

Thrombospondin-4 expression in the infrapatellar fat pad and its contribution to osteoarthritis pain

Frank Zaucke¹, Sebastian Braun², Patrizia Pollinger¹, Rebecca Sohn¹, Gundula Rösch¹, Zsuzsa Jenei-Lanzl¹

¹Department of Trauma Surgery and Orthopedics, University Hospital Frankfurt, Goethe University, Frankfurt/Main Germany, ²Center for Musculoskeletal Surgery, Charité - Universitätsmedizin Berlin, Berlin, Germany
frank.zaucke@ukffm.de

Disclosures: The authors have nothing to disclose

INTRODUCTION: Osteoarthritis (OA) is a chronic joint disease characterized by progressive degradation of articular cartilage as well as by pathological changes in the surrounding tissues¹. During OA pathogenesis fibrotic changes occur in the infrapatellar fat pad (IFP), which might contribute to the genesis of pain². In addition, recent studies suggested that the glycoprotein thrombospondin-4 (TSP-4), originally detected in the extracellular matrix of cartilage and expressed during progressive cartilage degradation³, is involved in genesis of pain⁴ after mechanical joint injury⁵ and contributes to spinal sensitization and neuropathic pain⁶. In the present study, the relationship between IFP fibrosis, TSP-4 expression in the IFP and knee joint pain was investigated.

METHODS: IFP tissue and synovial fluid samples were collected from 20 patients undergoing knee total knee replacement surgery at the University Hospital Frankfurt, Germany. All experiments were conducted in accordance with the Declaration of Helsinki, with informed consent and approved by the Ethics Committee of the Department of Medicine of the Goethe University Frankfurt (vote nr.: 19-347). Paraffin-embedded IFP sections were systematically stained with standard HE and Masson trichrome to assess fibrotic changes as well as immunohistochemically for TSP-4. The degree of fibrosis was evaluated by quantifying fibrotic areas, number of vessels and lymphocytic infiltration. In addition, the TSP-4 stained area was quantified in adjacent sections. The concentration of TSP-4 in synovial fluid samples was quantified using a commercially available ELISA. The pain level of OA patients was assessed preoperatively using the WOMAC Score.

RESULTS: A clear correlation between the degree of fibrosis, vascularization and lymphocytic infiltration was detected. This was independent of patient-specific parameters such as BMI, age and sex. The expression of TSP-4 could be detected in human IFP tissue at the protein level (Fig. 1). Moreover, the degree of fibrosis correlated with positively TSP-4 stained areas (Fig. 1). Regarding the degree of fibrosis and TSP-4 stained areas, four patient subgroups could be distinguished. Interestingly, a moderate TSP-4 expression could already be detected in samples with a low degree of fibrosis. There was no significant correlation between TSP-4 staining intensity in IFP and pain, however, it is striking that high TSP-4 values were never associated with low pain levels. The concentration of TSP-4 in synovial fluid ranged from 655 -1988 ng/ml (150-500% higher than in serum). There was no correlation between TSP-4 staining intensity and synovial fluid TSP-4 concentrations. However, a significant relationship between synovial fluid TSP-4 concentration and pain intensity was found in female OA patients (Fig. 2).

DISCUSSION: TSP-4 expression was demonstrated for the first time in the IFP. The correlation between TSP-4 staining intensity and degree of fibrosis and in particular the fact that increased TSP-4 intensity was observed in sections with minor fibrotic changes suggests that TSP-4 might play a role in the development of IFP fibrosis. While TSP-4 in the IFP does not seem to contribute to the genesis of pain, TSP-4 in the synovial fluid might play an important role in this regard.

SIGNIFICANCE/CLINICAL RELEVANCE: The analysis of synovial fluid and serum samples in larger patient cohorts will show if TSP-4 could be used as a pain biomarker or even represent a novel analgesic target in OA therapy.

REFERENCES:

[1] Loeser RF et al (2012) Arthritis Rheum 64:1697-1707. [2] Braun S et al. (2022) Biomedicines 10:1071. [3] Maly K et al (2019) Int J Mol Sci 20:447. [4] Park J et al (2016) J Biol Chem 291: 13335-13348. [5] Crosby ND et al (2015) Exp Neurol 264:111-120. [6] Kim DS et al (2012) J Neurosci 32:8977-8987.

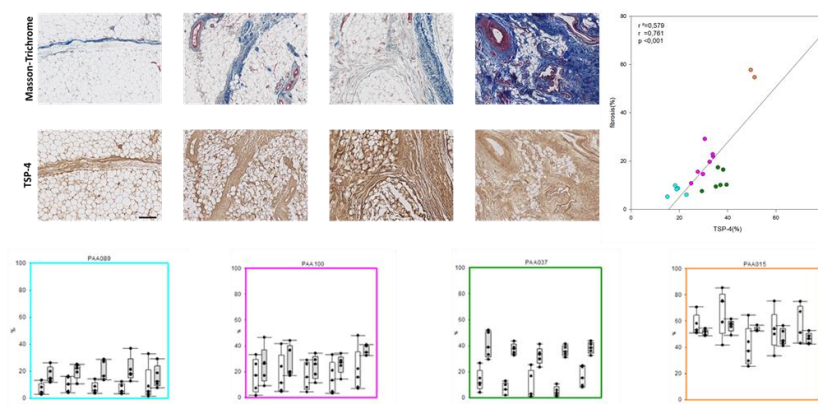


Fig. 1 Representative images of fibrosis (Masson Trichrome) and TSP-4 staining on sections of IFP from human OA patients
Strong positive correlation between the degree of fibrosis and the extent of TSP-4 ($r=0.579$, $r=0.761$, $p<0.001$). Each circle represents one individual patient. Patient samples were categorized into four groups based on the degree of fibrosis (white bars) and level of TSP-4 staining (grey bars): light blue circles - low fibrosis and medium TSP-4 expression; pink circles - medium levels of both fibrosis and TSP-4; green circles - low fibrosis but high strong TSP-4 expression; orange circles - very high pronounced fibrosis and strong TSP-4 expression.

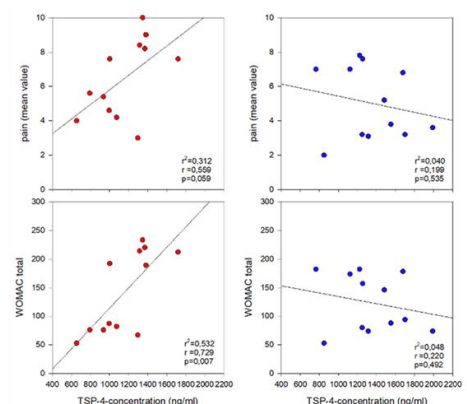


Fig. 2 Sex-dependent association of synovial fluid TSP-4 with WOMAC score
Positive correlation between synovial TSP-4 concentrations and WOMAC pain and total WOMAC scores in female (red circles) but not male patients (blue circles).