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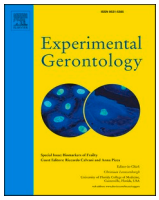

Therapeutic potential of sulfasalazine for sarcopenia: Insights from mouse models and clinical data

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ABSTRACT

Sarcopenia, a disease marked by a progressive loss of muscle mass, increases the risks of disability and metabolic disorders, and decreases quality of life. Current therapeutic options are limited. YY1 transcriptional activity is augmented through an interaction with PHF20 at its promoter region, suppressing muscle differentiation. This study screened sulfasalazine, a medication for managing inflammatory bowel diseases (IBD), using the PHF20-YY1 promoter assay in C_2C_{12} myoblasts from an FDA-approved drug library. Sulfasalazine effectively inhibited PHF20-induced YY1 promoter activity (IC_{50} = 24 μM), reducing YY1 expression and enhancing muscle-specific gene expression. In mouse models of muscle atrophy, sulfasalazine not only enhanced muscle strength and function but also mitigated muscle loss. Clinical data from patients with IBD revealed that those treated with sulfasalazine had a significantly higher TPI (total psoas index), used as a muscle mass marker, suggesting enhanced muscle preservation. In conclusion, this study suggests the potential for repurposing sulfasalazine to manage sarcopenia, especially associated with IBD.

1. Introduction

Sarcopenia is a progressive condition characterized by a generalized decline in skeletal muscle mass and function, leading to increased risks of disability, metabolic dysfunction, a poor quality of life, and death (Rosenberg, 2011; Cruz-Jentoft et al., 2010). Although it is primarily associated with aging (Cruz-Jentoft et al., 2010; Larsson et al., 2019), other factors contribute to muscle mass reduction, including a sedentary lifestyle (Larsson et al., 2019; Mo et al., 2023), immobilization (Larsson et al., 2019), malnutrition (Larsson et al., 2019; Muscaritoli et al., 2010), diabetes (Lisco et al., 2023), obesity (Benz et al., 2024), and acute or chronic inflammatory diseases like inflammatory bowel disease (IBD) (Nardone et al., 2021; Fatani et al., 2023), which in turn can exacerbate these conditions.

There are no drugs approved by the Food and Drug Administration (FDA) to address sarcopenia (Jang et al., 2023). However, the potential efficacies of growth hormone, anabolic, or androgenic steroids, selective androgen receptor modulators, protein anabolic agents, appetite stimulants, myostatin inhibitors, Type II receptor activators, β-receptor blockers, angiotensin-converting enzyme inhibitors, and troponin activators, are currently being evaluated (Cesari et al., 2022; Suh and Lee, 2020).

Yin Yang 1 (YY1), a transcription factor with a DNA binding domain, negatively regulates myogenesis YY1 forms a complex with Ezh2 and HDAC1, thereby inhibiting the transcription of muscle genes and myotube formation (Caretti et al., 2004). Its expression is downregulated by miR-29 during muscle differentiation (Wang et al., 2008). In addition to its role in myogenesis, YY1 represses insulin/IGF signaling of skeletal muscle that is crucial for muscle growth and repair (Blättler et al., 2012). Previously, we showed that PHD finger protein 20 (PHF20), a transcription factor with that is part of a lysine acetyltransferase complex, impedes muscle differentiation by augmenting YY1 transcription (Lee et al., 2020).

This study screened sulfasalazine, a medication commonly used to treat inflammatory bowel disease (IBD), from a library of FDA-approved drugs using a PHF20 induced-YY1 promoter assay. Sulfasalazine is known as an NF-κB inhibitor (Wahl et al., 1998; Weber et al., 2000). NF-κB signaling is involved in muscle wasting through regulation of YY1

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transcription (Wang et al., 2007; Li et al., 2008; Bakkar and Guttridge, 2010). The efficacy of sulfasalazine on myogenic differentiation and its impact on muscle strength, function, and mass was evaluated via both in vitro and in mouse muscle atrophy models. Finally, we assessed how sulfasalazine might influence muscle mass in IBD patients.

2. Materials and methods

2.1. Cell culture

C_2C_{12} myoblasts were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal bovine serum (WELGENE) and 1 % antibiotic–antimycotic (AA, Gibco) and differentiated in DM (DMEM containing 2 % horse serum and 1 % AA) for 5 or 6 days.

2.2. Construction of a PHF20 inducible pYY1-GFP C_2C_{12} stable cell line

Tet-On-inducible PHF20 C_2C_{12} cells previously reported (Lee et al., 2020) were transfected with GFP reporter plasmid containing YY1 promoter (pYY1-GFP) (pEZX-LvPF02, GeneCopoeia) using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). After transfection, the cells were selected using a combination of antibiotics (250 μg/ml of hygromycin, 1 mg/ml of G418, and 2 μg/ml of puromycin). This selection process lasted for about a month to ensure that only successfully transformed cells survived. To confirm the establishment of stable cell lines, the cells were treated with doxycycline (250 ng/ml) to induce the expression of PHF20 for 24 h, and then YY1 promoter activation was assessed by measuring the fluorescence intensity of the GFP reporter using a GloMax microplate reader (Promega, Madison, Wisconsin, USA).

2.3. High throughput screening

High throughput screening (HTS) of FDA-approved drug library (APExBIO, Discovery Probe) were performed using PHF20- pYY1-GFP-C_2C_{12} stable cells. Cells were plated at a density of 0.5×10^5 cells per well in a 96-well plate and incubated overnight in growth medium. After the overnight incubation, the cells were treated with 250 ng/ml of doxycycline (Doxy) for 24 h to induce the expression of the PHF20-pYY1-GFP construct and then the cells were exposed to 10 μM of various FDA-approved drugs for an additional 24 h. Primary hit compounds were identified based on their ability to inhibit GFP intensity by more than 30 % compared to Doxy-treated cells without any compound. The collected primary hits were then re-tested in a separate set of 96-well plates. The compounds were ranked based on the order of reduction in GFP intensity to select the final hits. Each compound was tested in duplicate in each experiment.

2.4. Luciferase assay

Gaussian luciferase reporter plasmid containing YY1 promoter (pYY1-Gluc, pEZX-LvPG02) and Secrete-Pair Gaussian Luciferase Assay Kit (LF062) were from GeneCopoeia. Following plating and overnight incubation in 96-well plate, C_2C_{12} myoblasts were co-transfected with YY1-GLuc (50 ng) and Flag-PHF20 plasmid (200 ng) using

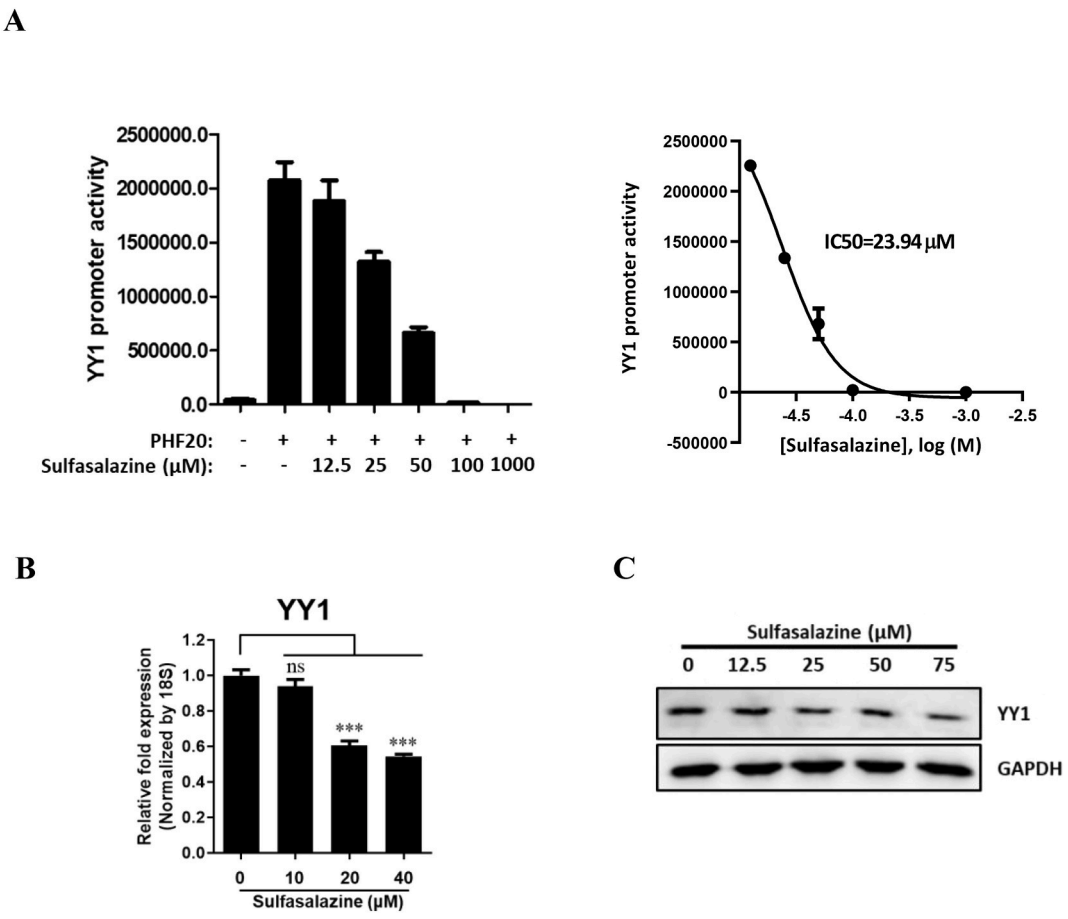

Fig. 1. **(A)** *Left*, YY1 promoter activity was assayed after 24 h of treatment of the indicated dose of sulfasalazine in C_2C_{12} myoblasts transfected with pYY1-Gluc in the absence and presence of Flag-PHF20 plasmids. Data are shown as mean ± SD of four wells. *Right*, Curve fitting (GraphPad Prism v.8.0 software) to determine IC50 value of sulfasalazine against PHF20-pYY1 activity. C_2C_{12} myoblasts were treated with the indicated concentrations of sulfasalazine for 24 h, and then expression of YY1 at mRNA and protein was detected by Q-PCR **(B)** and western blotting **(C)**. Data are shown as mean ± SD ($n = 4$). ns; no significance, $^{***}P < 0.001$ vs. untreated.

Lipofectamine 3000 for 24 h. Control cells were transfected only with the YY1-GLuc plasmid. At 24 h post-transfection, the cells were treated with sulfasalazine at the concentration shown in Fig. 1. Following incubation, luciferase activity was measured with GloMax microplate readers explorer using a Gaussia Luciferase Assay Kit according to the manufacturer's protocol. Data are expressed as mean ± SD of four wells.

2.5. Western blotting

Proteins were extracted from cells using lysis buffer (PRO-PREP™ Protein Extraction Solution, iNtRON) and were fractionated using 10–12 % SDS–PAGE. Western blotting was performed as previously described (Lee et al., 2020) with myogenin (DSHB, F5D), YY1 (Santa Cruz, sc-7341), GAPDH (Santa Cruz, sc-5174), and β-tubulin (Santa Cruz, sc-2128) antibodies.

2.6. Quantitative real-time PCR

Total RNA was extracted using an AccuPrep® universal RNA extraction kit (Bioneer), and cDNA was synthesized using SuperScript II (Invitrogen, 18064022). Quantitative real-time PCR (Q-PCR) was performed using a GoTaq qPCR master mix (Promega) on an AriaMx real-time PCR instrument (Agilent). PCR was carried out under the conditions of initial denaturation at 95 °C for 3 min, followed by denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, and extending at 72 °C for 30 s (40 cycles). Relative mRNA expression was calculated by normalization with reference genes such as GAPDH or 18 s rRNA and then by the standard curve method of cycle threshold value (Cq). Primer sequences are shown in Table 1.

2.7. Myotube formation

C_2C_{12} myoblasts were differentiated in DM with or without 20 μM sulfasalazine for 5 days. The cells were fixed in 4 % paraformaldehyde for 20 min and permeabilized with 0.2 % Triton X-100/PBS for 15 min, followed by myotube staining as previously described (Lee et al., 2020). Myotube number and length, and the fusion index (the ratio of nucleus to total nucleus in MHC-positive myotube) were analyzed using ImageJ software.

2.8. Design of animal studies

C57BL/6 J mice were purchased from DooYeol Biotech (Seoul, South Korea). Mice were acclimated to their environment for one week before the experiments began. Velcro-fixation model was established by immobilizing the left hindlimb of 7-week-old C57BL/6 J mice using a non-elastic bandage (Multipore™ Sports White Athletic Tape, 3 M Japan, Japan) and Velcro tape for 14 days (Suwankanit and Shimizu, 2022; Xie et al., 2021; Nakamura et al., 2020; Tando et al., 2016). After the immobilization period, the Velcro was removed, and mice were administered with PBS (vehicle) or sulfasalazine (50 mg/kg for muscle tissue analysis or 5, 50, and 500 mg/kg for muscle function test) via oral gavage daily for 6 days (for muscle tissue analysis) or 14 days (for muscle function tests). CTX-injury model was established as described by Glynnis et al. (Garry et al., 2016). Briefly, 7-week-old mice were injected with 10 μM cardiotoxin into the tibialis anterior (TA) muscles. Following a 24-h period, the mice received either PBS or sulfasalazine daily for 14 days. In the aging model (Xie et al., 2021; Kadoguchi et al., 2020), 60-week-old mice were treated daily with either PBS or sulfasalazine for 28 days. Each group in the muscle atrophy models consisted of five mice, except for the muscle tissue analysis, which had three mice per group. All animal experiments were conducted in accordance with procedures approved by the Institutional Animal Care and Use Committee of the Chungnam National University (202109 A-CNU-147).

2.9. H&E and immunofluorescence staining

Gastrocnemius (GA) muscle tissues from mice with or without damage by Velcro-fixation were collected on days 0, 2, 4, and 6 after given PBS or sulfasalazine (50 mg/kg). The tissues were prepared with paraffin-embedded sections, followed by hematoxylin-eosin (H&E) staining as previously described (Lee et al., 2020), and taken under a microscope (EVOSTM M5000, Invitrogen). For immunofluorescence staining, GA tissues were prepared by cryosections as previously described (Fra-Bido et al., 2021). The cryosections were incubated with anti-PAX7 antibody (Santa Cruz, sc-81,648) overnight at 4 °C. After washing with PBS, the sections were treated with an Alexa Fluor 488 secondary antibody (Invitrogen, A-10680) for 1 h at room temperature. The stained sections were analyzed using Image J software.

2.10. Treadmill

The treadmill (TM) exercise (Panlab, Harvard Apparatus) was measured on days 0, 3, 6, 10, and 14 of sulfasalazine administration. TM condition was as follows (Castro and Kuang, 2017); mice were warmed up at 15 cm/s for 3 min, and the speed was changed to 25 cm/s without incline, followed by an increase of 15 cm/s every minute until exhaustion (defined as exhaustion when electrical stimulation (0.2 AM) occurs 3 times). Treadmill exercise capacity was assessed as running distance (m).

2.11. Grip strength and balance ability

Grip strength and balance ability were measured on days 0, 7, and 14 using a grip strength meter (Bioseb, Harvard Apparatus) and rotarod device (Harvard & Panlab, LE8205), respectively. *Grip strength* (Castro and Kuang, 2017); The mice were placed with their forelimbs on a grid and the grip strength was measured immediately before mice fell from the bar. Each mouse undergoes five trials, and the grip strength is defined as the mean of these trials. *Balance ability* (Deacon, 2013); The accelerated rotarod test (4 to 40 rpm for 300 s) was conducted three times, with a test interval of 2 min. Balance capacity was determined by the lag time falling from the rotarod.

2.12. Clinical analysis of patients with inflammatory bowel diseases

The patients with inflammatory bowel diseases (IBD) such as ulcerative colitis (UC) and Crohn's disease (CD) were retrospectively enrolled by the Severance Clinical Research Analysis Portal for Anonymous (SCRAP). Initially, all patients diagnosed and treated for UC and CD

Table 1
Primer sequences for Q-PCR.

Gene	Species	Forward (5′ → 3′)	Reverse (5′ → 3′)
18 s rRNA	Mouse	GTAACCCGTTGAACC CCATT	CCATCCAATCGGTAGCG
YY1	Mouse	CAGAAGCAGGTGCAGATC AGACCCT	GCACCACCACCCACGGAATCG
MYF5	Mouse	AAGGCTCCTGTATCCCCTCAC	TGACCTTCTTCAGGCGTCTAC
MYH1	Mouse	AAGGGTCTGCGCAAACATGA	TTGGCCAGGTTGACATTGGA
MYH2	Mouse	ATTCTCAGGCTTCAGGATTTGGTG	CTTGCGGAACTTGGATAGATTTGTG
MYH4	Mouse	GAGTTCATTGACTTCGGGATGG	TGCTGCTCATACAGCTTGTTCTTG
MYH7	Mouse	ATGAGCTGGAGGCTGAGCA	TGCAGCCGCAGTAGGTTCTT

were extracted ($N = 7651$). Among them, patients prescribed the investigational drug sulfasalazine ($n = 4635$) were separated from those not prescribed ($n = 3016$) for baseline data investigation. The baseline characteristics of the recruited patients were as follows: 1) disease: UC and CD, 2) gender, age at diagnosis, duration of observation until the final assessment, duration of illness, 3) body mass index (BMI) and muscle quantity index (Total Psoas Index, TPI). To assess muscle mass, we utilized the image data from abdominal computer tomography (CT) and estimated TPI. Briefly, TPI was calculated based on the cross-sectional area of the psoas muscle at the level of the third lumbar vertebra using ImageJ. Exclusion criteria included recent diagnosis and prescription without sufficient observation period, prescription duration deemed insufficient to manifest the drug's adequate effects, uncertainty in diagnosis (e.g., unspecified UC and CD), and inadequate radiological examinations for determining TPI. Through this process, 50 participants were confirmed for the sulfasalazine treatment group, and 28 for the control group (Fig. 5A). For comparative analyses, we applied propensity score matching (1:5 matching) to adjust for any potential confounding factors between sulfasalazine-treated and untreated cohorts. This method allowed us to create a comparable cohort and control for selection biases that could influence clinical outcomes. The study protocol was reviewed and approved by the institutional review board of Severance hospital and the informed consent was waived because of its retrospective nature (IRB No. 2023–1688-001).

2.13. Statistical analysis of clinical data

In longitudinal analysis of clinical outcome changes over time, paired *t*-tests (or Wilcoxon signed-rank tests) were utilized within each group to determine the significance of changes pre- and post-treatment. Additionally, ANCOVA models were employed to account for covariates like baseline BMI and CRP levels, which may influence treatment efficacy. Differences were assessed by Student's unpaired t-test or Mann Whitney *U* test using GraphPad Prism v.8.0 software, accordingly, and chi-square tests were conducted to compare continuous and categorical variables, respectively, between the two groups. Differences were denoted as follows: ns; no significance. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

3. Results

3.1. Sulfasalazine inhibits YY1 transcription

To find drugs that promote myogenic differentiation via the PHF20-YY1 axis (Lee et al., 2020), we performed HTS on an FDA-approved library (1670 compounds) using C_2C_{12} myoblasts transformed with a PHF20-inducible pYY1-GFP reporter. Among the compounds screened, 13 sulfa-based drugs were identified, with sulfasalazine showing a significant reduction in GFP intensity, indicating inhibition of YY1 activation. Given our interest in commercially available drugs, we focused on sulfasalazine. Sulfasalazine is a medication commonly used to treat inflammatory bowel diseases (IBD) and rheumatoid arthritis (RA) (Choi et al., n.d.). The efficacy of sulfasalazine was further validated using a pYY1-GLuc assay. As shown in Fig. 1A, sulfasalazine inhibited PHF20-induced YY1 promoter activity in a dose-dependent manner, with an IC50 of approximately 24 μM. Consistent with this result, it decreased YY1 mRNA (Fig. 1B) and protein expression (Fig. 1C) in a dose-dependent manner. These data suggest that sulfasalazine may be a promising candidate for promoting myogenic differentiation by targeting the PHF20-YY1 axis.

3.2. Sulfasalazine promotes myogenic differentiation

To evaluate the effect of sulfasalazine on myogenic differentiation, the expression levels of myogenic markers such as myogenin (Chen et al., 2015) and myosin heavy chain (MyHC) (Agarwal et al., 2020) were assessed using western blotting or Q-PCR. Sulfasalazine treatment enhanced myogenin expression compared to control treatment with DMSO during a differentiation period of 0 to 6 days (Fig. 2A). MyHC genes such as MYH-1, 2, 4, and 7 exhibited significant increases at day 5 of differentiation in the sulfasalazine-treated cells compared to the DMSO (Fig. 2B). MYF5, a marker expressed in proliferating myoblasts and reduced in myotubes (Chen et al., 2015), showed significantly lower mRNA expression in the sulfasalazine-treated cells compared to the DMSO. Immunostaining with an anti-MyHC antibody revealed that sulfasalazine treatment resulted in a higher number of myotubes compared to the DMSO control (Fig. 2D). Morphological quantification indicated that sulfasalazine increased the number of myotubes (Fig. 2E), their length (Fig. 2F), and the myoblast fusion index (Fig. 2G). Overall, these results suggest that sulfasalazine promotes myogenic differentiation by enhancing the expression of myogenic markers and facilitating myotube formation.

3.3. Sulfasalazine improves muscle strength and function in mouse models of sarcopenia

To investigate the effect of sulfasalazine on muscle strength and function in both age-related and independent factors contributing to muscle atrophy, we used three models of muscle atrophy in mice: Velcro-fixation (Fig. 3) (Xie et al., 2021; Nakamura et al., 2020; Tando et al., 2016) and CTX-injury (Supplementary Fig. S1) models (Garry et al., 2016; Wang et al., 2022) in mice aged 7 weeks and aging models in mice aged 60 weeks (Supplementary Fig. S2) (Xie et al., 2021; Kadoguchi et al., 2020). The doses of sulfasalazine (5 and 50 mg/kg) were selected based on our in vitro IC50 value of 24 μM (equivalent 10 mg/kg) and previous studies indicating typical doses ranging from 30 mg/kg to 320 mg/kg (Verbruggen et al., 2021; Shin et al., 2017). A higher dose of 500 mg/kg was included to assess potential dose-dependent effects and safety. During the treatment period, we measured the grip strength, treadmill running distance, and rotarod balance ability commonly used for the assessment of muscle strength and muscle function in sarcopenia mouse models (Xie et al., 2021). As shown in Velcro-fixation model (Fig. 3), grip strength, running distance, and balance ability were increased in the groups given sulfasalazine compared to the PBS (vehicle) group in dose- and time-dependent manners. The positive effects of sulfasalazine were also confirmed in supplementary models (Supplementary Figs. S1 and S2), indicating a consistent benefit across different experimental setups. Overall, these results suggest that sulfasalazine may enhance muscle strength and function, potentially offering a therapeutic avenue for addressing sarcopenia.

3.4. Sulfasalazine promotes muscle recovery in mouse Velcro-induced muscle atrophy

Next, the effect of sulfasalazine on muscle loss recovery was investigated using a mouse Velcro-induced muscle atrophy model. To this end, GA muscle tissues from mice with or without damage caused by Velcro were collected 0, 2, 4, and6 days after administering PBS or sulfasalazine and then the cross-sectional area (CSA) of muscle fibers, representing the histological muscle mass (Nilwik et al., 2013), was measured after H&E staining. As shown in Fig. 4A, Velcro injury significantly reduced the CSA of muscle fibers compared to the control (no injury). At 6 days post-injury, the administration of sulfasalazine significantly increased the CSA of muscle fibers compared to the control group that received PBS, demonstrating sulfasalazine-induced muscle recovery (Fig. 4B). To further confirm the effects of sulfasalazine, Pax7 immunofluorescence staining was performed. Pax7 is a marker for satellite cells, which are crucial for muscle regeneration (von Maltzahn et al., 2013; McKenna and Fry, 2017; Careccia et al., 2023). The results showed a considerable increase in Pax7-expressing cells (green) in the sulfasalazine-treated group compared to the PBS group (Fig. 4C). Taken together, these data suggest that sulfasalazine promotes recovery from

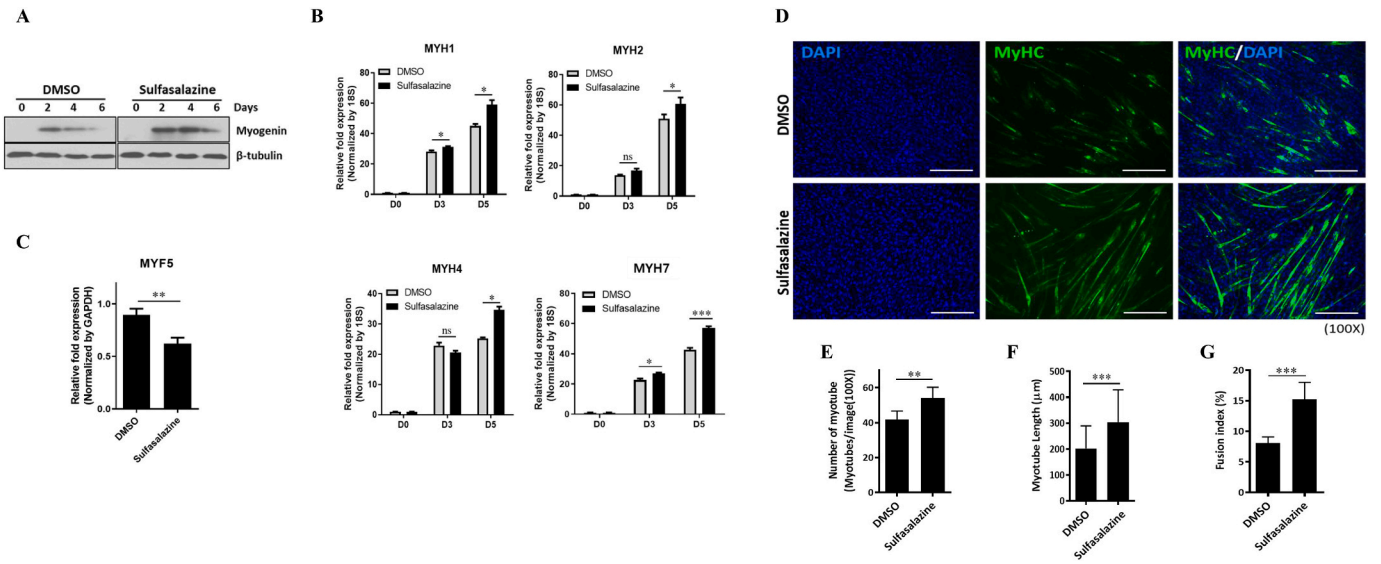

Fig. 2. C_2C_{12} myoblasts were differentiated in DM containing 20 μM sulfasalazine or DMSO (0.01 %) for 5 or 6 days. Cell were harvested on the indicated day or day 6 and then western blotting **(A)** or Q-PCR **(B, C)** was performed. mRNA expression was presented as fold induction for DMSO after normalization with internal control, 18S rRNA. Data are shown as mean ± SD (n = 4). ns; no significance, $^{*}P < 0.05$; ***P < 0.001 vs. DMSO. **(D)** Myotubes were detected via immunostaining with MyHC antibody (green) and nuclei were counterstained with DAPI (blue). magnification = 100×, scale bar = 100 μm. Quantitative analysis of myotube number **(E)**, myotube length **(F)**, and nuclei fusion index **(G)**. Data are represented as mean ± SD ($n = 6$). ***P < 0.001, $^{**}P < 0.01$ vs. DMSO. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

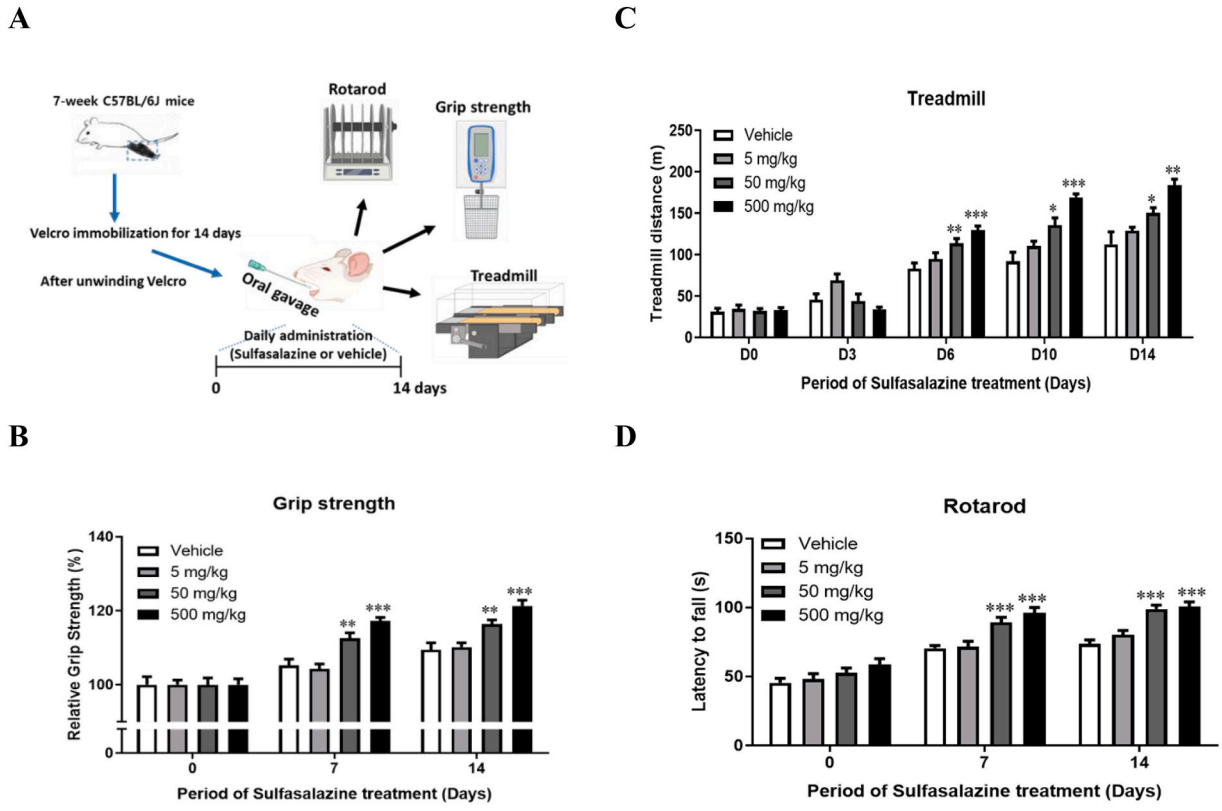

Fig. 3. **(A)** Schematic of experimental procedure. Details are descripted in materials and methods. **(B)** Grip strength (days 0, 7, and 14), **(C)** running distance (days 0, 3, 6, 10, and 14), and **(D)** balance ability (days 0 7, and 14) **(D)** were measured on specified days during the treatment period. Data are shown as mean ± SE ($n = 5$). ***P < 0.001, $^{**}P < 0.01$, $^{*}P < 0.05$ vs. control (vehicle; PBS) on each day of measurement.

muscle atrophy by increasing muscle fiber CSA and enhancing the activation of satellite cells involved in muscle regeneration.

3.5. Effect of sulfasalazine on muscle preservation in patients with inflammatory bowel diseases

Sarcopenia is particularly prevalent among patients with

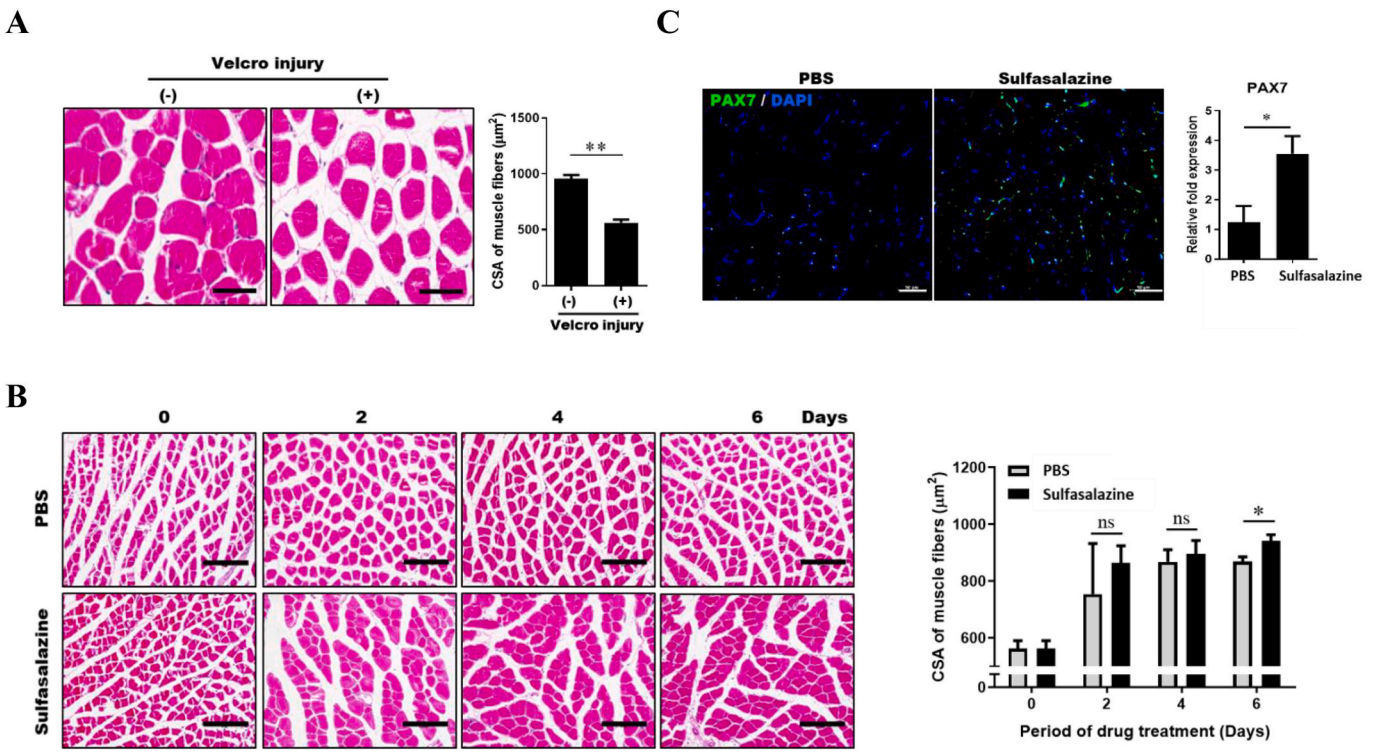

Fig. 4. **(A)** *Left*, H&E staining of the cross-sectional area (CSA) of GA tissues from mice without or with Velcro injury. Magnification = 200×, scale bar = 150 μm. *Right*, Quantification of the muscle fibers CSA. Data are shown as the mean ± SE ($n = 3$). **P < 0.01 vs. without injury. **(B)** *Left*, H&E staining of GA tissues from mice given PBS or 50 mg/mg of sulfasalazine on days 0, 2, 4, and 6. magnification = 200×, scale bar =50 μm. *Right*, Quantification of muscle fibers CSA. Data are shown as the mean ± SE (n = 3). ns; no significant difference, *P < 0.05 vs. PBS. **(C)** *Left*, Immunofluorescence staining of PAX7 (green) and counterstaining of the nuclei (DAPI, blue). scale bar = 50 μm. *Right*, Quantification of PAX7 expressing cells. Quantification was carried out using Image J software. Data are shown as the mean ± SE (n = 3). *P < 0.05 vs. PBS. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

inflammatory bowel disease (IBD), such as crohn's disease (CD) and ulcerative colitis (UC) (Nardone et al., 2021; Fatani et al., 2023). The chronic inflammation associated with IBD can lead to muscle wasting and loss of function, exacerbating the already significant health challenges faced by these patients. To assess the clinical efficacy of sulfasalazine, we analyzed total psoas index (TPI) in patients with IBD, such as UC or CD. TPI is a reliable biomarker for assessing muscle preservation (Xu et al., 2020; Minawala and Faye, 2024). Among 7651 IBD patients, 4635 were prescribed sulfasalazine and 3016 were not prescribed. After exclusion criteria, 50 patients in the sulfasalazine treatment group and 28 in the control group were included in the final analysis (Fig. 5A). The mean observation period was 93.54 ± 54.41 months in the sulfasalazine treatment group. Interestingly, TPI was elevated in the sulfasalazine-treated group compared to the non-treated group, while there were no significant changes in body weight or BMI between the two groups, suggesting that sulfasalazine administration enhanced muscle preservation in patients with UC and CD.

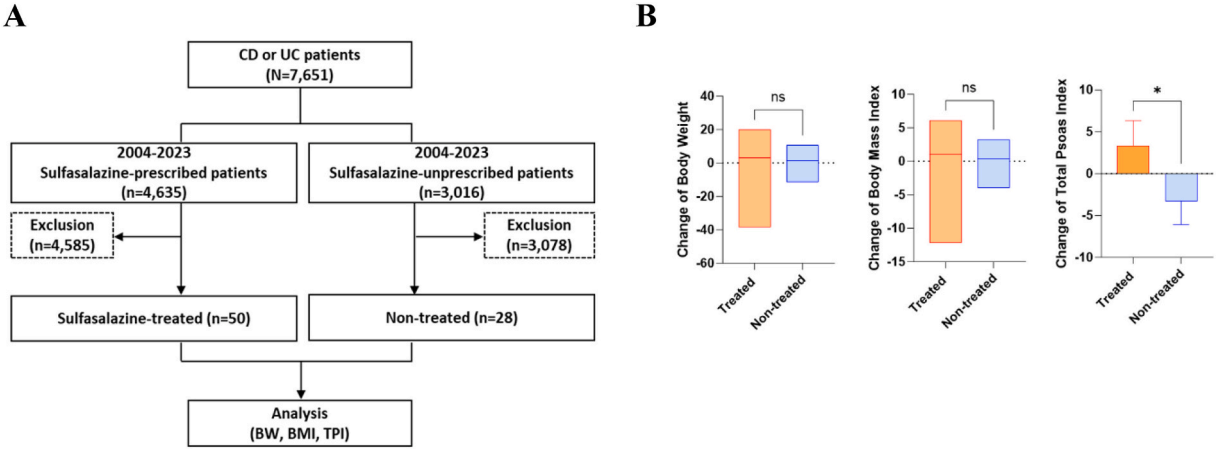

Fig. 5. **(A)** Schematic protocol of our retrospective cohort study. Details are descripted in materials and methods. **(B)** Comparison of the changes in body weight, body mass index and total psoas index between sulfasalazine treated group and non-treated group. The changes were obtained from pretreatment to last follow up period in sulfasalazine treated group (93.54 ± 54.41 months). For the non-treated group, the changes were calculated from same periods (96.36 ± 10.82 months) with that of sulfasalazine treated group. Two-tailed *P*-values were calculated by Mann-Whitney *U* test. *$P < 0.05$.

4. Discussion

The prevalence of sarcopenia in patients with IBD is 17–42 %, and IBD patients with sarcopenia have greater needs for surgery and more postoperative complications (Fatani et al., 2023; Pedersen et al., 2017). Accordingly, the importance of sarcopenia treatment in IBD patients is increasing. This study presents the potential of sulfasalazine, drug traditionally used for IBD, as a novel treatment for sarcopenia, particularly in patients suffering from IBD.

Sulfasalazine inhibits the activation of the nuclear factor kappa B (NF-κB) (Wahl et al., 1998; Weber et al., 2000), which is typically activated by pro-inflammatory cytokines such as TNF-α and IL-6. The activation of NF-κB is known to promote muscle protein degradation and inhibit myogenic differentiation by increasing YY1 transcription by binding to the YY1 promoter directly (Wang et al., 2007; Li et al., 2008; Bakkar and Guttridge, 2010). In NF-κB subunit p65−/− mice, YY1 mRNA levels are reduced compared to p65+/+ mice, indicating a link between NF-κB activity and YY1 expression. (Wang et al., 2007). PHF20 appears to be a critical regulator in this signaling pathway, maintaining NF-κB in an active state (Zhang et al., 2013) and promoting YY1 transcription (Blättler et al., 2012). We identified sulfasalazine as an inhibitor of the PHF20-YY1 interaction, with an IC50 value of 24 μM, indicating its potency in disrupting this interaction (Fig. 1A). The treatment with sulfasalazine resulted in a reduction in YY1 expression (Fig. 1B and C), and enhanced muscle differentiation (Fig. 2A and B) and myotube formation (Fig. 2D). These findings suggest that, inhibition of the NF-κB pathway by sulfasalazine may lead to a reduction in PHF20 activity, which in turn decreases YY1 transcription. This reduction in YY1 levels promotes myogenic differentiation and enhances muscle regeneration in C_2C_{12} myoblasts. Tracking inflammatory markers and YY1 expression levels, PHF20 and NF-κB activity, myogenic differentiation, and muscle regeneration in response to sulfasalazine treatment in p65−/− and p65+/+ mouse models will help validate the mechanism of action of sulfasalazine in the NF-κB/PHF20/YY1 signaling.

In mouse models of muscle atrophy, both the 50 mg/kg and 500 mg/kg doses of sulfasalazine produced similar outcomes in functional tests (Figs. 3 and Supplementary Figs. S1 and S2). The similar outcomes suggest that sulfasalazine may reach a saturation point in its mechanism of action. This could mean that once a certain threshold concentration of sulfasalazine is achieved in the body, additional increases in dosage do not yield further benefits in muscle strength or function. This could indicate that the therapeutic effects are maximized at lower doses, which is a common phenomenon with many pharmacological agents (Hoog et al., 2024). Individual variability among the mice, including genetic factors, baseline muscle function, and the extent of muscle atrophy, could also contribute to the observed outcomes. Some mice may respond more favorably to the lower dose, while others may not show significant differences at higher doses.

The 50 mg/kg dose given to mice (Figs. 3 and 4, and Supplementary Figs. 1 and 2) translates to a total dose of 1.25 mg per mouse for approximately 25 g (0.025 kg) of mice. Extrapolating to human dosing, a 60 kg adult could receive 240 mg once daily (Nair and Jacob, 2016); Human Equivalent Dose, HED (mg/kg) = Animal Dose (mg/kg) × (Human Km/Animal Km), Km is approximately 3 and 37, for mice and humans, respectively. Therefore, 50 mg/kg is calculated to approximately 4.05 mg/kg of HED. The total daily dose for a 60 kg adult: 4.05 mg/kg x 60 kg ≒ 240 mg. This dosage falls below the recommended doses for UC (500–1000 mg every 6 to 8 h) (Singh et al., 2019) or RA (500–1000 mg once or twice a day in divided doses) in adults (Suarez-Almazor et al., 2000), suggesting it may be safe for use in treating conditions like sarcopenia. However, it will be important to consider individual variability in response to drug, as factors such as genetics, baseline health status, and the specific condition being treated can influence efficacy and safety.

The treatment with sulfasalazine resulted in a significant increase in muscle fiber CSA and the number of satellite cells in the GA tissues of Velcro-fixation injured mice. (Fig. 4). This suggests that sulfasalazine enhances muscle regeneration capabilities. A decrease in satellite cells is often linked to muscle atrophy (McKenna and Fry, 2017; Careccia et al., 2023) and activation of NF-κB in satellite cells can lead to telomere shortening, which is associated with cellular aging and reduced regenerative capacity, especially in aged muscles (Tichy et al., 2021). Fengyuan Chen et al. have demonstrated the significant role of YY1 in metabolic reprogramming within muscle stem cells, suggesting that YY1 may also interact with NF-κB signaling (Chen et al., 2019). Therefore, sulfasalazine promotes muscle regeneration by modulating NF-κB/PHF20/YY1 signaling and preventing telomere shortening. To validate this hypothesis, the following experimental approaches will be helpful: 1) to assess the effects of sulfasalazine on muscle regeneration and telomere length in p65−/− and p65+/+ mice after muscle injury, 2) to measure the mRNA and protein levels of YY1, PHF20, and NF-κB in satellite cells from p65−/− and p65+/+ mice, and 3) to assess differentiation markers between satellite cells treated with sulfasalazine and untreated controls.

TPI (Total Psoas Index) is increasingly recognized as a valuable indicator of muscle mass and plays a significant role in identifying sarcopenia, particularly in clinical settings (Xu et al., 2020; Minawala and Faye, 2024). A low TPI has been associated with worse prognostic outcomes in various conditions, including IBD and longer recovery times and higher rates of complications after surgery (Minawala and Faye, 2024). We found a significant increase in TPI levels in patients with IBD treated with sulfasalazine compared to those who did not receive treatment (Fig. 5), indicating sulfasalazine's muscle preservation efficacy. Considering the pharmacological action of sulfasalazine, by decreasing the levels of cytokines through NF-κB inhibition, sulfasalazine may help alleviate the inflammation associated with IBD, which is often linked to muscle wasting. This could lead to a decrease in muscle catabolism (the breakdown of muscle tissue) and an enhancement in muscle protein synthesis, ultimately contributing to better muscle preservation in IBD patients. To better understand the effects of sulfasalazine on muscle health in IBD patients, further studies are needed to: 1) track muscle mass and inflammatory markers in IBD patients before and after starting sulfasalazine treatment, 2) compare the effects of sulfasalazine on muscle health with other anti-inflammatory treatments, and 3) determine the optimal dose of sulfasalazine for muscle preservation in relation to its effects on inflammatory markers.

Traditional treatments for sarcopenia, such as androgen modulators and anabolic steroids, have limited effectiveness in cases complicated by inflammation and raise long-term safety concerns, especially for elderly or chronically ill patients (Basualto-Alarcón et al., 2014). Inflammatory conditions can worsen muscle wasting and diminish the benefits of these treatments, which also carry risks of side effects like cardiovascular issues, liver damage, and hormonal imbalances (Najm et al., 2024). These risks are heightened in older patients due to existing comorbidities and age-related hormonal changes. Additionally, access to these treatments may be restricted in some regions (Kuzuya, 2024). While myostatin inhibitors show promise, their clinical results have been inconsistent and they remain under investigation (Suh and Lee, 2020). By contrast, the established anti-inflammatory properties of sulfasalazine and its ability to inhibit NF-κB uniquely position it as a potential therapeutic for sarcopenia in patients with inflammatory origins. Its effect on the PHF20/YY1 pathway provides an innovative approach that directly targets mechanisms involved in both muscle degradation and inflammation, underscoring its potential utility beyond traditional sarcopenia treatments. Further clinical research should confirm the efficacy and safety profile of sulfasalazine in this context, particularly in populations with chronic inflammatory conditions such as IBD.

In conclusion, our findings suggest that sulfasalazine is a promising candidate for managing sarcopenia, particularly in populations affected by chronic inflammatory conditions. To substantiate these findings, future research should conduct prospective clinical trials to evaluate its efficacy in larger, diverse patient groups, while closely monitoring long-

term safety, including potential adverse effects, variability in patient populations, and drug interactions.

Adverse events associated with sulfasalazine, such as blood dyscrasias (Narayan et al., 2017), gastrointestinal issues such as nausea, vomiting, diarrhea, and abdominal pain (Abhishek et al., 2024), hepatotoxicity (Jobanputra et al., 2008), and renal function impairment (Niknahad et al., 2017), necessitate regular monitoring of patients. Rarely, sulfasalazine can cause pulmonary toxicity, including interstitial lung disease (Kerget et al., 2018). Patients should be monitored for respiratory symptoms.

Combining sulfasalazine with other therapies, including corticosteroids (Bruscoli et al., 2021), anti-TNF agents like infliximab or adalimumab (Gordon et al., 2024), and immunomodulators (e.g., Azathioprine, Mercaptopurine) (Löwenberg et al., 2023) can improve treatment outcomes by more comprehensively mitigating inflammation and reducing muscle wasting. Probiotics may help restore gut microbiota balance and reduce inflammation, potentially working synergistically with sulfasalazine (Roy and Dhaneshwar, 2023).

Investigating sulfasalazine's effects on muscle strength, endurance, and overall physical performance is crucial for understanding its impact on quality of life in sarcopenic patients. Long-term studies are needed to confirm the safety and efficacy of sulfasalazine in individuals with sarcopenia, particularly those with IBD and other chronic inflammatory conditions.

CRediT authorship contribution statement

Meehee Park: Validation, Writing – review & editing, Writing – original draft, Methodology, Data curation, Formal analysis, Conceptualization. **Seungju Cho:** Conceptualization, Methodology, Writing – original draft, Validation, Investigation, Formal analysis, Visualization, Data curation. **Seonggyu Choi:** Visualization, Methodology, Investigation, Validation, Formal analysis, Writing – original draft. **Hwayoung Lee:** Formal analysis, Conceptualization, Validation, Data curation, Methodology, Writing – original draft, Resources, Investigation. **Jandee Lee:** Writing – original draft, Methodology, Data curation, Validation, Investigation, Formal analysis, Conceptualization, Visualization, Resources. **Youngsuk Jo:** Writing – review & editing, Investigation, Conceptualization, Writing – original draft, Project administration, Formal analysis, Visualization, Supervision, Data curation, Validation, Resources, Methodology. **Jisoo Park:** Writing – review & editing, Writing – original draft, Validation, Resources, Investigation, Visualization, Funding acquisition, Methodology, Data curation, Project administration, Supervision, Conceptualization. **Jongsun Park:** Writing – review & editing, Writing – original draft, Project administration, Conceptualization, Supervision, Resources, Data curation, Investigation, Validation, Software, Methodology, Visualization.

Ethical compliance

This research was carried out in alignment with ethical standards, having obtained approval from the Institutional Review Board (IRB) at Chungnam National University and Severance Hospital. This approval ensures the safeguarding of both animal and human subjects participating in the study.

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Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exger.2025.112883.

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