Detection of an Involvement of the Human Mismatch Repair Genes hMLH1 and hMSH2 in Nucleotide Excision Repair Is Dependent on UVC Fluence to Cells

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ABSTRACT

There is conflicting evidence for the role of the mismatch repair (MMR) genes hMLH1 and hMSH2 in nucleotide excision repair. In the present work, we have examined the role of these MMR genes in nucleotide excision repair using two reporter gene assays. AdHCMVlacZ is a replication-deficient recombinant adenovirus that expresses the β-galactosidase reporter gene under the control of the human cytomegalovirus immediate early promoter. We have reported previously a reduced host cell reactivation (HCR) for β-galactosidase expression of UVC-irradiated AdHCMVlacZ in TCR-deficient Cockayne syndrome (CS) fibroblasts compared with normal human fibroblasts, indicating that HCR depends, in at least part, on TCR. In addition, we have reported that UVC-enhanced expression of the undamaged reporter gene is induced at lower UV fluences to cells and at higher levels after low UV fluences in TCR-deficient compared with normal human fibroblasts, suggesting that persistent damage in active genes triggers increased activity from the human cytomegalovirus-driven reporter construct. We have examined HCR and UV-enhanced expression of the reporter gene in hMLH1-deficient HCT116 human colon adenocarcinoma cells and HCT116-chr3 cells (the MMR-proficient counterpart of HCT116) as well as hMSH2-deficient LoVo human colon adenocarcinoma cells and their hMSH2-proficient counterpart SW480 cells. We show a greater UV-enhanced expression of the undamaged reporter gene after low UV exposure in HCT116 compared with HCT116-chr3 cells and in LoVo compared with SW480 cells. We show also a reduced HCR in HCT116 compared with HCT116-chr3 cells and in LoVo compared with SW480 cells. However, the reduction in HCR was less or absent when cells were pretreated with UVC. These results suggest that detection of an involvement of hMLH1 and hMSH2 in TCR is dependent on UV (254 nm) fluence to cells.

INTRODUCTION

Nucleotide excision repair (NER) is a highly conserved DNA repair pathway that removes lesions from the genome induced by UV exposure, such as cyclobutane pyrimidine dimers (CPDs) and 6–4 photoproducts. Its clinical importance is especially evident in human skin cells, which are exposed to the omnipresence of UV radiation in the environment, potentially damaging the DNA in these cells. Additional elucidation that NER of CPDs occurs at different rates throughout the genome has revealed two related but distinct subpathways: transcription-coupled repair (TCR), which is characterized by the rapid repair of transcribed strands of active genes, and global genome repair (GGR), which involves slower removal of UV-induced lesions in the nontranscribed strand of active genes as well as transcriptionally inactive regions of the genome (genomic heterogeneity of NER reviewed in Ref. 1).

It has been reported also that the significance of TCR for the removal of UVC-induced DNA lesions depends not only on the type of lesion but also on the UVC exposure used. After a UVC exposure of 10 J/m², repair of CPDs in normal human fibroblasts is accelerated in the transcribed compared with the nontranscribed strand of active genes. In contrast, normal fibroblasts exposed to 30 J/m² lack strand-specific repair of both 6–4 photoproducts and CPDs, suggesting that TCR is overruled by GGR at this higher dose (2). In addition, Li and Ho (3) reported that normal human fibroblasts respond differently to low compared with high exposures of UVB irradiation. Pretreatment of human fibroblasts with 50 J/m² of UVB resulted in enhanced NER of a UVC-damaged reporter gene, whereas pretreatment with higher exposures of 100 and 200 J/m² did not result in enhanced NER. Taken together, these studies suggest that the relative contribution of TCR to NER of UVC-induced DNA lesions is dependent on the magnitude of the UV exposure used.

Due to the complex nature of both GGR and TCR in mammalian cells, examination of the role of TCR and GGR can be a difficult one. Because many experiments investigating cellular repair involve damaging the cell in some manner, it is difficult to ensure that cellular DNA is the only target because other cellular components such as membranes and signal transduction pathways may also be affected by UV irradiation. To address this problem, we have used two reporter gene assays involving the recombinant adenovirus vector AdHCMVlacZ, which is a nonreplicating virus that expresses the β-galactosidase (β-gal) reporter gene under the control of the human cytomegalovirus (HCMV) immediate early promoter (4). The first assay is a host cell reactivation (HCR) assay, in which UV irradiation of the virus express-produces lesions in the viral DNA similar to that produced in mammalian cells. The irradiated adenovirus construct is then used to infect the cell line of interest. Because proper β-gal expression in mammalian cells is expected to occur only from transcription of a lesion-free lacZ gene, HCR of the reporter gene is thought to be reflective of the NER capacity of the infected cell. We have reported previously a reduced HCR for β-gal expression of UVC-irradiated AdHCMVlacZ in TCR-deficient Cockayne syndrome (CS) cells compared with normal human fibroblasts, indicating that HCR depends, in part at least, on TCR (5). The second reporter gene assay involves infecting UV-pretreated cells with the undamaged AdHCMVlacZ vector. We have reported previously that UV-enhanced expression of the undamaged reporter gene is induced at lower UV fluences to cells and at higher levels for low UV fluence in TCR-deficient fibroblasts, but not in GGR-deficient fibroblasts, compared with normal human fibroblasts, suggesting that persistent damage in active genes triggers increased activity from the HCMV-driven reporter construct (6, 7).

DNA mismatch repair (MMR) constitutes another important, relatively conserved DNA repair pathway found in mammalian cells involving the recognition and repair of bases incorrectly incorporated during DNA replication. In human cells, this repair process involves the expression of two principal repair genes, namely hMLH1 (human mutL homologue 1) and hMSH2 (human mutS homologue 2). As their names suggest, these two proteins are functional homologues of the mutL and mutS bacterial proteins, respectively. At the present time, there appears to be conflicting evidence for the role of these MMR genes in the TCR pathway of NER in human cells. Using a strand-specific Southern blot-based assay, Mellon et al. (8) reported that the
MMR-deficient HCT116 and LoVo human adenocarcinoma cells (carrying mutations in the hMLH1 and hMSH2 genes, respectively) were defective in TCR of CPDs at the active dihydrofolate reductase gene locus. In contrast, using a ligation-mediated PCR that measures CPD removal at nucleotide resolution, Rochette et al. (9) have reported recently that these same MMR-deficient adenocarcinoma cells are fully proficient in TCR. In addition, Sonneveld et al. (10) reported that hMSH2-deficient murine embryonic fibroblasts are able to remove CPDs much more rapidly from the transcribed compared with the nontranscribed strand of active genes suggesting proficient TCR in murine embryonic fibroblasts, and Admioolam et al. (11) reported that hMLH1-deficient HCT116 cells are fully competent in TCR at the DHFR locus. In the present work, we have examined HCR of a UVC-damaged reporter gene and UVC-enhanced expression of the undamaged reporter gene in hMLH1-deficient HCT116 human colon adenocarcinoma cells and HCT116-chr3 cells (a derivative of HCT116 wherein wild-type expression of hMLH1 expression has been restored via chromosome 3 transfer), as well as hMSH2-deficient LoVo human colon adenocarcinoma cells and their hMSH2-proficient counterpart SW480 cells.

MATERIALS AND METHODS

Cell Lines and Virus Strains. SW480 and LoVo colon adenocarcinoma cells were purchased from the American Type Culture Collection. HCT116-chr3 and HCT116 human colon adenocarcinoma cell lines were kindly provided by Dr. Thomas Kunkel (National Institute of Environmental Health Sciences, Research Triangle Park, NC). The repair-proficient human fibroblast GM 9503 as well as the deficient cell line GM 739A (CS-B) were obtained from NIGMS (Camden, NJ). All of the cell cultures were grown in a humidified incubator at 37°C in 5% CO2. Fibroblast cell lines were cultured in Eagle’s α-MEM; SW480, HCT116-chr3, and HCT116 carcinomas were cultured in McCoy’s modified medium; and LoVo's were cultured in a 1:1 mixture of D-MEM and F-12 media (with 4500 mg/liter glucose). All of the cell culture media were supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. AdHCMVlacZ, also called AdCa17lacZ (12), is a nonreplicating, recombinant adenovirus containing the lacZ gene inserted under the control of the HCMV-IE promoter inserted in the deleted E1 virus gene. Virus stocks were prepared as described previously (13).

UVC Irradiation of Cells and Virus. UVC irradiation used a General Electric germicidal lamp (model G8T5) emitting predominantly at 254 nm. Irradiation of cells has been reported previously (4). Fibroblasts were seeded at a density of 2 x 105 cells/well in 96-well plates (Falcon, Lincoln Park, NJ), and carcinoma cell lines were seeded at 4 x 103 cells/well. Between 18 and 24 h after seeding, media were replaced with 40 μl of PBS and UVC irradiated at a fluence rate of 1 J/m2/s and then refed with appropriate supplemented growth medium. Irradiation of the virus by UVC has also been described previously (14). Briefly, viral suspensions were prepared in 1.8 ml of PBS in 35-mm Petri dishes on ice. With continuous stirring, virus suspensions were irradiated with UVC fluences up to 600 J/m2 at a fluence rate of 1 J/m2/s. Aliquots of 200 μl were removed after each exposure to the virus and diluted appropriately in unsupplemented α-MEM.

HCR of the AdHCMVlacZ Reporter Gene. For HCR experiments, untreated and UVC-treated cells were (within 30 min) infected immediately with a 40-μl volume of irradiated or nonirradiated virus at a multiplicity of infection of 20 plaque-forming units/cell. After viral absorption for 90 min, cells were refed with the appropriate supplemented medium and allowed to incubate at 37°C for 40–44 h before harvesting. At harvesting, the infected cell monolayers were incubated with 1 mm chlorononolored-β-β-galactopyranoside (Boehringer-Mannheim, Indianapolis, IN) in 0.01% Triton X-100, 1 mM MgCl2, and 100 mM phosphate buffer (pH 8.3). Absorbance readings at 570 nm were taken several times after the addition of chlorononolored-β-β-galactopyranoside solution using a 96-well plate reader (EL340 Bio Kinetics Reader; Bio-Tek Instruments).

Enhanced Expression of the AdHCMVlacZ Reporter Gene. Cells were UVC irradiated or left untreated and subsequently infected with nonirradiated AdHCMVlacZ at a multiplicity of infection of 20 plaque-forming units/cell. Human fibroblasts and the colon adenocarcinoma cell lines were infected 6 h after UVC irradiation and harvested 12–14 h after infection and also infected 24 h after UVC irradiation and harvested 24 h after viral infection.

RESULTS

HCR of the Reporter Gene Expression of UVC-Irradiated AdHCMVlacZ in MMR-Proficient and -Deficient Cell Lines. Typical survival curves of β-gal activity for UV-irradiated AdHCMVlacZ in untreated (left) and UVC-treated (right) cells are shown in Fig. 1 for TCR-deficient CS-B and normal human fibroblasts. It can be seen that HCR of the UV-damaged reporter gene in untreated cells is significantly reduced in the CS-B strain compared with normal as reported previously (5), indicating that HCR depends, in part at least, on TCR. It can also be seen that UVC pretreatment of cells results in an enhancement of HCR in both normal and CS-B cells, consistent with an up-regulation of GGR. The UVC enhancement of HCR is greater in the CS-B compared with the normal fibroblasts, such that the difference in HCR between the TCR-deficient CS-B strain and the normal strain is not significant in the pre-UVC-treated cells.

Survival curves of β-gal activity for UVC-irradiated AdHCMVlacZ in MMR-proficient and MMR-deficient colon carcinoma cells are presented in Fig. 2. In experiments involving no UVC pretreatment of cells (Fig. 2, left panels), HCR of β-gal activity for UVC-irradiated AdHCMVlacZ was significantly reduced in the MMR-deficient LoVo and HCT116 cells compared with their MMR-proficient SW480 and HCT116-chr3 cell counterparts, respectively. These results indicate some NER deficiency in the MMR-deficient cells compared with their MMR-proficient counterparts.

In contrast, there was no significant difference in HCR for the MMR-deficient cells compared with their MMR-proficient counterparts for cells pretreated with 12 J/m2 UVC (Fig. 2, right panels). Additional survival curves of β-gal activity for UVC-irradiated AdHCMVlacZ were obtained using other pre-UVC fluences to cells. The UV fluence to virus required to reduce β-gal activity to 37% of that for nonirradiated virus (D37) was calculated for each survival curve (Table 1). Relative D37 values in MMR-proficient compared with MMR-deficient cells are plotted as a function of UVC fluence to cells in Fig. 3. It can be seen that the difference in HCR for the MMR-
deficient and MMR-proficient cell lines detected in untreated cells was reduced or absent when cells were pretreated with UVC.

It can also be seen that 12 J/m² UVC pretreatment of cells resulted in a significant enhancement of HCR for LoVo cells, but not for SW480 cells, such that there was no significant difference in the HCR curves for LoVo and SW480 cells pretreated with 12 J/m² (Fig. 2; Table 1). In contrast, pre-UVC irradiation actually reduced HCR for SW480, HCT116, and HCT116-chr3 cells, although this reduction was only significant for HCT116 and HCT116-chr3 cells, and the reduction was greater in the MMR-proficient HCT116-chr3 cells, such that there was no significant difference in the HCR curves for HCT116 and HCT116-chr3 cells pretreated with 12 J/m². It appears that pre-UVC treatment of cells actually inhibits repair in the transcribed strand of the reporter gene in HCT116 and HCT116-chr3 cells and that this inhibition is greater for the MMR-proficient HCT116-chr3 cells.

Enhanced Expression of the Undamaged Reporter Gene in MMR-Proficient and -Deficient Cell Lines. We have previously shown that UVC-enhanced expression of the undamaged reporter gene is an indirect measurement of lesion removal by TCR in the transcribed strand of cellular genes (6, 7). UVC-enhanced expression of the undamaged reporter gene is induced at lower UVC fluences to cells and at higher levels after low UVC fluence to cells in TCR-deficient CS fibroblasts but not in xeroderma pigmentosum (XP)-C and XP-E fibroblasts that are deficient only in GGR, compared with normal human fibroblasts, suggesting that persistent damage in active genes, which is not repaired by TCR, triggers increased activity from the HCMV-driven reporter construct (6, 7). The expression of β-gal after infection of nonirradiated AdHCMVlacZ was examined in pre-UVC-treated MMR-proficient and MMR-deficient colon carcinoma cells. Results for normal and CS-B fibroblasts carried out under identical conditions are shown for comparison purposes. Mellon et al. (8) have reported a difference in repair of the transcribed strand of the active dihydrofolate reductase gene in HCT116 compared with HCT116-chr3 cells and in LoVo compared with SW480 cells. On the basis of this data, we chose carefully the time between UV exposure to cells and infection as well as the time between infection and scoring for β-gal to maximize the difference in repair of CPDs from the transcribed strand of active cellular genes in the MMR-deficient compared with the MMR-proficient cells.

Fig. 4 shows results for CS-B fibroblasts, normal fibroblasts, LoVo, SW480, HCT116, and HCT116-chr3 cells infected 6 h after UVC irradiation and subsequently scored for β-gal activity 12–14 h after infection. It can be seen that persistent damage in the active genes of the TCR-deficient CS-B cells correlated with a UVC-enhanced expression of the undamaged reporter gene at lower UVC fluences to cells and at higher levels compared with that in normal human fibroblasts as reported previously (6, 7). In addition, UVC-enhanced expression of the reporter gene was observed also at higher levels in LoVo compared with SW480 cells and in HCT116 compared with HCT116-chr3 at equivalent low UVC fluences to cells, suggesting a TCR deficiency in the MMR+ cells. Fig. 5 shows results for CS-B fibroblasts, normal fibroblasts, LoVo, SW480, HCT116, and HCT116-chr3 cells infected 24 h after UVC irradiation and subsequently harvested 24 h after infection. It can be seen that under these conditions, higher levels of UVC-enhanced expression of the reporter gene were detected in CS-B compared with normal fibroblasts and in LoVo compared with SW480 but not in HCT116 compared with HCT116-chr3 cells at equivalent low UVC fluences to cells.

These results suggest a similar level of repair in the transcribed strand of active genes in HCT116 compared with HCT116-chr3 cells by 24 h after UVC to cells and a reduced rate of repair in HCT116 compared with HCT116-chr3 cells at earlier times after UVC, because a difference in UV-enhanced expression was detected for cells infected at 6 h after UVC treatment (Fig. 4). In contrast, an increased

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**Table 1** $\delta$C of β-galactosidase activity for UVC-irradiated AdHCMVlacZ virus in MMR-proficient and MMR-deficient colon carcinoma cells pretreated with increasing UVC exposure

<table>
<thead>
<tr>
<th>Cell line</th>
<th>$0\ J/m^2$</th>
<th>$6\ J/m^2$</th>
<th>$12\ J/m^2$</th>
<th>$18\ J/m^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW480</td>
<td>227.75 ± 99.58</td>
<td>218.96 ± 61.21</td>
<td>94.29 ± 24.35</td>
<td>123.52 ± 26.59</td>
</tr>
<tr>
<td>LoVo</td>
<td>35.76 ± 2.29</td>
<td>46.60 ± 9.95</td>
<td>81.11 ± 17.09</td>
<td>97.47 ± 27.80</td>
</tr>
<tr>
<td>HCT116-chr3</td>
<td>415.03 ± 76.03</td>
<td>376.24 ± 76.12</td>
<td>145.44 ± 35.35</td>
<td>180.28 ± 59.56</td>
</tr>
<tr>
<td>HCT116</td>
<td>298.09 ± 27.17</td>
<td>230.37 ± 77.33</td>
<td>149.12 ± 37.27</td>
<td>208.02 ± 45.97</td>
</tr>
<tr>
<td>Relative HCR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SW480/LoVo</td>
<td>6.37 ± 2.84b</td>
<td>4.73 ± 1.41</td>
<td>1.22 ± 0.30</td>
<td>1.34 ± 0.28</td>
</tr>
<tr>
<td>HCT116-chr3/HCT116</td>
<td>1.37 ± 0.18b</td>
<td>2.22 ± 0.71</td>
<td>1.05 ± 0.17</td>
<td>0.87 ± 0.24</td>
</tr>
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a HCR, host cell reactivation; MMR, mismatch repair.

b Relative HCR significantly $>1$ by one-tailed $t$ test ($P < 0.05$).
UVC-enhanced expression of the unirradiated reporter gene was detected in LoVo compared with SW480 cells for infection at both 6 h and 24 h after infection, suggesting that TCR deficiency in LoVo cells is not just a deficiency in repair rate. These results are consistent with the difference in repair reported by Melon et al. (8) for the transcribed strand of the active dihydrofolate reductase gene in HCT116 compared with HCT116-chr3 and in LoVo compared with SW480 cells.

DISCUSSION

UV-induced CPDs are removed more rapidly from the transcribed strand compared with the nontranscribed strand of actively transcribing cellular genes. Although both GGR and TCR contribute to NER in the transcribed strand of active cellular genes, the TCR pathway acts only on transcription blocking CPDs in the transcribed strand, resulting in more rapid removal from the transcribed compared with the nontranscribed strand. However, the relative contribution of TCR and GGR to repair in the transcribed strand of an active gene appears to be dependent on the magnitude of the UV exposure to the cells (2).

Assessment of TCR in cellular DNA generally involves a comparison of lesion removal from the transcribed compared with the nontranscribed strand of an active gene or a comparison of lesion removal in an active compared with an inactive gene (15–17). In contrast, the assessment of TCR in the current work involved an indirect measurement of lesion removal by NER from the transcribed strand of the reporter gene in the HCR assay and an indirect measurement of lesion removal by TCR in the transcribed strand of cellular genes in the assay for enhanced expression of the undamaged reporter gene (4).

We have reported previously that HCR for expression of the UVC-damaged reporter gene in unirradiated cells is significantly reduced in TCR-deficient Cockayne syndrome fibroblasts as well as in GGR-deficient XP-C fibroblasts compared with that in normal human fibroblasts (5), indicating that both TCR and GGR contribute to expression of the UVC-damaged reporter gene in untreated human cells. Using a quantitative PCR technique, we have reported previously a significant removal of UVC-induced photoproducts from the reporter gene in normal human fibroblasts but a reduced removal in TCR-deficient CS-B and GGR deficient XP-C fibroblasts (18). These previous reports suggest that differences in HCR for expression of the UVC-damaged reporter gene reflect more likely differences in the removal of UVC-induced photoproducts by NER rather than differences in the rate of transcription and/or lesion bypass by polymerase II. The reduced HCR of the UVC-damaged reporter gene in the untreated MMR-deficient cells compared with their MMR-proficient counterparts reported here (Fig. 2, left panels; Table 1) indicates that the human colon adenocarcinoma cells bearing homozygous mutations in hMLH1 or hMSH2 are deficient in the TCR and/or GGR pathway of NER.

We have reported previously that UVC-enhanced expression of the undamaged reporter gene is induced at lower UV fluences to cells and at higher levels after low UV fluence in TCR-deficient fibroblasts, but not GGR-deficient fibroblasts, compared with normal human fibroblasts, suggesting that persistent damage in active genes, which is not repaired by TCR, triggers increased activity from the HCMV-driven reporter construct (4, 6). Current models of NER suggest that it is the persistent stalling of RNA polymerase II at sites of DNA damage, which are not repaired by the TCR pathway, that acts as a trigger for this response. We have reported also that p53 does not play an essential role in mediating UVC-induced expression from the CMV-IE-driven reporter gene, such that the UVC-enhanced expression is independent of the p53 status of the cell (7). Enhanced expression of...
β-gal for untreated AdHCMVlacZ virus was significantly greater at lower UVC fluences to cells in LoVo compared with SW480 cells and in HCT116 compared with HCT116-chr3 cells (Fig. 4). These results suggest that the efficiency of removal of UV lesions in the transcribed strand of active cellular genes by the TCR pathway is less in the MMR-deficient compared with the MMR-proficient cells.

There is evidence that some of the p53-regulated gene products are involved in inducible NER, including the p53-mediated and DNA-damaged induced GADD45 gene (19, 20), the p48-XPE gene (21, 22), and the XPC gene (23, 24). In particular, transcription from the p48 gene, which is mutated in GGR-deficient, damage-specific DNA binding protein-deficient XP-E cells (21), is up-regulated (in a p53-dependent manner) in response to UV treatment in human cells. This is consistent with a p53-dependent up-regulation of GGR in cellular DNA reported for human cells (22). We have reported previously that wild-type p53 is required for the expression of enhanced HCR of a UVC-damaged reporter gene in UVC-pretreated human fibroblasts (25). In addition, using a quantitative PCR technique, we have reported an enhanced removal of UVC-induced photolesions from the reporter gene in pre-UVC-treated human fibroblasts (18). In the present work, we show that UVC pretreatment of cells results in an enhancement of HCR for expression of the UV-damaged reporter gene in TCR-deficient CS-B cells, consistent with an enhanced removal of lesions from the transcribed strand of the reporter gene due to a p53-dependent up-regulation of GGR. UV-induced DNA lesions efficiently block transcription elongation and induce the p53 response, indicating that persistent UV lesions in the transcribed strand of active genes trigger the p53 response (26–28). Therefore, cells with impaired TCR induce the p53 response at lower UVC fluences compared with normal cells with functional TCR (27–29). This suggests that the p53-dependent up-regulation of GGR would occur at lower UVC exposures and to a greater extent in TCR-deficient compared with TCR-proficient cells. Consistent with this suggestion, we found that the UVC enhancement of HCR was greater in the CS-B compared with normal fibroblasts for pretreatment of cells with 12 J/m². The net result was that HCR of the UVC-damaged reporter gene in the TCR-deficient CS-B strain compared with the normal strain, although substantially different in untreated cells (Fig. 1A), was not significantly different in cells pretreated with 12 J/m² of UVC (Fig. 1B).

UVC pretreatment of cells with 12 J/m² resulted in enhanced HCR for the MMR− LoVo, similar to that observed for the TCR-deficient CS cells, consistent with an up-regulation of GGR and the p53 wild-type (+/+) status of LoVo cells (30). In contrast, no UVC-enhanced HCR was detected in SW480 cells consistent with their mutant p53 (−/−) status (30) or in HCT116 and HCT116-chr3 cells (Fig. 2; Table 1). Although, HCT116 cells have a p53 wild-type (+/+) status (30), we have reported previously a lack of both heat-shock and UVC-enhanced HCR in this human colon cancer cell line (31), indicating that genetic alteration in tumor cells, other than inactivation of p53, can inhibit enhanced HCR.

It has been reported that normal fibroblasts exposed to high UVC exposure (30 J/m²) lack strand-specific repair of both 6–4 photoproducts and CPDs, suggesting that TCR is overruled by GGR after high UVC exposure to cells (2). The reduction in TCR could result from a severe inhibition of transcription and/or the induction or inhibition of others factors affecting the TCR pathway after high UVC exposure. This suggests that there would be a greater reduction in the repair of the transcribed strand by this mechanism in TCR-proficient cells (compared with TCR-deficient cells) after high UVC exposure, which is consistent with the greater reduction in HCR for HCT116-chr3 cells compared with HCT116 cells. It appears that the level of repair in the transcribed strand of the reporter gene in pre-UVC-treated cells results from the net effect of the p53-dependent up-regulation of GGR (which would result in a greater enhancement in repair for TCR-deficient cells) together with a reduced contribution of TCR at high exposures (which would tend to result in a greater reduction in repair for TCR-proficient cells).

Our results are consistent with a role for both hMSH2 and hMLH1 in the TCR of UVC-induced lesions in human colon adenocarcinoma cells. We suggest that their role in TCR is not detectable in cells pretreated with 12 J/m² UVC and greater, due in part to a p53-dependent up-regulation of GGR together with an inhibition of TCR in the transcribed strand of the reporter gene at this high UV fluence to cells.

For no UVC exposure to cells, HCR of β-gal activity for UVC-irradiated AdHCMVlacZ virus was significantly reduced in LoVo cells compared with SW480 cells and in HCT116 cells compared with TCT116-chr3 cells. This indicates a NER deficiency in the MMR-deficient LoVo and HCT116 cells compared with their MMR-proficient counterparts and is consistent with a TCR deficiency in these same MMR-deficient cells as reported by Mellon et al. (8) for repair in the active dihydrofolate reductase gene after a UVC exposure of 10 J/m² to cells. The reduction in HCR was greater for the hMSH2-deficient LoVo cells compared with the hMSH2-proficient SW480 cells than for the hMLH1-deficient HCT116 cells compared with the hMLH1-proficient HCT116-chr3 cells, indicating a greater effect of hMSH2 deficiency compared with hMLH1 deficiency on NER in the transcribed strand of the reporter gene. These results for HCR in nontreated cells are consistent with the greater effect of hMSH2 deficiency compared with hMLH1 deficiency on removal of CPDs

Fig. 5. Enhanced expression of β-galactosidase in UVC-irradiated human fibroblasts and colon carcinoma cell lines after infection with unirradiated AdHCMVlacZ virus. Cells were infected 24 h after UVC irradiation and subsequently harvested 24 h after infection. Results are shown for GM 9503 ( ), GM 739A ( ), SW480 ( ), LoVo ( ), HCT116-chr3 ( ), and HCT116 ( ). Each point is the average of three to four independent experiments, each performed in triplicate; bars, ±SE.
from the transcribed strand of the active dihydrofolate reductase gene after a UVC exposure of 10 J/m² to these cells (8). In contrast, Adimoolam et al. (11) report that hMLH1-deficient HCT116 cells are fully competent in TCR at the dihydrofolate reductase locus after a similar UVC exposure of 10 J/m² to cells. The reason for the contradictory TCR results with the HCT116 cells remains unclear, although it has been suggested that the cell clones used by Adimoolam et al. (11) may have acquired a secondary mutation that either restores TCR or relieves the inhibition of TCR due to the primary hMLH1 mutation. It is also possible that the contradictory TCR results for HCT116 cells arise from differences in UV dosimetry and/or differences in the UV spectrum used, because the results of the present work suggest that relatively small differences in UVC exposure to cells can affect the relative contribution of TCR and GGR.

The difference in HCR of β-gal activity for UVC-irradiated AdHCMVlacZ virus detected in untreated cells for the MMR-deficient compared with the MMR-proficient cell lines was reduced or absent when cells were pretreated with UVC. For pre-UVC treatment of cells with 12 and 18 J/m², there was no significant difference between the HCR in the MMR-deficient compared with the MMR-proficient cell lines (Fig. 3). This is consistent with the results of Rochette et al. (9), showing a similar removal of CPDs from the transcribed strand of the c-jun and/or p53 gene in the MMR-deficient compared with the MMR-proficient cell lines after a UVC exposure of 15 J/m² to these same cell lines.

Taken together, the HCR and UVC-enhanced expression results of the present study indicate that human colon adenocarcinoma cells bearing homozygous mutations in hMLH1 or hMSH2 are deficient in NER of UVC-induced DNA lesions, and suggest that the ability to detect the involvement of these MMR genes in TCR is dependent on UVC fluence to cells. We suggest that the apparent discrepancy in results published previously regarding the role of hMLH1 and hMSH2 in TCR results, in part at least, from differences in the UV fluence to cells used to examine TCR.

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