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RECQL4-deficient cells are hypersensitive to oxidative stress/damage: Insights for osteosarcoma prevalence and heterogeneity in Rothmund-Thomson syndrome

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Abstract

Rothmund-Thomson syndrome (RTS) is a heterogeneous disease, associated with increased prevalence of osteosarcoma in very young patients with a mutated RECQL4 gene. In this study, we tested the ability of RECQL4 deficient fibroblasts, derived from a RTS patient to recover from hydrogen peroxide (H_2O_2)-induced oxidative stress/damage. Immunoperoxidase staining for 8-oxo-deoxyguanosine (8-oxo-dG) formation in RTS and normal human fibroblasts were compared to assess DNA damage. We determined DNA synthesis, cell growth, cell cycle distribution, and viability in RTS and normal human fibroblasts before and after H_2O_2 treatment. H_2O_2 induces 8-oxo-dG formation in both RTS and normal fibroblasts. In normal human fibroblasts, RECQL4 was predominantly localized to cytoplasm; nuclear translocation and foci formation occurred in response to oxidant stimulation. After recovery from oxidant exposure, viable RTS fibroblasts showed irreversible growth arrest compared to normal fibroblasts. DNA synthesis decreased significantly in treated RTS cells, with concomitant reduction of cells in the S-phase. These results suggest that enhanced oxidant sensitivity in RECQL4 deficient fibroblasts derived from RTS patients could be attributed to abnormal DNA metabolism and proliferation failure. The ramifications of these findings on osteosarcoma prevalence and heterogeneity in RTS are discussed.

Keywords: RECQL4; Rothmund-Thomson syndrome; DNA damage; Osteosarcoma

Diseases of genetic instability are associated with increased incidence of cancer (for review see [1]). Enzymes involved in DNA metabolism play a critical role in these diseases. For example, DNA helicases play essential roles in DNA replication, transcription, recombination, and repair. The RecQ helicase family has been implicated in double-strand break repair and homologous recombination and is important for the maintenance of genomic integrity (for review see [2]). Mutations in three of the five RecQ helicases identified in humans, *BLM*, *WRN*, and *RECQL4* have been associated with Bloom's syndrome (BS), Werner's syndrome (WS), and Rothmund-Thomson syndrome (RTS), respectively [2]. BS is a genetic instability disease associated with an increased risk of developing various cancers in multiple tissues [3,4]. WS is a genetic disease characterized by the early onset of aging signs and a high incidence of sarcomas [2]. RTS is characterized by chromosome fragility, skin and skeletal defects, cataracts, and typically associated with an increased predisposition to osteosarcoma [5–7]. BLM and WRN helicases are extensively studied compared to RECQL4, whose function remains largely unknown [5–7].

The association of RTS with genetic instability suggests that the cells derived from these patients have deficiencies in DNA processing abilities. Recent reports have shown that RECQL4 play essential roles in DNA metabolism [8–10]. RECQL4 was found to have a DNA-dependent ATPase function, and to participate in the repair of

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DNA double-strand breaks by homologous recombination [11]. The direct connection of RECQL4 with DNA processing is emphasized in recent studies showing that RECQL4 is indispensable for initiation of DNA replication and cell proliferation [12]. We recently demonstrated that low bone mass in RECQL4-deficient mice was associated with defects in osteoblast progenitors [13].

In this study, we investigated the response of RECQL4-deficient fibroblasts derived from a RTS patient and normal human fibroblasts to H_2O_2-induced-8-oxo-deoxyguanosine (8-oxo-dG), a widely accepted marker of reactive oxygen species (ROS) mediated DNA damage. H_2O_2 is a common ROS intermediate, generated by various forms of oxidative stress. ROS are generated by multiple cellular pathways [14]. During some physiological processes, they can reach sublethal concentrations up to 0.66 mM in eye lens, and is likely to be the major cause of cataract formation in humans [14]. We evaluated changes in subcellular distribution of RECQL4 in response to H_2O_2-induced damage in human fibroblasts, and the ability of RTS and human fibroblasts to recover from oxidant stress/damage. We demonstrate that RECQL4-deficient fibroblasts are hypersensitive to H_2O_2-induced oxidative stress with defects in DNA metabolism. The implications of these findings on the possible development of osteosarcoma in heterogeneous RTS disease are presented.

Materials and methods

Cell culture. All cells were maintained at 37 °C, 5% CO_2 in the presence of 2 mM L-glutamine (Mediatech, Herndon, VA), 100 U/ml penicillin, 100 μg/ml streptomycin (Invitrogen, Carlsbad, CA), and 10% FBS (Invitrogen). The AG18371A fibroblasts, from a patient with RTS, were obtained from Coriel Cell Repositories (Camden, NJ). They were grown in the minimal essential medium (MEM), supplemented with deoxynucleotides and ribonucleotides (Invitrogen, Carlsbad, CA). AG18371A cells bear an 11 bp deletion at nucleotide g2746 (intron 8) of *RECQL4*, resulting in truncated RECQL4 protein, which is not detected by anti-RECQL4 antibody in Western analysis (Fig. 1). Normal human fibroblasts (GM637, a gift from Dr. Suk-Hee Lee, Indiana University Cancer Center, Indianapolis, IN) were grown in MEM. All cells were routinely passaged before reaching confluence.

Western blot analysis of RECQL4. Cell lysates from GM637 normal and AG18371 RTS fibroblasts were obtained by adding whole cell lysis buffer (62.5 mM Tris–HCl, pH 6.8, 2% w/v SDS, 10% glycerol, and 0.2 mM DTT) directly to PBS washed adherent cells for 10 min on ice. Cells were scraped and collected in microcentrifuge tubes. Protein concentrations were determined by BCA assay (Bio-Rad Laboratories, Hercules, CA). Cells were diluted in SDS sample buffer to a final concentration of 0.5 μg/μl. Cell extracts were electrophoresed on 10% SDS–polyacrylamide gels at 100 V, and transferred to nitrocellulose overnight at 4 °C. Membranes were blocked with 5% non-fat dried milk for 2 h at room temperature, and then incubated in anti-RECQL4 (sc-16924, Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4 °C. Proteins were detected by enhanced chemiluminesence development using Super Signal West Pico substrate (Pierce, Rockford, IL). Anti-pan actin (MS-1295, Neomarkers, Fremont, CA) was used to determine protein loading.

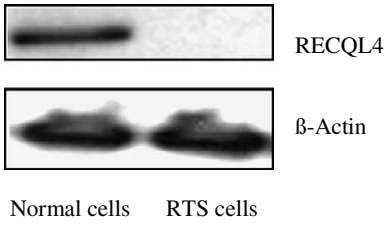

Fig. 1. RECQL4 protein expression in human GM637 normal and RTS fibroblasts used in this study. Whole cell extracts were analyzed by Western blot using anti-RECQL4 antibody. β-Actin was used as a control to verify equal protein loading.

Oxidative stress/damage induced by H_2O_2 treatment. H_2O_2 was obtained from Sigma–Aldrich (St. Louis, Mo). Exponentially growing cells (similar confluence) were incubated with H_2O_2 (for concentrations, please see Results section and figure legends) diluted in MEM for 15 min at 37 °C and 5% CO_2. After 15 min of damage, cells were collected and processed as described in the following Materials and methods section.

Immunoperoxidase staining for 8-oxo-deoxyguanosine. Immunocytochemistry for 8-oxo-dG was carried out in GM637 normal human and RTS fibroblasts, using anti-8-oxo-dG (Abcam, ab10802). Cells were treated with 0.5 mM H_2O_2 for 15 min at 37 °C and 5% CO_2. After damage, the cells were washed twice with 1× PBS and fixed for 10 min in methanol at −20 °C. Slides were then washed in PBST and blocked for 1 h at 37 °C in 5% non-fat dried milk, 0.1% BSA in PBS. Incubation with anti-8-oxo-dG was performed overnight at 4 °C. Following incubation the sample slides were washed three times for 15 min each in PBS. After secondary antibody incubation for 1 h at room temperature, followed by PBS wash, colorimetric detection was carried out using diaminobenzamide tetrahydrochloride (DAB) peroxidase system (Vector Laboratories, Burlingame, CA), and counterstained for 2 min with methyl-green (Vector Laboratories). Images from stained slides were collected using a Leitz DMR microscope (Leica, Wetzlar, Germany), equipped with a SPOT-RT color digital camera (Molecular Diagnostics, Burlingame, MI). Images were analyzed using Spot Advanced 4.0.8 software (Molecular Diagnostics, Burlingame, MI), and processed using Paint Shop Pro 7 software (Corel Minneapolis, Eden Prairie, MN). Darkly stained nuclei with no detectable methyl-green were counted as positive.

Subcellular localization of RECQL4 and BLM helicase proteins. To determine RECQL4 and BLM helicase cellular localization, GM637 normal fibroblasts were grown on tissue culture chamber slides (Nalge Nunc, Naperville, IL). Cells were treated with H_2O_2 (0.1 mM) for 15 min at 37 °C and 5% CO2. After damage, the cells were washed twice with 1× PBS and fixed for 10 min in methanol at −20 °C. The fixed cells were washed 3 times in PBS and then dried over a heat block at 40 °C. Slides were washed in PBST and blocked for 1 h at 37 °C in 5% non-fat dried milk, 0.1% BSA in PBS. Incubation with either anti-RECQL4 antibody 0103 (a generous gift from Dr. Jinhu Yin) or anti-BLM (ab476, Novus, Littleton, CO) was performed overnight at 4 °C. Following incubation, the samples were washed 3× for 15 min each in PBS; incubated in HRP conjugated anti-rabbit IgG antibody for 1 h at room temperature; then washed 3× 15 min in PBS. Counterstaining for nuclei was carried out by incubating the slides in 4′,6-diamidino-2-phenylindole (DAPI) for 1 h at room temperature, followed by three washes in PBS. Data shown are representative of three independent experiments.

Cell proliferation assay. Oxidative stress/damage was induced by overlaying GM637 normal and RTS fibroblasts with serum free medium containing 0.1 to 1 mM concentrations of H_2O_2. Untreated cells served as control. Cells at a density of 2×10^4 cells/ml were plated in 24-well plates. Cells were incubated with H_2O_2 for 15 min at 37 °C. Cells were then washed and incubated in complete medium at 37 °C. After recovery, cell growth was determined in surviving cells by counting at least 100 cells using a hemocytometer at the indicated time points. Cell proliferation experiments were performed in triplicate.

BrdU incorporation assay for cellular DNA synthesis. To measure DNA synthesis, untreated and 0.1 mM H_2O_2-treated GM637 normal and RTS fibroblasts were grown in 70 μM 5′-bromo-2′-deoxyuridine (BrdU) obtained from Sigma (St. Louis, MO) for 12 and 24 h. Cells were fixed in methanol at −20 °C for 7 min, washed 2× in PBS, followed by

denaturation of DNA in 2 N HCl for 30 min at 37 °C. The acid was neutralized by 2 washes in 0.1 M borate buffer, pH 8.5. Following neutralization, the cells were washed twice in PBS containing 0.05% Tween. Endogenous peroxidase activity was quenched with 3% H_2O_2. Antibody incubation and sample imaging was carried out as described above. To determine the percent of BrdU positive nuclei, 200 cells from three random regions of each slide were counted. Data shown are the summary of three independent experiments.

Cell viability assay. Cell viability was determined using the live/dead assay (Molecular Probes, Eugene, OR), as described in the manufacturer's protocol. Briefly, untreated and 0.1 mM H_2O_2-treated MG637 normal and RTS fibroblasts were incubated in solution containing cell permeant green fluorescent SYTO 10 and cell impermeant DEAD Red (ethidium homodimer-2). Live cells were differentiated from dead cells by exclusion of red stain. Images were collected as described above. The percent of dead cells was determined by counting at least 100 cells from three random regions of each slide ($n = 3$).

Flow cytometry for cell cycle distribution. Cell cycle distribution was determined by flow cytometric analysis. Cells were grown in T-25 culture flasks to 60–70% confluence and treated with 0.1 mM H_2O_2. Untreated cells served as control. Following a 24 h recovery, cells were trypsinized and washed with PBS. The cell pellets were resuspended in PBS, containing 6 μl/ml NP-40, 0.1 mg/ml propidium iodide, 1 μg/ml RNase at a concentration, 1×10^6 cells/ml. Cells were incubated on ice for 30 min before analysis. Flow cytometric analysis was performed on a FACSCalibur flow cytometer (BD Bioscience, San Jose, CA) and data collected using Cell Quest Pro software (BD Bioscience). Analysis of 10,000 events ($n = 3$) was performed on each cell line using ModFit LT (BD Bioscience) software.

Statistical analysis. Data were analyzed using JMP Statistical Discovery Software 4.1 (SAS Institute, Cary, NC). Data are expressed as means ± standard errors of mean (SEM). Data were analyzed by one-way analysis of variance, followed by pairwise t test. A p value <0.05 denoted statistically significant difference.

Results

RECQL4 protein is expressed in human fibroblasts

To show the RECQL4 protein expression in normal cells compared to RTS fibroblasts, we subjected whole cell extracts of GM637 normal human fibroblasts and AG18371A fibroblasts derived from a patient with RTS to SDS-PAGE, followed by Western analysis using anti-RECQL4 antibody. The anti-RECQL4 antibody detected RECQL4 protein in normal fibroblasts, but not in the RTS fibroblasts (Fig. 1).

H_2O_2 induces DNA damage/8-oxo-dG formation in RTS and normal fibroblasts

Fig. 2A shows a representative immunoperoxidase staining for 8-oxo-dG in GM637 normal and RTS fibroblasts treated with 0.5 mM H_2O_2 concentrations for 15 min. Although background nuclear staining for 8-oxo-dG was apparent in the control cultures, treatment with H_2O_2 increased the overall intensity and the number of 8-oxo-dG positive nuclei in both GM637 normal human and RTS fibroblasts. Quantitatively, the treatment of cells with H_2O_2 significantly increased the number of 8-oxo-dG positive nuclei in both normal and RTS fibroblasts compared to control cultures (Fig. 2B).

RECQL4 translocates and forms nuclear foci in response to H_2O_2 induced damage in human fibroblasts

In asynchronous GM637 normal human fibroblasts, RECQL4 was primarily localized to cytoplasm, with detectable perinuclear staining (Fig. 3A). RECQL4 foci formation in the nucleus was evident within 15 min after 0.1 mM H_2O_2 treatment and persisted 60 min following removal (Fig. 3B). BLM helicase was found to translocate from diffused nuclear localization to nuclear foci after H_2O_2 treatment (Fig. 3C and D). Induction of BLM nuclear foci was also observed in RTS fibroblasts suggesting that BLM foci formation is independent of RECQL4 protein (data not shown).

RTS cells show decreased cell growth after recovery from H_2O_2-induced damage

After recovery from H_2O_2 treatment, normal human GM637 fibroblasts grown in fresh complete medium exhibited delayed increase in cell number. As shown in Fig. 4A, 120 h following recovery from H_2O_2, normal human fibroblasts increased twofold (194 ± 5%, $p < 0.01$ vs. day 0). In contrast, treated RTS fibroblasts increased 39 ± 23% ($p < 0.001$ vs. untreated), demonstrating significant reduction in growth rate. Fig. 4B illustrates growth response of RTS fibroblasts after recovery from exposures to different concentrations of H_2O_2. Control untreated RTS fibroblasts showed approximately five-fold increase (487 ± 52.7 %), while RTS fibroblasts treated with 0.1 or 1.0 mM H_2O_2 did not increase significantly in 120 h cultures (Fig. 4B). These results suggest that RTS fibroblasts are hypersensitive to H_2O_2-induced oxidative stress/damage, and exhibit irreversible cell growth arrest.

Viable RTS cells exhibit reduced DNA synthesis after recovery from H_2O_2-induced damage

Changes in cell number after recovery from H_2O_2-induced DNA damage could be due to either reduced proliferation or an increased rate of cell death. To understand the mechanisms involved, we stained fibroblasts recovered from oxidant damage for BrdU incorporation, as a marker for DNA synthesis. Percentage BrdU positive nuclei were reduced in the treated RTS fibroblasts, when compared to normal human fibroblasts (Fig. 5A). In the untreated RTS fibroblasts, 45.9 ± 2.3% showed positive for BrdU incorporation at 12 h; a reduction of 26.8 ± 3.8% was observed in treated RTS fibroblasts. When RTS cells were incubated in BrdU beginning 24 h after treatment, a further reduction was observed (12.5 ± 2.2% BrdU positive nuclei). These results suggest that DNA synthesis continued to decrease over time, concomitant with reduced cell proliferation. Normal fibroblasts treated with H_2O_2 did not exhibit reduced BrdU uptake immediately after treatment (84.2 ± 0.4%

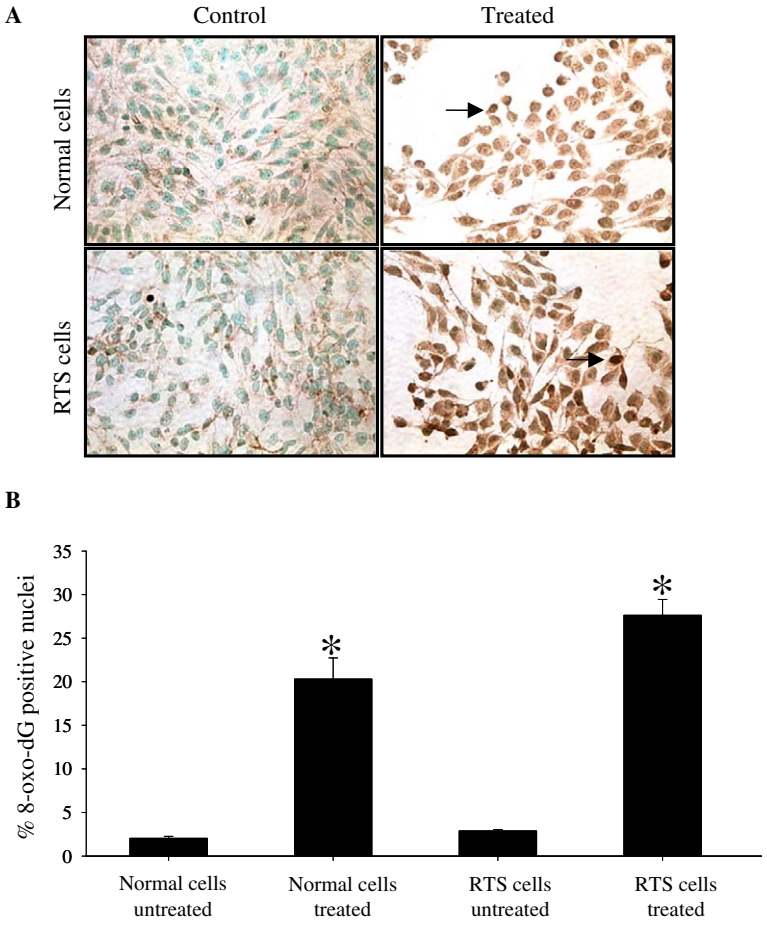

Fig. 2. Immunoperoxidase staining for the detection of 8-oxo-dG formation in normal and RTS fibroblasts. (A) RTS and normal fibroblasts were stained before and after 0.5 mM H_2O_2 treatment for the presence of 8-oxo-dG as described in Materials and methods. Darkly stained nuclei were identified as positive for 8-oxo-dG, as indicated by arrows. Nuclei were counterstained with methyl-green. (B) Quantification of positively stained nuclei for 8-oxo-dG in normal and RTS fibroblasts treated with H_2O_2, compared to their respective controls. Data are represented as means ± SEM ($n = 3$), $^*p < 0.05$ compared to untreated control cultures.

positive nuclei). However, there was a reduction in BrdU uptake, when the normal fibroblasts were measured 24 h post recovery (65.5 ± 2.3%). In contrast to the cell proliferation data, no significant difference in cell viability was observed in post H_2O_2 treated RTS fibroblasts over time (Fig. 5B).

RTS cells display a reduction in the S-phase cells after recovery from H_2O_2-induced damage

The cell cycle profile of treated RTS cells differed from that of GM637 normal human fibroblasts (Table 1). When treated with H_2O_2 for 15 min and analyzed for cell cycling 24 h after recovery, normal human fibroblasts exhibited a 17.7 ± 1.4% increase in the G_2/M-phase population. In RTS fibroblasts, a 2.7-fold reduction in the S-phase population from 13.0 ± 0.4% to 4.8 ± 0.4% occurred, with simultaneous small increases in both G_0/G_1 and G_2/M-phase populations. In concordance with the cell viability data, no sub-G_1 or apoptotic population changes were detected in the H_2O_2 treated or untreated RTS fibroblasts.

Discussion

RecQ helicases maintain genomic stability [2,15]. Individual family members may be specific to the type of DNA structures or damage with which they interact [14]. While mutation in *RECQL4* has been implicated in the etiology of RTS [5], the molecular function of RECQL4 protein remains to be elucidated [16,17]. In this study, we demonstrate that RECQL4 may be important in the cellular response to oxidative DNA damage. Oxidant treatment induces rapid translocation of RECQL4 to form nuclear foci in normal human fibroblasts. These foci may represent sites of DNA damage in response to oxidant stress. Lack of RECQL4 foci formation might be an indication for defective DNA repair process.

We demonstrate with this study that RECQL4-deficient fibroblasts from RTS patient are hypersensitive to H_2O_2-induced oxidative stress/damage with defects in DNA metabolism. H_2O_2 can traverse the plasma and nuclear membranes, thereby contributing to 8-oxo-dG formation and DNA damage as it enters the nuclear compartment

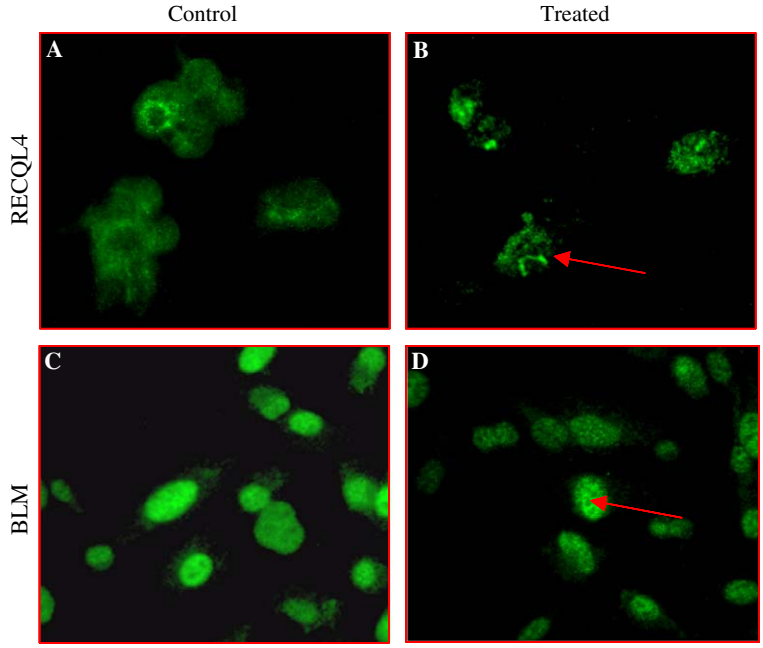

Fig. 3. Determination of subcellular localization of RecQL4 and BLM proteins in normal GM637 human fibroblasts as described in Materials and methods. Arrows indicate immunofluorescence images for nuclear foci formation by RECQL4 and BLM in response to 0.1 mM H_2O_2 treatment.

[18]. H_2O_2 induces DNA damage in both normal and RTS fibroblasts, as assessed by the 8-oxo-dG formation. Although 8-oxo-dG formation is one measure of oxidative damage, treatment of cells with H_2O_2-mediated DNA oxidation produces a wide spectrum of damage, including base lesions, oligonucleotide strand breaks, and abasic sites or sites of base loss [19]. In this study, we examined 8-oxo-dG as oxidative stress/damage end point because this DNA lesion is a widely accepted marker of oxidative damage [18]. Cell proliferation was reduced in both the cell lines tested following oxidant recovery. Although, normal fibroblasts exhibited delayed growth response after recovery from oxidant challenge, the ability of these cells to proliferate and grow remained unchanged. In contrast, following oxidant recovery, RTS fibroblasts did not proliferate and exhibited reduced DNA synthesis. The differences were also apparent in the cell cycle profile of RTS compared to normal fibroblasts. The immediate effect in normal fibroblasts after oxidant exposure recovery was accumulation of cells in the G_2/M-phase. This effect did not occur in RTS fibroblasts, where the primary effect was a reduced S-phase population, associated with increases in both G_1 and G_2/M-phase populations. Interestingly, cell viability remained the same in the treated and untreated RTS fibroblasts. These differences suggest that the lack of increase in cell number in RTS cells after recovery from oxidant exposure may be due to reduced and/or impaired proliferation. The direct connection of RECQL4 in DNA processing is emphasized in a recent study showing that RECQL4 is indispensable for initiation of DNA replication and cell proliferation [12].

Given the genomic instability associated with RTS patients, it is likely that processing of DNA in response to damage or during replication is defective. Because defects in RTS patients are restricted to limited tissues [7], the role of RECQL4 in this process is not likely across all cell types and tissues. RECQL4 function may be important in specific proliferating tissues such as developing bone and skin [20,21]. In fact, clinical features of all RTS manifest with a characteristic skin rash and skeletal defects, and approximately 80% of RTS patients develop osteosarcoma when very young [7]. Recently, we demonstrated that low bone mass phenotype in RECQL4-deficient mice was associated with defects in osteoblast progenitors [13]. Predisposition to osteosarcoma in RTS was recently linked to patients who carry at least one truncating mutations in the catalytic helicase domain of RECQL4 [6].

It has been increasingly evident that RTS is a clinically heterogeneous disease [6,22]. Mutations in *RECQL4* have been reported in a subset of RTS patients [5–7]. Discrepant results in the literature on sensitivity to ultraviolet or γ-irradiation may reflect true heterogeneity in the disease. These studies were primarily carried out with cells from RTS patients solely based on clinical phenotype; molecular analyses for *RECQL4* mutations were unknown at the time. For example, some studies report enhanced photosensitivity of RTS cells [23,24], while other study report no such sensitivity [25]. Fibroblasts derived from RTS patient showed enhanced radiosensitivity to hypoxic γ-irradiation by the colony-formation assay [23]. RTS fibroblasts also showed hypersensitivity to ultraviolet radiation with reduced amount of unscheduled DNA synthesis (UDS). The reduced cell survival and UDS may reflect the reduced DNA repair capacity in these cells [24]. In this study, hypersensitivity to H_2O_2, associated with reduced and/or impaired cell proliferation in RTS fibroblasts with

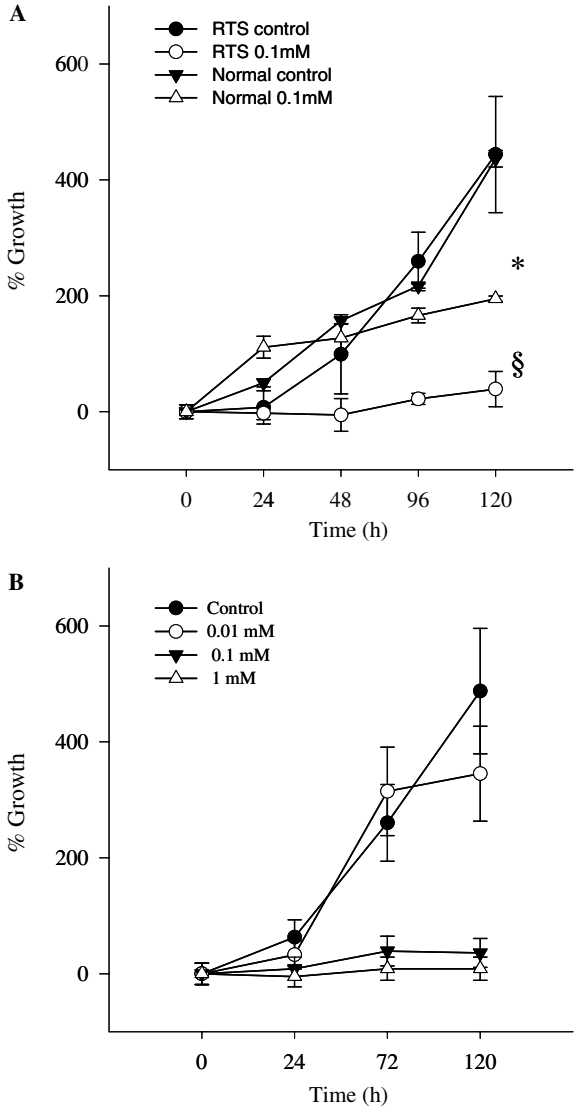

Fig. 4. RTS fibroblasts are hypersensitive to H_2O_2-induced oxidative stress/damage showing irreversible cell growth arrest. Cells were incubated with either H_2O_2 (as indicated) or medium alone (control) for 15 min at 37 °C. Cells were then washed and incubated in complete medium. After recovery, cell growth was determined in surviving cells by counting cell number at 48, 96, and 120 h. (A) Normal fibroblasts control (▼), 0.1 mM H_2O_2 treated normal fibroblasts (△), RTS fibroblasts control (●) and 0.1 mM H_2O_2 treated RTS fibroblasts (○). (B) Growth response of RTS fibroblasts after recovery from exposures to different concentrations of H_2O_2. After recovery, cell growth was determined in surviving cells by counting cell number at the indicated time points. Control (●), and treatments: 0.01 (○), 0.1 (▼), 1.0 (△). Data are represented as means ± SEM ($n = 3$), *$p < 0.05$ vs. 0 h; §$p < 0.001$ vs. control.

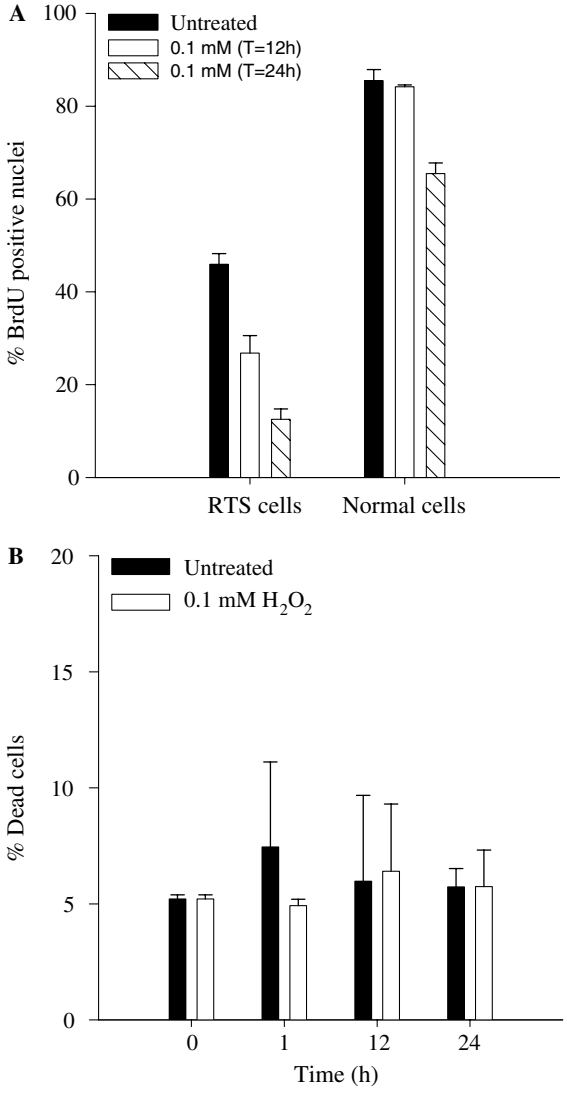

Fig. 5. DNA synthesis and cell viability in RTS and normal fibroblasts after recovery from H_2O_2-induced damage. (A) BrdU incorporation in RTS and normal fibroblasts was measured by anti-BrdU immunocytochemistry at 12 and 24 h after treatment with 0.1 mM H_2O_2. (B) Cell viability in RTS fibroblasts was determined in untreated control and H_2O_2-treated RTS fibroblasts at 0, 1, 12, and 24 h after treatment. Data are represented as means ± SEM ($n = 3$).

truncated RECQL4, could be due to defective or reduced DNA repair capacity.

In conclusion, our findings suggest that DNA lesions formed in RTS fibroblasts may be fixed, and are not removed from the viable cell population following oxidant

Table 1
Cell cycle analysis of human fibroblasts[a]

Cell cycle phase	Normal cells untreated	Normal cells 0.1 mM H_2O_2	RTS cells untreated	RTS cells 0.1 mM H_2O_2
G1	54.5 ± 1.2	29.8 ± 1.6	55.2 ± 0.3	61.4 ± 1.2
S	23.5 ± 0.7	30.5 ± 5.4	13.0 ± 0.4	4.8 ± 0.4
G_2/M	22.0 ± 1.4	39.7 ± 4.6	31.8 ± 0.7	33.8 ± 1.4

[a] Cell cycle analysis was carried out using flow cytometry in GM637 normal and RTS cells before and 24 h after H_2O_2 treatment. Data are represented as mean ± SEM ($n = 3$).

treatment. If these lesions are not repaired, or if repair introduces errors in the DNA sequence would, lead to mutations and increase the risk of tumor occurrence [26]. Because of irreversible cell growth arrest or senescence associated with viable RTS fibroblasts following oxidant damage, further activating mutations may be required in these cells for the oncogenic process to propagate. Alternatively, growth impaired RTS fibroblasts like senescent fibroblasts may secrete factors that favor tumor cell growth [27]. Taken together, it is reasonable to assume that enhanced oxidant sensitivity associated with abnormal DNA metabolism and repair deficiency in RTS patients with defective RECQL4 could be related to the increased risk of osteosarcoma in this syndrome.

Acknowledgments

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