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Identification of antibodies against extracellular matrix proteins in human osteoarthritis

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ABSTRACT

We investigated the presence of autoantibodies against the extracellular matrix proteins thrombospondin-4 (TSP-4), cartilage oligomeric matrix protein (COMP), C-type lectin domain family 3 member A (CLEC3A), collagen II, collagen VI, matrilin-3, and fibrillin-2 in the serum of osteoarthritis (OA) patients. We compared those results with the presence of such antibodies in rheumatoid arthritis (RA) patients and in healthy donors (HD). Our study examines whether antibodies against extracellular proteins can be used as potential biomarkers to support the clinical diagnosis of OA. 10 OA, 10 RA patients and 10 HD were enrolled in this explorative cross-sectional study. SDS-PAGE and immunoblot were used to investigate the presence of antibodies against extracellular matrix proteins. The serum of 5/10 OA patients but 0/10 HD exhibited TSP-4 IgG isotype antibodies ($P = 0.033$). The serum of 8/10 OA patients but only 1/10 HD exhibited IgG isotype antibodies against TSP-4 or COMP ($P = 0.005$). The serum of 9/10 OA patients but only 1/10 HD exhibited IgG isotype antibodies against TSP-4, COMP or CLEC3A ($P = 0.005$). We found strong evidence for the presence of IgG isotype autoantibodies against the cartilage extracellular matrix proteins TSP-4, COMP and CLEC3A in OA. The detection of IgG isotype autoantibodies against TSP-4, COMP and CLEC3A may support the clinical diagnosis of OA. OA with autoantibodies against cartilage extracellular matrix proteins defines a new OA subgroup suggesting that patients with high concentrations of autoantibodies may benefit from an immune suppressive therapy.

1. Introduction

OA diagnosis is currently based on clinical symptoms, especially articular pain, radiographic findings and laboratory data [1]. In early OA, X-ray imaging often remains negative [2]. Magnetic resonance imaging (MRI) assessment of OA patients has provided improved diagnostic features, especially for early OA [3]. Through ultrasound imaging, thickening and inflammation of the synovial membrane has recently been shown to be associated with OA [4]. However, in cases when X-ray imaging reveals no signs of OA and MRI assessment is not accessible, the diagnosis of OA relies on clinical features. In these situations, the identification of an OA biomarker could support the clinical diagnosis of OA.

Recent research has provided evidence that the immune system plays an important role in OA. In the course of OA, fragments of cartilage extracellular matrix proteins are released into the synovial fluid and the blood [5]. Specific cartilage protein fragments released have been shown to activate the complement system [6], which in turn plays a pivotal role in OA [7]. Further, it has been shown that activated professional antigen presenting cells (APC) ingest and process extracellular matrix proteins and play a role in OA pathophysiology [8]. Activated APCs release IL-6 and other pro-inflammatory factors [9]. IL-6, a major OA cytokine [10]

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associated with increased IgG concentrations in synovial fluid [11], leads to secretion of IL-21 [12]. IL-21 has been shown to be elevated in OA patients [13] and to induce antibody production [12]. If autoantibodies against extracellular matrix proteins could be detected, these could possibly be used to simplify the clinical diagnosis of OA.

The purpose of this study was to analyze the serum of OA patients for the presence of autoantibodies against extracellular matrix proteins (matrilin-3, C-type lectin domain family 3 member A (CLEC3A), cartilage oligomeric matrix protein (COMP), thrombospondin-4 (TSP-4), fibrillin-2) and against network-forming cartilage collagens (collagen II, collagen VI). Furthermore, we compared the results with the presence of the autoantibodies in the serum of rheumatoid arthritis (RA) patients and healthy donors (HD). We discuss whether antibodies against extracellular matrix proteins can be used as potential biomarkers to support the clinical diagnosis of OA, to stratify patients suffering from OA and to identify OA patients who would benefit from an immune modulatory therapy.

2. Materials and methods

Subjects - The study was performed at the Universities of Düsseldorf and of Cologne. This explorative cross-sectional study includes 10 OA and 10 RA patients and 10 HD. Inclusion criterion for the OA group was a diagnosed OA; exclusion criteria for the OA group were RA and other autoimmune diseases or cancer. Inclusion criterion for the RA group was a diagnosed RA; exclusion criteria were OA and other autoimmune diseases or cancer. Inclusion criterion for the HD group were no symptoms of a joint disease; exclusion criteria were OA, RA and other autoimmune diseases or cancer. The average age of the OA group was 63 (±8) years, that of the RA group was 54 (±11) years and for the HD group 40 (±9) years. The proportion of women and men was evenly distributed in all groups. The study was approved by the ethics committee of the University of Düsseldorf (No. 2018-88-KFogU, No. 3828) and Charité Berlin (EA1/193/10, No. 3483).

3. Methods

Blood samples - Blood samples were centrifuged at 2772 × g for 10 min and stored in aliquots at −20 °C.

Proteins - The cDNA of human tetranectin, human monomeric matrilin 3 (lacking the C-terminal coiled coil domain) [14], human CLEC3A [15] and a human fibrillin-2 fragment [16] were cloned into modified pCEP-Pu vectors and transfected into HEK-293 EBNA cells. The recombinant proteins were purified using affinity chromatography from the supernatant of the cells. Human collagen II was purified with pepsin from human cartilage [17]. Native human collagen VI (Abcam, Cambridge, UK), recombinant human TSP-4 (R&D Systems, Minneapolis, USA) and recombinant human COMP (R&D Systems, Minneapolis, USA) were commercially available.

SDS-polyacrylamide gel electrophoresis (SDS–PAGE) and immunoblot - To detect antibodies against extracellular matrix proteins in the serum samples, extracellular matrix proteins (1 μg/lane) were separated using SDS-PAGE and transferred to a PVDF membrane (0.45 μm, Invitrogen, Carlsbad, USA). Free binding sites were blocked with milk powder (5%) and bovine serum albumin (1%) in Tris-buffered saline with 0.1% Tween (TBS-T) and incubated with patient's serum (1:200 in 5% milk powder and 1% bovine serum albumin in TBS-T) followed by an HRP-conjugated anti-human IgG antibody (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). Signals were detected after incubation using ECL Plus (GE Healthcare, Buckinghamshire, UK) and the ChemiDoc XRS + imaging system (BioRad, Munich, Germany).

Statistical analysis - Categorical data analysis for contingency tables was performed using the chi-square or Miller-Fisher-Exact test using IBM SPSS-software. Odds ratios and 95% confidence intervals (95% CI) were calculated using Medcalc® software (Ostend, Belgium).

4. Results

The purity and migration pattern of the human extracellular proteins TSP-4, COMP, CLEC3A, matrilin-3, fibrillin-2, collagen II, and collagen VI was analyzed using SDS-PAGE and Coomassie Brilliant Blue staining at the beginning and end of the study (Fig. 1). All proteins showed a specific band pattern and no contaminations.

Using SDS-PAGE followed by immunoblot, we examined the serum of 10 OA, 10 RA patients and 10 HD for the presence of IgG isotype antibodies against extracellular matrix cartilage proteins with patient's serum as primary antibody. Human tetranectin, a serum protein, was used as negative control. Signals in the appropriate position that were distinguishable from the background were assessed as positive (Fig. 2).

The presence of IgG antibodies against extracellular matrix proteins in the different groups is summarized in Tables 1a and 1b. Noteworthy, the serum of 5/10 OA patients but 0/10 HD ($P = 0.033$) exhibited TSP-4 IgG isotype antibodies (Table 1a).

The odds ratio for OA and the presence of TSP-4 IgG isotype antibodies was 21 (95% CI: 1-454). The serum of 9/10 RA patients but 0/10 HD ($P < 0.001$) exhibited TSP-4 IgG isotype antibodies (Table 1b). The odds ratio for RA and the presence of TSP-4 IgG isotype antibodies was 133 (95% CI: 5-3674).

TSP-4, COMP, and CLEC3A were more frequently found in the OA group. The serum of 8/10 of the OA group but only 1/10 of that of the HD group exhibited IgG isotype antibodies against TSP-4 or COMP ($P = 0.005$). The odds ratio for OA and the presence of TSP-4 or COMP IgG isotype antibodies was 36 (95% CI: 3-476). The serum

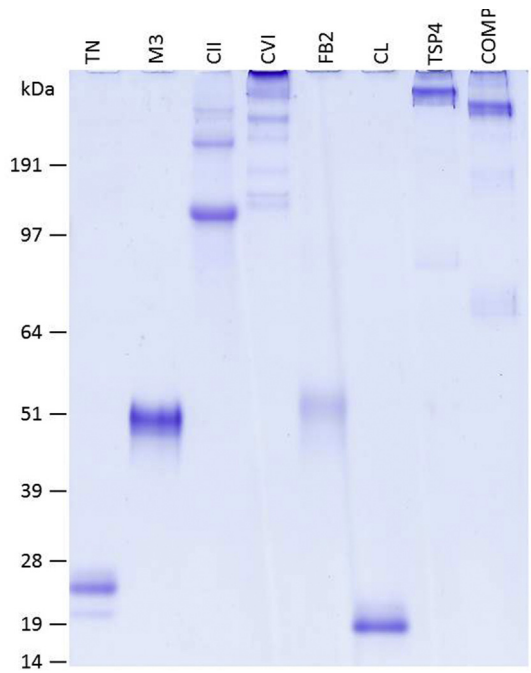

Fig. 1. Quality control of the proteins used in this study. Human tetranectin (TN), matrilin-3 (M3), collagen II (CII), collagen VI (CVI), fibrillin-2 (FB2), CLEC3A (CL), TSP-4 (TSP4), and COMP (COMP) were separated using SDS-PAGE under non-reducing conditions and stained with Coomassie Brilliant Blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

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