J Hum Genet (2004) 49:134–140
DOI 10.1007/s10038-003-0122-3

ORIGINAL ARTICLE

Kyong-Ah Yoon · Yusuke Nakamura
Hirofumi Arakawa

Identification of *ALDH4* as a p53-inducible gene and its protective role in cellular stresses

Received: 14 November 2003 / Accepted: 5 December 2003 / Published online: 25 February 2004

Abstract To identify additional targets of p53, we used a cDNA microarray system to examine gene-expression patterns in response to enforced expression of exogenous p53 in p53-deficient cancer cells, and identified the aldehyde dehydrogenase 4 (*ALDH4*) gene as a direct target of p53. ALDH4 is a mitochondrial-matrix NAD^+-dependent enzyme catalyzing the second step of the proline degradation pathway. Expression of *ALDH4* mRNA was induced in HCT116 cells in response to DNA damage caused by adriamycin treatment, in a p53-dependent manner. *ALDH4* contains a potential p53 binding sequence in intron1 and the interaction of p53 with the site was shown by EMSA and ChIP assays. We confirmed p53-dependent transcriptional activity of the binding site by means of a reporter assay. Inhibition of *ALDH4* expression by antisense oligonucleotides was able to enhance cell death induced by infection with Ad-p53. H1299 cells transformed to overexpress ALDH4 showed significantly lower intracellular reactive oxygen species (ROS) levels than parental or control cells after treatment with hydrogen peroxide or UV. Those cells were also resistant to cell damage caused by hydrogen peroxide. These results suggest that p53 might play a protective role against cell damage induced by generation of intracellular ROS, through transcriptional activation of *ALDH4*.

Keywords p53 · Target-gene · *ALDH4* · POX · Proline · Apoptosis

K.-A. Yoon · Y. Nakamura · H. Arakawa
Human Genome Center, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan

Present address: H. Arakawa (✉)
Cancer Medicine and Biophysics Division, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
E-mail: harakawa@gan2.res.ncc.go.jp
Tel.: +81-3-3547-5273
Fax: +81-3-3546-1369

Introduction

A major function of p53 is to provide cellular-growth checkpoints to protect against development of cancer. For example, p53 suppresses progression through the cell cycle in response to DNA damage to allow cells to repair; however, p53 induces apoptosis to eliminate dangerous cells if the damage is too severe. When cells are exposed to various stresses including DNA-damaging agents or irradiation, p53 protein is stabilized and activated to enhance transcription of genes involved in growth arrest, DNA repair, apoptosis, and other processes. The multiplicity of p53 functions presumably reflects the combined effects of several target genes, and hundreds of possible p53-binding sequences may be present in the human genome. Therefore, identification of additional p53-target genes would seem to be an absolute requirement for a full understanding of the physiological behavior of p53. We have already reported the discovery of several novel transcriptional targets of p53, including *p53AIP1*(p53-regulated Apoptosis Inducing Protein 1), which leads to apoptotic cell death (Oda et al. 2000); *p53R2*(a ribonucleotide reductase), involved in a cell-cycle checkpoint for DNA damage (Tanaka et al. 2000); and *p53DINP1*, an activator of p53-dependent apoptosis (Okamura et al. 2001). We believe that dozens of p53-target genes in the human genome still remain to be identified.

Aldehyde dehydrogenases are a superfamily of $NAD(P)^+$-dependent enzymes that metabolize aliphatic and aromatic aldehydes generated from numerous endogenous and exogenous precursors (Hsu et al. 1988; Yoshida et al. 1998). ALDH4, also known as glutamate-γ-semialdehyde dehydrogenase or pyrroline-5-carboxylate (P5C) dehydrogenase, is a mitochondrial-matrix NAD^+-dependent enzyme that catalyzes the second step of the proline degradation pathway (Hu et al. 1996). Although it is a member of the classical family of human ALDHs, this enzyme is not highly similar (<20%) to any of others.

Deficiency of *ALDH4* causes type II hyperprolinemia. Germline mutations in the gene have been reported in patients with this disorder (Geraghty et al. 1998; Vasiliou and Pappa 2000), which is characterized by accumulation of proline and P5C in plasma and shows neurological manifestations such as seizures and mental retardation. However, the role of ALDH4 has not been clarified yet at the molecular level.

We have lately been trying to isolate additional p53-target genes by means of microarray technology (Iiizumi et al. 2002; Mori et al. 2002a,b; Ochi et al. 2002). Here we report that ALDH4 is yet another target of transactivation by p53. Furthermore, we have demonstrated a role of ALDH4 in protecting cells from oxidative stress and the cell death that results, suggesting that p53 has a previously unrecognized role in cell survival.

Materials and methods

Cell lines

The cell lines used in these experiments, including U373MG (human glioblastoma) and H1299 (human non-small cell lung carcinoma) were purchased from the American Type Culture Collection (ATCC). HCT116 ($p53^{+/+}$ and $p53^{-/-}$) cell lines were gifts from B. Vogelstein (Johns Hopkins University, Baltimore, MD). All cell lines were cultured in conditions recommended by their respective depositors.

Northern blotting

Total RNAs were extracted from U373MG cells infected with Ad-p53 (80MOI) and HCT116 ($p53^{+/+}$ and $p53^{-/-}$) cells exposed to DNA-damaging conditions including gamma (50 Gy) or UV (10 J/m^2) irradiation, or treatment with 1 μg/ml adriamycin. Cells were harvested at 0, 6, 12, 24, and 48 h after treatment. Poly(A)+RNA was prepared using mRNA purification kits (Amersham Pharmacia Biotech, Piscataway, NJ). A 3 μg aliquot of each total mRNA was separated on a 1% agarose gel and transferred onto a nitrocellulose membrane, which was hybridized with a radiolabeled ALDH4 probe in a solution of 50% formamide, 10× Denhardt solution, 5× SSPE, 2% SDS, and 100 μg/ml of salmon sperm DNA at 42°C. Hybridized membranes were washed in 1× SSC/0.05% SDS at room temperature and 0.05× SSC/0.1% SDS at 50°C, then exposed to X-ray film.

Semiquantitative RT-PCR analysis

Total RNAs were isolated from HCT116 cells 24 h after adriamycin treatment using RNeasy spin-column kits (Qiagen) according to manufacturer's instructions. cDNAs were synthesized from 5 μg total RNAs with the SuperScript Preamplification System (Life Technologies, Inc.). The RT-PCR exponential phase was determined on 16–30 cycles to allow semiquantitative comparisons among cDNAs developed from identical reactions. Each PCR protocol involved a 4-min initial denaturation step at 94°C, followed by 25 cycles (for *ALDH4*), 32 cycles (for *POX*), 19 cycles (for $p21^{WAF1}$) or 16 cycles (for *GAPDH*) at 94°C for 30 s, 55–62°C for 30 s, and 72°C for 1 min on a Gene Amp PCR system 9600 (Perkin Elmer). Primer sequences were, for *ALDH4*: F, CCT GAA GCC TAT TGC AGA CC and R, TGA AGT TGA TGC CAC AGA GG; for *POX*: F, GCC ATT AAG CTC ACA GCA CTG GG and R, CTG ATG GCC GGC TGG AAG TAG. PCR products were separated by electrophoresis on 2% agarose gels.

Luciferase assay

A reporter plasmid, pGL3-ALDH4, was constructed to contain one or two copies of an oligonucleotides or a 550-bp genomic fragment containing the site. Sense and antisense strands corresponding to oligonucleotide 5′-AGGCATGTGC CACCATGTCC-3′ were annealed and cloned into pGL3-promoter vector. pGL3-ALDH4 or a control reporter plasmid were transfected into H1299 cells along with either wild-type or mutant p53 expression vector. Cells were harvested 24 h after transfection, and luciferase activity was measured using the Dual-Luciferase Reporter Assay system (Promega, Madison, WI) as previously described (Shiraishi et al. 2000).

Chromatin immunoprecipitation (ChIP) assay

HCT116 ($p53^{+/+}$ and $p53^{-/-}$) cells treated with adriamycin (1 μg/ml) were subjected to ChIP assay (Upstate Biotechnology, Lake Placid, NY) according to recommendations of the manufacturer. At 24 h after adriamycin treatment, cells were treated with 1% formaldehyde for 10 min to cross-link genomic DNA and protein. Cells were lysed with SDS-lysis buffer containing a protease-inhibitor cocktail and sonicated to generate DNA fragments 300–800 bp long. The supernatant of the cell lysate was immunoprecipitated with anti-p53 antibody (Do-7, Oncogene Science) for 16 h. Immunoprecipitates produced by anti-FLAG (M2, Sigma-Aldrich) antibody, or a supernatant without antibody, served as controls. After immunoprecipitation, DNA-protein cross-links were reversed by

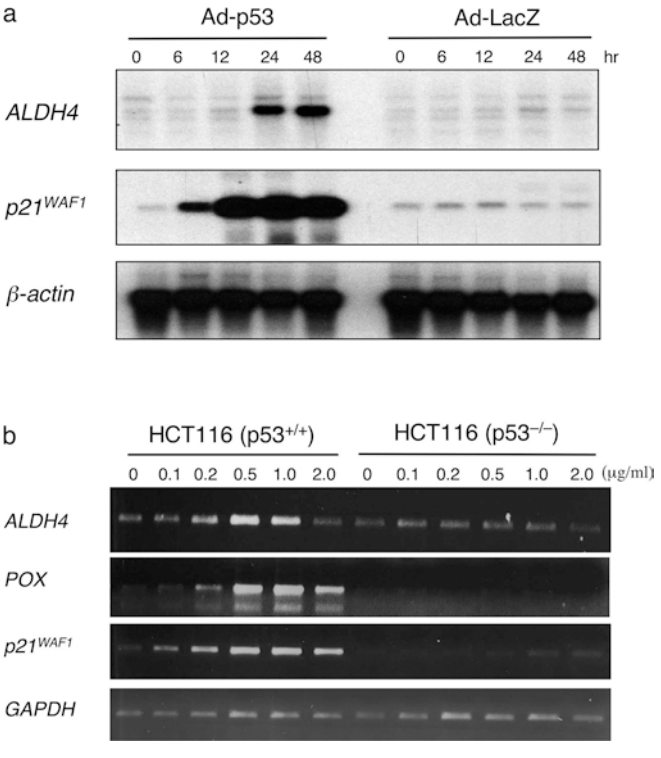

Fig. 1 Activation of *ALDH4* expression in response to p53. **a** Northern-blot showing induction of *ALDH4* and $p21^{waf1}$ by exogenous p53. U373MG cells were infected with adenovirus containing *p53*or *LacZ*, and mRNAs were harvested at the indicated time points after infection. Expression of β-actin was shown as a quantity control. **b** RT-PCR showing induction of *ALDH4*, *POX*, and $p21^{waf1}$ by endogenous p53. Expression of *GAPDH*was shown as a quantity control. HCT116 ($p53^{+/+}$and $p53^{-/-}$) cells were subjected to the DNA-damaging condition of treatment with the indicated concentrations of adriamycin

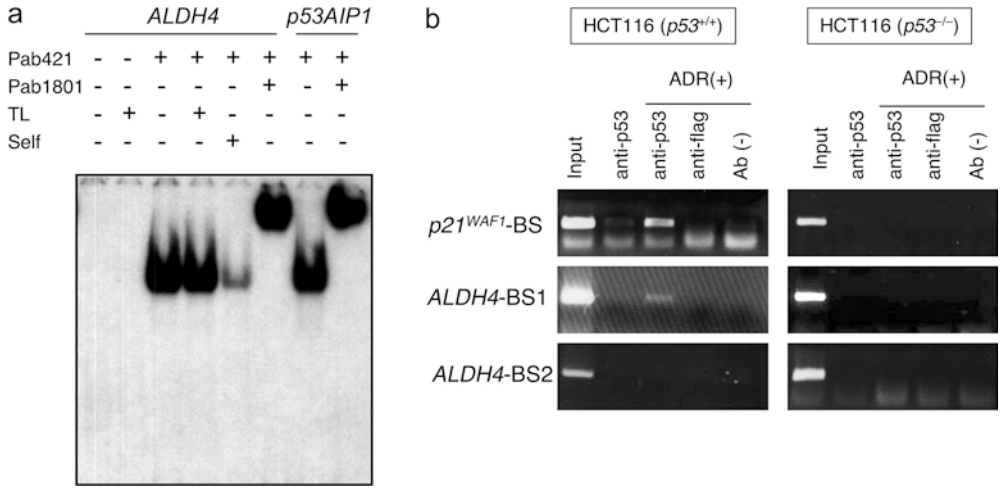

Fig. 2 *ALDH4* as a direct target of p53. **a** EMSA using a nuclear extract of H1299 cells infected with Ad-p53. The band representing the ^{32}P-labeled oligonucleotide of the binding site was shifted by anti-p53 antibodies (Pab 421, Pab1801). Unlabeled oligomer (*self*) and TL oligomer served as competitors, and the p53-binding site of the *p53AIP1* gene was used as a positive control. **b** ChIP assay of *ALDH4* and $p21^{waf1}$ in HCT116 ($p53^{+/+}$ and $p53^{-/-}$) cells. Cross-linked p53 protein-DNA complexes were immunoprecipitated with anti-p53 antibody or anti-FLAG antibody. "Input" indicates a portion of the sonicated chromatin before immunoprecipitation

incubation at 65°C for 4 h and then genomic DNA was extracted. PCR amplification of genomic fragments containing p53BS1 and p53BS2 was performed with specific primers flanking the suspected binding sites of *ALDH4* and for the promoter region of $p21^{WAF1}$ as a positive control (Morimoto et al. 2002).

Electrophoretic mobility-shift assay

Electrophoretic mobility-shift assay (EMSA) was carried out using H1299 cells infected with an adenovirus vector containing wild-type *p53*. Nuclear extracts of these cells were incubated with ^{32}P-labeled double-stranded oligomer and monoclonal anti-p53 antibodies (Pab421 from Oncogene Science and/or Pab1801 from Santa Cruz Biotechnology). Unlabeled oligomer or oligonucleotides without the consensus (TL) oligomer were used as competitors, and the binding site of the *p53AIP1* gene (5′-TCTCTTGCCC GGGCTTGTCG-3′) served as a positive control.

Antisense oligonucleotide

To suppress induction of endogenous *ALDH4*, we designed an antisense oligonucleotide (AS; GCGCCGGCAGCAGCAT) and as a control, a sense oligonucleotide (SE; ATGCTGCTGCCG-GCGC). Antisense or sense oligonucleotides were transfected to U-373MG cells using Lipofectin reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. After 4 h of incubation at 37°C, U-373MG cells were infected by adenovirus vector containing wild-type *p53*. Cells were harvested after incubation and subjected to RT-PCR experiments and analysis of the cell cycle.

Cell-cycle analysis

After 72 h of infection by Ad-p53, cells were washed with PBS, trypsinized, and fixed in 70% ethanol in PBS. Fixed cells were collected by centrifugation and treated with 1 mg/ml of RNase for

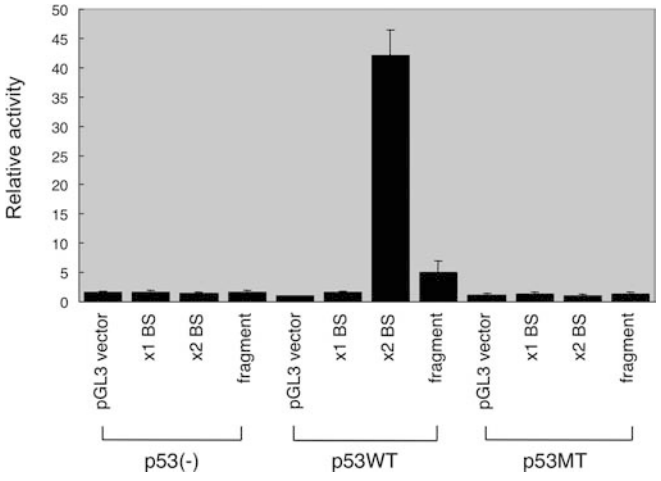

Fig. 3 Transcriptional activation of *ALDH4* by p53. Luciferase assay of the putative p53-binding site of *ALDH4*. Reporters were constructed to contain either one (1×BS) or two copies (2×BS) of the binding sequence, or a 550-bp genomic fragment (*fragment*) containing the binding site, and co-transfected with expression vectors of wild-type *p53*(p53WT) or mutant *p53* (p53MT)

30 min. Cells were then stained with 100 μg/ml of propidium iodide. Stained cells were analyzed on a FACScan flow cytometer (Beckton, Dickinson Biosciences).

Establishment of a cell line over-expressing ALDH4

H1299 cells were transfected with 8 μg of the ALDH4 expression vector (pcDNA3.1(+)/ALDH4) or control vector (pcDNA3.1(+)) for 48 h and incubated in culture medium containing 800 μg/ml G418. Two weeks later, G418-resistant colonies were selected and the expression level of *ALDH4* in each colony was examined by RT-PCR.

Measurement of ROS generated during H_2O_2treatment or UV radiation

H1299 cells and its derivative cells (H1299-ALDH4, H1299-vector) were grown in 6-well culture plates (2×10^5cells/plate) and treated with 100 μM $H_2O_2$for 1 h or 30 J/m^2 UV, then incubated in fresh

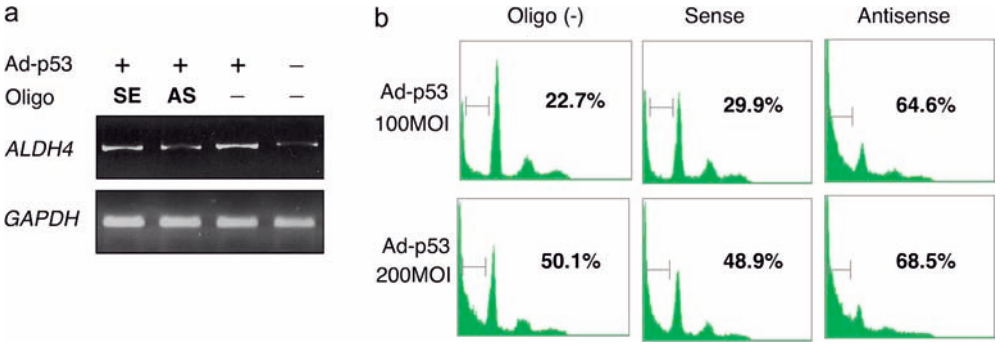

Fig. 4 Enhancement of p53-induced apoptosis when expression of *ALDH4* was inhibited by antisense oligonucleotide. U373MG cells had been transfected with antisense (AS) or sense (SE) oligonucleotides for 4 h and then infected with Ad-p53. **a** Expression level of *ALDH4* examined by RT-PCR. **b** FACS analysis. At 72 h after infection cells were subjected to FACS analysis. Apoptotic cells were indicated as a sub-G1 fraction (%) of the whole cells. The experiments were performed in triplicate and representative data were shown

culture medium. After 24 h the cells were incubated with 10 μM 2′,7′-dichlorodihydrofluoresceine diacetate (H_2DCF-DA; Molecular Probes, Eugene, OR) for 20 min at 37°C to measure the production of reactive oxygen species (ROS). Cell-permeable H_2DCF-DA was metabolized by non-specific esterases to the nonfluorescent product, 2′,7′-dichlorodihydrofluoresceine, which is oxidized to the fluorescent product, DCF, by ROS. The DCF was detected using 488 and 525 nm as excitation and emission wavelengths. After incubation the cells were washed with PBS, trypsinized, resuspended in PBS, and analyzed with a FACScan flow cytometer (Beckton, Dickinson Biosciences).

Measurement of cell viability

Stable transformants of *ALDH4* or vector were treated with 50 or 100 μM $H_2O_2$for 1 h and then cultured in fresh medium. After 72 h, cells were incubated for 4 h with 5 mg/ml MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma, St. Louis, MO). Then 0.04N HCl in isopropanol was added to dissolve the dark blue crystals, and absorbance of each sample was measured at 570 nm.

Results

We applied a cDNA microarray system consisting of 23,000 genes for screening p53-inducible genes. In brief, mRNAs were extracted at various time points from U373MG cells infected either with Ad-p53 or Ad-lacZ. Microarray analyses indicated that expression of *ALDH4* was remarkably elevated by infection with Ad-p53 in a time-dependent manner, but not by Ad-lacZ (data not shown), a result was also confirmed by northern-blot analysis (Fig. 1a). *ALDH4* mRNA expression was significantly increased 24 h after infection by Ad-p53, although its induction was slower than that of $p21^{WAF1}$ (Fig. 1a).

To determine whether endogenous p53 could activate transcription of *ALDH4*, HCT116 ($p53^{+/+}$ and $p53^{-/-}$) cells were damaged by the treatment of various doses of adriamycin (0–2 μg/ml). Then mRNAs were isolated from these cells at selected times and analyzed expression of *ALDH4*. Northern-blot analysis revealed that *ALDH4* mRNAs were strongly induced in HCT116 ($p53^{+/+}$), but not HCT116 ($p53^{-/-}$), in response to DNA damage by adriamycin (ADR) and reached a maximum at the concentration of 0.5 μg/ml of ADR (Fig. 1b), suggesting that induction of *ALDH4* mRNAs totally depends on endogenous p53.

To investigate whether transcription of *ALDH4* was regulated directly by p53, we searched for p53-binding site(s) (p53BS) in the genomic sequence of *ALDH4* and found a candidate site (5′-AGGCATGTGC CACCATGTCC-3′) in intron1 that revealed an 85% match to the p53-binding consensus (El-Deiry et al. 1992). To examine the binding of p53 protein to the candidate sequence, we performed an EMSA and a chromatin immunoprecipitation assay (ChIP). As shown in Fig. 2a, the p53BS bound to a molecule in the nuclear extract of H1299 cells infected with Ad-p53; the bands were shifted by anti-p53 antibodies, indicating that the molecule bound to the putative p53BS was in fact p53 protein. Furthermore, unlabeled self-oligonucleotides, but not non-specific oligonucleotides, were able to inhibit binding of p53 to the p53BS, supporting a conclusion that specific interaction between p53 and p53BS had occurred in vitro.

To examine binding of the p53BS of *ALDH4* to p53 in vivo, we carried out a ChIP assay. Using specific primers amplifying a 300-bp DNA fragment of *ALDH4* that included the p53BS, we were able to detect DNA that was immunoprecipitated by an anti-p53 antibody, but not by an anti-FLAG antibody (Fig. 2b). Since this result suggested that p53 interacted with the p53BS in vivo, we performed a reporter (luciferase) assay to evaluate p53-dependent transcriptional activity of the site. The reporter vectors were constructed by insertion of either one or two copies of the binding site or a 550-bp genomic fragment containing the site. As shown in Fig. 3, when wild-type p53 expression vector was co-transfected into H1299 cells along with each of the reporter vectors, luciferase activity was strongly enhanced in case of two copies of the p53BS and the genomic fragment of binding site. However, no enhancement was observed when mutant p53 was co-transfected. These results, taken together, suggested that *ALDH4* is a bona-fide target of p53.

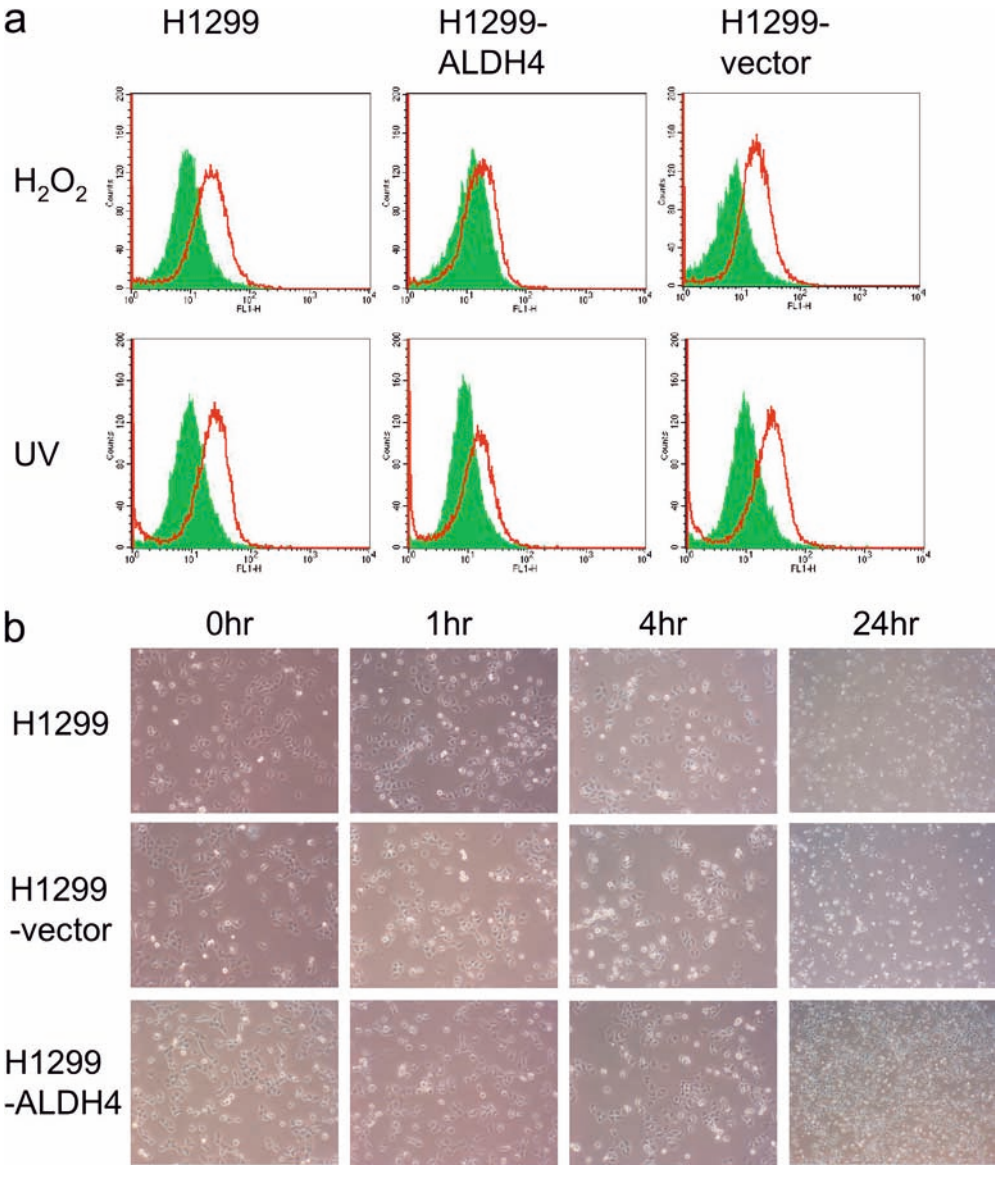

Fig. 5 Cellular ROS levels and morphological changes brought about by DNA-damaging agents. **a** Stable transformants of H1299-ALDH4, or control (H1299-vector) and parent cells (H1299) were exposed to 100 μM hydrogen peroxide for 1 h or to UV radiation (30 J/m^2). Intracellular levels of ROS were measured 24 h after H_2O_2treatment (*red*) or without H_2O_2treatment (*green*). **b** Morphological changes after treatment with 100 μM hydrogen peroxide. Photographs were taken at 0, 1, 4 h (×100) and 24 h (×40) after treatment

To examine the biological role of ALDH4, we transfected antisense-oligonucleotides (AS) or sense-oligonucleotides (SE) corresponding to *ALDH4* cDNA sequences into U373MG cells, and then infected the cells with Ad-p53 at 80 MOI. RT-PCR experiments 36 h later indicated suppression of *ALDH4* mRNA transcription by AS, but not by SE (Fig. 4a). We then used the FACS method to evaluate induction of apoptosis by Ad-p53, 72 h after infection. As shown in Fig. 4b, the sub-G1

▶

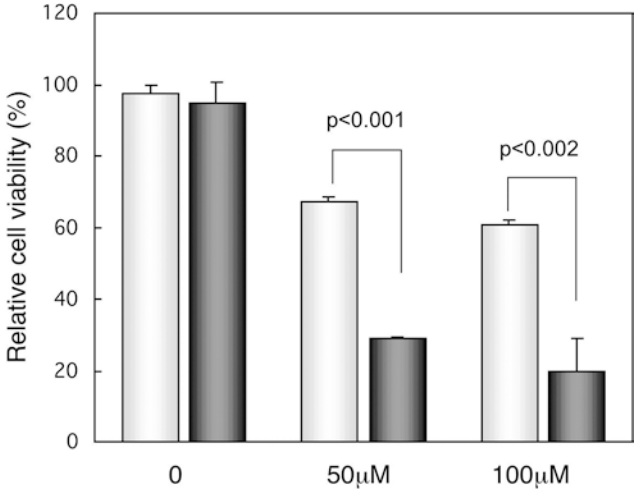

Fig. 6 Protective role of ALDH4 against oxidative stress. ALDH4-over-expressing cells (*white bar*) and control cells (*Shaded bar*) were treated with 50 or 100 μM of H_2O_2. Relative cell viability was measured by MTT assay after three days of treatment. The MTT assay was performed three times and data are presented as means ± SD

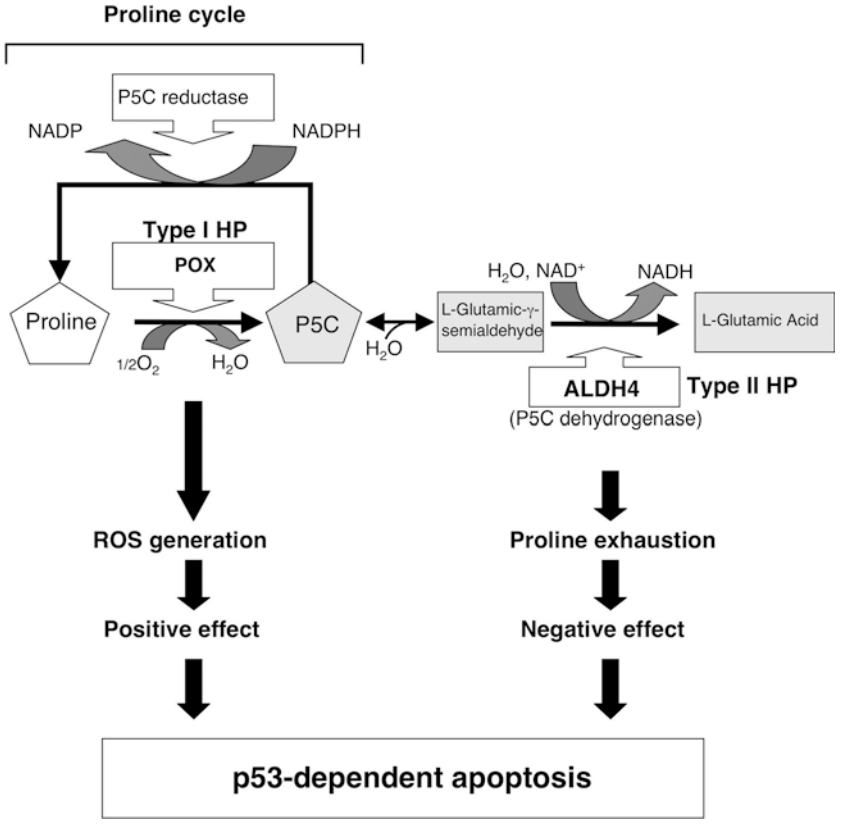

Fig. 7 Hypothetical mechanism of ALDH4 in p53-dependent apoptosis. The transcriptions of POX and ALDH4 are regulated by p53. POX catalyzes the conversion of proline to P5C with the concomitant transfer of electrons to cytochrome c, which supports the generation of ROS by donating reducing potential to an electron transport chain (Donald et al. 2001). P5C is converted back to proline by P5C reductase, completing a proline cycle. On the other hand, P5C is irreversibly converted to L-Glutamic acid via L-Glutamic-γ-semialdehyde by ALDH4, resulting in exhaustion of proline pool. This might negatively affect the generation of ROS in POX-mediated proline metabolism. We speculate that the regulation of ROS generation through proline metabolism by POX and ALDH4 might be one of the mechanisms of p53-dependent apoptosis. *POX* proline oxidase, *ALDH4* aldehyde dehydrogenase 4, *Type I HP* type I hyperprolinemia, *Type II HP* type II hyperprolinemia, *P5C*pyrroline-5-carboxylate, *ROS* reactive oxygen species

population was increased significantly in cells treated with AS, but not with SE. These results suggested that ALDH4 negatively regulated the cell death induced by Ad-p53.

To investigate the role of ALDH4 further, we transformed H1299 cells to over-express ALDH4 constitutively (H1299-ALDH4) and treated them with hydrogen peroxide (100 μM) or UV radiation (30 J/m^2). Twenty-four hours after either treatment, the H1299-ALDH4 cells and control cells transfected with vector alone (H1299-vector) were incubated with H_2DCF-DA to measure ROS levels. ROS increased in control cells as well as in parental H1299 cells, but were significantly lower in H1299-ALDH4 cells (Fig. 5a). The results of this experiment also reflected morphological alterations of the cells under oxidative stress; the parental cells or the vector-transfected cells became rounded after the H_2O_2 treatment but we observed no apparent morphological changes in H1299-ALDH4 cells (Fig. 5b). To investigate the apparent ability of ALDH4 to protect cells against oxidative stress, we measured the extent of cell death by MTT assay after 72 h of H_2O_2treatment. As Fig. 6 shows, the viability of H1299-ALDH4 cells was much higher than that of mock-transfected cells.

Discussion

Proline oxidase (*POX*), encoded by a p53-inducible gene (*PIG6*), appears to participate in p53-dependent apoptosis by catalyzing the proline-dependent generation of ROS (Polyak et al. 1997; Maxwell and Davis 2000; Donald et al. 2001). Oxygenation of proline by POX can contribute to the energy supply of the cell and enhance generation of ROS, resulting in induction of apoptosis. ALDH4 is a mitochondrial-matrix NAD^+-dependent enzyme that catalyzes irreversible conversion of P5C derived either from proline or ornithine, to glutamate, whereas POX mediates the reversible conversion of proline to P5C, forming a proline cycle (Hu et al. 1996). Mutations of *ALDH4* are responsible for type II hyperprolinemia; mutations of *POX* cause type I hyperprolinemia. Since p53 regulates transcription of both *ALDH4* and *POX*, it is clear that there are some similarities as well as distinctions in the functional behaviors of ALDH4 and POX.

We speculate that ALDH4 might regulate p53-dependent apoptosis negatively, and POX positively, according to processes illustrated in Fig. 7. That is, by oxygenating proline and reversibly converting proline to P5C through a proline cycle, POX could supply an alternate electron for supporting an apoptotic paradigm by providing the required ROS. However, ALDH4 might exhaust the pool of proline by catalyzing irreversible conversion of P5C, thus preventing the proline-dependent generation of ROS that is mediated by POX. Hence, a balance between ALDH4 and POX activities might be critical for p53-dependent apoptosis. In fact, our experiments have clearly shown that over-production of ALDH4 in p53-null cells inhibits H_2O_2-induced generation of ROS and the resulting apoptosis. Our findings provide evidence that one role of p53 might be to protect cells against oxidative stress; if p53 indeed plays an important role in cell survival, our results clearly impart a novel aspect to p53 function. Additional research should be undertaken to define the role of proline metabolism and the mechanisms involving ROS decline in p53-regulated cellular responses.

Acknowledgements This work was supported in part by Grant #14028018 from the Ministry of Education, Culture, Sports, Science and Technology (to H.A.), and in part by "Research for the Future" Program Grant #00L01402 from the Japan Society for the Promotion of Science (to Y.N.).

References

Donald SP, Sun XY, Hu CA, Yu J, Mei JM, Valle D, Phang JM (2001) Proline oxidase, encoded by p53-induced gene-6, catalyzes the generation of proline-dependent reactive oxygen species. Cancer Res 61:1810–1815

El-Deiry WS, Kern SE, Pietenpol JA, Kinzler KW, Vogelstein B (1992) Definition of a consensus binding site for p53. Nat Genet 1:45–49

Geraghty MT, Vaughn D, Nicholson AJ, Lin WW, Jimenez-Sanchez G, Obie C, Flynn MP, Valle D, Hu CA (1998) Mutations in the delta 1-pyrroline-5-carboxylate dehydrogenase gene cause type II hyperprolinemia. Hum Mol Genet 7:1411–1415

Hsu LC, Bendel RE, Yoshida A (1988) Genomic structure of the human mitochondrial aldehyde dehydrogenase gene. Genomics 2:57–65

Hu CA, Lin WW, Valle D (1996) Cloning, characterization and expression of cDNAs encoding human delta 1-pyrroline-5-carboxylate dehydrogenase. J Biol Chem 271:9795–9800

Iiizumi M, Arakawa H, Mori T, Ando A, Nakamura Y (2002) Isolation of a novel gene, CABC1, encoding a mitochondrial protein that is highly homologous to yeast activity of bc1 complex. Cancer Res 62:1246–1250

Maxwell SA, Davis GE (2000) Differential gene expression in p53-mediated apoptosis-resistant vs. apoptosis-sensitive tumor cell lines. Proc Natl Acad Sci USA 97:13009–13014

Mori T, Anazawa Y, Iiizumi M, Fukuda S, Nakamura Y, Arakawa H (2002a) Identification of the interferon regulatory factor 5 gene (IRF-5) as a direct target for p53. Oncogene 21:2914–2918

Mori T, Anazawa Y, Matsui K, Fukuda S, Nakamura Y, Arakawa H (2002b) Cyclin K as a direct transcriptional target of the p53 tumor suppressor. Neoplasia 4:268–274

Morimoto I, Sasaki Y, Ishida S, Imai K, Tokino T (2002) Identification of the osteopontin gene as a direct target of TP53. Genes Chromosomes Cancer 33:270–278

Ochi K, Mori T, Toyama Y, Nakamura Y, Arakawa H (2002) Identification of semaphorin3B as a direct target of p53. Neoplasia 4:82–87

Oda K, Arakawa H, Tanaka T, Matsuda K, Tanikawa C, Mori T, Nishimori H, Tamai K, Tokino T, Nakamura Y, Taya Y (2000) p53AIP1, a potential mediator of p53-dependent apoptosis, and its regulation by ser-46-phosphorylated p53. Cell 102:849–862

Okamura S, Arakawa H, Tanaka T, Nakanishi H, Ng CC, Taya Y, Monden M, Nakamura Y (2001) p53DINP1, a p53-inducible gene, regulates p53-dependent apoptosis. Mol Cell 8:85–94

Polyak K, Xia Y, Zweier JL, Kinzler KW, Vogelstein B (1997) A model for p53-induced apoptosis. Nature 389:300–305

Shiraishi K, Fukuda S, Mori T, Matsuda K, Yamaguchi T, Tanikawa C, Ogawa M, Nakamura Y, Arakawa H (2000) Identification of fractalkine, a CX3C-type chemokine, as a direct target of p53. Cancer Res 60:3722–3726

Tanaka H, Arakawa H, Yamaguchi T, Shiraishi K, Fukuda S, Matsui K, Takei Y, Nakamura Y (2000) A ribonucleotide reductase gene involved in a p53-dependent cell-cycle checkpoint for DNA damage. Nature 404:42–49

Vasiliou V, Pappa A (2000) Polymorphisms of human aldehyde dehydrogenases. Consequences for drug metabolism and disease. Pharmacology 61:192–198

Yoshida A, Rzhetsky A, Hsu LC, Chang C (1998) Human aldehyde dehydrogenase gene family. Eur J Biochem 251:549–557