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Omeprazole exacerbates intervertebral disc degeneration through Caspase-3 mediated apoptosis of nucleus pulposus cells: a Mendelian randomization, network toxicology, and in vitro experimental study

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Abstract

Objective To investigate the causal correlation and toxicological mechanisms of omeprazole in intervertebral disc degeneration (IVDD), alongside a particular emphasis on Caspase-3 (CASP3) mediated apoptosis of nucleus pulposus cells (NPCs).

Methods Mendelian randomization (MR): GWAS data was employed to assess causal associations between proton pump inhibitors (PPIs) and IVDD. Network toxicology: Shared omeprazole-IVDD targets were identified using STRING, SwissTargetPrediction, and GeneCards databases. Functional enrichment analysis: Biological pathways were explored by employing Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). Molecular docking: Omeprazole-CASP3 binding affinity was assessed by employing AutoDock Vina. Experimental validation: Rat NPCs were subjected to CCK-8 assay viability, flow cytometry apoptosis, Western blot, and immunofluorescence.

Results MR analysis suggested omeprazole substantially augmented IVDD risk (OR = 1.058, 95% CI = 1.004–1.115, $P = 0.034$), with no association observed for esomeprazole or lansoprazole. Network toxicology identified 11 overlapping targets, with CASP3 as the hub gene. Molecular docking revealed strong omeprazole-CASP3 binding (free energy: −6.725 kcal/mol) via hydrogen bonds, π—π stacking, and π—S interactions. Enrichment analysis highlighted the response to reactive oxygen species, caveolae, endopeptidase activity, and IL-17 signaling pathway as key pathways. As revealed by in vitro experiments, omeprazole dose-dependently lessened NPCs viability (300 μM) and heightened apoptosis (28.99% apoptosis rate). Western blot showed significant upregulation of Cleaved-CASP3/pro-CASP3 ratios ($P < 0.001$), and immunofluorescence demonstrated CASP3 nuclear translocation in omeprazole-treated NPCs.

Conclusions This study found that taking omeprazole may exacerbate IVDD, and its potential mechanism is through CASP3 leading to apoptosis of NPCs. These findings advocate cautious long-term omeprazole use in clinical practice and suggest alternative PPIs.

Keywords Omeprazole, Intervertebral Disc Degeneration, Mendelian Randomization, Network Toxicology, Caspase-3

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Introduction

As a prevalent condition associated with significant public health and economic burdens [1], intervertebral disc degeneration (IVDD) compromises spinal flexibility and shock absorption through structural deterioration of the intervertebral disc. Even though frequently asymptomatic in early stages, progressive IVDD potentially brings about chronic pain and spinal dysfunction [2]. In particular, the pathogenesis involves Caspase-3 (CASP3) mediated apoptosis of nucleus pulposus cells (NPCs) and dysregulated extracellular matrix (ECM) degradation [2, 3]. As the terminal executor of apoptosis, CASP3 activation has proven to be crucial in driving IVDD progression [4]. As evidently demonstrated by mechanistic studies, multiple stimuli, comprising TNF-α [5], IL-1β [6], and oxidative stress [7], converge to activate CASP3 signaling. This activation cascade facilitates NPC apoptosis and hinders ECM metabolism, ultimately exacerbating disc degeneration.

Despite significant advancements in surgical and pharmacological management, contemporary therapeutic strategies focus on symptom alleviation rather than arresting disease progression [8]. This unmet clinical need underscores the urgent necessity for identifying modifiable risk factors, particularly those attributable to iatrogenic sources like commonly prescribed pharmaceuticals. Omeprazole, a proton pump inhibitor (PPI), is extensively employed in managing gastric acid-related disorders. Emerging findings have prompted concerns over its musculoskeletal effects [9]. Recent epidemiological studies suggest that prolonged omeprazole use may heighten the risk of osteoporotic fractures and osteoarthritis, potentially via mechanisms including impaired calcium absorption due to gastric acid suppression, magnesium malabsorption, and vitamin B12 deficiency [9, 10]. In particular, mechanistic studies have demonstrated omeprazole's capacity to activate CASP3, encompassing apoptosis in renal tubular cells [11]. Nevertheless, it remains controversial about the potential influence of omeprazole on IVDD pathogenesis, among which three major limitations have confused the general public: (1) Inherent confounding bias in observational designs preventing causal inference; (2) Potential cell-type specificity of apoptotic mechanisms in NPCs residing within hypoxic, nutrient-deficient microenvironments [2]; (3) Insufficient integration of bioinformatics predictions with experimental validation.

As an advanced epidemiological approach, Mendelian randomization (MR) has garnered significant attention for its capacity to infer causal correlations from observational data. By leveraging genetic variants, particularly single nucleotide polymorphisms (SNPs), as instrumental variables, MR enables robust causality assessment between exposures and outcomes [12]. Renowned as an emerging discipline rooted in network biology and network pharmacology, network toxicology systematically characterizes and predicts drug toxicity by meticulously constructing integrative network models [13]. Grounded in preliminary evidence linking apoptosis to IVDD, this study puts forth a hypothesis that omeprazole exacerbates IVDD via CASP3-dependent apoptotic pathways. To validate this hypothesis, we implemented a tripartite strategy integrating: (1) MR analysis to establish genetic causality between omeprazole exposure and IVDD risk; (2) Network toxicology screening to identify shared omeprazole-IVDD targets and construct a"drug-target-disease"interaction network; (3) Molecular docking coupled with in vitro NPCs models to elucidate CASP3-mediated apoptotic mechanisms. These findings are anticipated to lay a solid theoretical foundation for optimizing PPI administration in IVDD patients.

Method

Mendelian randomization analysis of PPI and IVDD

The STROBE-MR framework was applied as the methodological foundation. From a theoretical standpoint, MR principally hinges on three core assumptions: (1) Instrumental variables demonstrate a conspicuous association with the exposure of interest; (2) Instrumental variables independent of confounders influence both exposure and outcome; (3) Instrumental variables do not directly affect the outcomes [14].

The GWAS summary data for PPI and IVDD are sourced from the IEU Open GWAS project (https://gwas.mrcieu.ac.uk/datasets/). Table 1 presents specific data. The process of filtering instrumental variables involved: (1) selecting SNPs with a significance level of 5×10^{-6}, (2) eliminating SNPs in linkage disequilibrium with an r2 threshold of <0.001 within 10,000 kb, (3) excluding palindromic SNPs through harmonization, and (4) using the LDtrait tool to exclude SNPs linked to confounders [12, 15].

Two-sample MRs were employed to gauge the influence of PPI on IVDD. Supplemented by MR Egger, weighted median, simple mode, and weighted mode analyses for robustness, our methodology principally employed the Inverse Variance Weighted (IVW) method to ensure accurate causal estimates. Additionally,

Table 1 Detailed information on the GWAS summary data

Phenotype	Consortium	Population	Case	Control	GWAS ID
omeprazole	MRC-IEU	European	27,277	435,656	ukb-b-14960
esomeprazole	MRC-IEU	European	1,546	461,387	ukb-b-17371
lansoprazole	MRC-IEU	European	16,448	446,485	ukb-b-19156
IVDD	NA	European	4,690	356,504	ukb-d-M51

sensitivity analysis was conducted accordingly, which primarily encompassed Cochrane's Q-test and MR Egger intercept test to examine heterogeneity and horizontal pleiotropy in MR results, and the MR-PRESSO test was used to identify and exclude outliers [12].

Omeprazole and IVDD gene target acquisition

The chemical structure of omeprazole was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [16] and subjected to target gene identification through computational analysis. Structural data were imported into the STRING database (https://cn.string-db.org/) [17] and SwissTargetPrediction (http://www.swisstargetprediction.ch) [18] for systematic pharmacological target prediction. In parallel, the GeneCards database (https://www.genecards.org) was interrogated using the search term"intervertebral disc degeneration,"with genes scoring above 10 selected as high-confidence candidates for IVDD-associated targets.

Construction of protein interaction networks and identification of Hub gene

Common target genes between omeprazole and IVDD were determined via Venn diagram analysis. Subsequent protein–protein interaction (PPI) network construction was constructed by utilizing the STRING database (https://cn.string-db.org/) with Homo sapiens as the reference organism, employing a stringent interaction confidence threshold (> 0.4) to ensure high-quality interactions. The resultant PPI data were imported into Cytoscape v3.7.0 for network visualization, topological metric calculation, and identification of hub genes through node centrality analysis [19].

GO and KEGG enrichment analysis

Functional and pathway enrichment analyses were methodically executed on 11 candidate genes implicated in omeprazole-induced IVDD. Gene Ontology (GO) analysis characterized biological processes (BP), cellular components (CC), and molecular functions (MF) to delineate their mechanistic roles in IVDD pathogenesis. Complementary Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis identified key signaling cascades associated with omeprazole-mediated IVDD [20].

Molecular docking of hub genes and omeprazole

The protein structure of CASP3 (PDB ID: 1 NMS) originated from the RCSB Protein Data Bank (https://www.rcsb.org) and was fabricated by utilizing MGLtools 1.4.2 [21]. The preparation process encompassed adding hydrogen atoms, eliminating crystallographic water molecules, and assigning Gasteiger partial charges through the prepare_receptor4 script. The binding site was defined as the centroid of the co-crystallized ligand within the original PDB structure.

The three-dimensional structure of omeprazole (CAS: 73,590–58-6) was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) and processed by utilizing the prepare_ligand4 script in MGLtools 1.4.2, which included structural optimization and Gasteiger charge assignment. Molecular docking simulations were performed by adopting AutoDock Vina v1.2.5 [22] with the following parameters: twenty ligand conformations were generated through systematic search algorithms, and the optimal pose was selected based on the lowest calculated binding free energy for subsequent interaction analysis.

Cell acquisition and culture

NPCs were procured from 6-week-old male Sprague–Dawley rats following humane euthanasia. Under aseptic conditions, the caudal vertebrae were dissected to reveal the intervertebral discs. The annulus fibrosus was incised circumferentially, and NP tissue was meticulously separated. Tissue digestion was performed using 5% type II collagenase (Solarbio, CAS: 9001–12-1) in a 37 °C shaking incubator for 4 h to materialize single-cell suspension.

Following enzymatic digestion, isolated NPCs were seeded in an F12 medium containing 10% fetal bovine serum (FBS) and maintained in a incubator (37 °C, 5% CO2). Cellular proliferation was periodically monitored via microscopy until the cells reached 80% confluence while maintaining morphological integrity. Subculturing was performed at this point. Cells from passages 3–4 were selected for subsequent experimental procedures.

CCK-8 assay for NPC viability

NPCs were seeded in 96-well plates at 5×10^3 cells/well and pre-cultured for 24 h (37 °C, 5% CO_2) to facilitate adhesion. Upon adhesion stabilization, cells were subjected to omeprazole (100–400 μM) for 24-, 48-, and 72-h durations. After the above treatments, 10% CCK-8 reagent (GLPBIO, GK10001) was introduced to each well and incubated for 2 h. Optical density (OD) at 450 nm was quantified by adopting a multimode microplate reade. Cell viability was then calculated based on these readings.

Flow cytometry apoptosis

Based on CCK-8 results, an optimal omeprazole concentration was selected for subsequent apoptosis assays.

Cells were harvested by centrifugation (300 ×g, 5 min) and washed with PBS. After washing, cells were resuspended in 500 µL of 1× Annexin V binding buffer. Staining was initiated by sequentially adding 5 µL Annexin V-FITC Reagent and 5 µL PI Reagent with gentle vortex mixing, accompanied by dark incubation. Flow cytometric analysis was immediately employed to quantify apoptotic populations: Annexin V^+/PI^- (early apoptotic), Annexin V^+/PI^+ (late apoptotic), and Annexin V^-/PI^+ (Necrotic cells) subpopulations.

Western blot

NPCs were assigned to two experimental groups: an untreated control group and an omeprazole-treated group, with the latter's optimal drug concentration predetermined through CCK-8 cytotoxicity assays. After a 24-h intervention, cells were lysed with RIPA buffer for protein extraction. Afterward, protein lysates were resolved on 12% SDS-PAGE gels and transferred to PVDF membranes. After blocking with 5% non-fat milk, the membrane was incubated overnight at 4 °C with the following primary antibodies: anti-CASP3 (1:1000, A19664, ABclonal) and β-actin (1:2000, BSM-33036 M, Bioss). Subsequently, the membrane was washed thrice with TBST and incubated with HRP-conjugated secondary antibodies (1:5000) at room temperature for 1 h. Following three additional TBST washes, chemiluminescent signals were detected using a gel imaging system. Band intensities were quantified via ImageJ grayscale densitometry and normalized to β-actin expression.

Immunofluorescence

NPCs were allocated into control and omeprazole-treated groups (concentration optimized via CCK-8 results). Following PBS washing, fixation was performed using 4% paraformaldehyde (PFA) for 15 min at room temperature. Subsequently, permeabilization with 0.1% Triton X-100 proceeded for 15 min, followed by blocking with 3% bovine serum albumin (BSA) for 1 h. Primary anti-CASP3 antibody (1:200, A11319, ABclonal) was applied overnight at 4 °C. After washing, Alexa Fluor 488-conjugated secondary antibody (1:500) incubation occurred for 50 min at room temperature. Nuclear counterstaining was performed with DAPI staining solution by incubating in the dark at room temperature for 10 min. After washing with PBS (PH7.4), fluorescence imaging was conducted using an inverted fluorescence microscope with DAPI and FITC channels.

Statistical analysis

Statistical analyses were performed using GraphPad Prism. Western blot quantification data are presented as mean ± SD, and an unpaired Student's t-test assessed comparisons between groups. Statistical significance was $p < 0.05$, with asterisks indicating significance levels. Detailed statistical parameters and figure legends are provided in the Results section.

Result

Causal effects of PPI on IVDD

IVW analysis illustrated that omeprazole exposure was tightly correlated with substantially augmented IVDD risk (GWAS ID: ukb-dM5, OR = 1.058, 95%CI = 1.004–1.115, $P = 0.034$), whereas esomeprazole and lansoprazole exhibited no significant associations (Fig. 1, Supplementary Table S1). Sensitivity analyses revealed no detectable heterogeneity or horizontal pleiotropy, confirming the result's robustness (Supplementary Table S2).

Target genes of omeprazole and IVDD

The chemical structure of omeprazole (SMILES: CC1 =CN =C(C(= C1OC)C)CS(= O)C2 =NC3 =C(N2) C =C(C =C3)OC) was retrieved from PubChem for target prediction. Using the STRING database, the structural analysis identified 10 putative omeprazole-associated genes (Fig. 2A), and SwissTargetPrediction further expanded the candidate pool with an additional 100 genes (Supplementary Table S3). Merging these datasets yielded 110 targets. Concurrently, GeneCards screening for"intervertebral disc degeneration"(score >10) generated 317 IVDD-related genes (Supplementary Table S4), establishing the foundation for subsequent network analysis.

Drug-target-disease network construction

Venn diagram analysis was employed to identify shared toxicology targets between omeprazole and IVDD, revealing 11 intersecting genes: MMP1, SIRT1, CSF1R, LRRK2, MAPK1, PIK3 CA, MMP9, CASP3, MMP13, HMOX1, and CASP9 (Fig. 2B). These overlapping targets were incorporated into a network toxicology framework by constructing a"drug-target-disease"interaction network with Cytoscape. The network revealed omeprazole's potential mechanisms in IVDD pathogenesis (Fig. 2C).

Functional enrichment analysis

To explore the molecular mechanisms underlying the pathological effects of omeprazole on IVDD, GO and KEGG enrichment analyses were conducted based on its potential targets. As shown in Fig. 2D, the top 10 significantly enriched terms across BP, CC, and MF are summarized. The BP analysis highlighted a predominant enrichment in the response to reactive oxygen species. The CC analysis revealed a strong association with caveolae. Furthermore, the MF analysis identified

exposure	nsnp	method	pval	OR(95% CI)
omeprazole	20	MR Egger	0.391	1.079 (0.911 to 1.278)
omeprazole	20	Weighted median	**0.048**	1.076 (1.001 to 1.156)
omeprazole	20	Inverse variance weighted	**0.034**	1.058 (1.004 to 1.115)
omeprazole	20	Simple mode	0.191	1.106 (0.956 to 1.279)
omeprazole	20	Weighted mode	0.208	1.102 (0.952 to 1.274)
esomeprazole	5	MR Egger	0.586	0.010 (0.000 to 26735.899)
esomeprazole	5	Weighted median	0.714	0.901 (0.515 to 1.577)
esomeprazole	5	Inverse variance weighted	0.694	0.918 (0.598 to 1.407)
esomeprazole	5	Simple mode	0.757	1.132 (0.545 to 2.348)
esomeprazole	5	Weighted mode	0.752	1.152 (0.508 to 2.612)
lansoprazole	13	MR Egger	0.894	1.017 (0.799 to 1.295)
lansoprazole	13	Weighted median	0.680	0.976 (0.872 to 1.093)
lansoprazole	13	Inverse variance weighted	0.845	1.008 (0.930 to 1.093)
lansoprazole	13	Simple mode	0.667	0.954 (0.776 to 1.174)
lansoprazole	13	Weighted mode	0.621	0.956 (0.804 to 1.137)

Fig. 1 Forest plot shows the effects of three PPIs on IVDD. OR, odds ratio; CI, confidence interval

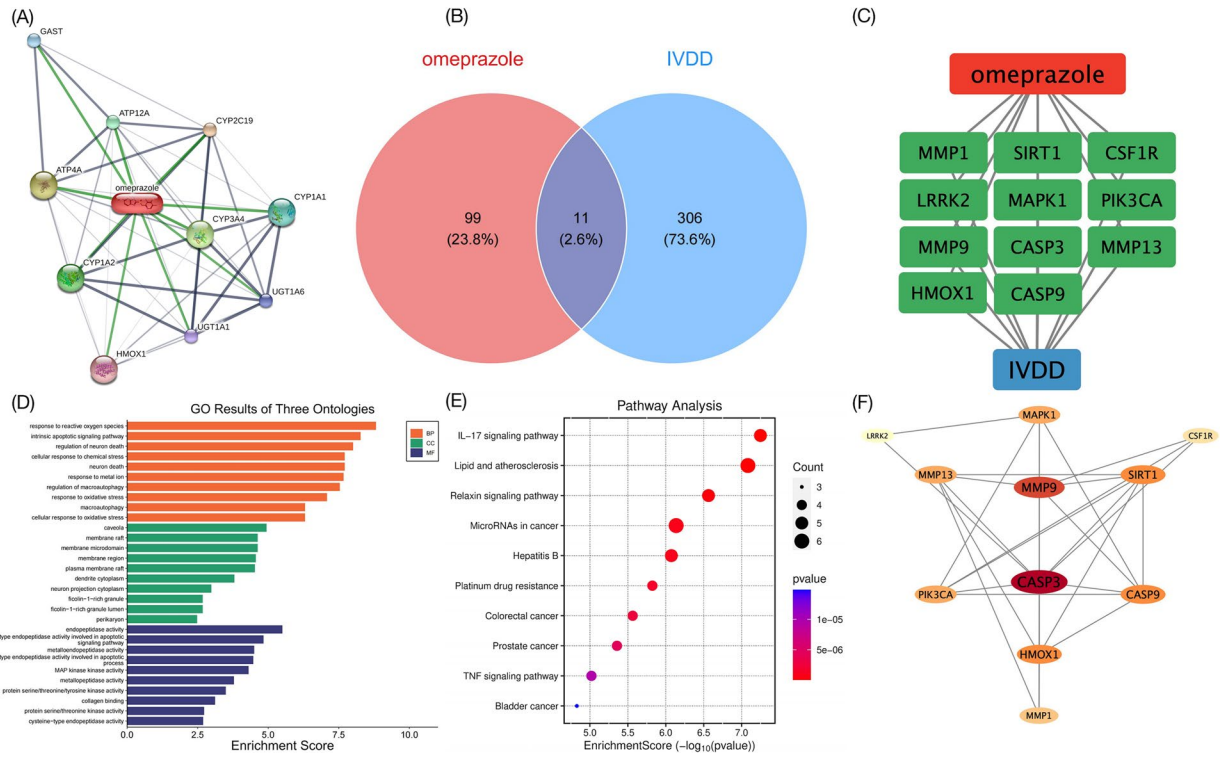

Fig. 2 Integrative network analysis of omeprazole-IVDD interactions: **A** STRING-based protein interaction network of omeprazole targets; **B** Venn diagram illustrating target gene overlap between omeprazole and IVDD; **C** Drug-target-disease interaction network mapping omeprazole-IVDD pathological associations; **D** Top 10 enriched Gene Ontology terms (BP: biological processes; CC: cellular components; MF: molecular functions) for shared targets; **E** KEGG pathway enrichment analysis of intersecting genes; **F** Protein–protein interaction (PPI) network highlighting hub genes in omeprazole-induced IVDD

critical functional activities, notably endopeptidase activity. For pathway-specific exploration, KEGG analysis identified 10 significantly enriched signaling pathways, among which the IL-17 signaling pathway showed the strongest relevance to IVDD pathogenesis (Fig. 2E, Supplementary Table S5-8).

Protein–protein interaction network construction and hub gene identification

Using the STRING database with the human genome as the reference, 11 overlapping genes were analyzed to construct a PPI network. The resulting network was subsequently imported into Cytoscape for hub gene identification. Rooted in node degree analysis, CASP3 demonstrated the most striking connectivity. Under such circumstances, it was identified as the hub gene mediating omeprazole's effects on IVDD (Fig. 2F).

Molecular docking

The analysis of the top-ranked conformation revealed that the small molecule forms hydrogen bond interactions with ARG-207 (bond distances: 1.8/2.6 Å) and TRP-214 (bond distance: 2.6 Å). Additionally, it engages in a π-π stacking interaction with TRP-206 and exhibits a π-S interaction with PHE-356. This binding pattern, combined with a calculated binding free energy of −6.725 kcal/mol, indicates a strong binding affinity (Fig. 3).

Omeprazole targets CASP3 to promote apoptosis of NPCs

The CCK-8 assay demonstrated that omeprazole diminished NPCs viability in a dose- and time-dependent manner, with striking suppression of cell proliferation observed at 300 μM across 24-, 48-, and 72-h treatment groups (Fig. 4A). Annexin V-FITC/PI staining combined with flow cytometry revealed a 28.99% apoptosis rate (23.00% early and 5.99% late apoptosis) in cells treated with 300 μM omeprazole for 24 h (Fig. 4B). As evidenced by Western blot analysis, the Cleaved-CASP3/pro-CASP3 protein ratio was significantly increased in the omeprazole-treated group compared to the control group (Fig. 4D-E). Immunofluorescence results suggested cytoplasmic localization of CASP3 in cells cultured in the control group, whereas omeprazole-treated cells exhibited nuclear and cytoplasmic shrinkage with partial nuclear translocation of CASP3 (Fig. 4C). CASP3, initially as pro-CASP3 in the cytoplasm, undergoes proteolytic cleavage into Cleaved-CASP3 during apoptosis, followed by nuclear translocation [23]. Collectively, findings summarized from CCK-8, flow cytometry, Western blot, and immunofluorescence analyses substantiate that omeprazole triggers NPCs apoptosis through CASP3 activation.

Discussion

For patients suffering from IVDD or chronic lower back pain, oral nonsteroidal anti-inflammatory drugs (NSAIDs) are routinely administered to alleviate discomfort in clinical practice [24]. Nonetheless, gastrointestinal disturbances frequently manifest as a common adverse effect. On that account, it is advocated to take PPIs orally simultaneously to protect the gastric mucosa. In particular, the most frequently employed one is omeprazole, which is also considered safe [25]. Nevertheless, emerging evidence has underscored that long-term use may augment the risk of osteoarthritis [10], a disease that shares pivotal pathophysiological pathways with IVDD, such as inflammation and extracellular matrix dysregulation [26]. As these overlapping mechanisms suggest, omeprazole may exacerbate the progression of IVDD, a hypothesis supported by our MR analysis. On top of that, we noticed that other PPIs, such as esomeprazole

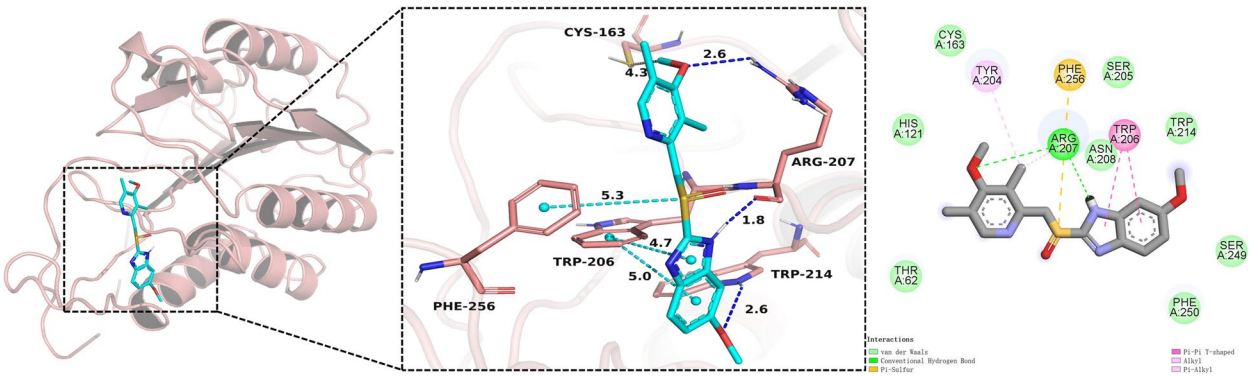

Fig. 3 In the molecular docking diagram of CASP3 and omeprazole, the cartoon represents a protein, cyan sticks represent small molecules, blue dashed lines represent hydrogen bonding interactions, and cyan dashed lines represent π—π interactions

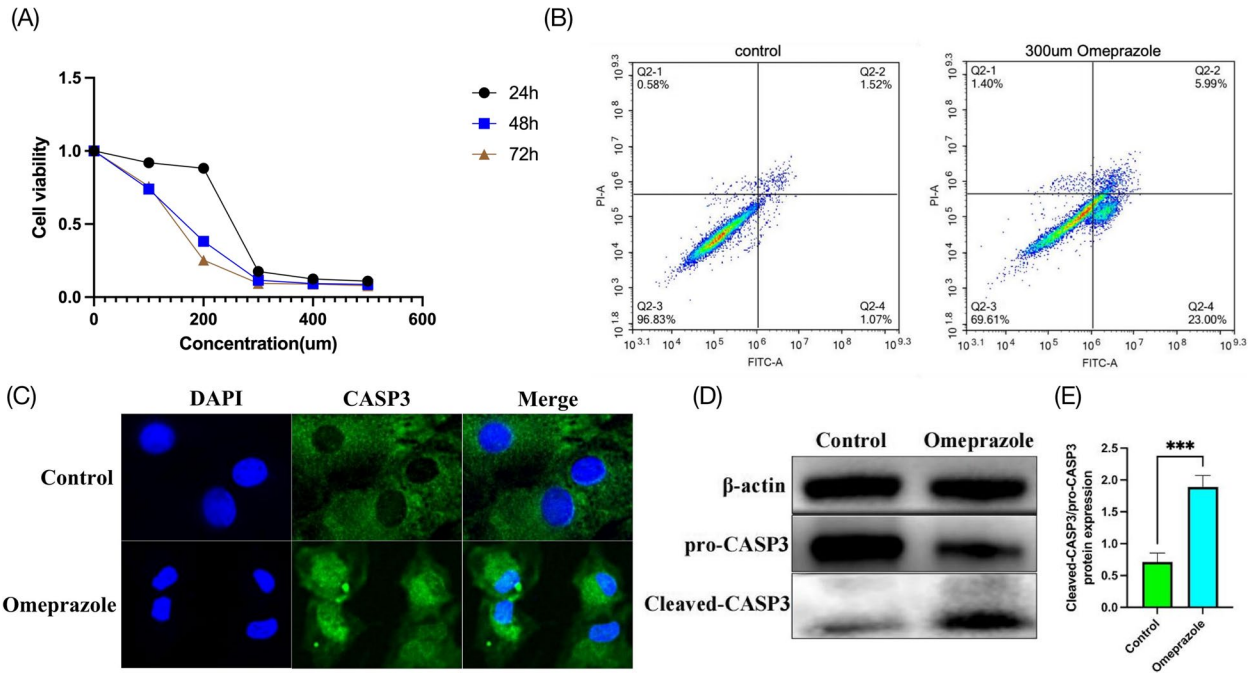

Fig. 4 Omeprazole induces apoptosis in NPCs through CASP3 activation. **A** CCK-8 assay; **B** Immunofluorescence staining of CASP3; (D, E) Western blot analysis of Cleaved-CASP3/pro-CASP3 protein expression

and lansoprazole, were not remarkably associated with IVDD risk, which evidently demonstrates that prioritizing their use as a substitute for omeprazole may reduce the potential risk of IVDD. Aside from that, for individuals continually enduring IVDD or lower back pain who require long-term use of NSAIDs, using drugs such as misoprostol, COX-2 selective inhibitors, and H (2) blockers instead of omeprazole to protect the gastric mucosa may serve as a satisfactory option [27].

As a proton pump inhibitor, omeprazole disrupts intestinal TRPM6/7 channel-mediated magnesium absorption, giving rise to hypomagnesemia in chronic users [28]. Magnesium holds indisputable significance for intervertebral disc cell function, safeguarding NPCs against ROS, inflammation, and aging to delay IVDD [29]. Magnesium deficiency impedes DNA replication, worsening inflammation and oxidative stress in NPCs, thereby expediting degeneration [30, 31]. Omeprazole-induced vitamin B12 malabsorption elevates homocysteine levels, negatively influencing collagen metabolism [32–34]. Abnormal collagen metabolism directly imposes a disadvantageous impact on the mechanical stability of the annulus fibrosus [35]. On top of that, gastric acid suppression alters gut microbiota, fostering systemic translocation of gram-negative bacterial lipopolysaccharides (LPS) [36, 37]. LPS activates Toll-like receptors, upregulates pro-inflammatory factors in discs, and activates matrix metalloproteinases (MMPs) to degrade the extracellular matrix [38].

We identified 11 genes that exhibited overlap between omeprazole and IVDD, comprising MMP1, SIRT1, CSF1R, LRRK2, MAPK1, PIK3 CA, MMP9, CASP3, MMP13, HMOX1, and CASP9. Subsequent BP analysis of these genes revealed predominant enrichment in ROS response pathways. Heightened ROS levels and lessened antioxidant enzyme activity in degenerative discs induce oxidative stress, which in turn accelerates disc degeneration by promoting cellular senescence, apoptosis, and ECM degradation [39]. Chronic omeprazole use exacerbates oxidative stress [40], which sufficiently demonstrates its enormous potential to aggravate disc cell dysfunction. CC analysis underscored caveolae—plasma membrane invaginations known to mediate signal transduction, endocytosis, and mechanotransduction [41]. Furthermore, caveolae regulate inflammatory and oxidative stress pathways, and their dysfunction may disrupt intervertebral disc cell mechanosensitivity and homeostasis, thereby accelerating extracellular matrix degradation and apoptosis [42]. MF analysis implicated endopeptidase activity in disc degeneration. ECM degradation, a hallmark of IVDD, is driven by MMPs, a family of endopeptidases mediating ECM remodeling [43]. Therefore, omeprazole may contribute to IVDD through MMP-mediated ECM disruption. KEGG analysis unveiled a pronounced enrichment of overlapping genes in the IL-17 signaling pathway. As a 35 kDa pro-inflammatory cytokine, IL-17 binds to IL-17RA/C receptor

heterodimers to activate NF-κB, MAPK/AP-1, and C/EBP pathways while synergistically amplifying inflammatory gene expression via the NOTCH pathway [44]. In IVDD, heightened IL-17 levels correlate with degeneration severity and drive pathology through: (1) ECM degradation via MMP-3, MMP-13, and ADAMTS-7 upregulation; (2) inflammatory cascade amplification (TNF-α, IL-1β, PGE2, NO); (3) JAK/STAT/VEGF overexpression and pathological angiogenesis; (4) PI3 K/Akt/Bcl-2-dependent autophagy suppression; (5) concentration-dependent inhibition of NPCs proliferation. These mechanisms hold back disc homeostasis and speed up degeneration [44].

Afterward, the PPI network identified CASP3 as a pivotal gene for omeprazole-mediated IVDD. The molecular docking findings demonstrated that omeprazole forms hydrogen bonds, π—π stacking, and π—S interactions with crucial residues of CASP3, and the binding free energy (−6.725 kcal/mol) further confirms its strong binding affinity. CASP3 plays a paramount role in apoptosis, which can be initiated by extrinsic stimuli (e.g., steroid hormones and TNF receptor ligands) [45] or intrinsic cellular stressors (e.g., viral infection, hypoxia, oxidative stress) [46]. The extrinsic pathway is activated through ligand binding to death receptors, eventually leading to caspase-8 activation and subsequent triggering of downstream effector caspases [45]. In contrast, the intrinsic pathway involves increased mitochondrial outer membrane permeabilization, releasing cytochrome c. Cytochrome c forms apoptosomes that activate effector caspases, comprising CASP3 [46]. In general, CASP3 exists as an inactive pro-CASP3 zymogen. Upon apoptotic induction, CASP3 undergoes proteolytic cleavage to form an active heterotetramer composed of p17 and p12 subunits. Activated CASP3 translocates to the nucleus, directly targeting nuclear proteins and inducing morphological changes such as chromatin condensation and DNA fragmentation [47, 48]. As illustrated by our experimental findings, 300 μM omeprazole treatment strikingly inhibited NPCs proliferation and brought about early apoptosis within 24 h. Evidenced by an elevated Cleaved-CASP3/pro-CASP3 ratio, Western blot analysis suggested noticeable CASP3 activation, which aligned with canonical caspase cascade dynamics during apoptosis. Immunofluorescence further demonstrated cytoplasmic CASP3 localization in cells cultured in the control group, whereas omeprazole-treated cells exhibited cytoplasmic shrinkage and partial nuclear translocation of CASP3. These subcellular redistribution patterns suggest active CASP3 enters the nucleus via membrane permeation or transport mechanisms to cleave nuclear substrates and initiate apoptosis. The aforementioned experimental observations confirm that omeprazole induces NPCs death through CASP3-dependent apoptosis. CASP3 plays a paramount role in IVDD. Inflammatory factors and mechanical stress activate CASP3, which not only lessens cell counts, holds back ECM homeostasis through upregulating MMPs and ADAMTS but also amplifies inflammation via cytokine release (IL-1β, TNF-α), thereby exacerbating IVDD [49–52]. Therapeutic strategies targeting CASP3, such as liraglutide (via the PI3 K/Akt signaling pathway) [53] and miR-155 (inhibiting FADD/CASP3) [54], have demonstrated impressive potential in mitigating apoptosis. Moreover, the connection between CASP3 and NLRP3 inflammasome activation and HIF-1α-mediated autophagy underscores its broader regulatory role in IVDD progression [50, 55].

In previous studies, omeprazole mediated multiple modes of cell death. In renal cells, omeprazole induces oxidative stress-driven necrotic death characterized by Annexin V/7-AAD positivity, LDH release, cytoplasmic vacuolization, and irregular chromatin condensation. This process is mediated by mitochondrial ROS accumulation and lipid peroxidation, which alter membrane permeability and disrupt energy metabolism, operating independently of apoptosis, necroptosis, ferroptosis, or autophagy pathways [11]. In human neutrophils, acidified omeprazole triggers CASP3-dependent apoptosis in a time- and dose-dependent manner, as evidenced by DEVD-CHO-mediated suppression of apoptosis [56]. These findings align with our observations in NPCs. Beyond apoptosis and necrosis, omeprazole may modulate additional cellular pathways. PPIs can induce cellular autophagy. After treatment with esomeprazole, the levels of autophagy markers LC3-II and p62 in EAC cells conspicuously heightened. The augment of LC3-II is universally acknowledged as a marker of autophagy activation, while the accumulation of p62 may indicate obstruction of autophagic flux [57]. More importantly, omeprazole can also heighten intracellular copper ion concentration and oxidative stress by inhibiting the copper transporter ATP7 A. The above effect can prevent the efflux of copper ions and give rise to cuprotosis and ferroptosis [58]. In NPCs, our study confirms omeprazole-induced apoptosis; however, the potential involvement of other death modalities (e.g., autophagy, cuprotosis, necroptosis) remains unresolved. Future studies are imperative to clarify these mechanisms.

Notwithstanding the contributions above, this study is imperfect in several facets. First and foremost, MR findings are restricted to European populations, limiting generalizability to other ethnic groups. Future academic and practical endeavors are warranted to conduct independent cohort or case–control studies in non-European populations to validate the association between omeprazole and IVDD. In addition, the stringent genome-wide

significance threshold ($P < 5 \times 10^{-8}$) lessened the number of eligible SNPs, necessitating reliance on a less rigorous threshold ($P < 5 \times 10^{-6}$), which may render the result reliability less satisfactory. Further validation is insufficient throughout in vitro and in vivo experiments. In this regard, future studies should validate omeprazole's role in disc degeneration, particularly its effects on apoptotic regulators (e.g., Bcl-2, Bax) beyond CASP3.

Conclusion

This study establishes that omeprazole accelerates IVDD progression via CASP3-mediated apoptosis of NPCs. These findings advocate cautious long-term omeprazole use in clinical practice and suggest alternative PPIs.

Abbreviations

IVDD	Intervertebral disc degeneration
CASP3	Caspase-3
NPCs	Nucleus pulposus cells
PPIs	Proton pump inhibitors
MR	Mendelian randomization
SNPs	Single nucleotide polymorphisms
GWAS	Genome-wide association studies
IVW	Inverse variance weighted
OR	Odds ratio
CI	Confidence interval
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
BP	Biological processes
CC	Cellular components
MF	Molecular functions
PPI	Protein-protein interaction

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13018-025-05863-4.

Supplementary Material 1.

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Not applicable

Authors' contributions

YJ: conceptualization; YJ and HZ: methodology; YJ and SH: writing—original draft preparation; YJ and HZ: formal analysis and investigation; All authors: resources; All authors: writing—review and editing; All authors: funding acquisition; BX: supervision. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Data availability

The supplementary material can be obtained in the supplementary table.

Declarations

Ethics approval and consent to participate

The animal experiment of the study was approved by the Tianiin Jinke Bona Biotechnology Co.,LTD (GENINK-20250006).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Dagenais S, Caro J, Haldeman S. A systematic review of low back pain cost of illness studies in the United States and internationally. Spine J. 2008;8(1):8–20. https://doi.org/10.1016/j.spinee.2007.10.005.
2. Kirnaz S, Capadona C, Wong T, Goldberg JL, Medary B, Sommer F, et al. Fundamentals of Intervertebral Disc Degeneration. World Neurosurg. 2022;157:264–73. https://doi.org/10.1016/j.wneu.2021.09.066.
3. Cazzanelli P, Wuertz-Kozak K. MicroRNAs in Intervertebral Disc Degeneration, Apoptosis, Inflammation, and Mechanobiology. Int J Mol Sci. 2020;21(10):3601. https://doi.org/10.3390/ijms21103601.
4. Fang Y, Qiu J, Jiang ZB, Xu SR, Zhou ZH, He RL. Associations of Caspase-3 gene polymorphism with lumbar disc herniation. Kaohsiung J Med Sci. 2016;32(11):552–8. https://doi.org/10.1016/j.kjms.2016.08.010.
5. Qiu C, Cheng L, Di D, Xiang Z, Wang C, Li J, et al. TNFα-reliant FSP1 up-regulation promotes intervertebral disc degeneration via caspase 3-dependent apoptosis. Genes Dis. 2024;12(1):101251. https://doi.org/10.1016/j.gendis.2024.101251.
6. Gao S, Wang C, Qi L, Liang S, Qu X, Liu W, et al. Bushen Huoxue Formula Inhibits IL-1β-Induced Apoptosis and Extracellular Matrix Degradation in the Nucleus Pulposus Cells and Improves Intervertebral Disc Degeneration in Rats. J Inflamm Res. 2024;17:121–36. https://doi.org/10.2147/JIR.S431609.
7. Guo D, Yu M, Guo H, Zeng M, Shao Y, Deng W, et al. Panax notoginseng saponins inhibits oxidative stress- induced human nucleus pulposus cell apoptosis and delays disc degeneration in vivo and in vitro. J Ethnopharmacol. 2024;319(Pt 1):117166. https://doi.org/10.1016/j.jep.2023.117166.
8. Xin J, Wang Y, Zheng Z, Wang S, Na S, Zhang S. Treatment of Intervertebral Disc Degeneration. Orthop Surg. 2022;14(7):1271–80. https://doi.org/10.1111/os.13254.
9. Lespessailles E, Toumi H. Proton Pump Inhibitors and Bone Health: An Update Narrative Review. Int J Mol Sci. 2022;23(18):10733. https://doi.org/10.3390/ijms231810733.
10. Cao S, Wei Y, Yue Y, Li G, Wang H, Lin J, et al. Omeprazole and risk of osteoarthritis: insights from a mendelian randomization study in the UK Biobank. J Transl Med. 2024;22(1):504. https://doi.org/10.1186/s12967-024-05255-y.
11. Fontecha-Barriuso M, Martín-Sanchez D, Martinez-Moreno JM, Cardenas-Villacres D, Carrasco S, Sanchez-Niño MD, et al. Molecular pathways driving omeprazole nephrotoxicity. Redox Biol. 2020;32:101464. https://doi.org/10.1016/j.redox.2020.101464.
12. Birney E. Mendelian Randomization. Cold Spring Harb Perspect Med. 2022;12(4):a041302. https://doi.org/10.1101/cshperspect.a041302.
13. Li X, Lin L, Pang L, Pu K, Fu J, Shen Y, et al. Application and development trends of network toxicology in the safety assessment of traditional Chinese medicine. J Ethnopharmacol. 2025;343:119480. https://doi.org/10.1016/j.jep.2025.119480.
14. Skrivankova VW, Richmond RC, Woolf BAR, Yarmolinsky J, Davies NM, Swanson SA, et al. Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization: The STROBE-MR Statement. JAMA. 2021;326(16):1614–21. https://doi.org/10.1001/jama.2021.18236.
15. Fu H, Jiang S, Song S, Zhang C, Xie Q. Causal associations between chronic viral hepatitis and psychiatric disorders: a Mendelian

randomization study. Front Psychiatry. 2024;15:1359080. https://doi.org/10.3389/fpsyt.2024.1359080.
16. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, et al. PubChem 2025 update. Nucleic Acids Res. 2025;53(D1):D1516–25. https://doi.org/10.1093/nar/gkae1059.
17. Szklarczyk D, Nastou K, Koutrouli M, Kirsch R, Mehryary F, Hachilif R, et al. The STRING database in 2025: protein networks with directionality of regulation. Nucleic Acids Res. 2025;53(D1):D730–7. https://doi.org/10.1093/nar/gkae1113.
18. Gfeller D, Grosdidier A, Wirth M, Daina A, Michielin O, Zoete V. SwissTargetPrediction: a web server for target prediction of bioactive small molecules. Nucleic Acids Res. 2014;42(Web Server issue):W32-8. https://doi.org/10.1093/nar/gku293.
19. Xie R, Li B, Jia L, Li Y. Identification of Core Genes and Pathways in Melanoma Metastasis via Bioinformatics Analysis. Int J Mol Sci. 2022;23(2):794. https://doi.org/10.3390/ijms23020794.
20. Chen L, Zhang YH, Wang S, Zhang Y, Huang T, et al. Prediction and analysis of essential genes using the enrichments of gene ontology and KEGG pathways. PLoS ONE. 2017;12(9):e0184129. https://doi.org/10.1371/journal.pone.0184129.
21. Arcon JP, Modenutti CP, Avendaño D, Lopez ED, Defelipe LA, Ambrosio FA, et al. AutoDock Bias: improving binding mode prediction and virtual screening using known protein-ligand interactions. Bioinformatics. 2019;35(19):3836–8. https://doi.org/10.1093/bioinformatics/btz152.
22. Tang S, Chen R, Lin M, Lin Q, Zhu Y, Ding J, et al. Accelerating AutoDock Vina with GPUs. Molecules. 2022;27(9):3041. https://doi.org/10.3390/molecules27093041.
23. Asadi M, Taghizadeh S, Kaviani E, Vakili O, Taheri-Anganeh M, Tahamtan M, et al. Caspase-3: Structure, function, and biotechnological aspects. Biotechnol Appl Biochem. 2022;69(4):1633–45. https://doi.org/10.1002/bab.2233.
24. Migliorini F, Maffulli N, Eschweiler J, Betsch M, Catalano G, Driessen A, et al. The pharmacological management of chronic lower back pain. Expert Opin Pharmacother. 2021;22(1):109–19. https://doi.org/10.1080/14656566.2020.1817384.
25. Vakil N. Peptic Ulcer Disease: A Review. JAMA. 2024;332(21):1832–42. https://doi.org/10.1001/jama.2024.19094.
26. Fine N, Lively S, Séguin CA, Perruccio AV, Kapoor M, Rampersaud R. Intervertebral disc degeneration and osteoarthritis: a common molecular disease spectrum. Nat Rev Rheumatol. 2023;19(3):136–52. https://doi.org/10.1038/s41584-022-00888-z.
27. Targownik LE, Metge CJ, Leung S, Chateau DG. The relative efficacies of gastroprotective strategies in chronic users of nonsteroidal anti-inflammatory drugs. Gastroenterology. 2008;134(4):937–44. https://doi.org/10.1053/j.gastro.2008.01.010.
28. Kampuang N, Thongon N. Mass spectrometric analysis of TRPM6 and TRPM7 from small intestine of omeprazole-induced hypomagnesemic rats. Front Oncol. 2022;12:947899. https://doi.org/10.3389/fonc.2022.947899.
29. Tang Y, Zhang K, Zhou H, Zhang C, Liu Z, Chen H, et al. Transplantation of active nucleus pulposus cells with a keep-charging hydrogel microsphere system to rescue intervertebral disc degeneration. J Nanobiotechnology. 2023;21(1):453. https://doi.org/10.1186/s12951-023-02226-1.
30. Maguire D, Neytchev O, Talwar D, McMillan D, Shiels PG. Telomere Homeostasis: Interplay with Magnesium. Int J Mol Sci. 2018;19(1):157. https://doi.org/10.3390/ijms19010157.
31. Zheltova AA, Kharitonova MV, Iezhitsa IN, Spasov AA. Magnesium deficiency and oxidative stress: an update. Biomedicine (Taipei). 2016;6(4):20. https://doi.org/10.7603/s40681-016-0020-6.
32. Choudhury A, Jena A, Jearth V, Dutta AK, Makharia G, Dutta U, et al. Vitamin B12 deficiency and use of proton pump inhibitors: a systematic review and meta-analysis. Expert Rev Gastroenterol Hepatol. 2023;17(5):479–87. https://doi.org/10.1080/17474124.2023.2204229.
33. Langan RC, Goodbred AJ. Vitamin B12 Deficiency: Recognition and Management. Am Fam Physician. 2017;96(6):384–9.
34. Saito M, Marumo K. The Effects of Homocysteine on the Skeleton. Curr Osteoporos Rep. 2018;16(5):554–60. https://doi.org/10.1007/s11914-018-0469-1.
35. Raj PP. Intervertebral disc: anatomy-physiology-pathophysiology-treatment. Pain Pract. 2008;8(1):18–44. https://doi.org/10.1111/j.1533-2500.2007.00171.x
36. Elias E, Targownik LE. The Clinician's Guide to Proton Pump Inhibitor Related Adverse Events. Drugs. 2019;79(7):715–31. https://doi.org/10.1007/s40265-019-01110-3.
37. Huang Z, Kraus VB. Does lipopolysaccharide-mediated inflammation have a role in OA? Nat Rev Rheumatol. 2016;12(2):123–9. https://doi.org/10.1038/nrrheum.2015.158.
38. Cai Z, Li K, Yang K, Luo D, Xu H. Suppression of miR-203-3p inhibits lipopolysaccharide induced human intervertebral disc inflammation and degeneration through upregulating estrogen receptor α. Gene Ther. 2020;27(9):417–26. https://doi.org/10.1038/s41434-019-0118-z.
39. Wang Y, Cheng H, Wang T, Zhang K, Zhang Y, Kang X. Oxidative stress in intervertebral disc degeneration: Molecular mechanisms, pathogenesis and treatment. Cell Prolif. 2023;56(9):e13448. https://doi.org/10.1111/cpr.13448.
40. Dries LS, Haefliger R, Seibert BS, de Lima AG, Cardoso CO, Perassolo MS. Cognition, oxidative stress and vitamin B12 levels evaluation on patients under long-term omeprazole use. J Pharm Pharmacol. 20220;74(4):547–55. https://doi.org/10.1093/jpp/rgab001
41. Parton RG, Tillu VA, Collins BM. Caveolae. Curr Biol. 2018;28(8):R402–5. https://doi.org/10.1016/j.cub.2017.11.075.
42. Wu Y, Lim YW, Parton RG. Caveolae and the oxidative stress response. Biochem Soc Trans. 2023;51(3):1377–85. https://doi.org/10.1042/BST20230121.
43. Zou X, Zhang X, Han S, Wei L, Zheng Z, Wang Y, et al. Pathogenesis and therapeutic implications of matrix metalloproteinases in intervertebral disc degeneration: A comprehensive review. Biochimie. 2023;214(Pt B):27–48. https://doi.org/10.1016/j.biochi.2023.05.015.
44. Tan JH, Li ZP, Liu LL, Liu H, Xue JB. IL-17 in intervertebral disc degeneration: Mechanistic insights and therapeutic implications. Cell Biol Int. 2022;46(4):535–47. https://doi.org/10.1002/cbin.11767.
45. Aram L, Yacobi-Sharon K, Arama E. CDPs: caspase-dependent non-lethal cellular processes. Cell Death Differ. 2017;24(8):1307–10. https://doi.org/10.1038/cdd.2017.111.
46. Julien O, Wells JA. Caspases and their substrates. Cell Death Differ. 2017;24(8):1380–9. https://doi.org/10.1038/cdd.2017.44.
47. Han Z, Hendrickson EA, Bremner TA, Wyche JH. A sequential two-step mechanism for the production of the mature p17:p12 form of caspase-3 in vitro. J Biol Chem. 1997;272(20):13432–6. https://doi.org/10.1074/jbc.272.20.13432.
48. Del Rosario O, Suresh K, Kallem M, Singh G, Shah A, Zheng L, et al. MK2 nonenzymatically promotes nuclear translocation of caspase-3 and resultant apoptosis. Am J Physiol Lung Cell Mol Physiol. 2023;324(5):L700–11. https://doi.org/10.1152/ajplung.00340.2022.
49. Wang B, Wang D, Yan T, Yuan H. MiR-138-5p promotes TNF-α-induced apoptosis in human intervertebral disc degeneration by targeting SIRT1 through PTEN/PI3K/Akt signaling. Exp Cell Res. 2016;345(2):199–205. https://doi.org/10.1016/j.yexcr.2016.05.011.
50. He R, Wang Z, Cui M, Liu S, Wu W, Chen M, et al. HIF1A Alleviates compression-induced apoptosis of nucleus pulposus derived stem cells via upregulating autophagy. Autophagy. 2021;17(11):3338–60. https://doi.org/10.1080/15548627.2021.1872227.
51. Wang Y, Che M, Xin J, Zheng Z, Li J, Zhang S. The role of IL-1β and TNF-α in intervertebral disc degeneration. Biomed Pharmacother. 2020;131:110660. https://doi.org/10.1016/j.biopha.2020.110660.
52. Chen ZH, Jin SH, Wang MY, Jin XL, Lv C, Deng YF, et al. Enhanced NLRP3, caspase-1, and IL- 1β levels in degenerate human intervertebral disc and their association with the grades of disc degeneration. Anat Rec (Hoboken). 2015;298(4):720–6. https://doi.org/10.1002/ar.23059.
53. Ming-Yan Y, Jing Z, Shu-Qin G, Xiao-Liang B, Zhi-Hong L, Xue Z. Liraglutide inhibits the apoptosis of human nucleus pulposus cells induced by high glucose through PI3K/Akt/caspase-3 signaling pathway. Biosci Rep. 2019;39(8):BSR20190109. https://doi.org/10.1042/BSR20190109.
54. Wang HQ, Yu XD, Liu ZH, Cheng X, Samartzis D, Jia LT, et al. Deregulated miR-155 promotes Fas-mediated apoptosis in human intervertebral disc degeneration by targeting FADD and caspase-3. J Pathol. 2011;225(2):232–42. https://doi.org/10.1002/path.2931.
55. Chen S, Wu X, Lai Y, Chen D, Bai X, Liu S, et al. Kindlin-2 inhibits Nlrp3 inflammasome activation in nucleus pulposus to maintain homeostasis of the intervertebral disc. Bone Res. 2022;10(1):5. https://doi.org/10.1038/s41413-021-00179-5.

56. Capodicasa E, Cornacchione P, Natalini B, Bartoli A, Coaccioli S, Marconi P, et al. Omeprazole induces apoptosis in normal human polymorphonuclear leucocytes. Int J Immunopathol Pharmacol. 2008;21(1):73–85. https://doi.org/10.1177/039463200802100109.
57. Chueca E, Apostolova N, Esplugues JV, García-González MA, Lanas Á, Piazuelo E. Proton Pump Inhibitor Display Antitumor Effects in Barrett's Adenocarcinoma Cells. Front Pharmacol. 2016;7:452. https://doi.org/10.3389/fphar.2016.00452.
58. Gu L, Sun Y, Bai T, Shao S, Tang S, Xue P, et al. Functional nanozyme system for synergistic tumor immunotherapy via cuproptosis and ferroptosis activation. J Nanobiotechnology. 2025;23(1):212. https://doi.org/10.1186/s12951-025-03284-3.

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