Inheritance of Abnormal Expression of SOS-like Response in Xeroderma Pigmentosum and Hereditary Cancer-prone Syndromes

Peter J. Abrahams, Ada Houweling, Paulien D. M. Cornelissen-Steijger, Fré Arwert, Fred H. Menko, Herbert M. Pinedo, Carrol Terleth, and Alex J. van der Eb

Laboratory for Molecular Carcinogenesis, Leiden University, P.O. Box 9503, 2300 RA Leiden [P. J. A., A. H., P. D. M. C-S., C. T., A. J. v. d. E.]; Department of Human Genetics, Faculty of Medicine, Free University of Amsterdam [F. A.]; and Departments of Clinical Genetics [F. H. M.] and Clinical Oncology [H. M. P.] of the University Hospital, Faculty of Medicine, Free University of Amsterdam, Amsterdam, the Netherlands

ABSTRACT

The time course of induction of SOS-like stress responses such as enhanced reactivation (ER) and enhanced mutagenesis (EM) has been investigated in UV-C-irradiated skin fibroblasts from a xeroderma pigmentosum (XP) family, using herpes simplex virus type 1 as a probe. Similar ER studies were performed in a Li-Fraumeni syndrome (LFS) family and in a family with a high incidence of breast, ovarian, and colon cancer. In two XP (complementation group B) patients, with a striking absence of skin tumors even at an age of >40 years, only induction of EM was observed, whereas ER was absent (XP-PER). The ER- phenotype was inherited from the father, whereas cells from the mother exhibited normal expression of ER and EM. This suggests that the absence of ER is a hereditary trait that is not correlated with a repair-deficient phenotype. Abnormally high levels of ER were observed in UV-C-exposed skin fibroblasts from five LFS patients. The inheritance of the ER response was studied in one LFS family. High levels of ER were observed only in cells derived from affected individuals carrying one mutated p53 allele, whereas cells from unaffected family members, carrying two wild-type p53 alleles, exhibited normal ER levels. This result shows that abnormally high levels of ER positively correlate with the occurrence of cancer in affected individuals from an LFS family. Interestingly, abnormally high levels of ER were observed in cells from affected as well as from unaffected members of a family with a high incidence of breast, ovarian, colon, and stomach cancer. This suggests that these latter individuals have inherited a mutated, putative predisposing gene, resulting in abnormal expression of ER, but that cancer had not yet developed. The results indicate that the ER response can possibly be used as a prognostic marker to identify carriers in various hereditary cancer-prone syndromes at an early age.

INTRODUCTION

Carcinogenesis appears to be a multistage process in which normal cells progress from benign to fully malignant cancer cells through an accumulation of genetic errors. Errors can take the form of point mutations or chromosomal aberrations such as translocations, deletions, insertions, or gene amplification (1, 2). This has led to the hypothesis that one of the underlying causes of cancer may be the induction of mutations in certain genes that in turn cause genetic instability, increasing the chance that oncogenic mutations accumulate in the same cell (3). The induction of an error-prone state may be caused by alterations in gene products involved in the maintenance of the chromatin structure, in cell cycle regulation, or DNA repair. Various DNA repair syndromes have been described that are characterized not only by hypersensitivity to one or more DNA-damaging agents or mutagens but also by genomic instability and predisposition to cancer (4). For example, cells from Bloom’s syndrome patients are hypersensitive to UV light, frequently show chromosomal abnormalities, and are susceptible to many types of spontaneous cancer (4). Cells from XP patients are not only hypersensitive to UV light and some other DNA-damaging agents but also exhibit increased levels of sister chromatid exchange and chromosome breaks (5–7). In addition, XP patients show a strong predisposition to cancer in sunlight-exposed areas of the skin. Other DNA repair deficiencies, such as ataxia telangiectasia and Fanconi’s anemia, also exhibit a higher cancer incidence than normal individuals (4–6). In hereditary nonpolyposis colorectal cancer, the predisposition to cancer is associated with a defect in mismatch repair and with genetic instability (8–13). These observations demonstrate that DNA repair processes are central in counteracting the mechanisms that give rise to spontaneous or environmentally induced cancers.

In Escherichia coli, UV light and other DNA-damaging agents transiently induce a number of phenomena, which are collectively called SOS functions. These functions cause higher survival rates but also higher levels of spontaneous mutations in phages infecting the treated bacteria (14–16). SOS-like responses, such as ER and EM, are also observed in fibroblasts from normal individuals and from XP, ataxia telangiectasia, or Cockayne’s syndrome patients (17–19). Whether these phenomena contribute to UV-stimulated cancer is not clear. On the other hand, some XP patients did not show induction of the ER response in their fibroblasts (20). Interestingly, the absence of ER in such cases correlated with the lack of tumors in sunlight-exposed skin areas, although generally the skin of XP patients is extremely tumor prone (4–6). The data suggested that induction of this SOS-like response might somehow be involved in UV-induced carcinogenesis. A relationship between ER and cancer was also suggested by the observation that ER levels after UV treatment of skin fibroblasts from various hereditary cancer-prone syndromes were much higher than those found in normal UV-treated cells (21).

In this study, we addressed the question whether the correlation between abnormal ER response and either absence of cancer or predisposition to cancer is inherited as a genetic trait. To that end, we have investigated the pattern of inheritance of abnormal ER for an XP family in which expression of the XP phenotype was associated with the absence of skin tumors and in cancer-prone families in which cancer predisposition is caused by a germ line mutation in a tumor suppressor gene or by an as yet unknown gene mutation.

MATERIALS AND METHODS

Cell Strains. Normal diploid human skin fibroblasts and skin fibroblasts derived from individuals from the XP-B family, LFS families, and the breast/ovarian family were grown in Ham’s F-10 medium (Life Technologies, Inc.) supplemented with 10% FCS. The normal diploid skin fibroblast cell lines VH10 and VH16 were used as control cells. Cell strains XPCS1BA and XPCS2BA from two XP brothers (complementation group B; Ref. 7) and 90RD395, 90RD397, and 90RD398, derived from their sister, father, and

3 The abbreviations used are: XP, xeroderma pigmentosum; ER, enhanced reactivation; EM, enhanced mutagenesis; LFS, Li-Fraumeni syndrome; HSV-1, herpes simplex virus type 1.
Fig. 1. Time course of induction of ER and EM of UV-C-irradiated HSV-1 in UV-C-exposed XPCS1BA, XPCS2BA, 90RD395, 90RD397, and 90RD398 cells. Cultures of XPCS1BA and XPCS2BA were exposed to 1.0 J m\(^{-2}\) UV-C and infected with UV-C-irradiated HSV-1 (40 J m\(^{-2}\)) to study induction of ER or with unirradiated HSV-1 to investigate induction of EM. Cultures of 90RD395, 90RD397, and 90RD398 were exposed to 10.0 J m\(^{-2}\) UV-C and infected with UV-C-irradiated HSV-1 (150 J m\(^{-2}\)) to study induction of ER or with unirradiated HSV-1 to investigate induction of EM. The experiments were performed as described previously (17).

mother, respectively, were kindly provided by Dr. W. J. Kleijer (University of Rotterdam, Rotterdam, the Netherlands). The LFS cell lines 317T, 378T, 2800T, 2859T, and 3223T were derived from five different LF families, and cell lines 1872T, 1873T, 2525T, 2674T, and 2675T from a single LFS family were obtained from Dr. McPaterson (Cross Cancer Center, Edmonton, Alberta, Canada). A partial pedigree and the clinical description of the latter LFS family have been published by Srivastava et al. (22). LFS cell line 4393 was kindly provided by Dr. S. Friend (MGH Cancer Center, Massachusetts General Hospital, Charlestown, MA).

Fig. 2. Partial pedigree of the XP-B family. XPCS1BA and XPCS2BA belong to complementation group B. Cell lines 90RD395, 90RD397, and 90RD398 are derived from the sister, father, and mother, respectively.

Virus. Wild-type HSV-1, Glasgow strain 17 syn\(^+\), was grown as described previously (17). To eliminate experimental variation, the same virus stock was used in all experiments.
Infection with unirradiated or UV-irradiated HSV-1 (150 J/m2) was carried out 24 h after UV exposure of the cells. The infectious center assay was performed as described previously (17).

**RESULTS**

**Two XP-B Patients without Skin Cancer Have an ER− Phenotype.** We have demonstrated previously that the absence of ER in fibroblasts of certain XP patients (XPER−) correlates with the absence of tumors in the patients, even on sunlight-exposed skin areas (20). This suggested that the ER response might somehow be involved in the process of oncogenic transformation. To extend these observations, we have investigated the induction of these stress responses in fibroblasts from individuals belonging to five different LFS families. In cell lines 2800T, 2859T, and 3223T, one allele of p53 is mutated. No data are available concerning mutations in p53 for cell lines 317T, 378T, and 4394. Unexpectedly, the standard dose range of UV-C light (0–25 J/m2; Ref. 21) was found to be too high for LFS cells; therefore, somewhat lower UV-C doses had to be used. As can be seen in Table 1, LFS cells exhibit variation in the levels of ER. Higher ER levels than those in normal cell lines are predominantly observed after exposure above 10 J/m2 UV-C. Variability in ER levels has also been observed in other hereditary cancer-prone syndromes. In contrast, normal cell lines exhibit less variation in ER levels (21).

To study the inheritance of the abnormal ER phenotype, the dose-response of ER was studied in skin fibroblasts from a LFS family whose partial pedigree is shown in Fig. 3. In cell lines 2525T and 1872T, both alleles of p53 are wild type, whereas in cell lines 1873T, 2674T, and 2675T, one allele of p53 is mutated (22). Table 2 shows that the cells derived from the individuals 2525T and 1872T exhibited normal levels of ER, whereas much higher ER levels are induced in cells from afflicted individuals (1873T, 2674T, and 2675T). Apparently, one mutated allele of p53 can cause the induction of abnormally high levels of ER, indicating that this stress response follows the inheritance pattern of the mutant tumor suppressor gene.

**INHERITANCE OF ABNORMAL SOS-LIKE RESPONSE**

**Table 1. Dose-response of ER of HSV-1 in skin fibroblasts derived from LFS patients.**

<table>
<thead>
<tr>
<th>UV dose (J/m2)</th>
<th>VH16</th>
<th>317T</th>
<th>378T</th>
<th>2800T</th>
<th>2859T</th>
<th>3223T</th>
<th>4393</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>1.1 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>2.1 ± 1.2</td>
<td>2.1 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>2.8 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.7 ± 0.2</td>
<td>2.5 ± 0.3</td>
<td>5.2 ± 1.2</td>
<td>3.6 ± 0.5</td>
<td>1.6 ± 0.3</td>
<td>3.5 ± 1.1</td>
<td>6.7 ± 0.7</td>
</tr>
<tr>
<td>15</td>
<td>2.0 ± 0.2</td>
<td>3.6 ± 0.3</td>
<td>17.7 ± 5.8</td>
<td>5.8 ± 0.8</td>
<td>5.9 ± 1.3</td>
<td>6.2 ± 2.1</td>
<td>14.5 ± 3.8</td>
</tr>
<tr>
<td>20</td>
<td>2.5 ± 0.8</td>
<td>4.1 ± 0.6</td>
<td>24.4 ± 5.6</td>
<td>7.8 ± 3.9</td>
<td>10.0 ± 1.6</td>
<td>19.0 ± 3.5</td>
<td>12.9 ± 1.8</td>
</tr>
</tbody>
</table>

**Fig. 3. Partial pedigree of a LFS family (22).** Open symbols, unaffected members; closed symbols, affected members. 2525T was affected with colon cancer, and 1865T suffered from breast cancer. 1873T developed astrocytoma; 2674T and 2675T suffered from osteosarcoma.
Table 3 Dose-response of ER of HSV-1 in skin fibroblasts derived from a family with high cancer incidence

<table>
<thead>
<tr>
<th>UV dose (J/m²)</th>
<th>Cell strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>303</td>
</tr>
<tr>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>10</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>15</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>20</td>
<td>3.9 ± 1.1</td>
</tr>
<tr>
<td>25</td>
<td>7.4 ± 3.0</td>
</tr>
</tbody>
</table>

DISCUSSION

In this study, we show that the induction of an abnormal ER response is inherited in XP-B and LFS patients and in individuals belonging to a family with a high incidence of breast and ovary cancer, as well as colon and stomach cancer. Abnormally high levels of ER are observed in cells from the afflicted individuals, but in this case, high ER is also found in cells from individuals 306, 502, and 503, who were still tumor free when the biopsy was taken. Normal close-to-normal ER levels are found in 93VU191 and 504, who were also tumor free at the time the skin biopsies were taken. Individuals 502 and 503, who show high ER responses, are still rather young for developing cancer. Thus far, the only exception in this study seems to be individual 306, who at old age shows a high ER response but is still tumor free.

Earlier observations that XP patients who are unusually "resistant" to the induction of skin cancer are consistently ER⁺, suggesting that the lack of an ER response somehow protects against this type of cancer. Further work showed that the ER⁺ phenotype was inherited from the normal heterozygotic father, who also transmitted the mutant XP-B/ERCC3 gene (7). No wild-type allele or other mutant allele has been found in the patients, suggesting that the second allele, which is presumably inherited from the mother, may be silent (7).

These data could also be interpreted to indicate that the lack of an ER response and possibly the absence of skin tumors are caused directly or indirectly by the mutation in the ERCC3 excision repair gene. In this interpretation, the non-tumor-prone phenotype would manifest itself only in association with a repair deficiency, not in a phenotypically normal individual such as the father of the two XP brothers. However, this interpretation appears to be unlikely, in view of the fact that the ER⁻ non-tumor-prone phenotype is also found in other XP complementation groups: XP groups A, C, D, and G (20); and F. Thus, the available data suggest that the ER⁻ and non-tumor-prone phenotype may be caused by a mutation or polymorphism of a genetic trait that is not related to an XP gene.

The situation may be different for the combination of ER⁺ and tumor proneness. In such cases, the abnormal stress response may be a consequence of the presence of a mutant allele of a tumor suppressor gene or of a decrease in the level of a suppressor gene product, as the segregation of the ER⁺ phenotype in the presence of a mutated p53 allele in the LFS family indicates.

4 F. Arwert, unpublished data.

5 P. J. Abrahams, unpublished observations.
The induction of abnormally high levels of ER were also found in cells from members of a family with a high incidence of breast and ovarian cancer, accompanied by colon or stomach cancer. The occurrence of different types of cancer would suggest that this family exhibits a LFS syndrome. However, no mutations could be detected in the p53 exons 5, 6, 7, 8, or 9 in the cell lines investigated in this study. In view of the high incidence of breast and ovarian cancer, a possible linkage with the hereditary breast cancer locus BRCA-1 on chromosome 17q21 was investigated (27). Segregation analysis of DNA markers known to be linked to the BRCA-1 gene on 17q21 (THRA-1, D17S183, D17S409, and D17S588) did not support linkage of the cancer-prone phenotype to the BRCA-1 locus. In this cancer-prone family, no linkage to any other specific locus or gene has been detected yet. Possibly, the disease has to be assigned to Lynch type 2 syndrome, which is associated with breast, ovarian, colon, and other cancers (28).

Interestingly, in this case abnormally high levels of ER were induced in cells not only from afflicted but also from unaffected persons. This was the case for individuals 502 and 503, who, at an age of about 25 years, may still be too young for the onset of breast or ovarian cancer and may still incur the disease at an older age. The only exception in this family is individual 306, who is still tumor free at an age of 75 but is strongly ER^{super+}.

In conclusion, the results, obtained in the present and in our previous studies indicate that abnormal expression of ER behaves like a genetic trait. In the case of the ER^{super+} phenotype, it appears that the abnormal ER response is directly correlated with the presence of a germline mutation in certain tumor suppressor genes. This suggests that loss of one functional allele or the presence of a mutant allele of a tumor suppressor gene can predispose normal diploid cells to induction of abnormally high levels of a stress response after exposure to radiation. Whether this ER response also induces genetic instability is an interesting possibility that is currently under investigation. The ER^- phenotype has thus far been observed only in cells from non-tumor-prone XP patients and, in one instance, in cells from a normal individual (the father of ER^- XP-B patients). The combined experimental evidence suggests that the ER^- character is not associated with a mutation in an XP gene and, hence, may reflect a non-XP-linked polymorphism, possibly of a gene involved in the induction of the ER response. If the ER response is synonymous or coregulated with genetic instability, it may explain why a lack of the ER response correlates with less cancer induction in response to DNA damage.

The results obtained in this study suggest that an abnormally high ER response may in itself, directly or indirectly, lead to genomic instability and hence to cancer induction. The process can be triggered by UV irradiation and probably by other DNA-damaging agents and requires the mutation of one of the two alleles of certain tumor suppressor genes. If this supposition is correct, the ER response may possibly be used as a biological prognostic marker to identify carriers of certain hereditary cancer-prone syndromes that are characterized by germline mutations in tumor suppressor genes.

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