Early Differential Diagnosis of Arthritis

Quantification of HDJ2 (DNAJA1) in Synovial Fluid

Albert-Ludwigs-Universität Freiburg

UNI FREIBURG

Technology

We studied Hdj2 (DNAJA1) - a member of the J-protein family of chaperones - in a large number of synovial fluids (SF) taken before or after diagnosis of arthritis. Hdj2 occurred in high frequency in samples of patients with rheumatoid arthritis, including very early samples taken long before diagnosis. In contrast, samples from patients with other forms of arthritis contained Hdj2 less frequently, and only later in the course of the disease. We suggest an assay for the detection and quantification of synovial Hdj2 in early arthritis. This assay may contribute to the differential diagnosis of rheumatoid arthritis, thus allowing efficient treatment in due time.

Innovation

- new marker for differential diagnosis of rheumatoid arthritis at an early stage
- easy and reliable method
- efficient treatment at an early stage
- may allow prediction of the course of disease
- possibility to prevent severe disease

Application

- to develop mAbs for specific detection of Hdj2
- to develop specific detection assays (ELISA and other methods)
- to develop kits for early differential diagnosis of rheumatoid arthritis

Market Potential

Arthritis occurs in more than 20 % of the adult population, leading to activity limitations in 40 % of the patients. Without treatment, severe disability may follow. Specific markers for early differential diagnosis are required to ensure the best possible treatment.

Responsible Scientist

Dr. Inga Melchers

Department of Rheumatology and Clinical Immunology

Branch

Pharma

Patent Status

EP Appl. No. EP 2103942 A1

Filed (PRD): March 20th 2008

WO 2009115190 A1

National Appl.: US, CA, AUS, IN, RUS

Reference Number

ZEE20080310

Stand: Mai-11

Contact

Campus Technologies Freiburg GmbH | Stefan-Meier-Str. 8 | D-79104 Freiburg
Email: Claudia.Skamel@campus-technologies.de
Tel: +49 (0)761 203-4987
Fax:+49 (0)761 203-5021

CAMPUS TECHNOLOGIES FREIBURG

signo Hochschulen
Schutz von Ideen für die gewerbliche Nutzung

EARLY DIFFERENTIAL DIAGNOSIS OF ARTHRITIS BY QUANTIFICATION OF HDJ2 (DNAJA1) IN SYNOVIAL FLUID

Claudia Lehr, Rita Rzepka, Christoph Schick, Andrea Zgaga-Griesz and Inga Melchers
Clinical Research Unit for Rheumatology, University Medical Center, Freiburg, Germany

BACKGROUND

The J-protein Hdj2 (DNAJA1, Hsj2, Hsdj, dj-2) is one of ~40 known human co-chaperones. Its highly conserved J-domain shows homology to *E. coli* DnaJ and cooperates with HSP70 family members. HSP70/J-protein chaperone machines not only act as „foldases" during protein synthesis and transport, but are also involved in many physiological processes like signal transduction, apoptosis or antigen presentation.

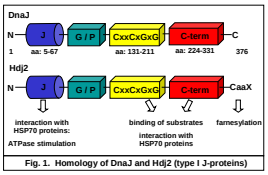

Fig. 1. Homology of DnaJ and Hdj2 (type I J-proteins)

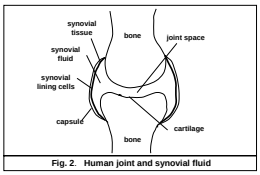

Fig. 2. Human joint and synovial fluid

In healthy human joints (Fig. 2), the surface of synovial tissue (ST) is covered by a thin layer of synovial lining cells (SLC), and the joint space between cartilage and ST is filled with little synovial fluid (SF). During arthritis, the amount of ST, SLC and SF increases, leading to joint effusions which may be aspirated for therapy and analyses.

Earlier we described an enhanced expression of Hsc70 (but not of the inducible Hsp70) and undefined J-proteins in ST from patients with rheumatoid arthritis (RA), but not from patients with osteoarthritis (OA) (1, 2). Here we show data on the expression of Hdj2 in SF and ST of patients with RA and other forms of arthritis.

MATERIAL & METHODS

Patients were diagnosed in the Department of Rheumatology and Clinical Immunology, University Medical Center, Freiburg. All diagnoses fulfilled the appropriate criteria, e. g. RA patients were characterized according to the ACR-criteria. All procedures were approved by the local ethics committee, and patients gave their informed consent to the study.

SF were collected from 130 patients with clinically apparent joint effusions. Samples were analysed for the presence of Hdj2 by 10 % SDS-PAGE, blotting onto nitrocellulose membranes, and detection with a specific murine monoclonal antibody (KA2A5.6) using HRP-labelled anti-IgG, substrate (Super Signal; Pierce) and Hyperfilm (Amersham). In addition, detailed clinical and laboratory parameters of the patients were collected. SPSS15.0 was used for statistical analyses.

CONCLUSIONS

The presence of Hdj2 was significantly correlated with the diagnosis of rheumatoid arthritis (p = 0.021; I). Also synovial tissue of patients with rheumatoid arthritis showed overexpression of Hdj2, which was not detected in ST from patients with OA (data not shown).

Extracellular Hdj2 was also detected in synovial fluids of patients with other joint diseases (I). However, detailed analyses showed that it occured with a different time course: it appeared EARLY in RA and LATE in other forms of arthritis (II). Hdj2 was also detected in the SF of patients, before the diagnosis of RA was possible according to ACR criteria (II). These patients were negative for rheumatoid factor (RF) and anti-CCP (data not shown).

In RA patients, the amount of extracellular Hdj2 correlated with the degree of inflammation, and its presence was significantly associated with the presence of autoantibodies typical for the disease (RF, anti-CCP; III).

Using immunoblots, Hdj2 was only detected in SF, but not in peripheral blood drawn at the same time (data not shown).

REFERENCES

1. Schick C et al. *Continuous enhanced expression of Hsc70 but not Hsp70 in rheumatoid arthritis synovial tissue.* Arthritis Rheum, 2004. 50: 88-93.

2. Kurzik-Dumke U et al. *Overexpression of human homologs of the bacterial DnaJ chaperone in the synovial tissue of patients with rheumatoid arthritis.* Arthritis Rheum, 1999. 42: 210-20.

RESULTS

I. The presence of Hdj2 in synovial fluid is significantly correlated with rheumatoid arthritis

Disease	All N (%)	Hdj2 pos. N (%)	Hdj2 neg. N (%)
Unselected	130 (100)	80 (61.5)	50 (38.5)
Rheumatoid arthritis	50 (38.5)	37 (74.0) *	13 (26.0) *
Other diseases	80 (61.5)	43 (53.8) *	37 (46.3) *
Reactive arthritis	24 (18.5)	12 (50.0)	12 (50.0)
Psoriatic arthritis	14 (10.8)	8 (57.1)	6 (42.9)
M. Bechterew	4 (3.1)	3 (75.0)	1 (25.0)
M. Reiter	4 (3.1)	3 (75.0)	1 (25.0)

Tab. 1. Expression of Hdj2 in SF of patients with RA or other diseases. The presence of Hdj2 is significantly correlated to the diagnosis RA (*p = 0.021).

II. Early detection of Hdj2 in synovial fluid of patients with rheumatoid arthritis

Patients (N)	Hdj2	Diagnosis	Time of detection
6	Present	RA	11-36 months before diagnosis
5	Present	RA	at diagnosis
8	Absent	No RA	at diagnosis
7	Present	RA	1-24 months after diagnosis
3	Absent	No RA	1-24 months after diagnosis

III. In RA patients, presence and amount of Hdj2 correlate with the degree of inflammation and the presence of autoantibodies

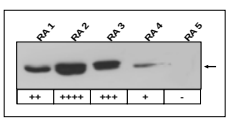

Fig. 3. Detection of Hdj2 (position marked by the arrow) in SF from 5 patients with RA. A semiquantitative graduation is used to classify the amounts (detection limit: 0.1 ng).

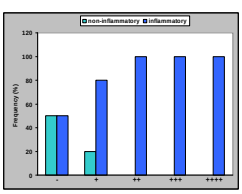

Fig. 4. Correlation between the quantity of Hdj2 detected and the degree of inflammation (based on the number of infiltrating leukocytes). The frequency of inflamed and non-inflamed SF in each category is given ($C_{corr.}$ = 0.894).

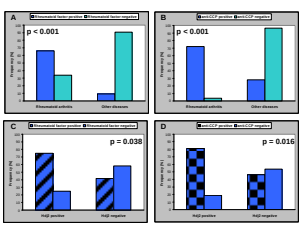

Fig. 5. Correlations between the presence of rheumatoid factor (A + C) or anti-CCP (cyclic citrullinated peptide) antibodies (B + D) with diagnosis (A + B; RA vs. other diseases) or Hdj2 presence (C + D). In C + D only RA patients are shown.