Vulnerability to oxidative stress and different patterns of senescence in human peritoneal mesothelial cell strains

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Submitted 26 May 2008; accepted in final form 25 November 2008

KSIAZEK K, MIKULA-PIETRASIK J, OLIJSLAGERS S, JÖRES A, VON ZGLINICKI T, WITOWSKI J. Vulnerability to oxidative stress and different patterns of senescence in human peritoneal mesothelial cell strains. Am J Physiol Regul Integr Comp Physiol 296: R374–R382, 2009. First published November 26, 2008; doi:10.1152/ajpregu.90451.2008.—Both the ascites fluid-derived mesothelial cell line LP-9 and primary cultures of human omentum-derived mesothelial cells (HOMCs) are commonly used in experimental studies. However, they seem to have a different replicative potential in vitro. In the present study, we have attempted to determine the causes of this discrepancy. HOMCs were found to divide fewer times and enter senescence earlier than LP-9 cells. This effect was coupled with earlier increases in the expression of senescence-associated-β-galactosidase and cell cycle inhibitors p16INK4a and p21WAF1. Moreover, almost 3 times as many early-passage HOMCs as LP-9 cells bore senescence-associated DNA damage foci. In sharp contrast to LP-9 cells, the foci present in HOMCs localized predominantly outside the telomeres, and the HOMC telomere length did not significantly shorten during senescence. Compared with LP-9 cells, HOMCs were found to enter senescence with significantly lower levels of lipofuscin and damaged DNA, and markedly decreased glutathione contents. In addition, early-passage HOMCs generated significantly more reactive oxygen species either spontaneously or in response to exogenous oxidants. These results indicate that compared with LP-9 cells, HOMCs undergo stress-induced telomere-independent premature senescence, which may result from increased vulnerability to oxidative DNA injury.

DNA damage; peritoneal mesothelial cells

Mesothelial cells cover with a single layer the parietal and visceral aspects of the serosal cavities. The main function of mesothelial cells is to protect internal organs and allow their frictionless movements (see Refs. 30 and 31 for comprehensive reviews). Mesothelial cells are also involved in the transport of body fluids, inflammation, wound healing, and prevention of adhesion formation. Mesothelial cells of the peritoneal cavity are the most extensively studied subpopulation of the mesothelium. They can be isolated from omentum, parietal peritoneum, ascites fluid, and spent peritoneal dialysate (28, 50). As pointed out by Kenny et al. (21), despite the diversity of sources, all peritoneal mesothelial cells have been regarded as being the same. This paradigm implies that apart from some minor differences in morphology (33), all peritoneal mesothelial cells share the same biological properties irrespective of their origin. To our knowledge, however, this assumption has not been formally tested, at least as far as mesothelial cell senescence is concerned. In this respect, some data may point to differences between various mesothelial cell strains. It has been reported that human peritoneal mesothelial cells of the ascites fluid-derived line LP-9 display features of the senescence phenotype after as many as 40–60 population doublings (PD) (6, 42). In contrast, human omentum-derived peritoneal mesothelial cells (HOMCs) have been reported to senesce already after 6–10 PD (26). Moreover, Zhang et al. (51) have observed the differences in the growth potential of apparently normal peritoneal mesothelial cells isolated from patients with or without advanced ovarian malignancy.

In the present study, we have systematically analyzed the patterns of senescence in HOMCs and LP-9 cells to determine the cause of their different replicative life span. In particular, we have assessed the role of oxidative stress as a factor contributing to senescence.

MATERIALS AND METHODS

Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tissue culture plastics were from Nunc (Roskilde, Denmark).

Cell culture. Human peritoneal mesothelial cell line LP-9 was obtained from the Aging Cell Repository (catalog number AG07086) of the Coriell Institute for Medical Research (Camden, NJ). The culture was initiated in 1981 by plating an ascites fluid suspension obtained from a 26-year-old ovarian cancer patient (47). The cells were delivered at PD=24 and entered the experiments at PD=26. These numbers could be calculated quite accurately since after the
isolation, LP-9 cells grew out of countable colonies (J. G. Rheinwald, personal communication). The cells were maintained in medium M199 supplemented with L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), hydrocortisone (0.4 μg/ml) and 10% (vol/vol) fetal bovine serum.

HOMCs were isolated by enzymatic disaggregation of omentum, as described in detail elsewhere (40, 43, 50). Briefly, following the study approval by the institutional ethics committee, the specimens of omentum were obtained from consenting patients undergoing abdominal surgery at the Department of Surgery at the Poznan University of Medical Sciences. The donors did not suffer from any peritoneal malignancy. The tissue was incubated in a solution of 0.05% trypsin and 0.02% EDTA for 30 min at 37°C with gentle shaking. The cells isolated were cultured in the same medium as used for LP-9 cells. Cells were identified as pure mesothelial by uniform positive staining for cytokeratins (36) and HMBE-1 antigen (51). To minimize the potential impact of donor’s age on HOMC proliferative capacity (27) compared with LP-9 cells, the specimens of omentum were obtained from donors of similar age (22 to 37 years).

In contrast to LP-9 cells, an initial yield and growth rate of HOMCs was difficult to estimate due to the contamination with blood cells and tissue debris. Therefore, the number of cell divisions before the formation of easily recognizable mesothelial cell clusters could only be approximated on the basis of known population doubling time for early-passage cells (personal observations and Ref. 5), and the time to reach first confluence. These calculations revealed that HOMCs usually entered the experiments not later than at PD = 3.

Induction of cell senescence. Cell senescence was induced by serial passages. Both cell types were passaged at 5-day intervals, using a fixed seeding density of 3 x 10^4 cells/cm² (26), until their proliferative capacity was exhausted. The number of cells at each passage was determined using a Bürker chamber, and the number of population doublings was estimated, as described previously (26). Cells were considered senescent when they failed to increase in number during 4 wk and showed enlarged morphology, and when >70% cells stained positively for SA-β-Gal (35).

In some experiments, cells were subjected to sublethal oxidative stress following several cycles of exposure to tert-butyl hydroperoxide (t-BHP) at nontoxic doses (13). In brief, proliferating early-passage cells were treated with 30 μM t-BHP for 1 h during 7 consecutive days. Between the stresses, the cells were allowed to recover in standard medium. Control group underwent the same procedure, but in the absence of t-BHP. At the end of the experiment, the cells were harvested with trypsinization, counted, and assayed for the generation of reactive oxygen species (ROS).

Detection of SA-β-gal. SA-β-Gal was assessed according to Dimri et al. (11). Briefly, cells were grown on Lab-Tek Chamber Slides (Nunc), fixed with 3% formaldehyde, washed, and exposed for 16 h at 37°C to a solution containing 1 mg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 150 mM NaCl, 2 mM MgCl₂ and 40 mM citric acid, pH 6.0.

Detection of p16INK4a and p21WAF1. Expression of p16INK4a was assessed by immunocytochemistry. Briefly, cells were cultured in Lab-Tek Chamber Slides, fixed with 3% formaldehyde and exposed to a specific anti-p16INK4a antibody (Pharmingen, BD Biosciences, San Jose, CA), diluted 1:10. Bound antibodies were detected by immunoperoxidase staining using the EnVision+ System (Dako, Glostrup, Denmark). Five hundred cells in four randomly selected areas were counted at 200 magnification, and the number of positively stained cells was recorded.

Expression of p21WAF1 was assessed with the use of p21WAF1 immunoassay kit (Calbiochem, Merck Biosciences, Darmstadt, Germany). Cell extracts were prepared and assayed according to the manufacturer’s protocol. One unit/ml of p21WAF1 was defined as the amount of p21WAF1 originating from 2.7 x 10^5 human breast adenocarcinoma cells (of the MCF7 line) harvested in a standardized manner.

Expression of p16INK4a and p21WAF1 mRNAs was assessed by RT-PCR. Briefly, total RNA was extracted with the RNA Bee (Tel-Test, Friendswood, TX), purified and reverse transcribed into cDNA with random hexamer primers, as described previously (19). Primer sequences and PCR conditions are shown in Table 1.

Detection of DNA damage foci. Cells were fixed with formaldehyde and incubated with monoclonal antibody against γ-H2A.X (Upstate Biotechnology, Lake Placid, NY). Next, cells were washed and treated with Alexa Fluor 594 goat anti-mouse IgG (Molecular Probes, Eugene, OR). The specimens were then washed to perform immunofluorescence. When immuno-FISH was done, the incubation with the secondary antibody was repeated, and cells were stained with DAPI. The preparations were inspected in a Zeiss LSM 510 Meta Confocal Microscope (Carl Zeiss, Jena, Germany). For colocalization studies, confocal images of individual nuclei were deconvoluted, and a Pearson’s correlation coefficient (R) was determined by the Costes approximation method using ImageJ v1.37a software (http://rsb.info.nih.gov/ij/), with the plug-in bundle from the Wright Cell Imaging Facility (http://www.uhnresearch.ca/facilities/wcil/imagej/), as previously described (24). The Pearson coefficient determines the degree of colocalization on a scale of +1 to -1, representing perfect colocalization to no colocalization at all, respectively. The pixels found to colocalize were indicated in a separate image as a yellow pixel overlay.

Assessment of telomere length. Telomere length was measured by real-time PCR. Genomic DNA was extracted using QIAamp DNA mini kit (Qiagen, West Sussex, UK). PCR reaction mixture contained 100 nM primer Tel A (5’CGGTGGTGCATGTTGGTGGTGTTGTTGGTTGTTGGT-3’), 300 nM primer Tel B (5’GGCTGCCTATCCCTTTACCCCT-3’), 10 ng genomic DNA, and 1× Sigma SYBR Green I JumpStart Taq Ready Mix (Sigma). The PCR began with an initial denaturation step at 95°C for 3 min, followed by 40 cycles of 95°C for 15 s and 60°C for 30 s. PCRs were carried out using the iCycler Real-Time PCR system (Bio-Rad Laboratories, Hercules, CA) (24).

Detection of oxidative DNA damage. Oxidative DNA damage was assessed by measuring the level of 8-hydroxydeoxyguanosine (8-OH-
dG) using a competitive ELISA-based kit (Cell Biolabs, San Diego, CA). DNA was extracted with a GenElute Mammalian Genomic DNA Miniprep Kit (Sigma), and all of the procedures were performed as per the manufacturers’ instructions.

Measurement of lipofuscin levels. Lipofuscin levels were assessed by measuring autofluorescence of viable unstained cells suspended in PBS. The measurements were made using a spectrophotometer (Perkin-Elmer, Turku, Finland) with excitation at 485 nm and emission at 535 nm (12).

Detection of ROS. ROS production was assessed with 2’,7’-dichlorodihydrofluorescein diacetate (H2DCFDA) probe. Cells were incubated in the presence of 5 μM H2DCFDA (Molecular Probes, Eugene, OR) for 45 min at 37°C and then solubilized. Sample fluorescence was monitored in a spectrofluorometer with excitation at 485 nm and emission at 535 nm.

Antioxidant measurements. Cellular antioxidant levels were assessed in cell lysates prepared in 0.1% (vol/vol) Triton X-100 in PBS. Total superoxide dismutase (SOD) activity was measured with a commercially available kit (R&D Systems Europe, Abingdon, UK) as per manufacturer’s instructions. Reduced glutathione (GSH) was determined according to the method of Akerboom and Sies (1).

Statistics. Results are presented as means ± SD. The results derived from all assays were normalized per 10⁵ cells. The data obtained from HOMCs and LP-9 cells were analyzed with the unpaired t-test using GraphPad Prism 4.00 software (GraphPad Software, San Diego, CA). Differences with a P value <0.05 were considered to be statistically significant.

RESULTS

Replicative life span of HOMCs and LP-9 cells. Replicative capacity of HOMCs and LP-9 cells was tested under the same culture conditions. As previously observed (26), the period of vigorous proliferation of freshly isolated HOMCs was relatively short, and the cells became senescent after reaching 7.5 ± 1.2 PD (the range 6.3–9.2). In contrast, the phase of exponential growth of LP-9 cells was markedly longer so that cells reached 15.3 ± 0.7 PD before senescence (Fig. 1). Given that LP-9 cells entered the experiment at PD = 26, the cumulative number of PD mounted to 41.3 ± 0.7 PD.

Senescence markers in HOMCs and LP-9 cells. As illustrated in Fig. 2A, the fraction of cells staining positively for SA-β-Gal increased progressively in both HOMCs and LP-9 cells, reaching similar values in senescent cultures (86 ± 7% vs. 82 ± 5%, respectively). Interestingly, however, HOMCs showed increased expression of SA-β-Gal already at very early stages. When assessed after the first passage, 11.3 ± 3.3% of HOMCs stained for SA-β-Gal compared with 5.5 ± 0.6% of LP-9 cells. Furthermore, the fraction of senescent cells increased faster in HOMCs than in LP-9 cells.

As expected (26), the increase in SA-β-Gal expression in HOMCs coincided with a progressive increase in the expression of both p16INK4a protein and mRNA (Fig. 2, B–D). The fraction of p16INK4A-expressing cells was high already in early-passage HOMCs and reached 39 ± 2% at senescence. In contrast, expression of p16INK4A in LP-9 cells was markedly lower, either at early stages or at senescence. Furthermore, the rise in p16INK4A protein in senescent LP-9 cells was not associated with a significant increase in p16INK4A mRNA.

The differences between HOMCs and LP-9 cells were less apparent as far as p21WAFI was concerned (Fig. 2, E–G). Senescence of both cell types was associated with a rise in p21WAFI protein but with no significant changes in p21WAFI mRNA. The levels of p21WAFI protein in HOMCs and LP-9 cells were similar either at early stages (3.1 ± 0.6 vs. 2.6 ± 0.3 mU/10⁵ cells, respectively) or at senescence (8.1 ± 3.5 vs. 6.4 ± 1.9 mU/10⁵ cells, respectively). Consistently with the pattern of growth, however, HOMCs arrived at these values much earlier compared with LP-9 cells.

Frequency and localization of senescence-associated DNA damage foci. To determine the extent of senescence-associated DNA damage, cells were stained for the phosphorylated variant of histone H2A.X (γ-H2A.X). The accumulation of γ-H2A.X reflects a DNA damage response that might be triggered either by DNA double-strand breaks at sites distinct from telomeres or by uncapped telomeres. The percentage of cells bearing γ-H2A.X foci increased during consecutive passages in both HOMCs and LP-9 cells, reaching similar peak levels at senescence (73 ± 8% and 78 ± 3%, respectively) (Fig. 3A). There was, however, a marked difference in the fraction of γ-H2A.X-positive cells at very early stages of the experiment, when 48 ± 13% of HOMCs contained γ-H2A.X foci compared with 16 ± 5% of LP-9 cells (P < 0.002) (Fig. 3, A and B).

The analysis of individual γ-H2A.X-positive nuclei revealed that in HOMCs, the fraction of DNA damage foci that colocalized to telomeres was similar in early-passage cells and in senescent cells (26 ± 6% vs. 21 ± 3%, respectively) (Fig. 3, C and D). In contrast, the telomeric localization of DNA damage foci in LP-9 cells increased from 19 ± 6% in early-passage cells to 37 ± 6% at senescence, (P < 0.01). The levels observed in senescent LP-9 cells were significantly higher compared with senescent HOMCs (P < 0.004). To confirm these observations, the Pearson’s correlation coefficient (which reflects the degree of colocalization between the two fluorescent images) was calculated in 20 randomly chosen nuclei in each group. The analysis revealed that while in HOMCs the correlation coefficients were similar in early-passage and senescent cells (0.11 ± 0.02 vs. 0.12 ± 0.02, respectively), they increased significantly in LP-9 cells from 0.08 ± 0.02 in early-passage cells to 0.32 ± 0.21 in senescent cells (P < 0.02). Importantly, the Pearson’s coefficient in senescent LP-9 cells was significantly higher than in senescent HOMCs.

Fig. 1. Replicative capacity of human omentum-derived mesothelial cells (HOMCs) and LP-9 cells. Cells were cultured under the same conditions as described in MATERIALS AND METHODS. The data were derived from five independent experiments performed with HOMCs from different donors.
Telomere length in HOMCs and LP-9 cells. In agreement with previous observations (24), we found that telomere length in HOMCs did not shorten significantly during senescence (Fig. 4). In contrast, the telomeres in LP-9 cells did shorten during senescence by \( \Delta \text{438 base pairs (bp)} \) \( (P < 0.04) \). Interestingly, early-passage LP-9 cells were found to have longer telomeres than early-passage HOMCs \( (4,339 \pm 227 \text{ vs. } 3,522 \pm 207; P < 0.02) \).

Oxidative DNA and lipid injury in HOMCs and LP-9 cells. The presence of the oxidative DNA adduct, 8-OH-dG, and the lipid peroxidation product, lipofuscin, reflected the extent of oxidative injury to DNA and lipids. The levels of 8-OH-dG in early-passage cells were similar in HOMC and LP-9 cultures (Fig. 5A). These levels increased with time, as the cells progressed into senescence. A sharp rise in 8-OH-dG accumulation began much earlier in HOMCs and resulted in an \( \Delta \text{8-fold} \)
increase in senescent cells. An increase observed in LP-9 cells occurred later but was equally sharp and led to even greater accumulation of 8-OH-dG in senescent cells. These levels were about 12 times higher than in early passage LP-9 cells and significantly greater than in senescent HOMCs ($P < 0.001$).

Senescence of both HOMCs and LP-9 cells was also associated with increased accumulation of lipofuscin (Fig. 5B). Compared with 8-OH-dG, accumulation of lipofuscin was less pronounced and occurred more gradually. Similarly to 8-OH-dG, however, the levels of lipofuscin in cultures entering senescence were higher in LP-9 cells than in HOMCs ($412 \pm 78$ vs. $298 \pm 40$ relative light units/$10^5$ cells, $P < 0.05$).

**Generation of ROS by HOMCs and LP-9 cells.** Generation of ROS by HOMCs and LP-9 cells rose progressively during consecutive passages (Fig. 6A). The values measured in HOMCs at early stages were $1.5$-fold higher than those in LP-9 cells ($P < 0.04$). The levels recorded in senescent cells did not differ significantly. To assess the generation of ROS in response to exogenous oxidative stress, early-passage HOMCs and LP-9 cells were exposed to sublethal doses of t-BHP. The

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**Fig. 3.** DNA damage response in HOMCs and LP-9 cells. A: fraction of cells with DNA damage foci. B: representative images showing the presence of DNA damage foci in early-passage and senescent HOMCs and LP-9 cells, as assessed by $\gamma$H2A.X immunostaining (magnification $\times 400$). The arrows identify cells bearing $\gamma$H2A.X (green pixels). C: fraction of $\gamma$H2A.X foci colocalizing to telomeres. D: representative images showing colocalization of $\gamma$H2A.X foci (green pixels) and telomeres (red pixels) in senescent cells. Exemplary colocalization sites identified by the Pearson’s correlation analysis (yellow pixels) are shown in squares. The data presented in A and C were obtained from four experiments with HOMCs from separate donors. *Significant difference between early-passage cells and senescent cells of the same type.
exposure resulted in increased production of ROS in both cell types. The degree of stimulation was greater in HOMCs compared with LP-9 cells (4.5 ± 0.5 vs. 3.4 ± 0.7-fold increase, respectively). In absolute values, the generation of ROS by t-BHP-treated HOMCs was 2.3 ± 0.2 times greater compared with LP-9 cells (P < 0.0001) (Fig. 6B).

Antioxidant defense in HOMCs and LP-9 cells. The activity of SOD in either HOMCs or LP-9 cells did not change markedly during senescence (Fig. 7A). However, at all time points investigated, the activity of SOD in LP-9 cells was significantly higher than that in HOMCs. As previously reported (22), GSH levels in HOMCs decreased during senescence. In contrast, GSH content in LP-9 cells was higher and remained elevated as the cells progressed toward senescence. At this stage, GSH level in LP-9 cells was more than twice as high as in HOMCs (P < 0.001) (Fig. 7B).

DISCUSSION

Various cell types undergo senescence after having spent different times in culture. For instance, fibroblasts can usually divide many more times than epithelial cells before getting senescent (3, 37). The differences may exist even between cells of the same type but derived from different anatomical sites, as exemplified by MRC-5 lung fibroblasts and BJ skin fibroblasts (39) or various porcine fibroblast subpopulations (52). In the present study, we have attempted to shed more light on earlier observations, suggesting that the replicative potential of the mesothelial cell line LP-9 may be different from that of omentum-derived mesothelial cells (26, 42). To this end, we have compared several senescence-associated parameters in LP-9 cells and HOMCs under standardized culture conditions. We have been able to confirm that, indeed, HOMCs enter senescence after significantly fewer divisions compared with LP-9 cells. In addition, while both cell strains ubiquitously expressed SA-β-Gal at senescence, a significantly higher percentage of HOMCs expressed SA-β-Gal already at early stages in culture. In this respect, Zhu et al. (52) have observed that—from several porcine fibroblast subpopulations—those with the lowest proliferative capacity showed the earliest increase in the proportion of SA-β-Gal-positive cells and the earliest entry into senescence. Furthermore, there is large heterogeneity in the doubling potential of individual cells within a population. As a result, senescent cells may emerge in apparently young cultures and potentially impact on the proliferative capacity of the population as a whole (7). In this respect, we have recently observed faster senescence of those HOMC cultures that contained increased numbers of senescent cells at early-passage stages (23). Consequently, one may hypothesize that a lower percentage of senescent cells in LP-9 cultures contributes partially to their increased life span.

It is now well recognized that senescence may be a form of cell response to extensive and/or irreparable DNA damage (8, 46). We have therefore assessed HOMCs and LP-9 cells for the presence and the extent of DNA damage. In agreement with a previous report (24), we have observed a high fraction of early-passage HOMCs carrying γ-H2A.X, a molecular indicator of DNA damage response. Remarkably, the relative number of HOMCs affected was almost three times as large as that seen in early-passage LP-9 cells. The fraction of LP-9 cells with DNA damage foci was similar to that reported in early-passage fibroblasts and thought to result from ongoing replication (8). The difference between HOMCs and LP-9 cells in the fraction

![Fig. 4. Telomere length in HOMCs and LP-9 cells. Telomere length was measured in early-passage and senescent cells. The data were derived from three experiments with HOMCs from different donors. *Significant difference between early-passage cells and senescent cells of the same type.](http://ajpregu.physiology.org/)

![Fig. 5. Accumulation of 8-OH-dG (A) and lipofuscin (B) in HOMCs and LP-9 cells. The data were obtained from four experiments with HOMCs from separate donors. *Significant difference between HOMCs and LP-9 cells at the same time point. Bidirectional arrows compare HOMCs and LP-9 cells at senescence.](http://ajpregu.physiology.org/)
of early-passage cells with γ-H2A.X corresponded to a difference in SA-β-Gal expression at this stage, which suggested that DNA damage response played a key role in inducing HOMC senescence.

We have previously reported that HOMCs undergo senescence largely in a telomere-independent and p16INK4a-mediated manner (24, 26). In contrast, our current data point to a more significant involvement of telomeres in senescence of LP-9 cells. First, we found that the levels of p16INK4a mRNA and protein expression in LP-9 cells were significantly lower than in HOMCs. Next, we detected that senescence of LP-9 cells, but not of HOMCs, was associated with a marked increase in DNA damage foci localizing to telomeres. Moreover, LP-9 cells displayed a gradual increase in p21WAF1 expression that is thought to accompany telomere-dependent senescence. Finally, we observed that telomeres of LP-9 cells shortened during consecutive passages by 18–34 bp/PD, which approximated the lowest rate of telomere shortening estimated to occur in fibroblasts (44). These findings are in keeping with those of Dickson et al. (9), who have demonstrated that LP-9 cells transduced to express the telomerase catalytic subunit, hTERT, continued to divide beyond their natural life span (9).

Finding previously unaltered p21WAF1 mRNA expression, as opposed to markedly increased p16INK4a mRNA expression, we did not consider p21WAF1 to be a key regulator of HOMC senescence (26). However, our current data show that the levels of p21WAF1 protein in HOMCs may increase without a corresponding rise in mRNA expression. Nevertheless, this increase in p21WAF1 expression does not have to undermine the concept of HOMCs undergoing predominantly p16INK4a-mediated senescence. Simultaneous increases in p16INK4a and p21WAF1 have been reported in various strains of senescent fibroblasts (2). This effect may reflect different sensitivities of some cells within a population to stochastic environmental stresses (18). Although senescence-associated DNA injuries in HOMCs were found predominantly in nontelomeric sequences, they were also detected within telomeres (25), which in some cells might trigger p21WAF1, telomere dependent senescence and contribute to the rise of p21WAF1 expression observed.

Since oxidative stress has been implicated as a prominent causal factor of cellular senescence in vitro (44), we measured how parameters of oxidative stress changed during senescence of HOMCs and LP-9 cells. We found that the accumulation of 8-OH-dG and lipofuscin increased significantly over time in both HOMCs and LP-9 cells. Interestingly, when assessed in
cells entering senescence, the levels of 8-OH-dG and lipofuscin were found to be significantly higher in LP-9 cells than in HOMCs. This observation could be interpreted as reflecting greater vulnerability of HOMCs compared with LP-9 cells so that less extensive oxidative DNA and lipid damage may trigger senescence in response to oxidative stress.

We then went on to examine the production of ROS in HOMCs and LP-9 cells. We found that ROS levels increased during senescence of both HOMCs and LP-9 cells. Consistently, with the time course of progression into senescence, the rise in ROS generation occurred earlier in HOMCs than in LP-9 cells. In this respect, Lorenz et al. (29) have shown that MRC-5 lung fibroblasts, which stop dividing earlier than BJ skin cells, display at that time significantly increased production of ROS. Moreover, induction of ROS by prolonged exposure to hyperoxia caused an early senescence-like growth arrest in MRC-5 cells but not in BJ fibroblasts (29). These results suggest that cells characterized by high proliferative capacity are more effectively protected against oxidative stress. To test whether the same effects occur in HOMCs and LP-9 cells, we assessed the cell response to exogenous t-BHP-induced oxidative stress. We found that, indeed, exposure of HOMCs to an exogenous oxidant resulted in a massive induction of intracellular ROS. In contrast, generation of ROS by t-BHP-treated LP-9 cells was considerably lower.

To assess antioxidant defenses in HOMCs and LP-9 cells, we measured the levels of enzymatic (SOD) and nonenzymatic (GSH) antioxidants. Both the activity of SOD and the GSH content were considerably higher in LP-9 cells and did not fall during senescence. In this respect, Serra et al. (38) have documented increased SOD activity in fibroblasts with longer replicative life span compared with fibroblasts with lower proliferative potential. Similarly, GSH levels were found to decrease during senescence of MRC-5 fibroblasts (22) that seem to have a relatively short replicative life span (49). In contrast, GSH levels did not significantly decline throughout the replicative history of well-proliferating TIG-3S skin fibroblasts (20).

Although HOMCs were obtained from various donors, the pattern of HOMC senescence observed both in the present and in our earlier study (26) was remarkably consistent and therefore could be viewed as typical for this cell type. Yet, it remains to be determined whether mesothelial cells derived from the ascites fluid associated either with cancer or with nonmalignant lesions, display features similar to those of the LP-9 line. Another factor that may potentially contribute to the differences observed is the isolation procedure. HOMCs were released from a tissue specimen by enzymatic digestion, while LP-9 cells were simply harvested from the peritoneal transudate. One may hypothesize that the stress connected with the surgical removal of omentum and subsequent exposure to trypsin added to extensive DNA damage and an early-onset of senescence. One could also argue that peritoneal mesothelial cells found in the ascites fluid are not representative of the normal mesothelium but are instead dysfunctional cells that detached from a diseased peritoneal surface. On the other hand, an elegant study by Foley-Comer et al. (15) has demonstrated that a small population of normal mesothelial cells floating freely in the peritoneal cavity plays a key role in regeneration of the mesothelium after injury. It has, therefore, been suggested (17) that these cells may have progenitor cell-like properties and increased replicative capacity.

In conclusion, we show that two commonly used models of the peritoneal mesothelium differ significantly in their patterns of senescence. Although phenotypic features of senescent HOMCs and LP-9 cells are very similar, the dynamics of their appearance and the underlying mechanisms are significantly different. These observations may have some practical implications, e.g., during peritoneal mesothelial cell transplantation. The procedure has been proposed to enhance mesothelial regeneration after surgery- or peritoneal dialysis-induced trauma and thus prevent peritoneal adhesion formation (32). In addition, transplantation of mesothelial cells has been used to improve vascularization of the ischemic myocardium (14) and to reconstruct the bladder (48). There is an ongoing debate on how to optimally isolate and propagate cells to be transplanted (32). In this respect, the fact that peritoneal mesothelial cell strains exhibit different senescence rates may be of importance if such procedures are to be effective.

Furthermore, our observations may impact on peritoneal dialysis fluid biocompatibility testing. It has been observed that peritoneal mesothelial cells exposed chronically to dialysis fluids develop a senescence-like phenotype (16). We have previously demonstrated that glucose (a basic component of dialysis solutions) may accelerate the development of senescence features in mesothelial cells, largely by exacerbating oxidative stress (22). Therefore, the assessment of whether new dialysis fluid formulations induce a senescence response in mesothelial cells may be used as an additional parameter in biocompatibility testing. In this respect, the demonstration that mesothelial cell strains differ in their vulnerability to oxidative stress and senescence may help standardize such assays.

GRANTS

This work was partially supported by The Royal Society Travel Grant to K. Ksiazek and T. von Zglinicki, by the Research into Ageing Programme Grant to T. von Zglinicki, and by the grant from the Polish Ministry of Science and Higher Education (N401 094 31/2181) to K. Ksiazek and J. Witowski.

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