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Resistance to ultraviolet-induced apoptosis in DNA repair deficient growth arrested human fibroblasts is not related to recovery from RNA transcription blockage

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Abstract

The impact of ultraviolet (UV-C) photoproducts on apoptosis induction was investigated in growth arrested (confluent) and proliferating human primary fibroblasts. Confluent fibroblasts were more resistant to UV-C-induced apoptosis than proliferating cells, and this was observed for normal human cells and for cells from patients with Cockayne and trichothiodystrophy syndromes, deficient in transcription coupled repair. This resistance was sustained for at least seven days and was not due to DNA repair efficiency, as the removal of CPDs in the genome was similar under both growth conditions. There was no correlation between reduced apoptosis and RNA synthesis recovery. Following UV-C treatment, proliferating and confluent fibroblasts showed a similar level of RNA synthesis inhibition and recovery from transcription blockage. These results support the hypothesis that the decrease of DNA replication, in growth arrested cells, protects cell from UV-C-induced apoptosis, even in the presence of DNA lesions.

Keywords: Nucleotide excision repair; Apoptosis; Ultraviolet; Transcription coupled repair; Replication; Quiescent cells

1. Introduction

DNA damage may result in cellular dysfunctions, such as genetic instability, mutagenesis or cell death, considered hallmarks of biological processes such as cancer and aging. However, cells are equipped with efficient systems that are responsible for maintaining the integrity of their DNA. These mechanisms are responsible for removing and tolerating DNA damage, and failures are very hazardous for cells, their consequences being observed in several human diseases.

Xeroderma pigmentosum (XP), Cockayne syndrome (CS) and trichothiodystrophy (TTD) patients present photosensitivity, although at different levels, due to failure in removing UV-induced DNA lesions by the nucleotide excision repair (NER) mechanism [1]. XP patients denote strong pigmentation, actinic keratoses, loss of elasticity and multiple skin cancers, in sun exposed areas of the skin. Seven complementation groups (XPA-G) and one variant (XPV), which is deficient in lesion bypass by the translesion synthesis DNA polymerase eta, have been identified. CS and TTD patients are not particularly susceptible to skin cancer development, and their clinical features have little in common with XP patients. CS patients are characterized by reminiscent traits of normal aging, such as systemic growth failure, neurological degeneration and cataracts. Moreover, a small number of XP patients (from XP-B, XP-D and XP-G complementation groups) display a combination of cutaneous abnormalities together with the severe neurological and developmental anomalies of Cockayne's syndrome. Two complementation groups have been identified (CS-A and CS-B), and besides their importance on lesion removal, the corresponding proteins have an important role in RNA transcription. TTD is very heterogeneous, and groups different patients with

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sulphur-deficient brittle hair. About half of TTD patients exhibit photosensitivity, due to their deficiency in removing UV-induced DNA lesions, ichthyotic skin, small stature and mental retardation. There are three complementation groups of photosensitive TTD patients (TTD-A, XP-B and XP-D), and all of the corresponding proteins are components of the transcription factor H of RNA polymerase II (TFIIH) [2].

NER is considered the most flexible and versatile DNA repair pathway, as it deals with a wide range of structurally unrelated DNA lesions. The major injuries induced by UV light are cyclobutane pyrimidine dimers (CPDs) and 6–4 photoproducts (6–4 PPs), which are removed by NER. The NER process involves the coordinated action of at least 30 proteins, and its basic mechanism consists of the removal of the DNA segment containing the lesion and gap polymerization using the intact strand as template [3]. Two NER subpathways have been identified: transcription-coupled repair (TCR), selectively acting on lesions present in the transcribed strand of expressed genes, and the global genome repair (GGR) that acts upon the rest of the genome. The transcribed strand of expressed genes is repaired faster than other genomic regions, including the non-transcribed strand of active genes [3]. CS cells are exclusively deficient in TCR of CPD but are proficient for GGR. On the other hand, XP-C and XP-E cells are exclusively deficient in GGR of both CPD and 6–4 PPs. The other XP and TTD cells are deficient for both subpathways, TCR and GGR [1].

NER-deficient cells are hypersensitive to UV light, specially those defective in TCR (XP-A, XP-B, XP-D, XP-F, XP-G, CS and TTD), which show high levels of apoptosis induction following exposure to low doses of this genotoxic agent [4,5]. The TCR-deficient cells do not recover normal transcription following UV exposure, probably as a consequence of blockage of RNA polymerase II by the remaining lesions on the transcribed strand of active genes [4,6]. Based on this evidence, transcription was proposed to act as a "damage dosimeter", where the severity of the DNA damage, the ability to remove the lesions and the kinetics of mRNA synthesis recovery, determine whether the cell lives or dies [7]. However, this is not a universal consensus. Results obtained with serum starvation and high density seeding protocols indicate that DNA replication plays a role in UV-induced apoptosis [8]. Accordingly, McKay et al. [9] have shown that the repair of UV-induced lesions is of particular importance in proliferating fibroblasts before DNA replication in the S phase. Similar results were found in human keratinocytes. Rapidly proliferating keratinocytes were more susceptible to UV-light-induced apoptosis, whereas those induced to undergo growth arrest/early differentiation were relatively resistant to apoptosis [10,11]. In agreement with the idea that replication of damaged DNA also signals for apoptosis, it was recently shown that hamster cells that had their DNA replication blocked by aphidicolin were more resistant to UV-induced apoptosis [12]. Most probably the replication of UV-C lesions generates secondary lesions that potentially lead to cell death. In fact, a balance between transcription and replication blockage must be involved in DNA damage signaling pathways responsible for the decision as to whether a cell dies or lives after DNA insult.

The impact of growth arrest and RNA transcription in apoptosis induction by UV-C-lesions was investigated in this work. These studies were performed in primary human skin fibroblasts, which sustain a contact inhibited proliferative state. This condition has the advantage of keeping low levels of DNA replication without disturbing the cells with chemicals or starvation, which certainly interfere in their normal metabolism. Moreover, most of the cells in the human body are probably in a quiescent state, similar to confluent cells. Previous experiments have indicated that XP-C confluent cells were more resistant to UV-C-induced apoptosis than proliferating ones [13]. The present report extends these findings to TCR-deficient cells (CS and TTD cells) which are also more resistant to UV-C-induced apoptosis when irradiated in confluent conditions at low doses. Protection was observed for long periods, indicating that it does not postpone cell death. NER efficiency was evaluated, and no significant difference was found for CPD removal from DNA under both growth conditions. Moreover, RNA synthesis inhibition by UV-C-light was similar in confluent and proliferating cells, despite the different apoptosis levels. Altogether, these results show a correlation between UV-C-induced apoptosis and DNA replication, and calls attention to the different apoptotic responses depending on the cell cycle status.

2. Materials and methods

2.1. Cell culture

Human primary fibroblasts derived from skin biopsies were employed in this study: normal (gently donated by Dr. Claudimara Lotfi, ICB, USP-SP, Brazil), CS1AN (mutated in *CSB*, gently donated by Dr. Jaspers NG, Department of Cell Biology and Genetics, Erasmus University Rotterdam, Netherlands), CS1VI (mutated in *CSB*), TTD1VI (mutated in *XPD*). Cells were grown in Dulbecco's modified Eagle's medium (DMEM, Invitrogen Corporation, Carlsbad, USA) supplemented with 10% fetal calf serum (FCS, Cultilab, Campinas, SP, Brazil), 100 U/ml penicillin G sodium, 100 μg/ml streptomycin and 0.25 mg/ml amphotericin (Invitrogen Corporation). An atmosphere of 5% CO_2 was maintained in a humidified incubator at 37 °C. Proliferating cells corresponded to cells plated in 1.2×10^5 cells per 60 mm Petri dishes, and after 3 days approximately, with less than 80% of confluence, they were irradiated. Confluent cells were obtained after seeding 2.4×10^5 cells in 30 mm Petri dishes, and they were employed only 5 days after reaching confluency.

2.2. Cell irradiation

Confluent and proliferating cells were washed with PBS and irradiated with UV-C, using a germicidal lamp (emitting mainly at 254 nm, dose rate of 1 J/m^2 s). UV-C dose was monitored by the radiometer VLX 3W, monochromatic sensor CX-254 (Marne la Vallee, France). It should be noted that due to a change in the dosimeter, the values of UV-C doses of this work correspond to approximately 4.0 times the values previously published in this laboratory [13]. After irradiation, the cell medium was replenished and cells were incubated under normal conditions for the desired period.

2.3. Cell cycle flow cytometry

Apoptosis was measured by quantifying a sub-G1 population, following cell cycle analysis of propidium iodide staining. Briefly, floating and attached cells were harvested by trypsinization, collected and resuspended with 70% ethanol/PBS and stored at 4 °C. Cells were then stained with 50 μg/ml propidium iodide for 1 h in the presence of 40 μg/ml of RNAse A (DNAse-free). Measurements were carried out in a FACScalibur flow cytometer (Becton Dickinson,

Franklin Lakes, NJ). Results were analyzed with CellQuest software (Becton Dickinson).

2.4. Cell viability assay

Cell viability of human fibroblasts was performed 7 days after UV-C-irradiation, by employing a XTT assay (Cell Proliferation Kit II, XTT; Roche Molecular. Biochemicals, Mannheim, Germany). The procedure was followed as recommended by the supplier.

2.5. DNA synthesis quantification

Proliferating and confluent cells were pulse-labeled by incubating in medium containing 4.0 μCi/ml of [^{3}H]-methyl-thymidine (specific activity of 89.0 Ci/mmol, GE Healthcare, USA) for 1 h. Cells were then washed once with PBS, 5% trichloroacetic acid and twice with hydrated alcohol, before 0.3 M NaOH was added. Part of this cell lysate (50% of the total volume) was applied on Whatman 17 paper, which was again washed once with 5% trichloroacetic acid, twice with ethanol and once with acetone. Radioactivity was measured with a liquid scintillation spectrometer (Beckman LS 7000). The other part of the lysate was used to measure absorbance at 260 nm, for data normalization. The ratio between radioactivity and absorbance expresses the amount of [^{3}H]-methyl-thymidine incorporated by cells during DNA synthesis.

2.6. Immuno-slot-blot analysis of CPD

Proliferating and confluent fibroblasts were UV-C-irradiated with 15 J/m^2. At different times following irradiation (0, 3 and 24 h) their genomic DNA was extracted as described [14]. Genomic DNA was loaded onto nylon membrane under a vacuum, and the DNA was fixed by 2 h of incubation in 80 °C. The membranes were immersed in PBS containing 5% low-fat dried milk for 1 h, and then incubated overnight with anti-CPD (MBL, Japan) monoclonal antibody (dilution 1:1000). Chemifluorescent detection was performed with ECL western blotting kit (GE Healthcare) via alkaline phosphatase anti-mouse secondary antibody. Quantification of signals was obtained using Molecular Analyst software (Bio-Rad Laboratories, California, USA). The percentage of repair is expressed as a function of residual intensity of bands at post UV-C irradiation times over samples processed. Results are the average of four different assays, corresponding to two different DNA samples.

2.7. RNA synthesis recovery assay

RNA synthesis was determined based on a previously described method [15]. Cells were plated in Petri dishes containing normal medium. After reaching the desired confluency, cells were UV-C irradiated, incubated for a certain period of time, and then pulse-labeled for 1 h in DMEM with 3% dialyzed FCS and [5-^{3}H]uridine (4.0 μCi/ml, specific activity of 27.0 Ci/mmol, GE Health Care). Cells were then harvested and separated into two samples. In the first sample, cells were lysed (NaCl 0.3 M; Tris–HCl pH 8.0 20 mM; EDTA 2 mM; SDS 1% and K proteinase 200 μg/ml), and transferred to Whatman 17 paper, then being washed twice with 15% trichloroacetic acid and hydrated ethanol for 30 min, for radioactivity measurement. The second sample was used to determine absorbance at 260 nm for data normalization. The ratio between radioactivity and absorbance expresses RNA synthesis of these cells.

3. Results

3.1. Proficient and deficient NER cells are resistant to UV-C radiation under confluent conditions

The UV-C-induced apoptosis was determined in proliferating and confluent human primary fibroblast strains. The fraction of cells with a sub-G1 DNA content was quantified by flow cytometry in TCR proficient cells (FHN), GGR and TCR defective

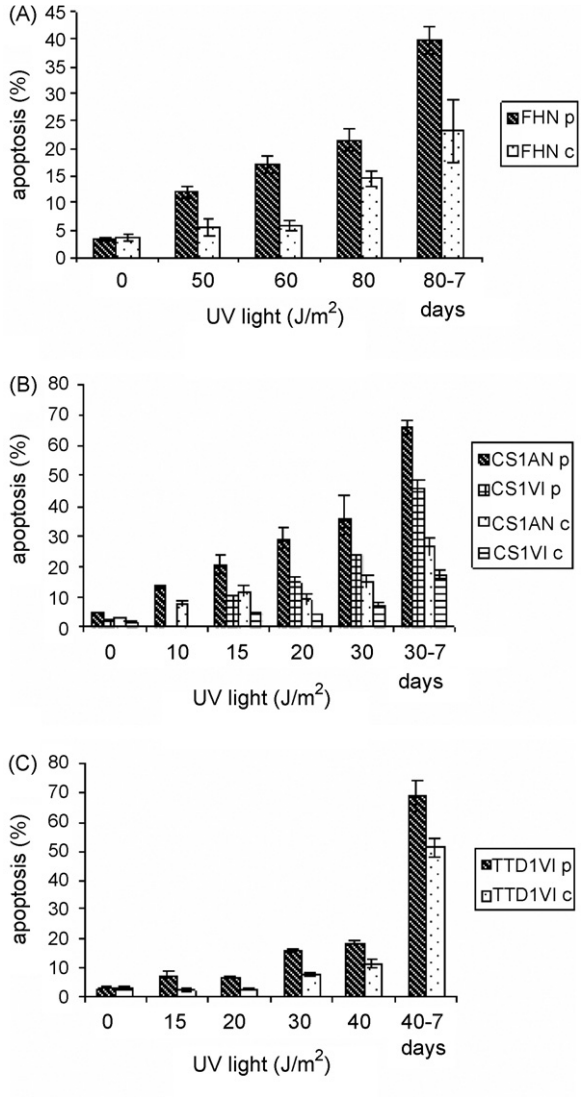

Fig. 1. Resistance of confluent cells to UV-C-induced apoptosis. Induction of apoptosis (sub-G1, as measured by flow cytometry) in normal (FHN) (A), CS-B (CS1AN and CS1VI) (B) and TTD (TTD1VI) (C) cells, 3 and 7 days (when stated) after UV-C exposure. Each result represents the mean of three duplicated independent experiments. Proliferating (p) and confluent (c) cells were employed, as indicated in the inset.

TTD (TTD1VI) cells, and TCR defective cells from two CS patients, both mutated at the *CSB* gene (CS1AN and CS1VI) (Fig. 1). Sub-G1 cells were observed only 72 h following UV-C exposure, and as cell ability to remove DNA lesions is different among the cells tested, different UV-C doses were used, corresponding to comparable cell death induction. As expected, lower UV-C doses were necessary to induce similar levels of apoptosis in NER deficient fibroblasts than normal cells. Different responses were observed in the two CS-B cell strains, probably due to either the genetic background of the cells or the effect of the different CSB mutations.

The sensitivity of these cells to UV-C-induced DNA damage was significantly reduced when they were kept in confluence. Lower levels of apoptosis were also observed in TCR deficient cells, when they are not proliferating. Similar results were

observed in XP-C fibroblasts [13], and in normal keratinocytes [10], thus indicating this to be a general phenomenon, although the protection in XP-C confluent fibroblasts seems to be stronger. Apoptosis was also checked, for the higher doses, considering seven days after UV-C irradiation. Although in both cases the levels of apoptosis increased, confluent cells were still more resistant than proliferating cells (Fig. 1). Importantly, this reduction of apoptosis in confluent cells was observed for low and high doses of UV-C. Cell viability was also investigated seven days after UV-C exposure (Fig. 2). Confirming the results for apoptosis, the confluent growth conditions dramatically increased the level of cell survival. These data indicate that the status of the cell cycle plays an important role in the decision whether a cell dies or lives after DNA insult.

The rate of DNA replication in proliferating and confluent cells was obtained by direct measurement of [H^3]-thymidine incorporation in the genome of cells under both culture conditions. Confluent cells had approximately 10% of the thymidine incorporation of proliferating cells, confirming their low rate of DNA synthesis.

3.2. CPD removal in proliferating and confluent cells

One possible explanation for the difference in cell survival observed for proliferating and confluent cells is their ability to remove DNA lesions by NER. The removal of the major DNA photoproduct, CPD, from the genome, was measured by immunoblot assays. Genomic DNA from normal (FHN), CS-B (CS1AN) and TTD (TTD1VI) cells was extracted immediately and several hours following UV-C irradiation (15 J/m^2), and CPDs were detected using specific monoclonal antibody (TDM-2) (Fig. 3). As expected, TTD cells displayed lower CPD repair levels, when compared to normal and CS-B cells. It is important to recall that this type of assay is not appropriate for distinguishing lesion removal in the transcribed strand by TCR. This explains the similar rates of repair in both normal and CS1AN cells, although this seems to be slightly lower in CS1AN cells. Curiously, all cell strains displayed slightly faster CPD removal under confluent conditions, at lower times following UV-C exposure. However, it is difficult to correlate these results with the higher survival of growth arrested cells, whereas after 24 h the percentage of remaining CPDs in TTD1V1 (∼60%) and CS1AN (∼20%) was similar in both proliferating and confluent cells. On the other hand, only growth arrested normal cells removed almost 100% of CPDs from their genome compared to 80% from proliferating cells.

3.3. Quantification of RNA synthesis

It is well known that UV-C-induced lesions cause transient inhibition of RNA synthesis [7]. The more efficiently the cell removes these lesions from actively transcribed genes, the faster it recovers its RNA synthesis. We were interested in evaluating the relationship between apoptosis and RNA synthesis recovery, as growth arrested cells were shown to be more resistant to UV-C-induced cell death. The RNA synthesis was measured through the incorporation of ^{3}H-uridine in different periods of time following low and high UV-C exposures in normal (FHN), CS-B (CS1AN) and TTD (TTD1VI) cells. The low-dose condition (15 J/m^2) does not induce significant apoptosis in TTD1VI and normal cells, whereas it induces approximately 20% of apoptosis

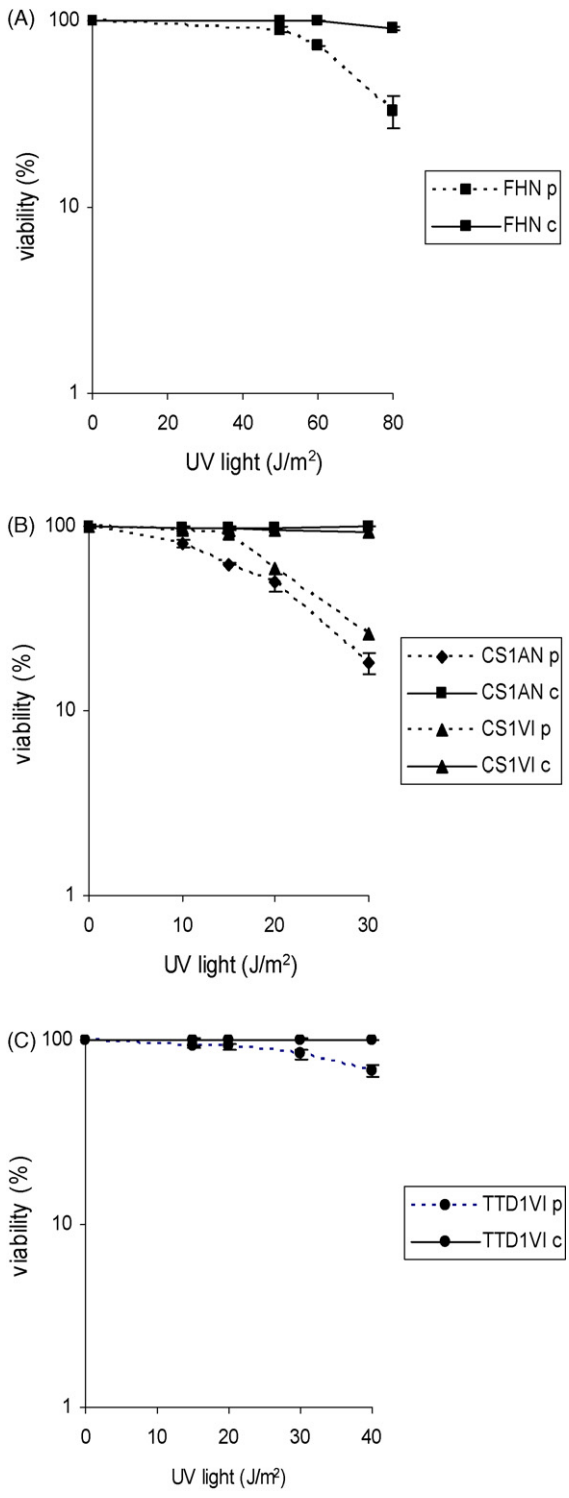

Fig. 2. Higher cell viability of confluent cells to UV-C treatment. Viability was assessed by XTT assay seven days following UV-C exposure in normal (FHN) (A), CS-B (CS1AN and CS1VI) (B) and TTD (TTD1VI) (C) fibroblasts. Each result represents the mean of three duplicated independent experiments. Proliferating (p) and confluent (c) cells were employed, as indicated in the inset.

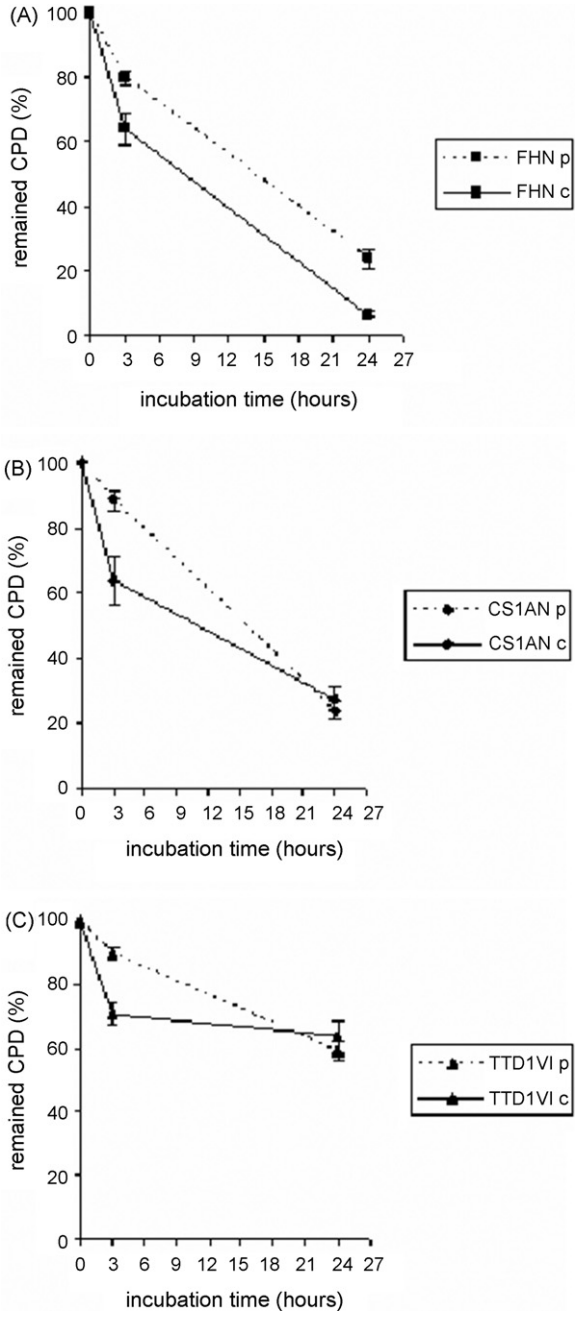

Fig. 3. Kinetics of CPD removal in proliferating and confluent fibroblasts. Cells were harvested at the indicated time after UV-C irradiation (15 J/m^2), genomic DNA was extracted, and CPDs were detected by immuno-slot blot, as described in Material and Methods: normal (FHN) (A), CS-B (CS1AN) (B) and TTD (TTD1VI) (C) fibroblasts. Experimental data are from two independent experiments, each performed in duplicate. Proliferating (p) and confluent (c) cells were employed, as indicated in the inset.

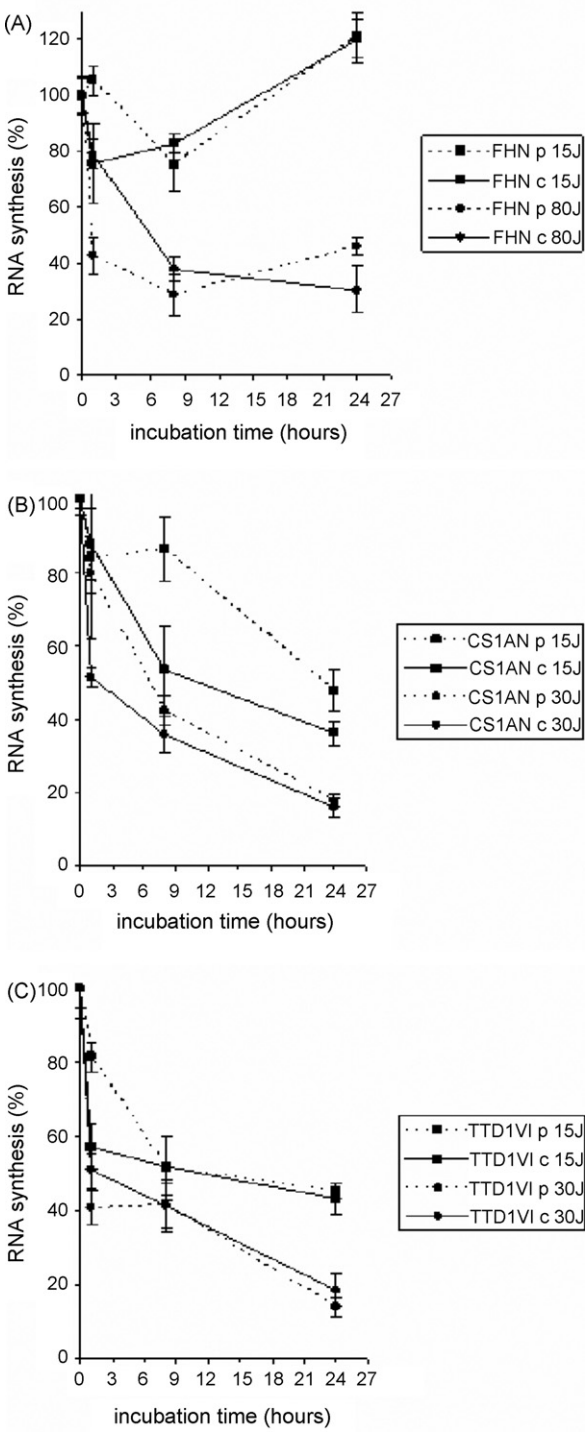

Fig. 4. RNA synthesis recovery following UV-C treatment. Fibroblasts were exposed to different UV-C doses and the RNA synthesis measured at the indicated times after irradiation. (A) Normal (FHN) fibroblasts were exposed to 15 and 80 J/m^2; and (B) CS-B (CS1AN) or (C) TTD (TTD1VI) fibroblasts were exposed to 15 and 30 J/m^2. Each result represents the mean of two duplicated independent experiments. Proliferating (p) and confluent (c) cells were employed, as indicated in the inset.

in CS1AN proliferating cells (Fig. 1). The high-dose employed for normal cells (80 J/m^2) was considered too high for TTD1VI and CS1AN cells, thus a different dose (30 J/m^2) was chosen as the high-dose for these cells, as the reduction of RNA synthesis was roughly similar (∼60%, 9 h after UV-C).

The level of RNA synthesis reduction was dependent both on the UV-C dose employed and on the ability of cells to remove DNA lesions (Fig. 4), confirming that defective TCR cells, TTD1VI and CS1AN, were inefficient to recover the RNA synthesis following low (15 J/m^2) and high (30 J/m^2) UV dose irradiation. Curiously, the RNA transcription inhibition and lack of recovery was similar in TTDIVI and CS1AN, although

TTDIVI showed lower levels of UV-C-induced apoptosis. On the other hand, normal cells efficiently recovered RNA synthesis following 24 h only after a low UV dose (15 J/m^2). Surprisingly, all cell strains did not display any significant difference of UV-C-induced RNA synthesis inhibition or recovery when proliferating or confluent cells were compared. Therefore, these results indicate that the reduction of UV-C-induced apoptosis observed in confluent cells is independent on RNA synthesis recovery.

4. Discussion

Apoptosis induction by UV irradiation has been associated with the persistence of unrepaired DNA lesions. This has been confirmed in NER deficient fibroblasts expressing CPD and 6–4-PP photolyases, where photoremoval of these lesions prevented UV-induced apoptosis [16,17]. However, the mechanisms through which DNA lesions trigger the apoptosis cascade are not completely understood. This work investigated apoptosis induction by UV-C irradiation in normal and TCR-deficient cells (CS-B and TTD), in two different cell culture conditions, proliferating and growth arrested by confluence.

The results indicate a relationship between UV-C-induced apoptosis and DNA replication. Confluent fibroblasts, which have a reduced DNA synthesis rate, were more resistant to UV-C-induced apoptosis than proliferating cells. This protection was also observed in cell survival experiments, indicating that it could not result from a simple delay of cell death. The kinetics of CPD removal for proliferating and confluent cells was also analyzed. Although all lineages displayed a slightly faster removal of CPD under confluent condition, this was not enough to explain the higher survival of growth arrested cells, whereas after 24 h the percentage of remaining CPDs was similar in proliferating and confluent cells. The impact of DNA replication on CPD removal under the analyzed condition must be low, as it has been observed by several authors that high UV-C doses causes a strong blockage of DNA replication [18–20]. Therefore, the effects of DNA lesion dilution by cell division and DNA synthesis should also be low in these cells, and most of CPD reduction in Fig. 3 is due to damage removal by DNA repair.

The link between susceptibility to apoptosis and cell proliferating activity was also observed in DNA repair proficient keratinocytes [10]. Keratinocytes that were under cell cycle arrest or senescence became resistant to UV-C-induced apoptosis. The results presented here extend these observations to TCR defective fibroblasts, which were also less susceptible to apoptosis induction when arrested. Studies using human cells with TCR defects showed higher inability to restore normal transcription following UV irradiation, leading to p53 accumulation and apoptosis induction after low UV dose irradiation [4–6,21–23]. The results linking RNA synthesis inhibition and apoptosis induction favor the hypothesis that transcription acts as a damage dosimeter. However, the data presented here do not support this concept, at least for cells maintained quiescent by confluence. The inhibition of RNA synthesis following UV-C treatment was evaluated in growth arrested cells, and the results clearly indicated that the lower apoptosis was not accompanied by a more efficient RNA synthesis recovery. In fact, the kinetics of RNA synthesis inhibition and recovery were similar in growth arrested and proliferating cells. Moreover, TTD1VI cells, which were more resistant to UV-C-induced apoptosis than CS1AN cells, were similarly unable to recover RNA synthesis. Preliminary studies showed that normal and CS1AN cells also accumulated p53 under non proliferative conditions after UV-C irradiation, this accumulation not being associated with apoptosis induction (data not shown). However, the participation of transcription blockage in UV-C-induced apoptosis, especially in proliferating cells, cannot be discarded, as TCR defective cells were more sensitive to UV-C-induced apoptosis than normal cells.

Recently, further evidence pointing to the involvement of DNA duplication in UV-C-induced apoptosis was reported. The decrease of DNA synthesis by serum starvation treatment leads to a reduction of 50% in UV-C-induced apoptosis for both NER deficient and proficient rodent cells [8]. Moreover, inhibition of DNA replication by aphidicolin, following UV-C treatment, prevented apoptosis induction when compared to proliferating cells [12]. McKay et al. [9] have also observed that apoptosis induction in UV-irradiated fibroblasts only occurred after cells have entered S-phase and replicated their DNA. Working with synchronized hamster cell lines, proficient and deficient in TCR, Proietti De Santis et al. [18] have observed that at high doses of UV-C the cells underwent apoptosis without the entry into S-phase. Although this is in apparently contrast with the role of DNA replication in UV-C-induced apoptosis, the increase of G1-phase cells, observed in that work, could be due to a strong blockage of DNA replication at the beginning of S-phase. This blockage would lead to a decrease on the number of cells in S-phase, but could still be triggering apoptosis. Again, it should be emphasized that the confluent cells were more resistant even when irradiated at low UV-C doses.

The UV-C-induced apoptosis may result from DNA replication machinery blockage by DNA photoproducts, or by secondary lesions caused by this obstacle. It has been proposed that the collapsed replication fork in response to UV photoproducts generates double strand breaks (DSBs) [8], and non repaired DSBs may trigger apoptosis [24]. The ATR kinase, a member of the superfamily of phosphatidylinositol-3-kinase-related kinase (PIKK), appears to be the major activator of the replication stress response [25]. This kinase plays an essential role during normal replication [26] and is required for cellular checkpoint responses to stalled replication forks [27]. When activated, this kinase displays a series of signals which control cell cycle transition, DNA repair activity and, in some cases, the induction of apoptosis [28]. Therefore, it is possible that DNA replication of a damaged template would first activate ATR, and subsequent cell events would lead to an active program of cell death. In confluent cells, the lower levels of DNA synthesis should reduce ATR activation, thus increasing cell resistance to UV-C irradiation. Another possibility is that the apoptotic signals depend on both DNA replication and RNA synthesis, consistent with the model proposed by the Ljungman group [7,9], where UV-induced apoptosis results from the collision of transcription blocked complexes with oncoming replication forks. This model fits in with the observations of this work, as the levels of DNA

replication in confluent cells are low, and there would be lower levels of collisions, even though RNA synthesis is still highly inhibited in the growth arrested conditions.

5. Conclusions

These results demonstrate that normal or TCR-deficient fibroblasts are more resistant to UV-C-light when kept in a growth arrested state, a condition which did not significantly affect DNA damage removal, or RNA synthesis recovery. This strongly supports the idea that DNA synthesis of damaged template is necessary for apoptosis induction by UV-C light. Considering that most of the cells in the skin are normally quiescent, and not replicating their DNA, the results observed for confluent cells may correlate as to how cells respond to the UV component (especially UV-B) of sunlight *in vivo*. Although this work was performed in fibroblasts, it is possible that other cell types (such as keratinocytes) may present similar behavior, and thus the differences in apoptosis induction observed in growth arrested cells may have direct implications with photocarcinogenesis. A more extensive work on how growth arrested cells deal with DNA damages is necessary for the understanding of genetic instability and apoptosis mechanisms.

Acknowledgments

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