Increase of mitochondria and mitochondrial DNA in response to oxidative stress in human cells

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Mitochondrial respiratory function is impaired in the target tissues of patients with mitochondrial diseases and declines with age in various human tissues. It is generally accepted that respiratory-chain defects result in enhanced production of reactive oxygen species and free radicals in mitochondria. Recently, we have demonstrated that the copy number of mitochondrial DNA (mtDNA) is increased in the lung tissues of elderly human subjects. The mtDNA copy number was suggested to be increased by a feedback mechanism that compensates for defects in mitochondria harbouring mutated mtDNA and a defective respiratory system. However, the detailed mechanism remains unclear. In this study, we treated a human lung fibroblast cell line, MRC-5, with H_2O_2 at concentrations of 90–360 μM. After the treatment for 24–72 h, we found that cells were arrested at G_0 and G_1 phases but that mitochondrial mass and mtDNA content were significantly increased in a concentration- and time-dependent manner. Moreover, the oxidative stress induced by buthionine sulphoximine was also found to cause an increase in mitochondrial mass of the treated cells. Increased uptake of a vital mitochondrial dye Rhodamine 123 and enhanced tetrazolium [MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide] reduction revealed that the mitochondria increased by H_2O_2 treatment were functional. In addition, the increase in the mitochondrial mass was also observed in cell-cycle-arrested cells induced by mimosine, lovastatin and genistein. Taken together, these findings suggest that the increase in mitochondrial mass and mtDNA content are the early molecular events of human cells in response to endogenous or exogenous oxidative stress through cell-cycle arrest.

Key words: aging, cell-cycle arrest, hydrogen peroxide, mtDNA copy number, reactive oxygen species.

INTRODUCTION

Mitochondria are the intracellular organelles responsible for ATP synthesis through the coupling of oxidative phosphorylation to respiration in human and animal cells. Under normal physiological conditions, a small fraction of oxygen consumed by mitochondria is converted to superoxide anions, H_2O_2 and other reactive oxygen species (ROS) [1]. Moreover, mitochondrial respiratory function has been demonstrated to decline in various human tissues during the aging process [2,3]. This is thought to be caused, at least partly, by oxidative damage and mutation of mitochondrial DNA (mtDNA) in somatic tissues of aged individuals [3].

Recently, we found that mtDNA copy number is increased in the lung tissues of elderly human subjects [4]. Moreover, we showed that mtDNA mutation, oxidative DNA damage and lipid peroxidation are increased during aging in the human lung [4–7] and other tissues [8,9]. These findings are consistent with a previous report that mtDNA contents are increased in the tissues of old rats [10] and of elderly subjects [11]. This increase in the content of mtDNA was thought to compensate for respiratory-function decline during the aging process [12]. However, the molecular mechanism underlying the increase of mtDNA associated with respiratory-function decline in human aging remains unclear.

Mitochondria are the major intracellular source and primary target of ROS, which are generated under normal conditions as by-products of aerobic metabolism in animal and human cells. It has been established that defects in the respiratory chain lead to enhanced production of ROS and free radicals in mitochondria [13–15]. Moreover, H_2O_2 has been proposed to play a role as a secondary messenger in signal-transduction pathways [16,17]. The increase of ROS and enhanced oxidative stress stimulates the expression of early growth-related genes such as c-*fos* and c-*jun* [18]. Recently, H_2O_2 was proposed to be involved in the communication between mitochondria and the nucleus [19,20]. Therefore, we hypothesized that the age-dependent increase in the production of superoxide anions and H_2O_2 from mitochondria is one of the factors involved in feedback compensation for the decline of respiratory function during the aging process [21].

To test this hypothesis, we exposed MRC-5, a human lung fibroblast cell line, to oxidative stress by treatment with low concentrations of H_2O_2 or buthionine sulphoximine (BSO). The effect of oxidative-stress treatment on the copy number of mtDNA and mitochondrial mass in the cell was investigated.

MATERIALS AND METHODS

Cell culture

Human lung fibroblasts, from the MRC-5 cell line, were cultured at 37 °C in humidified 5% CO_2/95% air in Dulbecco's modified Eagle's medium (Gibco/BRL, Bethesda, MD, U.S.A.) supplemented with 10% fetal bovine serum, 50 units/ml penicillin G

Abbreviations used: mtDNA, mitochondrial DNA; ROS, reactive oxygen species; BSO, buthionine sulphoximine; NAO, 10-*n*-Nonyl-Acridine Orange; DCF, 2′,7′-dichlorofluorescein; DCFH-DA, 2′,7′-dichlorofluorescin diacetate; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide; $DiOC_6(3)$, 3,3′-dihexyloxacarbocyanine iodide.

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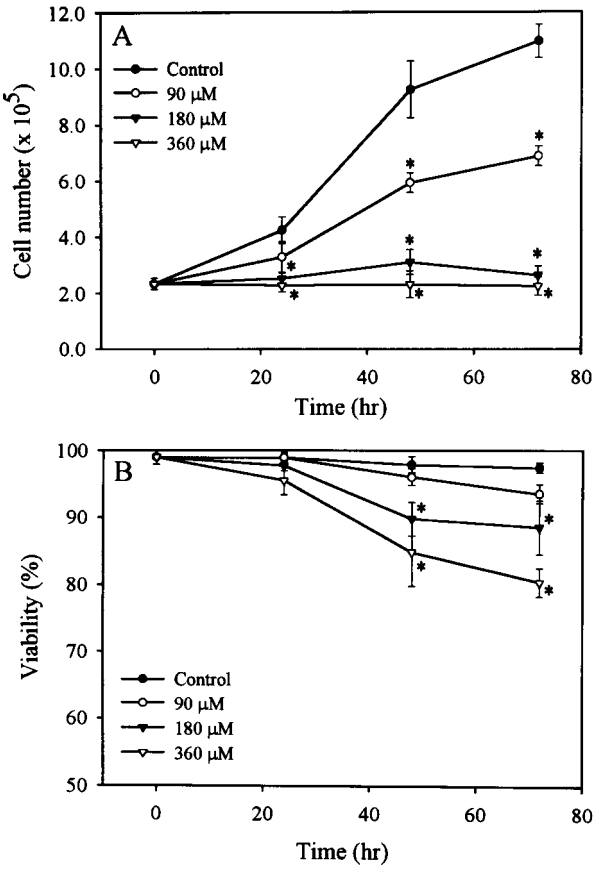

Figure 1 Cell growth and viability of MRC-5 cells treated with H_2O_2

MRC-5 cells were treated with H_2O_2 at concentrations from 90 to 360 μM. After treatment for 24–72 h, cells were enumerated using a haemocytometer (**A**), and the number of viable cells was determined by Trypan Blue exclusion (**B**). Data points represent measured values $\pm$ S.D. of two independent experiments performed on cells from three separate dishes. The differences in growth kinetics and viability between untreated and H_2O_2-treated cells were found to be statistically significant by using one-way ANOVA (*, $P < 0.05$).

and 50 mg/ml streptomycin sulphate. Cells were subjected to a 1:2 split every 4 days.

Oxidative-stress treatment

Oxidative-stress treatment was performed on cells that had all been cultured at the same density. An aliquot of 2.5×10^5 cells at 25–35 passages was plated in triplicate in 25-cm^2 flasks 24 h before treatment. For H_2O_2 treatment, a suitable aliquot of 30% H_2O_2 solution was freshly diluted into the culture medium before the experiment. Cells were exposed to the indicated concentrations of H_2O_2 for a fixed period of time under normal culture conditions as described above. To deplete intracellular glutathione, cells were treated with 1 or 2 mM BSO (Sigma, St. Louis, MO, U.S.A.) for 72 h. The treated cells were then washed once with Hanks' balanced salt solution (Gibco/BRL) and were harvested by trypsinization at various time points for further analysis.

Treatment with drugs affecting the cell cycle

To arrest the cell cycle, cells were treated with 200 μM mimosine, 2.5 μM lovastatin or 50 μM genistein (Sigma) for 24 h. The treated cells were then washed once with Hanks' balanced salt solution and were harvested by trypsinization at various time points for cell-cycle analysis and mitochondrial-mass determination.

Cell growth and viability assay

Cells were harvested by trypsinization and resuspended in 1 ml of Medium 199 (Gibco/BRL). The cells were enumerated using a haemocytometer, and the number of viable cells was determined on the basis of their exclusion of 0.4% Trypan Blue.

Cell-cycle analysis

After treatment with H_2O_2 or other chemicals, cells were trypsinized and resuspended in 70% ethanol. The cells were incubated on ice for at least 1 h and resuspended in 1 ml of cell-cycle assay buffer (0.38 mM sodium citrate, 0.5 mg/ml RNase A and 0.01 mg/ml propidium iodide) at a concentration of 5×10^5 cells/ml. Samples were stored in the dark at 4 °C until cell-cycle analysis, which was carried out by use of a flow cytometer and ModFit LT 2.0 software (Verity Software, Topsham, ME, U.S.A.).

Determination of mitochondrial mass

The fluorescent dye 10-*n*-Nonyl-Acridine Orange (NAO; Molecular Probes, Eugene, OR, U.S.A.), which binds specifically to cardiolipin at the inner mitochondrial membrane independently of membrane potential ($\Delta\Psi_m$) [22,23], was used to monitor the mitochondrial mass [24]. Cells were trypsinized and resuspended in 0.5 ml of Medium 199 containing 10 μM NAO or 10 μM MitoTracker Green FM (Molecular Probes), which is preferentially accumulated in mitochondria regardless of mitochondrial membrane potential. After incubation for 10 min at 25 °C in the dark, cells were transferred immediately to a tube on ice for flow-cytometric analysis.

Dichlorofluorescin staining

H_2O_2 production by cultured cells was measured using the probe 2′,7′-dichlorofluorescin diacetate (DCFH-DA; Molecular Probes) [24–26]. This probe is accumulated by cells and hydrolysed by cytoplasmic esterases to become 2′,7′-dichlorofluorescin, which then reacts with H_2O_2 to give the fluorescent product 2′,7′-dichlorofluorescein (DCF) [25]. Cells were incubated with 5 μM DCFH-DA in culture medium for 30 min at 37 °C and then washed, resuspended in 0.5 ml of PBS, and submitted to flow-cytometric analysis.

Measurement of mitochondrial membrane potential

Cells were trypsinized and resuspended in 0.5 ml of Medium 199 containing 0.5 μg/ml Rhodamine 123 (Molecular Probes) or 3,3′-dihexyloxacarbocyanine iodide [$DiOC_6(3)$; Molecular Probes]. After incubation for 30 min at 37 °C in the dark, cells were transferred immediately to a tube on ice for analysis of the fluorescence intensity by flow cytometry.

Flow-cytometric analysis

A FACScan flow cytometer (Becton Dickinson, Bedford, MA, U.S.A.) equipped with a 488-nm argon laser was used for the flow-cytometric analysis. Forward and side scatters were used to establish size gates and exclude cellular debris from the analysis.

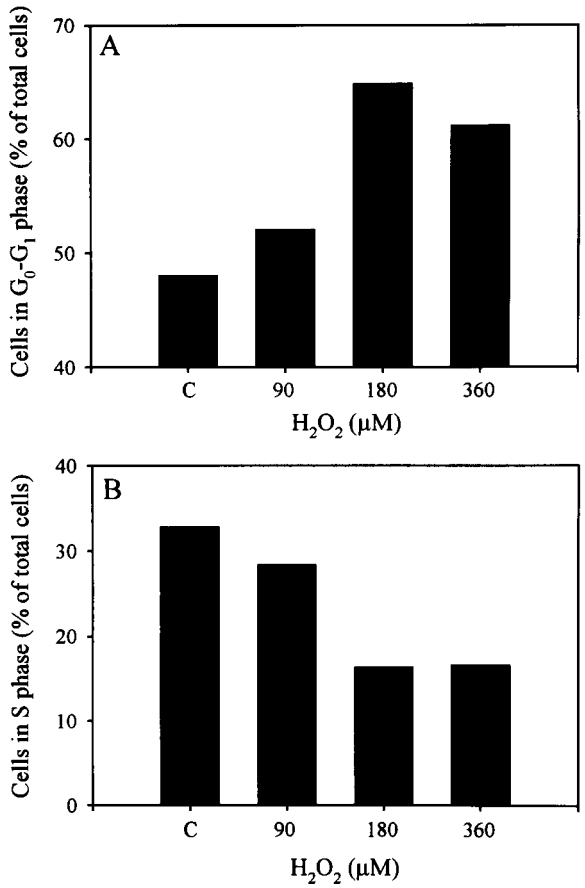

Figure 2 Cell-cycle arrest induced by H_2O_2

MRC-5 cells were treated with 90–360 μM H_2O_2. After treatment for 24 h, the percentages of cells in G_0–G_1 phase (**A**) and S phase (**B**) were analysed on a flow cytometer as described in the Materials and methods section. C, control.

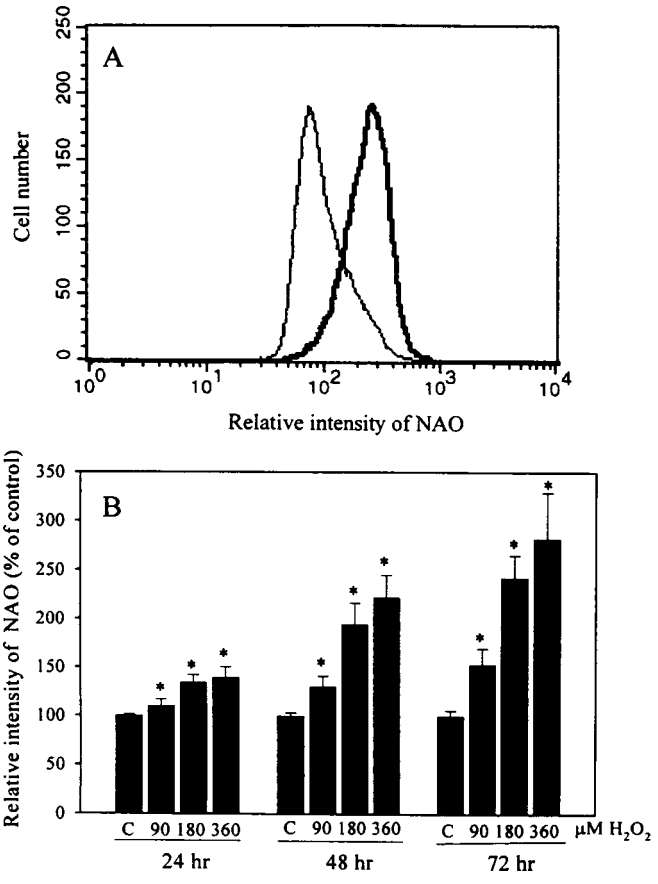

Figure 3 Increase of mitochondrial mass in MRC-5 cells treated with H_2O_2

(**A**) MRC-5 cells were treated with 180 μM H_2O_2. After 48 h of treatment, the mitochondrial mass of the cells stained with NAO was analysed by flow cytometry. The area under the thin (left-hand) curve represents the population of control cells, and the area under the thick (right-hand) curve represents the population of H_2O_2-treated cells. (**B**) MRC-5 cells were treated for 24–72 h with 90–360 μM H_2O_2, and the relative NAO intensity of treated cells was analysed by flow cytometry. Results are expressed graphically as percentages of the fluorescence intensity of the untreated control (C) cells. Values are means $\pm$ S.D. of results from six independent experiments performed in triplicate. Statistical analysis showed that the differences in the NAO intensity between the untreated and H_2O_2-treated cells were statistically significant (*, $P < 0.05$ versus control).

The excitation wavelength was set at 488 nm. The observation wavelength of 530 nm was chosen for green fluorescence and 585 nm for red fluorescence and the intensities of emitted fluorescence were collected on FL1 and FL2 channels, respectively. In each measurement, a minimum of 20000 cells were analysed. Data were acquired and analysed using the Cell Quest software (Becton Dickinson). Relative change in the mean fluorescence intensity was calculated as the ratio between mean fluorescence intensity in the channel of the treated cells and that of the control cells.

Southern-blot hybridization

For Southern-blot analysis, 5 μg of total DNA of the treated and control cells were linearized by digestion with *Bam*HI (Boehringer Mannheim, Mannheim, Germany), and the digested DNA was electrophoresed at 50 V for 12 h in a 1.0% agarose gel. DNA bands in the gel were denatured and transferred on to a Hybond N^+ nylon membrane (Amersham, Little Chalfont, Bucks., U.K.). Hybridization with a [α-^{32}P]dCTP-labelled human mtDNA probe of the D-loop region (spanning nucleotide positions 16455–1462) was carried out at 65 °C for 2 h in a rapid hybridization buffer (Amersham). The filter was washed with 2 × SSC/0.1% SDS (where 1 × SSC is 0.15 M NaCl/0.015 M sodium citrate) for 15 min at room temperature and then washed twice with 0.1 × SSC/0.1% SDS for 15 min at 65 °C. The washed filter was wrapped with Saran Wrap ('clingfilm') and subjected to autoradiography.

MTT reduction assay

Cells were treated with H_2O_2 at the concentrations from 90 to 360 μM as described above. After 48 h of incubation, cells were harvested and cell viability was determined by the Trypan Blue-exclusion method. For determination of mitochondrial function, we incubated the cells with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT; Sigma) at a final concentration of 2.0 μg/ml during the last 2 h of the incubation period. The cells were lysed with DMSO and the absorbance at 570 nm was measured. An MTT reduction index was calculated

as the ratio between the absorbance at 570 nm and the number of viable cells [27].

Statistics

Data are presented as means ± S.D. except where indicated. Comparisons among multiple groups were made by a one-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference *post hoc* test. The differences with $P < 0.05$ were considered significant.

RESULTS

After treatment for 24–72 h with 90–360 μM H_2O_2, growth kinetics and viability of MRC-5 cells were affected in a dose-dependent manner as shown in Figure 1(A) and 1(B), respectively. We found that the untreated cells and the cells treated with 90 μM H_2O_2 kept growing steadily, but growth retardation was observed in the cells treated with 180 or 360 μM H_2O_2. Moreover, the rate of increase in the number of the cells treated with 90 or 180 μM H_2O_2 was significantly lower than that of the untreated cells ($P < 0.05$, Figure 1A). Most of the cells treated with 180 or 360 μM H_2O_2 appeared enlarged, flattened, and less spindle-shaped as compared with the control cells at the end of 24-h treatment (results not shown). We also found that the viability of the cells treated with 180 or 360 μM H_2O_2 was lower than that of the control cells ($P < 0.05$), but still remained 80% after 72-h treatment (Figure 1B). Furthermore, we found that 24 h after treatment the percentage of cells in G_0 and G_1 phases was increased (Figure 2A), and that the percentage of cells in the S phase decreased as a function of H_2O_2 concentration (Figure 2B).

In an attempt to determine whether H_2O_2 leads to the proliferation of mitochondria, we used the fluorescent dye NAO to monitor the mitochondrial mass [22–24]. After treatment for 24–72 h with the indicated concentrations of H_2O_2, the relative NAO intensity of the treated cells was found to be significantly higher than that of the untreated cells ($P < 0.05$, Figure 3). The relative NAO intensity of the treated cells was increased to 1.3–2.5-fold as compared with that of the control cells, depending on the H_2O_2 concentration and the duration of incubation (Figure 3). We observed a similar increase in the relative intensity of another fluorescent dye, MitoTracker Green FM (results not shown). These results indicate that the oxidative stress induced by H_2O_2 can lead to an increase in the number of mitochondria in MRC-5 cells in a concentration- and time-dependent manner.

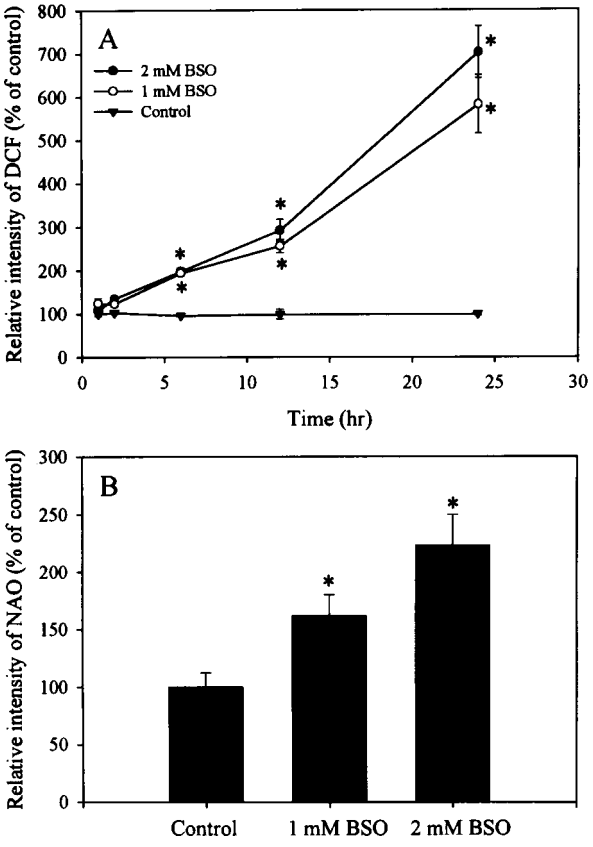

Figure 4 Increase of mitochondrial mass in MRC-5 cells treated with BSO

After treatment of MRC-5 cells with 1 or 2 mM BSO, the production of ROS in the cells, monitored by DCF fluorescence, increased with time (**A**). After 72 h of treatment, the mitochondrial mass of the cells stained with NAO was analysed by flow cytometry (**B**). Results are expressed graphically as percentages of the fluorescence intensity of the untreated (C) cells. Values are means ± S.D. of the results from two independent experiments performed in triplicate. Statistical analysis showed that the differences in the DCF or NAO intensity between the untreated and BSO-treated cells were statistically significant (*, $P < 0.05$ versus control).

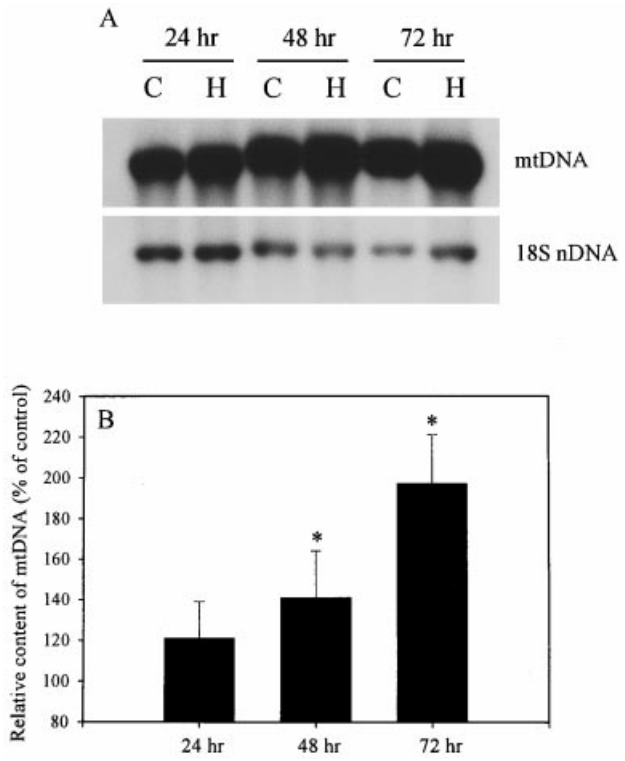

Figure 5 Increase of mtDNA content in MRC-5 cells treated with H_2O_2

After treatment of MRC-5 cells with 180 μM H_2O_2 for 24–72 h, the relative mtDNA content of the H_2O_2-treated cells (H) was determined by Southern-blot hybridization (**A**). The DNA band intensities were analysed by scanning densitometry and mtDNA content was corrected for variation in sample loading by normalization with the DNA band intensity of the 18 S rRNA gene. (**B**) Results are expressed graphically as percentages of the mtDNA band intensity of the untreated (C) cells. Values are means ± S.D. of the results from three independent experiments. Statistical analysis showed that the differences in the mtDNA band intensity between the untreated and H_2O_2-treated cells were statistically significant (*, $P < 0.05$ versus control).

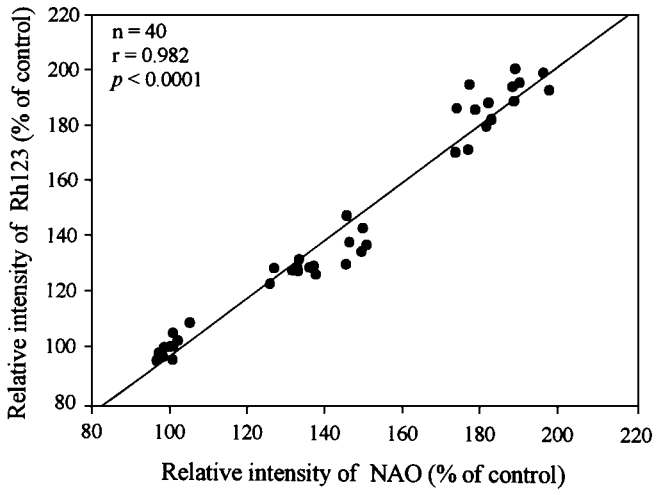

Figure 6 Membrane potential is normal in the extra mitochondria of the H_2O_2-treated cells

After treatment of MRC-5 cells with 90 or 180 μM H_2O_2 for 48 h, the mitochondrial mass and membrane potential were measured by flow cytometry after staining the cells with NAO and Rhodamine 123 (Rh123), respectively. The relative intensity is expressed graphically as a percentage of the fluorescence intensity of the untreated cells. The increase in mitochondrial membrane potential and mitochondrial mass were correlated throughout the 48-h period of the experiment ($r = 0.982$, $P < 0.0001$).

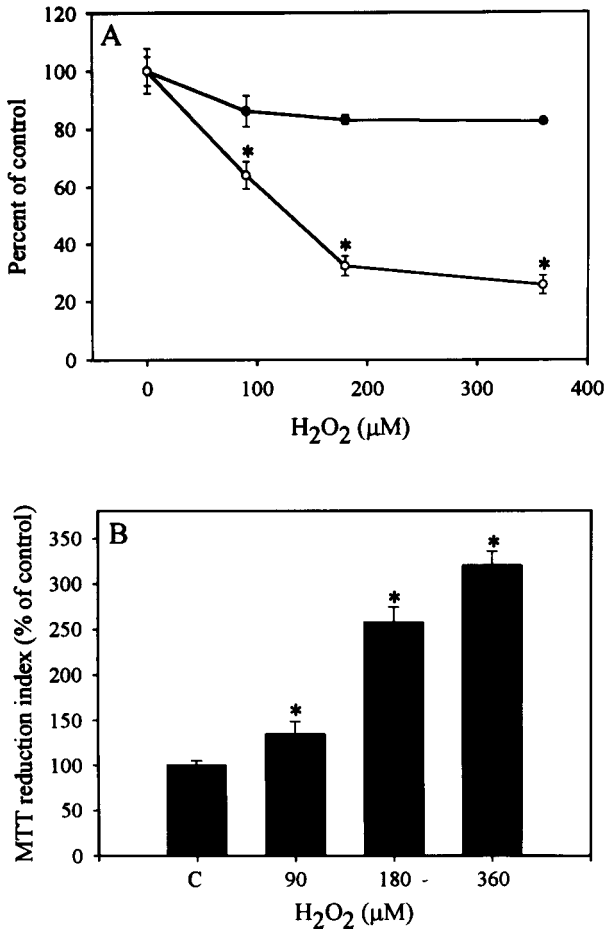

Figure 7 The increased mitochondria in the H_2O_2-treated cells are functional

Cells were treated with H_2O_2 at concentrations from 90 to 360 μM as described in the text. After 48 h of incubation, the capacity of the treated cells to reduce MTT was analysed as described in the Materials and methods section. The absorbance at 570 nm of the formosan formed in the MTT assay (●) and the number of viable cells (○) were determined concurrently after incubation of the MRC-5 cells for 48 h with the indicated concentrations of H_2O_2, and are expressed as percentages of the control (**A**). MTT reduction index was calculated as the ratio between the absorbance at 570 nm and the number of viable cells counted using a haemacytometer after 48 h of incubation with indicated concentrations of H_2O_2 (**B**). The data are expressed as the percentage of controls (C; means ± S.D.) from two independent experiments in triplicate. Statistical analysis showed that the differences in MTT reduction index between H_2O_2-treated and control cells are statistically significant (*, $P < 0.05$ versus control).

In addition to the oxidative stress induced by H_2O_2, BSO, an inhibitor of the glutathione biosynthetic pathway, was used to treat the cultured cells. After treatment with 1 or 2 mM BSO, we found that the concentration of ROS in the cells, as revealed by staining with DCFH-DA, increased with time ($P < 0.05$, Figure 4A). After treatment for 72 h with 1 or 2 mM BSO, the relative intensity of NAO of the treated cells was increased to 1.6- or 2.2-fold as compared with that of the control cells, respectively ($P < 0.05$, Figure 4B). These results indicate that not only H_2O_2 but also glutathione depletion can lead to an increase in the number of mitochondria in MRC-5 cells.

Using Southern-blot hybridization, we determined the mtDNA content of the MRC-5 cells after treatment with 180 μM H_2O_2 for 24–72 h. We found that the mtDNA content of the treated cells was higher than that of the untreated cells and increased with the duration of incubation (Figure 5). This increase of mtDNA content was consistent with the observation of proliferation of mitochondria in the H_2O_2-treated cells as revealed by NAO staining.

To analyse whether the extra mitochondria in the H_2O_2-treated cells were functional, we measured mitochondrial membrane potential of the treated cells by staining with Rhodamine 123 [25]. The relative intensity of Rhodamine 123 of the H_2O_2-treated cells was significantly higher than that of the control cells and was increased with the duration of incubation (results not shown). The increase in mitochondrial membrane potential correlated well with the increase in mitochondrial mass throughout the 48 h period of the experiment ($r = 0.982$, $P < 0.0001$, Figure 6). The mitochondrial membrane potential per unit mitochondrial mass, expressed as the ratio of fluorescence intensity of Rhodamine 123 to that of NAO, appeared to be similar throughout the 48 h of the experiment (Figure 6). Moreover, we observed similar results using another fluorescent dye, $DiOC_6(3)$, the uptake of which is sensitive to mitochondrial membrane potential (results not shown).

For the assessment of mitochondrial function, we analysed the capacity of H_2O_2-treated cells to reduce MTT. We found that although the number of the viable cells after H_2O_2 treatment was significantly decreased ($P < 0.05$), the absorbance at 570 nm of the remaining cells was only slightly decreased as compared with the untreated cells (Figure 7A). An arbitrary MTT reduction index, calculated as the ratio between the absorbance at 570 nm and the corresponding number of viable cells, revealed a significant increase in the relative MTT activity ($P < 0.05$, Figure 7B). These results indicate that the mitochondria increased in the H_2O_2-treated cells were functional.

To test whether the alteration of mitochondrial mass and mtDNA copy number in the cell might be uncoupled from the cell-cycle-controlled biosynthesis of chromosomal DNA, we treated MRC-5 cells with mimosine, lovastatin or genistein,

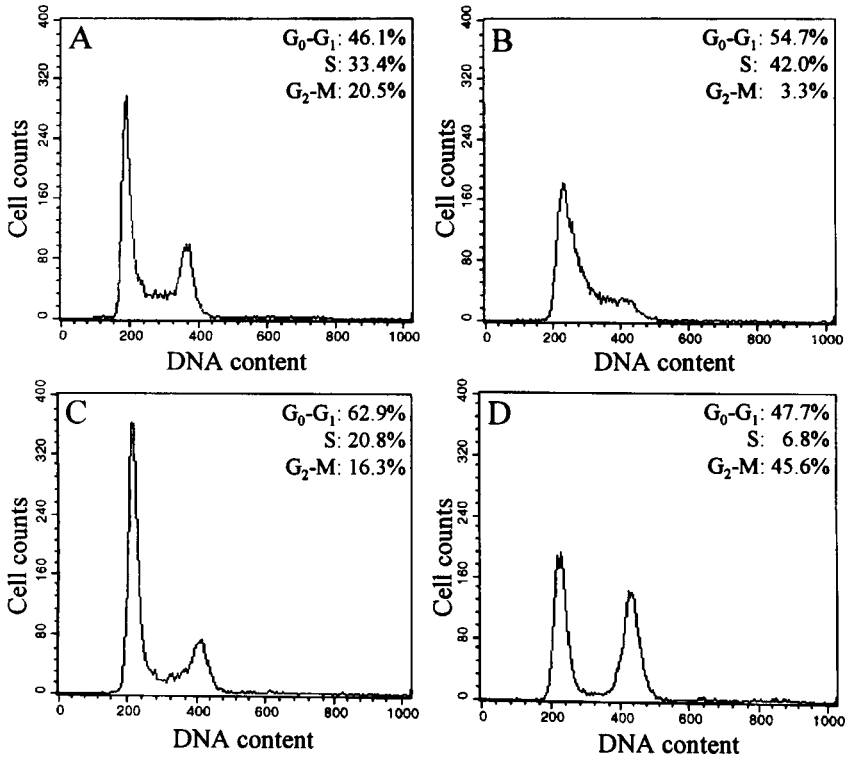

Figure 8 Cell-cycle arrest induced by mimosine, lovastatin and genistein

MRC-5 cells were treated with 200 μM mimosine, 2.5 μM lovastatin and 50 μM genistein in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. After 24 h of incubation at 37 °C, the cell cycle was analysed on a flow cytometer as described in the Materials and methods section. Typical results from untreated cells (**A**) and cells treated with mimosine (**B**), lovastatin (**C**) and genistein (**D**) are presented.

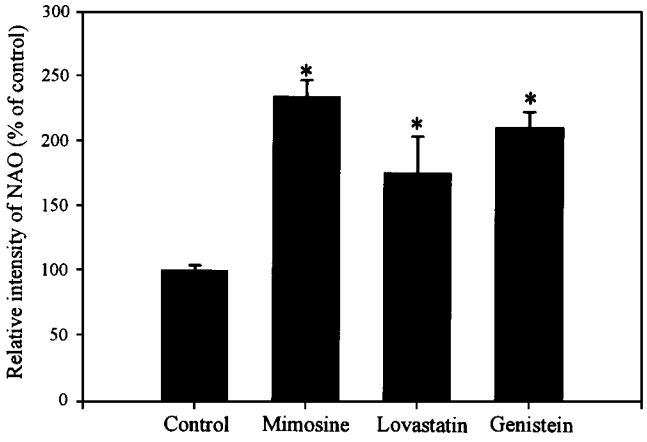

Figure 9 Increase of mitochondrial mass in MRC-5 cells treated with drugs affecting the cell cycle

MRC-5 cells were treated with 200 μM mimosine, 2.5 μM lovastatin and 50 μM genistein to cause cell-cycle arrest. After 48 h of incubation at 37 °C, the relative NAO intensity of treated cells was analysed by flow cytometry as described in the Materials and methods section. The results are expressed graphically as a percentage of the fluorescence intensity of the untreated MRC-5 cells. Values are means $\pm$ S.D. of the results obtained from three independent experiments. Statistical analysis showed that the differences in the NAO intensity between the untreated and drug-treated cells were statistically significant (*, $P < 0.05$ versus control).

which cause cell-cycle arrest. We found that after 24 h of treatment the cell cycle was arrested at the late G_1, G_0/G_1 and G_2/M phases by mimosine, lovastatin and genistein, respectively (Figure 8). Moreover, after 48 h of treatment the relative intensity of NAO of the treated cells was increased to 2.3-, 1.7- and 2.1-fold, respectively, as compared with that of the control cells ($P < 0.05$, Figure 9).

DISCUSSION

Each human and animal cell contains between several hundred and over a thousand mitochondria, each carrying 2–10 copies of mtDNA. The mitochondrial mass and mtDNA copy number of individual cells vary with the type of the cell and tissue, and are changed during cell differentiation, hormone treatment and exercise [28–31].

In the present study, we observed growth arrest in human lung fibroblasts treated with low concentrations of H_2O_2 (Figures 1 and 2). It is apparent that H_2O_2 at concentrations of between 90 and 180 μM increased the population-doubling time and that 360 μM H_2O_2 caused a permanent growth arrest. Most of the treated cells exhibited significant alterations in morphology as compared with the control cells. The results are consistent with the previous observations that H_2O_2 at sublethal concentrations can induce senescence-like growth arrest in human cells [32,33]. Moreover, we found in this study that the oxidative stress induced by sublethal concentrations of H_2O_2 caused an increase in the amount of mitochondria and mtDNA of the treated cells and that the increased mitochondria were functional. Furthermore, the oxidative stress induced by reduction of the intracellular glutathione level with BSO was also found to increase mitochondria in the treated cells (Figure 4). In a separate experiment,

we exposed MRC-5 cells to 20 μM antimycin A and observed that the ROS generation in the treated cells was increased 2-fold compared with that of the untreated cells after 30 min of treatment. Moreover, after treatment for 72 h, we found that the relative intensity of NAO in the treated cells was increased to 2.6-fold compared with that of the control cells. This is consistent with the observation that by partial inhibition of respiratory function by antimycin A, the expressions of both cytochrome c_1 and cytochrome *b* are increased in human fibroblasts [34]. Moreover, intracellular H_2O_2 level was found to increase within 3 h prior to expression of these genes. These results indicate together that mild oxidative stress can trigger cell-cycle arrest and lead to an increase of mitochondrial mass and mtDNA in human cells.

Increases of mitochondria and mtDNA synthesis have been observed to occur in the absence of nuclear DNA replication [35]. The alteration of mitochondrial mass and mtDNA in the cell might be uncoupled from the cell-cycle-controlled biosynthesis of chromosomal DNA. To confirm this, we treated MRC-5 cells with lovastatin, genistein or mimosine and observed that these drugs caused cell-cycle arrest (Figure 8). In addition, the mitochondrial mass was increased, to a different extent, in the cells after 48 h of treatment (Figure 9). It is worth noting that the amplitude of increase of the mitochondrial mass in the cells treated with the cell-cycle-arresting drugs was similar to that in the H_2O_2-treated cells within the same treatment period. The results imply that whereas overall cell division was arrested by H_2O_2 or the drugs, mitochondrial biogenesis was unaffected and that mitochondria continued to proliferate as if the cells were going to divide. Thus, mild oxidative stress may induce an increase of mitochondria and mtDNA via a pathway that bypasses cell-cycle control.

Increase of mitochondria has also been observed in some tumour cells treated with herbimycin A and genistein [24,27]. Mancini et al. [24] found that herbimycin A, a tyrosine kinase inhibitor, induces cell-cycle arrest in G_2/M and results in a marked accumulation of mitochondria in a human colon carcinoma cell line (Colo-205). In addition, the G_2/M cell-cycle arrest and increase of mitochondrial number and/or function were also observed in three tumour cell lines (MCF-7, human breast tumour; Jurkat cells, human T-cell leukaemia; and L-929, mouse transformed fibroblasts) after treatment of genistein [27]. The results obtained in these studies are consistent with our findings and support the notion that the cell-cycle arrest induced by oxidative stress plays a critical role in the increase of mitochondria and mtDNA.

Furthermore, it has been found that the relative content of mtDNA is increased with age and that the mtDNA content is correlated with the level of oxidative damage to DNA in human lung tissues [4]. Both oxidative DNA damage and lipid peroxidation are enhanced during aging in the human lung [4–7], indicating that there is a higher oxidative stress in the tissues of aged individuals. Therefore, the endogenous and/or exogenous oxidative stress in the lung of elderly subjects may cause cell-cycle arrest and thereby increase the amount of mitochondria and mtDNA in lung tissues.

In summary, we have demonstrated clearly for the first time that oxidative stress induced by H_2O_2 leads to an increase of mitochondria and mtDNA in human lung fibroblasts. Moreover, the mitochondria increased by H_2O_2-elicited oxidative stress are functional. We therefore suggest that the increase in mitochondrial mass and mtDNA content may be one of the early molecular events gearing the human cells up to respond to endogenous or exogenous oxidative stress through cell-cycle arrest.

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