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Tetranectin is significantly elevated in the synovial fluid of patients with full-thickness rotator cuff tears

Chae-Eun Moon[1,6], Woo-Sung Do[2,6], Young Mi Kang[2], Yong Woo Ji[3]✉ & Joon Ryul Lim[2,4,5]✉

Rotator cuff tears are one of the most common shoulder disorders and can lead to pain, weakness, and limited mobility. Although the clinical condition has been widely studied, its molecular mechanisms are still not fully understood. In this exploratory study, we analyzed the proteomic profile of synovial fluid in patients with full-thickness rotator cuff tears and compared the results to those from a control group. Proteomic analysis was performed on synovial fluid samples from seven patients with full-thickness tears and three patients with partial-thickness tears, who served as the control group. Label-free quantification using the MaxQuant platform identified 284 proteins at 1% FDR. Among them, CLEC3B (tetranectin) showed the most significant difference, with a fold change of 40.35 and a p-value of 2.6×10^{-3}. Tetranectin exhibited a large effect size (Hedges' g = 2.53), suggesting that it could be a potential candidate protein associated with full-thickness rotator cuff tear severity. Gene ontology enrichment analysis revealed biological processes related to extracellular matrix remodeling, fibrinolysis, and inflammation. Given its known roles in extracellular matrix remodeling, tetranectin was identified as a potential candidate protein associated with full-thickness rotator cuff tear, providing insight into pathological processes such as tendon degeneration and fatty infiltration.

Keywords Full-thickness rotator cuff tears, Synovial fluid, Tetranectin, Proteomics

Rotator cuff tears(RCTs) are among the most prevalent musculoskeletal disorders affecting the shoulder joint, causing pain, functional impairment, and reduced quality of life. The etiology of full-thickness RCTs is multifactorial, involving both intrinsic and extrinsic factors such as age-related degeneration, mechanical overload, ischemia, and inflammation[1]. Despite extensive research, the molecular mechanisms underlying rotator cuff pathology remain incompletely understood[2]..

Recently, proteomic analysis has emerged as a powerful tool for identifying potential biomarkers that may aid in the diagnosis, prognosis, and treatment of musculoskeletal disorders. By characterizing the protein composition of biological fluids, proteomics enables a deeper understanding of disease-specific molecular pathways[3]. Especially, in knee-related disorders, such as anterior cruciate ligament injury and osteoarthritis, proteomic profiling has identified key biomarkers that contribute to early joint degeneration[4,5]. However, studies investigating the proteomic profile of synovial fluid in shoulder pathology remain limited. Compared to the knee joint, synovial fluid analysis in the shoulder is technically challenging due to the smaller joint space and lower fluid volume, contributing to a relative scarcity of proteomic studies. Consequently, the identification of synovial biomarkers specific to RCTs remains an unmet need.

In this study, we aimed to conduct an exploratory characterization of the proteomic profile of synovial fluid in patients with full-thickness RCTs to enhance our understanding of the secreted protein composition specific to full-thickness rotator cuff tear. We hypothesized that a comparative analysis between full-thickness rotator cuff tear patients and a control group would reveal specific biomarkers indicative of disease severity. Through proteomic analysis, we sought to identify candidate proteins that could serve as diagnostic or prognostic biomarkers for rotator cuff pathology.

[1]Institute of Vision Research, Department of Ophthalmology, Severance Hospital, College of Medicine, Yonsei University, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea. [2]Arthroscopy and Joint Research Institute, Department of Orthopaedic Surgery, Severance Hospital, Yonsei University College of Medicine, Seoul, Korea. [3]Department of Ophthalmology, Yongin Severance Hospital, Yonsei University College of Medicine, Yongin-si 16995, Gyeonggi-do, Republic of Korea. [4]Department of Orthopaedic Surgery, Gangnam Severance Hospital, Yonsei University College of Medicine, 211, Eounju-ro, Gangnam-gu, Seoul 03722, Republic of Korea. [5]AI and Robotics Institute, MEDISBY Co., Ltd., SeodaemunGu Yonsei-Ro 50, Engineering park 311, Yonsei University, Seoul 03715, Korea. [6]Chae-Eun Moon and Woo-Sung Do contributed equally to this work. ✉email: lusita30@yuhs.ac; yalleeyallee@yuhs.ac

Results

Baseline characteristics

Comprehensive profiling of the synovial fluid proteome was conducted in patients with full-thickness rotator cuff tears ($n=7$) and controls with partial-thickness tears ($n=3$). Baseline clinical and imaging characteristics were comparable between the two groups, with no statistically significant differences observed (Table 1). Variables such as age, sex, comorbidities (hypertension, diabetes, dyslipidemia), tear size, and the degree of fatty infiltration on MRI were similar between groups.

Comprehensive profiling of synovial fluid proteome in patients with full-thickness rotator cuff tears and control groups

The label-free quantification (LFQ) proteomics analysis using the MaxQuant platform identified a total of 284 proteins in synovial fluid samples at the 1% false discovery rate (FDR). Hierarchical clustering analysis demonstrated clear segregation of protein expression profiles between the rotator cuff tear group and the control group, suggesting the presence of distinct disease-associated proteomic signatures in the synovial fluid (Fig. 1B). Principal component analysis (PCA) was performed to evaluate overall variance in the proteomic profiles between groups. The analysis revealed partial separation along PC2, accounting for 17.1% of the total variance, while substantial overlap was observed along PC1, which explained 41.5% of the variance (Fig. 1C). These results suggest that proteomic alterations in rotator cuff tear patients are confined to specific subsets of proteins rather than a global shift across the entire proteome. To further elucidate biological implications, gene ontology (GO) enrichment analysis was performed using ClueGO software. The analysis revealed significant enrichment of proteins involved in diverse biological processes, complement activation, immune regulation, inflammatory response, stress response, wound healing, and lipoprotein metabolism. Collectively, these findings indicate that multiple biological processes are associated with the molecular landscape of rotator cuff pathology, as indicated by GO biological process (GOBP) enrichment analysis (Fig. 1D).

Identification of differentially expressed proteins and GO functional characterization

Differentially expressed proteins (DEPs) between the rotator cuff tear and control groups were identified based on label-free quantification data. A total of 22 DEPs were identified, including 21 upregulated and 1 downregulated protein in the rotator cuff tear group. The complete list of DEPs is provided in Table 2. For transparent reporting beyond p-values, effect estimates were standardized and reported as log2 fold change (log2FC; tear vs. control) based on log2-transformed LFQ intensities (Table 2). To visualize the overall distribution and statistical significance of the identified proteins, a volcano plot was generated using $\log_2$ fold-change and $-\log_{10}$ p-value (Fig. 2A). Benjamini-Hochberg FDR correction was applied to Welch's t-test p-values across all proteins with available p-values (m = 278 of 284 identified proteins); adjusted q-values are reported in Supplementary Tables 1, and no proteins reached $q < 0.05$ in this exploratory dataset. As a sensitivity analysis, Mann-Whitney U test p-values (two-sided, unadjusted) are also provided in Supplementary Tables 1, and CLEC3B remained significant in the Mann-Whitney U test ($p=0.017$). Among the upregulated proteins, CLEC3B showed the smallest nominal p-value (Welch's t-test $p=0.003$), suggesting potential biological relevance to rotator cuff tear pathology. Among the upregulated proteins, CLEC3B showed the smallest nominal p-value (Welch's t-test $p=0.003$), suggesting potential biological relevance to rotator cuff tear pathology.

	Full-thickness Tear ($N=7$)	Control ($N=3$)	*p*-value
Age (years)*	63.0 [56.0;68.5]	50.0 [45.0;51.0]	0.067
Sex (Male)	4/7 (57.1%)	1/3 (33.3%)	1
HTN	3/7 (42.9%)	1/3 (33.3%)	1
DM	6/7 (85.7%)	3/3 (100.0%)	1
Dyslipidemia	7/7 (100.0%)	2/3 (66.7%)	0.645
SLAP	6/7 (85.7%)	2/3 (66.7%)	1
Supraspinatus tear	5/7 (71.4%)		
Subscapularis tear	4/7 (57.1%)		
AP tear size (mm)*	9.0 [3.0;14.5]		
ML tear size (mm)*	14.0 [4.0;17.0]		
SST fatty infiltration (Grade)	0: 3 (42.9%), I: 2 (28.6%), II: 1 (14.3%), IV: 1 (14.3%)	0: 2 (66.7%) I: 1 (33.3%)	0.774
IST fatty infiltration (Grade)	0: 5 (71.4%) I: 1 (14.3%) II: 1 (14.3%)	0: 2 (66.7%) I: 1 (33.3%)	0.665
SSC fatty infiltration (Grade)	0: 5 (71.4%) I: 2 (28.6%)	0: 2 (66.7%) I: 1 (33.3%)	1
Tangent sign (+)	1/7 (14.3%)	0/3 (0.0%)	1

Table 1. Baseline clinical and imaging characteristics of patients with full-thickness rotator cuff tears and controls. *Continuous variables are presented as medians with interquartile ranges [*Q* 1; *Q* 3].

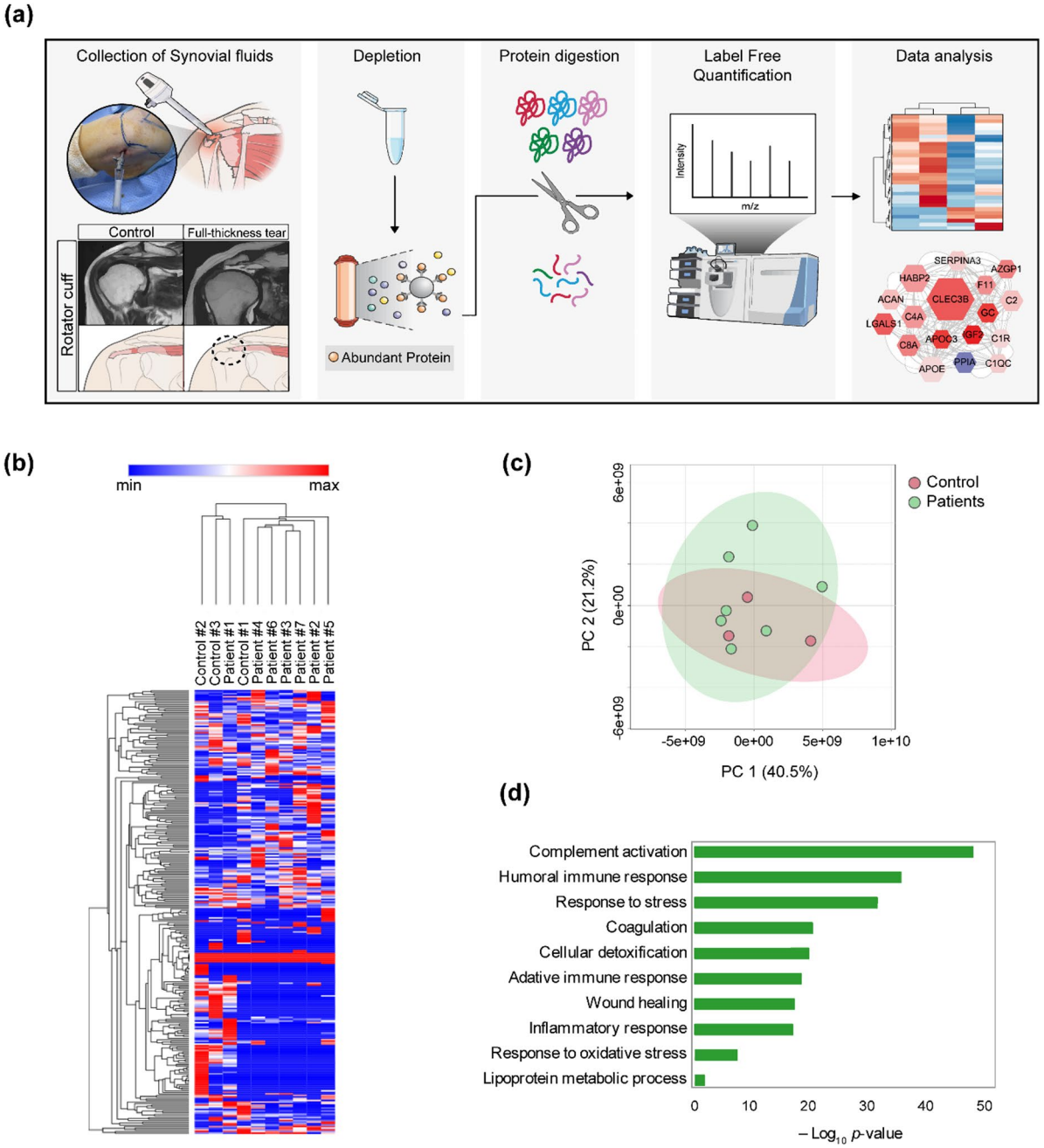

Fig. 1. (**A**) Schematic representation of the proteomics workflow used for synovial fluid analysis, including sample preparation, LC-MS/MS acquisition, and data processing. (**B**) Hierarchical clustering heatmap showing distinct protein expression profiles between the full-thickness tear and control groups. (**C**) Principal component analysis (PCA) plot illustrating sample distribution based on protein expression variance. (**D**) Bar graph of the top 10 enriched Gene Ontology Biological Process (GOBP) terms identified from the synovial fluid proteome.

To gain functional insight into the DEPs, Gene Ontology (GO) enrichment analysis was performed across three categories: Biological Process (GOBP), Molecular Function (GOMF), and Cellular Component (GOCC). The top five enriched GO terms in each category are presented in Fig. 2B. This analysis revealed overrepresentation of terms such as “extracellular region” and “collagen-containing extracellular matrix” (GOCC); “complement activation”, “immune system process”, and “defense response” (GOBP); and “serine-type peptidase activity”, “serine hydrolase activity”, and “immunoglobulin receptor binding” (GOMF), implicating immune-related and extracellular processes. Together, these results indicate that the DEPs are predominantly involved in immune modulation, extracellular localization, and proteolytic activity, highlighting their potential roles in rotator cuff pathology.

DEPs		
Protein	**Log2 Fold change**	**p-value**
IGHG2	12.6	0.078
APOC3	11.8	0.087
GC	8.74	0.086
IGF2	8.0	0.084
IGHV3-74	7.2	0.083
CLEC3B	5.3	0.003
LGALS1	5.1	0.058
C8A	4.0	0.060
AZGP1	3.9	0.094
F11	3.4	0.091
C4A	3.1	0.050
HABP2	3.0	0.026
IGKV3D-7	2.6	0.090
IGHG4	2.4	0.056
IGKV3D-11	2.2	0.078
ACAN	2.0	0.094
C2	1.9	0.068
SERPINA3	1.6	0.085
C1QC	1.5	0.085
C1R	1.2	0.092
APOE	1.2	0.032
PPIA	−1.3	0.070

Table 2. List of differentially expressed proteins (DEPs) identified in synovial fluid samples from full-thickness rotator cuff tear patients compared to controls. Candidate proteins were prioritized based on exploratory screening criteria (Fold change| ≥ 1.2 and *p*-value < 0.1).

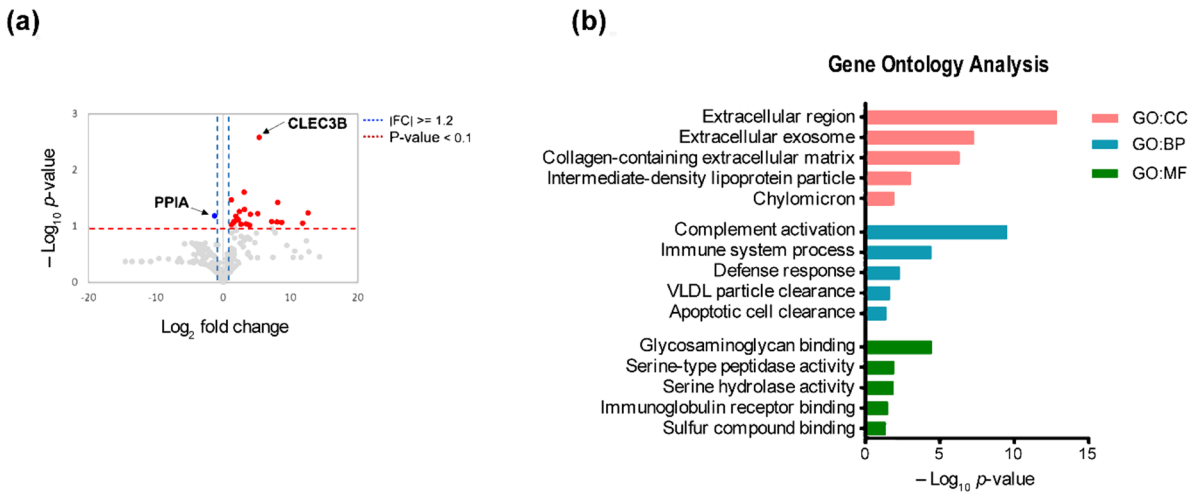

Fig. 2. (**A**) Volcano plot of differentially expressed proteins (DEPs) between groups. Vertical dashed lines indicate the fold-change threshold (|FC| ≥ 1.2), and the horizontal dashed line marks the significance cutoff (p-value < 0.1). (**B**) Bar chart showing the top five enriched Gene Ontology (GO) terms across Biological Process (GOBP), Molecular Function (GOMF), and Cellular Component (GOCC) categories. Enrichment analysis highlights biological pathways and molecular functions associated with rotator cuff pathology.

Protein–protein interaction network analysis of differentially expressed proteins

To investigate the interaction landscape of DEPs, a protein-protein interaction network was constructed using STRING database and visualized in Cytoscape (Fig. 3A). The network consisted of upregulated (red) and downregulated (blue) proteins, with edges representing validated interactions. CLEC3B, a prioritized DEP with the smallest nominal p-value, was found to interact with several other upregulated proteins, suggesting potential involvement within the rotator cuff tear–associated proteomic network. Although CLEC3B did not rank among

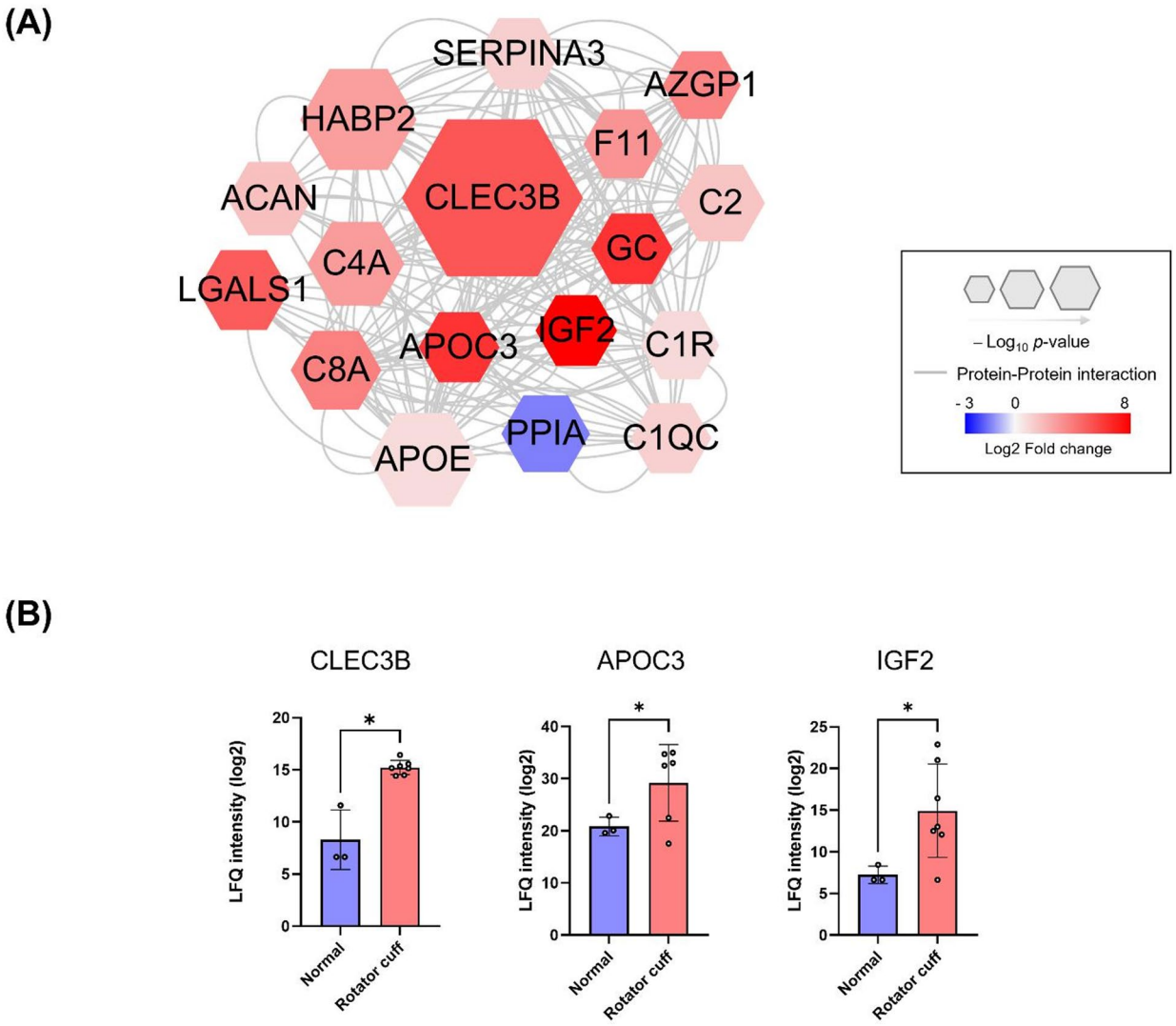

Fig. 3. (**A**) Protein-protein interaction (PPI) network of proteins prioritized under exploratory differential-expression criteria between the full-thickness rotator cuff tear group ($n = 7$) and the partial-thickness tear control group ($n = 3$). Interactions were retrieved from the STRING database and visualized in Cytoscape. Node color indicates the direction and magnitude of change (log2 fold change; full-thickness vs. control; red, upregulated; blue, downregulated). Node size is proportional to –log10 (nominal p-value), reflecting relative strength of the group difference within the prioritized set. Edges represent known or predicted protein-protein associations. (**B**) Log2-transformed LFQ intensities (LOD-based imputation for visualization/sensitivity analysis) are shown as mean ± SD with individual values; Welch's t-test was used (* $p < 0.05$). BH-adjusted q-values are reported in Supplementary Table 1.

the top nodes based on maximal clique centrality, several of the highest-ranking proteins—such as APOE, AZGP1, GC, and SERPINA3—are involved in lipid metabolism and immune regulation, pathways known to contribute to rotator cuff pathology. These results provide network-level context for the prioritized candidates in this exploratory dataset. CLEC3B, which is secreted by adipocytes and promotes lipogenesis through ERK signaling, shares functional relevance with these hub proteins. These findings suggest that CLEC3B may act as a context-specific regulator within the disease-associated proteomic network, despite not ranking among the top nodes in centrality analysis (Supplementary Fig. 1).

To validate and visualize group-specific expression patterns, relative abundance levels of representative DEPs were compared between the control and rotator cuff tear groups based on log2-transformed LFQ intensities (Fig. 3B). CLEC3B APOC3, and IGF2 exhibited a increased abundance in the rotator cuff tear groupThese proteins displayed distinct group-specific expression profiles, supporting their characterization as representative DEPs associated with rotator cuff pathology.

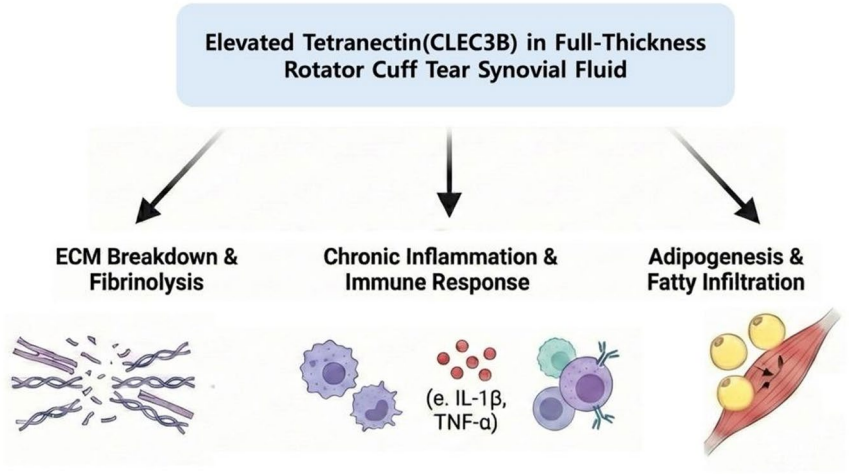

Fig. 4. Schematic illustration of Tetranectin's potential roles in Rotator Cuff Tear pathology. Elevated synovial tetranectin (CLEC3B) contributes to disease progression through three primary pathways: ECM breakdown and fibrinolysis promoting matrix turnover; chronic inflammation modulating immune responses; and adipogenesis driving fatty infiltration within the rotator cuff muscle. *ECM* extracellular matrix, *IL-1β* interleukin-1 beta, *TNF-α*, tumor necrosis factor-alpha.

Discussion

The primary finding of this study is that tetranectin is increased in the synovial fluid of patients with full-thickness RCTs compared to those with partial-thickness tears. The observed elevation of tetranectin in full-thickness tears compared to partial-thickness controls suggests it reflects structural tear severity rather than age-related changes. To our knowledge, this is the first study to perform synovial fluid proteomic analysis for rotator cuff tears, and it is also the first to report the relevance of tetranectin in the shoulder joint. Tetranectin is known to play a role in extracellular matrix (ECM) remodeling and tissue homeostasis,[6,7] raising the possibility that its upregulation in synovial fluid reflects underlying pathological changes in tendon integrity and healing responses. Given the small cohort size ($n = 10$), the present findings should be interpreted as exploratory candidate prioritization rather than confirmatory biomarker discovery.

The increased tetranectin levels in full-thickness RCTs may reflect altered ECM turnover within the joint microenvironment Tetranectin has been reported to promote plasminogen activation and fibrinolysis, which facilitate ECM remodeling[8,9]. Given that full-thickness tears exhibit greater tendon matrix degradation than partial tears, the observed tetranectin upregulation may indicate a compensatory response to excessive ECM turnover(Fig. 4). Furthermore, chronic RCTs are often accompanied by fibrosis and fatty infiltration[10], both of which are processes in which tetranectin has been implicated. Tetranectin deficiency has been associated with abnormal ECM organization in spinal disorders, suggesting a conserved role in connective tissue maintenance. Additionally, tetranectin has been identified as an adipogenic factor that enhances adipocyte differentiation and lipogenesis through ERK signaling[11]. Given the increased fatty infiltration seen in full-thickness RCTs, the observed upregulation of tetranectin may also reflect adipogenic remodeling within rotator cuff muscle[12]. This aligns with previous findings that associate tetranectin with early adipocyte expansion and metabolic activity.

Although CLEC3B, which encodes tetranectin, did not rank among the top nodes when hubs were ranked by maximal clique centrality (MCC) in the DEP-derived protein-protein interaction network, several of the highest-ranking proteins—such as APOE, AZGP1, GC, and SERPINA3—are functionally linked to lipid metabolism and immune regulation, both of which are implicated in rotator cuff degeneration. CLEC3B has been reported in adipose-related contexts and linked to adipogenic pathways, including ERK-associated signaling; however, this interpretation is literature-informed and hypothesis-generating in the context of the present synovial fluid dataset. Therefore, despite its lower network centrality, CLEC3B may be interpreted as a prioritized candidate in this exploratory dataset. These findings emphasize the need to interpret protein-protein interaction networks alongside disease-specific expression and biological function.

In addition to tetranectin, we observed two other proteins, IGF1 and TIMP2, that were exclusively detected in the full-thickness rotator cuff tear group and not in the control group, with p-values below 0.05. While these proteins did not meet fold-change thresholds due to their absence in the control group, their group-specific detection suggests potential biological involvement. IGF1 is well known for promoting musculoskeletal tissue

regeneration and has been shown to enhance proliferation and migration of bursa-derived cells in the shoulder tissue[13,14]. In vivo studies further support its contribution to tendon-bone interface healing following chronic rotator cuff injury[15]. TIMP2 (tissue inhibitor of metalloproteinases 2) regulates extracellular matrix turnover by inhibiting MMP activity, and its upregulation in torn rotator cuff tendon tissue has been associated with fibrotic remodeling and chronic tendon degeneration[16]. Together, these proteins may contribute to the chronic degenerative processes characteristic of full-thickness RCTs and represent exploratory candidates worthy of further validation.

In recent years, proteomic research on various shoulder joint diseases, such as frozen shoulder and shoulder instability, has gradually expanded[3,17]. Unlike classical molecular biology techniques such as Western blot, ELISA, and qPCR, which target specific molecules, proteomics enables high-throughput, unbiased analysis of thousands of proteins simultaneously, identifying novel biomarkers and molecular pathways that might otherwise be overlooked[18]. This capability has recently accelerated the growth of proteomic research in shoulder joint diseases, compared to knee osteoarthritis and anterior cruciate ligament injuries, where proteomic applications have been extensively explored for a longer period. We speculate that this discrepancy may stem from the fact that synovial fluid is relatively easy to obtain in knee joint compared to shoulder joint. Indeed, a review of shoulder proteomics studies reveals the use of various types of specimens[3,19,20]..

Tissue selection plays a critical role in proteomics outcomes, as different sample types offer distinct insights into joint pathology[21]. Synovial fluid, compared to tissue biopsies, provides a more uniform protein distribution, making it advantageous for biomarker discovery. In this study, we collected native synovial fluid without lavage, preserving its physiological concentration. Although this approach made large-scale sample collection more difficult, it ensured that the obtained specimens were well-suited for proteomic analysis, strengthening the validity of our findings. Clinically, tetranectin may represent a potential diagnostic candidate for monitoring structural integrity or a candidate marker for evaluating tendon healing progress.

This study has several limitations. The small cohort ($N = 10$) and lack of a healthy control group due to ethical constraints are acknowledged. Additionally, the potential influence of age and metabolic comorbidities (e.g., diabetes or hypertension) as confounders was not fully controlled for in this preliminary study. Accordingly, the findings should be interpreted as exploratory candidate prioritization rather than confirmatory evidence. To compensate for the limited statistical power in this exploratory phase, we integrated effect estimates and sensitivity analyses alongside nominal p-value rather than relying on p-values alone. The effect size for tetranectin (Hedges' $g = 2.53$; Supplementary Table 1) supports its prioritization as a candidate for follow-up; however, validation in larger, well-balanced cohorts using orthogonal quantitative assays is required.

Conclusion

Despite these limitations, our findings highlight tetranectin as a promising candidate for further prioritization for full-thickness rotator cuff tears, offering insights into the pathological changes associated with tendon degeneration and fatty infiltration. Future studies with larger cohorts and longitudinal analyses will be essential to validate these findings and further explore tetranectin's clinical utility in diagnosing and monitoring rotator cuff pathology.

Materials and methods

Participants and sample collection

This study was conducted in compliance with the ethical principles specified in the Declaration of Helsinki and Good Clinical Practice Guidelines. Prior to study initiation, ethical approval was obtained from Yonsei University Health System, Severance Hospital, Institutional Review Board(4–2022-1297). Written informed consent was obtained from all participants before their enrollment in the study. All collected samples were immediately stored at −80˚C until further analysis. An average of 3–4 ml of native synovial fluid was collected. All samples were immediately stored at −80˚C within 10 min of collection to ensure protein stability.

For synovial fluid collection, patients were positioned in the beach chair position, and a standard posterior portal was initially created. After the insertion of a 4.5 mm sheath with a trocar, followed by a 5.5 mm sheath using a switching stick, the obturator was removed. Considering the heavy viscosity of the synovial fluid, a collection time of at least 10 s was maintained through the trocar's cannula to ensure adequate sample volume. Among the 32 patients, synovial fluid was successfully collected from 10 patients.

Patient data collection

Clinical and imaging variables were recorded to assess potential factors that could influence rotator cuff pathology and synovial fluid proteomic profiles. Comorbidities such as hypertension, diabetes mellitus, and dyslipidemia were documented due to their known associations with tendon degeneration and extracellular matrix remodeling. Preoperative MRI findings, including tear size, fatty infiltration graded by the Goutallier classification, and the tangent sign, were collected to evaluate disease severity, while concurrent SLAP lesions were assessed intraoperatively.

Because synovial fluid aspiration from truly healthy shoulders is ethically infeasible, patients with partial-thickness rotator cuff tears undergoing arthroscopy were used as the reference group.

Sample preparation for proteomic analysis

Protein concentrations in synovial fluid samples were determined in triplicate using the bicinchoninic acid (BCA) protein assay, following the manufacturer's protocol (Thermo Scientific Pierce, U.S.). To facilitate the detection of low-abundance proteins, highly abundant proteins were selectively depleted using Seppro IgY spin columns (Sigma-Aldrich, St. Louis, MO, USA), in accordance with previously reported methods. For in-solution

digestion, 100 µg of total protein from each sample was denatured in a 10 M urea solution prepared in 100 mM ammonium bicarbonate (v/v, 1:1), ensuring a final urea concentration of at least 5 M. The mixture was incubated at room temperature for 30 min to promote denaturation. Reduction was subsequently performed using 10 mM dithiothreitol (DTT), followed by alkylation with 30 mM iodoacetamide in darkness for 1 h. Proteolytic digestion was carried out overnight at 37 °C using sequencing-grade trypsin at a protein-to-protease ratio of 50:1 (w/w) to ensure efficient enzymatic cleavage. The digestion reaction was quenched by adding 0.4% trifluoroacetic acid (TFA), and peptides were subsequently purified via C18 Harvard macro spin columns to remove residual salts. The purified peptides were lyophilized and stored at −80 °C until MS analysis.

Protein identification via LC-MS/MS

For label-free quantification analysis, peptides were reconstituted in 0.1% formic acid (FA) in water and subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a Q Exactive HF-X mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) coupled with an EASY-nLC 1000 nano-LC system (Thermo Fisher Scientific, Seattle, WA, USA). A total of 2 µg of peptide sample was loaded onto a trap column (Acclaim PepMap 100, 75 µm × 2 cm, 3 µm, C18, 100 Å) using buffer A (0.1% formic acid in water), followed by separation on an analytical C18 column (PepMap RSLC C18, 50 cm × 75 µm I.D., 2 µm particle size) with an electrospray voltage of 1.8 kV. Peptides were eluted with a linear gradient of buffer B (0.1% FA in acetonitrile), increasing from 5% to 80% over the chromatographic run at a flow rate of 300 nL/min. Full MS spectra were acquired within a scan range of 400–2,000 Th at a resolution of 60,000, with an automated gain control(AGC) target of 3.0×10^6 and a maximum ion injection time of 100 ms. The top 20 most abundant precursor ions were selected for data-dependent acquisition (DDA), with a 2.0 m/z isolation window, high-energy collision dissociation (HCD) at a normalized collision energy of 27, and a dynamic exclusion duration of 30 s. The MS/MS spectra were acquired with an AGC target of 1.0×10^6 and a maximum ion injection time of 100 ms.

Raw data processing

Mass spectrometry data were processed using MaxQuant software (version 1.5.7.4). Spectra were searched against the UniProt human database (release May 2024, containing 20,205 entries). Carbamidomethylation of cysteine residues was set as a fixed modification, while N-terminal acetylation and methionine oxidation were considered variable modifications. A false discovery rate (FDR) threshold of 1% was applied at both the peptide-spectrum match (PSM) and protein levels to ensure high-confidence identifications. Mass tolerance parameters were defined with an initial precursor mass deviation of up to 4.5 parts per million (ppm) and a fragment mass deviation of up to 20 ppm. Protein identification required at least one peptide, utilizing the "razor plus unique peptides" setting in MaxQuant. Quantification was performed using the extracted ion chromatogram (XIC)-based LFQ algorithm implemented in MaxQuant. The 'match between runs' feature was enabled to align retention times nonlinearly across different runs, with a match time window of 0.7 min and an alignment time window of 20 min. To account for differences in overall signal intensity and reduce systematic bias, sum normalization was performed following LFQ quantification in MaxQuant.

Functional enrichment analysis and protein–protein interaction network

To visualize and interpret GO enrichment analysis, a functional interaction network was constructed using EnrichmentMap in Cytoscape, highlighting enriched biological processes. To generate a protein interaction network for genes commonly identified in differentially expressed proteins (DEPs), protein-protein interaction data were obtained from the STRING database (version 11). The network was then assembled and analyzed in Cytoscape to explore functional relationships among identified proteins. The MS-based proteomics data of all identified peptides and proteins list have been deposited in the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository (4): dataset identifier PXD.

Statistical analysis

Differential expression analysis was performed on $\log_2$-transformed LFQ intensity values obtained from MaxQuant. Following LFQ quantification, protein intensities were sum-normalized to minimize systematic variation between samples. For exploratory DEP screening and downstream functional analyses, we used the non-imputed LFQ intensity matrix as exported from MaxQuant, where non-quantified values were retained as reported, and proteins with fold-change ≥ 1.2 and nominal p-value < 0.1 were designated as DEPs. To provide assumption-robust inference and transparent multiple-testing control in this small dataset, we additionally conducted sensitivity analyses using log2-transformed LFQ intensities after LOD-based imputation (50% of the minimum non-zero LFQ intensity observed across all samples). Between-group comparisons were conducted using two-tailed unpaired Welch's t-tests on log2-transformed LFQ intensities. Benjamini-Hochberg false discovery rate (FDR) correction was applied to Welch's t-test p-values across all proteins with available p-values (m = 278 of 284 identified proteins), and adjusted q-values are reported in Supplementary Table 1. As sensitivity analysis, two-sided Mann-Whitney U test p-values were also calculated and are provided in Supplementary Table 1. To prioritize candidates for this exploratory pilot study and capture broader biological sinals, proteins with fold-change ≥ 1.2 and nominal p-value < 0.1 were designated as DEPs. Principal component analysis (PCA) was conducted using MetaboAnalyst (version 6.0) on $\log_2$-transformed intensity data. Statistical analyses were performed using GraphPad Prism (version 9.0).

Data availability

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD064264.

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Author contributions

C.E.M. contributed to conducting the experiments, data acquisition, data analysis, and manuscript writing. W.S.D. contributed to data acquisition, data analysis, and manuscript writing. Y.M.K contributed to data analysis. Y.W.J. and J.R.L. contributed to the study design, conducted experiments, acquired and analyzed the data, and wrote the manuscript. All authors have read and approved the final version of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to Y.W.J. or J.R.L.