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RESEARCH

Sodium fluoride promotes myopia progression via the activation of the ferroptosis pathway by PIEZO1 and pharmacological targeting PIEZO1 represents an innovative approach for myopia treatment

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Abstract Sodium fluoride-induced ocular damage constitutes a significant public health concern globally; however, the precise molecular mechanisms underlying this issue remain obscure. This study aims to investigate the effects of sodium fluoride on myopia and to offer novel theoretical foundations for future strategies in myopia prevention and control. The experimental data showed that sodium fluoride could promote myopia progression, and through bioinformatics analysis, we found that sodium fluoride could affect the ferroptosis pathway. Western blotting and redox kit assays further confirmed that sodium fluoride activates the ferroptosis pathway. We also demonstrated that PIEZO1 plays a crucial role in sodium fluoride-induced myopia, and that the PIEZO1 inhibitor (GsMTx4) can inhibit the ferroptosis pathway. Subsequently, we identified PIEZO1 as a potential target of baicalin, which inhibited PIEZO1 expression in vivo and in vitro, as confirmed by molecular docking modeling and CETSA assays. Finally, we found that baicalin inhibited sodium fluoride-induced myopia via PIEZO1. Taken together, our findings indicate that sodium fluoride can promote myopia progression by activating the ferroptosis pathway through PIEZO1, and that targeting PIEZO1 expression can delay myopia progression, which may provide a new drug target for myopia treatment in the future.

Keywords Myopia · Sodium fluoride · PIEZO1 · Ferroptosis · Baicalin

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10565-025-10020-y.

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Introduction

Myopia has become a significant global public health concern, with projections indicating that nearly 50% of the world's population will be affected by 2050. While genetic predisposition and near-work activities remain established risk factors, recent epidemiological studies have identified environmental pollutants as critical modulators of ocular pathophysiology (Baird et al. 2020; Zhang et al. 2024). Of particular concern are airborne contaminants generated through industrial processes (including fluoride compounds), which exhibit both systemic toxicity and ocular tropism. The dual mechanistic capacity of these pollutants to simultaneously induce oxidative stress and disrupt ocular developmental signaling pathways emphasizes their potential role as drivers of myopiagenesis (Wei et al. 2019; Yuan and Zou 2022; Zhang et al. 2023), underscoring the critical need for targeted investigation into their pathophysiological interactions.

Sodium fluoride (NaF) is a versatile inorganic compound that has garnered considerable attention across various scientific disciplines due to its unique chemical properties and broad range of applications (da Silva et al. 2022). Although clinically utilized for dental caries prevention through enamel remineralization (Rajendiran et al. 2021), its industrial applications in metallurgy, glass production, and semiconductor manufacturing contribute significantly to atmospheric fluoride pollution (da Silva et al. 2022). Compelling evidence reveals that chronic fluoride exposure induces multi-organ toxicity via oxidative damage and apoptotic pathways, particularly affecting hepatic and neural tissues (Lu et al. 2017). Of particular concern is fluoride's demonstrated capacity to traverse critical biological barriers, including the blood-retinal barrier (BRB), which has substantial implications for its ocular bioavailability and potential to directly modulate retinal homeostasis (Guth et al. 2020; Lech 2011). While no direct evidence links NaF to myopia pathogenesis, parallel studies have shown that particulate matter (PM2.5) and gaseous pollutants promote axial elongation through retinal oxidative stress and dysregulation of dopamine/IGF-1 signaling (Wei et al. 2019; Zhang et al. 2017). Thus, we hypothesize that ocular surface-deposited NaF may translocate to posterior ocular tissues, where it could disrupt retinal signaling pathways, thereby potentially accelerating myopiagenesis through mechanisms involving oxidative stress and inflammatory modulation.

To test this hypothesis, we examined the myopic parameters (refraction and axial length) of mice after administering sodium fluoride solution to their ocular surface for one month, collected retinal tissues for proteomic analysis, and verified the possible mechanism of NaF-induced myopia using bioinformatics analysis. Our data indicated that sodium fluoride can activate the ferroptosis pathway through PIEZO1, thereby promoting myopia progression. To further explore whether pharmacological inhibition of PIEZO1 has clinical translational value in myopia, we selected baicalin for further research through extensive literature review. The selection of baicalin, a bioactive flavonoid compound derived from Scutellaria baicalensis Georgi, as our primary therapeutic candidate is supported by its unique dual mechanism of action (Huang 2022; Wen et al. 2023). This botanical agent exhibits two distinct yet complementary pharmacological properties: (1) potent free radical scavenging activity through its flavonoid antioxidant capacity, and (2) specific modulation of mechanosensitive ion channel function. Our findings that baicalin can target and inhibit the expression of PIEZO1 and delay myopia progression offer novel theoretical insights for future clinical treatment of myopia.

Material and methods

Mice

Male C57BL/6 J mice, aged 4 weeks, were procured from Charles River Laboratories (China) and housed under controlled environmental conditions with a 12-h photoperiod and unrestricted access to standard rodent chow and water. Animals were randomly divided into experimental and control groups before intervention, and then refractive status and axial length measurements were conducted using infrared photorefraction and spectral-domain optical coherence tomography, respectively. Sodium fluoride (HY-B1766, MCE) was prepared as a sterile saline solution at a concentration of 80 mg/mL and administered to the ocular surface of mice in 10 µL drops, three times per day at 8-h intervals for 30 consecutive days. Baicalin (HY-N0197, MCE) was delivered through oral gavage at a dosage of 50 mg/kg body

weight once daily for the same duration. For intravitreal administration, GsMTx4 (HY-P1410, MCE) and Yoda1 (HY-18723, MCE) were prepared as 10 μM solutions and injected in 1 μL volumes at 5-day intervals. Biochemical analyses were performed using commercial assay kits for glutathione (GSH, S0053) and malondialdehyde (MDA, S0131S) quantification, obtained from Beyotime Biotechnology. All experimental procedures were designed to minimize animal discomfort and optimize the utilization of experimental subjects in accordance with the principles of the 3Rs (Replacement, Reduction, and Refinement).

Cell lines and culture conditions

The 661W photoreceptor cell line was maintained in Dulbecco's Modified Eagle Medium (DMEM) enriched with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. Cells were incubated at 37 °C in a humidified environment with 5% CO_2 to ensure optimal growth conditions. For experimental procedures, cells were plated at a density of 1×10^5 cells per well in 12-well culture plates and allowed to attach for approximately 12–16 h prior to treatment initiation. To investigate the effects of sodium fluoride, 661W cells were treated with a concentration gradient of sodium fluoride (0, 20, 40, and 60 mg/L) for 24 h. Concurrently, baicalin was administered to the cells at a final concentration of 40 μM for the same duration.

Western blotting and immunofluorescence

Proteins were extracted with ice-cold RIPA buffer supplemented with protease/phosphatase inhibitors. Protein quantification was performed via BCA assay, with equal aliquots resolved by 10% SDS-PAGE and electroblotted onto PVDF membranes. Membranes were blocked with 5% skim milk/TBST (1 h, 25 °C), then probed with primary antibodies (4 °C, overnight) and HRP-conjugated secondary antibodies (1 h, 25 °C) after TBST washes. Standard protocols were followed as previously established (Liu et al. 2024; Liu et al. 2023a). The primary antibodies used were anti-GAPDH (Bioss, bsm-0978 M), anti-PIEZO1 (Abcam, ab128245), anti-COX2 (Abcam, ab179800), anti-GPX4 (Abcam, ab125066). Retinal tissues were fixed in 4% paraformaldehyde (PFA) for 24 h, then dehydrated, embedded, and cryosectioned at 5–10 μm thickness. Sections were blocked with 5% BSA and 0.3% Triton X-100 in PBS for 1 h to minimize nonspecific interactions and improve antibody accessibility. Primary antibodies were applied and incubated at 4 °C overnight. Following PBS rinses, fluorophore-labeled secondary antibodies were added and incubated for 1 h at RT under light-protected conditions. Nuclei were counterstained with DAPI (5 min), PBS-washed, and mounted using antifade medium. Fluorescence imaging was performed using confocal microscopy.

QPCR

Total RNA was isolated with TRIzol reagent following the manufacturer's guidelines. cDNA synthesis was performed using a commercial reverse transcription kit. qPCR analysis was carried out on a StepOnePlus instrument with SYBR Green Master Mix as the detection system. Gene expression quantification was determined through the 2^-ΔΔCt method, with GAPDH serving as the endogenous control. Specific experimental conditions were consistent with established protocols (Li et al. 2021; Liu et al. 2024). The primers for qPCR were as follows: *Gapdh*: F: ACC ACAGTCCATGCCATCAC, R: TCCACCACCCTG TTGCTGTA; *Piezo1*: F: CACAAAGTACCGGGCG, R: AAAGTAAATGCACTTGACG.

CCK8 and cellular ROS detection

Cells were seeded in 96-well plates (2×10^3 cells/well) and cultured overnight at 37 °C with 5% CO_2. Following sodium fluoride treatment at specified concentrations, 100 μL of fresh medium and 10 μL CCK-8 solution were added to each well. Plates were incubated at 37 °C for 2 h under light-protected conditions. Optical density at 450 nm was measured using a microplate reader to assess cellular viability. Reagents including CCK-8 (C0037) and ROS detection kits (S0033S) were obtained from Biyuntian (China).

Proteomics analysis

Retinal tissues from mice were homogenized in urea/SDS buffer containing protease and phosphatase inhibitors. Proteins were extracted by centrifugation, quantified using a BCA assay, and subjected to

reduction, alkylation, and tryptic digestion. Peptides were acidified, desalted using C18 solid-phase extraction (SPE), and lyophilized prior to reconstitution in 0.1% formic acid. Peptides were separated using nano-liquid chromatography (nano-LC) and analyzed by high-resolution tandem mass spectrometry (MS/MS). Raw data were processed against the UniProt mouse database for protein identification and quantification. Differential expression analysis was performed using the limma package in R. Volcano plots were generated to visualize significantly upregulated and downregulated proteins ($|\text{Log}_2$ fold change $|>2$, adjusted p-value <0.05). Functional enrichment analysis was conducted using the clusterProfiler package. Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were examined to identify biological processes, molecular functions, and pathways significantly enriched in the differentially expressed proteins. Visualization of GO and KEGG results was performed using the ggplot2 and enrichplot packages.

Statistical analysis

Experimental results were expressed as the mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by post hoc comparisons was employed for between-group analyses, with multiplicity adjustment implemented through the Bonferroni post hoc test. The Bonferroni post hoc test is a widely used statistical method for controlling the family-wise error rate in multiple comparisons. It is particularly useful when conducting multiple hypothesis tests simultaneously, as it helps to reduce the likelihood of false positives that can occur when comparing multiple groups or conditions. A corrected p-value threshold of 0.05 was established as the significance criterion throughout the study. All computational procedures were executed using GraphPad Prism software.

Results

Sodium fluoride promotes myopia progression

To investigate the effect of sodium fluoride on myopia, we divided 4-week-old mice equally into three groups, namely the control group, saline-treated group, and sodium fluoride-treated group. Before initiating the experiment, we measured the axial length and refraction of the mice and excluded those with significant variations. Subsequently, we administered sodium fluoride solution at a concentration of 80 mg/ml onto the ocular surface of the mice, 10 µl each time, once every 8 h for 4 weeks, with the same procedure applied to the saline-treated group. The experimental protocol is detailed in Fig. 1A. After 4 weeks, the refraction of mice in the sodium fluoride-treated group shifted in the direction of myopia, and the axial length increased compared to the control group, indicating that sodium fluoride promotes myopia progression (Figs. 1B and C).

Sodium fluoride activates the ferroptosis pathway

To further investigate the molecular mechanism of sodium fluoride in the promotion of myopia, we collected retinal tissues from the saline-treated group and sodium fluoride-treated group for proteomic analysis. As illustrated in Fig. 2A, differentially expressed proteins were identified using stringent screening criteria (adjusted p-value <0.05 and $|\log2(\text{fold change})|>2$), revealing 181 significantly up-regulated and 496 down-regulated candidates. These proteins were subsequently subjected to functional annotation through Gene Ontology (GO) and pathway enrichment analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Notably, we found that a large number of differential proteins were enriched in the ferroptosis pathway, suggesting that sodium fluoride may modulate the ferroptosis pathway (Figs. 2B-C). To elucidate the potential involvement of ferroptosis in sodium fluoride-mediated myopia pathogenesis, we conducted a series of mechanistic investigations to assess pathway activation and its functional consequences. We utilized a ferroptosis assay kit to detect biochemical indicators in mouse tissues and Western blotting to assess ferroptosis-related proteins. Glutathione (GSH) is the most abundant intracellular antioxidant, and its levels are negatively correlated with ferroptosis. Malondialdehyde (MDA), a well-established biomarker of lipid peroxidation, exhibited a significant positive correlation with ferroptotic activity, further supporting the involvement of oxidative lipid damage in this form of regulated cell death. Numerous studies have shown that GPX4 expression is down-regulated and

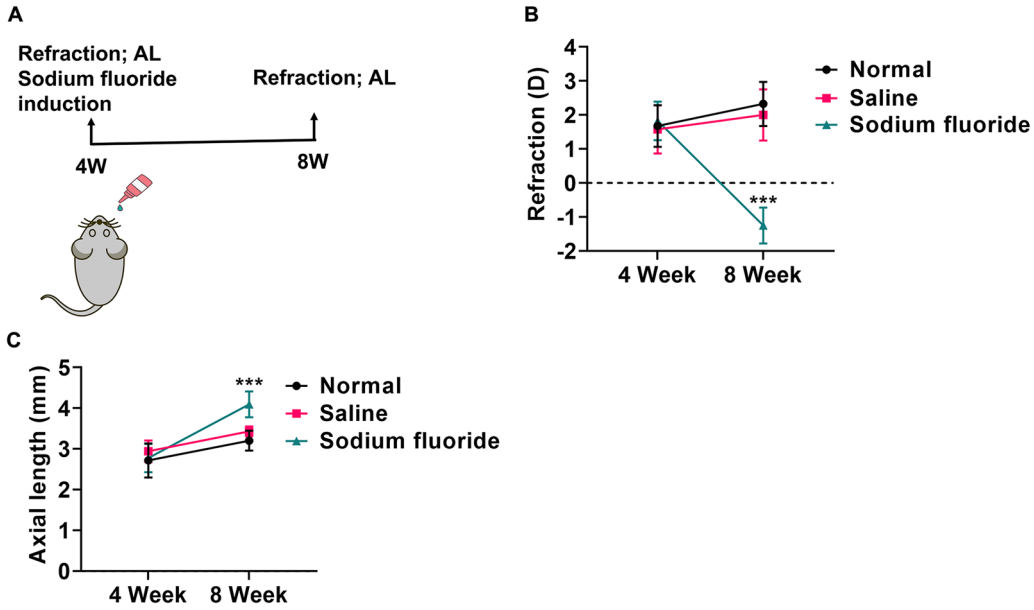


Fig. 1 Sodium fluoride promotes myopia progression. **A** The schematic diagram of the model of sodium fluoride-induced myopia; **B** Detection of refraction in mice after treatment with saline or sodium fluoride; **C** Examination of axial length in mice after treatment with saline or sodium fluoride. Datas were presented as the mean ± SD of ten replicates in (**B** and **C**). *P* values were determined using one-way ANOVA with Bonferroni post hoc testing. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

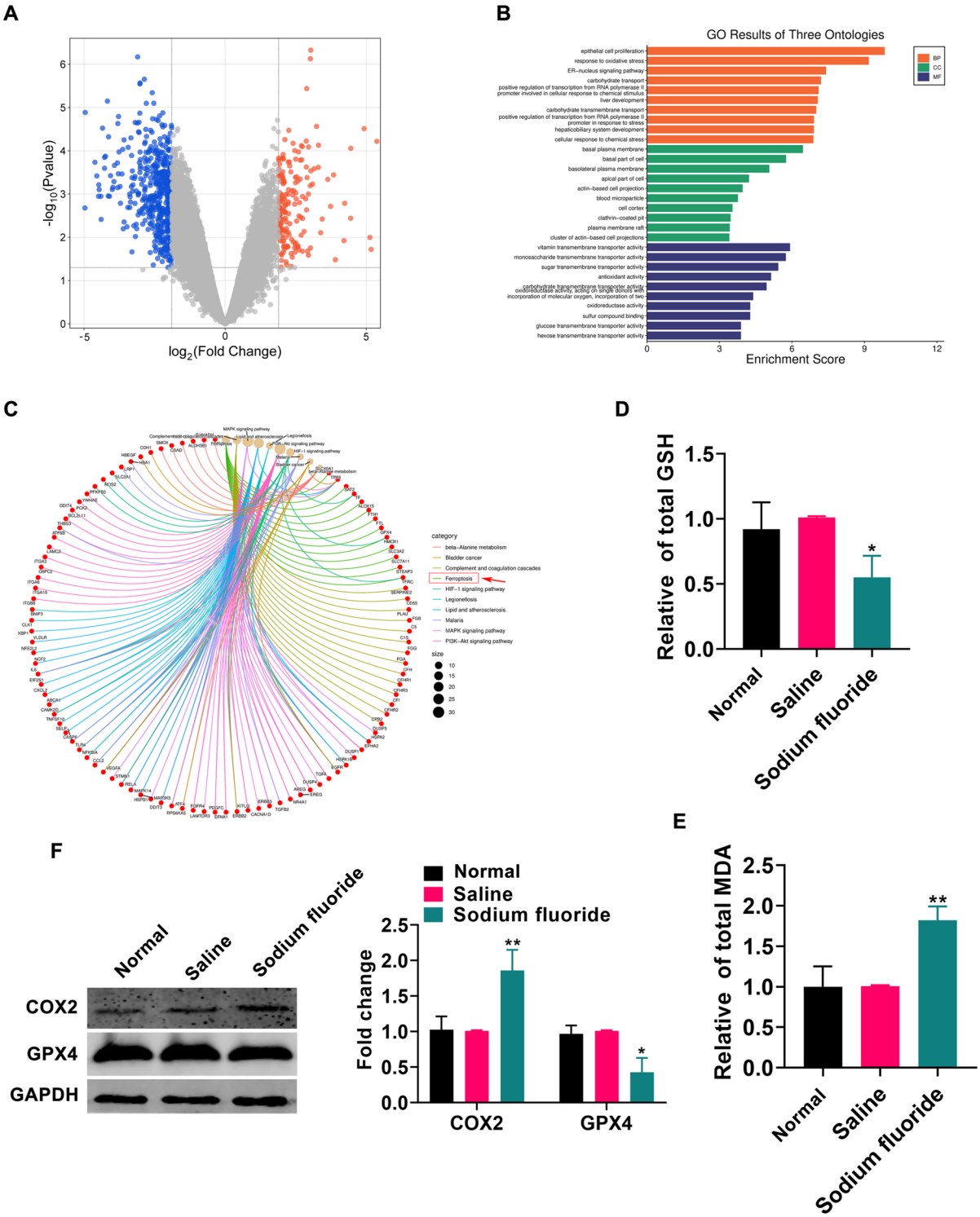
COX2 expression is up-regulated in cells or tissues where ferroptosis occurs. As shown in Figs. 2D-F and S1B-C the sodium fluoride-treated group, compared to the saline-treated group, showed a decrease in GSH content, an increase in MDA content, a reduction in GPX4 expression, and an increase in COX2 expression, indicating that the ferroptosis pathway was activated. Concurrently, we conducted comprehensive assessments of cellular activity and ROS levels in 661W cells following exposure to varying concentrations of sodium fluoride, as presented in Figs. S4A-C. Quantitative analysis demonstrated a concentration-dependent relationship, wherein sodium fluoride significantly enhanced intracellular ROS accumulation while concurrently suppressing cellular viability. In summary, sodium fluoride activates the ferroptosis pathway in the process of promoting myopia.

Sodium fluoride promotes PIEZO1 expression in vivo and in vitro

Protein enrichment analysis within the ferroptosis pathway revealed that PIEZO1 is significantly implicated in sodium fluoride-induced ferroptosis, suggesting a potential regulatory role in this process (Fig. 3A). This is because PIEZO1 is strongly associated with ferroptosis (Hirata 2023) and has been shown to promote myopia progression in guinea pigs (Zhong et al. 2023). We quantitatively evaluated PIEZO1 expression dynamics post-sodium fluoride exposure, employing a multimodal analytical approach to assess both transcriptional and translational profiles. As depicted in Figs. 3B, D and S1A, quantitative analyses demonstrated significant upregulation of PIEZO1 expression in sodium fluoride-exposed samples, with concomitant elevation in both mRNA and protein levels relative to untreated controls. Meanwhile, we treated 661W cells with varying concentrations of sodium fluoride and collected their RNA and protein to assess PIEZO1 expression. As depicted in Figs. 3C and E, the mRNA and protein expression levels of PIEZO1 increased in a dose-dependent manner. These data suggest that sodium fluoride promotes PIEZO1 expression.

Sodium fluoride promotes myopia progression by activating ferroptosis via PIEZO1

To further investigate the function of PIEZO1 in myopia, we injected the PIEZO1 inhibitor (GsMTx4)


A
-log10(Pvalue)
log2(Fold Change)
B
GO Results of Three Ontologies
BP
CC
MF
epithelial cell proliferation
response to oxidative stress
ER−nucleus signaling pathway
carbohydrate transport
positive regulation of transcription from RNA polymerase II promoter involved in cellular response to chemical stimulus
liver development
carbohydrate transmembrane transport
positive regulation of transcription from RNA polymerase II promoter in response to stress
hepaticobiliary system development
cellular response to chemical stress
basal plasma membrane
basal part of cell
basolateral plasma membrane
apical part of cell
actin−based cell projection
blood microparticle
cell cortex
clathrin−coated pit
plasma membrane raft
cluster of actin−based cell projections
vitamin transmembrane transporter activity
monosaccharide transmembrane transporter activity
sugar transmembrane transporter activity
antioxidant activity
carbohydrate transmembrane transporter activity
oxidoreductase activity, acting on paired donors, with incorporation of molecular oxygen, incorporation of two
oxidoreductase activity
sulfur compound binding
glucose transmembrane transporter activity
hexose transmembrane transporter activity
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category
beta−Alanine metabolism
Bladder cancer
Complement and coagulation cascades
Ferroptosis
HIF−1 signaling pathway
Legionellosis
Lipid and atherosclerosis
Malaria
MAPK signaling pathway
PI3K−Akt signaling pathway
size
D
Relative of total GSH
Normal
Saline
Sodium fluoride
E
Relative of total MDA
F
Normal
Saline
Sodium fluoride
COX2
GPX4
GAPDH
Fold change

◂**Fig. 2** Sodium fluoride activates the ferroptosis pathway. **A** The volcanic map of differential proteins; **B** The GO analysis of differential proteins; **C** The KEGG analysis of differential proteins; **D** Detection of the GSH levels in the retina of mice after treatment with saline or sodium fluoride; **E** Detection of the MDA levels in the retina of mice after treatment with saline or sodium fluoride; **F** Western blotting analysis of COX2 and GPX4 in the retina of mice after treatment with saline or sodium fluoride. Datas were presented as the mean ± SD of three replicates in (**D**-**F**). *P* values were determined using one-way ANOVA with Bonferroni post hoc testing. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

into the vitreous cavity of mice, employed a morphologic deprivation model of myopia, and subsequently examined the refraction and axial length of the mice. As shown in Fig. S2B, GsMTx4 inhibited PIEZO1 expression compared to the control group. As depicted in Figs. S2C and S2D, compared to the saline group, the refraction of mice in the GsMTx4 group shifted towards orthokeratology, and axial length growth was delayed. Conversely, pharmacological activation of PIEZO1 using Yoda1 significantly exacerbated myopia progression, as evidenced by the experimental data presented in Fig. S3. These collective findings strongly suggest that PIEZO1 plays a promotive role in myopia pathogenesis. Next, we investigated whether sodium fluoride activates the ferroptosis pathway via PIEZO1, thereby promoting myopia progression. We treated mice in the saline and sodium fluoride groups with either saline or the PIEZO1 inhibitor (GsMTx4) and measured ferroptosis markers, including glutathione (GSH) and malondialdehyde (MDA). As shown in Figs. 4A-C, compared to the control, retinal tissues from GsMTx4-treated mice demonstrated decreased expression of PIEZO1 and COX2, increased expression of GPX4, elevated GSH content, and reduced MDA content. These findings suggest that sodium fluoride activates the ferroptosis pathway through PIEZO1. Subsequently, we examined the refraction and axial length of mice in the four groups: Saline/Saline, Sodium fluoride/Saline, Saline/GsMTx4, and Sodium fluoride/GsMTx4. As shown in Figs. 5B and C, the shift in refraction towards orthokeratology and delayed axial length growth in the Sodium fluoride/GsMTx4 group compared to the Sodium fluoride/Saline group demonstrated that sodium fluoride can influence myopia progression via PIEZO1. In conclusion, sodium fluoride promotes myopia progression by activating ferroptosis via PIEZO1.

PIEZO1 is a drug target of baicalin

To pharmacologically investigate whether sodium fluoride affects the development of myopia through PIEZO1, we reviewed extensive literature and found that baicalin can affect the ferroptosis pathway (Wen et al. 2023). However, the relationship between baicalin and PIEZO1, as well as its role in myopia, remains unknown. Therefore, we hypothesized that PIEZO1 is the drug target of baicalin. To test this hypothesis, we conducted molecular docking modeling and cellular experiments to explore whether PIEZO1 is the target of baicalin. As shown in Fig. 6A, the molecular docking model indicated that PIEZO1 can bind to baicalin. Then, we found that the degradation rate of PIEZO1 protein was reduced in the baicalin group compared to the saline group, as determined by CETSA assay (Fig. 6B), indicating that baicalin targets PIEZO1. Finally, we treated mice and 661W cells with baicalin and, compared to the control group, observed reduced mRNA and protein expression levels of PIEZO1 both in vivo and in vitro (Figs. 6C-F). These data suggest that PIEZO1 is a potential target of baicalin and that baicalin inhibits PIEZO1 expression both in vivo and in vitro.

Baicalin inhibits myopia progression through PIEZO1

Finally, we administered saline and baicalin treatments to the mouse morphologic deprivation myopia model, respectively, and examined the refraction and axial length of the mice to investigate whether baicalin could delay myopia progression. As shown in Figs. S5B and S5C, the refraction shifted towards orthokeratology and the ocular axial length decreased in the baicalin group compared to the control group, suggesting that baicalin could delay myopia progression. We conducted a comparative analysis of myopia-related parameters between the GsMTx4-treated and Baicalin-treated groups, as illustrated in Fig. S6. Quantitative assessment revealed that the Baicalin treatment group demonstrated superior efficacy in myopia suppression compared to the GsMTx4 treatment group. Meanwhile, we administered sodium fluoride and saline drops to the ocular surface of the mice, followed by gavage administration of baicalin and saline, respectively. As shown in Fig. 7B, baicalin attenuated the sodium fluoride-induced upregulation

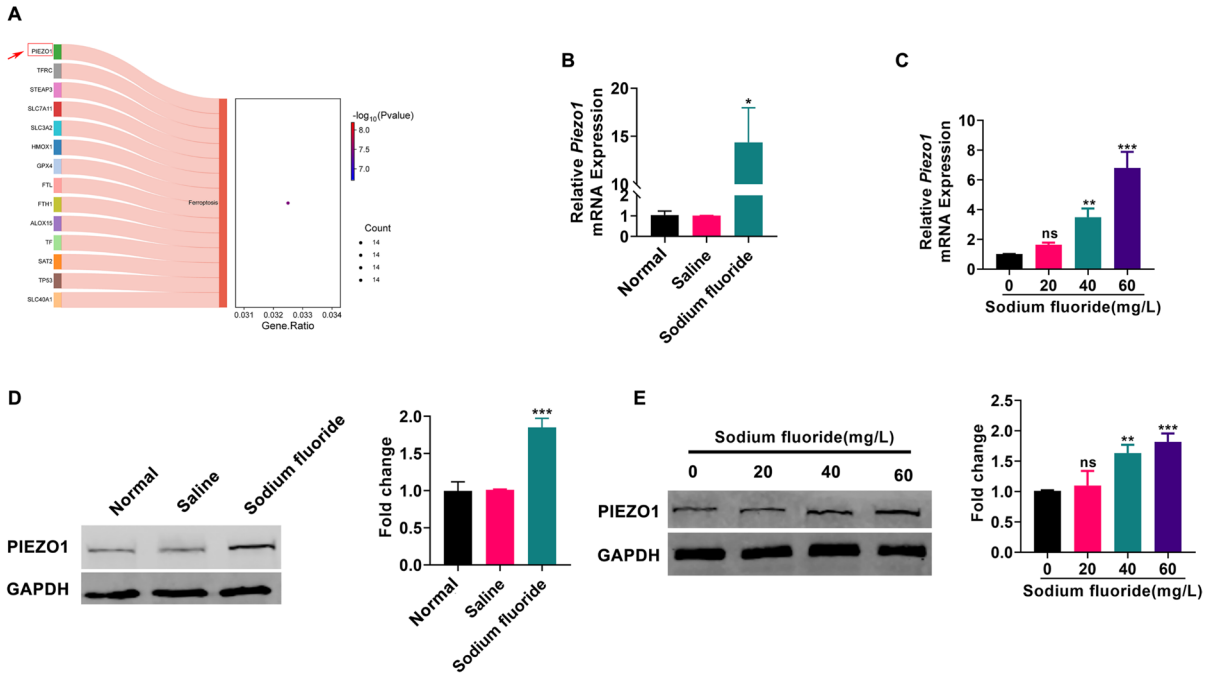

Fig. 3 Sodium fluoride promotes PIEZO1 expression in vivo and in vitro. **A** The visual presentation of genes enriched into ferroptosis pathway; **B** The detection of *Piezo1* mRNA expression in mice after saline or sodium fluoride treatment; **C** The detection of *Piezo1* mRNA expression in 661W cells after saline or sodium fluoride treatment; **D** The expression of PIEZO1 in mice after saline or sodium fluoride treatment; **E** The expression of PIEZO1 in 661W cells after saline or sodium fluoride treatment; Datas were presented as the mean ± SD of three replicates in (**B**-**E**). *P* values were determined using one-way ANOVA with Bonferroni post hoc testing. (*$P<0.05$, **$P<0.01$, ***$P<0.001$; #$P<0.05$, ##$P<0.01$, ###$P<0.001$)

of PIEZO1 compared to the control group. Finally, we examined the myopic parameters of mice in the different treatment groups described above. As shown in Fig. 7C and D, baicalin attenuated sodium fluoride-induced myopia progression compared to the control group. In summary, baicalin inhibited sodium fluoride-induced myopia via PIEZO1.

Discussion

Ferroptosis is a form of regulated cell death characterized by the accumulation of reactive oxygen species (ROS) and subsequent oxidative damage to cellular components. This process is distinct from other forms of cell death, such as apoptosis, necroptosis, and autophagy, playing a critical role in the maintenance of tissue homeostasis and the prevention of diseases associated with oxidative stress (Gao et al. 2022; Jiang et al. 2021). One of the key players in ferroptosis is the enzyme glutathione peroxidase 4 (GPX4), which is responsible for reducing lipid hydroperoxides to their corresponding alcohols, thereby preventing the propagation of oxidative damage. Disruption of GPX4 activity or depletion of its cofactor, reduced glutathione (GSH), triggers ferroptosis, highlighting the importance of maintaining GSH levels for cellular survival (Lei et al. 2021). The discovery of ferroptosis has significant implications for our understanding of disease pathogenesis. There is growing evidence that ferroptosis plays a role in a variety of diseases, including neurodegenerative disorders such as Parkinson's (Mahoney-Sánchez et al. 2021) and Alzheimer's disease (Lane et al. 2021), ischemic stroke (Cui et al. 2021), and certain types of cancer (Tong et al. 2022). The ability of ferroptosis to selectively eliminate damaged or transformed cells without causing inflammation makes it an attractive target for therapeutic intervention. In cancer research,

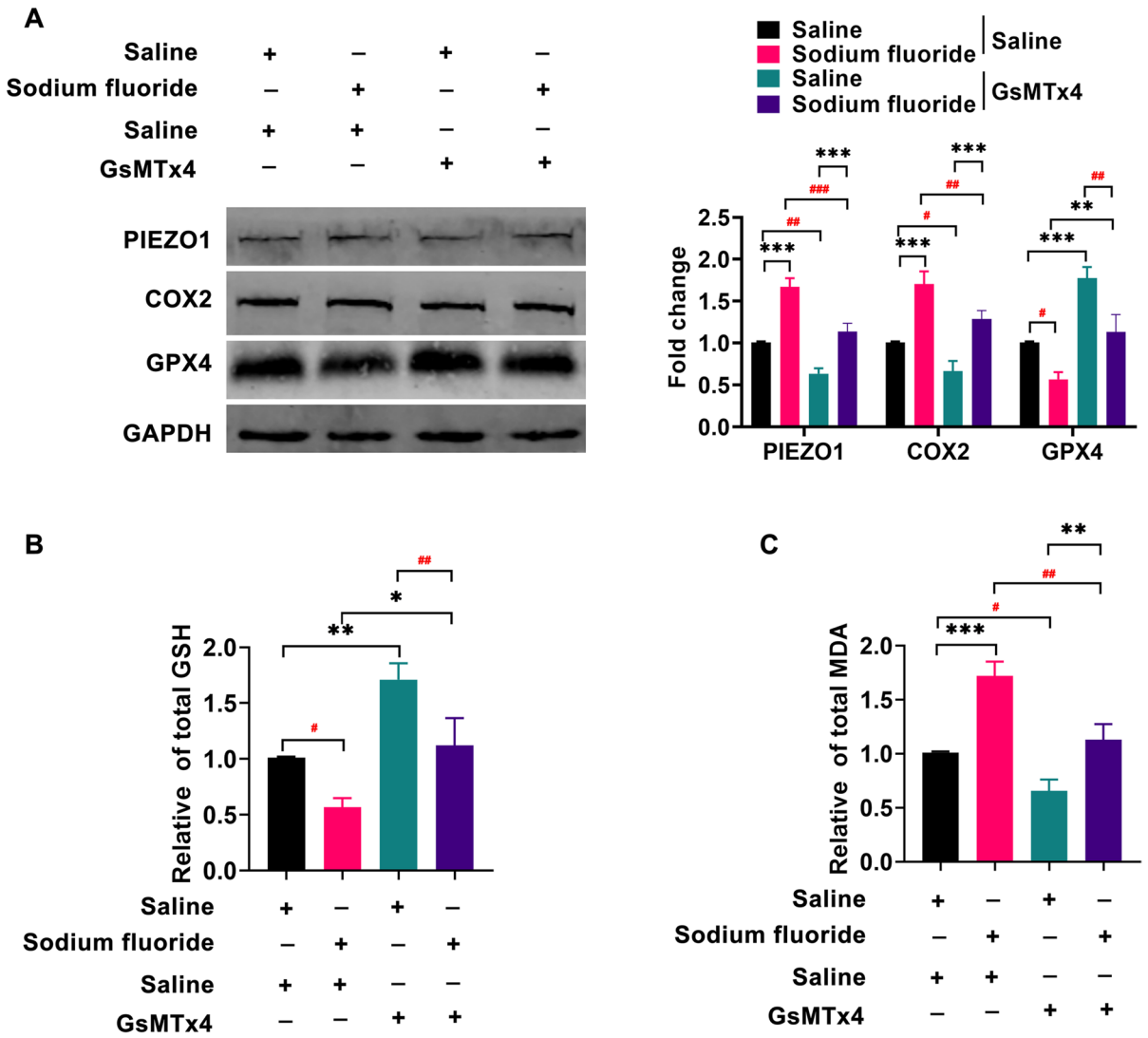

Fig. 4 Sodium fluoride activates the ferroptosis pathway via PIEZO1. **A** Western blotting analysis of PIEZO1, COX2 and GPX4 in the retina of mice after different groups of treatments (saline/saline; sodium fluoride/saline; saline/GsMTx4; sodium fluoride/ GsMTx4); **B** Detection of the GSH levels in the retina of mice after different groups of treatments (saline/saline; sodium fluoride/saline; saline/GsMTx4; sodium fluoride/ GsMTx4); **C** Detection of the MDA levels in the retina of mice after different groups of treatments (saline/saline; sodium fluoride/saline; saline/GsMTx4; sodium fluoride/ GsMTx4); Datas were presented as the mean ± SD of three replicates in (**A**-**B**). *P* values were determined using one-way ANOVA with Bonferroni post hoc testing. (*$P<0.05$, **$P<0.01$, ***$P<0.001$; #$P<0.05$, ##$P<0.01$, ###$P<0.001$)

ferroptosis has emerged as a potential strategy for cancer therapy. Tumors often exhibit high levels of oxidative stress, and cancer cells are more susceptible to ferroptosis due to their increased dependence on iron and lipid metabolism (Tong et al. 2022). Several compounds have been identified that can induce ferroptosis in cancer cells, including erastin, which inhibits the system xc- transporter, and RSL3, which directly binds to and inactivates GPX4, a key enzyme in ferroptosis (Lei et al. 2021; Li et al. 2020). In conclusion, ferroptosis is a novel and promising area of research with significant implications for advancing our understanding of cell death mechanisms and their roles in human health and disease. With continued investigation, we can expect to gain deeper insights into the molecular

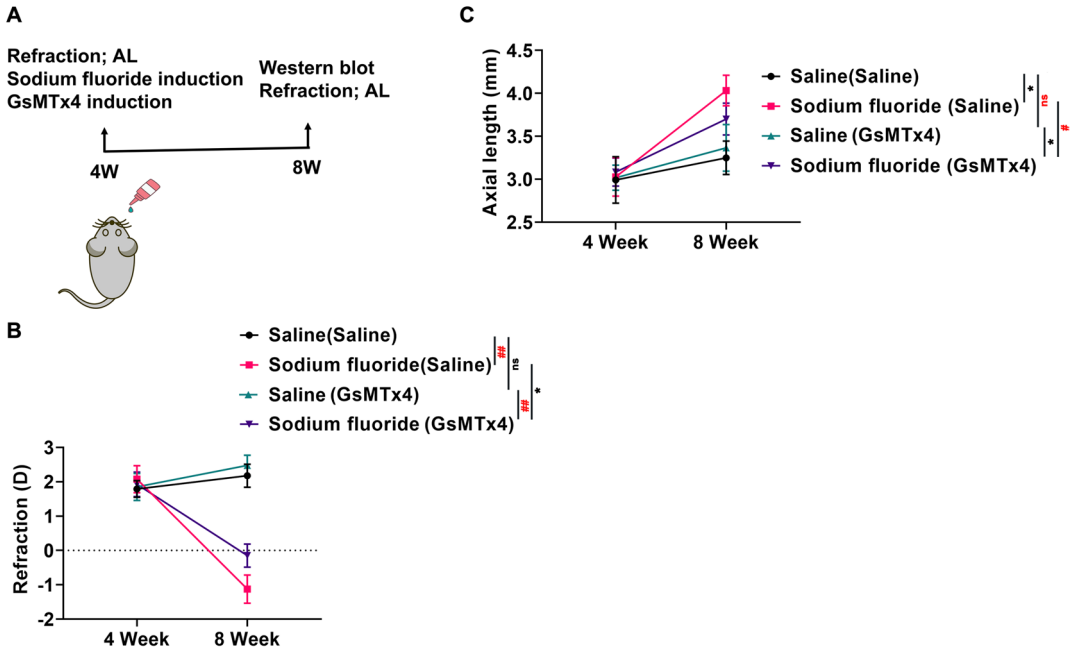

Fig. 5 Sodium fluoride promotes myopia progression by activating ferroptosis. **A** The schematic diagram of the model of sodium fluoride-induced myopia; **B** Detection of refraction in mice after treatment with sodium fluoride or GsMTx4; **C** Examination of axial length in mice after treatment with sodium fluoride or GsMTx4. Datas were presented as the mean ± SD of ten replicates in (B and C). *P* values were determined using one-way ANOVA with Bonferroni post hoc testing. (PIEZO1 inhibitor: GsMTx4, $*P<0.05$, $**P<0.01$, $***P<0.001$; $\#P<0.05$, $\#\#P<0.01$, $\#\#\#P<0.001$)

underpinnings of ferroptosis and its potential applications in the diagnosis, prevention, and treatment of various pathologies.

Research into ferroptosis in ophthalmic diseases has gained considerable attention recently, as this novel form of regulated cell death (RCD) has been implicated in the pathogenesis of several vision-threatening conditions. The unique characteristics of ferroptosis, particularly its reliance on iron-dependent lipid reactive oxygen species (ROS) and the resultant membrane damage, make it an intriguing target for understanding and treating ocular disorders (Chen et al. 2022a; Liu et al. 2023b). In the context of eye diseases, ferroptosis has been linked to retinal degeneration (Liu et al. 2022), glaucoma (Yao et al. 2023), and age-related macular degeneration (AMD) (Gupta 2023; Tang et al. 2021), among others. Retinal degeneration, for instance, involves the progressive loss of photoreceptor cells, a process potentially exacerbated by iron accumulation and oxidative stress. Studies have suggested that the inhibition of ferroptosis pathways could slow or halt this degenerative process, offering a promising therapeutic avenue (Liu et al. 2022; Tang et al. 2021). Glaucoma, another leading cause of blindness worldwide, is characterized by the loss of retinal ganglion cells (RGCs) and their axons. Recent research has highlighted the role of ferroptosis in RGC death, with evidence suggesting that oxidative stress and glutamate excitotoxicity can trigger ferroptosis in these cells (Qin et al. 2022; Yao et al. 2023). This finding underscores the potential of ferroptosis inhibitors as neuroprotective agents in glaucoma therapy. Moreover, AMD, a complex and multifactorial disease, has also been associated with iron accumulation and oxidative stress. The accumulation of iron-containing pigments, such as lipofuscin, in retinal pigment epithelium (RPE) cells has been linked to the generation of ROS and subsequent cell death, possibly via ferroptosis-related mechanisms (Tang et al. 2021; Xiang et al. 2024). Thus, targeting ferroptosis pathways in RPE cells may represent a novel therapeutic strategy for AMD. In this article, we identified through proteomic analysis that sodium fluoride activates the ferroptosis pathway in the promotion of myopia. We then extracted the retinas of mice for molecular analysis and observed that sodium

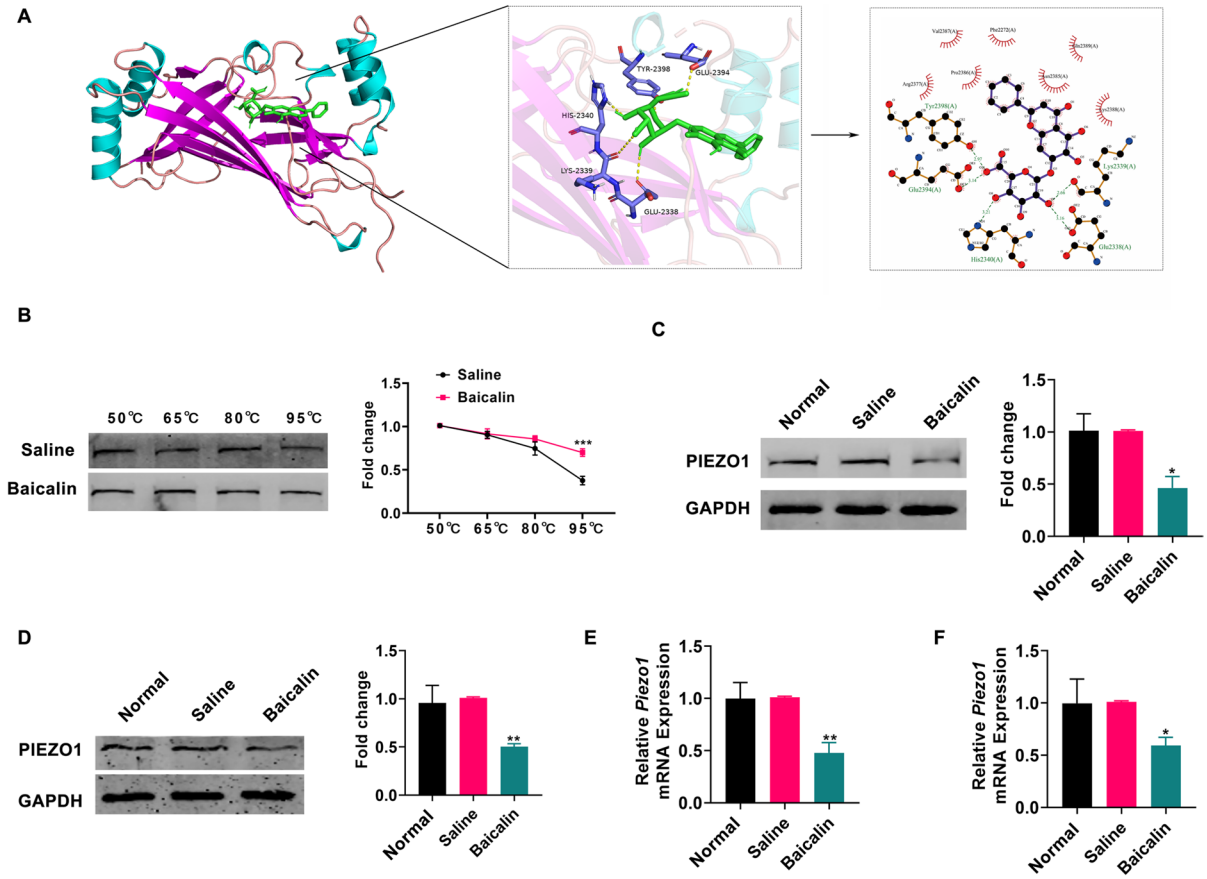

Fig. 6 PIEZO1 is a drug target of Baicalin. **A** The molecular docking simulation of Baicalin binding to PIEZO1; **B** The detection of PIEZO1 expression in 661W cells after treatment with Saline or Baicalin at different temperature; **C** The detection of PIEZO1 expression in 661W cells after Saline or Baicalin treatment; **D** The detection of PIEZO1 expression in mice after Saline or Baicalin treatment; **E** The detection of *Piezo1* mRNA expression in 661W cells after Saline or Baicalin treatment; **F** The detection of *Piezo1* mRNA expression in mice after Saline or Baicalin treatment. Datas were presented as the mean ± SD of three replicates in (**B**-**F**). *P* values were determined using one-way ANOVA with Bonferroni post hoc testing. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

fluoride promotes a decrease in GPX4 expression, an increase in COX2 expression, reduced GSH content, and increased MDA content (Fig. 2), suggesting that ferroptosis plays an important role in the progression of myopia. In summary, the study of ferroptosis in ophthalmic diseases is still in its infancy, but the preliminary findings are promising. As we continue to unravel the molecular mechanisms underlying ferroptosis and its role in ocular pathologies, we can expect to develop more effective and targeted therapies for these vision-threatening conditions.

PIEZO1 is a large transmembrane protein encoded by the FAM38A gene and comprises 2,100 to 4,700 amino acids. It belongs to the mechano-gated cation channel family, which includes PIEZO1 and PIEZO2, and is characterized by its ability to convert mechanical stimuli into electrical or biochemical signals. This unique property makes PIEZO1 a vital player in a wide range of cellular processes, including those related to cell biomechanics and mechanotransduction (Lai et al. 2022; Qin et al. 2021). In recent years, PIEZO1 has garnered significant attention for its potential role in multiple diseases and conditions. For instance, studies have shown that PIEZO1 mediates the mechanosensitivity of osteoblasts, osteoclasts, and chondrocytes, thereby regulating bone formation and remodeling (Xu 2021). This finding has important implications

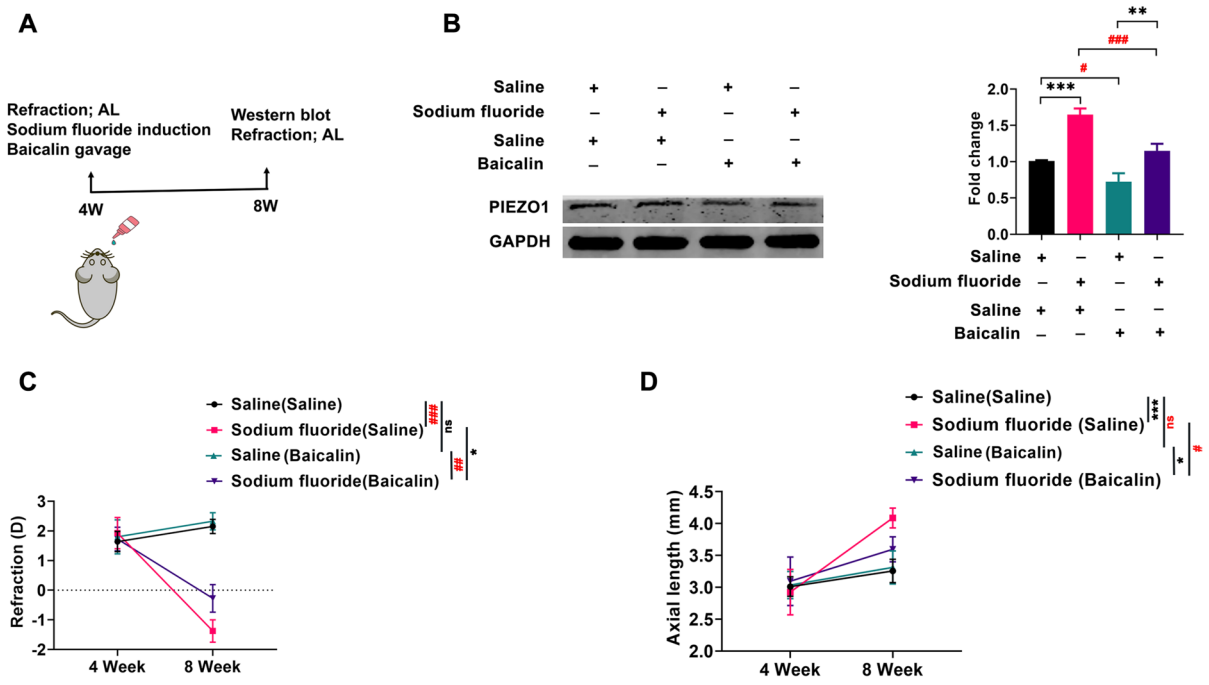

Fig. 7 Baicalin inhibits myopia progression through PIEZO1. **A** The schematic diagram of sodium fluoride-induced myopia model; **B** Detection of PIEZO1 expression after different groups of treatments (saline/saline; sodium fluoride/saline; saline/baicalin; sodium fluoride/ baicalin); **C** Detection of refraction in mice after different groups of treatments (saline/saline; sodium fluoride/saline; saline/baicalin; sodium fluoride/ baicalin) **D** Examination of axial length in mice after different groups of treatments (saline/saline; sodium fluoride/saline; saline/baicalin; sodium fluoride/ baicalin). Datas were presented as the mean ± SD of three replicates in (**B**) and ten replicates in (**C** and **D**). *P* values were determined using one-way ANOVA with Bonferroni post hoc testing. (*$P<0.05$, **$P<0.01$, ***$P<0.001$; #$P<0.05$, ##$P<0.01$, ###$P<0.001$)

for the treatment of bone-related disorders, such as osteoporosis and degenerative joint diseases. Additionally, PIEZO1 has been implicated in iron metabolism and cardiac function, further underscoring its versatility and significance in maintaining physiological homeostasis (Beech and Kalli 2019). In the context of eye diseases, PIEZO1 has been implicated in a range of conditions that involve alterations in ocular mechanics and mechanotransduction. For example, glaucoma, a leading cause of blindness worldwide, is characterized by the progressive loss of RGCs and their axons, often due to elevated intraocular pressure (IOP) (Chen et al. 2022b). Studies have suggested that PIEZO1 may be involved in the mechanosensation of RGCs and their supporting tissues, potentially contributing to the pathogenesis of glaucoma. By modulating PIEZO1 activity, it may be possible to protect RGCs from mechanical stress and delay the progression of the disease (Morozumi et al. 2020). Another area of interest is the role of PIEZO1 in corneal mechanotransduction. The cornea, the outermost layer of the eye, is highly sensitive to mechanical stimuli and plays a crucial role in maintaining the clarity and refractive power of the eye. PIEZO1 has been detected in corneal epithelial cells and stromal fibroblasts, suggesting that it may play a role in regulating corneal biomechanics and homeostasis (Morozumi et al. 2020). Abnormalities in PIEZO1 function may contribute to the development of corneal diseases, such as keratoconus, a degenerative disorder of the cornea that leads to progressive thinning and distortion of the tissue (Sonkodi et al. 2023). Our literature review suggests that PIEZO1 is closely related to myopia development (Zhong et al. 2023) and the ferroptosis pathway (Hirata 2023), leading us to investigate whether sodium fluoride activates ferroptosis through PIEZO1, thereby promoting myopia progression. As shown in Fig. 3, sodium fluoride can promote the expression of PIEZO1. Using the PIEZO1 inhibitor

GsMTx4 in a morphologic deprivation mouse myopia model, we found that GsMTx4 retarded myopia progression (Fig. S2). In a sodium fluoride-induced mouse model of myopia, the sodium fluoride/GsMTx4 group showed reduced myopic parameters and inhibited ferroptosis compared to the control group (Figs. 4 and 5). We further explored PIEZO1 as a target of baicalin in delaying myopia progression (Figs. 6 and 7). All of these data suggest that sodium fluoride can promote myopia progression by activating the ferroptosis pathway through PIEZO1. In conclusion, PIEZO1 represents a pivotal player in mechanosensation and mechanotransduction, with diverse roles in various physiological and pathological processes. As we continue to unravel the mysteries of PIEZO1, we can expect to gain deeper insights into its function and develop more effective strategies for harnessing its potential in the clinic.

Conclusion

Our study demonstrated that sodium fluoride can activate the ferroptosis pathway via PIEZO1 to promote myopia progression, and that PIEZO1 inhibition, using a PIEZO1 inhibitor and a morphologic deprivation mouse model, delayed myopia progression. We also found that PIEZO1 is a direct target of baicalin through molecular docking modeling and CETSA assays, and that baicalin inhibits PIEZO1 expression to delay myopia progression. In conclusion, PIEZO1 may be a novel drug target for myopia treatment.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval All animal experiments were approved by the Animal Ethics Committee of Zhongshan Hospital Affiliated to Fudan University.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

Clinical trial number Not applicable.

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