Poly(ADP-ribose) Synthesis following DNA Damage in Cells Heterozygous or Homozygous for the Xeroderma Pigmentosum Genotype*

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Treatment of normal human cells with DNA-damaging agents such as UV light or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) stimulates the conversion of NAD to the chromosomal polymer poly(ADP-ribose) (1, 2), which in turn results in a rapid depletion of the cellular NAD pool. We have studied the effect of UV light or MNNG on the NAD pools of seven cell lines of human fibroblasts either homozygous or heterozygous for the xeroderma pigmentosum genotype. Xeroderma pigmentosum cells of genetic complementation groups A, C, and D are deficient in the excision repair of DNA damage caused by UV light. Following UV treatment, the NAD content of these cells was unchanged (complementation groups A and D) or only slightly reduced (complementation group C). Xeroderma pigmentosum cells with the variant genotype have normal excision repair and UV treatment caused a large reduction in the size of the NAD pool. Cell lines derived from asymptomatic, parental heterozygotes of xeroderma pigmentosum complementation groups A and D showed an amount of lowering of NAD following UV treatment that was approximately one-half that of the control cell line. All of the cell lines are able to excise DNA damage caused by MNNG and all of the cell lines had a greatly reduced content of NAD following MNNG treatment. The results demonstrate a close relationship between the conversion of NAD to poly(ADP-ribose) and DNA excision repair in human cells.

NAD is the substrate for the synthesis of the chromosomal polymer poly(ADP-ribose) (1, 2). Recent evidence has associated poly(ADP-ribose) synthesis with the occurrence and repair of molecular damage to DNA. Agents that cause DNA damage stimulate the synthesis of poly(ADP-ribose) both in vitro (3-11) and in vivo (12) and cause a rapid depletion of the cellular NAD pool (11, 13-21). When 3T3 cells are nutritionally depleted of NAD, unscheduled DNA synthesis induced by MNNG1 is greatly reduced while replicative DNA synthesis remains normal or near normal (22, 23). In L1210 cells, inhibitors of poly(ADP-ribose) polymerase inhibit rejoining of DNA strand breaks caused by dimethyl sulfate (24). Lugger et al. (25) have reported that UV treatment of lymphocytes from normal individuals results in a 1.5- to 3-fold increase in the rate of poly(ADP-ribose) synthesis in vitro but that lymphocytes from patients with xeroderma pigmentosum show either no increase or a delayed increase following UV treatment. Xeroderma pigmentosum is a genetic disease in which affected individuals develop skin cancers following exposure to sunlight since the repair of UV light-induced pyrimidine dimers in DNA is reduced or absent (see Ref. 26 for review). Most xeroderma pigmentosum cells are deficient in the removal of pyrimidine dimers from DNA and seven different genetic complementation groups have been identified. An additional type, termed xeroderma pigmentosum variant (27), has normal dimer removal but is defective in another aspect of repair (28-30). Xeroderma pigmentosum cells are repair-proficient for the DNA damage caused by MNNG (31).

Our previous studies have shown that treatment of cells with DNA-damaging chemicals results in a selective depletion of the cellular NAD pool due to a rapid conversion of NAD to poly(ADP-ribose) (11, 12, 32). We report here the effect of UV and MNNG on the NAD pools of human fibroblasts carrying the xeroderma pigmentosum genotype.

EXPERIMENTAL PROCEDURES

Normal human fibroblasts (IMR-90), xeroderma pigmentosum, xeroderma pigmentosum variant, and parental xeroderma pigmentosum cell lines were obtained from the American Type Culture Collection and were cultured as described previously for 3T3 cells (33). For experiments, cells were seeded in 35-mm dishes and grown for 3 to 7 days with replacement of fresh medium at 48-h intervals. All cultures were in the log phase of growth when treated with UV light or MNNG. To treat with UV light, the medium was removed and the cells were washed with 10 mM sodium phosphate buffer, pH 7.2, 0.14 M NaCl. The cells were placed under a General Electric germicidal lamp (G15T8) with an incident dose, principally 254 nm, of 0.6 J/cm²-s. Control cultures were treated in the same way except that the UV lamp was not turned on. Following treatment, medium was replaced and the cells were returned to the incubator. To treat with MNNG, medium was removed and replaced with medium containing MNNG. The cells were then returned to the incubator until extraction. The total NAD pool was extracted as described previously (34). NAD was measured by an enzymatic cycling assay described in detail elsewhere (33).

RESULTS AND DISCUSSION

We have previously studied the mechanism by which MNNG causes a rapid lowering of cellular NAD pools in 3T3 cells (11, 12, 32). These studies have ruled out effects on the biosynthesis of NAD or nonspecific effects on nucleotide pools and have shown that the lowering of NAD is due to a rapid conversion of NAD to poly(ADP-ribose) in response to DNA damage. We have also shown that many other DNA-damaging chemicals cause a rapid lowering of NAD levels in human lymphocytes (21). The data in Fig. 1 show that treatment of normal human fibroblasts with UV light also causes a rapid time- and dose-dependent lowering of cellular NAD levels. Fig. 1 also shows that the lowering can be largely blocked by addition of theophylline to the culture medium following UV treatment. Theophylline is a potent inhibitor of poly(ADP-ribose) polymerase (35). Thus, the measurement of cellular NAD levels following UV treatment provides a sensitive
and parental heterozygotes of xeroderma pigmentosum. The seven cell lines carrying the xeroderma pigmentosum genotype include xeroderma pigmentosum complementation groups A, C, and D, xeroderma pigmentosum variant, and parental heterozygotes of xeroderma pigmentosum. The data are expressed as the percentage of total cellular NAD content present at time zero. The absolute levels of NAD were similar in all of the cell lines, ranging from 2.7 to 3.5 pmol/µg of protein. Doses of MNNG or UV light were chosen that cause a large amount of reduction of the NAD content of the control cell line. Treatment with MNNG also caused a large decrease of NAD content in each of the cell lines carrying the xeroderma pigmentosum genotype, however, the levels of NAD following UV treatment showed a very different pattern. Xeroderma pigmentosum cells of complementation groups A and D did not show a significant change in NAD levels, and xeroderma pigmentosum cells of complementation group C showed a significant but greatly reduced amount of lowering of NAD relative to normal cells. The xeroderma pigmentosum variant cells showed a decrease in NAD quite similar to that of normal cells. In each case, the parental heterozygotes of xeroderma pigmentosum complementation groups A, C, and D showed an amount of lowering of NAD that was intermediate between the corresponding xeroderma pigmentosum homozygotes and normal cells. These data provide a highly specific correlation between the ability of the cells to convert NAD to poly(ADP-ribose) and the known ability of these cell lines to excise damage from DNA. Xeroderma pigmentosum cells are proficient in the excision of damage caused by MNNG (31) and all of the xeroderma pigmentosum cell lines deplete their NAD pools following MNNG treatment. The xeroderma pigmentosum cell lines of complementation groups A, C, and D have greatly reduced rates of removal of UV-induced pyrimidine dimers and subsequent repair replication relative to normal cells (26, 36) and following UV treatment the conversion of NAD to poly(ADP-ribose) in either undetectable (complementation groups A and D) or significantly reduced (complementation group C). We note that the cell lines of complementation groups A and D are from patients with a more severe form of xeroderma pigmentosum than the patient of complementation group C (36). The xeroderma pigmentosum variant cell line examined has rates of dimer removal and repair replication similar to normal cells (36) and we observe a depletion of the NAD pool similar to that observed for the control cell line following UV treatment. The xeroderma pigmentosum heterozygotes are asymptomatic and in general have rates of repair replication following UV treatment that are normal or slightly below normal (37). Although we do observe a depletion of the NAD pool in these cells, it is of interest that the amount of lowering observed in the heterozygotes for complementation groups A and D was very nearly one-half that of the control cell line. Since the rate of lowering of NAD following UV treatment is nearly linear for 5 h (Fig. 1), the decreased amount of lowering observed in the xeroderma pigmentosum heterozygotes reflects a decreased rate of conversion of NAD to poly(ADP-ribose).

Xeroderma pigmentosum fibroblasts are able to synthesize poly(ADP-ribose) following the induction of DNA damage with MNNG as evidenced by the lowering of NAD pools following MNNG treatment. This result agrees with that of Berger et al. (25) who reported that although UV pretreatment of xeroderma pigmentosum lymphocytes did not stimulate poly(ADP-ribose) synthesis in vitro, pretreatment with MNNG did cause a stimulation. In an experiment in which XP12BE cells (complementation group A) were first treated with UV and then with MNNG, a lowering of NAD was observed that was similar to cells treated with MNNG alone. This result makes it unlikely that the lack of lowering of NAD in xeroderma pigmentosum cells following UV treatment is due to inactivation of poly(ADP-ribose) polymerase. Thus, we feel that the best interpretation of the data presented here is that the synthesis of poly(ADP-ribose) normally takes place during DNA excision repair and that poly(ADP-ribose) synthesis is a step in repair distal to the step that is defective in xeroderma pigmentosum. The excision repair of UV light-induced pyrimidine dimers requires an endonucleolytic incision into DNA near the dimer. Xeroderma pigmentosum cells are known to be deficient in the introduction of endonucleolytic incisions into DNA (38) although the exact nature of the defect is poorly understood. Poly(ADP-ribose) polymerase is known to be stimulated in vitro by DNA strand breaks (3, 4, 7). Thus, a working hypothesis that is consistent with all of the available data is that poly(ADP-ribose) synthesis normally occurs during one or more steps in DNA repair that follow the occurrence of a strand break. The molecular details of this involvement await further study.

The observation that the parental heterozygotes of xeroderma pigmentosum show a rate of lowering of NAD following UV that correlates with the gene dosage of the xeroderma pigmentosum defect may also be of clinical interest. The observation that relatives of xeroderma pigmentosum patients

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**Table I**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Control</th>
<th>UV-treated</th>
<th>MNNG-treated</th>
</tr>
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<tbody>
<tr>
<td>IMR90</td>
<td>94.5 ± 0.7 (2)</td>
<td>17.4 ± 2.8 (5)</td>
<td>6.5 ± 1.3 (4)</td>
</tr>
<tr>
<td>Parent of XP12BE</td>
<td>94.8 ± 5.0 (10)</td>
<td>89.0 ± 4.7 (13)</td>
<td>15.7 ± 0.5 (6)</td>
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<tr>
<td>Parent of XP2BE</td>
<td>97.4 ± 3.4 (8)</td>
<td>54.8 ± 4.9 (6)</td>
<td>11.0 ± 0.6 (4)</td>
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<tr>
<td>Parent of XP6BE</td>
<td>98.3 ± 4.4 (7)</td>
<td>31.9 ± 10.3 (9)</td>
<td>2.2 ± 0.5 (4)</td>
</tr>
<tr>
<td>XP6BE (variant)</td>
<td>95.4 ± 2.0 (5)</td>
<td>89.6 ± 4.4 (5)</td>
<td>8.5 ± 2.0 (2)</td>
</tr>
<tr>
<td>Parent of XP6BE</td>
<td>95.8 ± 2.3 (6)</td>
<td>48.7 ± 1.5 (4)</td>
<td>5.5 ± 0.5 (6)</td>
</tr>
<tr>
<td>XP4BE (variant)</td>
<td>100.2 ± 3.7 (4)</td>
<td>28.7 ± 2.2 (4)</td>
<td>10.0 ± 1.4 (4)</td>
</tr>
</tbody>
</table>
who receive considerable sun exposure are more likely to develop skin cancers than the general population (39) suggests that individuals who are heterozygous for the xeroderma pigmentosum defect may be at an increased risk of developing skin cancer. At present, it is not possible to identify a family at risk for the xeroderma pigmentosum genotype until a skin cancer. At present, it is not possible to identify a family that is an increased risk of developing skin cancer, providing a convenient method for identification of such individuals.

REFERENCES