also tested inflammation and fibrosis related indicator proteins. Compared with young mice, aging mice exhibited significantly increased α-SMA, NLRP3, Collagen III, p-SMAD2/3 and TGF-β1 expression. Compared with aging mice, the expression of α-SMA, NLRP3, Collagen III, p-SMAD2/3 and TGF-β1 was significantly increased in the hearts of MT1/2 knockout mice (Fig. 2F).

3.2. Knockout of MT1 and MT2 promotes oxidative stress in the hearts of aging mice

Aging disrupts the balance between oxidation and antioxidant systems in the body, leading to an accumulation of free radicals or a decrease in scavenging capacity, thereby promoting aging. First, we determined the oxidative stress level in each group of mice by dihydroethidium (DHE) staining. The results showed that compared with young mice, the DHE expression level was significantly increased in aging mice. Compared with aging mice, DHE expression level in MT1/2 knockout mice were significantly increased. Compared with MT2 knockout mice, the DHE expression level were significantly increased in MT1 knockout mice (Fig. 3A). Next, we measured γ-H2AX by immunofluorescence to observe DNA damage in the hearts of mice in each group. The results showed no significant difference in γ-H2AX expression levels between aged and young mice. Compared with aging mice, the γ-H2AX expression

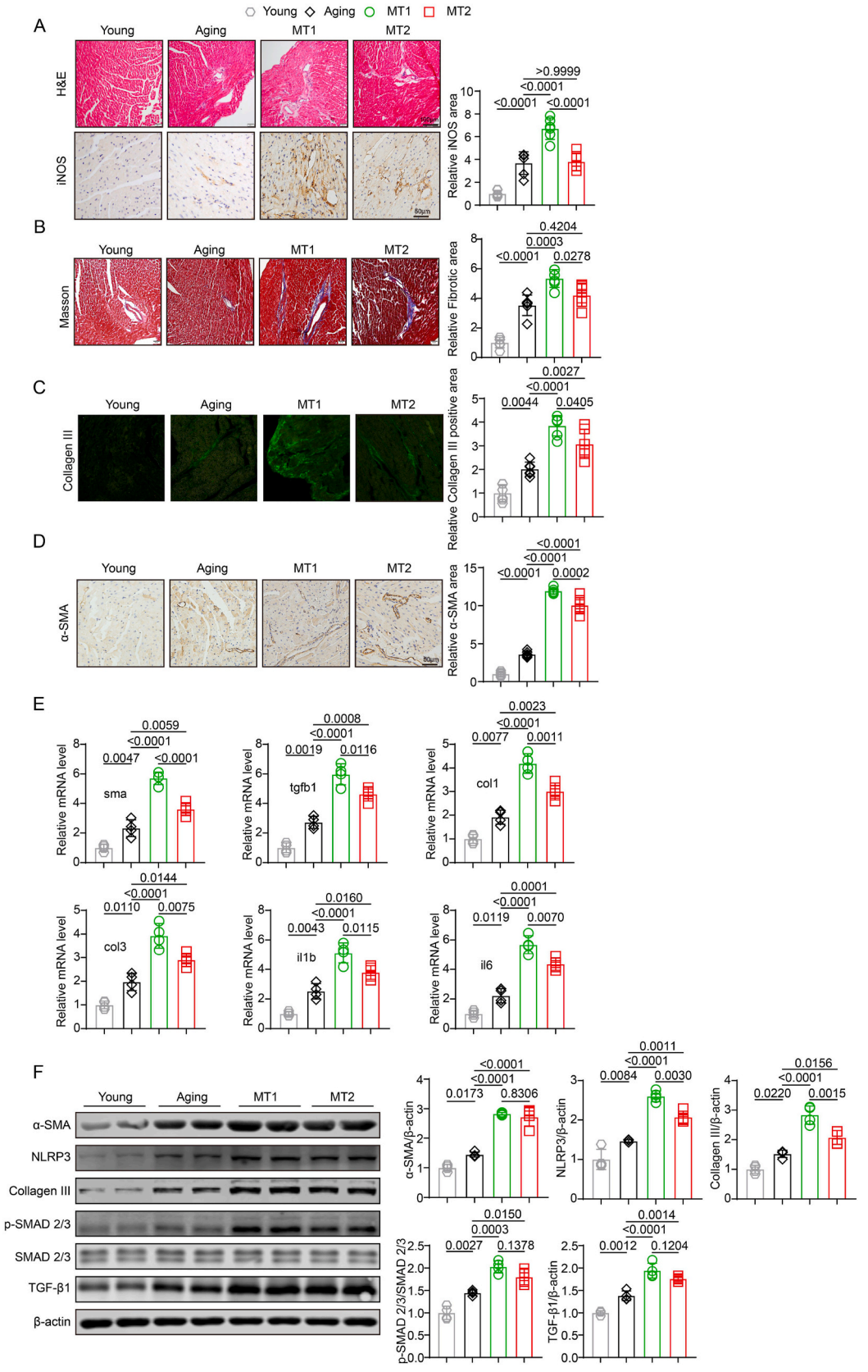

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Fig. 2. Deletion of MT1 and MT2 promotes myocardial inflammation and fibrosis in aging mice. (A) Representative H&E staining (UP) and iNOS (DOWN); (B) Representative images of Masson trichrome staining; (C) Representative images of Collagen III staining; (D) Representative α-SMA immunohistochemistry; (E) The mRNA expression of α-SMA, tgfb1, Col1, Col3, il1b, and il6 in each group; (F) The expression of α-SMA, NLRP3, Collagen III, p-SMAD 2/3, SMAD 2/3, TGF-β1 and β-actin in each group.

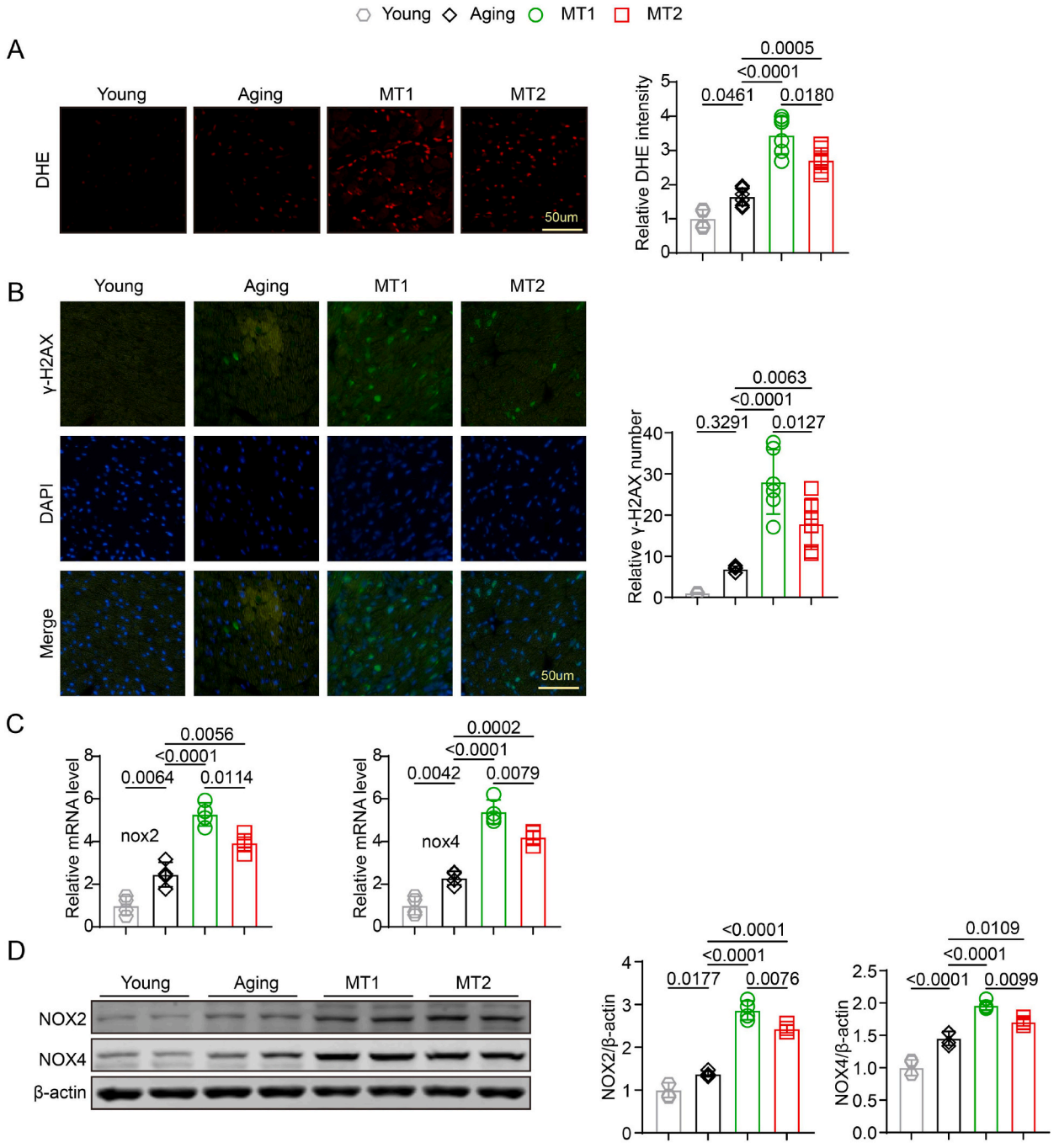

Fig. 3. Knockout of MT1 and MT2 promotes oxidative stress in the hearts of aging mice (A) Representative DHE staining; (B) Representative γ-H2AX and DAPI staining; (C) The mRNA expression of Nox2 and Nox4 in each group; (D) The protein expression of NOX2, NOX4 and β-actin in each group.

levels were significantly increased in MT1/2 knockout mice. Compared with MT2 knockout mice, the γ-H2AX expression levels were significantly increased in MT1 knockout mice (Fig. 3B). We also tested for oxidative-stress-related indicator genes, and the results showed that compared with young mice, the mRNA expression of Nox2 and Nox4 in aging mice was significantly increased. In contrast, compared with aging mice, the mRNA expression of Nox2 and Nox4 was significantly increased in the hearts of MT1/2 knockout mice (Fig. 3C). We also tested oxidative stress related indicator proteins. The results showed that compared with young mice, the expression of NOX2 and NOX4 in aging mice was significantly increased. In contrast, compared with aging mice, NOX2 and NOX4 expression was significantly increased in the hearts of MT1/2 knockout mice (Fig. 3D).

3.3. Knockout of MT1 and MT2 promoted cardiac senescence and myocardial apoptosis in aging mice

Oxidative stress promoted cardiomyocyte apoptosis. We observed cardiomyocyte apoptosis in each group by measuring the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) expression. The results showed that compared with young mice, the TUNEL expression levels were significantly increased in aging mice. TUNEL levels were significantly increased in MT1/2 knockout mice compared with aging mice. Compared with MT2 knockout mice, the TUNEL levels in MT1 knockout mice were significantly increased (Fig. 4A). p53 and p16 serve as crucial signaling molecules in mediating and sustaining cellular senescence. The expression of p16 was evaluated via immunohistochemistry. The results revealed a significant difference in the p16 expression levels between aged and young mice. Compared with aging mice, the p16 expression levels were significantly increased in MT1/2 knockout mice. Compared with MT2 knockout mice, the p16 expression levels was significantly increased in MT1 knockout mice (Fig. 4B). We measured the p53 protein expression levels in mice from each group using Western blot. The results showed that compared with the control group, the p53 expression level was significantly increased in the aging group. Compared with the aging group, the p53 expression in MT1/2 knockout mice was significantly increased (Fig. 4C).

Leukocyte differentiation antigen 14 (CD14) may be a downstream regulatory factor of the melatonin receptor in the cardiac aging signaling pathway.

Through pathological experiments, we found that the effect of MT1 knockout on the heart was significantly greater than that of

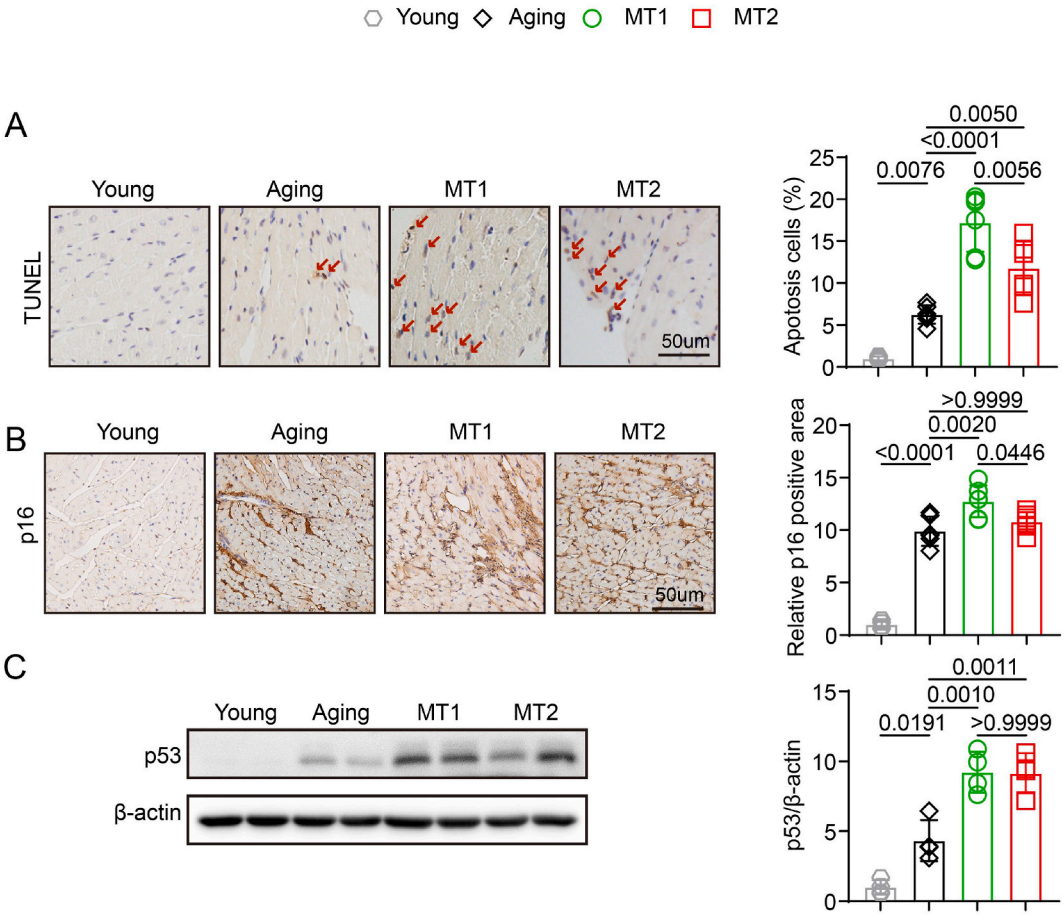

Fig. 4. Knockout of MT1 and MT2 promoted cardiac senescence and myocardial apoptosis in aging mice. (A) Representative TUNEL; (B) Representative p16 immunohistochemistry in each group; (C) The expression of p53 and β-actin were detected by western Blot.

MT2 knockout and that MT1 knockout was significantly greater than that on the atria. Therefore, we selected MT1 tissues for analysis and screened for significant genes using an MT1 knockout. Five mice from each group were selected for aging polymerase chain reaction (PCR) analysis. The results are displayed using a heatmap and a volcano map. The results showed that compared with the aging group, the expression levels of Zmpste24, Cd14, Lmnb1, Vps13c, and Snap23 were significantly increased in MT1 knockout mice. Compared with the aging group, Mrpl43, C1qc, C4a, and Hsp27 expression levels were significantly reduced in MT1 knockout mice (Fig. 5A and B). Next, we performed in vitro experiments to verify these results. We first simulated aging using D-galactose, followed by

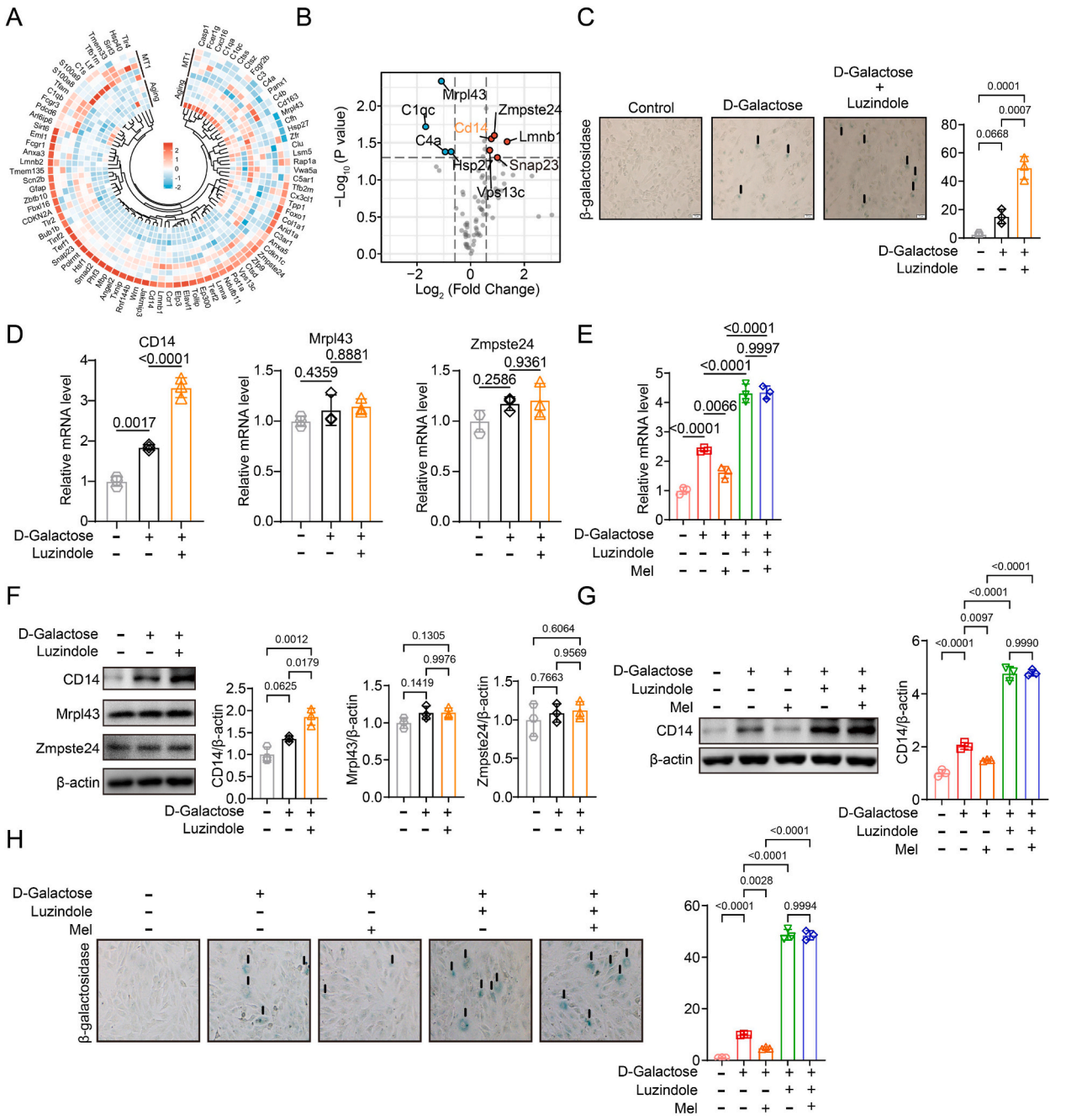

Fig. 5. Leukocyte differentiation antigen 14 (CD14) may be a downstream regulatory factor of the melatonin receptor in the cardiac aging signaling pathway. (A) Heat map showing gene expression in each group; (B) Volcano plot showing gene expression in aging group and MT1 group; (C) the level of cell senescence in each group detected by β-galactosidase staining; (D) The mRNA expression of cd14, Mrpl43 and Zmpste24 in each group; (E) The mRNA expression of CD14 in each group; (F) The protein expression of cd14 and β-actin in each group; G) The protein expression of CD14 and β-actin in each group; (H) the level of cell senescence in each group detected by β-galactosidase staining.

treatment with a melatonin receptor inhibitor (luzindole). Compared with the control group, the β-galactosidase staining increased in the D-galactose group and was more pronounced after luzindole treatment (Fig. 5C). In cardiomyocytes, we verified whether significantly different genes changed significantly as well. The results showed that the mRNA and protein CD14 expression increased in the aging group, whereas, the expression of Mrpl43 and Zmpste24 did not. CD14 expression further increased after luzindole treatment (Fig. 5D and F). Finally, by stimulating cells with melatonin, we found that it could inhibit the increase in CD14 and β-galactosidase caused by aging (induced by D-galactose). In contrast, inhibition of the melatonin receptor could inhibit the effect of melatonin (induced by luzindole) (Fig. 5E–G and H).

3.4. *Inhibiting MT1/2 interferes with the protective effect of melatonin on the aging heart*

We demonstrated that melatonin receptor function could inhibit the effects of melatonin in vitro. Further, in vivo experiments were performed. WGA staining showed that the size of cardiomyocytes in aged mice was larger than that in young mice, and the addition of

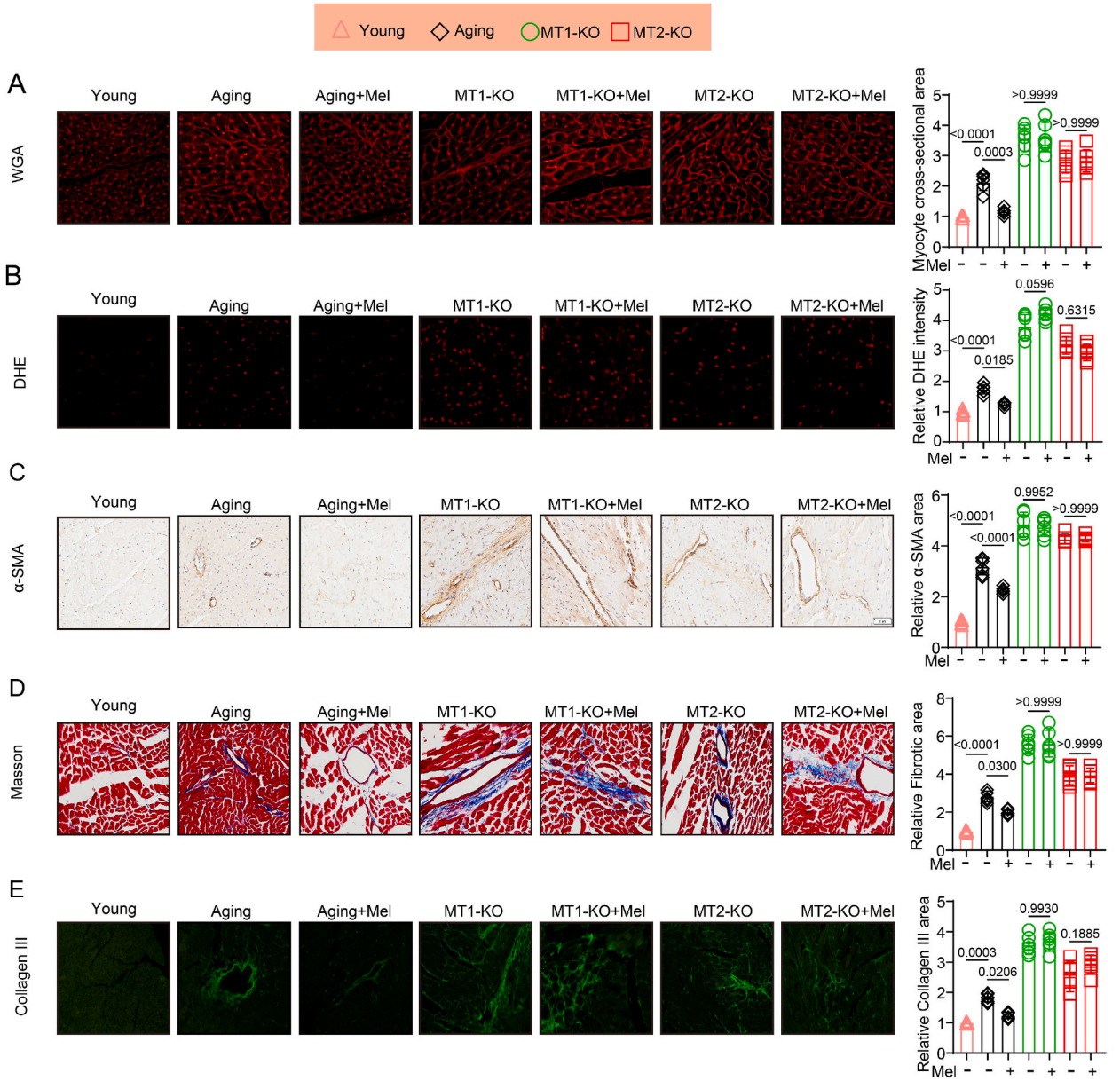

Fig. 6. Inhibiting MT1/2 interferes with the protective effect of melatonin on aging heart. (A) Heart cross-sections were stained with WGA; (B) Representative DHE staining; (C) Representative α-SMA immunohistochemistry; (D) Representative images of Masson trichrome staining; (E) Representative images of Collagen III staining.

melatonin inhibited the degree of cardiac hypertrophy in aging mice. However, after MT1/2 knockout, melatonin did not reduce the degree of hypertrophy in aging mice (Fig. 6A). Similarly, DHE staining (Fig. 6B), α-SMA staining (Fig. 6C), Masson staining (Fig. 6D), and Collagen III staining (Fig. 6E) showed that aging mice inflammation, fibrosis and oxidative stress were significantly higher than in young group, and the addition of melatonin inhibited oxidative stress and fibrosis in the hearts of aging mice. After MT1/2 knockout, melatonin did not reduce the degree of oxidative stress or fibrosis in the hearts of aging mice. We confirmed that melatonin regulates the progression of cardiac senescence through MT1/2.

4. Discussion

Heart failure is a globally life-threatening condition. Cardiac aging is a complex pathophysiological process with unique histological and biochemical features, including cardiac remodeling and dysfunction. The precise mechanisms underlying cardiac aging are still not fully understood. Melatonin and its receptors are known to be associated with aging [33,34]. In this study, MT1/2 knockout mice were used to investigate the relationship between melatonin receptors and age-related CVDs. It was observed that MT1/2 knockout mice had a mortality rate of approximately 50 % when reared until 12 months of age. A 12-month-old mouse is equivalent to a 40-year-old human. MT1/2 knockout has a strong effect in mice. It was observed that there was no change in heart function, perhaps because of the HF with preserved EF, or because the mice were harvested at 12 months of age when only some pathological changes occurred in the heart. The functional alterations did not show significant variance.

Research has shown that melatonin receptors also play an essential role in the cardiovascular system, mainly by regulating cardiac output, blood pressure, and heart rate, among other functions. It has been found that melatonin receptors are distributed in the vascular system [35], and can regulate the constriction and dilation of arteries [36,37]. When melatonin is below 0.1 μM, it controls vascular constriction [38]; when its concentration is above 0.1 μM, it controls vascular dilation [39]. MT1 primarily regulates vascular constriction, whereas MT2 regulates vascular dilation. Clinical data have indicated that patients with CVDs often exhibit abnormalities in melatonin secretion, and clinical evidence has confirmed that melatonin has a good auxiliary therapeutic effect on CVDs such as hypertension [40–42]. MT1/2 play vital roles in CVDs. However, in this study, we found that MT1/2 knockout worsened the aging of mouse hearts. At the same time, supplementing with melatonin did not alleviate the aging of mouse hearts after MT1/2 knockout.

In older adults, monocytes/macrophages typically increase pro-inflammatory cytokine production and decrease the responsiveness to external stimuli in a quiescent state. Immunosenescence manifests as the M1 polarization of certain monocytes to promote an inflammatory phenotype [43]. The accumulation of tumor necrosis factor, IL-6, and IL-1β in the myocardium and peripheral tissues plays an vital role in the occurrence and development of HF. Our study revealed a significant increase in inflammation levels in aging mice after MT1/2 gene deletion. At the same time, we found that the degree of immune cell infiltration by H&E staining was much higher in MT1/2 group than that in the Aging group. Inflammation is the primary cause of cardiac aging. Therefore, MT1/2 may affect the aging of the mouse heart by regulating inflammation.

Cardiomyocyte death activates the inflammatory cascade, leading to fibrosis and scarring. Although fibrosis is a normal repair mechanism that preventing myocardial rupture, a robust inflammatory response, and excessive fibrosis may contribute to the developing of HF. Fibrosis is involved in the pathogenesis of various chronic diseases and age-related organ failure and is the ultimate hallmark of myocardial pathological remodeling [44]. Tissue fibrosis is closely related to aging and triggers and induces its progression. Normal aging is usually accompanied by reduced left ventricular wall compliance and left atrial dilatation, both of which are associated with increased myocardial fibrosis. In addition, fibrosis can cause left and right cardiac load [23]. In this study, using Masson staining and collagen III immunofluorescence staining, we found that the fibrosis levels in 12-month-old mice were significantly higher than those in young mice. The level of myocardial fibrosis in MT1/2 knockout mice was more apparent than in other mice. Fibrosis causes myocardial tissue stiffness and reduces flexibility, leading to cardiac systolic dysfunction and an increased the risk of HF [45]. Therefore, reducing the occurrence of myocardial fibrosis is the goal of treating HF, and the negative regulation of the endogenous pathways involved in myocardial fibrosis is the key to inhibiting cardiac aging.

With aging, the balance between the oxidation and antioxidant systems in the body is gradually destroyed, which can produce too many free radicals or reduce the scavenging ability, thereby inducing aging [46]. ROS production significantly increases in the hearts of aging animals, as seen in animal models. ROS can leak into cells from the mitochondria, damage proteins, lipids, and DNA, and further induce heart damage by activating inflammatory cytokines that enhance inflammatory responses. Simultaneously, the activities of antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase, decrease, and the balance between oxidation and antioxidant function is disrupted, leading to increased in oxidative stress.Previous literature reported that Melatonin can directly detoxify the ROS and RNS by a receptor-independent mechanism. melatonin mainly entraps the radicals By the single electron transfer and hydrogen transfer [47,48]. But there are also some experiments prove that melatonin by melatonin receptor downstream the levels of oxidative stress. Melatonin can inhibit the oxidative stress injury of endothelial cells induced by high glucose, but the protective function of melatonin disappeared after the addition of melatonin receptor inhibitor Luzindole. Also in the myocardial infarction model, the anti-oxidative stress function of melatonin was inhibited after the addition of Luzindole [49,50]. In this article, we study found after knockout of melatonin receptor, can inhibit the aging of melatonin on oxidative stress removal effect. ROS can also cause DNA damage. Double-strand breaks cause DNA damage responses that activate and recruit ATM- and ATR-associated kinases to the site of damage and lead to the phosphorylation of histone γH2AX at Ser139 [51–53]. In this experiment, compared with young mice, we found that p-γH2AX expression in 12-month-old mice was increased, while p-γH2AX expression in MT1/2 knockout mice was significantly increased. p-γH2AX induces phosphorylation of Chk1 and Chk2, activating the p53/p21 and p16/Rb signaling pathways, where the p53 protein is the main transcription factor mediating the DNA damage response. Previous studies have shown that mice overexpressing p53 live shorter lives and exhibit signs of premature aging. DNA damage activates p53 and directly or

indirectly inhibits insulin signaling through the JNK/IKK-mediated pathways [54,55]. In this study, we found that p53 and p16 expression was increased in 12-month-old mice compared with young mice, whereas p53 and p16 expression was significantly increased in MT1/2 knockout mice. It can be concluded that deleting the melatonin receptor can cause DNA damage and defects in the DNA repair mechanism, leading to accelerated aging in mice.

Currently, melatonin has two main receptors: membrane and nuclear. The two types of membrane receptors are MT1 and MT2, belonging to the G protein-coupled receptor superfamily [10]. The nuclear receptor belongs to the retinoic-acid-related orphan nucleus receptor/retinoic acid Z receptor family. Melatonin receptors play an important role in reducing CVD risk [27,56]. Moreover, with increasing mouse age, MT1/2 expression in the heart decreases, indicating that MT1 and MT2 play important roles in the heart.

Interestingly, previous reports have indicated that MT2 knockout, rather than MT1 knockout, plays an important role in ischemia-reperfusion [20,21]. However, in an aging model, MT1 plays a greater role in cardiac aging [57]. This finding aligns with our own experimental results, where we observed that the deletion of these two membrane receptors exacerbated fibrosis, inflammation, apoptosis, HF, and other cardiac aging effects in mice. Melatonin can also play a direct physiological role via non-receptor pathways. The effect of melatonin on anti-cardiac aging in mice was evident; however, when MT1/2 was knocked out, melatonin function diminished. Melatonin was orally administered at 10 mg/kg/day during the last 2 months before the early- (12 months) and old-aged (24 months) mice were sacrificed [58]. Melatonin therapy restored age-induced changes in mitochondrial dynamics but had little effect on cardiac autophagy. Therefore, we speculated that melatonin plays an important role through the melatonin receptors (MT1/2) during mouse aging, especially during heart aging.

Following this, we investigated the impact of specific genes on heart aging in aging MT1 mice through PCR analysis. Cell senescence was induced using D-galactose, revealing a significant increase in CD14 expression levels in senescent cells. CD14 was initially believed to be a membrane receptor for lipopolysaccharide in macrophages and to mediate the host cell cytokine response to sepsis [59,60]. In addition to macrophages, CD14 has been detected in various cell types, including human intestinal epithelial cells, human and mouse liver cells, and pancreatic cells [61–63]. In this study, we found that cardiomyocytes also expressed CD14, indicating that CD14 may induce cardiomyocyte aging. CD14 expression further increased after the melatonin receptor inhibitor was used to inhibit melatonin receptor expression. CD14 is a polymorphic inflammatory cytokine mainly involved in regulating inflammation and immune responses in the body [64]. In atherosclerotic lesions, CD14 expression in macrophages was significantly higher than that in the normal vascular intima. CD14 activation in cancer cells induces MyD88 and TRIF signaling to orchestrate tumor-promoting inflammation and drive tumor cell proliferation to promote tumor growth [65].

Increasing evidence has emphasized the role of the NLRP3 inflammasome in aging. Cardiomyocytes can also generate inflammasomes, secrete inflammatory factors, and promote cardiomyocyte aging [66]. CD14 can activate NLRP3-mediated inflammasome assembly, leading to the release of IL-1β and IL-18 [67]. We hypothesize that melatonin modulates CD14 expression through MT1/2 in cardiomyocytes, influencing inflammasome assembly, suppressing IL-18 secretion, and ultimately mitigating cardiomyocyte aging.

In conclusion, our study demonstrates that the knockout of MT1 and MT2 exacerbates cardiac damage in aging mice. Additionally, the regulation of CD14 by melatonin receptors during cardiac aging is highlighted. Further investigation is required to determine if CD14 can impede the anti-aging properties of melatonin and its receptors.

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Data availability statement

The datasets used and/or analyzed during the study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All procedures were approved by the Animal Care and Use Committee of Dalian Medical University (AEE21079). We performed the procedures in accord with the Guidelines. Animal handling, care, killing, and other procedures involving animals were conducted in compliance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

CRediT authorship contribution statement

Zhenyu Feng: Data curation. **Yang Liu:** Data curation. **Yijin Yang:** Data curation. **Jie Bai:** Data curation. **Qiu-yue Lin:** Data curation. **Yun-long Xia:** Writing – original draft. **Yunpeng Xie:** Writing – review & editing, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e38098.

References

[1] S. Dogra, D.W. Dunstan, T. Sugiyama, A. Stathi, P.A. Gardiner, N. Owen, Active aging and public health: evidence, implications, and opportunities, Annu. Rev. Publ. Health 432022) 439-459, https://doi.org/10.1146/annurev-publhealth-052620-091107.

[2] L. Yanez-Bisbe, A. Garcia-Elias, M. Tajes, et al., Aging impairs reverse remodeling and recovery of ventricular function after isoproterenol-induced cardiomyopathy, Int. J. Mol. Sci. 23 (1) (2021), https://doi.org/10.3390/ijms23010174.

[3] Y. Gong, Y. Luo, S. Liu, et al., Pentacyclic triterpene oleanolic acid protects against cardiac aging through regulation of mitophagy and mitochondrial integrity, Biochim. Biophys. Acta, Mol. Basis Dis. 1868 (7) (2022) 166402, https://doi.org/10.1016/j.bbadis.2022.166402.

[4] H. Fang, S. Ghosh, L.C. Sims, et al., Fgf21 prevents low-protein diet-induced renal inflammation in aged mice, Am. J. Physiol. Ren. Physiol. 321 (3) (2021) F356–F368, https://doi.org/10.1152/ajprenal.00107.2021.

[5] E. Quarles, N. Basisty, Y.A. Chiao, et al., Rapamycin persistently improves cardiac function in aged, male and female mice, even following cessation of treatment, Aging Cell 19 (2) (2020) e13086, https://doi.org/10.1111/acel.13086.

[6] A.B. Newman, B.L. Naydeck, D.G. Ives, et al., Coronary artery calcium, carotid artery wall thickness, and cardiovascular disease outcomes in adults 70 to 99 years old, Am. J. Cardiol. 101 (2) (2008) 186–192, https://doi.org/10.1016/j.amjcard.2007.07.075.

[7] A.V. Durkina, O.G. Bernikova, M.A. Gonotkov, et al., Melatonin treatment improves ventricular conduction via upregulation of nav1.5 channel proteins and sodium current in the normal rat heart, J. Pineal Res. (2022), https://doi.org/10.1111/jpi.12798.

[8] S.F. Lo, P. Muti, G. Blandino, S. Strano, Melatonin and hippo pathway: is there existing cross-talk? Int. J. Mol. Sci. 18 (9) (2017) https://doi.org/10.3390/ijms18091913.

[9] M. Baeeri, T. Didari, M. Khalid, et al., Molecular evidence of the inhibitory potential of melatonin against naaso2-induced aging in male rats, Molecules 26 (21) (2021), https://doi.org/10.3390/molecules26216603.

[10] M. Tobeiha, A. Jafari, S. Fadaei, et al., Evidence for the benefits of melatonin in cardiovascular disease, Front. Cardiovasc. Med. 92022) 888319, https://doi.org/10.3389/fcvm.2022.888319.

[11] C. Xu, Z. He, J. Li, Melatonin as a potential neuroprotectant: mechanisms in subarachnoid hemorrhage-induced early brain injury, Front. Aging Neurosci. 142022) 899678, https://doi.org/10.3389/fnagi.2022.899678.

[12] Y. Wang, W. Guo, H. Xu, K. Tang, L. Zan, W. Yang, Melatonin suppresses milk fat synthesis by inhibiting the mtor signaling pathway via the mt1 receptor in bovine mammary epithelial cells, J. Pineal Res. 67 (3) (2019) e12593, https://doi.org/10.1111/jpi.12593.

[13] H. Bae, C. Yang, J.Y. Lee, et al., Melatonin improves uterine-conceptus interaction via regulation of sirt1 during early pregnancy, J. Pineal Res. 69 (2) (2020) e12670, https://doi.org/10.1111/jpi.12670.

[14] M.L. Dubocovich, P. Delagrange, D.N. Krause, D. Sugden, D.P. Cardinali, J. Olcese, International union of basic and clinical pharmacology. Lxxv. Nomenclature, classification, and pharmacology of g protein-coupled melatonin receptors, Pharmacol. Rev. 62 (3) (2010) 343–380, https://doi.org/10.1124/pr.110.002832.

[15] M.L. Dubocovich, M. Markowska, Functional mt1 and mt2 melatonin receptors in mammals, Endocrine 27 (2) (2005) 101–110, https://doi.org/10.1385/ENDO:27:2:101.

[16] R. Jockers, P. Delagrange, M.L. Dubocovich, et al., Update on melatonin receptors: iuphar review 20, Br. J. Pharmacol. 173 (18) (2016) 2702–2725, https://doi.org/10.1111/bph.13536.

[17] S.M. Hill, C. Cheng, L. Yuan, et al., Declining melatonin levels and mt1 receptor expression in aging rats is associated with enhanced mammary tumor growth and decreased sensitivity to melatonin, Breast Cancer Res. Treat. 127 (1) (2011) 91–98, https://doi.org/10.1007/s10549-010-0958-0.

[18] J. Leon, J. Casado, A. Carazo, et al., Gender-related invasion differences associated with mrna expression levels of melatonin membrane receptors in colorectal cancer, Mol. Carcinog. 51 (8) (2012) 608–618, https://doi.org/10.1002/mc.20832.

[19] M. Stroethoff, L. Goetze, C. Torregroza, et al., The melatonin receptor agonist ramelteon induces cardioprotection that requires mt2 receptor activation and release of reactive oxygen species, Cardiovasc. Drugs Ther. 34 (3) (2020) 303–310, https://doi.org/10.1007/s10557-020-06972-4.

[20] K. Singhanat, N. Apaijai, T. Jaiwongkam, S. Kerdphoo, S.C. Chattipakorn, N. Chattipakorn, Melatonin as a therapy in cardiac ischemia-reperfusion injury: potential mechanisms by which mt2 activation mediates cardioprotection, J. Adv. Res. 292021) 33-44, https://doi.org/10.1016/j.jare.2020.09.007.

[21] D. Han, Y. Wang, J. Chen, et al., Activation of melatonin receptor 2 but not melatonin receptor 1 mediates melatonin-conferred cardioprotection against myocardial ischemia/reperfusion injury, J. Pineal Res. 67 (1) (2019) e12571, https://doi.org/10.1111/jpi.12571.

[22] D. Ren, J. Fedorova, K. Davitt, et al., Activated protein c strengthens cardiac tolerance to ischemic insults in aging, Circ. Res. 130 (2) (2022) 252–272, https://doi.org/10.1161/CIRCRESAHA.121.319044.

[23] X. Luo, W. Yu, Z. Liu, et al., Ageing increases cardiac electrical remodelling in rats and mice via nox4/ros/camkii-mediated calcium signalling, Oxidative Med. Cell. Longev. 20222022) 8538296, https://doi.org/10.1155/2022/8538296.

[24] H. Wang, K.M. Muthu, D. Devadoss, M. Nair, H.S. Chand, M.K. Lakshmana, Tfeb protein expression is reduced in aged brains and its overexpression mitigates senescence-associated biomarkers and memory deficits in mice, Neurobiol. Aging 1062021) 26-36, https://doi.org/10.1016/j.neurobiolaging.2021.06.003.

[25] L. Chen, R. Yang, W. Qiao, et al., 1,25-dihydroxyvitamin d exerts an antiaging role by activation of nrf2-antioxidant signaling and inactivation of p16/p53-senescence signaling, Aging Cell 18 (3) (2019) e12951, https://doi.org/10.1111/acel.12951.

[26] Y. Suzuki, K. Takaya, S. Watanabe, M. Otaki, H. Kono, K. Kishi, Evaluation of the effect of age of the younger mice on the rejuvenation of the older mice by heterochronic parabiosis, Aging (Albany NY) 14 (6) (2022) 2507–2512, https://doi.org/10.18632/aging.203966.

[27] K. Singhanat, N. Apaijai, T. Jaiwongkam, S. Kerdphoo, S.C. Chattipakorn, N. Chattipakorn, Melatonin as a therapy in cardiac ischemia-reperfusion injury: potential mechanisms by which mt2 activation mediates cardioprotection, J. Adv. Res. 292021) 33-44, https://doi.org/10.1016/j.jare.2020.09.007.

[28] Y.P. Xie, S. Lai, Q.Y. Lin, et al., Cdc20 regulates cardiac hypertrophy via targeting lc3-dependent autophagy, Theranostics 8 (21) (2018) 5995–6007, https://doi.org/10.7150/thno.27706.

[29] M. Ezeani, A. Noor, K. Alt, et al., Collagen-targeted peptides for molecular imaging of diffuse cardiac fibrosis, J. Am. Heart Assoc. 10 (18) (2021) e22139, https://doi.org/10.1161/JAHA.121.022139.

[30] P. Wang, M. Wang, Y. Hu, et al., Isorhapontigenin protects against doxorubicin-induced cardiotoxicity via increasing yap1 expression, Acta Pharm. Sin. B 11 (3) (2021) 680–693, https://doi.org/10.1016/j.apsb.2020.10.017.

[31] C. Maneechote, T. Khuanjing, B. Ongnok, et al., Promoting mitochondrial fusion in doxorubicin-induced cardiotoxicity: a novel therapeutic target for cardioprotection, Clin. Sci. (Lond.) 136 (11) (2022) 841–860, https://doi.org/10.1042/CS20220074.

[32] Y. Wang, J. Han, S. Zhan, et al., Fucoidan alleviates doxorubicin-induced cardiotoxicity by inhibiting ferroptosis via nrf2/gpx4 pathway, Int. J. Biol. Macromol. 276 (Pt 1) (2024) 133792, https://doi.org/10.1016/j.ijbiomac.2024.133792.

[33] M. Majidinia, R.J. Reiter, S.K. Shakouri, B. Yousefi, The role of melatonin, a multitasking molecule, in retarding the processes of ageing, Ageing Res. Rev. 472018) 198-213, https://doi.org/10.1016/j.arr.2018.07.010.

[34] J. Vriend, R.J. Reiter, Melatonin feedback on clock genes: a theory involving the proteasome, J. Pineal Res. 58 (1) (2015) 1–11, https://doi.org/10.1111/jpi.12189.

[35] M. Viswanathan, J.T. Laitinen, J.M. Saavedra, Expression of melatonin receptors in arteries involved in thermoregulation, Proc. Natl. Acad. Sci. U. S. A 87 (16) (1990) 6200–6203, https://doi.org/10.1073/pnas.87.16.6200.
[36] D.N. Krause, V.E. Barrios, S.P. Duckles, Melatonin receptors mediate potentiation of contractile responses to adrenergic nerve stimulation in rat caudal artery, Eur. J. Pharmacol. 276 (3) (1995) 207–213, https://doi.org/10.1016/0014-2999(95)00028-j.
[37] H. Girouard, C. Chulak, M. Lejossec, D. Lamontagne, J. de Champlain, Vasorelaxant effects of the chronic treatment with melatonin on mesenteric artery and aorta of spontaneously hypertensive rats, J. Hypertens. 19 (8) (2001) 1369–1377, https://doi.org/10.1097/00004872-200108000-00004.
[38] S. Doolen, D.N. Krause, M.L. Dubocovich, S.P. Duckles, Melatonin mediates two distinct responses in vascular smooth muscle, Eur. J. Pharmacol. 345 (1) (1998) 67–69, https://doi.org/10.1016/s0014-2999(98)00064-8.
[39] M.I. Masana, S. Doolen, C. Ersahin, et al., Mt(2) melatonin receptors are present and functional in rat caudal artery, J. Pharmacol. Exp. Therapeut. 302 (3) (2002) 1295–1302, https://doi.org/10.1124/jpet.302.3.1295.
[40] N.J. Prado, L. Ferder, W. Manucha, E.R. Diez, Anti-inflammatory effects of melatonin in obesity and hypertension, Curr. Hypertens. Rep. 20 (5) (2018) 45, https://doi.org/10.1007/s11906-018-0842-6.
[41] E. Grossman, M. Laudon, R. Yalcin, et al., Melatonin reduces night blood pressure in patients with nocturnal hypertension, Am. J. Med. 119 (10) (2006) 898–902, https://doi.org/10.1016/j.amjmed.2006.02.002.
[42] F.A. Scheer, G.A. Van Montfrans, E.J. van Someren, G. Mairuhu, R.M. Buijs, Daily nighttime melatonin reduces blood pressure in male patients with essential hypertension, Hypertension 43 (2) (2004) 192–197, https://doi.org/10.1161/01.HYP.0000113293.15186.3b.
[43] J. Jimenez, K.J. Lavine, The dynamic role of cardiac macrophages in aging and disease, Curr. Cardiol. Rep. (2022), https://doi.org/10.1007/s11886-022-01714-4.
[44] A. Shirakabe, Y. Ikeda, S. Sciarretta, D.K. Zablocki, J. Sadoshima, Aging and autophagy in the heart, Circ. Res. 118 (10) (2016) 1563–1576, https://doi.org/10.1161/CIRCRESAHA.116.307474.
[45] M.D. Tallquist, Cardiac fibroblast diversity, Annu. Rev. Physiol. 822020) 63-78, https://doi.org/10.1146/annurev-physiol-021119-034527.
[46] H. Zhu, X. Wang, X. Meng, et al., Selenium supplementation improved cardiac functions by suppressing dnmt2-mediated gpx1 promoter dna methylation in age-induced heart failure, Oxidative Med. Cell. Longev. 20222022) 5402997, https://doi.org/10.1155/2022/5402997.
[47] K.B. Megha, A. Arathi, S. Shikha, R. Alka, P. Ramya, P.V. Mohanan, Significance of melatonin in the regulation of circadian rhythms and disease management, Mol. Neurobiol. (2024), https://doi.org/10.1007/s12035-024-03915-0.
[48] R.J. Reiter, S.D. Paredes, L.C. Manchester, D.X. Tan, Reducing oxidative/nitrosative stress: a newly-discovered genre for melatonin, Crit. Rev. Biochem. Mol. Biol. 44 (4) (2009) 175–200, https://doi.org/10.1080/10409230903044914.
[49] H.F. Pei, J.N. Hou, F.P. Wei, et al., Melatonin attenuates postmyocardial infarction injury via increasing tom70 expression, J. Pineal Res. 62 (1) (2017), https://doi.org/10.1111/jpi.12371.
[50] C.S. Kuo, C.Y. Chen, H.L. Huang, et al., Melatonin improves ischemia-induced circulation recovery impairment in mice with streptozotocin-induced diabetes by improving the endothelial progenitor cells functioning, Int. J. Mol. Sci. 23 (17) (2022), https://doi.org/10.3390/ijms23179839.
[51] Y. Cai, H. Liu, E. Song, et al., Deficiency of telomere-associated repressor activator protein 1 precipitates cardiac aging in mice via p53/pparalpha signaling, Theranostics 11 (10) (2021) 4710–4727, https://doi.org/10.7150/thno.51739.
[52] L.J. Mah, A. El-Osta, T.C. Karagiannis, Gammah2ax as a molecular marker of aging and disease, Epigenetics 5 (2) (2010) 129–136, https://doi.org/10.4161/epi.5.2.11080.
[53] L. Beels, K. Bacher, D. De Wolf, J. Werbrouck, H. Thierens, Gamma-h2ax foci as a biomarker for patient x-ray exposure in pediatric cardiac catheterization: are we underestimating radiation risks? Circulation 120 (19) (2009) 1903–1909, https://doi.org/10.1161/CIRCULATIONAHA.109.880385.
[54] W. Feng, X. Xu, G. Zhao, et al., Increased age-related cardiac dysfunction in bradykinin b2 receptor-deficient mice, J. Gerontol. Ser. A-Biol. Sci. Med. Sci. 71 (2) (2016) 178–187, https://doi.org/10.1093/gerona/glu210.
[55] L.Z. Liao, Z.C. Chen, S.S. Wang, W.B. Liu, C.L. Zhao, X.D. Zhuang, Nlrp3 inflammasome activation contributes to the pathogenesis of cardiocytes aging, Aging (Albany NY) 13 (16) (2021) 20534–20551, https://doi.org/10.18632/aging.203435.
[56] M. Segovia-Roldan, E.R. Diez, E. Pueyo, Melatonin to rescue the aged heart: antiarrhythmic and antioxidant benefits, Oxidative Med. Cell. Longev. 20212021) 8876792, https://doi.org/10.1155/2021/8876792.
[57] M. Sanchez-Hidalgo, M.J. Guerrero, M.P. Carrascosa-Salmoral, G.M.C. Naranjo, P.J. Lardone, R.C. de la Lastra, Decreased mt1 and mt2 melatonin receptor expression in extrapineal tissues of the rat during physiological aging, J. Pineal Res. 46 (1) (2009) 29–35, https://doi.org/10.1111/j.1600-079X.2008.00604.x.
[58] M. Fernandez-Ortiz, R. Sayed, J. Fernandez-Martinez, et al., Melatonin/nrf2/nlrp3 connection in mouse heart mitochondria during aging, Antioxidants 9 (12) (2020), https://doi.org/10.3390/antiox9121187.
[59] S.D. Wright, R.A. Ramos, P.S. Tobias, R.J. Ulevitch, J.C. Mathison, Cd14, a receptor for complexes of lipopolysaccharide (lps) and lps binding protein, Science 249 (4975) (1990) 1431–1433, https://doi.org/10.1126/science.1698311.
[60] D. Sharygin, L.G. Koniaris, C. Wells, T.A. Zimmers, T. Hamidi, Role of cd14 in human disease, Immunology 169 (3) (2023) 260–270, https://doi.org/10.1111/imm.13634.
[61] D.P. Funda, L. Tuckova, M.A. Farre, T. Iwase, I. Moro, H. Tlaskalova-Hogenova, Cd14 is expressed and released as soluble cd14 by human intestinal epithelial cells in vitro: lipopolysaccharide activation of epithelial cells revisited, Infect. Immun. 69 (6) (2001) 3772–3781, https://doi.org/10.1128/IAI.69.6.3772-3781.2001.
[62] G.L. Su, K. Dorko, S.C. Strom, A.K. Nussler, S.C. Wang, Cd14 expression and production by human hepatocytes, J. Hepatol. 31 (3) (1999) 435–442, https://doi.org/10.1016/s0168-8278(99)80034-8.
[63] M. Vives-Pi, N. Somoza, J. Fernandez-Alvarez, et al., Evidence of expression of endotoxin receptors cd14, toll-like receptors tlr4 and tlr2 and associated molecule md-2 and of sensitivity to endotoxin (lps) in islet beta cells, Clin. Exp. Immunol. 133 (2) (2003) 208–218, https://doi.org/10.1046/j.1365-2249.2003.02211.x.
[64] A. Ciesielska, M. Krawczyk, H. Sas-Nowosielska, A. Hromada-Judycka, K. Kwiatkowska, Cd14 recycling modulates lps-induced inflammatory responses of murine macrophages, Traffic 23 (6) (2022) 310–330, https://doi.org/10.1111/tra.12842.
[65] A. Grahnert, R. Weiss, E. Schilling, N. Stanslowsky, U. Sack, S. Hauschildt, Cd14 counterregulates lipopolysacharide-induced tumor necrosis factor-alpha production in a macrophage subset, J. Innate Immun. 11 (4) (2019) 359–374, https://doi.org/10.1159/000495528.
[66] Y. Shi, L. Zhao, J. Wang, S. Liu, Y. Zhang, Q. Qin, The selective nlrp3 inflammasome inhibitor mcc950 improves isoproterenol-induced cardiac dysfunction by inhibiting cardiomyocyte senescence, Eur. J. Pharmacol. 9372022) 175364, https://doi.org/10.1016/j.ejphar.2022.175364.
[67] Z. Jiang, P. Georgel, X. Du, et al., Cd14 is required for myd88-independent lps signaling, Nat. Immunol. 6 (6) (2005) 565–570, https://doi.org/10.1038/ni1207.