

p53-Dependent ICAM-1 overexpression in senescent human cells identified in atherosclerotic lesions

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Most normal somatic cells enter a state called replicative senescence after a certain number of divisions, characterized by irreversible growth arrest. Moreover, they express a pronounced inflammatory phenotype that could contribute to the aging process and the development of age-related pathologies. Among the molecules involved in the inflammatory response that are overexpressed in senescent cells and aged tissues is intercellular adhesion molecule-1 (ICAM-1). Furthermore, ICAM-1 is overexpressed in atherosclerosis, an age-related, chronic inflammatory disease. We have recently reported that the transcriptional activator p53 can trigger ICAM-1 expression in an nuclear factor-kappa B (NF- κ B)-independent manner (Gorgoulis *et al*, *EMBO J*. 2003; 22: 1567–1578). As p53 exhibits an increased transcriptional activity in senescent cells, we investigated whether p53 activation is responsible for the senescence-associated ICAM-1 overexpression. To this end, we used two model systems of cellular senescence: (a) human fibroblasts and (b) conditionally immortalized human vascular smooth muscle cells. Here, we present evidence from both cell systems to support a p53-mediated ICAM-1 overexpression in senescent cells that is independent of NF- κ B. We also demonstrate in atherosclerotic lesions the presence of cells coexpressing activated p53, ICAM-1, and stained with the senescence-associated β -galactosidase, a biomarker of replicative senescence. Collectively, our data suggest a direct functional link between p53 and ICAM-1 in senescence and age-related disorders.

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Most normal human cells do not divide indefinitely. In contrast, they exhibit a limited proliferative potential. When cultured *in vitro* they can undergo only a finite number of cell divisions before entering a nondividing state, referred to as replicative senescence,^{1–3} which has been suggested to potentially contribute to the aging process and the development of age-related diseases.¹ The presence of senescent cells in tissues remained for

long a matter of debate. Recently, however, a senescence-associated biomarker (senescence-associated β -galactosidase staining—SA- β -Gal) has been described, that allows the identification of senescent cells not only in culture, but also in several tissues with increasing age or in age-related disorders.^{4–6} Besides the loss of proliferative capacity, replicative senescence is accompanied by a series of specific alterations in cellular morphology and function.³ Changes in gene expression patterns indicate that senescent cells are characterized by a less fibrogenic and a pronounced inflammatory phenotype,^{7,8} which could be responsible for age-related alterations *in vivo*. However, the mechanism underlying the connection between the senescent state and inflammation awaits elucidation.

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Among the molecules involved in the inflammatory response that are overexpressed in senescent cells and aged tissues is intercellular adhesion molecule-1 (ICAM-1 or CD54).^{6,7} ICAM-1 is a member of the immunoglobulin gene superfamily, which binds to the $\beta 2$ leukocyte integrins, leukocyte function antigen-1 (LFA-1, CD11a/CD18), Mac-1 (CD11b/CD18), and is also expressed in other cells including antigen-presenting cells, where it functions as a costimulatory molecule for T-cell activation (reviewed in Cotran and Mayadas-Norton⁹). Its importance is evident from the phenotype of ICAM-1^{-/-} mice that exhibit several immune defects.⁹ Furthermore, in humans, it is overexpressed in atherosclerosis, an age-related, chronic inflammatory disease.¹⁰ ICAM-1 is induced by several cytokines and stress stimuli such as hypoxia, ultraviolet and ionizing radiation¹¹⁻¹⁴ and the nuclear factor-kappa B (NF- κ B) signalling cascade plays an important role in its activation.¹⁵ However, several reports indicate that NF- κ B-independent pathways may also participate in ICAM-1 stimulation.¹⁶⁻¹⁸ In this vein, we have recently demonstrated that the transcriptional activator p53 can trigger ICAM-1 expression in an NF- κ B-independent manner.¹⁹ This induction is abolished in the presence of the specific p53 inhibitor pifithrin- α and is abrogated in p53-deficient cell lines. In addition, we have shown that p53 most probably mediates its effect on ICAM-1 directly by binding on two p53-responsive elements to the introns of ICAM-1 gene.¹⁹

In senescent cells, p53 exhibits an increased DNA-binding and transcriptional activity and, when overexpressed, it can induce a senescent-like phenotype.²⁰ Moreover, mice that express a modestly hyperactive p53 form display a premature onset of a spectrum of age-related features and reduced longevity, hence implicating p53 also in organismal aging.²¹ Bearing in mind that ICAM-1 is overexpressed in senescent cells and aged tissues,^{6,7,22} we explored whether p53 activation is responsible for the overexpression of ICAM-1 in cellular senescence. Furthermore, this hypothesis was addressed in a classical age-related disorder, such as atherosclerosis.⁶

Materials and methods

Cells and Culture Conditions

Human fetal foreskin fibroblasts (strain HFFF₂) were obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK), and they were routinely cultured in minimum essential medium (MEM) supplemented with penicillin (100 U/ml), streptomycin (100 μ g/ml) and 10% fetal bovine serum (all media and antibiotics from Biochrom KG, Berlin, Germany). The cells were serially subcultured using trypsin-citrate (0.25–0.3%, respectively) until they reached proliferative senescence; the number of cumulative population

doublings (CPDs) achieved was recorded as previously described.²³

The conditionally immortalized human vascular smooth muscle cell line HVTs-SM1 was previously developed in our laboratories.²⁴ The cells were routinely cultured at the permissive temperature of 36°C in Dulbecco's modified Eagle's medium (DMEM) supplemented with neomycin (G418 200 μ g/ml, Gibco-Invitrogen, Paisley, UK), as well as, penicillin (100 U/ml), streptomycin (100 μ g/ml) and 10% fetal bovine serum. HVTs-SM1 cells when being at approx. 80–90% confluency were subcultured with trypsin-citrate, as described above. Senescence was induced by shifting HTVs-SM1 cultures to the nonpermissive temperature of 39°C.^{24,25}

For both cell types, cellular senescence was identified microscopically by their altered morphology and their inability to proliferate, and was further confirmed by using bromodeoxyuridine labelling, as well as p21^{WAF1} expression and SA- β -Gal staining.

Effect of Specific Inhibitors and of TNF- α on ICAM-1 Expression

Based on our previous findings,¹⁹ we have studied the effect of p53 and NF- κ B on ICAM-1 expression in senescent cells. To this end, the cultures were treated with the specific inhibitors pifithrin- α hydrobromide (PFT- α —Tocris, Ellisville, MO, USA)^{19,26} at the indicated concentrations or BAY 11-7082 (Sigma, St Louis, MO, USA)²⁷ at 10 μ M, respectively, and for the indicated time-points. Finally, ICAM-1 expression was also measured 24 h after the addition of tumor necrosis factor- α (TNF- α —R&D, Systems, Minneapolis, MN, USA) (10 ng/ml).

Tissue Specimens

A total of 20 atherosclerotic plaques of human carotid arteries were obtained from patients undergoing carotid endarterectomy. The lesions were type V (fibroatheroma) and VI (complicated) according to the American Heart Association classification.²⁸ Two samples of each plaque were obtained: one washed with PBS and immersed in SA- β -Gal staining solution (see below) and the other one fixed in 4% buffered paraformaldehyde, embedded in paraffin (FFPE) and prepared according to standard methods. Serial sections were stained for hematoxylin and eosin, and used for immunohistochemistry. Moreover, three histologically normal internal mammary arteries from patients undergoing aorto-coronary bypass surgery were included as control tissue.

SA- β -Gal Staining

SA- β -Gal staining in cells and tissues was performed as described by Dimri *et al*,⁴ modified as follows:

Cell cultures were fixed for 5 min in neutral formalin (approx. 4% formaldehyde), washed in

PBS and incubated for 12–24 h at 37°C with 1 mg/ml X-Gal (5-bromo-4-chloro-3-indolyl β -D-galactoside, Sigma) in a buffer containing 40 mM citric acid/sodium phosphate pH: 6.0, 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 150 mM sodium chloride and 2 mM magnesium chloride (SA- β -Gal staining solution).

Carotid plaques were incubated for 24 h at 37°C in the SA- β -Gal staining solution (see above). After incubation, the tissue was fixed in 4% paraformaldehyde, embedded in paraffin and treated as FFPE material.

mRNA Analysis

The mRNA levels of the studied genes were assessed using a comparative multiplex RT-PCR method, as previously described.¹⁹ Briefly, RNA was extracted using the GenElute extraction kit (Sigma) and subsequently cDNA was generated using the M-MLV Superscript II RT according to the manufacturer's instructions (Life Technologies-Invitrogen). The GAPDH gene was used as reference gene for all PCRs. The following amplimers were designed using the Oligo 4.01 software (National Biosciences Inc., Plymouth, MN, USA): GAPDH (Accession No. XM_033263) forward (F), CAT CTC TGC CCC CTC TGC TG (position 830), reverse (R), CGA CGC CTG CTT CAC CAC CT (position 411), product length 438 bp; p21^{WAF1} (Accession No. U03106) F, CTG CCG CCG CCT CTT C (position 126), R, CTG AGC GAG GCA CAA GGG TA (position 426), product length 319 bp, annealing temperature 61°C, plus 5% dimethylsulfoxide (DMSO); ICAM-1 (Accession No. X06990) F, TGG TAG CAG CCG CAG TCA TA (position 1469), R, CTC CTT CCT CTT GGC TTA GT (position 1829), product length 377 bp, annealing temperature 57°C. PCR products were electrophoresed in a nondenaturing 8% acrylamide/*bis*-acrylamide (19:1) gel. Gels were stained with ethidium bromide and images were captured with a CCD camera connected to a PC, using the BioProfil gel documentation software (Vilber Lourmat, Torcy-Paris, France).

Western Immunoblot Analysis

For the collection of whole cell-lysates, the cell-monolayers were washed with ice-cold tris buffered saline (TBS: 10 mM Tris-HCl pH 7.4, 150 mM NaCl) and scraped immediately in hot SDS-PAGE sample buffer, that is, 62.5 mM Tris pH 6.8, 6% w/v SDS, 2% v/v glycerol, 5% v/v 2-mercaptoethanol, 0.0125% w/v bromophenol blue, protease- and phosphatase-inhibitor cocktails (Sigma). Following sonication for 15 s, the samples were clarified by centrifugation and stored at –80°C until use.

Samples were separated on 9% SDS-PAGE and the proteins were transferred to PVDF membranes (Amersham Biosciences, Buckinghamshire, UK).

The membranes were blocked with 5% nonfat milk in TBS containing 0.1% Tween-20 (TTBS) and incubated with the primary mouse monoclonal antibodies, that is, anti-ICAM-1 (G-5) (class IgG2a; epitope, amino acids 258–365, human origin, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-p21^{WAF1} (Class: IgG, epitope: residues 1–159 of p21^{WAF1}, Santa Cruz Biotechnology), anti- α -tubulin (Sigma) or PanActin antibody (NeoMarkers, Lab Vision Corporation, Fremont, CA, USA). Following incubation with horseradish peroxidase-conjugated secondary anti-mouse antibody (Sigma), immunoreactive bands were visualized on Kodak-X-OMAT AR film by chemiluminescence (ECL kit) according to the manufacturer's (Amersham) instructions. The intensity of the bands was quantified as described above.

Immunohistochemistry

Antibodies

For immunohistochemical analysis, the following antibodies (Abs) were used: anti-ICAM-1 and anti-p21^{WAF1} (see above), as well as DO7 (Class: IgG2b, mouse monoclonal, epitope: residues 1–45 of p53) (Dako, Kalifronas Athens Greece).

Method

Paraffin sections, 5- μ m thick, of the lesions were mounted on poly-a-lysine-coated slides, dewaxed, rehydrated and incubated for 30 min with 0.3% hydrogen peroxide to quench the endogenous peroxidase activity. Unmasking of ICAM-1, p53, and p21^{WAF1} proteins was carried out with the heat-mediated antigen retrieval method using 1mM EDTA pH 8.0 in a steamer. The sections were incubated with the antibodies at a 1:100 dilution at 4°C overnight. Biotin-conjugated secondary antibody (Dako) was added at a 1:200 dilution for 1 h at room temperature (RT). Strept-Avidin-Biotin complex (Dako) at a dilution of 1:100 was applied to enhance the signal. For color development, we used 3,3'-diaminobenzidine tetrahydrochloride (DAB) and hematoxylin as counterstain.

Controls

Mouse IgG1 MAb of unrelated specificity and the IgG fraction of normal rabbit serum were used as negative controls.

Results

ICAM-1 Overexpression in Senescent Human Fibroblasts is Mediated by p53

Initially, we investigated the expression of ICAM-1 in normal human fibroblasts that have undergone senescence after serial subculturing *in vitro*, in comparison to young (early-passage) cells. Figure 1a depicts the typical appearance of senes-

cent fibroblasts. The cells, in contrast to the spindle-like appearance of young fibroblasts, are highly enlarged, irregular in shape and with an intense perinuclear SA- β -Gal staining. We observed that senescent fibroblasts strongly overexpress ICAM-1, both at the mRNA and protein levels, compared to young cells. An ~ 2.5 -fold augmentation was found at the RNA levels, while at the protein level this effect was more intense: an ~ 5 -fold increase was detected (Figure 1b,c).

Next, we used a novel low-molecular-weight compound that inhibits p53-mediated transcription, namely PFT- α . This agent has been shown to inhibit the activation of p53-responsive genes, including *p21^{WAF1}* and *mdm2*.²⁶ PFT- α blocks the activation of

p53 in cells after a variety of treatments, for example, doxorubicin, etoposide, Taxol, cytosine arabinoside, UV- and γ -irradiation, and there are already suggestions for potential clinical applications (for a review, see Komarova and Gudkov²⁹). In addition, we have recently shown, that PFT- α effectively inhibits the p53-mediated *ICAM-1* and *p21^{WAF1}* induction after γ -irradiation of human fibroblasts.¹⁹ Here, we demonstrate that in the presence of 20 μ M PFT- α , the observed ICAM-1 overexpression in senescent fibroblasts was markedly impaired (Figure 1b,c). Interestingly, PFT- α has the same effect on the expression of *p21^{WAF1}*, a classical p53 target gene and a marker of cellular senescence.³ As expected, *p21^{WAF1}* was found to be

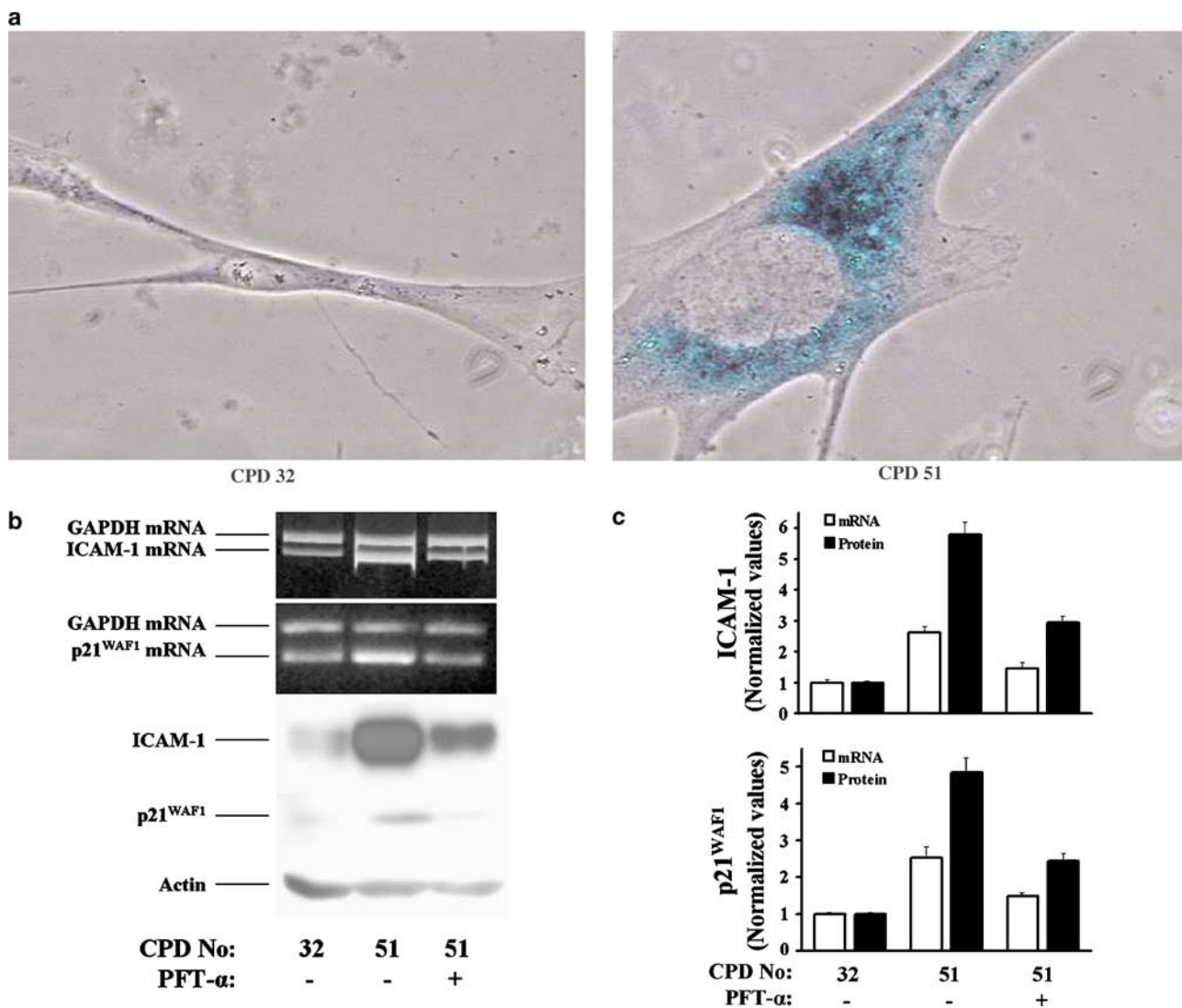


Figure 1 ICAM-1 expression in senescent human skin fibroblasts. The human fibroblast cell strain HFFF₂ has been passed until it reached replicative senescence, as described under Materials and methods. In (a) young (CPD 32) and senescent (CPD 51) cells were stained with the SA- β -Gal staining (magnification $\times 1000$). In (b) young and senescent cells, as well as, senescent cells treated with 20 μ M PFT- α for 12 h (for mRNA analysis) or 24 h (for protein analysis) were studied for the expression of ICAM-1 and *p21^{WAF1}* at the mRNA level by RT-PCR and at the protein level by Western analysis; GAPDH and actin levels were used as loading controls, respectively. In (c) the intensity of the bands was assessed by densitometric analysis and it is presented after normalization according to the intensity of the loading controls (mean of three experiments; error bars indicate standard deviation).

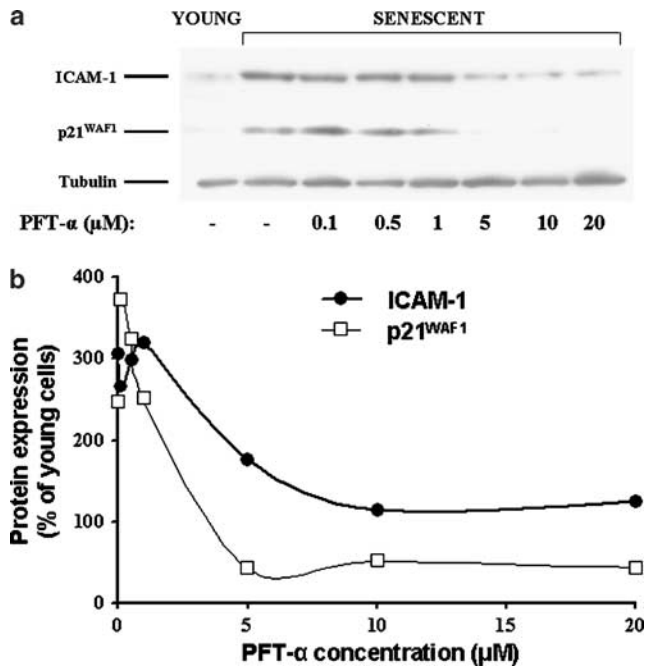


Figure 2 Dose-dependent inhibition of ICAM-1 expression in senescent human skin fibroblasts by PFT- α . In (a) senescent human skin fibroblasts treated with the indicated concentrations of PFT- α for 24 h were studied for the expression of ICAM-1 and p21^{WAF1} by Western analysis; tubulin levels were used to ensure equal loading, while the lysate of young fibroblasts was included as control. In (b) the intensity of the bands was assessed by densitometric analysis, normalized according to the intensity of the loading controls and it is presented as a percentage of the expression levels in young cells.

overexpressed in senescent cells, at the mRNA and protein levels, and this effect was largely reversed by PFT- α (Figure 1b,c). Furthermore, the inhibitory effect of PFT- α on the overexpression of ICAM-1 in senescent human fibroblasts was found to be dose-dependent: an approximately 50% inhibition was obtained with 5 μ M PFT- α , while over 10 μ M the senescence-induced ICAM-1-overexpression was almost completely annulled (Figure 2). A similar pattern was also observed in the expression of p21^{WAF1} in senescent cells in the presence of PFT- α (Figure 2).

p53 Activation in a Model of Senescent Human Vascular Smooth Muscle Cells Leads to Enhanced ICAM-1 Expression

We next studied ICAM-1 expression in senescent human smooth muscle cells (SMCs). It is well known that the *in vitro* study of SMCs, especially from human origin, is hindered by the fact that their responses vary considerably among donors.^{30,31} To circumvent this, we used an immortalized cell line that we have recently developed, designated as HVTs-SM1. This cell line was developed from human vascular SMCs, stably transfected with a

nonreplicative retroviral vector containing a temperature-sensitive (tsA58) mutant of the SV40 large T-antigen.²⁴ SV40 large T-antigen targets and inactivates pivotal cell-cycle regulators, like p53.³² Accordingly, at the permissive temperature of 36°C, these cells can proliferate indefinitely, while at the nonpermissive temperature of 39°C (where large T-antigen expression is downregulated, hence p53 is active) they acquire a senescent phenotype.²⁴ As shown in Figure 3, HVTs-SM1 cells exhibit an intense expression of the senescence markers p21^{WAF1} and SA- β -Gal staining only at the nonpermissive temperature. Senescent HVTs-SM1 cells strongly overexpress also ICAM-1, at the mRNA and protein levels. An ~3-fold increase was observed in comparison to HVTs-SM1 maintained at the permissive temperature (Figure 3b,c). Once again, in the presence of 20 μ M of the p53 inhibitor PFT- α , this ICAM-1 overexpression in senescent cells was severely reduced (Figure 3b,c). An analogous expression pattern was obtained for p21^{WAF1}: the overexpression observed in senescent HVTs-SM1 cells at the nonpermissive temperature, due to p53 activation, was largely reversed by PFT- α (Figure 3b,c).

ICAM-1 Overexpression in Senescent Cells is not Mediated by the NF- κ B Pathway

It is well appreciated that NF- κ B is the main transcriptional regulator of ICAM-1 expression. Accordingly, we examined whether the overexpression of ICAM-1 in senescent cells is also mediated through this signalling cascade. First, we studied the response of young and senescent cells to TNF- α , as the latter is a potent stimulator of ICAM-1 expression, acting through the NF- κ B pathway.^{9,11,15} Figure 4a reveals that TNF- α potentiates ICAM-1 expression significantly in both young and senescent fibroblasts and HVTs-SM1 cells, implying that the TNF- α -NF- κ B pathway is functional in these cells. Next, we employed a specific NF- κ B inhibitor, BAY 11-7082.²⁷ This compound, when used at a concentration of 10 μ M, has been shown to selectively inhibit the TNF- α -induced surface expression of ICAM-1 by blocking the phosphorylation of I κ B- α .²⁷ As demonstrated in Figure 4b, BAY 11-7082 can severely suppress the TNF- α -mediated ICAM-1 expression in fibroblasts. Interestingly, it slightly suppresses the basal levels of ICAM-1 expression in young cells, possibly indicating that these are controlled by the NF- κ B pathway. On the other hand, PFT- α has no effect either to the basal or TNF- α -induced ICAM-1 expression, indicating that it does not interfere with the TNF- α -NF- κ B pathway. Finally, the presence of 10 μ M BAY 11-7082 was incapable of reverting ICAM-1 overexpression in both senescent human fibroblasts and senescent HVTs-SM1 cells (Figure 4c).

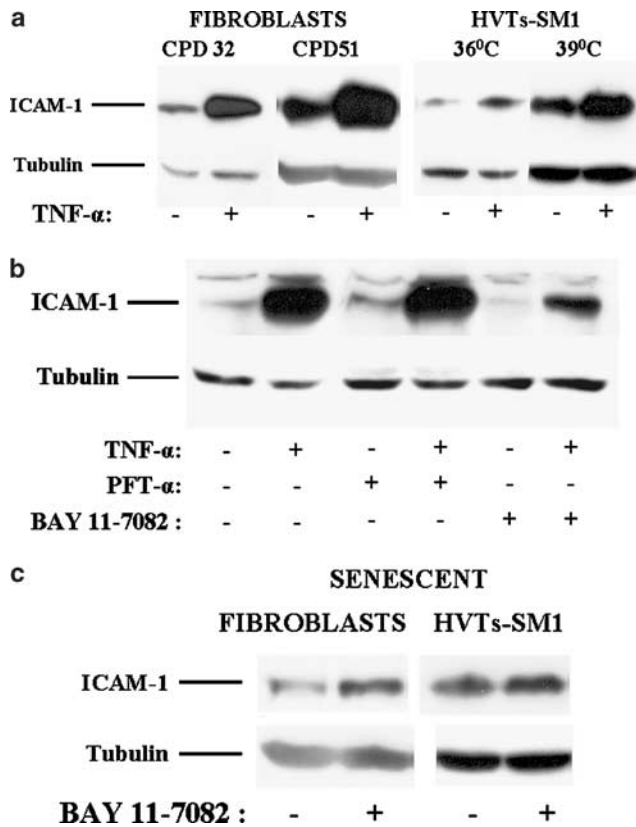


Figure 4 ICAM-1 expression in senescent cells is not mediated by NF- κ B. ICAM-1 expression was monitored by Western analysis 24 h after stimulation with TNF- α in (a) young (CPD 32) and senescent (CPD 51) human fibroblasts, as well as HVTs-SM1 cells at the 36 and 39°C, and in (b) young fibroblasts (CPD 32) pretreated with BAY 11-7082 or pifithrin- α . (c) ICAM-1 expression in senescent human fibroblasts and HVTs-SM1 cells treated for 24 h with BAY 11-7082.

observed an increased number of positive cells (with perinuclear blue staining) (Table 1). Interestingly, this percentage is even more enhanced in the ICAM-1-rich areas of the lesions (Table 1). By contrast, in normal arteries, as expected, we found little ICAM-1 staining and only occasionally p53 activation, whereas no SA- β -Gal-positive cells were observed (Table 1). Therefore, our data indicate the presence of a subpopulation of senescent cells in the atheromatous plaques, characterized by the coexpression of p53 and ICAM-1.

Discussion

One of the principal differences between normal and tumor cells is that only the former exhibit a limited proliferative lifespan, while the latter are immortalized, that is, they can proliferate indefinitely.^{1-3,34} This led to the interpretation that the biological role of cellular senescence is a mechanism for restricting tumor progression.³⁴ However, several lines of evidence support also an analogy

between senescence and aging *in vivo*. For example, numerous cell types such as epithelial cells, osteoblasts, chondrocytes or smooth muscle cells, when isolated from older donors have very limited or no proliferative capacity.³⁵ In addition, cells derived from patients suffering from progeroid syndromes, such as Werner syndrome, exhibit a significantly shorter proliferative lifespan, compared to cells originating from normal donors.³⁶ For both biological processes the transcriptional activator p53 seems to be of paramount importance. The role of p53 as a tumor suppressor is well established: nearly 80% of human cancers display defects in p53 signalling, while half of all cancers harbor structural alterations in the *p53* gene.^{20,37} On the other hand, in senescent cells—characterized by their inability to proliferate—although the levels of p53 protein are not elevated, its DNA-binding and transcriptional activation capacity is highly enhanced.³⁸ Moreover, the increased expression of a classical p53-target gene, *p21^{WAF1}*, a cyclin-dependent kinase inhibitor, is a ‘hallmark’ of the initiation of the senescence programme.³⁹ Finally, it has been recently shown that mice expressing a hyperactive form of p53 are (as expected) resistant to spontaneous tumorigenesis, but they also develop prematurely several phenotypes associated with normal aging (eg osteoporosis, sarcopenia and skin atrophy, reduced body mass, impaired wound healing, reduced stress tolerance and depletion of hematopoietic stem cells) and their lifespan is approx. 20% shorter, thus implicating p53 in organismal aging.²¹ These findings are in concert with the hypothesis of ‘antagonistic pleiotropy’, which predicts that some genes that have been selected during evolution to ensure the maintenance of homeostasis (like cancer prevention) in young organisms, can have deleterious effects in aged individuals.^{40,41}

To fully realize the connection between cellular senescence and aging *in vivo*, it must be mentioned that the senescence phenotype is not restricted to the exhaustion of the proliferative potential. Classical biochemical approaches, as well as, cDNA microarray analyses unraveled that senescent cells are characterized by a less fibrogenic and a pronounced inflammatory phenotype,^{7,8} probably contributing to the aging process. One of the molecules that have been found to be overexpressed in several cell types during senescence, as well as in aged tissues is ICAM-1.^{6,7} The latter is an adhesion molecule implicated in vital aspects of the immune response,⁹ and a well-established NF- κ B target.¹⁵ Nevertheless, we have recently shown that ICAM-1 can also be induced by p53 in an NF- κ B-independent manner and thus we hypothesized that a p53-ICAM-1 pathway may operate in senescent cells.¹⁹ Indeed, the data presented here support this hypothesis: we demonstrated that in senescent human cells ICAM-1 is overexpressed at the mRNA and protein levels in parallel with the classical p53-target *p21^{WAF1}* (Figures 1 and 2), and moreover, this

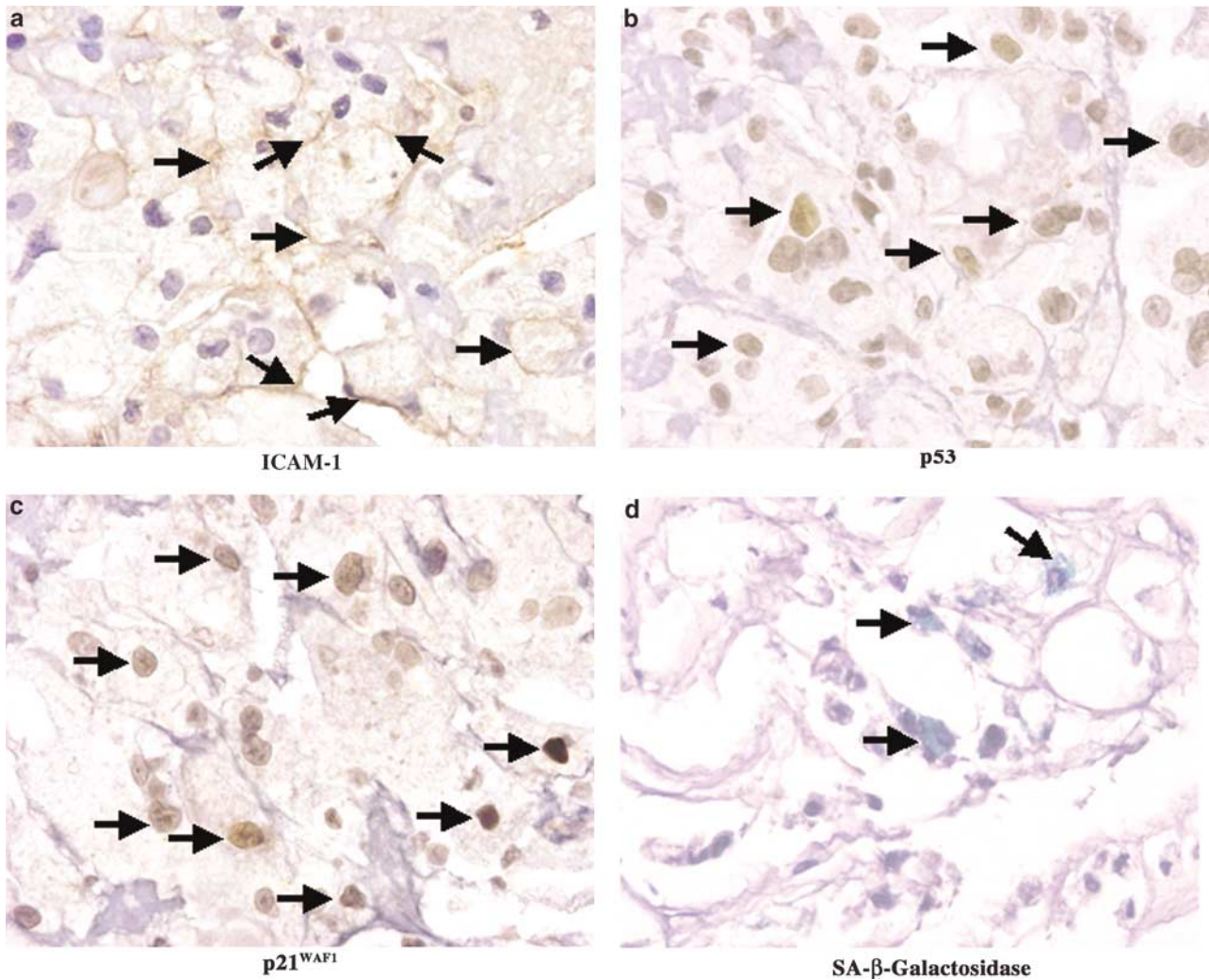


Figure 5 Identification of p53, p21^{WAF1}, ICAM-1 and SA-β-Gal positive cells in semiserial sections of atherosclerotic lesions. (a) The foam cells exhibit positive anti-ICAM-1 (membranous) staining (arrows). In the same region, colocalization of p53 and p21^{WAF1} (nuclear immunoreactivity, see arrows) in the aforementioned cells is shown (b and c, respectively). The foam cells undergoing senescence are depicted by the positive cytoplasmic SA-β-Gal staining, indicated by arrows (d). (magnification × 400).

Table 1 Percentages of positive cells for p53, p21^{WAF1}, MDM2, ICAM-1 and SA-β-Gal in the atheromatous lesions and controls

	<i>Atheromatous lesions (± s.d.)</i>	<i>Controls (± s.d.)</i>
p53	69.2 (± 7.4)	0.3 (± 0.4)
p21 ^{WAF1}	65.1 (± 9.3)	3.2 (± 1.8)
MDM2	58.7 (± 12.9)	1.0 (± 1.4)
ICAM-1	32.5 (± 10.2)	0.5 (± 0.7)
SA-β-gal	13.6 (± 5.8)	0.0
SA-β-gal in the ICAM-1-rich region	37.6 (± 7.3)	0.0

overexpression is reversed in a dose-dependent manner by the p53-inhibitor PFT-α (Figures 1–3) but not by the NF-κB inhibitor, BAY 11-7082 (Figure 4). Hence, it seems that p53 activation is responsible

for this aspect of the proinflammatory phenotype of senescent cells. Notably, we demonstrated this functional link in two human cellular systems, fibroblasts and smooth muscle cells, the latter being of major importance in the development of atherosclerosis.⁴²

Atherosclerosis is an age-related chronic inflammatory disease.⁴³ Immunocytochemical studies have revealed an ICAM-1 overexpression in human atherosclerotic plaques.¹⁰ Although the absence of p53 accelerates atherosclerosis by increasing cell proliferation in apoE-knockout mice,⁴⁴ it has been shown that wild-type p53 accumulates in human atherosclerotic tissues.^{33,45} Moreover, and in agreement with the results presented here (Figure 5, Table 1), it has been reported that the majority of these p53 immunoreactive cells express p21^{WAF1}.⁴⁵ These cells are not proliferating, while only few of them exhibit

the nuclear morphology of apoptosis.^{33,45} The above, in conjunction with our previous observation that ICAM-1 can be induced by p53,¹⁹ suggest a mechanism for the direct involvement of p53 in atherogenesis. Moreover, aging is a major risk for the development of vascular diseases, such as atherosclerosis. In accordance, we document the presence of a subset of senescent cells in the atherosclerotic lesions, coexpressing activated p53 and ICAM-1, indicating another involvement of p53 in this disorder. This is in concert with previous data showing that human plaque-derived vascular smooth muscle cells (VSMCs) exhibit in culture lower levels of proliferation and an earlier senescence, because of a defect in retinoblastoma protein phosphorylation, the latter being regulated by p53.⁴⁶ In this route, it has been shown that repeated balloon catheter denudation of carotid arteries in experimental animals can lead to accumulation of senescent VSMCs in the neointima and in the media. This is most likely the result of a denudation-induced proliferation in the injured area.⁴⁷ These observations are in symphony with the aforementioned hypothesis of 'antagonistic pleiotropy'. In this context, the p53-mediated ICAM-1 expression reported here may be a paradigm of a beneficial stress response that activates immune reaction; however, the continuous ICAM-1 overexpression can contribute to age-related pathologies, such as atherosclerosis. A similar involvement of 'antagonistically pleiotropic' mechanisms has been proposed also for several other age-related diseases, such as benign and malignant prostate hypertrophy, Alzheimer's disease or even the reciprocal relationship between cellular senescence and carcinogenesis.^{48,49}

In conclusion, we report here that p53 activation in senescent cells is responsible for an enhanced expression of ICAM-1, which can play a role in certain age-related aberrations.

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