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ORIGINAL ARTICLE

Disease-specific expression patterns of proteases in synovial tissues ☆

Akira Kido[a,*], Geza Pap[b], Kenji Kawate[a], Albert Roessner[c], Yoshinori Takakura[a]

[a]*Department of Orthopedic Surgery, Nara Medical University, Nara, Japan*
[b]*Department of Orthopedic Surgery, Otto-von-Guericke University Magdeburg, Germany*
[c]*The Institute of Pathology, Otto-von-Guericke University Magdeburg, Germany*

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Abstract

To assess whether protease expression patterns can be discriminated according to matrix degradation mechanisms in aseptic prosthesis loosening (APL), rheumatoid arthritis (RA), and osteoarthritis (OA), we immunohistochemically examined the expressions of matrix metalloproteinase-1 and cathepsins B, D, and L in periprosthetic synovial-like interface tissues from 32 patients with failed prosthetic hips, from 29 RA-patients with hip synovial membranes, and from 35 patients with primary OA. Numerical values, calculated for the positivity of each protease, were used to rank the staining patterns, and a multivariate analysis was carried out to examine the discriminant probabilities. As a result of stepwise linear discriminant analyses, the three groups were successfully discriminated with probabilities of 100%, 62.1%, and 77.1%, respectively. Cathepsin L was significantly related to the discrimination of APL from RA and primary OA. Disease-specific protease activation pathways might exist, and cathepsin L can be a key enzyme for APL pathogenesis.

Level of evidence: Prognostic study, level III (retrospective study).

Keywords: Aseptic prosthesis loosening; Osteoarthritis; Rheumatoid arthritis; Cathepsins; Matrix metalloproteinases

Introduction

Periprosthetic osteolysis, a key mechanism in aseptic prosthetic loosening (APL), is one of the most common reasons for revision surgery [3,26]. Previous studies have demonstrated that the cellular host response to implants is one of the most important features in the osteolytic process [8,22,24,23,28] in addition to mechanical factors, such as cyclic loading or micromotion of the implants [1,5,10]. We have previously reported that cysteine and aspartic proteases and matrix metalloproteinase-1 (MMP-1) are strongly involved in accelerating tissue destruction around loose arthroplasties [13]. Accordingly, we and several other researchers have demonstrated similarities between a synovial-like interface membrane (SLIM) and hyperplastic synovium in rheumatoid arthritis (RA) [8,13,24,23]. In addition, recent data indicate that prosthesis loosening fibroblasts (PLFs) share some characteristic features of RA synovial fibroblasts, including anchorage-independent proliferation [14,27], escape of contact inhibition [9], and activation of tumor-associated pathways, including protooncogenes [27] and alterations in apoptosis [7].

☆ Each authors certifies that he has no commercial associations that might pose a conflict of interest in connection with the submitted articles. Each authors certifies that his institution has approved the human protocol for the investigation and all investigation were conducted in conformity with ethical principles of research.

*Corresponding author. Tel.: +81 744 22 3051; fax: +81 744 25 6449.
E-mail address: akirakid@naramed-u.ac.jp (A. Kido).

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In this study, we focused on expression patterns of MMP-1, cathepsins B, D, and L in proliferative synovial tissues. MMP-1 is the best characterized member of the MMP family, also referred to as collagenase-1 [19]. MMP-1 cleaves collagens I, II, VII, and X. MMP-1 is the most prominent MMP, although most other MMPs have also been detected in activated synovium. Cathepsins represent a second important family of proteolytic enzymes in activated synovium, especially because the expression of cysteine protese is linked to the activation of numerous oncogenes observed in synovial fibroblasts. As cathepsins B and L are capable of degrading collagens and proteoglycans, the upregulation of these cysteine proteases in proliferating synovial cells is thought to play a major role in matrix degradation [17,19]. The aspartic protease cathepsin D is a key mediator of induced apoptosis [16]. In our previous study, cathepsin D was frequently detected in samples from RA or APL, suggesting its possible contribution to induced-cell death in activated synovial fibroblasts [13].

However, in spite of the morphologic and biologic similarity in synoviocytes of RA and APL, the pathogenesis of these two diseases seems different. Proposed causes for RA are genetic alterations, pathogenic responses triggered by infectious agents, autoimmunity against synovium and cartilage, disordered regulation of proinflammatory cytokines, and transfomation of synovial fibroblasts, while originally, APL is a local biologic reaction against orthopedic implants. Thus, in this study, our hypothesis is that (1) matrix degradation progresses differently in each disease, and therefore, (2) expression patterns of the four proteases may discriminate RA and APL. To gain deeper insights into the matrix degradation mechanism, we performed a comparative immunohistiochemical study using RA synovium and SLIMs, followed by multivariate analyses. Because of the histologic similarity and well-clarified pathology, OA synovium was assessed as a control.

Materials and methods

Samples of interface tissue around loose cemented hip arthroplasties containing fibrous tissue and attached bone were obtained from 32 patients with failed prosthetic hips at revision surgery performed at our institutions, and designated the APL group (Table 1). All samples were taken at the cement–bone interface in the femur. The mean time from primary hip arthroplasty to revision surgery was 11 years (SD, ±6.3 years; range, 2–25 years). All patients had prosthesis implantation for primary OA of the hip. In addition, synovial membrane tissue samples were obtained from 29 patients with stage IV RA (RA group) according to Larson et al. [15], and from 35 patients with primary OA of the hip (OA group), who were all designated for hip arthroplasty. After immediate fixation in 4% formalin for 6 h and decalcification in 10% EDTA solution, the tissue samples were embedded in paraffin. The use of all human material was approved according to the relevant laws and regulations of our institutes. Informed consent for the experimental analyses was obtained from all the patients.

Table 1. Summary of the clinicopathologic data

Group	Number of patients	Age in years (mean ± SD)	Males/females
Aseptic prosthetic loosening (APL)	32	63 ± 10	15/17
Rheumatoid arthritis (RA)	29	57 ± 12	8/21
Primary osteoarthritis (OA)	35	61 ± 15	16/19

We analyzed the expressions of the two cysteine proteases cathepsins B and L, the aspartic protease cathepsin D, and the metalloproteinase MMP-1. Immunohistochemical analyses were carried out on sections of formalin-fixed and paraffin-embedded tissue samples using the avidin–biotin–complex (ABC) method with either horseradish peroxidase (HRP) or alkaline phosphatase (ALP). Briefly, the paraffin sections (4-μm thick) were deparaffinized, rehydrated, and incubated with the following primary antibodies: monoclonal anti-MMP-1 (Oncogene Research Products, Cambridge, MA), polyclonal anti-cathepsin B (Oncogene Research Products), monoclonal anti-cathepsin D (Novocastra Laboratories Ltd., Newcastle Upon Tyne, UK), and polyclonal anti-cathepsin L (Biogenesis, Poole, UK).

The primary antibodies were diluted 1:100 (anti-MMP-1), 1:50 (anti-cathepsins B and D), or 1:80 (anti-cathepsin L) in a buffer composed of 50 mL RPMI 1640 (GIBCO, Eggenstein, Germany), 450 mL distilled water, 50 mL fetal bovine serum (GIBCO), and 0.5 g sodium azide (Sigma, Deisenhofen, Germany) at pH 7.4–7.6. The sections were incubated with the primary antibodies for 32 min at 42 °C (anti-MMP-1 and anti-cathepsin D) or 60 min at 37 °C (anti-cathepsin B and anti-cathepsin L).

Paraffin-embedded tissue sections of human breast carcinomas (for anti-MMP-1, anti-cathepsin B, and anti-cathepsin L) and tonsils (for anti-cathepsin D) with well-established positivities for the corresponding antigens were used as positive controls. Sections containing tissues with known positivities for the antigens were exposed to all reaction components, except for the primary antibodies, and used as negative controls. Sections exposed to primary antibodies that had been