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The role of nucleotide excision repair in protecting embryonic stem cells from genotoxic effects of UV-induced DNA damage

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ABSTRACT

In this study the role of nucleotide excision repair (NER) in protecting mouse embryonic stem (ES) cells against the genotoxic effects of UV-photolesions was analysed. Repair of cyclobutane pyrimidine dimers (CPD) in transcribed genes could not be detected whereas the removal of (6–4) photoproducts (6–4PP) was incomplete, already reaching its maximum (30%) 4 h after irradiation. Measurements of repair replication revealed a saturation of NER activity at UV doses >5 J/m^2 while at a lower dose (2.5 J/m^2) the repair kinetics were similar to those in murine embryonic fibroblasts (MEFs). Cytotoxic and mutagenic effects of photolesions were determined in ES cells differing in NER activity. ERCC1-deficient ES cells were hypermutable (10-fold) compared to wild-type cells, indicating that at physiologically relevant doses ES cells efficiently remove photolesions. The effect of the NER deficiency on cytoxicity was only 2-fold. Exposure to high UV doses (10 J/m^2) resulted in a rapid and massive induction of apoptosis. Possibly, to avoid the accumulation of mutated cells, ES cells rely on the induction of a strong apoptotic response with a simultaneous shutting down of NER activity.

INTRODUCTION

Mammalian embryonic stem (ES) cells are rapidly dividing cells of the inner cell mass of embryos at the blastocyst stage of development. ES cells are pluripotent and will differentiate into the various different cell types of the embryo proper. This characteristic pluripotency of mouse ES cells has been exploited to generate genetically altered mice (1,2).

It has been estimated that the inner cell mass of the mouse blastocyst contains between 20 and 40 cells (3). The small size of the population of cells that are at the basis of early embryonal development implies that ES cells should be equipped with efficient cellular defence mechanisms to cope with DNA damage, to avoid the production of mutated daughter cells that will have detrimental effects on embryogenesis. Such cellular defence systems include the removal of DNA damage, either endogenous or exogenous in source, by DNA repair mechanisms and the elimination of damaged ES cells via apoptosis (4). The latter process has been shown to be efficiently induced in ES cells within 9 h after UV-C exposure (5,6).

The nucleotide excision repair (NER) pathway is an important DNA repair pathway involved in the removal of a wide variety of DNA lesions, including photoproducts induced by ultraviolet (UV) light and chemically-induced bulky lesions. Mammalian NER has been studied extensively in rodent and human diploid fibroblasts as well as established cell lines. These studies revealed that mammalian NER can be subdivided into two subpathways. The transcription coupled repair (TCR) pathway removes DNA damage preferentially from the transcribed strands of active genes, while the global genome repair (GGR) pathway removes DNA damage indiscriminate of the transcriptional status of the DNA (7).

Despite vast knowledge of NER in somatic cells, little is known of NER in cells of early embryos. Measurements of unscheduled DNA synthesis (UDS) which is indicative of repair of DNA lesions have been performed on pre-implantation stage embryos such as morulae and blastocysts, showing that cells of the inner cell mass perform little UV-induced UDS in comparison to trophoblast cells or morula nuclei (8). Embryonic carcinoma (EC) cells resemble ES cells in many characteristics, such as the ability to differentiate in various cell types. Whereas undifferentiated EC cells were proficient in repair of UV-induced photolesions, the capacity to remove photolesions was significantly reduced after *in vitro* differentiation (9). It is unclear, however, whether NER activity in tumour-derived EC cells is representative for normal embryonic tissue.

The present study describes the analysis of NER activity in ES cells and the role of NER in protecting ES cells against genotoxic effects induced by DNA damaging agents using culture conditions that prevent differentiation of ES cells. Although a recent study has shown that undifferentiated ES cells perform

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UV-induced repair synthesis in the active dihydrofolate reductase (*Dhfr*) gene via TCR, it is unclear whether this synthesis is the result of the removal of cyclobutane pyrimidine dimers (CPD) and/or the removal of (6–4) photoproducts (6–4PP) (10).

Here, we determined the removal of CPD and 6–4PP from the transcriptionally active genes encoding *p53* and *Hprt* and the inactive *c-mos* gene. Moreover, UV-induced repair synthesis was measured in the genome overall. The contribution of NER in protecting undifferentiated ES cells against the cytotoxic and mutagenic effects of UV-C light was studied using wild-type ES cells and ES cells lacking the ERCC1 protein, which is required for the 5'-incision step in the NER pathway (11). In addition, induction of apoptosis was determined in ES cells exposed to UV-C light.

MATERIALS AND METHODS

Cell lines and cell culture

The E14 (129/Ola) derived ES cell lines IB10.51 and ERCC1$^{-/-}$ were cultured on a feeder cell layer of lethally X-ray irradiated (40 Gy) mouse embryonic fibroblasts (MEFs) in DMEM high glucose (Gibco BRL) supplemented with 10% ES-qualified foetal bovine serum (FBS), 0.1 M non-essential amino acids (MEM), 50 μM 2-mercaptoethanol, 500 U LIF/ml (ESGRO), 50 IU/ml penicillin, 50 μg/ml streptomycin (all obtained from Life Technologies, Breda, The Netherlands) and 3.33 μM nucleosides (Sigma, St Louis, MO) hereafter referred to as ES complete medium (12). When plated on 0.1% gelatin (Sigma, St Louis, MO) coated culture dishes, ES complete medium without nucleosides was made up with 50% BRL conditioned medium (DMEM high glucose with 10% FBS) (13), hereafter referred to as BRL-complete medium. MEFs were isolated from embryos at mid-gestation (14.5 d.p.c.) essentially as described (14) and cultured in DMEM high glucose supplemented with 10% ES-qualified FBS and 50 IU/ml penicillin, 50 μg/ml streptomycin hereafter referred to as MEF culture medium.

Analysis of CPD removal from defined sequences

The removal of CPD from the DNA was analysed as described by Bohr *et al.* (15) with slight modifications. Cells were plated at a density of 3×10^6 cells per 100 mm dish on culture dishes coated with gelatin and incubated in BRL complete medium, 16–20 h prior to UVC irradiation with a Philips T.U.V. lamp at a dose rate of 0.275 J/m^2/s. Following exposure to UV-C, it was observed that at the latest time points few cells remained attached to the culture dishes. For these time points the number of dishes plated with irradiated cells was increased 3-fold. Before irradiation, medium was removed and the cells were washed once with phosphate-buffered saline (PBS). After irradiation, cells were either immediately lysed in NET buffer (150 mM NaCl, 10 mM EDTA and 10 mM Tris–HCl pH 8.0) supplemented with 0.5% sodium dodecyl sulfate (SDS) and 100 μg/ml proteinase K or incubated for up to 24 h in BRL complete medium to allow repair of photolesions. Pilot experiments had shown that the proportion of ES cells in the population that undergo cell division after a dose of 20 J/m^2 UV-C light was very small (<10%). Therefore, CsCl density gradient centrifugation to separate parental from newly synthesised DNA was not required (16). After lysis of the cells, DNA was isolated and purified by phenol–chloroform extraction and ethanol precipitation before digestion with *Bam*HI or *Eco*RI. Equal portions of DNA were treated or mock-treated with T4 endonuclease V and separated by alkaline gel electrophoresis (15,16). The DNA was transferred to Hybond-N$^+$ (Amersham, Roosendaal, The Netherlands) and resulting blots were hybridised with strand-specific DNA probes for the *Hprt*, *p53* and *c-mos* genes as previously described (17,18). Quantification of the radioactive signal in full-size restriction fragments was performed by scanning of the filters using the InstantImager™ (Packard Instrument Company, Meriden, CT). The frequency of CPD in various restriction fragments was calculated by comparing the relative band intensities in lanes containing DNA treated with T4 endonuclease V or mock-treated, assuming a Poisson distribution of lesions.

Analysis of 6–4PP removal from defined sequences

Prior to analysis of the frequencies of 6–4PP in the *p53* gene, CPD were removed by treatment of the DNA with photolyase in the presence of photoreactivating light (425 nm) (19). The 6–4PPs were subsequently detected by incubating equal portions of *Eco*RI digested DNA with or without UvrABC complex (20). Alkaline electrophoresis, blotting and hybridisation with *p53* strand-specific probes was performed as described above for the analysis of CPD removal.

Measurements of DNA repair replication

DNA repair replication assays were performed with ES cells and MEFs essentially as described by Van Zeeland *et al.* (21). Briefly, ES cells were grown at a density of 3×10^6 cells per 100 mm on gelatin-coated culture dishes in BRL complete medium containing 0.3 μCi/ml ^{32}P-orthophosphate. For each UV dose, three dishes were used. After 24 h, medium was changed to ES complete medium without nucleosides or label to allow optimal incorporation of thymidine analogues. One hour before irradiation, 10 μM 5-bromodeoxyuridine (BrdU) and 1 μM fluorodeoxyuridine (FdU) were added to this medium. After irradiation with UV-C light at a dose rate of 0.275 J/m^2/s, the cells were incubated for 5 h in ES complete medium without nucleosides containing 10 μCi/ml [^{3}H]thymidine (82 Ci/mmol), 10 μM BrdU and 1 μM FdU.

MEFs were incubated at a density of 3×10^6 cells per 100 mm dish in MEF culture medium supplemented with 0.3 μCi/ml ^{32}P-orthophosphate. For each UV dose, three dishes were used. After 65 h, medium was changed to medium without label for another 24 h incubation. One hour before irradiation, 10 μM BrdU and 1 μM FdU were added to this medium. After irradiation with UV-C light, the cells were incubated for 5 h in MEF culture medium containing 10 μCi/ml [^{3}H]thymidine (82 Ci/mmol), 10 μM BrdU and 1 μM FdU.

ES cells, as well as MEFs cells, were lysed in NET buffer supplemented with 0.5% SDS and 100 μg/ml proteinase K and incubated at 37°C for 16 h. Genomic DNA was phenol-extracted, ethanol precipitated and sheared. Two rounds of neutral CsCl gradient centrifugation were performed to separate replicated DNA from parental DNA. Four or five fractions containing most of the parental DNA were pooled and alkaline CsCl gradient centrifugation was performed to remove remaining traces of replicated DNA. Fractions were collected, DNA was precipitated with trichloroacetic acid, and ^{3}H and ^{32}P counts were measured in each fraction by scintillation counting. The

specific activity of the ^{32}P-labelled DNA (^{32}P c.p.m./μg DNA) was determined from parallel cell cultures that were not UV-irradiated. Repair replication was expressed as ^{3}H c.p.m./μg of DNA.

Analysis of UV-induced cytotoxicity and induction of mutations at the *Hprt* gene

ES cells were seeded at a density of 5×10^6 cells per 100 mm gelatin-coated culture dish and incubated in BRL complete medium 16 h before UV-C irradiation. The cells were rinsed once with PBS and irradiated at a dose rate of 0.075 J/m^2/s using a Philips T.U.V. lamp. After UV-C irradiation, cells were trypsinised and seeded on gelatin-coated dishes in BRL complete medium without LIF at a density of 500–1000 cells per 100 mm dish (five dishes per dose) to determine cell survival. Mass cultures of UV-irradiated cells were propagated for 6 days on MEF feeder layers in ES complete medium at cell densities of 3–4 $\times$ 10^6 cells per 100 mm dish. Cells were passaged every 2 days and at least 9×10^6 cells were plated per dose after each passage. After the 6-day expression period, 1×10^6 cells per dose were plated for selection with 2.5 μg/ml 6-thioguanine (Sigma, St Louis, MO) in BRL complete medium without LIF at a density of 2×10^5 per 100 mm dish. Additionally, the cloning efficiency was determined by seeding 500 cells per dish (five dishes per dose) in medium without 6-thioguanine. Colonies were fixed, stained and counted 6–7 days after seeding of the cells.

Analysis of ploidy of cells exposed to UV-C light

ES cells were exposed to UV-C light and seeded as in the mutation induction experiments described above. Six days after exposure, cells were trypsinised, pelleted and subsequently fixed using ice-cold 0.75 M KCl. Metaphase spreads were prepared, stained with Giemsa and chromosomes were counted (3).

Analysis of UV-induced apoptosis

Cells were plated on gelatin-coated culture dishes and incubated as described for repair experiments in BRL complete medium. At 8, 12 and 24 h after UV-C irradiation, medium was collected from the plates and detached cells were pelleted by centrifugation (1000 r.p.m.) before lysis. Cells still attached to the culture dish were also lysed. After digestion with proteinase K, DNA from both lysates was purified by phenol–chloroform extraction followed by ethanol precipitation. After treatment with RNase (100 μg/ml) in TE for 1 h at 37°C, DNA was loaded on a 1.5% neutral agarose gel containing ethidium bromide. Electrophoresis was performed for 4 h at 60 V. The size of DNA fragments was estimated using a size marker generated by digestion of pUC13 DNA with *Hpa*II.

RESULTS

Induction of UV-C-induced photolesions in DNA of ES cells

The frequency of the two major UV-C-induced photoproducts in ES cells was determined in the *p53* gene as a function of the dose and compared to the frequency obtained at similar doses in V79 Chinese hamster cells. The induction of both CPD and 6–4PP in ES cells was consistently 2-fold lower than observed in V79 cells (results not shown). To obtain a lesion frequency of one CPD or 6–4PP/15 kb, ES cells had to be exposed to 20 and 60 J/m^2 UV-C irradiation, respectively, hereafter referred to as effective doses of 10 and 30 J/m^2.

Analysis of gene-specific removal of UV-C-induced photolesions

To determine NER activity in ES cells, removal of CPD in specific genes of ES cells exposed to an effective dose of 10 J/m^2 of UV-C was studied using T4 endonuclease V digestion and alkaline Southern blotting (15,16). Removal of CPD in time from the transcribed strand and non-transcribed strand of the active *p53* and *Hprt* genes was measured in 16 kb *Eco*RI and 11 kb *Bam*HI fragments respectively, using strand-specific probes. Removal of CPD from the inactive *c-mos* gene was measured in a 22 kb *Bam*HI fragment. Within a 24-h repair period no removal of CPD could be observed from the transcribed strand of either of the active genes. CPD were also not removed from the non-transcribed strand of the active genes or from the inactive *c-mos* gene (Fig. 1A; Table 1).

Since NER has been shown to act much more efficiently on 6–4PP than on CPD (22), the removal of 6–4PP was also measured. ES cells were exposed to an effective dose of 30 J/m^2 and allowed to repair photolesions for up to 12 h. After removal of CPD from the DNA by photoreactivation, the remaining 6–4PP were detected using UvrABC excinuclease digestion and alkaline Southern blotting (19,20). Using probes that recognised either the transcribed or the non-transcribed strand of the *p53* gene, it was observed that for both strands the removal of 6–4PP reached its maximum (30%) within a 4-h repair period (Fig. 1B; Table 1). No further increase in 6–4PP repair could be detected between 4 and 12 h after UV-C exposure. Taken together these results suggest that ES cells are proficient in NER although NER activity seemed to be inhibited shortly after UV-C treatment.

Repair replication in ES cells and MEFs

To determine whether the level of repair was affected by dose, we measured UV-C-induced repair synthesis in the genome-overall using the repair replication assay (21). In contrast to gene-specific repair measurements that require lesion frequencies of one CPD per 15 kb, i.e. 10 J/m^2, repair replication measurements can be performed at effective doses as low as 2.5 J/m^2. In these experiments, MEFs were included as a positive control, since these cells have been shown to remove UV-C-induced photolesions as efficiently as established rodent cell lines (19,23). The level of repair synthesis in MEFs showed a linear dose-relationship up to a dose of 15 J/m^2 which corresponds to a lesion frequency of one CPD per 10 kb. At higher doses some saturation of repair synthesis was observed. In contrast to MEFs, ES cells showed saturation of repair synthesis already at effective doses higher than 5 J/m^2 of UV-C. Only at the lowest dose tested was the level of repair synthesis in ES cells similar to that in MEFs (Fig. 2).

UV-induced cell killing and mutation induction in ES cells differing in NER activity

To extend the finding that ES cells exposed to low levels of UV-C light were capable of removing photolesions, the role of NER in protecting ES cells against cytotoxic and mutagenic effects of UV-C light was examined. In this study, ERCC1-deficient ES cells, which are unable to perform the 5'-incision

Table 1. Repair of CPD and 6–4PP in different genomic regions at various time points after UV-C irradiation

Repair of CPD						Repair of 6–4PP		
Time (h)	*Hprt* TS	*Hprt* NTS	*p53* TS	*p53* NTS	*c-mos*	Time (h)	*p53* TS	*p53* NTS
2	–0.5 ± 3.1	–0.9 ± 11.1	1.7 ± 3.2	13.5 ± 4.2	14.5 ± 8.7	2	22.4 ± 14.6	37.3 ± 7.2
4	9.6 ± 2.6	3.4 ± 10.9	6.4 ± 9.5	6.6 ± 5.8	11.8 ± 10.8	4	31.5 ± 2.2	27.4 ± 0.5
8	0.5 ± 8.4	–4.6 ± 3.8	11.5 ± 6.0	11.5 ± 5.3	9.7 ± 11.3	8	9.1 ± 2.3	12.9 ± 7.1
24	–24.2 ± 4.4	–6.6 ± 2.1	10.3 ± 16.8	1.7 ± 23.6	0.8 ± 14.5	12	10.8 ± 12.7	26.6 ± 5.1

Effective doses of 10 and 30 J/m^2 were used in studies of CPD and 6–4PP removal respectively. Percentage removal and standard error of the mean are given. TS, transcribed strand; NTS, non-transcribed strand.

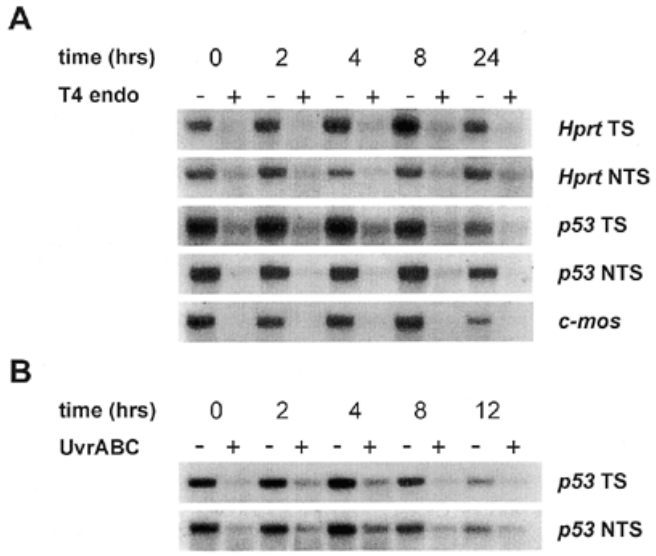

Figure 1. (**A**) Representative autoradiograms showing removal of CPD after an effective UV dose of 10 J/m^2 from the transcribed strand (TS) and non-transcribed strand (NTS) of the active *p53* and *Hprt* genes and the inactive *c-mos* gene. (**B**) Representative autoradiograms showing strand-specific removal of 6–4PP from the active *p53* gene after exposure to an effective UV dose of 30 J/m^2.

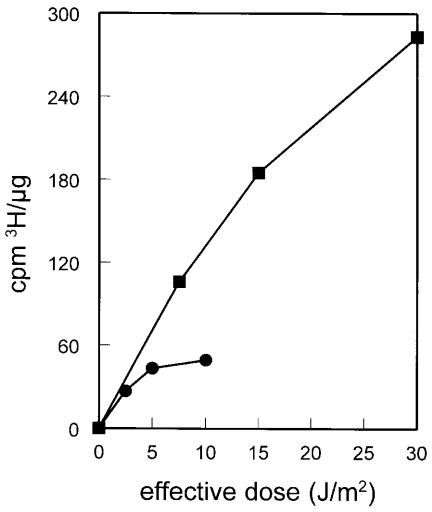

Figure 2. DNA repair replication in ES cells (circle) and MEFs (square) after irradiation with different UV-C doses. Representative experiments are shown.

step during NER, were used (11). Previous reports have shown that ERCC1-deficient mammalian cells are also disturbed in recombinational processes resulting in sensitivity to crosslinking agents such as Mitomycin C (24,25). Wild-type ES cells and ERCC1$^{-/-}$ ES cells were exposed to effective doses up to 2 J/m^2 UV-C light and assayed for cell survival and mutation induction. For ERCC1$^{-/-}$ ES cells the effective dose that results in 37% survival was 2-fold lower than for wild-type ES cells, clearly indicating that NER has a protective effect on UV-C-induced cytotoxicity (Fig. 3A). The frequencies of UV-C-induced mutants were measured at the X-chromosomal *Hprt* locus which is hemizygous in diploid male ES cells, therefore requiring only one mutational event to become functionally *Hprt* deficient. The mutation induction in ERCC1$^{-/-}$ ES cells was only 2–3-fold increased compared to wild-type ES cells. Analysis of the ploidy of untreated ERCC1$^{-/-}$ ES cells revealed however that ~30% of the cells in the population were tetraploid. This number increased up to 76% after exposure to an effective dose of 1 J/m^2 UV-C light while <2% tetraploid cells was observed either in untreated or UV-C-exposed wild-type ES cell populations. Since tetraploid ES cells contain two functional *Hprt* genes these cells will hardly contribute to the recovery of *Hprt* mutants. For direct comparison to wild-type ES cells, the mutation frequencies in ERCC1$^{-/-}$ ES cells were corrected for the number of diploid cells in the surviving population. Following this correction, a dramatic increase in *Hprt* mutant frequency was observed in the NER-deficient ERCC1$^{-/-}$ ES cells compared to the wild-type cells (Fig. 3B). For comparison, data from the literature were included on cell killing and mutation induction in repair proficient (CHO9) and ERCC1-deficient (43–3B) Chinese hamster cells (26,27). In conclusion, these data indicate that NER plays an important role in the removal of photolesions from the DNA of ES cells when these cells are exposed to relatively low doses of UV-C light.

Measurements of apoptosis induced by UV-C light

In the course of the gene-specific repair experiments it was observed that ES cells started to detach from the culture dish

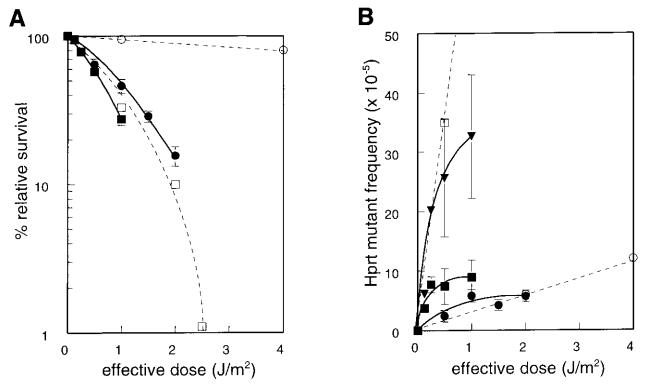

Figure 3. UV survival (**A**) and mutation induction at the *Hprt* locus (**B**) in wild-type ES cells (closed circle), ERCC1-deficient ES cells (closed square) and ERCC1-deficient ES cells that were corrected for ploidy (triangle). Each point represents the mean of at least three experiments. UV survival and mutation induction curves in CHO9 (open circle) and 43–3B (open square) cells have been published previously (27).

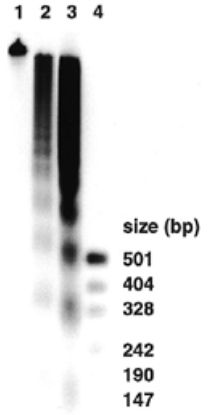

Figure 4. Detection of UV-induced nucleosomal degradation in DNA isolated from cells after irradiation with an effective dose of 10 J/m^2. Lane 1, DNA from cells that remained attached to the culture dishes 24 h after irradiation. Lanes 2 and 3, DNA from detached ES cells isolated from the medium at 8 and 24 h after irradiation respectively. The genomic DNA was separated on a 1.5% agarose gel and stained with ethidium bromide. Lane 4, the size-marker used is pUC13 digested with *Hpa*II.

only 8 h after irradiation with an effective dose of 10 J/m^2 UV-C light. To determine if this observed cell death resulted from UV-C-induced apoptosis, DNA was isolated from both attached and detached cells at 8 or 24 h after UV irradiation. Neutral agarose gel analysis of DNA isolated from the attached cells (~10% of the total cell population) revealed that this DNA was still intact after 24 h. DNA isolated from detached cells showed, however, the nucleosomal laddering pattern characteristic of apoptosis indicating that ES cells indeed undergo rapid and massive apoptosis after exposure to UV-C light (Fig. 4).

DISCUSSION

The small number of stem cells and their rapid cell division inherently sensitises the early developing embryo to the detrimental consequences of DNA damage. To avoid the induction of mutations and their transmittance to daughter cells, ES cells should be equipped with efficient cellular defence mechanisms. In this study, we have analysed the capacity of mouse ES cells to remove bulky lesions, such as UV-C-induced photoproducts, from their DNA via the NER pathway. Furthermore, the role of NER and apoptosis in protecting early embryonic cells from the cytotoxic and mutagenic effects of UV-C light was investigated.

The capacity of ES cells to remove UV-induced photolesions by NER was investigated in transcriptionally active and inactive genes. Removal of CPD could not be detected in either class of genes even 24 h after UV exposure. The repair of 6–4PP measured in both strands of the active *p53* gene was incomplete and reached its maximum (30%) only 4 h after UV irradiation. The level of repair observed in ES cells sharply contrasted to the NER activity measured using the same assays in established rodent cell lines and MEFs (19,23). The latter cells efficiently removed up to 80% of the CPD from actively transcribed DNA within 24 h after UV exposure whereas CPD were not removed from non-transcribed DNA. The removal of 6–4PP was more rapid than CPD and showed no influence of the transcriptional activity of the gene, resulting in the removal of 40–70% of the 6–4PP within 12 h after UV-C exposure (23). Therefore, the question remains as to what causes the low NER activity in ES cells observed in these gene-specific repair experiments?

To answer this question, we first determined the dose–response relationship of photolesion-repair in ES cells and MEFs using repair replication assays. In contrast to gene-specific repair experiments that require relatively high doses (10–30 J/m^2) of UV-C light, repair replication assays allow measurements of repair synthesis at effective doses as low as 2.5 J/m^2. MEFs showed a linear dose-relationship up to 15 J/m^2 similar to established rodent cell lines (28). In ES cells, repair synthesis was saturated after exposure to effective doses >5 J/m^2. However, at the lowest dose tested (2.5 J/m^2) the levels of repair replication in ES cells and MEFs were similar. The saturation of repair in ES cells with increasing dose might explain why only a fraction of the induced 6–4PP were removed 12 h after exposure to an effective dose of 30 J/m^2. The notion that ES cells efficiently repair photolesions induced at low UV doses is supported by a recent report (10) which shows that ES cells actively repair photolesions in the transcribed strand of the *Dhfr* gene after exposure to an effective dose of 5 J/m^2.

Additional evidence for efficient removal of photolesions in ES cells, induced at low UV doses, comes from the analysis of *Hprt* mutation frequencies in wild-type and NER-deficient ES cells. ERCC1-deficient ES cells that, apart from having a NER-defect are also defective in recombinational pathways (25,29), exhibited a 10-fold higher UV-induced mutation induction compared to wild-type ES cells. A similar hypermutability has been reported for the ERCC1-deficient rodent cell line 43–3B (26,27). Moreover, the mutability of wild-type ES cells is similar to that of NER proficient hamster cell lines following exposure to low doses of UV-C (26,27).

In contrast to UV-induced mutagenesis, ES cells differed markedly from established rodent cells with respect to UV-induced cytotoxicity, i.e. wild-type ES cells were much more

sensitive to the toxic effects of UV-C light compared to NER-proficient established rodent cell lines when exposed to a similar effective dose. The latter cells did not show any reduction in cell survival up to an effective dose of 2 J/m^2 (26,27) while only 20% of the ES cells survived at this dose.

The impact of a NER defect on UV-induced cytotoxicity is more pronounced (12-fold) in established hamster cell lines (43–3B versus CHO9) than in ES cells, which showed only a 2-fold reduction in cell survival after UV exposure (ERCC1$^{-/-}$ versus wild-type cells). This difference in UV sensitivity probably stems from differences between established cell lines and ES cells in their apoptotic response after exposure to UV-C light. ES cells were shown to rapidly undergo apoptosis after the induction of DNA damage (5,6,30). In this study, only 8 h after irradiation, nucleosomal degradation of DNA, a late step in the apoptotic process, was observed. Such rapid and extensive induction of apoptosis has not been observed in CHO cells after exposure to UV-C light (31), possibly as a result of a mutation in the *p53* gene in these cells (32,33).

Interestingly, a massive induction of apoptosis after UV-C exposure has been reported for mouse erythroid leukemia (MEL) cells and mouse GRSL 13-2 cells. These cells, like ES cells, also have (i) a low clonal survival, (ii) a normal mutability and (iii) a similar saturation of repair replication after UV-exposure (34). It has been proposed that the repair of photolesions was masked by the large fraction of cells undergoing apoptosis within the first few hours after UV exposure. MEL and GRSL cells that survived the treatment for 24 h and did not show DNA fragmentation efficiently removed photolesions from their DNA, as measured by gene-specific repair experiments (34). Thus, the induction of apoptosis and the poor removal of photolesions appear to be correlated suggesting an interfering role of apoptosis with NER in these cell types. This interference may be caused by double-strand breaks induced during nucleosomal degradation of the DNA which might directly inhibit NER. It has, for instance, been shown that DNA end-binding proteins inhibit the interaction of repair proteins with double-strand breaks *in vitro* (35).

In intact cells, inhibition of NER has also been reported to result from the induction of single-strand breaks that may lead to a loss of supercoiling (36). However, in ES cells, the inhibition of NER was complete 4 h after irradiation, while the nucleosomal degradation did not become apparent until 8 h after irradiation. NER was also severely inhibited in the subpopulation of ES cells that remained attached to the culture dish 24 h after UV-irradiation. These observations suggest that the inhibition of NER in ES may be mediated by cellular factors produced during the early stages in the process of apoptosis. The nature of these cellular factors remains to be determined. Several studies suggest that different signal transduction pathways operate in the process of DNA damage-induced apoptosis in ES cells (5,6,30). The induction of apoptosis in ES cells after UV-irradiation is probably mainly mediated by the p53 protein which is highly expressed in proliferating undifferentiated ES cells compared to differentiated ES cells and MEFs (5,30) and is induced upon exposure to UV-C (6). Accordingly, a significant reduction in the UV-induced apoptotic response has been observed in p53-deficient ES cells (5,6). The residual apoptotic response detected in these cells indicates that also p53-independent apoptotic pathways function in ES cells after UV-exposure (6). In addition, it should be noted that, in contrast to UV-irradiation, exposure to *N*-ethyl-*N*-nitrosourea (ENU) or X-rays did not elicit such a strong cytotoxic response in ES cells compared to established cell lines (unpublished observations; 37), suggesting that the induction of apoptosis in ES cells also depends on the type of DNA damaging agent.

In conclusion, the results obtained in this and other studies indicate that for protection against the deleterious effects of UV-induced DNA damage, early embryonal cells not only rely on processes that efficiently remove lesions from their DNA. In addition, the induction of apoptosis in cells with remaining lesions is very efficient and functions to avoid the expansion of mutated cells within the embryo. It will be interesting to further unravel the mechanisms by which ES cells limit the number of mutations induced by various DNA damaging agents and to gain insight in the mutational risks for embryos at the earliest stages of development. Somatic stem cells, like ES cells, are undifferentiated, proliferating cells that undergo apoptosis in response to DNA damage (38–40). Mutations in somatic stem cells with the potential to divide and self-renew during the whole life span of the organism could result in the initiation of tumour formation. Therefore, studies of ES cells may provide insight in the mechanisms employed by somatic stem cells to avoid the induction of mutations.

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