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The attempt of spontaneous repair of rotator cuff tear: The role of periostin

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ABSTRACT

The development of a periostin-rich microenvironment in areas associated with insult, orchestrating pathways of repair and rebuilding, is documented. Literature lacks information regarding the presence of periostin in the context of rotator cuff tear (RCT).

55 consecutive patients with RCT were enrolled. Immunohistochemical periostin detection was performed on tissue samples excised from tear margins.

Our study documented the presence of periostin in the margins of RCT. It is plausible that, when a tear occurs, multiple stimuli, both mechanical and inflammatory, lead to the development of a periostin-rich microenvironment as an attempt to tendon healing.

1. Introduction

Periostin is a matricellular protein; it belongs to the fasciclin family and shows a structure similar to the insect axon guidance fasciclin1 and transforming growth factor-β-inducible protein (βig-H3). It was first identified in a mouse osteoblastic cell line as a putative cell adhesion protein for preosteoblasts.[1] Originally known as osteoblast-specific factor, the name was changed into periostin due to its preferential location in the periosteum.[2] Periostin is encoded by the Postn gene (genebank D13664) located at locus 13q13.3 in humans and its expression is induced by transforming growth factor ?? (TGF-??)[3] and bone morphogenic protein 2 (BMP-2).[4]

Nakazawa et al.[5] observed, after cDNA microarray analysis, that periostin is largely involved in fracture healing; the role in accelerating the process of cutaneous wound repair by activating fibroblasts is also known.[6]

Kudo[7] showed that the expression of TGF-??, IL-4 and IL-13 is induced in macrophages and neutrophils by inflammation and mechanical stress. These cytokines trigger the expression of periostin as well as primarily splice variants of other extracellular matrix molecules (fibronectin and tenascin-C) in fibroblasts, by acting through transcription factors such as twist and c-fos. The splice variant of periostin is secreted and localized in the extracellular matrix, where it interacts with myofibroblasts to induce cell migration through downstream signals involving Akt and focal adhesion kinase (FAK) phosphorylation. These fibroblasts produce type I collagen to repair tissues.

Periostin expression is not limited to bone as it is predominantly expressed in collagen-rich fibrous connective tissues submitted to constant mechanical stress, such as periodontal ligament,[4,8,9] heart valves,[8,10,11] and tendons.[12]

Juneja et al.[13] demonstrated the correlation between periostin synthesis and the healing process of flexor digitorum longus tendon graft in mouse. Using a reverse transcriptase polymerase chain reaction (PCR-RT) analysis, periostin mRNA expression was increased from day 7 until day 28, indicating its role in tendon maturation, collagen fibril arrangement, and remodeling events.

Considering periostin properties, we hypothesized the presence of periostin in the edge of torn rotator cuff tendons and its hypothetical role in rotator cuff tendon healing.

2. Materials and methods

59 consecutive patients [31M-28F, mean age (SD): 64.6 (6.9)] with RCT were enrolled. Diagnosis was obtained after physical examination, standard X-Ray (true AP and axillary views) and MRI of the involved shoulder. Exclusion criteria were: traumatic RCT; anti-inflammatory drugs assumption during the two months before surgery; V-shaped or L-shaped lesions, gleno-humeral arthritis, diabetes, rheumatologic

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diseases, and prior surgery.

The Southern California Orthopedic Institute (SCOI) classification of complete RCTs was used to classify tendon tears intraoperatively. We considered the lesions belonging to type I as small, those of types II and III as large, and those of type IV as massive.

Samples from the anterior and posterior edges of the tear and the medial portion of subacromial bursa (SB) were excised during the arthroscopic operation. This procedure did not require patient consent because we usually perform debridement of cuff tear margins. Samples of uninjured subscapularis tendon were collected from 28 of these patients and used as control.

Immunohistochemical detection of periostin expression was performed with standard streptavidin–biotin– peroxidase on tissue sections.

Histological sections (3-μm) were deparaffinized and rehydrated in graded ethanol. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide for 10 min. Antigen retrieval was performed by three 5-min microwave cycles in sodium citrate buffer (pH: 6.0). The sections were incubated with primary antibody, mouse anti-human periostin (clone F-10, Santa Cruz Biotechnology, Inc.) 1:100, overnight at 4 °C in a humidified chamber. After incubation, sections were washed with phosphate-buffered saline (PBS), and the staining were detected using the Dako LSAB + System kit and DAB substrate–chromogen (DAKO Corp, Carpinteria, CA, USA) according to the manufacturer's instructions. The sections were counterstained with hematoxylin, dehydrated and enclosed.

Both positive and negative controls were used in each experiment.

Immunostaining was evaluated blindly by two pathologists without knowledge of the clinical details. The samples were scored semi-quantitatively using a score based on the intensity and distribution: 0, undetectable; 1+, weak staining; 2+, medium staining, 3+, strong staining.

All participants signed an informed consent form in accordance with the Declaration of Helsinki. Ethics committee approval was obtained.

2.1. Statistical analysis

Calculation of sample size was done using G*Power 3.1.9 software (Heinrich-Heine-University, Dusseldorf, Germany). According to a priori x^2 test, assuming an α-value of 0.05 (sensitivity of 95%) and a β-value of 0.2 (with a study power of 80%), 32 patients will be required.

The x^2 test with 1° of freedom was applied to verify whether patients with different sizes of cuff tear had a significant statistical difference in terms of presence of periostin on the cuff tear margins, on the bursa, and on the subscapularis. The significance level was set at p = 0.05.

3. Results

The study group was finally composed of 55 cases [29M-26F, mean age (SD): 63,4 (7.2)] and 28 controls [13M; 15F, mean age (SD): 65,1 (6.3)]. The right shoulder was involved in 34 cases. Right-handers were 46. Information regarding cuff tear dimension was shown in Table 1.

The percentage of positive responses to periostin in anterior tear margin, posterior tear margin, bursal tissue and healthy tendon was shown in Figs. 1 and 2. Significant differences were found comparing positive responses to periostin in anterior margin (x^2 = 9,17 p = 0,0025 p = 1%), posterior margin (x^2 = 6,43 p = 0,0112 p = 5%) and bursal tissue (x^2 = 6,43 p = 0,0112 p = 5%) with healthy tendon (subscapularis).

Considering the semi-quantitative score, a significant difference in the intensity and distribution of the response was found between bursal tissue and healthy tendon (x^2 = 8,36, p = 0,0391; Fig. 3).

Positive responses to periostin were shown in Figs. 4 and 5 relative to RCT size. Significant difference was found comparing anterior margin and healthy tendon in both small (x^2 = 5,74 p = 0,0166 p = 5%) and large tears (x^2 = 4,59 p = 0,0321) and bursal tissue with healthy tendon in small tears (x^2 = 5,74 p = 0,0166 p = 5%).

Table 1
Information regarding RCT sizes in the studied groups.

Rotator cuff tears	n	%
Small (C1 = 1 cm)	17	31%
Large (C2–C3 = 2–4 cm)	23	42%
Massive (C4 ≥ 5 cm)	15	27%
Healthy tendon	**n**	**%**
Small (C1 = 1 cm)	9	32%
Large (C2–C3 = 2–4 cm)	10	36%
Massive (C4 ≥ 5 cm)	9	32%

4. Discussion

The development of a periostin-rich environment with the aim to orchestrate pathways of repair and rebuilding in areas associated with insult, such as injury and/or inflammation, is documented.[11] However, literature lacks information regarding the presence of periostin in the context of rotator cuff tear.

Our study demonstrated that the prevalence of a positive response to periostin in tear margins and bursal tissue was significantly higher compared with controls.

4.1. Four hypothesis were formulated

- *Subacromial space response*: In the context of the rotator cuff tear, histological changes, consisting in degenerative and inflammatory conditions, were observed.[14] Gumina et al.[15] found that the lip bordering the tear margin in case of RCT consists in lymphocytes, macropheges, plasmacytes and young fibrocytes. We hypothesized that the strong inflammatory stimulation associated with rotator cuff tendon degeneration and tear, could be the primary cause leading to a high periostin production as a mechanism in order to attempt tendon repair. We found no significant difference in periostin presence between anterior and posterior margin of tendon tear, resulting in a homogeneous response to the damage. Furthermore, considering RCT size, a significant higher periostin presence was found in both small and large tears compared to massive ones; the greater inflammatory response associated to small and large tears could justify this finding.[16] In addition to the direct stimulation on the torn edges, our data demonstrated the role of the subacromial bursa in periostin secretion. In fact, considering the semi quantitative assessment, the highest intensity and distribution of periostin response was detected in the bursal tissue, underlying its coadjutant role in the inflammatory response.
- *Mechanical stimulation*: Perry et al.,[17] using an animal sample, demonstrated that alterations in the healthy tendon's viscoelastic properties occurred after RCT and increased with cuff tear size worsening, leading to a mechanical overturning. They also observed that the cross-sectional area of the remaining tendons was increased, with a greater structure but with a worse quality tendon. These mechanical changes may be result of the positive periostin response in our healthy subscapularis tendon sample. It is plausible that increasing in periostin prevalence, with the worsening of tear dimension, might be due to the attempt of the healthy tendon to change its viscoelastic properties to prevent increased damage. On the other side, we observed that periostin is significantly higher in injured tendon compared to the healthy one.
- *Articular fluid stimulation*: Tissue remodelling factors and synovial inflammation are increased during RCT. Abrams et al.[16] using an immunofluorescence analysis demonstrated a linear correlation

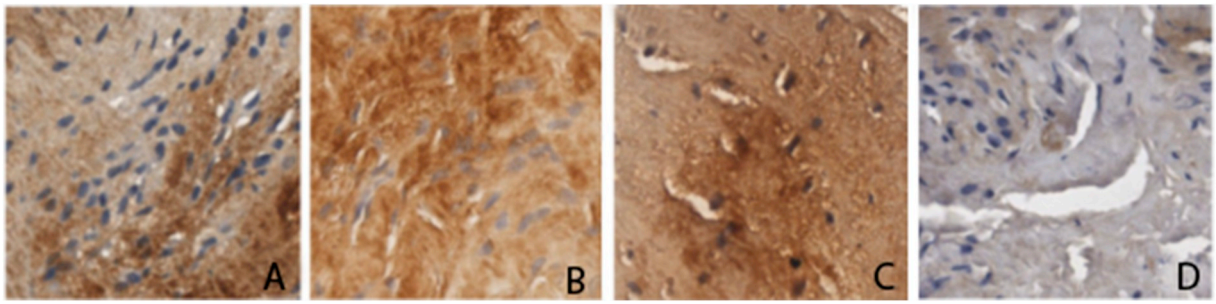

Fig. 1. High-power photomicrograph (magnification 40x) showing immunohistochemical expression of periostin (in brown) in: anterior margin (A); posterior margin (B); bursal tissue (C). D) No staining in subscapularis healthy tendon.

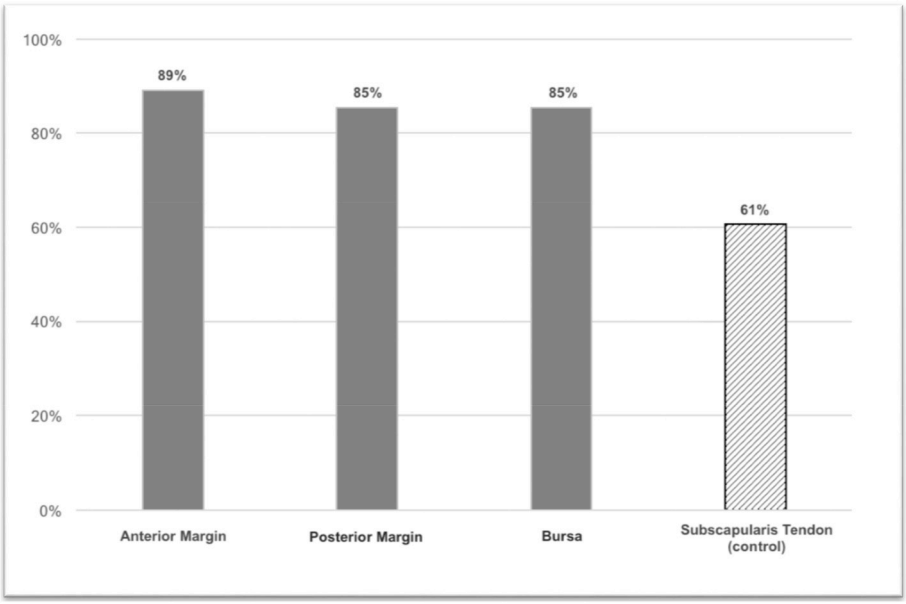

Fig. 2. Positive response to periostin in anterior and posterior margins, bursal tissue and healthy tendon.

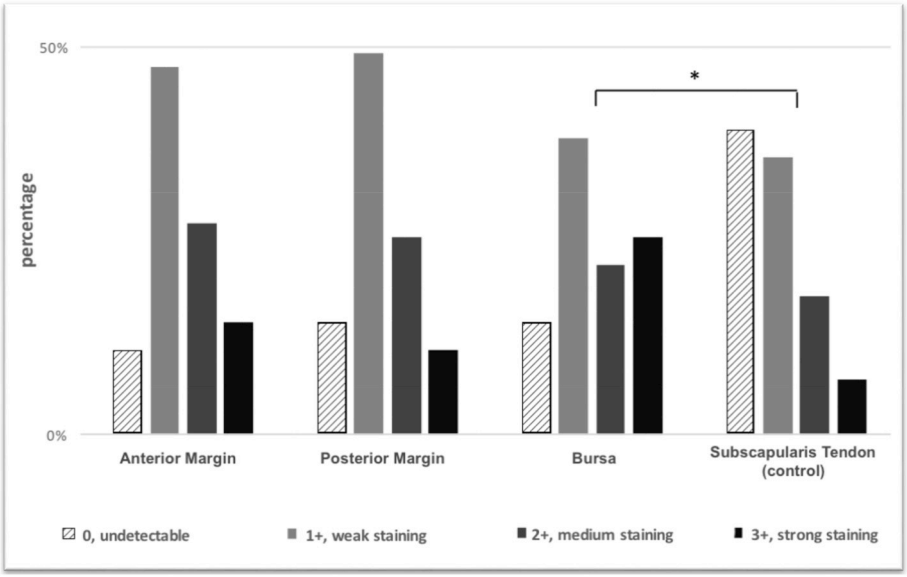

Fig. 3. Chi-square test was used to compare the IHC score in anterior margin/posterior margin/bursal tissue versus healthy tendon.

between the intra-articular level of matrix metalloproteinase-3 (MMP-3) and synovial inflammation in patients with full thickness RCT. An increased number of CD45 (common leukocyte antigen) and CD68 (istiocity markers) in the synovia was also detected. Shindle et al.[18] using an hematoxylin-eosin staining, found increased proinflammatory cells and vascularity in the synovium and,

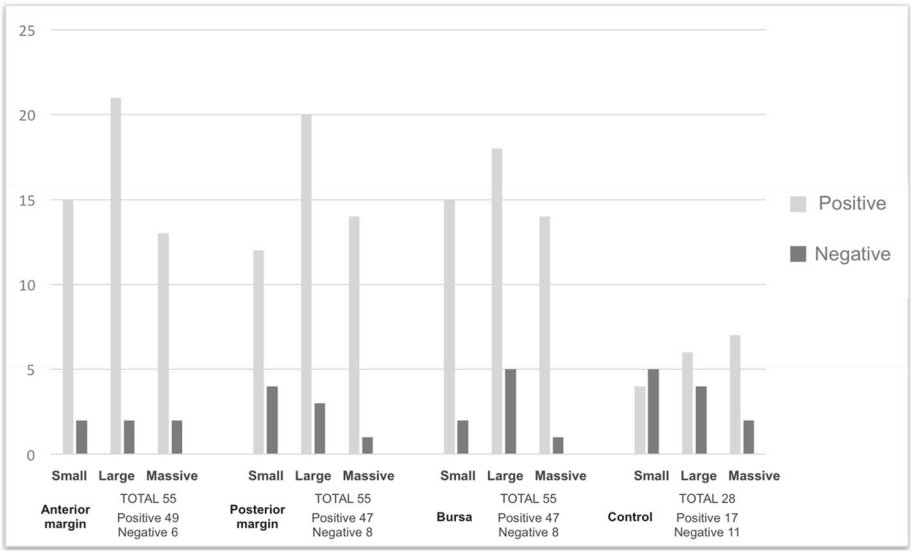

Fig. 4. Frequency of positive and negative responses to periostin, considering different rotator cuff tear sizes.

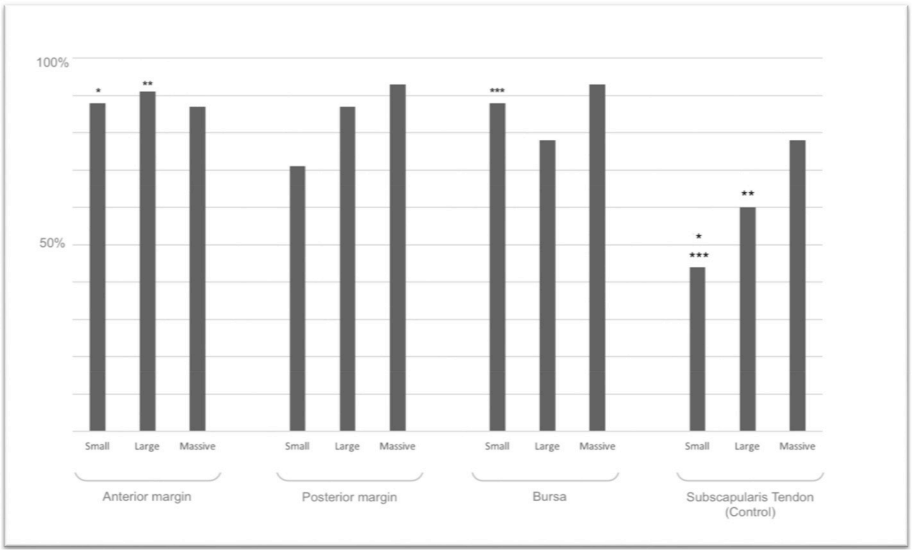

Fig. 5. Prevalence of positive responses to periostin, according to different rotator cuff tear sizes. */**/*** = significant differences when comparing with subscapularis tendon.

using real-time PCR, demonstrated that IL-1β were upregulated as well VEGF and TNF-α. Considering these findings, periostin production may be the result of biochemical stimulus induced in the synovial fluid by a full-thickness RCT.

- *Multifactorial effect*: Considering that RCT leads to a series of pathophysiological events, it is plausible that not only a single factor but different mechanical stresses and inflammation responses lead to an increasing in periostin synthesis.

The relative low number of studied patients is a limit of our study; however, the sample numerosity leads us to achieve significant information.

5. Conclusions

Our study demonstrated the relationship between periostin production and full thickness RCT. We hypothesized possible causes for the increasing in periostin synthesis after tendon damage: the subacromial bursa; -the mechanical stress and -the articular inflammation associated with full thickness RCT may all play a relevant role in leading to periostin production as an attempt to tendon healing.

Conflicts of interest

The authors declare that they have no conflict of interest.

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