

The codon 72 polymorphic variants of p53 in Italian rheumatoid arthritis patients

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Abstract

Objective

The p53 tumor suppressor protein plays an important role in cell apoptosis. The wild type p53 protein presents a common polymorphism at position 72 resulting in either a proline or an arginine residue at this position, leading to differences between the two variants in the induction of apoptosis. We examined the possible associations of this polymorphism with the occurrence of rheumatoid arthritis (RA) and its severity in a series of RA patients of Italian origin.

Methods

170 consecutive RA patients fulfilling the 1997 ACR criteria and seen over a 4-month period in our rheumatology centre were studied. The medical records of the patients were reviewed for demographic and clinical parameters. Radiographs of the hands and feet taken at disease onset and after 5 years were available for 122 of the patients and were used to determine the presence and number of erosions, which were scored according to the modified Sharp/van der Heijde method (S/vdH). All of the RA patients and controls were genotyped by the polymerase chain reaction and allele-specific oligonucleotide techniques for p53 gene polymorphism Arg/Pro at codon 72.

Results

The distribution of the polymorphism of Arg/Pro 72 did not differ significantly between patients and healthy controls (Arg/Arg 47.1 vs 48.5%, Arg/Pro 43.5% vs 42%, Pro/Pro 9.8 vs 9.5% respectively, $p=ns$).

Patients carrying the Pro/Pro genotype had a significantly higher percentage of erosive disease at year 5 compared with patients carrying the Arg/Arg genotype (Pro/Pro 93%, Arg/Arg 52%, $p=0.0001$). The mean number of eroded joints per patient at 5 years was higher in the Pro/Pro subgroup and significantly lower in the Arg/Arg subgroup (Pro/Pro 13.2, Arg/Arg 3.6, $p=0.0001$). The mean S/vdH erosive score, joint space narrowing score and total damage score were significantly higher in the Pro/Pro subgroup compared with the Arg/Arg and Arg/Pro subgroups.

Conclusions

In the Italian population there is no association between codon 72-p53 gene polymorphism and the occurrence of RA. However, this polymorphism is associated with the structural damage of the disease.

Key words

Rheumatoid arthritis, p 53 polymorphism, bone erosions.

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Introduction

Rheumatoid arthritis (RA) is a chronic and destructive disorder of the joints that may be associated with significant systemic involvement. Its primary characteristic is the growth of an inflamed and hyperplastic synovial tissue which progressively invades and destroys joint cartilage and subcondral bone (1). The mechanisms leading to hyperplastic synovitis are not fully understood, but it has been demonstrated that an alteration in apoptosis contributes to the pathogenesis of RA (2-4).

A variety of apoptosis-related factors have been investigated in RA such as the tumor suppressor gene p53 (5-8), Fas antigen and ligand (9, 10), and several molecules of the Bcl-2 family (11-13). Although the Fas antigen is expressed and functionally active in RA synoviocytes, intractable synovial hyperplasia is often observed during the course of RA, suggesting that Fas-mediated apoptosis may be incapable of fully eliminating cells in the proliferative RA synovium (8, 14). This defective apoptosis is not restricted to a particular cell type but affects the whole cellular network.

Underlining the importance of apoptosis in RA is the belief that several effective therapies currently in use to treat patients with RA may work, at least in part, through the induction of apoptosis (2). Moreover, work in experimental arthritis supports the potential role of p53 as a therapeutic tool, having demonstrated that the intra-articular injection of an adenoviral vector expressing wild-type p53 induces synovial apoptosis and reduces inflammation (15).

Two main apoptotic pathways have been identified in a variety of cell types (16). The type I pathway is mediated by the activation of a death receptor by extrinsic signalling and requires the activation of caspase 8 at the death-inducing signalling complex (DISC), followed by activation of caspase-3. In the type II intrinsic pathway, caspase-3 is activated by caspase-9 via activation of Bid by caspase-8, loss of mitochondrial membrane potential and cytochrome c release to the cytosol.

In this mitochondrial apoptotic pathway, p53 plays an important role in

mitochondrial membrane stability and promotes apoptosis (17). p53 is a tightly regulated transcription factor that induces cell cycle arrest or apoptosis in response to cellular stress such as DNA damage (18). The p53 gene is one of the most intensely studied human genes because of its role as a tumor suppressor gene.

The wild type p53 protein presents a common polymorphism at position 72, resulting in either a proline or an arginine residue at this position (19). This substitution leads to differences between the two variants in their binding of components of the transcriptional gene, the activation of transcription and the induction of apoptosis (20, 21). Recent studies in cell lines containing inducible version of alleles encoding the Pro72 and Arg 72 variants and in cells with endogenous p53 have shown that the Arg72 variant of p53 is able to induce apoptosis at least five times better than the Pro72 variant (21). This enhanced apoptotic potential seems to be correlated with the greater ability of the Arg72 variant to localize to the mitochondria and induce the release of cytochrome c into the cytosol (21).

In the present study we evaluated the frequency of p53 gene polymorphism at codon 72 in a consecutive series of 170 RA patients of Italian origin. They were compared with a group of 200 healthy people from the same geographic area. To test the hypothesis that there is an association between this gene polymorphism and the outcome of RA, we evaluated the presence and number of radiological erosions in the joints of the hands and feet at 5 years from disease onset.

Patients and methods

Study population

One hundred and seventy consecutive RA patients fulfilling the 1997 ACR criteria (22) and seen during a 4-month period in our rheumatological centre were studied. All of these patients were Caucasian, of Italian descent, and had been resident in Italy for at least 2 generations. No ethnic differences were found between the patients and controls. None of the study participants had a Jewish background. The medical

Competing interests: none declared.

records of the patients were reviewed for the presence of rheumatoid factor (patients were considered to be seropositive if on the Waaler-Rose test they had a titre > 1:64 or their nephelometric RF determination was > 40 IU/ml on two or more occasions), the presence of antinuclear antibody, age at disease onset, duration of the disease, and the presence of extra-articular features (EAFs).

As EAF we considered the following: subcutaneous nodules, pulmonary involvement (fibrosing alveolitis and pleuritis), cardiac involvement (pericarditis), cutaneous vasculitis, Sjögren's syndrome, Felty's syndrome, neuropathy, and amyloidosis. The diagnostic definition of each EAF has been reported in a previous study (23).

Radiographs of the hands and feet taken at disease onset and after 5 years were available for 122 patients and were read separately by two authors (PM, CS) without knowledge of the identity of the patients. A blind consensus result was given when necessary. We evaluated the wrists, metacarpophalangeal and proximal interphalangeal joints of the hands, and the metatarsophalangeal and interphalangeal joints of the big toes, for the presence of erosions and the number of eroded joints. The same observers evaluated the radiographs of the hands and feet according to the modified Sharp/van der Heijde (S/vdH) method and the mean score assigned by the two observers was used for the data analyses (24).

The control group consisted of 200 healthy subjects who were unrelated volunteers blood donors matched for age and sex with the patients.

Molecular analysis of p-53 gene polymorphism

Genomic DNA was isolated from 500 µl whole blood collected in edetic acid. DNA was extracted from whole peripheral blood of healthy volunteers and RA patients by a standard method with phenol, chloroform and isoamyl alcohol.

The primers for p53 codon 72 polymorphism, in accordance with Largey *et al.* (25), were:

Forward 5'GATGCTGTCCGCGGACGATATT 3'

Reverse 5' CGTGCAAGTCAGACTTGGC 3'.

The polymerase chain reaction (PCR) was carried out in a P.E. 9600 (Perkin Elmer, Cetus, Norwalk, CT, USA) thermal cycler in a 50 µl reaction volume containing 100 ng template DNA, 50 mM KCl, 10 mM Tris-HCl, 0.1% Triton X-100, 200 µM each of dATP, dCTP, dGTP, dTTP (Amersham Pharmacia Biotech, Piscataway, NJ, USA), 2.5 mM MgCl₂, 0.5 µM of each primer and 1 U Taq DNA polymerase (Perkin Elmer).

Following an initial denaturation step (2 min at 95°C), samples were subjected to 35 cycles of 95°C for 30 sec, 57°C for 30 sec, and 72°C for 30 sec with a final extension time of 5 min at 72°C.

The PCR products were digested with restriction endonuclease Bst UI (New England Biolabs, Ipswich, MA, USA) and restriction fragments were analyzed on 2% agarose gel. The fragment of homozygote Pro/Pro (C/C) gave only an undigested band of 259 bp, the Arg/Arg (G/G) homozygote gave 2 bands of 160 bp and 99 bp while the heterozygote Arg/Pro (C/G) gave 3 bands of 259 bp, 160 bp and 99 bp (Fig. 1).

Statistical analysis

Statistical analysis was carried out using the SPSS statistical package (SPSS Inc., Chicago, Illinois, USA). The Mann-Whitney test was computed to compare the means. The frequencies of the alleles and genotypes among the

case patients and control groups were determined and compared by the chi-square test. The odds ratios were calculated, together with their 95% confidence intervals.

Results

Table I shows the main demographic and clinical characteristics of the total group of 170 RA patients and of the 122 patients whose radiographs were available after 5 years of disease.

The allele and genotype frequencies of Arg/Pro 72 in the population and in the control group are shown in Table II. The distribution of the polymorphism of Arg/Pro 72 did not differ significantly between patients and healthy controls.

The disease duration, age at RA onset, percentage with a positive RF, percentage with ANA positivity, duration of follow-up and the use of second line anti-rheumatic drugs did not differ between the groups of patients with the carriage rate of Pro 72.

The percentage of patients with radiologically evident joint erosions on hand and feet x-ray examination after 5 years from disease onset differed significantly among the 3 genotype populations. The highest percentage of patients with at least one eroded joint were found among those carrying the Pro/Pro genotype. The mean number of eroded joints per patient was higher in the Pro/Pro subgroup and significantly lower in the Arg/Arg subgroup. The mean S/vdH erosive severity score,

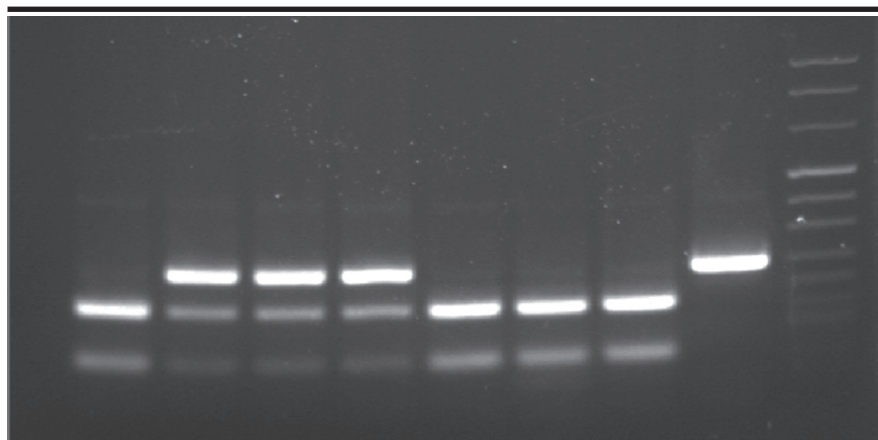


Fig. 1. Lane 1: PCR negative control; lanes 2-6-7-8: Arg72/Arg 72 (G/G genotype); lanes 3-4-5: Arg72/Pro72 (C/G genotype); lane 9: Pro72/Pro72 (C/C genotype); lane 10: Molecular Weight Marker VIII.

Table I. Main demographic and clinical characteristics of all 170 RA patients and of the 122 patients whose radiographs were available after 5 years of disease.

Characteristics	170 RA patients (SD)	122 patients with Rx (SD)
Age (yrs.)	61.4 (12.7)	61 (12.1)
Sex (% F/M)	78/22	76/24
Age at disease onset (yrs.)	51.8 (13.1)	51.9 (12.5)
Disease duration (mos.)	115.4 (83.2)	109.4 (62.5)
RF positive (%)	78.3	79.7
ANA positive (%)	31.9	29.7
Number of eroded joints		5.5 (6.5)
Patients with erosions (%)		69.7
S/vdH erosive score*		10.15 (14.2)
S/vdH joint narrowing score*		18.9 (19.5)
S/vdH total score*		29.1 (32.1)
Patients with EAFs (%)	36.6	34.1
EORA (%)	27.2	27.9

EAF: extra-articular features; EORA: elderly onset rheumatoid arthritis; RF: rheumatoid factor; S/vdH: Sharp/van der Heijde.

*97 patients

Table II. Allele and genotype frequencies of Arg/Pro 72 p53 gene polymorphism in the RA patients and in the control group.

	RA patients (170)	Healthy controls (200)	p-value	OR (CI)
<i>Alleles</i>				
Arg	234/340 (0.69)	278/400 (0.42)	ns	1.01 (0.5-2.0)
Pro	106/340 (0.31)	122/400 (0.58)	ns	1.05 (0.7-1.6)
<i>Genotypes</i>				
Arg/Arg	80/170 (47.1)	97/200 (48.5)	(ns)	
Arg/Pro	74/170 (43.5)	84/200 (42.0)		
Pro/Pro	17/170 (9.8)	19/200 (9.5)		

Table III. Demographic and clinical characteristics of the 122 patients according to the genotype of p53codon 72 Arg/Pro polymorphism.

	Genotype Arg/Arg (A) (54)	Genotype Arg/Pro (B) (54)	Genotype Pro/Pro (C) (14)	P AvsC	P BvsC	P AvsB
Age (yrs.)	60 (12)	63 (13)	59 (12)	ns	ns	ns
Female %	87	68	65	0.042	ns	ns
Disease duration (mos.)	124 (92)	117 (75)	137 (94)	ns	ns	ns
Disease onset (yrs.)	50 (14)	53 (14)	48 (12)	ns	ns	ns
EAF %	33	36	31	ns	ns	ns
EORA %	22	37	14	ns	ns	ns
RF+ %	73	83	87	ns	ns	ns
ANA+ %	34	28	22	ns	ns	ns
Patients with erosive disease %	52	81	93	0.0001	ns	0.001
Number of eroded joints (mean)	3.6 (5)	5.4 (6)	13.2 (8)	0.0001	0.0001	0.017
S/vdH erosive score	7.05 (10.7)	7.6 (9.4)	37.3 (14.8)	0.0001	0.0001	ns
S/vdH joint space narrowing score	15.7 (15.3)	17.3 (17.7)	48.9 (20.6)	0.0001	0.0001	ns
S/vdH total score	22.8 (24.8)	24.9 (23.1)	86.2 (34.9)	0.0001	0.0001	ns

EAF: extra-articular features; EORA: elderly onset rheumatoid arthritis; RF: rheumatoid factor; S/vdH: Sharp/van der Heijde.

joint space narrowing severity score, and total damage score were significantly higher in the Pro/Pro subgroup compared to the Arg/Arg and Arg/Pro genotype subgroups (Table III).

There was a significantly higher prevalence of females in the Arg/Arg genotype, with no differences for the other clinical and demographic characteristics among the 3 genotypes.

The group of patients carrying the Pro allele had a higher percentage of erosive disease and a higher mean number of eroded joints per patient when compared with the group that was negative for the Pro allele (Table IV). Furthermore, Pro/Pro homozygote patients had a higher mean number of eroded joints per patient, a higher S/vdH erosive score, a higher S/vdH JSN score, and a higher S/vdH total damage score when compared with the Arg allele positive group (Table IV).

Comparisons between the different subgroups of RA patients and the control population for the distribution of the allelic variant of codon 72 p53 polymorphism (RF positive, RF negative, with or without EAF, with or without erosive disease, with disease onset before or after the age of 60 years) did not produce any statistically significant data (data not shown).

Discussion

Only two other studies in diverse populations have evaluated the association between p53 codon 72 polymorphism and RA susceptibility (26, 27). In a Korean study no significant differences were found in the distribution of this polymorphism in 114 RA patients and 114 healthy controls. In a study conducted in Germany (27), the frequency of p53 Arg/Pro codon 72 polymorphism in a series of 16 patients with juvenile chronic arthritis (JCA) and 15 patients with RA was evaluated. The distribution of the 3 allelic types was similar to that in the normal healthy Caucasian controls and did not differ from the results of other published studies. A predominance of the Pro/Pro genotype was observed in the subgroup of JCA patients, but the difference was not significant (18.8% vs 10.2% in normal controls and 6.7% in RA). The results

Table IV. Demographic and clinical characteristics of 122 patients according to the carriage rate of p53codon 72 Arg/Pro polymorphism.

	Carriage rate Pro/Pro+ Pro/Arg (68)	Carriage rate Arg/Arg (54)	<i>p</i>	Carriage rate Pro/Pro (14)	Carriage rate Arg/Arg Pro/Arg (108)	<i>p</i>
Age (yrs.)	62 (13)	60 (12)	ns	59 (12)	62 (13)	ns
Female %	72	87	ns	65	79	ns
Disease duration (mos.)	122 (79)	124 (92)	ns	137 (94)	121 (85)	ns
Disease onset (yrs.)	52 (13)	50 (14)	ns	48 (12)	51 (14)	ns
EAF %	37	33	ns	31	36	ns
EORA %	30	22	ns	14	29	ns
RF+ %	83	73	ns	87	76	ns
ANA+ %	28	34	ns	22	34	ns
Patients with erosive disease %	84	52	0.0001	93	67	0.062
Number of eroded joints (mean)	7.1 (7.1)	3.6 (5.1)	0.003	13.3 (7.6)	4.5 (5.7)	0.0001
S/vdH erosive score	13.3 (16.6)	7.05 (10.7)	0.027	37.3 (14.8)	7.0 (9.9)	0.0001
S/vdH joint space narrowing score	22.9 (22.7)	15.7 (15.3)	0.044	48.9 (20.6)	15.4 (16.0)	0.0001
S/vdH total score	36.2 (37.0)	22.8 (24.8)	0.027	86.2 (34.9)	22.4 (23.6)	0.0001

EAF: extra-articular features; EORA: elderly onset rheumatoid arthritis; RF: rheumatoid factor; S/vdH: Sharp/van der Heijde.

of our study in an Italian RA population confirm the absence of an influence of the p53 codon 72 polymorphism on the occurrence of RA.

The association between the clinical features of RA and p53 codon 72 polymorphism was also investigated in the Korean study (26). No association with age at disease onset, functional class, extra-articular involvement, RF titre or the frequency of joint surgery was found. No significant differences were found in our RA Italian population when demographic, clinical or serological variables were compared among the 3 p53 codon 72 genotypes or the controls.

The most relevant conclusion of our study was the observation of an association between p53 codon 72 polymorphism and the development of joint erosions that seemed to be independent of any other clinical or demographic characteristic of the patient population. We can speculate that the differing apoptotic potential of the two p53 variant could lead to more aggressive disease in patients carrying the Pro/Pro genotype. To address this hypothesis, it would be of interest to conduct studies on the apoptotic sensitivity of synovio-

cytes from Pro/Pro patients. Defective apoptosis is considered to be one of the mechanisms of cellular accumulation in RA joints (2-4).

Mutations in the p53 molecules, induced by the long-standing inflammation and oxidative stress present in synovial RA tissue that reduces its pro-apoptotic activity, have been described in several cellular populations in the joints of RA patients and different lines of evidence have correlated these mutations with more erosive disease. In addition, a role of p53 mutation on the increased production of IL-6 by synovial lining and sublining cells has been suggested (28, 29). To our knowledge no study has analysed whether there are differing susceptibilities of the p53 variant molecules to damage induced by oxidative stress in inflamed joints. Several drugs used for the treatment of RA [steroids, methotrexate (MTX) and NSAIDs] were found to enhance p53 gene expression in different cellular models, and probably its apoptotic activity. Moreover, MTX (30, 31), sulphasalazine (32, 33), anti-tumor necrosis factor drugs (34) and rituximab (35, 36) may at least partially control synovial inflammation through the in-

duction of apoptosis in different cells. All of these reports confirm a role of p53 molecule on the clinical evolution of RA. As has been hypothesized in the case of cancer (21), response to treatment in RA could be in part dependent on the differing sensitivity of p53 codon 72 polymorphism to apoptosis.

Our study has some limitations because of the small number of patients carrying the Pro/Pro genotype in the cohort and also because we did not analyse other genes known to contribute to erosive disease (namely MHC genes and non-MHC genes located at chromosome 6p and at other chromosomes). We cannot exclude the possibility of a linkage with some of these genes.

In summary, the results of our study support the hypothesis that in the Italian population there is no association between codon Arg/Pro 72-p53 gene polymorphism and the occurrence of RA, but that this polymorphism is associated with the structural damage of RA. Further independent studies are needed to confirm the influence of this polymorphism on the progression of erosive RA.

References

1. HARRIS ED JR: Rheumatoid arthritis. Pathophysiology and implication for therapy. *N Engl J Med* 1990; 322: 1277-89.
2. LIU H, POPE RM: Apoptosis in rheumatoid arthritis: friend or foe. *Rheum Dis Clin North Am* 2004; 30: 603-25.
3. BAIER A, MEINECKEL I, GAY S, PAP T: Apoptosis in rheumatoid arthritis. *Curr Opin Rheumatol* 2003; 15: 274-9.
4. NISHIOKA K, HASUNUMA T, KATO T, KOBATA T: Apoptosis in rheumatoid arthritis: a novel pathway in the regulation of the synovial tissue. *Arthritis Rheum* 1998; 41: 1-9.
5. FIRESTEIN GS, NGUYEN K, AUPPERLE KR, YEO M, BOYLE DL, ZVAIFLER NJ: Apoptosis in rheumatoid arthritis: p53 overexpression in rheumatoid arthritis synovium. *Am J Pathol* 1996; 149: 2143-51.
6. TAK PP, SMEETS TJ, BOYLE DL *et al.*: p53 overexpression in synovial tissue from patients with early and longstanding rheumatoid arthritis compared with patients with reactive arthritis and osteoarthritis. *Arthritis Rheum* 1999; 42: 948-53.
7. MAAS K, WESTFALL M, PIETENPOL J, OLSEN NJ, AUNE T: Reduced p53 in peripheral blood mononuclear cells from patients with rheumatoid arthritis is associated with loss of radiation-induced apoptosis. *Arthritis Rheum* 2005; 52: 1047-57.
8. SIMELYTE E, ROSENGREN S, BOYLE DL, CORR M, GREEN DR, FIRESTEIN GS: Regulation of arthritis by p53. Critical role of adap-

- tive immunity. *Arthritis Rheum* 2005; 52: 1876-84.
9. KOBAYASHI T, OKAMOTO K, KOBATA T, HASUNUMA T, KATO T, HAMADA H, NISHIOKA K: Differential regulation of Fas-mediated apoptosis of rheumatoid synoviocytes by tumor necrosis factor alpha and basic fibroblast growth factor is associated with the expression of apoptosis-related molecules. *Arthritis Rheum* 2000; 43: 1106-14.
10. MATSUMOTO S, MULLER-LADNER U, GAY RE, NISHIOKA K, GAY S: Ultrastructural demonstration of apoptosis, Fas and Bcl-2 expression of rheumatoid synovial fibroblasts. *J Rheumatol* 1996; 23: 1345-52.
11. SIOUD M, MELLBYE O, FORRE O: Analysis of the NF-kappa B p65 subunit, Fas antigen, Fas ligand and Bcl-2-related proteins in the synovium of RA and polyarticular JRA. *Clin Exp Rheumatol* 1998; 16: 125-34.
12. SUGIYAMA M, TSUKAZAKI T, YONEKURA A, MATSUZAKI S, YAMASHITA S, IWASAKI K: Localisation of apoptosis and expression of apoptosis-related proteins in the synovium of patients with rheumatoid arthritis. *Ann Rheum Dis* 1996; 55: 442-9.
13. KUROWSKA M, RUDNICKA W, KONTNY E *et al.*: Fibroblast-like synoviocytes from rheumatoid arthritis patients express functional IL-15 receptor complex: endogenous IL-15 in autocrine fashion enhances cell proliferation and expression of Bcl-x(L) and Bcl-2. *J Immunol* 2002; 169: 1760-7.
14. PERLMAN H, PAGLIARI LJ, LIU H, KOCH AE, HAINES GK 3RD, POPE RM: Rheumatoid arthritis synovial macrophages express the Fas-associated death domain-like interleukin-1beta-converting enzyme-inhibitory protein and are refractory to Fas-mediated apoptosis. *Arthritis Rheum* 2001; 44: 21-30.
15. YAO Q, WANG S, GLORIOSO JC *et al.*: Gene transfer of p53 to arthritic joints stimulates synovial apoptosis and inhibits inflammation. *Mol Ther* 2001; 3: 901-10.
16. MARTIN DA, ELKON KB: Mechanisms of apoptosis. *Rheum Dis Clin North Am* 2004; 30: 441-54.
17. VOUSDEN KH: p53: death star. *Cell* 2000; 103: 691-4.
18. EVAN G, LITTLEWOOD T: A matter of life and cell death. *Science* 1998; 281: 1317-22.
19. MATLASHEWSKI GJ, TUCK S, PIM D, LAMB P, SCHNEIDER J, CRAWFORD LV: Primary structure polymorphism at amino acid residue 72 of human p53. *Mol Cell Biol* 1987; 7: 961-3.
20. THOMAS M, KALITA A, LABRECQUE S, PIM D, BANKS L, MATLASHEWSKI G: Two polymorphic variants of wild-type p53 differ biochemically and biologically. *Mol Cell Biol* 1999; 19: 1092-100.
21. DUMONT P, LEU JI, DELLA PIETRA AC 3RD, GEORGE DL, MURPHY M: The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. *Nat Genet* 2003; 33: 357-65.
22. ARNETT FC, EDWORTHY SM, BLOCH DA *et al.*: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-24.
23. MACCHIONI P, BOIARDI L, CASALI B, NICOLI D, FARNETTI E, SALVARANI C: Inter-cellular adhesion molecule 1 (ICAM-1) gene polymorphisms in Italian patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2000; 18: 553-8.
24. VAN DER HEIJDE DMFM: How to read radiographs according to the Sharp/van der Heijde method. *J Rheumatol* 1999; 26: 743-5.
25. LARGEY JS, MELTZER SJ, YIN J, NORRIS K, SAUK JJ, ARCHIBALD DW: Loss of heterozygosity of p53 in oral cancers demonstrated by the polymerase chain reaction. *Cancer* 1993; 71: 1933-7.
26. LEE YH, KIM YR, JI JD, SOHN J, SONG GG: p53 codon 72 polymorphism and rheumatoid arthritis. *J Rheumatol* 2001; 28: 2392-4.
27. TAUBERT H, THAMM B, MEYE A *et al.*: The p53 status in juvenile chronic arthritis and rheumatoid arthritis. *Clin Exp Immunol* 2000; 122: 264-9.
28. MARGULIES L, SEHGAL PB: Modulation of the human interleukin-6 promoter (IL-6) and transcription factor C/EBP beta (NF-IL6) activity by p53 species. *J Biol Chem* 1993; 268: 15096-100.
29. YAMANISHI Y, BOYLE DL, ROSENGREN S, GREEN DR, ZVAIFLER NJ, FIRESTEIN GS: Regional analysis of p53 mutations in rheumatoid arthritis synovium. *Proc Natl Acad Sci USA* 2002; 99: 10025-30.
30. GENESTIER L, PAILLOT R, FOURNEL S, FERRARO C, MIOSEC P, REVILLARD JP: Immunosuppressive properties of methotrexate: apoptosis and clonal deletion of activated peripheral T cells. *J Clin Invest* 1998; 102: 322-8.
31. NAKAZAWA F, MATSUNO H, YUDOH K, KATAYAMA R, SAWAI T, UZUKI M, KIMURA T: Methotrexate inhibits rheumatoid synovitis by inducing apoptosis. *J Rheumatol* 2001; 28: 1800-8.
32. RODENBURG RJ, GANGA A, VAN LENT PL, VAN DE PUTTE LB, VAN VENROOIJ WJ: The antiinflammatory drug sulfasalazine inhibits tumor necrosis factor alpha expression in macrophages by inducing apoptosis. *Arthritis Rheum* 2000; 43: 1941-50.
33. LIPTAY S, FULDA S, SCHANBACHER M *et al.*: Molecular mechanisms of sulfasalazine-induced T-cell apoptosis. *Br J Pharmacol* 2002; 137: 608-20.
34. CATRINA AI, TROLLMO C, af KLINT E *et al.*: Evidence that anti-tumor necrosis factor therapy with both etanercept and infliximab induces apoptosis in macrophages, but not lymphocytes, in rheumatoid arthritis joints: extended report. *Arthritis Rheum* 2005; 52: 61-72.
35. ALAS S, BONAVIDA B: Rituximab inactivates signal transducer and activation of transcription 3 (STAT3) activity in B-non-Hodgkin's lymphoma through inhibition of the interleukin 10 autocrine/paracrine loop and results in down-regulation of Bcl-2 and sensitization to cytotoxic drugs. *Cancer Res* 2001; 61: 5137-44.
36. SZODORAY P, ALEX P, DANDAPANI V *et al.*: Apoptotic effect of rituximab on peripheral blood B cells in rheumatoid arthritis. *Scand J Immunol* 2004; 60: 209-18.