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ORIGINAL INVESTIGATION

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Cell fusion corrects the 4-nitroquinoline 1-oxide sensitivity of Werner syndrome fibroblast cell lines

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Abstract We have shown that Werner syndrome (WRN) fibroblast cell lines are unusually sensitive to the DNA-damaging agent 4-nitroquinoline 1-oxide (4NQO), though not to gamma radiation or to hydrogen peroxide. The fusion of 4NQO-sensitive WRN and 4NQO-resistant control fibroblast cell lines generated proliferating WRN × control cell hybrids that expressed WRN protein and were 4NQO-resistant. These results establish the recessive nature of 4NQO sensitivity in WRN cell lines and provide a cellular assay for WRN protein function.

Introduction

Werner syndrome (WRN; MIM 277700) is an uncommon autosomal recessive disease whose phenotype mimics premature aging. WRN patients develop a prematurely aged appearance beginning in the second and third decades of life with the graying and loss of hair, bilateral cataract formation, osteoporosis, atherosclerosis, hypogonadism, diabetes mellitus, and scleroderma-like skin changes with ulceration. The most conspicuous laboratory finding is elevated urinary hyaluronic acid secretion (Epstein et al. 1966; Goto 1997). WRN patients also have an elevated risk of developing selected neoplasms, among which soft tissue and osteosarcomas, thyroid carcinoma, acral lentigenous melanoma, and meningioma predominate (Epstein et al. 1966; Goto et al. 1996). Cancer and atherosclerotic cardiovascular disease are the most common causes of death, at a mean age of 46 years, in WRN patients (Epstein et al. 1966; Goto et al. 1996; Goto 1997). The phenotypic overlap between WRN and normal aging is extensive, though incomplete: thus, WRN is often considered a "segmental" progeroid syndrome that may share mechanistic or pathogenetic overlap with normal aging (Martin 1978).

Consistent cellular phenotypes in WRN include reduced replicative potential of primary fibroblast cells (Martin et al. 1970; Tollefsbol and Cohen 1984) and genetic instability in cells and/or cell lines from the fibroblast, lymphoid, and erythroid cell lineages (reviewed in Moser et al. 1999). The mechanistic basis for genetic instability in WRN is not known, although cells and cell lines from WRN patients are unusually sensitive to 4-nitroquinoline 1-oxide (4NQO; Gebhart et al. 1988; Ogburn et al. 1997) and to the DNA topoisomerase I inhibitor camptothecin (Okada et al. 1998; Poot et al. 1999). These selective sensitivities are particularly intriguing, as WRN cells are not generally sensitive to DNA-damaging agents and do not appear to have rate-limiting deficits in any of the major DNA repair pathways (reviewed in Monnat 1992; Moser et al. 1999).

We have used a survival assay based on colony-forming efficiency in conjunction with cell fusion to investigate further the 4NQO sensitivity of WRN cell lines. We show that independently derived SV40-transformed WRN fibroblast cell lines are unusually sensitive to killing by 4NQO, although not by other DNA-damaging agents, and that the fusion of WRN and control cell lines generates proliferating cell hybrids that are 4NQO-resistant. These results confirm the recessive nature of 4NQO sensitivity in WRN cells and provide a cellular assay for WRN protein function.

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Table 1 WRN genotype of WRN cell lines used

Cell line	WRN mutation(s)[a]	Predicted WRN protein(s)[b]
Controls	None	1432 residues
WS780/AG11395	c.1336C → T	368 residues
W-V	c.3004delG[c]	972 residues
PSV811/AG07066	c.3453T → G (Phe1074Leu)	1432 residues
SYR10010 (*WRN* +/–)	Complex 4 bp del exon 32	1432/1245 residues
SYR10011 (*WRN* –/–)	Complex 4 bp del exon 32	1245 residues

[a] Mutation descriptions follow Nomenclature Working Group guidelines and use the human WRN cDNA as a reference (Yu et al. 1996; accession no. L76937). Complete details are available at URL http://www.pathology.washington.edu/werner/, the HUGO *WRN* Locus-specific Mutational Database server. A print version is also available (Moser et al. 1999)

[b] Lengths are given in amino acid residues

[c] The W-V cell line is either homozygous mutant or hemizygous for this allele and lacks detectable WRN protein

Materials and methods

Cell lines

Three independently derived SV40-transformed WRN fibroblast cell lines were used: W-V (Huschtscha et al. 1986), PSV811 (AG07066; Matsumura et al. 1985), and WS780 (AG11395; Saito and Moses 1991). Four independently derived control SV40 fibroblast cell lines, viz., GM637, GM638, GM639, and GM847, were obtained from the NIGMS Human Genetic Mutant Cell Repository (Camden, N.J.). Clonal derivatives of these WRN and control lines included: WV-37R1, a spontaneous HPRT-deficient mutant of W-V (Fukuchi et al. 1989) transfected with the hygromycin resistance plasmid pTG76 (Giordano and McAllister 1990); PSVL30, a clonal derivative of PSV811 transfected with the neomycin resistance/recombination reporter plasmid pLrec (Herzing and Meyn 1993); GM639–16R1, a spontaneous HPRT-deficient mutant of GM639 (Fukuchi et al. 1989) transfected with pTG76; and 639L22, a clonal derivative of GM639 transfected with pLrec. Seven DNA-repair-deficient or DNA-damage-response-deficient SV40-transformed fibroblast cell lines were obtained from the NIGMS Human Genetic Mutant Cell Repository and used as potential positive controls in 4NQO survival assays: GM05849A from an ataxia-telangiectasia (AT) patient, GM08505B from a Bloom syndrome (BS) patient, GM06914B from a Fanconi anemia (FA) complementation group A patient, and four xeroderma pigmentosum (XP) lines: GM04429E and GM04312B (XP-A), GM08207B (XP-D), and GM08437A (XP-F). Two lymphoblastoid cell lines from a Syrian WRN pedigree, viz., SYR10010 and SYR10011, were used as controls in Western analyses. The genotypes of the WRN cell lines are given in Table 1.

SV40-transformed cell lines were grown in high glucose (4500 mg/l) Dulbecco-modified Eagle's medium (Irvine Scientific, Santa Ana, Calif., or Gibco/BRL, Grand Island, N.Y.) supplemented with 10%–16% (v/v) fetal bovine serum (FBS; Hyclone, Logan, Utah), penicillin (100 U/ml), and streptomycin (100 μg/ml). Lymphoblastoid cell lines were grown in RPMI 1640 medium (Gibco/BRL) supplemented with 15% FBS and antibiotics. All cells were grown at 37 °C in a humidified 5% CO_2 incubator.

Cell transfection and fusion

Linearized plasmid (5 μg) was electroporated into 10^7 cells in 0.8 ml phosphate-buffered saline (PBS) by using a BRL Cell-Porator set at 400 V and 330 μF. Electroporated cells were resuspended in complete growth medium, then plated in five 10-cm dishes, and allowed to recover for 24 h prior to adding selective agents, viz., 150 μg/ml hygromycin B (Calbiochem, La Jolla, Calif.) or 500 μg/ml G418 (Gibco/BRL). Modified $CaCl_2$ transfections were performed by using 5 μg plasmid DNA and 2×10^5 cells/10-cm dish as previously described (Chen and Okayama 1987). Selective agents were added 48 h after transfection. Drug-resistant colonies generated by both methods were isolated after two weeks of continuous selection.

Cell lines for fusion (5×10^5 cells from each line) were plated and co-cultivated overnight in 6-cm dishes and then fused with two treatments of polyethylene glycol (PEG; 1.5 ml 1450 MW PEG solution; Sigma) for 20 s at room temperature. After each PEG incubation, cells were rinsed twice with a 1:1 (v/v) mix of Hank's balanced salt solution (Gibco/BRL)/PBS prior to re-feeding with complete growth medium. The following day, 5% of each fused culture was plated onto a 10-cm dish and allowed to recover for 24 h prior to selection. Pools of independent hygromycin/G418-resistant colonies were isolated after 19 days of growth in the presence of both antibiotics and then used in colony-forming assays as described below.

Clonal survival assays

Exponentially growing cells (5×10^4) in 25-cm^2 tissue culture flasks were treated with 4NQO (0.1–0.8 μg/ml) in complete medium for 24 h, then trypsinized, counted, and replated in 10-cm dishes at a low density (90–1000 cells/dish) for colony formation. Triplicate dishes were set up for each cell line and 4NQO dose in six experiments, and quadruplicate plates in three experiments. 4NQO (3 mg/ml) was prepared in dimethylsulfoxide (DMSO) and stored at –20 °C until use. DMSO alone was used to treat control cultures. The growth medium in dishes was changed once, five days after plating, and colonies were fixed and stained with 0.5% crystal violet in 20% ethanol after 10 days of growth. Colonies consisting of more than 50 cells were counted to determine survival.

Cell survival after H_2O_2 treatment or γ-irradiation was determined as described above. An H_2O_2 solution (30% w/w, Sigma) was diluted and added to freshly prepared medium to obtain the desired H_2O_2 concentration (10–80 μM). After growth in the presence of H_2O_2 for 24 h, treated and control cultures were trypsinized, counted, and replated in 10-cm dishes at a low density (205–2280 cells/dish) in triplicate for colony formation. Cultures were γ-irradiated (2–8 Gy, at 1 Gy/min) by using a Gammacell 40 ^{137}Cs source (Nordion International, Ontario, Canada). Immediately following irradiation, treated and control cultures were trypsinized, counted, and replated in 10-cm dishes at a low density (200 cells/dish) in triplicate for colony formation. Colonies consisting of more than 50 cells were counted after crystal violet staining to determine survival.

The survival of proliferating cell hybrids after 4NQO treatment was determined by using a colony-forming assay without replating: hybrid cells were plated at 5×10^4 or 5×10^5 cells/10-cm dish in duplicate for survival determinations or at a low density (1000 cells/dish) in duplicate to determine colony-forming efficiency. After incubation overnight, the growth medium was replaced with fresh medium containing 4NQO (0.05–0.15 μg/ml). Cells were grown for 24 h in the presence of 4NQO, then rinsed twice with PBS and re-fed with complete growth medium. The growth medium was changed once, 5–7 days after treatment, and colonies were fixed and stained with crystal violet after 10 days of growth.

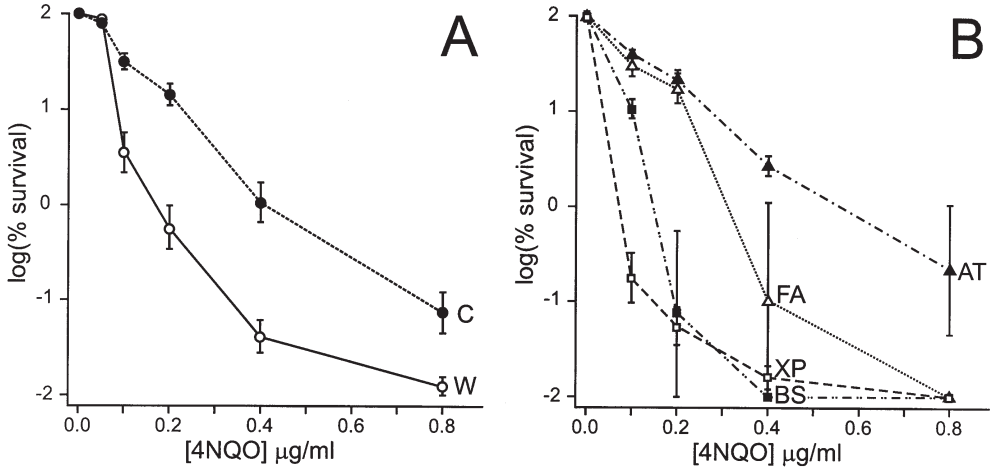

Fig. 1 A, B Survival of WRN, control, and repair-deficient cell lines after 4NQO exposure. **A** Mean log percentage survivals and standard errors are shown for three independent Werner syndrome (*open circles*) and four independent control (*closed circles*) fibroblast cell lines as a function of 4NQO dose. The survival curves summarize 434 individual data points derived from nine independent experiments. The mean number of data points per cell line was 62 (range 99–39), and the mean number of data points per cell line at each 4NQO dose was 12.4 (range 22–3). The difference in WRN and control dose-dependent survival curves is highly significant ($P < 0.0001$). **B** Mean log percentage survivals and standard errors are shown for seven fibroblast cell lines from four DNA-repair or DNA-damage response-deficiency syndromes as a function of 4NQO dose. The survival curves summarize 119 data points from four independent experiments. The mean number of data points per cell line was 16.6 (range 30–14), and the mean number of data points per cell line at each 4NQO dose was 2.6 (range 6–2). *AT* Ataxia-telangiectasia, *BS* Bloom syndrome, *FA* Fanconi anemia, *XP* xeroderma pigmentosum

Colonies consisting of more than 50 cells were counted to determine survival.

Statistical analysis of cell survival

The statistical significance of differences in survival as a function of cell line, agent, and dose was analyzed by using the general linear model (GLM) procedure in the SAS statistical software package (version 6.12, SAS Institute, Cary, N.C.). Survival at each dose, cell line, and experiment was defined as the colony-forming efficiency, normalized by the average colony-forming efficiency at the zero dose for that cell line and experiment. Log survival was modeled as a linear or quadratic function of mutagen dose by using survival data from independent experiments. A comparison of survival curves for individual cell lines and experiments revealed that the survival for a given cell type tracked along slightly different, parallel curves on different experimental dates. As there was no systematic effect of experimental date on survival, we adjusted for this variation by including a "random effect for date" correction in our GLM-based analytical model. Survival curves for the different cell types were then tested for equality by using likelihood ratio tests (Hartley and Rao 1967).

Western blot analysis of WRN protein

Western blot analysis with an affinity-purified rabbit anti-WRN polyclonal antiserum (Gray et al. 1998) was used to detect WRN protein in cell lines and hybrids. Cell extracts were prepared by resuspending 10^6 cells in 100 ml SDS sample buffer and then heating to 100 °C for 3 min. Extract equivalent to 2.5×10^5 cells was then size-fractionated on 6% SDS-polyacrylamide gels prior to transfer onto a nitrocellulose membrane (Coligan et al. 1995). The membrane was blocked with 500 mM NaCl, 25 mM TRIS-HCl pH 7.5, 0.1% Tween 20 containing 10% non-fat dry milk (NDM) prior to adding primary antisera diluted 1:500 in buffer containing 1% NDM. Bound primary antisera was visualized with a goat anti-rabbit IgG alkaline phosphatase conjugate (Sigma, St. Louis, Mo.) diluted 1:10,000 in buffer containing 1% NDM and Immun-Star chemiluminescent substrate (Bio-Rad, Hercules, Calif.). The alkaline phosphatase chemiluminescent signal was recorded with Bio-Max MR film (Kodak, Rochester, N.Y.).

Results

Survival of WRN and control cell lines after exposure to DNA-damaging agents

We used a colony-forming assay to determine the survival of independently derived WRN and control SV40 fibroblast cell lines after treatment with the DNA-damaging agents 4NQO, H_2O_2, or γ-irradiation. In nine independent experiments, WRN cell lines were more sensitive than controls to 4NQO at concentrations between 0.1 and 0.8 μg/ml. This difference in survival was highly significant ($P < 0.0001$). Composite survival curves for the WRN and control cell lines are shown in Fig. 1A.

We also compared the survival of different WRN and control lines as a function of 4NQO dose. The only paired comparison among WRN lines that was significant was W-V versus PSV811, with higher dose-dependent survivals in W-V than in PSV811 ($P = 0.002$). Several of the paired comparisons within the control group were also significant: three involved GM639, which showed significantly better survival than the other control lines, and one was represented by the better survival of GM638 versus GM637. None of the remaining paired comparisons of control lines was significant (additional results not shown). The mean colony-forming efficiency of cell lines in these experiments ranged from 14% to 47% (WRN: PSV811, 13.8%; WS780, 17.7%; W-V, 33.5%; controls: GM638, 24.3%; GM637, 26.3%; GM847, 34.7%; GM639, 47%).

We also examined the survival of seven SV40-transformed cell lines from patients with the DNA-repair-deficient or DNA-damage-response-deficient diseases AT, BS, FA, and XP after 4NQO exposure. An AT cell line had survival comparable to control cell lines at all 4NQO

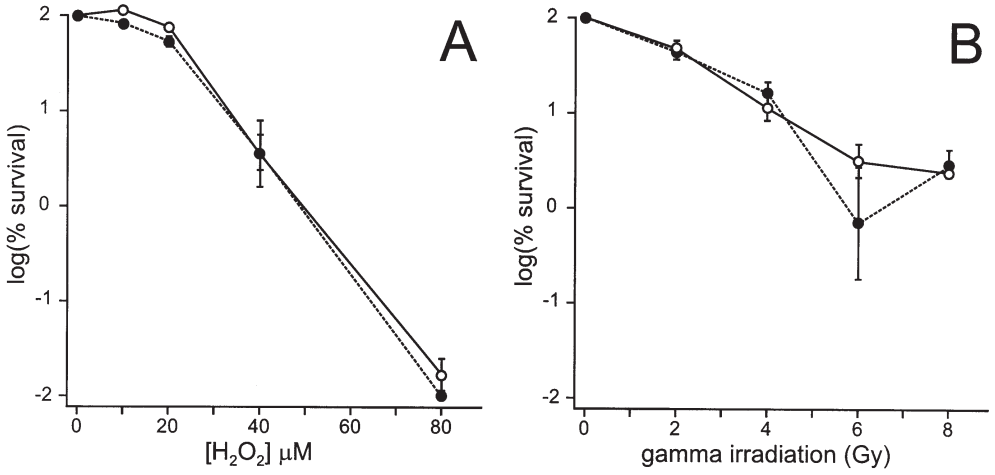

Fig. 2 A, B Survival of WRN and control cell lines after H_2O_2 exposure or γ-irradiation. Mean log percentage survivals and standard errors are shown for Werner syndrome (*open circles*) and control (*closed circles*) cell lines as a function of H_2O_2 dose (**A**) or γ-irradiation level (**B**). The survival curves summarize the results of two independent experiments with three WRN and two control cell lines in **A** and of one experiment with two WRN and two control cell lines in **B**. There was no significant difference between the survival of WRN and control cell lines for either agent (H_2O_2, P = 0.62; γ-irradiation, P = 0.32)

concentrations tested (Fig. 1B). The survival of one FA-A line was comparable to control cell lines at 4NQO concentrations of 0.1 or 0.2 μg/ml; survival at 4NQO concentrations greater than 0.2 μg/ml was comparable to WRN cell lines (Fig. 1B). In contrast, both the BS and XP cell lines showed lower survival than did the WRN or control cell lines at 4NQO concentrations of 0.1 or 0.2 μg/ml; survival at 4NQO concentrations greater than 0.2 μg/ml was comparable to that of WRN cell lines (Fig. 1B). We saw no significant survival difference among XP cell lines from three complementation groups (XP-A, XP-D, and XP-F; additional results not shown). Two WRN and three control cell lines treated with H_2O_2 showed virtually indistinguishable survival curves (P = 0.62; Fig. 2A), as did two WRN and two control lines that were γ-irradiated (P = 0.32; Fig. 2B).

Survival of WRN and control cell hybrids after 4NQO exposure

We used cell fusion followed by a colony-forming assay to determine whether the 4NQO sensitivity of WRN cell lines could be complemented in proliferating cell hybrids. Fusions were performed with hygromycin- or G418-resistant clonal derivatives of WRN and control cell lines that had significantly different survival after 4NQO treatment (Fig. 3). Four different fusions were performed: two "test" fusions of WRN and control cells, viz., GM639–16R1 × PSVL30 (fusion C1/W2) and 639L22 × WV1–37R1 (fusion C2/W1), and "selfing" fusions of the control (GM639–16R1 × 639L22; fusion C1/C2) and WRN fusion partners (WV1–37R1 × PSVL30; fusion W1/W2). These fusions were designed to associate alternately hygromycin- and G418-resistance with the WRN or control fusion partners.

The survival of pools of proliferating hybrids as a function of 4NQO dose was determined in four independent experiments (Fig. 3). The survival of WRN × control fusions following 4NQO exposure was significantly better than the survival of WRN × WRN hybrids (P < 0.0001) or of either WRN fusion partners (P < 0.0001; Fig. 3). However, the survival of WRN × WRN hybrids after 4NQO treatment did not differ from either WRN fusion partner (P = 0.97; Fig. 3). The mean colony-forming efficiency of cell lines ranged from 2.8% to 25% (PSVL30, 2.8%; WV1–37R1, 9.3%; GM639–16R1, 17.7%; 639L22, 25.2%)

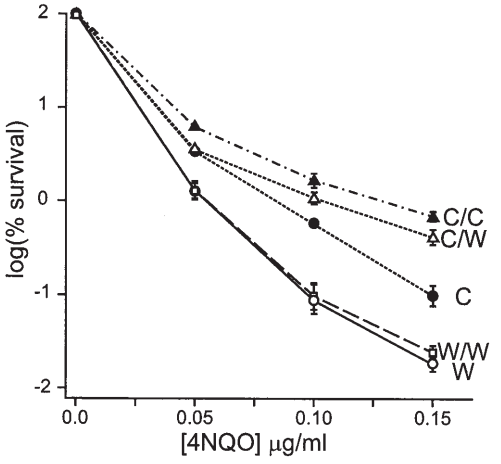

Fig. 3 Survival of WRN × control hybrids after 4NQO exposure. Mean log percentage survival and standard errors are shown for two WRN (*open circles*) and two control (*closed circles*) clones and for proliferating hybrids formed by fusing these, as a function of 4NQO dose (*closed triangles* C/C hybrids, *open triangles* C/W hybrids, *open squares* W/W hybrids). Survival curves summarize 168 individual data points from four independent experiments. The number of data points per cell line or hybrid pool was 21, and the mean number of data points per cell line or hybrid pool at each 4NQO dose was 4.5 (range 6–3). The survival difference for WRN and control clones prior to fusion was significant (P = 0.0007), as were differences in the survival of C/W hybrids as compared with W/W hybrids (P < 0.0001) or WRN clones (P < 0.0001). There was no significant difference in the dose-dependent survival of WRN clones versus the W/W hybrid generated by their fusion (P = 0.97)

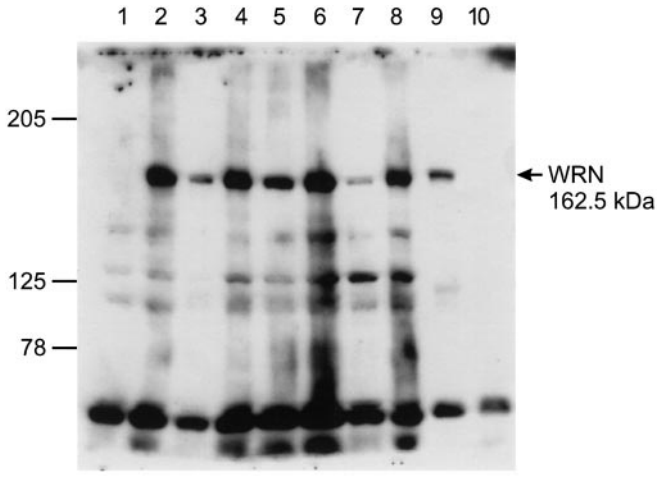

Fig. 4 Western blot analysis of WRN protein in WRN and control clones and hybrids. Rabbit anti-WRN polyclonal antiserum was used to detect WRN protein in cell lines and hybrids. A molecular weight standard (in kDa) is shown *left*, and the position of the native 162.5 kDa WRN protein is shown *right*. *Lane 1* WRN clone WV1–37R1 (W1), *lane 2* control clone GM639–16R1 (C1), *lane 3* WRN clone PSVL30 (W2), *lane 4* control clone 639L22 (C2), *lane 5* W2/C1 hybrids, *lane 6* C1/C2 hybrids, *lane 7* W1/W2 hybrids, *lane 8* W1/C2 hybrids, *lane 9 WRN* +/+ lymphoblastoid cell line SYR10010, *lane 10 WRN* –/– lymphoblastoid cell line SYR10011. The WRN antiserum detects several low-molecular-weight cross-reacting species that are prominent in fibroblast cell line extracts but that do not appear to be WRN-related

and of hybrids generated from these from 18% to 33% (639L22 × WV1–37R1, fusion C2/W1, 17.6%; V1–37R1 × PSVL30, fusion W1/W2, 20%; GM639–16R1 × PSVL30, fusion C1/W2, 31.4%; GM639–16R1 × 639L22, fusion C1/C2, 33%).

Western blot analysis with an affinity-purified anti-WRN antiserum revealed full-length WRN protein in hybrids and in several fusion partners (Fig. 4, lanes 2, 4–9). WRN protein was undetectable in the WRN fusion partner WV1–37R1 (W1; Fig. 4, lane 1) and in a homozygous mutant WRN lymphoblastoid cell line (Fig. 4, lane 10). The WRN cell line PSVL30 (W2) surprisingly contained WRN protein (Fig. 4, lane 3; additional data not shown).

Discussion

We have shown that independently derived WRN SV40 fibroblast cell lines are unusually sensitive to the DNA-damaging agent 4NQO, and that this sensitivity can be complemented by fusing WRN and control cell lines. The mechanism underlying the 4NQO sensitivity of WRN cells and cell lines is not known. 4NQO generates DNA adducts when reduced to 4-hydroxy-aminoquinoline1-oxide by pyridine dinucleotide oxidoreductases. These adducts are predominantly on guanine bases (Nagao and Sugimura 1976) and are mutagenic (Daubersies et al. 1992; Fronza et al. 1992, 1994; Inga et al. 1994). 4NQO could also be toxic and/or mutagenic by redox cycling to generate reactive oxygen species (Nagao and Sugimura 1976; Nunoshiba and Demple 1993; Ruiz-Laguna et al. 1994).

Experiments with plasmid DNA substrates indicate that 4NQO adducts can be formed preferentially at junctions between B- and Z-form DNA (Rodolfo et al. 1994). DNA sequences with the potential to form Z-DNA are widely dispersed in the human genome, and Z-DNA may play a physiologic role in modulating gene expression and/or genetic recombination (reviewed in Sinden 1994). The helicase and nuclease activities of WRN (Gray et al. 1997; Suzuki et al. 1997; Huang et al. 1998; Kamath-Loeb et al. 1998; Shen et al. 1998a, 1998b) could facilitate the repair of 4NQO adducts in B-Z junction DNA and thus limit the potential of unrepaired 4NQO damage to alter gene expression or promote recombination. It seems less likely that WRN acts to limit oxidative DNA damage generated by 4NQO redox cycling: WRN cell lines are not unusually sensitive to H_2O_2 or γ-irradiation (Fig. 2), and primary WRN fibroblasts are not unusually sensitive to cumene hydroperoxide (M. Poot, personal communication).

In addition to documenting the 4NQO sensitivity of WRN SV40 fibroblast cell lines, we have shown that this cellular phenotype is recessive and can be complemented by fusing WRN and control SV40 fibroblast cell lines to form proliferating hybrids. Several groups have previously found that primary WRN fibroblasts rarely generate proliferating hybrids in cell fusion experiments (Norwood et al. 1979; Tanaka et al. 1979, 1980; Salk et al. 1981). The ease with which we have generated WRN × control proliferating hybrids may in part reflect the better growth potential of SV40-transformed WRN fibroblasts. In our experiments, all of the hybrids showed better colony-forming efficiency than did either fusion partner. However, only those hybrids that contained at least one control fusion partner had enhanced dose-dependent survival following 4NQO exposure (Fig. 3). The simplest conclusion is that the WRN fusion partners that we have used share a common defect that confers 4NQO sensitivity and that can be complemented upon fusion with control cells.

Kodama et al. (1998) recently reported experiments in which a G418-tagged human chromosome 8 was transferred into WS780 cells. They documented a high proportion of deletions among spontaneous *HPRT* mutations in this WRN cell line, as we had previously shown in other SV40-transformed WRN fibroblast cell lines (Fukuchi et al. 1989). However, the one WS780 clone that they assayed and that contained an intact chromosome 8 remained 4NQO-sensitive. Kodama et al. (1998) have concluded from these and additional observations that 4NQO sensitivity results from secondary mutations in WRN cells. We think this conclusion is unlikely to be correct. 4NQO sensitivity has been consistently associated with the loss of WRN function in many independent fibroblast and lymphoid cells and cell lines (Gebhart et al. 1988; Ogburn et al. 1997). If secondary mutations confer 4NQO sensitivity, they would have to occur both consistently and shortly after the loss of WRN function in many independent patients, cells, and lineages. This seems implausible. A simpler and more likely explanation is that WRN protein modulates the response of human cells to 4NQO damage. The continued 4NQO sensitivity of the single WS780/chromosome 8 clone examined by Kodama et al.

(1998) might be explained by clone-to-clone variation in 4NQO sensitivity of the WS780 cell line or by additional mutations in the transferred *WRN* gene that lies outside the 20% of the open reading frame that they have characterized.

An unexpected and surprising finding was that the 4NQO-sensitive PSV811 cell line contained WRN protein (Fig. 3). This was also noted, although not discussed, by Kodama and colleagues (1998) and has been observed independently by Marciniak et al. (1998). Our group and Marciniak et al. (1998) have sequenced the complete open reading frame of PSV811 as a reverse transcription/polymerase chain reaction product and have found a common polymorphism (Phe → Leu at residue 1074) but no mutations. We have confirmed this absence of mutations by amplifying and sequencing several commonly mutated exons and the *WRN* promoter region of PSV811 (J. Oshima and G.M. Martin, unpublished results).

There are three plausible explanations for the presence of apparently normal WRN protein in PSV811 cells in conjunction with 4NQO sensitivity and a failure to complement other WRN cell lines upon fusion. The first, suggested by Marciniak et al. (1998), is phenotypic misclassification or misdiagnosis. This seems unlikely on two accounts: PSV811 was derived from a patient with an unambiguous clinical diagnosis of WRN (Matsumura et al. 1985; Goto et al. 1978), and primary fibroblasts and the SV40-transformed PSV811 cell line from this patient display cellular phenotypes that are characteristic of WRN cell lines (Matsumura et al. 1985; Fukuchi et al. 1989). A second explanation is that there may simply be too little WRN protein in PSV811 or in tetraploid proliferating hybrids derived from PSV811 to confer 4NQO resistance. Although our previous work indicates that 4NQO resistance can be correlated with the level of WRN expression (Ogburn et al. 1997), this explanation in its simplest form is unlikely to be correct: Western blot data indicate that WRN is readily detected in PSV811 (Fig. 4; see also Bennett et al. 1999), although the survival of PSV811 and derivatives was consistently lower than the survival of WRN cell lines that lacked WRN protein.

A third possibility is that PSV811 cells lack a gene product that acts with or that acts on WRN as part of a common functional pathway. The failure to restore 4NQO resistance upon fusion could thus be an example of non-allelic noncomplementation. Non-allelic noncomplementation, also known as unlinked, false, or second-site noncomplementation, is the failure of mutations in two different genes to complement. Non-allelic noncomplementation has been described in yeast, *Caenorhabditis elegans*, *Drosophila*, and mice, and most instances involve mutations in genes that encode interacting proteins (Rine and Herskowitz 1987; Stearns and Botstein 1988; Heitman et al. 1991; Varkey et al. 1993; Rancourt et al. 1995; Harris and Juriloff 1998). Two general models have been proposed to explain non-allelic noncomplementation (Stearns and Botstein 1988): a "subunit level" model, in which too little functional heterodimer or heteromultimer is formed to allow the recovery of function, and a "poison subunit" model, in which mutant heterodimers disrupt native heterodimer or heteromultimer function. These models can be provisionally tested by expressing native heterodimer, which should reverse the non-complementing mutant phenotype selectively if the "subunit level" model is correct (Stearns and Botstein 1988).

The explanation that our cell hybrid results as an example of non-allelic noncomplementation is intriguing from at least three points of view: (1) it suggests that WRN is a genetically heterogeneous disease; (2) it is biologically plausible, as helicases rarely function in isolation (Matson et al. 1994); (3) it may provide a way to delineate and thus further study WRN-interacting proteins and functional pathways. The identity of at least two potential WRN-interacting proteins is suggested by the identification of camptothecin sensitivity in WRN lymphoblastoid cell lines (Okada et al. 1998; Poot et al. 1999) and by the ability of the human DNA single-strand binding protein RP-A to stimulate WRN helicase activity in vitro (Shen et al. 1998b). Further biochemical and genetic characterization of WRN and of WRN-interacting proteins should indicate the way in which WRN modulates the cellular response to 4NQO and the way in which the loss of WRN function promotes the cellular and clinical phenotype of WRN.

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