**MUTATIONS OF THE P53 TUMOR SUPPRESSOR GENE IN RHEUMATOID ARTHRITIS SYN_ovium**

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**Introduction:**
Rheumatoid arthritis (RA) is a chronic inflammatory disease, and the factors that contribute to its onset, perpetuation, and invasiveness have been extensively studied. However, the possible role of nonimmunologic factors in the progression of RA has not been rigorously addressed. Cultured RA synovial cells display malignant cell features, e.g., a tendency to grow in disorganized monolayers and form foci, ability to form colonies in soft agar, presence of many multi-nucleated cells, and tumorigenicity in nude mice. Several genes, particularly oncogenes and tumor suppressor genes, can be involved in the regulation of the cell cycle process. In recent studies of the RA synovium a discrepancy has been found between excessive DNA strand breaks and minimal apoptotic morphology in the intimal lining cells. Marked overexpression of the p53 tumor suppressor gene subsequently was observed in the region of DNA fragmentation. Because high p53 expression has been associated with point mutations and abnormal apoptosis, we hypothesized that alterations in the structure or function of p53 might contribute to the transformed phenotype of RA synoviocytes. We now present data demonstrating that somatic mutations occur in the p53 tumor suppressor gene in RA synovium and in cultured rheumatoid fibroblast-like synoviocytes.

**Materials and Methods:**
Synovial tissues were obtained from 13 patients diagnosed as having RA (according to the American College of Rheumatology) and 9 osteoarthritis (OA) patients. 4 adherent cell lines of 14 rheumatoid patients and 4 adherent cell lines of osteoarthritis patients were prepared by cultivating very small tissue pieces in culture plates and passing on three occasions in culture flasks, leading mainly to fibroblast-like synoviocytes. Genomic DNA was extracted from cell nuclei by standard methods using cesium chloride and guanidinium isothiocyanate, proteinase K/phenol-chloroform extraction and ethanol precipitation. The sequences of the primers were designed to amplify individual exons 5-8 together with parts of their adjacent introns. To sequence the p53, a DNA sample was diluted into 60 µl of solution containing 200 µM dNTP. Then, hot start PCR was performed by the AccuPower PCR PreMix. Direct genomic sequencing was performed for DNA samples that were thought to contain a mutant p53 gene. The resulting double-stranded DNA fragments were purified by the DNA PrepMate. Then, purified single strands were sequenced directly on the AccuPower DNA sequencing kit and the Silverstar DNA staining kit. For immunohistochemistry, deparaffinized tissue sections were washed in PBS and pre-incubated in 0.5% blocking serum. Incubation was then performed overnight at 4°C using the mouse monoclonal p53 antibody DO-1. Detection with a biotinylated sheep anti-mouse secondary antibody and streptavidine-alkaline phosphatase conjugate were each applied for 1 hr at 37°C.

**Results:**
After PCR amplification of various portions of conserved domains of the p53 gene, e.g., exon 2, and exons 5-9, we tried to detect point mutations using the direct p53 DNA sequencing technique, based on conformation of the single-strand DNA as a positive control. We used previously sequenced, mutated DNAs from tumor cell lines which presented a shifted band, thus indicating point mutations. There was no mutation in osteoarthritis samples, but there were 4 mutations of the 13 rheumatoid arthritis samples (out of the 4 adherent cell lines, 2 of them mutated). Mutations were found at the codon 177 (AGG to AGT, G→T), 277 (TGT to TGC, T→C), and, surprisingly, two patients at the codon 237 (CAT to CCT, A→C). Among base substitutions (n=4), all mutations were found to undergo transition, not transversion. These results are consistent with a common mechanism of mutation, such as oxidative deamination by NO or oxygen radicals. The mutations detected were clustered within 3 evolutionary highly conserved regions of the p53 gene. We found 1 mutation in domain III (codons 171 to 181), 2 mutations in domain IV (codons 234 to 258) and 1 mutation in domain V (codons 270 to 286). In immunohistological studies, the Do 1 monoclonal antibody stained 6 out of the 13 rheumatoid arthritis tissue biopsies analysed. The distribution of the p53-expressing cells is mostly located in the peripheral lining layer of the tissue.

**Conclusion:**
The predicted amino acid substitutions in p53 were similar or identical to those commonly observed in a variety of tumors and might influence growth and survival of rheumatoid synoviocytes. Thus, mutations in p53 and subsequent selection of the mutant cells may occur in the joints of patients as a consequence of inflammation and contribute to pathogenesis of the disease. The notion that somatic mutation occurs in apoptosis and cell cycle regulating genes in RA supports the hypothesis that chronic RA has a transformed, invasive component and suggests that novel therapeutic strategies might be applicable to arthritis and perhaps other inflammatory diseases. Such an approach might have the advantage of directly addressing tissue destruction and altering the natural history of the disease rather than simply suppressing inflammation.

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