Article

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Activation of TGF-β Smad Signaling Promotes Temporomandibular Joint Injury Repair in Aged Rats

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Background: Temporomandibular joint (TMJ) disorders are common in adults, leading to cartilage degradation and joint dysfunction. Transforming growth factor-beta (TGF-β) has been found to increase extracellular matrix synthesis and suppress inflammation in osteoarthritis; however, its specific role in TMJ disorders remains uninvestigated. Therefore, this study aims to explore the involvement of TGF-β in TMJ injury repair, particularly examining its effects in aged rats.
Methods: The Sprague-Dawley rats were assigned to young (n = 10) and aged (n = 30) groups, with TMJ injury induced using type II collagenase. TGF-β was injected to activate the TGF-β/Smad signaling pathway, while SB431542 was used as an inhibitor of the pathway. Histological analysis (hematoxylin and eosin [H&E] staining and Safranin O/Fast Green staining) was used to examine cartilage repair, and Western blot analysis was employed to determine changes in TGF-β/Smad pathway and cartilage matrix-related proteins. Furthermore, head withdrawal threshold (HWT) and feeding behavior were evaluated to determine the impact of TGF-β treatment on pain sensitivity and feeding behavior.
Results: TGF-β treatment significantly improved cartilage repair in aged rats following TMJ injury. Histological analysis revealed increased cartilage thickness, improved cell arrangement, and reduced proteoglycan depletion in the TGF-β group. Western blot analysis demonstrated substantially elevated phosphorylated-Smad2/Smad2 (p-Smad2/Smad2) and p-Smad3/Smad3 ratios in the TGF-β group ($p < 0.01$). Furthermore, TGF-β treatment upregulated the expression of Aggrecan and Collagen II, while downregulating the levels of matrix-degrading enzymes matrix metalloproteinase-13 (MMP-13), matrix metalloproteinase-3 (MMP-3), and A disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5) ($p < 0.01$). Additionally, the TGF-β group had significantly reduced pain sensitivity and improved feeding behavior compared to the injury group ($p < 0.05$). In contrast, SB431542 treatment aggravated cartilage degradation, increased proteoglycan loss, elevated matrix-degrading enzymes, and worsened pain sensitivity ($p < 0.05$).
Conclusion: TGF-β activation promotes cartilage repair in aged rats with TMJ injury by enhancing extracellular matrix synthesis and reducing degradation, offering a novel approach for treating age-related TMJ disorders.

Keywords: temporomandibular joint injury; TGF-β; Smad signaling; cartilage repair; aging

Introduction

Temporomandibular disorders (TMDs) are a group of musculoskeletal and neuromuscular conditions affecting the temporomandibular joint (TMJ) complex, its adjacent muscles, and osseous structures. Approximately 10–15% of adults experience TMDs, with the peak incidence occurring in early to middle adulthood (20–40 years) [1]. However, as the population ages, TMD prevalence in older individuals is increasing [2]. In elderly patients, it presents with accelerated cartilage degeneration, increased inflammatory responses, and impaired tissue repair capability, leading to progressive joint dysfunction [3]. Furthermore, age-related declines in cellular regeneration and increased sensitivity to inflammatory stimuli further complicate disease progression and hinder recovery in these patients [4,5].

The transforming growth factor-beta (TGF-β)/Smad signaling pathway is a crucial molecular axis involved in joint repair and homeostasis [6,7]. TGF-β maintains the structural integrity of cartilage tissue by regulating extracellular matrix (ECM) synthesis and turnover, particularly through its downstream effectors, Smad2 and Smad3 [8,9]. Upon TGF-β binding to its receptor, receptor-regulated Smads (R-Smads) such as Smad3 and Smad2 undergo phosphorylation, form complexes with Smad4, and then translocate to the nucleus to regulate the transcription of target genes involved in cartilage formation, inflammation control, and tissue remodeling [10,11].

TGF-β contributes to cartilage homeostasis by promoting anabolic processes, including the upregulation of essential ECM components such as Collagen II and Aggrecan [12]. Furthermore, this signaling pathway in-

hibits the expression of catabolic enzymes, such as matrix metalloproteinases (MMP-3 and MMP-13) and A disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5), thereby preventing ECM degradation [13,14]. Thus, TGF-β plays a dual protective role in cartilage preservation by promoting ECM synthesis while inhibiting its breakdown.

The response of the TGF-β/Smad signaling axis to injury in aged individuals remains poorly understood. Aging affects both TGF-β availability and cellular responsiveness to its signaling [15]. Studies have shown that aging is associated with reduced TGF-β receptor expression and diminished Smad signaling activity, leading to impaired tissue repair and increased vulnerability to inflammatory damage [16,17]. Moreover, aged tissues often exhibit a pro-inflammatory environment in which cytokines and other inflammatory mediators may antagonize the protective effects of TGF-β, further complicating the repair process after TMJ injury [18].

This study aims to investigate the involvement of TGF-β/Smad signaling in the recovery of TMJ injuries in aged rats. By comparing signaling responses between young and aged rats, this study intends to identify potential therapeutic pathways that could alleviate age-related joint degeneration and promote post-injury recovery.

Materials and Methods

Animal Model and Grouping

Sprague-Dawley (SD) rats, obtained from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China), were used to establish the TMJ injury model [19,20]. Rats were divided into young and aging groups. The young group consisted of 10 eight-week-old male SD rats weighing 200–250 grams, whereas the aging group included 30 eighteen-month-old male SD rats weighing 450–550 grams. All rats were housed under a standard 12-hour light/dark rhythm, with room temperature maintained at 22 $\pm$ 2 °C and humidity at 50–60%. Throughout the study, food and water were provided without restriction.

To evaluate the effects of injury and treatment, the rats were randomly assigned to eight groups, with five rats in each group [21,22]: (1) Young-normal control (NC) group: these rats received a unilateral 0.05 mL injection of normal saline into the TMJ cavity using a 27-gauge needle; (2) Young-injury group: these rats were injected unilaterally with 0.05 mL of type II collagenase (100 U/mL; C2-28-100MG, Sigma-Aldrich, St. Louis, MO, USA) into the TMJ cavity to induce inflammatory injury; (3) Aging-NC group: these rats received a unilateral 0.05 mL injection of normal saline; and (4) Aging-injury group: aged rats were injected with 0.05 mL of type II collagenase (100 U/mL).

Four additional treatment groups were established to assess therapeutic effects. (5) Control group: aged rats were injected with 0.05 mL normal saline into the TMJ cavity, followed by two weekly injections of saline for four consecutive weeks; (6) Injury group: aged rats were given an initial 0.05 mL injection of type II collagenase, followed by saline injections; (7) TGF-β group: these rats received an initial 0.05 mL injection of type II collagenase, followed by 0.05 mL biweekly injections of TGF-β (5 ng/mL; GMP-TG1H25, PeproTech, Rocky Hill, NJ, USA) for four weeks; and (8) SB431542 group: following the same collagenase-induce injury, these rats were treated with 0.05 mL SB431542 (5 mg/kg; S4317, Sigma-Aldrich, St. Louis, MO, USA) twice weekly for four weeks [23].

After treatment, anesthesia was induced with isoflurane as follows: rats were placed in an induction chamber containing 5% isoflurane until complete sedation, confirmed by loss of paw withdrawal reflex. Animals were constantly observed for signs of discomfort. All experimental procedures used in this study gained ethical approval from the Committee of Bengbu Medical University (2024-386) and followed relevant guidelines for the care and use of laboratory animals.

Tissue Collection

At the end of the 4-week treatment, rats designated for tissue collection were euthanized by overdose of pentobarbital (150 mg/kg). TMJ samples were harvested from the left side of each rat for histological analysis (hematoxylin and eosin [H&E] and Safranin O/Fast Green staining; n = 5 per group), while right TMJ tissues were collected for protein analysis using Western blotting (n = 3 per group). Cartilage was carefully separated from the subchondral bone and stored at –80 °C for subsequent experiments.

Behavioral Assessments

Mechanical allodynia was evaluated using the von Frey filament assay to determine sensitivity in the TMJ region [24]. Rats in the Control, Injury, TGF-β, and SB431542 groups were housed individually in mesh-floored cages. Calibrated von Frey filaments (57814, Stoelting, Wood Dale, IL, USA) were applied to the TMJ area, beginning with the lowest force and gradually increasing until a head withdrawal response was observed. The head withdrawal threshold (HWT) was recorded as the minimum force (in grams) required to elicit this response. Each rat was tested three times at 5-minute intervals, and the average HWT was used for group comparisons. Testing was conducted at the end of the 4-week treatment period.

Rats in the Control, Injury, TGF-β, and SB431542 groups were housed individually, and food consumption and feeding behavior were monitored over four weeks using an automated feeding system. These measurements included total food intake (in grams), feeding frequency (number of feeding events), and feeding duration (the time spent eating). These parameters were measured during multiple 15-hour observation windows across the four-week monitoring period, and the average values for each metric were calculated for each rat.

H&E Staining

For histological evaluation, TMJ cartilage tissues from each group underwent 48-hour fixation in 4% paraformaldehyde at room temperature, followed by decalcification in 10% Ethylenediaminetetraacetic acid (EDTA) solution (IE9030, Solarbio, Beijing, China). The specimens were dehydrated through a graded ethanol series, embedded in paraffin, and subsequently sectioned into 5 µm slices using a rotary microtome (Leica RM2235, Leica Biosystems, Nussloch, Germany). Tissue sections underwent hematoxylin staining for 5 minutes and counterstaining with eosin for 2 minutes following standard H&E staining protocols. After dehydration and clearing in xylene, the sections were mounted with neutral balsam. Histological analysis focused on the cartilage surface integrity, cell arrangement, subchondral bone structure, and inflammatory indicators. High-resolution images were captured using a digital slide scanner (NanoZoomer S360, Hamamatsu Photonics K.K., Hamamatsu, Japan) for subsequent analysis.

Safranin O/Fast Green Staining

Proteoglycan content in TMJ cartilage was evaluated using Safranin O/Fast Green staining. Sections underwent xylene-based deparaffinization and ethanol gradient rehydration before staining with 0.1% Safranin O (S8884, Sigma-Aldrich, St. Louis, MO, USA) for 5 minutes and counterstaining with 0.02% Fast Green (F7252, Sigma-Aldrich) for 2 minutes. After washing in 1% acetic acid, tissue sections were dehydrated, cleared in xylene, and mounted for microscopic examination. Cartilage degradation was assessed using the Osteoarthritis Research Society International (OARSI) grading system [25], based on combined H&E and Safranin O/Fast Green staining, evaluating parameters such as cartilage thickness, proteoglycan retention, and matrix integrity, with higher scores reflecting more severe damage.

Western Blot Analysis

Tissues were disrupted in ice-cold radioimmunoprecipitation assay (RIPA) buffer (P0013C, Beyotime, Shanghai, China) containing protease and phosphatase inhibitor cocktails (P2714, Sigma-Aldrich, St. Louis, MO, USA). Total protein content was quantified using a bicinchoninic acid (BCA) protein assay kit (23227, Thermo Fisher Scientific, Waltham, MA, USA). An equal amount of proteins (30 µg per sample) was resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (88518, Sigma-Aldrich, St. Louis, MO, USA). Following a 1-hour blocking step with 5% non-fat milk in tris-buffered saline (TBS) at ambient temperature, the membranes were incubated overnight at 4 °C with the following primary antibodies: TGF-β1 (1:1000, ab215715, Abcam, Cambridge, UK), Smad2 (1:10,000, ab40855, Abcam), phosphorylated-Smad2 (p-Smad2) (1:1000, ab316117, Abcam), Smad3 (1:10,000, ab40854, Abcam), p-Smad3 (1:2000, ab52903, Abcam), Collagen II (1:1000, ab307674, Abcam), Aggrecan (1:1000, ab315486, Abcam), SRY-box transcription factor 9 (Sox9) (1:1000, ab185966, Abcam), MMP-13 (1:3000, ab39012, Abcam), MMP-3 (1:10,000, ab52915, Abcam), ADAMTS5 (1:250, ab41037, Abcam), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:10,000, ab181602, Abcam). Following rinsing, membranes were incubated for one hour with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:5000, ab6721, ab205719, Abcam) at room temperature. Protein bands were visualized using an enhanced chemiluminescence (ECL) detection system (Thermo Fisher Scientific) and quantified through ImageJ software (version 1.53, NIH, Bethesda, MD, USA).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 9 software (GraphPad Software, La Jolla, CA, USA). Data was expressed as mean $\pm$ standard deviation (SD). For comparisons involving three or more groups, one-way analysis of variance (ANOVA) was performed, followed by Tukey's post hoc test. For non-normally distributed data, nonparametric tests such as the Kruskal–Wallis test, followed by Dunn's multiple comparisons test, were applied. A p-value < 0.05 was considered statistically significant.

Results

Histological Structure of TMJ Injury in Young and Aged Rats

H&E staining in the Young-NC group showed a smooth cartilage surface with significant thickness and a well-organized four-layer structure. Chondrocytes were orderly arranged, and subchondral trabeculae appeared dense. In contrast, the Young-injury group showed a rough cartilage surface, significantly reduced thickness, and loss of the four-layer structure. The superficial zone was absent, subchondral trabeculae were severely damaged, and there was significant inflammatory cell infiltration near dilated blood vessels. Furthermore, in the Aging-NC group, the cartilage surface remained smooth but was thinner and contained fewer cartilage cells compared to the Young-NC group. However, its four-layer structure remained evident, with moderately organized cells and dense subchondral bone trabeculae, although mild inflammation was observed near blood vessels.

The Aging-injury group had a rough cartilage surface, significantly thinner cartilage, and fewer chondrocytes than the Aging-NC group. The typical four-layer structure was poorly defined, with disorganized cell arrangement and loss of the superficial zone. There was severe destruction of the subchondral trabeculae accompanied by extensive inflammatory cell infiltration. Cartilage damage in the Aging-injury group was more pronounced than in the Young-injury group (Fig. 1A).

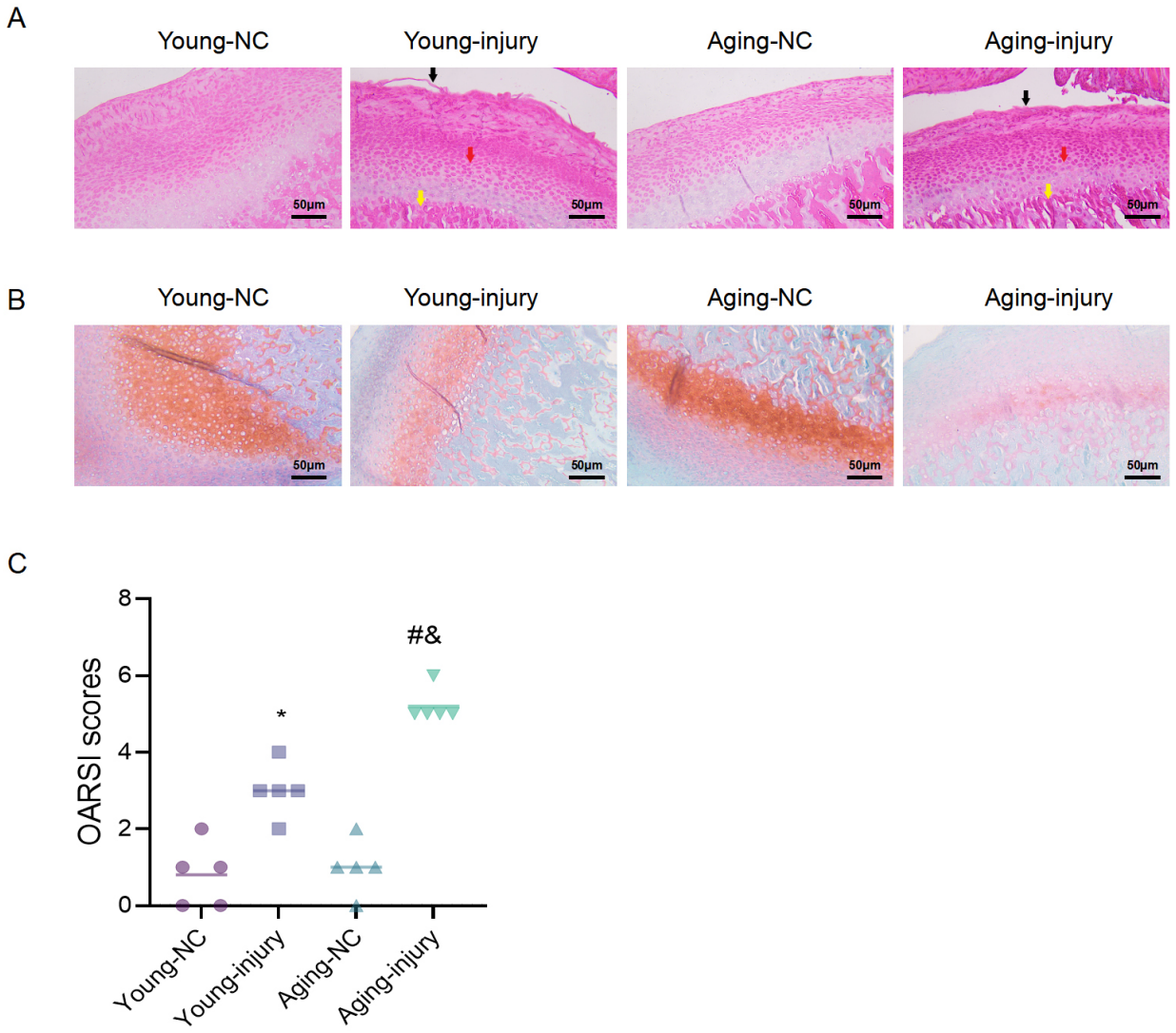

Fig. 1. Histology and proteoglycan staining of TMJ cartilage in young and aged rats. (A) H&E staining of cartilage in the Young-NC, Young-injury, Aging-NC, and Aging-injury groups. Black arrow: surface layer of cartilage, with a rough surface and thinning in the injury groups. Red arrow: transitional or deep cartilage layers, indicating disorganized cell arrangement in the injury groups. Yellow arrow: subchondral bone trabeculae, with severe damage and extensive inflammatory cell infiltration in the injury groups. (B) Safranin O/Fast Green staining indicated increased proteoglycan loss and cartilage thinning in the injury groups. Scale bars, 50 μm. (C) OARSI scores were significantly higher in the Aging-injury group than in the Aging-NC group. n = 5. $^{*}p < 0.05$ vs. the Young-NC group; $^{\#}p < 0.05$ vs. the Aging-NC group; $^{\&}p < 0.05$ vs. the Young-injury group. H&E, hematoxylin and eosin; TMJ, Temporomandibular joint; OARSI, Osteoarthritis Research Society International; NC, normal control.

Safranin O/Fast Green staining results were consistent with the H&E staining findings. Compared to the Young-NC group, the Young-injury group demonstrated a substantial proteoglycan depletion with a thinner cartilage matrix. Similarly, the Aging-injury group had significantly higher proteoglycan loss and a thinner cartilage matrix compared to the Aging-NC group, and these changes were more evident than in the Young-injury group (Fig. 1B). Additionally, OARSI scores were significantly higher in both the young and aging injury groups than their respective control groups, with the Aging-injury group exhibiting the highest scores ($p < 0.05$, Fig. 1C).

Expression of TGF-β/Smad Signaling Pathway in TMJ Injury of Young and Aged Rats

Western blot analysis revealed significant changes in TGF-β/Smad signaling proteins in both young and aged rats following TMJ injury. TGF-β1 levels were substantially reduced in the Young-injury group compared to the Young-NC group ($p < 0.01$, Fig. 2A), along with a significant decrease in p-Smad2/Smad2 and p-Smad3/Smad3 ratios ($p < 0.01$, Fig. 2B). Similarly, TGF-β1 levels were markedly lower in the Aging-injury group than in the Aging-NC group ($p < 0.01$, Fig. 2B), accompanied by significantly reduced p-Smad2/Smad2 and p-Smad3/Smad3 ratios.

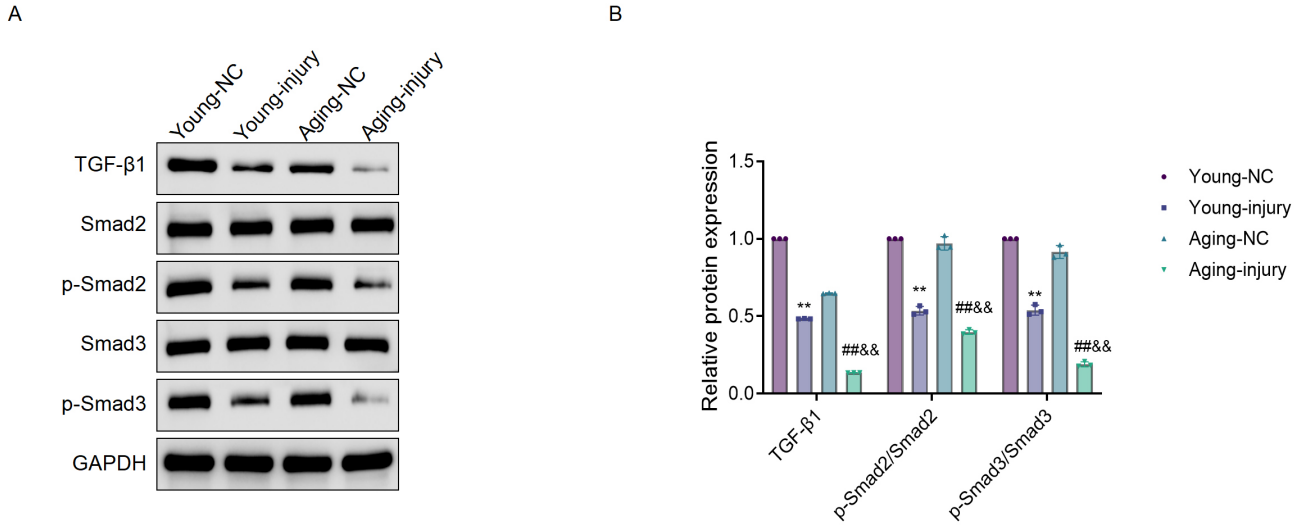

Fig. 2. Western blot analysis of TGF-β/Smad signaling proteins in TMJ cartilage of young and aged rats. (A) Representative Western blot images showing the expression levels of TGF-β1, Smad2, p-Smad2, Smad3, and p-Smad3 in the Young-NC (normal control), Young-injury, Aging-NC, and Aging-injury groups. (B) Relative protein expression levels of TGF-β1, p-Smad2/Smad2, and p-Smad3/Smad3 across the groups. n = 3. $^{**}p < 0.01$ vs. the Young-NC group; $^{\#\#}p < 0.01$ vs. the Aging-NC group; $^{\&\&}p < 0.01$ vs. the Young-injury group. TGF-β1, transforming growth factor-beta; p-Smad2, phosphorylated-Smad2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

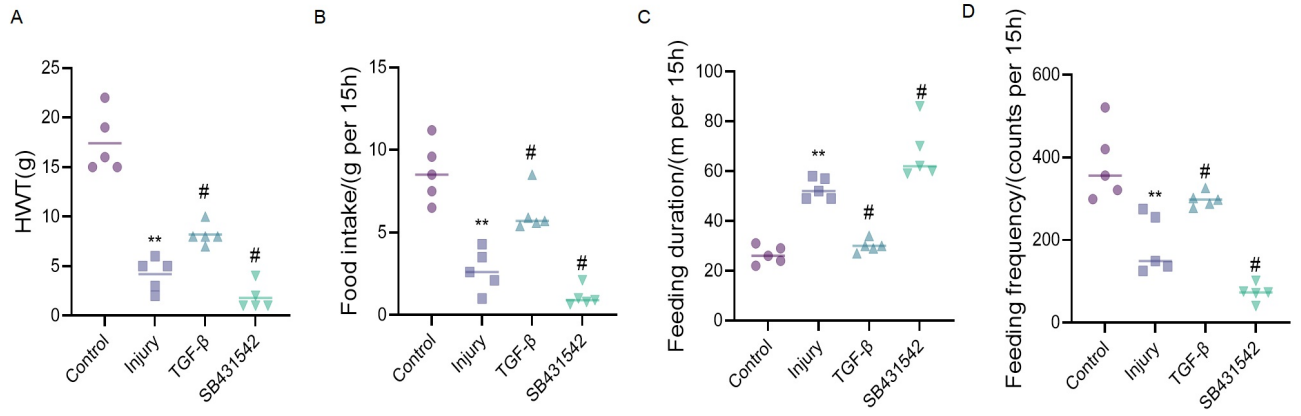

Fig. 3. Pain sensitivity and feeding behavior in aged rats with TMJ injury. (A) Head withdrawal threshold (HWT) reflecting pain sensitivity in each group. (B) Food intake per 15 hours. (C) Feeding duration per 15 hours. (D) Feeding frequency per 15 hours. n = 5. $^{**}p < 0.01$ vs. the Control group; $^{\#}p < 0.05$ vs. the Injury group.

TGF-β Reduced Pain and Improved Feeding Behavior in Aged Rats With TMJ Injury

The von Frey filament test showed a significant reduction in HWT in the Injury group, indicating increased pain sensitivity ($p < 0.01$). TGF-β treatment significantly increased HWT ($p < 0.05$), whereas SB431542 further reduced HWT ($p < 0.05$, Fig. 3A). Feeding behavior analysis revealed that the Injury group had significantly lower food intake and feeding frequency, along with prolonged feeding duration ($p < 0.01$). TGF-β treatment improved these changes by increasing food intake, food frequency, and shortening feeding duration ($p < 0.05$). Conversely, SB431542 administration aggravated feeding deficits, with further reductions in intake and frequency and extension of feeding duration ($p < 0.05$, Fig. 3B–D).

TGF-β Improved Histological Structure of TMJ Injury in Aged Rats

H&E staining in the Control group showed smooth cartilage surfaces, significant cartilage thickness, well-defined four-layer structure, well-organized cells, and dense subchondral bone trabeculae. In the Injury group, the cartilage surface was rough, with significantly reduced thickness, fewer cells, loss of the superficial zone, severe disruption of subchondral trabeculae, and substantial inflammatory cell infiltration. In the TGF-β group, carti-

lage integrity was significantly improved, with increased thickness, greater cell numbers, more organized structure, and reduced inflammatory cell infiltration. In contrast, the SB431542 group showed more severe cartilage damage, with thinner cartilage, fewer cells, disorganized cellular arrangement, loss of the superficial zone, and increased inflammatory cell infiltration (Fig. 4A).

Safranin O/Fast Green staining showed increased proteoglycan depletion and a thinner cartilage matrix in the Injury group compared to the Control group. TGF-β treatment significantly reduced proteoglycan loss than the Injury group, whereas SB431542 further increased this effect (Fig. 4B). The Injury group exhibited substantially elevated OARSI scores relative to the Control group ($p < 0.05$). TGF-β administration significantly alleviated cartilage damage, as evidenced by lower OARSI scores than those in the Injury group ($p < 0.05$), while the SB431542 group manifested significantly higher OARSI scores ($p < 0.05$, Fig. 4C).

TGF-β/Smad Signaling Pathway Activation in TMJ of Aged Rats

Relative to controls, TGF-β1 levels in TMJ cartilage were considerably reduced in the Injury group ($p < 0.01$) (Fig. 5A), along with a significant decrease in the p-Smad2/Smad2 and p-Smad3/Smad3 ratios ($p < 0.01$, Fig. 5B). TGF-β treatment significantly upregulated TGF-β1 expression and elevated the p-Smad2/Smad2 and p-Smad3/Smad3 ratios ($p < 0.01$). In contrast, SB431542 treatment reversed these effects, further reducing TGF-β1 expression and the phosphorylation ratios of Smad2 and Smad3.

TGF-β Upregulated Matrix Production Proteins and Inhibited Matrix Degradation Proteins in TMJ of Aged Rats

Compared to the Control group, the Injury group showed significantly reduced levels of Collagen II, Aggrecan, and Sox9 in TMJ cartilage ($p < 0.01$). However, TGF-β treatment significantly increased their expression ($p < 0.01$), whereas SB431542 administration reduced their levels compared to the Injury group ($p < 0.01$, Fig. 6A,B).

The Injury group had significantly higher levels of matrix degradation proteins (MMP-13, MMP-3, and ADAMTS5) compared to the Control group ($p < 0.01$). However, the TGF-β group displayed significantly lower levels of these proteins compared to the Injury group ($p < 0.01$), while the SB431542 group had substantial enhancement of MMP-13, MMP-3, and ADAMTS5 expression ($p < 0.01$, Fig. 6C,D).

Discussion

TMJ disorders are highly prevalent in the elderly population and frequently involve cartilage degeneration, chronic pain, and chewing dysfunction [26]. Age-related reduction in the regenerative capacity of cartilage significantly makes the treatment of such diseases more complex and less effective [5]. This study revealed that the TGF-β/Smad signaling pathway activation significantly promotes cartilage repair following TMJ injury in aged rats. TGF-β treatment improved cartilage structure, reduced proteoglycan depletion and inflammatory cell infiltration, and effectively alleviated mechanical hypersensitivity and feeding behavior impairments, demonstrating a significant enhancement in functional recovery.

TGF-β has been extensively reported to promote ECM synthesis and suppress inflammatory responses in osteoarthritis models [7,27]. Liu *et al.* [28] demonstrated that TGF-β upregulates key cartilage ECM components like Collagen II and Aggrecan, while effectively attenuating local inflammation. These effects are primarily mediated through the canonical Smad2/3 signaling pathway [29]. Upon binding to its receptors (TGF-βRI/II), TGF-β induces phosphorylation of Smad2 and Smad3, which subsequently form a complex with Smad4 and translocate to the nucleus to regulate the transcription of genes associated with cartilage homeostasis, including Collagen II, Aggrecan, and Sox9. This activation contributes to ECM synthesis and is crucial for cartilage protection and repair [10].

Although TGF-β/Smad signaling has been well characterized in osteoarthritis (OA), its involvement in TMJ disorders remains relatively underexplored. In this study, TGF-β treatment substantially elevated Smad2 and Smad3 phosphorylation, indicating Smad pathway activation in TMJ cartilage. Furthermore, TGF-β upregulated anabolic markers like Collagen II and Sox9, while downregulating catabolic enzymes including MMP-13, MMP-3, and ADAMTS5. These findings suggest that TGF-β promotes TMJ cartilage repair by simultaneously enhancing matrix synthesis and inhibiting matrix degradation, thereby restoring metabolic equilibrium and improving structural integrity and functional outcomes. In contrast, inhibiting the TGF-β/Smad signaling pathway with SB431542 exacerbated cartilage damage, as evidenced by increased OARSI scores.

Aged rats treated with TGF-β showed significantly reduced mechanical pain sensitivity in the HWT test and improved feeding behavior. Moreover, SB431542 treatment partially reversed these effects, further supporting the role of TGF-β/Smad signaling in TMJ functional recovery. However, TGF-β therapy may carry potential risks during cartilage repair, particularly the induction of fibrosis and osteophyte formation in adjacent joint tissues [30]. For example, Halofuginone has been reported to attenuate cartilage degradation and matrix breakdown in osteoarthritis by inhibiting TGF-β signaling, highlighting the pathological role of excessive TGF-β axis activation [31]. Therefore, precise regulation of TGF-β dosage and pathway-specific activation is essential to maximize therapeutic effects while minimizing adverse outcomes. Additional investigations are warranted to explain the context-dependent roles of TGF-

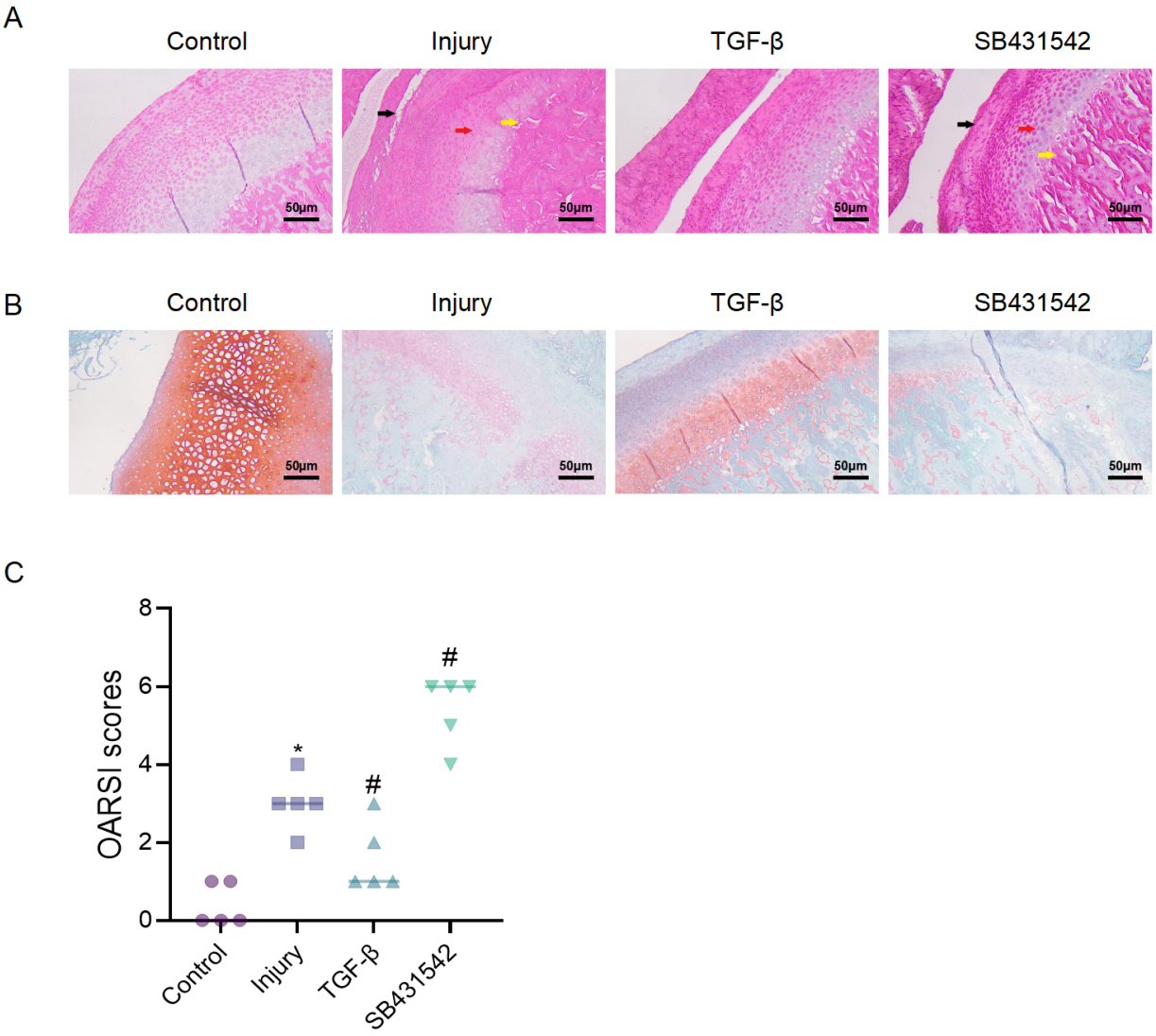

Fig. 4. Histological and proteoglycan staining in TMJ cartilage of aged rats. (A) H&E staining indicates smooth cartilage surfaces in the Control group, while the Injury group shows rough surfaces and disrupted structures. TGF-β treatment improved cartilage thickness and organization, while SB431542 administration worsened the damage. Black arrows indicate surface disruption or loss of the superficial cartilage layer. Red arrows indicate inflammatory cell infiltration. Yellow arrows indicate subchondral trabecular bone damage. (B) Safranin O/Fast Green staining indicates increased proteoglycan loss in the Injury group, with improvements in the TGF-β group and further degradation in the SB431542 group. (C) OARSI scores correlate with these histological findings. n = 5. *$p < 0.05$ vs. the Control group; $^{\#}p < 0.05$ vs. the Injury group.

β in various joint environments and to develop optimized strategies that balance its regenerative efficacy with safety.

Despite several promising findings, this study has certain limitations. First, it is a short-term investigation, leaving the long-term safety and potential adverse effects of TGF-β treatment unexamined. Second, as these findings were derived from the rat model, their applicability to human clinical settings or more complex physiological systems remains to be validated. Lastly, the study did not include *in situ* confirmation approaches such as immunohistochemistry. Further mechanistic studies are required to elucidate the regulation and role of the TGF-β/Smad pathway in TMJ repair.

Our findings demonstrate that TGF-β activation significantly improves both histological and functional recovery in aged rats with TMJ injury by enhancing ECM synthesis and inhibiting matrix degradation. These observations suggest that TGF-β could be used as a therapeutic strategy for TMJ disorders and other age-related joint injuries. Further investigations are warranted to explore long-term outcomes and potential clinical applications.

Conclusion

This study highlights the novel role of TGF-β/Smad signaling in promoting TMJ injury repair, particularly in

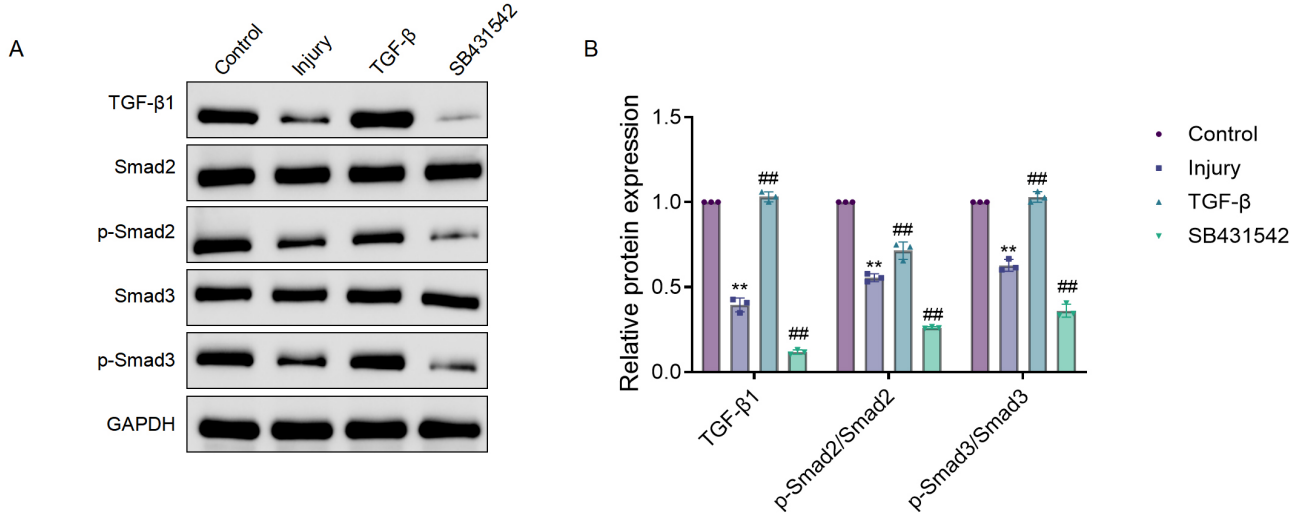

Fig. 5. Activation of TGF-β/Smad signaling pathway in TMJ cartilage of aged rats. (A) Western blot analysis of TGF-β1, Smad2, p-Smad2, Smad3, and p-Smad3 protein expression in each group. (B) Quantification of p-Smad2/Smad2 and p-Smad3/Smad3 ratios. n = 3. $^{**}p < 0.01$ vs. the Control group; $^{\#\#}p < 0.01$ vs. the Injury group.

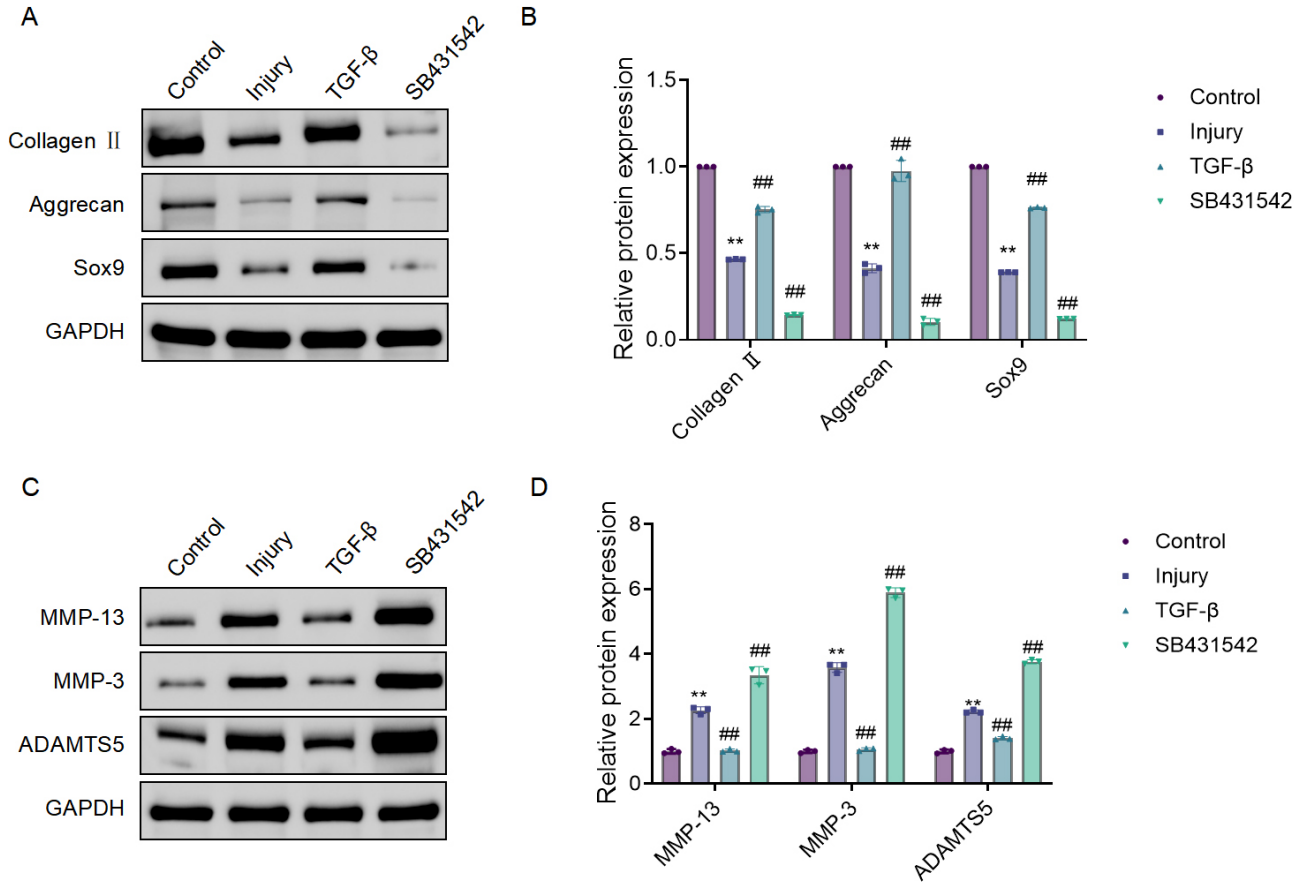

Fig. 6. Expression levels of matrix production and degradation proteins in TMJ cartilage of aged rats. (A) Western blot analysis presenting Collagen II, Aggrecan, and Sox9 expression levels. (B) Quantitative analysis of the Collagen II, Aggrecan, and Sox9 relative expression levels in each group. (C) Western blot analysis showing MMP-13, MMP-3, and ADAMTS5 expression levels. (D) The relative expression levels of MMP-13, MMP-3, and ADAMTS5. n = 3. $^{**}p < 0.01$ vs. the Control group; $^{\#\#}p < 0.01$ vs. the Injury group. Sox9, SRY-box transcription factor 9; MMP-13, matrix metalloproteinase-13; ADAMTS5, A disintegrin and metalloproteinase with thrombospondin motifs 5.

aged populations. By enhancing ECM synthesis and suppressing cartilage breakdown, TGF-β significantly improves both histological integrity and functional outcomes. These findings suggest that modulating the TGF-β/Smad pathway offers a promising therapeutic candidate for age-related TMJ disorders. Clinically, this strategy could improve the management of TMJ dysfunction and other degenerative joint diseases in elderly patients. Future studies should optimize dosing regimens, assess the long-term safety and efficacy of TGF-based therapies, and explore the translational applicability in human subjects.

Availability of Data and Materials

The data used to support the findings of this study are available from the corresponding author upon request.

Author Contributions

RT: Conceptualization, Formal Analysis, Writing - Original Draft, Writing - Review and Critical Revision; YG and XZ: Formal Analysis, Writing - Review and Critical Revision; XX, JL and YD: Data Curation, Writing - Review and Critical Revision; XL: Methodology, Writing - Review and Critical Revision, Project Administration, Funding Acquisition. All authors have given final approval of the version to be published; have agreed on the journal to which the article has been submitted; and have agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The animal experiments described in this study were authorized by the Committee of Bengbu Medical University (2024-386).

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Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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