Mechanistic Studies of Cyclooxygenase-2 (COX-2) in Skeletal Muscle Cells During Rotator Cuff Injury: An In Vitro Study

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Summary

The mechanism of rotator cuff injury remains to be elucidated. And COX-2 plays a dual role in skeletal muscle injury and regeneration, would be associated with the development of rotator cuff injury. Therefore, we chose human skeletal muscle cells (HSKMC) as an *in vitro* muscle tissue model and transfected lentivirus with overexpressed COX-2 to simulate the *in vitro* environment of rotator cuff injury. To investigate the specific molecular biological mechanism of COX-2, transcriptome sequencing (RNA-Seq) was used to analyze the differentially expressed mRNAs in HSKMC overexpressing COX-2. Enrichment analysis was performed to analyze these differentially expressed genes and real-time quantitative PCR (RT-qPCR) was used to examine the mRNA levels of genes induced by overexpression. Subsequently, the role of COX-2 in cell proliferation was confirmed by cell counting kit-8 (CCK-8), and focal adhesion kinase (FAK) and signal transducer and activator of transcription 3 (STAT3) phosphorylation induced by COX-2 was utilized by western blotting (WB). The results showed that total of 30,759 differentially expressed genes were obtained, and the expression of CYP4F3 and GPR87 was significantly increased. COX-2 could bind CYP4F3 and GPR87 and co-localize with them in the cytoplasm. Finally, COX-2 promoted the proliferation of human skeletal muscle cells by activating the FAK and STAT3 pathways.

Key words

COX-2 • Rotator cuff injury • Human skeletal muscle cells • GPR87 • CYP4F3

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Introduction

Rotator cuff injury is a tear of the rotator cuff tendon area, with shoulder pain, weakness, and activity limitation as the main manifestation of a disease [1]. Rotator cuff injury is more common in middle-aged and old people and shoulder joint trauma, and its incidence accounts for 17-41 % of shoulder joint diseases [2]. Factors causing rotator cuff injury are complicated, such as rotator cuff degeneration, external impact, inflammation infiltration, endocrine, metabolic, apoptosis and other factors can cause rotator cuff injury.

Rotator cuff injury can cause movement limitation and chronic pain, which may seriously affect patients' quality of life. On the one hand, chronic, massive rotator cuff tears cause muscle atrophy. Previous reports have shown that reductions in muscle weight, volume, and/or fiber length have been observed in both animal models and human cadavers suffering from rotator cuff injuries [3,4]. On the other hand, the process of rotator cuff injury progression is one of chronic inflammatory infiltration, and altered pain signaling pathways involving inflammatory mediators are the main cause of pain in patients with rotator cuff injuries. Studies have shown that rotator cuff injury result in a high release of inflammatory factors in the muscle fiber tissues, rotator cuff tissues and blood samples [5-7]. Cyclooxygenase (COX)-1 and COX-2 are essential enzymes in this inflammatory cascade, and COX enzymes convert arachidonic acid to prostaglandin H2, which can then be further converted to other prostaglandins with potent proinflammatory effects [8]. COX-2, a member of the cyclooxygenase family, plays an important role in inflammation and muscle damage.

Studies have shown that mRNA expression of COX-2 was upregulated while COX-1 was constitutively expressed in tendon cells co-cultured with IL-1β *in vitro*, and IL-1β caused changes in COX-2 by altering PGE2 expression in tendon cells [9]. Furthermore, COX-2 was involved in the ephrinBs/EphBs pain signaling pathway to produce nociception [10]. Silencing of COX-2 blocked PDK1/TRAF4-induced AKT pathway activation, thereby inhibiting fiber formation during skeletal muscle atrophy [11]. Down-regulation of COX-2 attenuated denervation-induced muscle atrophy by suppressing inflammation and oxidative stress and improved microcirculation [12]. The above findings suggest that inhibition of COX-2 reduces pain triggered by inflammatory factors and relieves skeletal muscle atrophy retardation. Therefore, COX-2, as an analgesic target, plays an important therapeutic role in clinical practice.

Signal transducer and activator of transcription 3 (STAT3) is a key gene regulated by COX-2 and has the dual functions of signal transduction and transcriptional activation [13,14]. Previous studies have shown that STAT3 mRNA levels were mainly expressed 2 weeks after exercise to promote muscle proliferation and differentiation after injury [15]. Interfering with STAT3 activity promoted smooth muscle cell apoptosis [16]. In addition, STAT3 activation promoted musculoskeletal differentiation [17]. Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase, and activation of FAK allowed cells such as primary myoblasts or embryonic stem cells to migrate more efficiently when transplanted into regenerating muscle, which could promote the muscle regeneration process [18]. These findings suggested the therapeutic potential of STAT3 and FAK in promoting regeneration of injured muscle.

However, additional literature has found that inhibition of COX-2 is detrimental to skeletal muscle regenerative differentiation. For example, NSAIDs and coxibs that inhibited COX-2 had detrimental effected on tendon and attachment point regeneration as well as muscle regeneration [19,20]. Skeletal muscle cell C2C12 proliferation was increased by inducing COX-1 and COX-2-dependent pathways [21]. These again suggest that COX-2 enhancement favors the regenerative differentiation of skeletal muscle. COX-2 has dual roles in the muscle injury phase, and there is an urgent need to elucidate the molecular mechanisms of COX-2 in skeletal muscle injury.

In this study, we screened for differentially expressed genes and enriched pathways caused by overexpression of COX-2 in human skeletal muscle cells through RNA-seq. COX-2 caused up-regulation of CYP4F3 and GPR87 expression by interacting with CYP4F3 or GPR87 proteins. Secondly, overexpression of COX-2 promoted skeletal muscle cell proliferation and activated downstream FAK as well as STAT3 pathways. These could provide new targets for clinical treatment of rotator cuff injury.

Materials and Methods

Cell culture

Human skeletal muscle cells (HSKMC, PCS-950-010) as well as epithelial-like cell 293T (CRL-3216) were acquired from American Type Culture Collection (ATCC, Manassas, VA, USA). HSKMC were cultured with fetal bovine serum (5 %, Gibco, New York, USA), skeletal muscle cell growth supplement (1 %, ATCC, Manassas, VA, USA), and penicillin/streptomycin (1 %, Gibco, New York, USA), while 293T was cultured in DMEM medium (Gibco, New York, USA) with 10 % fetal bovine serum and 1 % penicillin-streptomycin. Cells were all cultured at 37 °C and CO_2 (5 %) in a cell incubator.

Cell infection

The COX-2 gene was used as a template and primers were designed to amplify the sequence to construct a lentiviral vector for overexpression of COX-2. Meanwhile, the 106 or 341 mutation sites of COX-2 as templates and primers were designed to amplify the sequences to construct lentiviral vectors for the gene mutations. Lentiviral packaging vector LV-013, containing a green fluorescent protein (GFP) tag were purchased from GeneChem (Shanghai, China). Then, HSKMC were transfected with lentivirus-mediated human COX-2 overexpression vector (COX-2, 1 µl) or control vector (NC, 1 µl), while 293T cells were transfected with lentivirus-mediated COX-2 106 mutation vector (COX-2 mut-106, 1 µl), lentivirus-mediated COX-2 341 mutation vector (COX-2 mut-341, 1 µl) or COX-2 wildtype vector (COX-2, 1 µl). Then, cells were infected for 48 h with lentivirus in the presence of 5 µg/ml polybrene (Sigma-Aldrich, St. Louis, MO, USA), and then selected for 7 days with 2 µg/ml puromycin (Sigma-Aldrich, St. Louis, MO, USA). Cells resistant to puromycin were isolated for further studies.

RNA sequencing

Total RNA was isolated and analyzed by RNA-seq in HSKMC cells transfected with NC or COX-2 overexpressing lentivirus. Total RNA extraction was performed using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Total RNA was enriched for mRNA with polyA tails by Oligo(dT) magnetic beads and fragmented in Fragmentation Buffer with divalent cations. The fragmented mRNA was used as a template to synthesize first and second strand cDNA using random hexamer primers, followed by end repair, poly-A 3' ends, splice junctions, and DNA fragment enrichment to construct an RNA-seq library using the NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (Illumina, San Diego, CA, USA). Library quality was assessed on an Agilent Bioanalyzer 2100 system (Agilent Technologies, Palo Alto, CA, USA). Finally, Illumina NovaSeq 6000 platform was used for RNA-Seq data sequence analysis of 150-BP end-to-end model.

Identification of differentially-expressed genes and enrichment analysis

FeatureCounts (1.5.0-p3) was used to calculate the number of reads mapped to each gene. The FPKM of each gene was then calculated based on the gene's length, the FPKM of each gene was calculated and the number of reads mapped to that gene was computed. FPKM refers to the number of reads per million base The FPKM refers to the expected number of fragments per kilobase of the sequenced transcript sequence fragments. Differentially expressed genes (DEGs) were analyzed using the DESeq2 (v1.20.0) or edgeR (v3.22.5). The p-value was adjusted using the Benjamini & Hochberg method. Padj<=0.05 & | log2 (foldchange)|>= 1 was set as the threshold for significant differential expression. The volcano plot and heatmap were drawn by ggplot2 and heatmap R package, respectively. DisGeNET, KEGG, GO enrichment implemented by ClusterProfiler (v3.8.1).

Table 1. Antibody information.

Experiment	Name	LOT	Manufacturer	Dilution ratios
co-IP	COX-2	ab179800	abcam	1:1000
	CYP4F3	ab230709	abcam	1:1000
	GPR87	ab77517	abcam	1:1000
	GAPDH	AF7021	Affinity	1:3000
	Goat Anti-Rabbit	S0001	Affinity	1:3000
IF	COX-2	ab179800	abcam	1:200
	CYP4F3	ab230709	abcam	1:200
	GPR87	ab77517	abcam	1:200
	Goat Anti-Rabbit IgG (H+L) Fluor594-conjugated	S0006	Affinity	1:500
	Goat Anti-Rabbit IgG (H+L) FITC-conjugated	S0008	Affinity	1:500
WB	COX-2	ab179800	abcam	1:1000
	GPR87	ab77517	abcam	1:1000
	JAK1	ab125051	abcam	1:2000
	p-JAK1	ab138005	abcam	1:1000
	STAT3	ab68153	abcam	1:1000
	p-STAT3	ab76315	abcam	1:2000
	GAPDH	AF7021	Affinity	1:3000
	Goat Anti-Rabbit	A0208	Beyotime	1:3000

Co-IP – co-immunoprecipitation; IF – mean siImmunofluorescence; WB – Western blotting.

Co-immunoprecipitation (co-IP)

Cells were lysed in RIPA buffer containing a protease inhibitor mixture (cOmplet, EDTA-free, Roche). After removal of cellular debris by centrifugation, the extracts

were incubated with anti-COX-2 antibody or Goat Anti-Rabbit at 4 °C overnight. Dynabeads® Protein A Immunoprecipitation Kit (10006D, Thermo Fisher, Waltham, MA) was then added and incubated at 4 °C for 4 h. Proteins eluted from Protein A/G beads were detected by protein blotting. To detect the interaction between COX-2 and CYP4F3 or GPR87, 4 μg/ml anti-COX-2 antibody, anti-CYP4F3 antibody and anti-GPR87 antibody were used. Antibody information was summarized in Table 1.

Immunofluorescence (IF)

Cells were fixed with 4 % (MilliporeSigma, St. Louis, MO, USA) for 20 min and blocked with 5 % goat serum for 2 h at room temperature, then incubated with COX-2 and CYP4F3 or GPR87 primary antibodies at 4 °C. After overnight incubation, cells were washed with PBS and secondary antibody coupled with fluorescein isothiocyanate (FITC) or Fluor594. Cell nuclei were stained with DAPI (Beyotime, Beijing, China). Finally, cells were observed using a fluorescence microscope (Nikon, Tokyo, Japan) at 400× magnification for at least 5 fields of view. Antibody information was summarized in Table 1.

Cell Counting Kit-8 (CCK-8)

HSKMC cells transfected with lentiviral vector or treated with Acetaminophen (Adooq, Shanghai, China) were seeded in a 96-well plate. Then, 10 μl Cell Counting Kit 8 (CCK8; Beyotime Institute of Biotechnology, Beijing, China) solution was treated at 1-5th day. The OD values were detected at 570 nm at different time points by microplate reader (Bio-Rad, Hercules, CA, USA).

Real-time quantitative PCR (RT-qPCR) and Western blotting (WB)

For RT-qPCR, TRIzol (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA of cells. After the integrity was verified by electrophoresis, RNA was reversely transcribed into cDNA by reverse transcription kit (Takara, Dalian, China). SYBR Green reagent was used for qRT-PCR experiment [24]. The primer sequence was displayed in Table 2. Relative expression was calculated using the $2^{-\triangle Ct}$ method. GAPDH were amplified as controls for RNA integrity.

For WB, total cell protein from cells or tissue was extracted by total protein extraction kit (Goodbio Technology, Wuhan, China), and then protein loading buffer was added, denaturated at 95 ℃ for 15 min, and stored at -20 ℃. Protein was separated by 10 % SDS electrophoresis and transferred onto PVDF membrane. Nonspecific antigen sites were blocked with 5 % BSA and incubated at room temperature for 1 h, and then membranes were incubated with primary antibodies for overnight at 4 ℃. Primary antibodies used in WB were all listed on Table1. The following day, membranes were incubated with Goat Anti-Rabbit secondary antibody for 1 h at room temperature. Finally, the membranes were washed with Enhanced chemiluminescence reagent (Beyotime, Beijing, China) and image J was used for quantification.

Table 2. Primers sequence.

Primer	Forward sequence (5'-3')	Reverse forward sequence (5'-3')	Product size (bp)
GAPDH	TGACTTCAACAGCGACACCCA	CACCCTGTTGCTGTAGCCAAA	121
INHBA	GCAGTCGCACAGACCTTT	CGCAGTAGTTGGCATGATAG	194
IP6K3	AGCCTTCTCGCTGGTGGA	ACCGATGCCGCTTGTTCT	134
KR175	TCAACAGTGGATTTGGCTAT	GATTTGCAGGTGAAGAGGA	134
CYP4F3	GATTGAATGGGACGACCTG	GCTGGGTTGTGATGGGTTC	189
GLRA2	GGAATACGCAGCGGTGA	GTTGTGGGAGTGGGTTGG	200
PTCHD4	CAGGCTGGGTIGTGCGTGAG	TCGGGTGGAAGCGGTTGAG	105
CGA	ACATTGTCGGTGTTTCTG	ACCTTAGTGGAGTGGGATA	160
GPR87	GCATACGGCAGTCACCTA	CACAGCCACAACAACCCT	172
FAM217A	GAAATTAAGGCACCGAAGA	CAATAAGGGAAGGCGACA	118
TAS2R3	GGAATTGAGGCCACCAGG	CAGGGAGAAGATGAGCAAAGAG	144

Statistical analysis

Experimental results are presented as mean ± SD from at least three separate experiments. Statistical analyses were performed using GraphPad Prims (version 9.5.0) and one-way ANOVA was used to compare the different treatment groups. Statistical significance was set at $P<0.05$.

Results

RNA-Seq was employed to explore COX-2 induced aberrant gene expression in human skeletal muscle cells

COX-2 plays an important role in rotator cuff injury; however, its specific molecular biological mechanism remains to be demonstrated. Therefore, we transfected plasmids to overexpress COX-2 in HSKMC. Transcriptome sequencing (RNA-Seq) was used to analyze the differentially expressed mRNAs in overexpressing COX-2 in HSKMC compared to NC groups. After data filtering, a total of 30,759 differentially expressed genes were obtained. Among them, 1447 genes were up-regulated and 1607 genes were down-regulated (NC vs. COX-2, Fig. 1A). Heatmap was drawn with these differential genes and displayed in Supplementary Figure 1, and it was found that the expression of the two groups of genes can be well differentiated on a macroscopic level.

Subsequently, we performed enrichment analysis of these differentially expressed genes (DisGeNET, GO and KEGG). Figure 1B showed that these differential expression genes were mainly enriched in different diseases, including muscle-related diseases, such as Abnormal mitochondria in muscle tissue. Functional enrichment was shown in Figures 1C and 1D, where GO analysis revealed that these genes are involved in endoplasmic reticulum and RNA processing related biological processes, such as protein targeting to ER, protein localization to endoplasmic reticulum, rRNA processing, rRNA metabolic process and ribosome biogenesis. KEGG enrichment analysis revealed that the differential expression genes were involved in metabolism-related pathways, such as Pyruvate metabolism Glutathione metabolism, Pyruvate metabolism, Fatty acid degradation, *et al.*

COX-2 can bind CYP4F3 and GPR87 proteins

Next, qRT-PCR was performed to validate the RNA-Seq results. 10 genes (INHBA, IP6K3, KRT75, CYP4F3, GLRA2, PTCHD4, CGA, GPR87, FAM217A, and TAS2R3) were selected and subjected to qRT-PCR. qRT-PCR results showed that the gene expression propensity was consistent with the RNA-Seq results (Fig. 2A), indicating that the RNA-Seq results of this study were plausible. In addition, the expression of CYP4F3 and GPR87 was significantly increased in HSKMC which was overexpressed COX-2 ($P<0.001$), so these two were selected for the next analysis.

CO-IP was performed to verify the interaction between COX-2 and CYP4F3 or GPR87. The results indicated that in HSKMC cells, COX-2 protein bound CYP4F3 and GPR87 (Fig. 2B). In addition, immunofluorescence experiments detected that COX-2 protein localized with CYP4F3 or GPR87 protein in the cytoplasm of HSKMC cells (Fig. 2C). To summarize, overexpression of COX-2 in HSKMC cells significantly promoted the mRNA levels of CYP4F3 and GPR87 and it could bound the proteins of CYP4F3 and GPR87.

Exploration of COX-2 and GPR87 protein binding sites

In order to further investigate the binding site of COX-2 and the interacting protein GPR87, we constructed COX-2 binding site mutant plasmids, COX-2 amino acid 106 and 341 site mutant plasmids (COX-2 mut-106 or COX-2 mut-341) and the wild-type plasmid (COX-2 WT) respectively to transfect the tool cell 293T. The CO-IP results showed that the wild-type COX-2 could bind to GPR87 (Fig. 3A). The binding of COX-2 to GPR87 was not affected when the COX-2 amino acid 106 site was mutation (Fig. 3B). Whereas mutation of COX-2 amino acid 341 site resulted in non-binding of COX-2 to GPR87 (Fig. 3C). It suggested that COX-2 may bind to GPR87 at the site 341, not at the site 106. Moreover, co-localization experiments used by immunofluorescence were also verified that COX-2 and GPR87 proteins still co-localized in the cytoplasm when the COX-2 amino acid 106 site was mutated (Fig. 3D).

Effects of COX-2 on human skeletal muscle cell proliferation and downstream pathways

Finally, to further clarify whether the kinase activity of COX-2 affected cell proliferation and downstream pathways, we added the COX-2 inhibitor Acetaminophen to COX-2 overexpressing cells. As shown in Figure 4A, overexpression of COX-2 promoted the proliferation of HSKMC cells, whereas addition of Acetaminophen reversed the cell proliferation induced by overexpression of COX-2. Moreover, overexpression of COX-2 promoted not only GPR87 protein expression, but

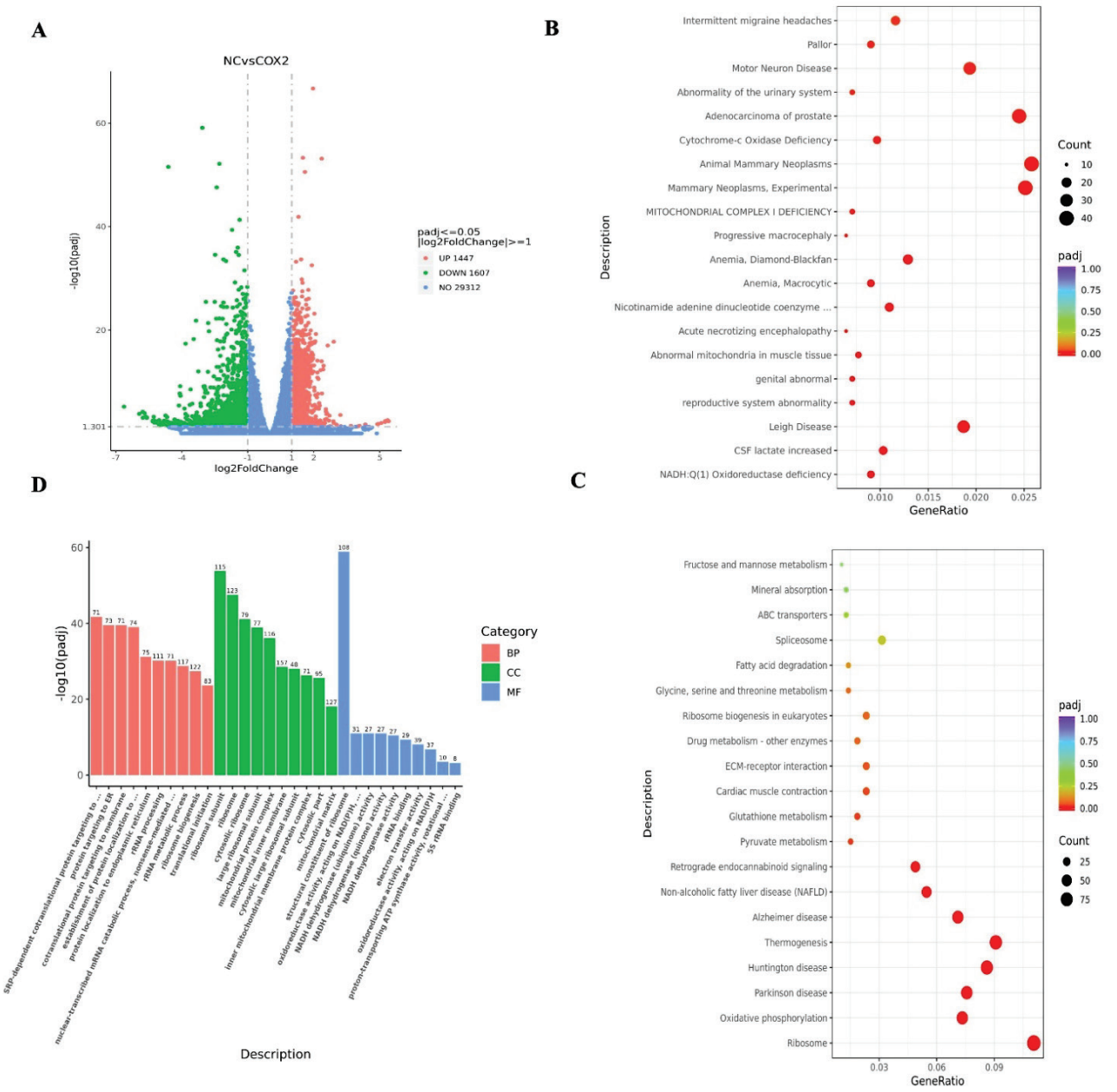

Fig. 1. RNA-Seq was employed to explore COX-2 induced aberrant gene expression in human skeletal muscle cells. (**A**) Volcano plots of differentially expressed genes induced by COX-2. Up means up-regulated expression genes. Down means down-regulated expression genes. No means genes with no statistical significance. (**B**) The main category terms of DisGeNET enrichment analysis. BP means Biological Process. CC means Cellular Component. MF means Molecular Function. (**C**) The main category terms of GO enrichment analysis. (**D**) The main category terms of KEGG enrichment analysis.

Discussion

Rotator cuff injury is a tear of the rotator cuff tendon area, with shoulder pain, weakness, and activity limitation as the main manifestation of a disease [1]. Rotator cuff injury can cause movement limitation and chronic pain, which may seriously affect patients' quality of life.

COX-2, a member of the cyclooxygenase family, plays an important role in inflammation and muscle damage. To investigate the specific mechanism of COX-2 in rotator cuff injury, we used RNA-seq to perform RNA sequencing of COX-2 in overexpressing human skeletal muscle cells, screened for differentially expressed genes and performed enrichment analysis. A total 30,759 genes were aberrant expression induced by overexpression of COX-2 and these genes were mainly enriched in different diseases, including muscle-related diseases, such as abnormal mitochondria in muscle tissue. This suggested that the overexpression of COX-2 may be

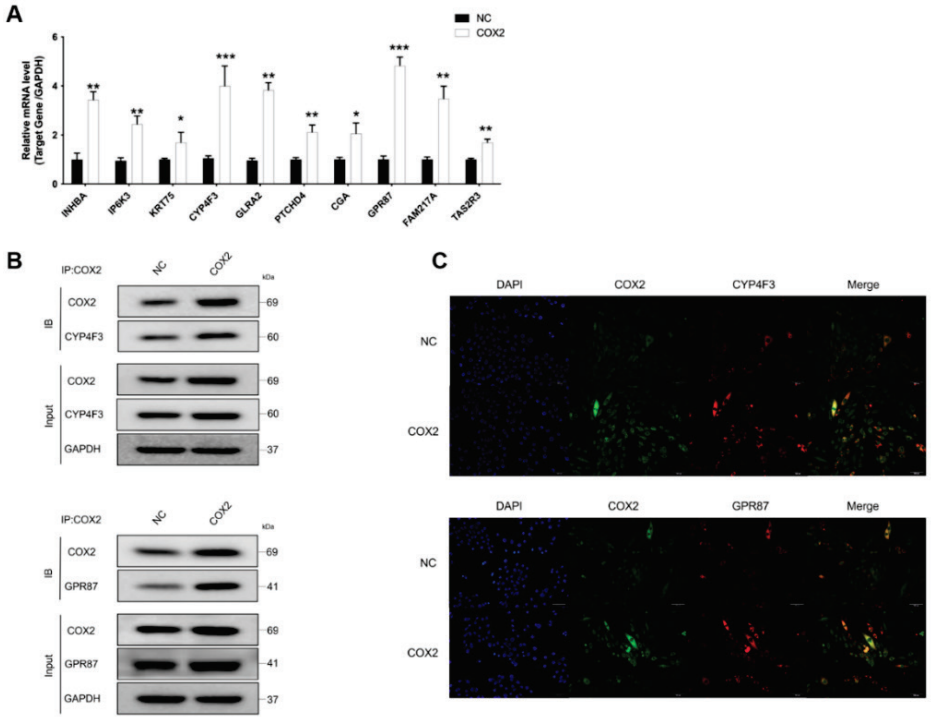

Fig. 2. COX-2 can bind CYP4F3 and GPR87 proteins. (**A**) The mRNA level of 10 genes (INHBA, IP6K3, KRT75, CYP4F3, GLRA2, PTCHD4, CGA, GPR87, FAM217A, and TAS2R3) were performed by qRT-PCR (n=3). (**B**) Interaction between COX-2 protein and CYP4F3 or GPR87 protein was performed by CO-IP (n=3). (**C**) Co-localization of COX-2 with CYP4F3 or GPR87 verified with immunofluorescence (Scale bar: 100 μm). Data are expressed as the mean ± SD. * P<0.05, ** P<0.01, *** P<0.001 compared with NC.

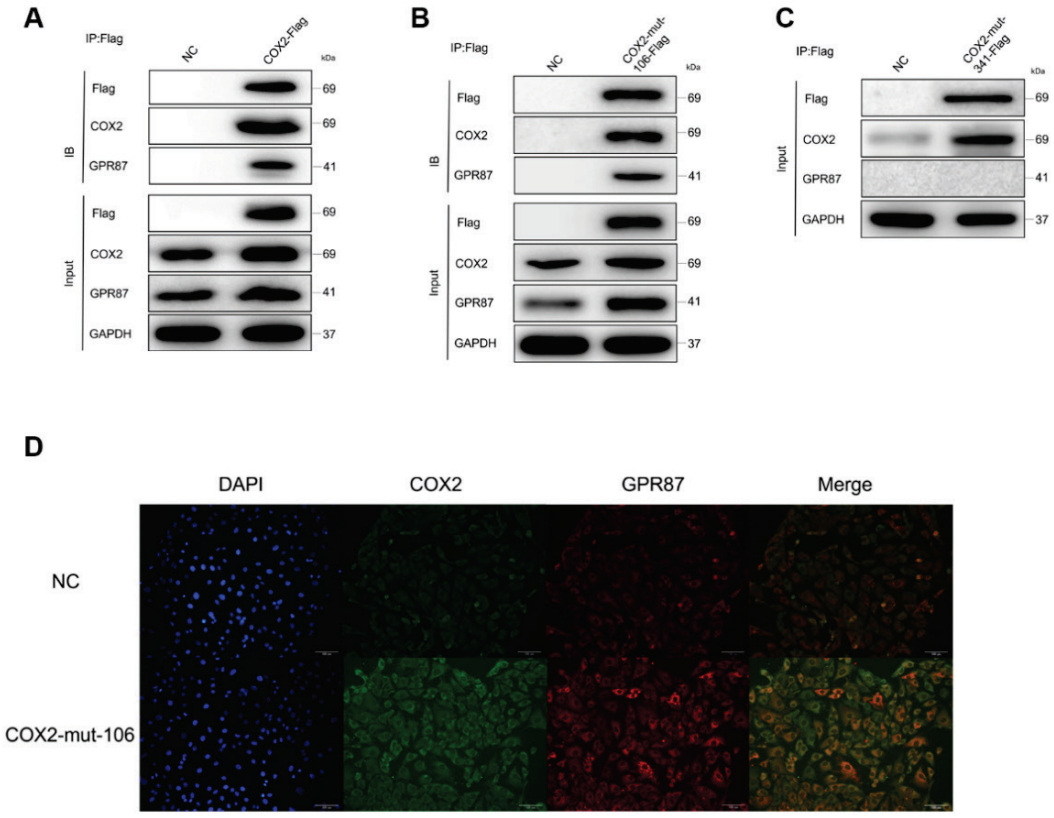

Fig. 3. Exploration of COX-2 and GPR87 protein binding sites. (**A**) Interaction between COX-2 WT protein and GPR87 protein was performed by CO-IP (n=3). (**B**) Interaction between COX-2 mut 106 protein and GPR87 protein was performed by CO-IP (n=3). (**C**) Interaction between COX-2 mut 341 protein and GPR87 protein was performed by CO-IP. (**D**) Co-localization of COX-2 mut 106 with CYP4F3 or GPR87 verified with immunofluorescence (Scale bar: 100 μm).

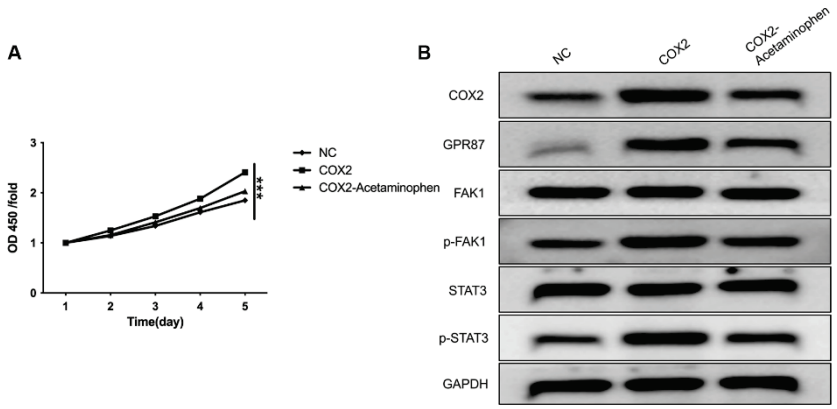

Fig. 4. Effects of COX-2 on human skeletal muscle cell proliferation and downstream pathways. (**A**) CCK-8 assay of OD_{450} in COX-2 and Acetaminophen groups (n=3). (**B**) The protein levels of COX-2, GPR87 and phosphorylation of FAK as well as STAT3 were performed by WB (n=3). Data are expressed as the mean ± SD. * P<0.05, ** P<0.01, *** P<0.001 compared with NC.

associated with skeletal muscle injury-related diseases. In addition, metabolic process, like Fatty acid degradation, were also significantly regulated in skeletal muscle cells overexpressing COX-2. As reported, fibrosis and fat accumulation into muscle tissue were common pathological changes occurring in rotator cuff injuries, and blocked the process of fat accumulation and promoted fat acid degradation could alleviate rotator cuff injury [22,23]. This indicates that fatty acid metabolism plays an important role in the process of rotator cuff injury, and our enrichment results also implied that COX-2 may regulate skeletal muscle injury by regulating fatty acid degradation.

RNA-seq and RT-qPCR results showed that overexpression of COX-2 in human skeletal muscle cells significantly increased CYP4F3 and GPR87 mRNA levels. In addition, this study demonstrated that COX-2 increased the expression of CYP4F3 and GPR87 by binding to both proteins. However, CYP4F3 and GPR87 have been less reported in regulating skeletal muscle injury, and only one study found that GPR87 was expressed in skeletal muscle [24]; Whereas, CYP4F3 ω-hydroxylated leukotriene B4 (LTB4), and further converted it to the inactive metabolite 20-carboxy-LTB4 [25,26]. While, LTB4 can also be produced in skeletal muscle, where it initiated chemotaxis to damaged tissues and enhances myoblast proliferation and differentiation [27]. This illustrated that CYP4F3 and GPR87 may be targets for the treatment of skeletal muscle-related diseases.

Finally, to further clarify whether COX-2 affected cell proliferation and downstream pathways, we found that overexpression of COX-2 promoted the proliferation of HSKMC cells, whereas addition of COX-2 inhibitor Acetaminophen to inhibit the cell proliferation induced by overexpression of COX-2. Moreover, overexpression of COX-2 promoted not only GPR87 protein expression, but also FAK and STAT3 phosphorylation. The administration of NSAIDs in the treatment of injury and muscle regeneration remains paradoxical in terms of effectiveness. This interferes with the production of prostaglandins derived from the inflammatory isoform COX-2, which is essential for regulating tissue regeneration [20]. Similar to previous findings, inhibition of COX-2 inhibited the proliferation of skeletal muscle cells, thereby interfering with the process of muscle regeneration. The STAT3 pathway is a downstream pathway regulated by COX-2. Previous research showed that STAT3 pathway activation restored myokine/cytokine Meteorin-like (Metrnl) expression in injured muscle and improved muscle repair, and that Metrnl was determined to be a key regulator of muscle regeneration [28]. In addition, the muscle regeneration process induced in muscle injury by activated STAT3 signaling to promote self-renewal and value-addition of adult muscle satellite cells [29]. In addition, activation of FAK (adhesion kinase) facilitated the muscle regeneration process by allowing cells such as primary adult myoblasts or embryonic stem cells to become characterized by more efficient migration when transplanted into regenerating muscle [18]. These results implied that the skeletal muscle regenerative effects of COX-2 might be associated with the activation of downstream STAT3 and FAK pathways.

Conclusions

In this study, we screened for differentially expressed genes and enriched pathways caused by overexpression of COX-2 in human skeletal muscle cells through RNA-seq. COX-2 caused up-regulation of CYP4F3 and GPR87 expression by interacting with CYP4F3 or GPR87 proteins. Secondly, overexpression of COX-2 promoted skeletal muscle cell proliferation and activated downstream FAK as well as STAT3 pathways.

These could provide new targets for clinical treatment of rotator cuff injury.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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Abbreviations

CO-IP, Co-immunoprecipitation; HSKMC, human skeletal muscle cells; RNA-Seq, transcriptome sequencing; RT-qPCR, Real-time quantitative PCR

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