American Journal of Pathology, Vol. 149, No. 6, December 1996
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Apoptosis in Rheumatoid Arthritis

p53 Overexpression in Rheumatoid Arthritis Synovium

Gary S. Firestein, Katherine Nguyen, Karlfried R. Aupperle, Michele Yeo, David L. Boyle, and Nathan J. Zvaifler

From the University of California, San Diego, School of Medicine, San Diego, California

DNA damage induces p53 tumor suppressor gene expression and protein production, which in turn facilitates DNA repair or apoptosis. Wild-type p53 protein has a short half-life, so it is rarely detected in non-neoplastic tissue. Because DNA fragmentation is abundant in the intimal lining in rheumatoid arthritis (RA) synovial tissue (ST) using* in situ *end-labeling (Firestein GS, Yeo M, Zvaifler NJ: Apoptosis in rheumatoid arthritis synovium. J Clin Invest 1995, 96:1631–1638), we assessed ST p53 expression. Immunohistochemical analysis of fixed RA synovium using antibody PAb 1801 showed prominent p53 staining in the cytoplasm and nuclei of intimal lining cells. Noninflammatory and osteoarthritis (OA) ST had significantly less p53 in the lining. These data were confirmed by Western blot analysis of ST extracts, with abundant p53 found in RA compared with OA. p53 expression in cultured fibroblast-like synoviocytes (FLS) was then examined. Flow cytometry on permeabilized cells showed that RA FLS constitutively express p53 protein. Western blots showed that RA FLS expressed significantly more p53 than either OA FLS or dermal fibroblasts. Immunohistochemistry of FLS cultured in chamber slides localized the p53 to the cytoplasm of most resting FLS, with nuclear staining in only 10.7 ± 2.4%. Exposure to hydrogen peroxide for increased nuclear staining to 70.7 ± 12.8% after 8 hours (P = 0.003). These data indicate that p53 is overexpressed in RA ST in the intimal lining, which is the primary site of DNA damage, and is constitutively expressed by FLS. (Am J Pathol 1996, 149:2143–2151)

The p53 tumor suppressor is a nuclear phosphoprotein that serves as a critical regulator of cell survival and proliferation. It functions as a transcriptional activator of genes that block progression from G1 to S phase in mammalian cells, inhibits angiogenesis, and regulates a variety of DNA repair mechanisms.[1] p53 expression increases in response to DNA damage, and the subsequent cell cycle prolongation permits DNA repair or, in the severe cases, leads to apoptosis. Loss of p53 function, either through mutation, deletion, or other mechanisms, is often associated with neoplasia, whereas reintroduction of the gene suppresses tumor growth.[2–4] Mutations of the p53 gene are found at high frequency in a large number of human cancers.[5,6] Such mutations often prolong the half-life of the protein (which is normally very short) and permit its detection in tumors using immunohistochemistry or Western blot analysis.[7–9] The very ability to detect the protein reportedly indicates missense mutations in epithelial tumors.[10–12]

We recently demonstrated that extensive DNA fragmentation occurs in the rheumatoid synovium, especially in the intimal lining.[13] Similar DNA strand breaks were induced in cultured fibroblast-like synoviocytes (FLS) when exposed to tumor necrosis factor (TNF)-α or anti-Fas antibody. Hence, the environment of the inflamed joint, with local cytokines and oxygen radicals, likely contributes to DNA damage in the rheumatoid arthritis (RA) synovial lining.[14] Despite the presence of DNA fragmentation in up to 50% of synoviocytes *in situ*, only rare cells actually complete apoptosis as determined by morphological criteria. Although the removal of apoptotic bodies is admittedly very fast, the extent of strand breaks is so great that this disparity suggests ineffective apoptosis.

Supported in part by National Institutes of Health grants AR40525, AR42200, 5 T32 AR07062, and 1 P60 AR40770 and grants from Pfizer Central Research and the Deutsche Akademische Austauschdienst.

Accepted for publication August 13, 1996.

Address reprint requests to Dr. Gary S. Firestein, Division of Rheumatology, 0656, UCSD School of Medicine, 9500 Gilman Drive, La Jolla, CA, 92093-0656.

These data led us to investigate the expression of p53 as a key regulator of DNA repair and apoptosis in the RA joint. Our studies demonstrate that p53 is expressed in RA synovium, especially in the intimal lining where abundant DNA strand breaks occur. Furthermore, cultured FLS, unlike dermal fibroblasts (DF), constitutively express p53, and reactive oxygen species alter the intracellular distribution of p53 protein. The presence of substantial amounts of p53 in a chronic inflammatory, non-neoplastic human disease may result from the toxic environment of the joint.

Materials and Methods

Reagents

The following reagents were purchased from Sigma Chemical Co. (St. Louis, MO): sodium citrate, sodium chloride, EDTA, Tris/HCl, glycine, collagenase, diaminobenzidine, bovine serum albumin (BSA), paraformaldehyde, Tween-20, saponin, methanol, and gelatin. Monoclonal antibodies PAb 240 and PAb 1801 were obtained from Oncogene, Cambridge, MA.

Patient Selection and Tissue Preparation

FLS were isolated from RA synovial tissues obtained at joint replacement surgery as previously described.[15] The diagnosis of RA conformed to the 1987 revised American College of Rheumatology criteria.[16] Tissue from nine RA patients (mean age, 66 ± 4 years; six females and three males), five OA patients (mean age, 69 ± 4 years; four females and one male), and three patients with no inflammatory disease (mean age, 52 ± 5 years; three males: one normal, one avascular necrosis, and one post-traumatic). The tissues were minced and incubated with 1 mg/ml collagenase in serum-free RPMI 1640 (Gibco, Grand Island, NY) for 2 hours at 37°C, filtered through a nylon mesh, extensively washed and cultured in DMEM supplemented with 10% fetal calf serum (FCS) in a humidified 5% CO_2 atmosphere. After overnight culture, non-adherent cells were removed and adherent cells were cultivated in Dulbecco's minimal essential medium (DMEM) plus 10% FCS. FLS were used from passages 3 through 10, during which time they were a homogeneous population of FLS (<1% CD11b positive, <1% phagocytic, and <1% FcR II and FcR III receptor positive). Dermal fibroblast lines were similarly prepared from enzymatically dispersed skin biopsies. All samples were obtained in accordance with the University of California, San Diego, Institutional Review Board.

Inflammation Scores

Synovial inflammation was analyzed as previously described.[17] Hematoxylin and eosin (H&E)-stained sections were coded and randomly analyzed. The inflammation score was determined by the sum of three components: synovial intimal lining thickness (0, 1 to 2 cells; 1+, 2 to 4 cells; 2+, 5 to 8 cells; 3+, >8 cells), subintimal mononuclear cell infiltration (0, <5% of field; 1+, 5 to 33%;, 2+, 33 to 67%; 3+, >67%), and lymphoid aggregates (0, 0 to 1/low-power field (lpf); 1+, 2 to 4/lpf; 2+, 5 to 7/lpf; 3+, >7/lpf).

Immunoperoxidase Assays

Tissue Sections

Five-micron paraffin-embedded sections of synovial tissue (ST) were mounted on glass slides, fixed in acetone, and then rehydrated in phosphate-buffered saline (PBS) plus 0.1% BSA. Slides were preincubated with 10% horse serum followed by 10% human AB serum. The primary antibody was then added for 45 minutes. The PAb 1801 anti-p53 antibody detects an epitope between amino acids 46 and 55 at the amino-terminal portion of both wild-type and mutant p53.[18,19] After 2 washes in PBS plus 0.1% BSA, biotinylated horse anti-mouse antibody (Vector Laboratories, Burlingame, CA) in 10% human AB serum was added for 30 minutes. The slides were washed and endogenous peroxidase was depleted with 0.3% hydrogen peroxide in PBS for 20 minutes. The sections were then incubated with ABC horseradish peroxidase complex for 30 minutes (Vector Laboratories). The peroxidase was developed with 0.5 mg/ml diaminobenzidine and 0.02% hydrogen peroxide and subsequently counterstained lightly with dilute hematoxylin. Intensity of staining in the synovial lining and sublining mononuclear cells was judged on a 0 to 4+ scale as follows: 0, no staining; 1+, rare positive cells or trace staining; 2+, scattered clusters of positive cells; 3+, moderate staining in a specific region; 4+, extensive staining throughout a region. This scale has been validated using several other synovial markers.[17]

Cultured Fibroblast-Like Synoviocytes

Isolated dermal or synovial fibroblasts from lines that have been passaged two to eight times were plated into four-well Lab-Tek chamber slides (Nunc, Naperville, IL) at approximately 6 × 10^3 cells/well. They were maintained at 37°C in complete medium (DMEM supplemented with 10% FCS) in a humidified

5% CO_2 atmosphere. During this time they adhered and spread. After 3 days, the medium was removed and replaced with 0.2 ml of DMEM/well for 18 hours to synchronize their cell cycles. They were then switched to 10% FCS in DMEM with medium or 0.2 mmol/L hydrogen peroxide. After 100 minutes, the cells were washed twice with DMEM and replaced with 200 μl of DMEM supplemented with 10% FCS. At predetermined time intervals, cells were washed three times in Hanks' balanced salt solution, vacuum dried for 1 hour, and fixed with 4% phosphate-buffered (pH 7.4) paraformaldehyde at 4°C for 15 minutes. They were stored in Hanks' balanced salt solution at 4°C until immunostaining. At this time, they were washed and then blocked with 0.1% BSA, 1% horse serum, and 1% human serum sequentially. The cells were then stained by the immunoperoxidase technique, except that all protein solutions, washing solutions, and antibodies contained 0.1% saponin as a permeabilizing agent. After immunostaining, the slides were counterstained lightly with hematoxylin, fixed with 95% ethanol, and mounted. The cell populations were analyzed at ×400 magnification. Approximately 600 to 800 cells per slide were examined and the proportion of cells with nuclear staining determined.

Flow Cytometry

FLS (1×10^5 to 2×10^5) were cultured in six-well plastic dishes (Costar Corp., Cambridge, MA) in DMEM/10% FCS. Cells were washed and harvested with 0.01% trypsin at 37°C, washed, and fixed for 15 minutes in 4% paraformaldehyde on ice. The cells were then washed with 0.1% BSA in PBS and stained with PAb 1801 or control antibody in 0.1% saponin in PBS for 40 minutes. Cells were then washed and preincubated with 1% goat serum for 5 minutes on ice and incubated with PAb 1801 or an isotype-matched monoclonal antibody for 40 minutes on ice. Longer incubations or varying the temperature of this process did not alter the results. The secondary antibody, phycoerythrin-conjugated Fab_2 goat anti-mouse IgG (Tago, Burlingame, CA) was then added for 30 minutes. The samples were then washed twice and fixed again in 1% paraformaldehyde. A total of 5×10^3 to 10×10^3 cells were analyzed by flow cytometry. A cell was defined as a positive when its mean fluorescence channel was >95% of cells stained with the control antibody.

Table 1. *Expression of Immunoreactive p53 in Synovium*

	Lining	Sublining mononuclear cells	Inflammation score
RA (n = 9)	2.9 ± 0.3*	0.9 ± 0.2	6.0 ± 0.5*
OA (n = 5)	1.8 ± 0.4	0.4 ± 0.2	2.6 ± 0.2†
Noninflammatory (n = 3)‡	0.7 ± 0.3	0.3 ± 0.3	1.3 ± 0.3

Semiquantitative scales for staining and inflammation are described in Materials and Methods.
*$P \leq 0.05$ compared with OA or noninflammatory ST.
†$P \leq 0.03$ compared with noninflammatory ST.
‡One normal, one avascular necrosis, and one post-traumatic.

Western Blot Analysis

Protein samples (20 μg/lane) from FLS (10^6 cells) or ST (100 mg of tissue) lysates were run on a 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis gel and transferred onto a nitrocellulose membrane at 140 mA in 25 mmol/L Tris/HCl, pH 8.3, 192 mmol/L glycine, 10% methanol. Filters were blocked with Tris-buffered saline plus 0.5% Tween-20 and 1.5% gelatin for 45 minutes. This was followed by incubation with PAb 240 (0.1 μg/ml)[20] at 4°C overnight. The membrane was washed three times and incubated with goat anti-mouse peroxidase antibody (Boehringer Mannheim, Indianapolis, IN) for 2 hours at room temperature. The proteins were visualized by chemiluminescence using hydrogen peroxide and luminol as a substrate (DuPont, Wilmington, DE) using Kodak X-AR film. As the gels were performed under denaturing conditions and PAb 240 detects a denatured conformational determinant, the results do not necessarily reflect detection of a p53 mutation.

Statistical Analysis

Data were analyzed using the unpaired Student's *t*-test.

Results

Expression of p53 Protein in Synovial Tissue

Immunohistochemistry

Fixed, permeabilized sections of RA, OA, and other noninflammatory STs were probed using the monoclonal antibody PAb 1801. Virtually all RA tissues stained intensely in the synovial intimal lining (see Figure 1 for a representative example and Table 1). Most often, the layer of cells in direct contact with the intra-articular space (and synovial fluid) was pos-

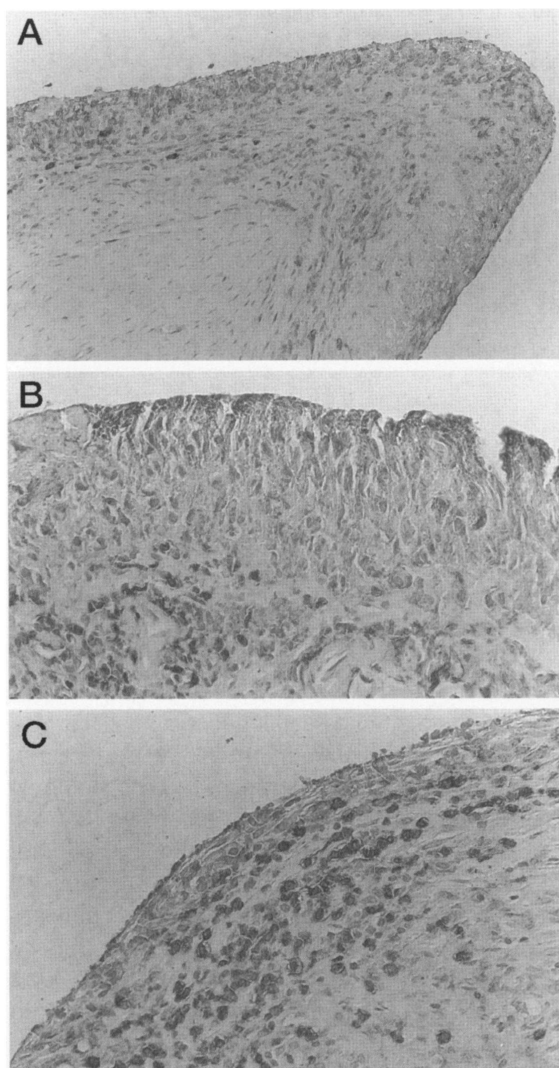

Figure 1. *p53 protein in RA synovial tissue.* **A**: *The synovial intimal lining is the area of most intense staining using the immunoperoxidase method. Magnification, ×100. Tissues were stained using PAb 1801 and lightly counterstained with hematoxylin.* **B**: *A higher-power view from the same patient shows that staining is cytoplasmic and nuclear in the intimal lining cells. Magnification, ×400.* **C**: *Another region from the same patient shows intimal lining staining as well as intense nuclear staining in mononuclear cells immediately below the lining. Magnification, ×400.*

itive. The p53 protein was detected in both the cytoplasm and nuclei of lining cells, although cytoplasmic staining tended to be more prominent in the intimal lining whereas nuclear staining occurred more often in the sublining layer (see Figure 1). Lesser amounts of immunoreactive p53 were detected in the sublining mononuclear cells. Occasional positive cells were identified within lymphoid aggregates, but the majority of p53-expressing sublining cells were scattered throughout the sublining or clustered around blood vessels.

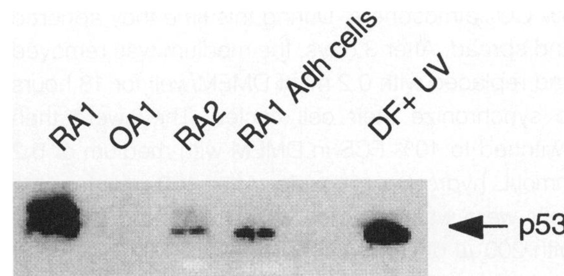

Figure 2. *Western blot analysis showing immunoreactive p53 in synovial tissue. Representative STs are shown for RA (RA1 and RA2) and OA (OA). In addition, the adherent cells from enzymatically dispersed RA1 ST contained p53. Ultraviolet-irradiated DFs are shown as a positive control.*

Samples from non-RA synovia were also examined. OA ST (n = 5) also contained some immunoreactive p53. As with RA, the primary site was the intimal lining, although the amount of staining was significantly less than rheumatoid tissues ($P \leq 0.05$; see Table 1). Minimal or no staining was observed in three noninflammatory STs (normal, post-traumatic arthritis, and avascular necrosis; $P \leq 0.05$ compared with RA; see Table 1).

Western Blot Analysis

To confirm the presence of immunoreactive p53 in RA ST, Western blot analysis was performed on extracts of synovial tissue that had been snap-frozen immediately after removal from the joint. As shown in Figure 2, prominent p53 bands were observed in the lanes containing RA extracts. Of the eight RA STs examined by Western blot analysis, seven contained significant amounts of immunoreactive p53 protein. Control antibodies did not exhibit binding at 53 kd (data not shown). Four RA STs were enzymatically digested and extracts were prepared from the adherent cell population. This population is composed of FLS and macrophage-like synoviocytes but is devoid of lymphocytes. In each case, the immunoreactive p53 was detected (see Figure 2 for an example). Six OA ST extracts were also examined. Four were negative and two contained faint bands, a finding consistent with the immunohistochemistry data (see Table 1).

Expression of p53 Protein in Cultured Fibroblast-Like Synoviocytes

Flow Cytometry

In light of prominent p53 staining in the RA intimal lining, p53 expression was then examined in cultured FLS using flow cytometry. Because p53 is an intracellular antigen, cells were permeabilized with

Table 2. *Expression of p53 Protein in FLS by Flow Cytometry*

	% positive	MFC
RA (n = 7)	50.9 ± 7.9	35.9 ± 7.7
OA (n = 4)	44.2 ± 2.9	24.4 ± 6.3

MFC, mean fluorescence channel

paraformaldehyde and saponin to increase antibody penetration. Table 2 shows that nearly 50% of RA FLS constitutively express immunoreactive p53 (n = 7). A representative histogram is shown in Figure 3. Although there was a trend toward greater amounts of p53 protein in RA FLS compared with OA FLS (n = 4), the difference did not reach statistical significance.

Western Blot Analysis

To confirm constitutive p53 expression by RA FLS, Western blot analysis was also performed on FLS lysates. All RA lines studied with this technique (n = 11) showed abundant immunoreactive p53 (see Figure 4 for representative examples). The intensities of the bands were similar at various passage numbers (from 2 to 8). In OA FLS (n = 4; passages 2 to 8), p53 was also detected, although the amount was substantially less than in RA (see Figure 4). Cultured DF contained only very small amounts of p53 (see Figure 4), although it could be induced to the level of RA FLS by ultraviolet exposure (see Figure 2). Using densitometry, the expression of p53 was 74.2 ± 4.5 absorption units in RA FLS, 24.0 ± 5.8 absorption units in OA FLS, and 4.0 ± 2.4 absorption units in DF ($P < 0.001$ for RA *versus* OA and RA *versus* DF; $P < 0.05$ for OA *versus* DF).

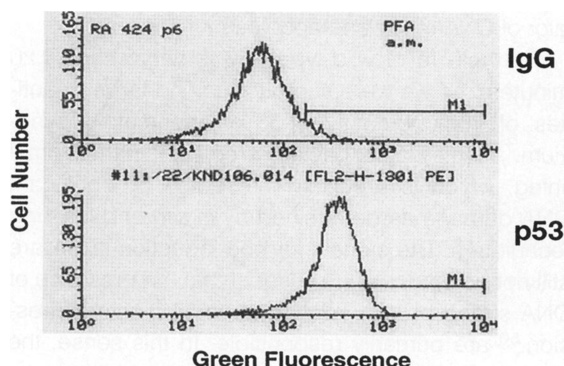

Figure 3. *Flow cytometry shows immunoreactive p53 in a RA FLS line. The* top histogram *shows an isotype-matched control antibody and the* lower histogram *shows PAb 1801.*

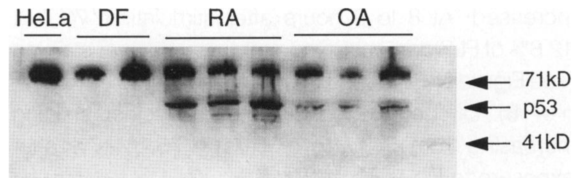

Figure 4. *Western blot analysis showing immunoreactive p53 in FLS. Both RA and OA FLS contain p53 protein, with greater amounts in RA cells. The top band at approximately 80 kd is nonspecific and appears in all lanes. DF contained very small amounts of p53, whereas HeLa cells (American Type Culture Collection, Rockville, MD) were negative.*

Immunohistochemistry

The intracellular location of p53 protein is an important determinant of its functional capabilities.[21] It is known that p53 can reside in the cytoplasm (generally bound to hsc70) but is transported to the nucleus after DNA damage or proliferative signals. Our studies of intact tissue showed both cytoplasmic and nuclear staining (see Figure 1). Immunohistochemistry was then performed on cultured RA FLS to determine the subcellular localization of p53. Faint cytoplasmic staining was detected in the majority of resting FLS, with 10.7 ± 2.4% of cells demonstrating nuclear staining (see Figure 5). If cells were exposed to H_2O_2 for 100 minutes, nuclear p53 protein expression was markedly

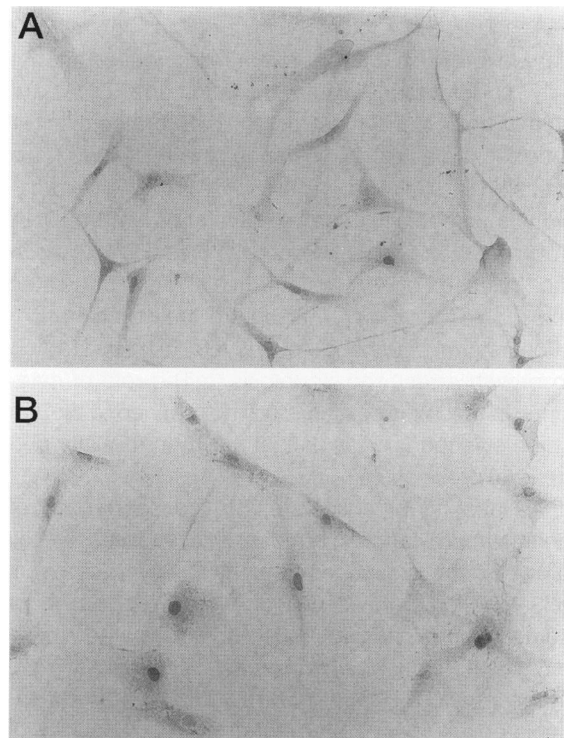

Figure 5. *Immunoperoxidase studies of cultured FLS. FLS were immunostained using PAb 1801 using the immunoperoxidase method. No counterstain was used.* A: *Resting FLS contained primarily cytoplasmic p53 protein.* B: *Hydrogen peroxide markedly increased nuclear staining.*

increased. At 8 to 9 hours after stimulation, 70.7 ± 12.8% of FLS demonstrated prominent nuclear staining (see Figure 5; $P = 0.003$ compared with medium alone; n = 3). DF behaved similarly, with nuclear staining increasing from 5 ± 1% of cells to 75 ± 17% after exposure to H_2O_2.

Discussion

The p53 tumor suppressor is a key regulator of DNA repair and cell replication. Although not an oncogene itself, p53 is under the transcriptional control of oncogenes like c-*myc*[22] and provides the critical signals to arrest cell growth and/or induce apoptosis. p53 has several domains that serve distinct functions. A transactivation region stimulates transcription of a number of genes, including p21^{waf1}, the ribosomal gene cluster, GADD45, and many others.[23–28] In contrast, a transrepression region decreases expression of genes like RB1 and PCNA.[29,30] Many of the suppressed genes regulate cell proliferation, and down-regulation arrests the cell cycle at the G1 phase.[1,31,32] This is especially prominent after cells are transformed by oncogenes like *ras*[33] or sustain DNA damage, thereby leading to apoptosis or providing sufficient time for DNA repair.

Although the regulatory factors that control p53 gene expression and/or protein redistribution are not fully understood, agents that damage DNA are clearly among the most potent p53 inducers.[34] Ultraviolet radiation, for instance, increases p53 expression in skin and in cultured DF.[35] p53 expression is especially high in skin sites where DNA fragmentation can be detected.[36] Hypoxic injury with the release of oxygen radicals also regulates p53.[37,38] Oncogenes that control cell proliferation, such as c-*myc*, enhance p53 levels, thereby leading to growth arrest and apoptosis.[22] The role of cytokines in these processes is still not defined, although some evidence suggests that they can regulate p53 gene expression.[39]

As one might anticipate, the consequences of defective p53 function are dire. As it suppresses cell growth in the presence of damaged DNA, abnormal p53 function permits cell division. Propagation of somatic mutations subsequently ensues when these mutations occur in oncogenes, with ultimate cell transformation. Hence, p53 mutants have been associated with cancer in perhaps as many as 10% of malignancies in humans.[40] This does not imply that p53 mutants cause cancer; more likely, an appropriate mutation is permissive of cell growth induced by other factors. p53 knockout mice also have an increased incidence of tumors and are especially sensitive to the transforming effects of radiation.[41]

Immunoreactive p53 (especially nuclear staining) has been detected in a variety of neoplastic conditions,[42] but the protein is rarely seen in sections of nonmalignant tissue unless mutations or other mechanisms of post-translational stabilization prolong its half-life.[7,8,9,18] Nevertheless, modestly increased amounts of p53 were identified in the acutely inflamed mucosa of two patients with ulcerative colitis,[43] and occasional p53 positive cells are present in reactive lymphoid tissue,[44] benign prostatic hyperplasia,[45] actinic keratoses,[46] and condyloma acuminata.[47] Increased expression of wild-type p53 has been reported in the family of two women with breast cancer.[48] As a rule, however, detectable p53 is associated only with cancer or diseases characterized by a high incidence of malignant transformation. The extent and intensity of p53 staining observed in nonmalignant RA synovium has no precedent in the literature.

Our interest in p53 stemmed from the observation that the RA synovial lining is rife with DNA strand breaks, and up to 50% of cells stain positively by *in situ* end labeling (ISEL). Although not proven, the toxic environment of the joint, with local production of nitric oxide, oxygen radicals, and cytokines, probably is responsible. The extent of DNA strand breaks was striking, especially when one considers that only approximately 1% of the lining cells show morphological evidence of apoptosis.[49] Although it is true that apoptotic cells are typically cleared quite rapidly, the shear magnitude of DNA fragmentation in RA implies that the lining would have to be completely replaced with unfathomable rapidity if the damaged cells truly progressed to complete apoptosis. Although we initially considered the abundant strand breaks as *prima facie* evidence of increased apoptosis, we could not exclude the possibility of an apoptosis defect in RA. Therefore, we examined p53 as a potential key regulator of DNA repair and apoptosis.

The half-life of wild-type p53 is very short ($<$20 minutes), so we were surprised to find large quantities of immunoreactive p53 in rheumatoid synovium.[7–9] This was especially prominent in the intimal lining, which is precisely the region of significant DNA damage as determined by *in situ* end labeling techniques. The signals for p53 induction in RA are still not defined, but we suspect that the presence of DNA strand breaks, along with local c-*myc* expression,[50] are primarily responsible. In this sense, the synovium parallels skin after ultraviolet irradiation, ie, p53 is induced and localized to sites of DNA fragmentation. Immunoreactive p53 was also detected in

some non-RA tissues, but the degree of staining was markedly less than in RA. This observation was confirmed by Western blot analysis of ST extracts. Our previous studies showed some DNA fragmentation in OA and normal synovium (albeit less than in RA),[13] so the presence of immunoreactive p53 in these tissues is consistent with this finding as well as the fact that FLS constitutively express the p53 gene.

The localization of p53 protein to the intimal lining suggested that type B synoviocytes might express the protein. Alternatively, it is also possible that the increased levels in RA could be due to p53-laden macrophages in the lining. Therefore, we studied p53 expression in cultured RA FLS, which are thought to originate from this region.[51] Surprisingly, flow cytometry studies on permeabilized FLS showed that they constitutively expressed p53. The level of expression was modestly higher than in OA synoviocytes. This method could potentially underestimate nuclear p53 expression if the antibodies do not fully penetrate into the nucleus. Western blot analysis on FLS lysates was also performed to quantify p53 protein. Using this technique, we confirmed that RA FLS contain substantially more p53 protein than the OA FLS (an observation consistent with Western blots of whole ST extracts). DF were examined as a control in Western blot experiments and, as expected, expressed little or no p53. If they were irradiated with ultraviolet light, the level of p53 expression increased to the amounts constitutively produced by resting RA FLS. Therefore, FLS are quantitatively different from DF in that constitutive p53 expression is much greater. The rank order of p53 expression in the fibroblasts examined in these studies was RA FLS > OA FLS > DFs. The reason for enhanced p53 expression in OA FLS compared with DF is not certain but could reflect either an inherent property of synoviocytes or an irreversible alteration in cell phenotype due to the presence of oxygen radicals and cytokines in OA synovium.

The subcellular distribution of p53 was also explored. The ability of p53 to localize to the nucleus is probably critical to its transactivating functions. Some p53 mutants are incapable of migrating to the nucleus, thereby accounting for their functional shortcomings. To determine the location of p53 in cultured FLS, we used an immunostaining technique on cells grown in chamber slides. Under resting conditions, the majority of FLS (both OA and RA) showed faint staining in the cytoplasm, most likely due to the interaction between p53 and the cytoplasmic protein hsc70.[52] After induction of DNA damage by hydrogen peroxide, p53 protein redistributed from the cytoplasm to the nucleus.

Given the abundance of p53 protein in the rheumatoid joint, the question still remains as to why the level of apoptosis in RA appears insufficient to limit expansion of the intimal lining cells. Moreover, the observed frequency of cells that actually progress to the late stages of apoptosis is surprisingly low given the extent of DNA fragmentation.[49] This suggests that DNA strand breaks induced by cytokines or reactive oxygen in RA synovium do not necessarily lead to cell deletion and that synoviocytes (either macrophage-like or fibroblast-like cells) can survive longer than normally anticipated. The mechanism of a potential apoptosis defect has not been fully elucidated but has stimulated us to investigate the machinery of apoptosis in RA to detect abnormalities. There are a variety of potential explanations, including the possibility that cell trafficking into the joint and local proliferation simply overwhelm the usual rate of cell death. However, a second explanation is that the some of the p53 protein expressed in the joint is abnormal. Similar phenomena are clearly associated with expanded cell populations in tumors where p53 mutations directly interfere with growth arrest and apoptosis. If present in RA, it could, in combination with other factors, lead to FLS transformation.

Several lines of investigation suggest that FLS are transformed in RA. They can, in some circumstance, proliferate in an anchorage-independent manner.[53] Cultured FLS as well as type B synoviocytes *in situ* express several oncogenes that are characteristic of cells that have escaped normal growth-regulatory mechanisms. One of the most important proto-oncogenes is c-*myc*, which is a critical signal that initiates cell proliferation and can independently induce p53 gene expression.[22] Also, enzymatically dispersed synovial tissue cells co-implanted with cartilage explants into SCID mice invade into the cartilage matrix, looking very much like destructive pannus.[54] Perhaps more important, this phenomenon still occurs even when pure populations of long-term cultured RA FLS are used.[55]

The foregoing findings led us to study the structure and function of p53 in RA. These studies provide evidence that the p53 in RA synovium is abnormal, with somatic mutations identified in synovial p53 cDNAs (manuscript in preparation). Such mutations, if they inactivate the p53 protein, could account for many of the abnormalities that we and others have observed in RA. As some of these mutations reside in previously identified hot spots that control p53 binding to DNA, there is a good chance that the proteins are functionally inactive. If true, then targeting an apoptosis defect in RA resulting from a specific gene defect could provide a novel and exciting therapeutic approach.

Acknowledgments

We thank Dr. Douglas Green for stimulating and useful discussions, Aung Lwin for technical assistance, and Dennis Young for flow cytometry work. We also thank Dr. Steven Baird for advice on histochemical techniques.

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