Synovial membrane p53 protein immunoreactivity in rheumatoid arthritis patients

C Soon Lee, Ian Portek, John Edmonds, Bruce Kirkham

Abstract

Objectives—To examine the expression of the p53 protein in synovial membrane of rheumatoid arthritis (RA) patients and to compare this with the expression in normal synovial tissues in subjects without RA.

Methods—Immunohistological expression of the p53 protein was studied using a streptavidin-biotin-peroxidase method and the monoclonal antibody DO-7, an antibody directed against both wild and mutant forms of p53 protein, in synovial tissues of RA patients (n=10) and from subjects with no known joint disease (n=4).

Results—p53 protein expression was present in a small percentage of synovial cells in the majority of the RA patients (n=8, 80%) and in half of the normal control cases with no inflammatory joint disease (n=2, 50%). No sample had more than 5% cells staining with intranuclear pattern. The difference in synvial p53 immunoreactivity between the RA patients and normal controls is not statistically significant (p=0.64; χ² contingency test).

Conclusions—This study has shown that p53 protein is only weakly expressed in the rheumatoid synovial membrane, with a low percentage of p53 protein immunostaining cells present, with intranuclear staining. These results suggest this is the wild type p53 protein rather than mutant protein. These findings suggest that synovial p53 protein expression may not be important in the pathogenesis of RA and may only represent a reactive repair process to DNA damage secondary to the immune and inflammatory reactions associated with the disease.


The 53 kDa nuclear phosphoprotein encoded by the p53 gene, located on human chromosome 17p13.1, is important in the control of the cell cycle. Wild type p53 protein inhibits cell proliferation and transformation. Muta

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Immunohistochemistry can demonstrate p53 protein overexpression in formalin fixed, paraffin wax processed tissue sections of tumours. Mutant p53 protein has a greatly extended half life compared with normal wild type p53 and is more easily detected by immunohistochemical methods. Immunohistoch

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of each tissue section was assessed and an average score calculated for each patient. For each subject two to six samples were available for examination, giving a total of 41 samples from 10 RA patients and 14 samples from the four subjects with no inflammatory joint disease.

Immunostaining was assigned a score of 0–4+: 0 = no staining; 1+ = intranuclear staining of <1% of cells; 2+ = 1–5% cells staining; 3+ = 5–20% cells staining; 4+ = >20% cells staining.

**STATISTICAL ANALYSIS**
Categorical variables were analysed with the $\chi^2$ contingency test. Only p values of less than 0.05 were considered significant.

**Results**

**SUBJECT DETAILS**
The 10 patients with RA varied in both disease duration and current treatments (table 1). Six patients had active RA, with at least two of these features: swollen joint count > 5; ESR > 28 or CRP > 8; early morning stiffness > 45 minutes. The four normal subjects with non-inflammatory synovium had arthroscopies for investigation of knee pain of less than six months duration. Two had no discernible cause found, and two had meniscal tears.

**P53 EXPRESSION**
Expression of the p53 protein was seen as nuclear immunostaining of cells scattered through the sub-synovial lining layer. Eight of the 10 RA patients (80%) had definite immunostaining (fig 1; table 1). Of the 41 samples from the 10 patients examined, most (28 of 41; 68%) showed staining for p53 protein, with similar expression at the different biopsy sites. However, staining was infrequent, with no subject showing more than 5% cells staining. p53 staining did not correlate with disease activity or duration.

In two subjects with no inflammatory joint disease (50%), p53 immunoactivity was also seen in a minority of samples (3 of 11; 27%) (fig 2), with less than 5% of cells staining in each sample. The difference in synovial p53 immunoreactivity between the RA patients and normal controls is not statistically significant ($p = 0.64$; $\chi^2$ contingency test).

**Discussion**
Studies of p53 expression in the RA synovium have been stimulated by the hypothesis that cellular accumulation occurs because of defective apoptosis. Abnormal function of a tumour suppressor, such as p53 could contribute to defective apoptosis and also to the transformed appearance of RA synovial fibroblasts. Initial reports of p53 protein expression demonstrated both high and low or absent levels. Tak et al has reported p53 expression in both early (< 6 months) and longer duration (> 5 years) RA. That study used a highly sensitive technique on frozen sections and detected 6–15% cells positive in the sublining leucocyte infiltrate, with less than 5% in the lining layer and lymphocyte aggregates. This differs from previous reports from that group of high levels in the intimal lining layer, and may be accounted for by different antibodies and staining techniques. However, a feature of both these reports is strong cytoplasmic staining. It has been reported in an unusually aggressive breast carcinoma using frozen section techniques and was shown to be a truncated form of the p53 protein, because of a non-sense mutation in exon 6.

<table>
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<th>Subject</th>
<th>Sex/age</th>
<th>DD (y)</th>
<th>Drugs</th>
<th>ESR/CRP</th>
<th>RF</th>
<th>SJC</th>
<th>EMS (min)</th>
<th>Maximum p53 score</th>
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<td>1</td>
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<td>M</td>
<td>43</td>
<td>GOLD</td>
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DD = duration of disease; PRED = oral prednisolone; MTX = methotrexate; GOLD = IM sodium aurothiomalate; SZ = sulfasalazine; SJC = swollen joint count; EMS = early morning stiffness.

**Figure 1** p53 protein nuclear immunostaining present in a small number of synovial cells in a case of clinically active rheumatoid arthritis. (Immunoperoxidase, DO-7; original magnification x 200).

**Figure 2** Absence of p53 protein immunostaining in the normal synovium of a subject with no inflammatory joint disease. (Immunoperoxidase, DO-7; original magnification x 200).

<table>
<thead>
<tr>
<th>Table 1 Clinical details and p53 immunostaining scores of subjects with rheumatoid arthritis</th>
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<td>Subject</td>
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<td>M</td>
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We used the DO7 monoclonal antibody, regarded by tumour pathologists as the most consistent antibody for paraffin wax sections. This antibody was used by Tak et al for western blot analysis. The DO1 and Pab 1801 antibodies have previously been shown to give no staining in RA synovium. In fact p53 staining varies widely depending on the antibody used. Studies of this variability have shown that staining with the Pab 1801 antibody was not repeatable even using a different batch of the antibody from the same manufacturer. In that report staining with the DO1 antibody was negative and caution about overinterpretation of p53 immunostaining was called for; particularly if unusual patterns such as cytoplasmic staining were seen. More recently a detailed investigation of p53 gene mutations failed to detect any mutations in synovial fibroblasts from European RA patients, but did detect mutations in synovial fibroblasts from three Californian patients.

Our results also show that p53 immunostaining is detectable in RA synovium with a frequency of positive cells generally higher than control tissue. The lack of statistical significance may relate to the small numbers of control tissue samples and this may be a biologically important increase in RA. Low p53 expression in various human cancers is strongly associated with wild type, rather than mutant expression in various human cancers. The lack of statistical significance may relate to the small numbers of control tissue. The lack of statistical significance may relate to the small numbers of control tissue.

Apoptosis in rheumatoid arthritis: p53 overexpression in the RA synovium represents a reactive process to DNA damage secondary to the immune and inflammatory mediators of the disease. We are unable to explain the Californian patients cytoplasmic staining of p53 and suggest it may relate more to the immunostaining techniques and monoclonal antibodies used. Our suggestion that p53 staining is not related to mutations is supported by the recent findings that mutation of the p53 gene was not found in European patients with RA.

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