Relationship of DNA Repair to Carcinogenesis in Xeroderma Pigmentosum

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SUMMARY

Radioautograms of intact ultraviolet (UV)-irradiated epidermis from a patient with xeroderma pigmentosum showed no detectable abnormality in UV-induced thymidine-3H incorporation. This result is consistent with findings in this patient's UV-irradiated skin fibroblasts and lymphocytes and contrasts with findings in cells from typical xeroderma pigmentosum patients, all of which exhibit an impaired ability to repair UV-damaged DNA. The development of numerous tumors in the presence of apparently normal DNA repair suggests that some mechanism other than enhancement of UV carcinogenesis by defective DNA repair may be responsible for skin tumor formation in this patient and perhaps in all patients with xeroderma pigmentosum.

INTRODUCTION

In patients with XP, a hereditary disease with cutaneous abnormalities including cancers on sun-exposed areas (12, 16), there is an impaired ability to remove UV-induced pyrimidine dimers from DNA (8, 22). This impairment, which has been attributed to decreased endonucleolytic activity (5, 6, 22), can be demonstrated by comparing the rates of UV-induced TdR-3H incorporation, a measure of repair replication (13, 15), into the nuclear DNA of cells from XP patients and normal donors. Thus, the rates of such incorporation were lower than normal in XP patients' skin fibroblasts (1, 3, 4, 14), epidermal cells (9, 10), and peripheral blood lymphocytes (2, 10, 19, 20). Several investigators (4, 9, 22) have suggested that the XP patients' repair defect may be etiologically related to their cancer via unrepaired UV-induced thymine dimers that cause gene mutation. However, the possibility that the repair defect might not be etiologically related to any of the patients' abnormalities was raised when we found an XP patient, designated Patient 4 of our XP series (2), who, since childhood, has had classical skin involvement, including ectodermally derived tumors (e.g., squamous and basal cell carcinomas of the epidermis and malignant melanomas), but who had a normal rate of UV-induced TdR-3H incorporation into his peripheral blood lymphocytes (2) and skin fibroblasts (3).

Demonstration that this patient's epidermis, the primary site of his clinical disease, also has an essentially normal rate of UV-induced TdR-3H incorporation would make it necessary to consider alternative etiological mechanisms for the link between sunlight and the abnormalities of XP at least for this patient and perhaps, therefore, for all the others. An important element of such a demonstration has been obtained from studies on this patient's trypsin-dissociated epidermal cells which were irradiated and incubated with TdR-3H in vitro (21). Radioautographic studies showed that his dissociated cells, as well as those from control donors, were comprised of 2 populations. One population had UV-induced unscheduled TdR-3H incorporation while the other showed no evidence of such incorporation, the latter unresponsive population presumably being composed of cells that had been damaged or killed during the preparative procedures. The responsive population from this Patient 4 had the same degree of unscheduled incorporation as the responsive population from the control donors and considerably more incorporation than dissociated epidermal cells from an XP patient, designated Patient 1 of our XP series, whose fibroblasts (3) and lymphocytes (2, 19) were known to have the defective DNA repair typical of XP. While these findings suggested that the epidermis of Patient 4 was similar to normal epidermis with regard to the capacity to perform unscheduled TdR-3H incorporation, the possibility remained that not all the cells or layers of his epidermis could perform such incorporation. Therefore, in order to determine whether he had such a mosaic epidermis, the following in vitro studies were performed on intact epidermis from this Patient 4, from Patient 1, and from normal donors.

MATERIALS AND METHODS

The techniques for obtaining and treating skin are modified from methods used by other investigators (10). Two separate experiments were performed. In Experiment 1, dermatome shavings 0.1 mm thick, containing epidermis including stratum corneum, were obtained from normal-appearing skin from the back of Patient 4 and from a normal donor and were placed dermal side down on phosphate-buffered-saline-moistened gauze sponges. Some of these specimens were then irradiated at room temperature from above with a 254 nm germicidal lamp (General Electric Lamp G15T8) delivering 50 ergs/sq mm/sec to the surface of the stratum corneum to deliver an incident dose of 105 ergs/sq mm. Irradiated and unirradiated specimens were then placed in a culture fluid...
was performed in an attempt to obtain and demonstrate more uptake of the TdR-3H. The dermatome was set to cut at a depth of 0.2 mm; the incident doses were 10^4 and 5 \times 10^4 ergs/sq mm delivered at a flux of 100 ergs/sq mm/sec; after the time of irradiation the tissues were embedded in paraffin and 4-um sections were cut perpendicular to the skin surface, mounted on glass slides, and coated with NTB-3 radioautographic emulsion (Eastman Kodak Co., Rochester, N. Y.). After varying times of exposure the radioautograms were developed, stained with hematoxylin, and examined for the presence of "heavily labeled" nuclei (resulting from the uptake of TdR-3H during scheduled, semiconservative DNA synthesis in preparation for mitotic division) (9, 11, 15, 23) and "lightly labeled" nuclei (resulting from the UV-induced, unscheduled TdR-3H incorporation of repair replication) (9, 15). Lightly labeled nuclei were detected only after a 9-month exposure. Therefore, a 2nd experiment was performed in an attempt to obtain and demonstrate more uptake of the TdR-3H.

For Experiment 2, skin was obtained from another normal volunteer, from Patient 1 who is known to have decreased DNA repair in her lymphocytes (2, 19) and fibroblasts (3), and from the low back of Patient 4 on the side contralateral to the one from which shavings were obtained for the 1st experiment. In an attempt to maintain the epidermal cells under conditions optimal for viability and to enable the TdR-3H to penetrate the skin and reach the epidermal cells, we instituted modifications in the experimental technique, including some of those used by Lachapelle and Gillman (11), in Experiment 2. These modifications consisted primarily of the following. The dermatome was set to cut at a depth of 0.2 mm; the incident doses were 10^4 and 5 \times 10^4 ergs/sq mm delivered at a flux of 100 ergs/sq mm/sec; after the time of irradiation the shavings were sliced into strips approximately 2 mm wide; the strips were then incubated at 38° for 4 hr with TdR-3H. After appropriate fixation, 4 slices from each part were sectioned serially, prepared for radioautography, and exposed for periods up to 4 months. The results are illustrated by the radioautograms of 48 days of exposure (Figs. 5 to 10). None of the slices from the unirradiated parts showed unscheduled TdR-3H incorporation although the scheduled TdR-3H incorporation of cells preparing for mitotic division was readily apparent (arrows in Fig. 5 for the normal donor, Fig. 7 for Patient 4, and Fig. 9 for Patient 1). These results indicate that the absence of unscheduled incorporation was due to the absence of UV irradiation and not to failure of the TdR-3H to penetrate the tissues.

Several of the 8 slices from each of the donors' irradiated epidermis had evidence of TdR-3H penetration by virtue of the presence of scheduled TdR-3H incorporation. In each of these slices (5 for the normal control, 6 for Patient 1, and 4 for Patient 4), there was also unscheduled TdR-3H incorporation. The remaining slices showed neither scheduled nor unscheduled TdR-3H incorporation. There was a marked degree of unscheduled incorporation found in certain areas of slices from the irradiated skin from both the normal donor and Patient 4. In certain areas of slices of irradiated skin from the normal donor (Fig. 6) and from Patient 4 (Fig. 8), virtually every epidermal cell showed evidence of unscheduled TdR-3H incorporation. The most deeply situated cells, e.g., the basal cells, generally had less incorporation than the overlying cells, presumably because most of the irradiation had been absorbed by the more superficial layers, thereby significantly reducing the dose reaching the deeper layers.

Radioautograms of irradiated epidermis from Patient 1, however, showed very little unscheduled TdR-3H incorporation despite the fact that the TdR-3H had penetrated the tissue as evidenced by scheduled TdR-3H incorporation. The section of skin from Patient 1 that showed the most unscheduled incorporation is shown in Fig. 10. In Fig. 10, 7 nuclei can be seen to show scheduled TdR-3H incorporation, indicating that the TdR-3H had penetrated the tissue. With regard to unscheduled incorporation, however, the other nuclei gave either no grains or, at best, gave so few grains that each grain could be distinguished individually. These results are in marked contrast to the intensity of labeling found over many of the epidermal nuclei in skin from the normal donor (Fig. 6) and from Patient 4 (Fig. 8), where the grains were so numerous that most of them cannot be individually distinguished.
DISCUSSION

These results with intact epidermis complement and extend the observations obtained with these XP patients' dissociated epidermal cells. In these latter experiments (21), the percentage of the 3 normal donors' epidermal cells that showed UV-induced unscheduled DNA synthesis ranged from 18 to 74%, while 40% of the cells from Patient 4 responded. The average of the mean grain numbers per cell nucleus for the control donors' responsive populations was 36.5, while the mean grain number for that of Patient 4 was 36.0. Thus, these dissociated cell experiments showed that the rate of unscheduled incorporation into the responding cells of Patient 4 was the same as that of the controls. However, since only a part of each donor's cell population underwent detectable unscheduled incorporation, the possibility remained that the lack of response in some or all of the cells of the unresponsive population (which comprised 60% of the epidermal cells of Patient 4) might be due not to cell death or damage but to an inherent inability to perform unscheduled incorporation. However, the radioautographic experiments with intact epidermis reported in this paper indicate that Patient 4 does not have a mosaic epidermis. Thus, sections from Patient 4 such as that in Fig. 8 show that among his responding cells there could be very few, if any, with a repair defect comparable to the defect shown by the epidermal cells of Patient 1 (Fig. 10). These studies on irradiated intact epidermis do not rule out the possibility that Patient 4 had a mosaic epidermis containing relatively rare small patches of unresponsive cells interspersed in an otherwise normal epidermis and that such patches after sunlight exposure gave rise to his clinical manifestations. However, we consider this possibility unlikely because this patient's skin had a distribution and density of the clinical cutaneous signs of XP which were very similar to those of Patient 1 and other typical XP patients and because we found no slices of his irradiated epidermis in which regions failed to show unscheduled incorporation under conditions in which adequate TdR-3H penetration of the slices was simultaneously evident as shown by the presence of scheduled incorporation.

We conclude, therefore, that the UV-induced unscheduled TdR-3H incorporation into intact epidermis of Patient 4 was indistinguishable from that into the epidermis of normal individuals and considerably greater than that into the epidermis from Patient 1. In previous radioautographic studies with lymphocytes (2), fibroblasts (3), and dissociated epidermal cells (21) from Patients 1 and 4, a similar difference in such unscheduled incorporation was also found. Thus, while these 2 patients had almost identical clinical histories and manifestations of XP (J. H. Robbins, K. H. Kraemer, and P. G. Burk, in preparation), none of the cell types tested from Patient 4 showed any evidence of an impairment in UV-induced, unscheduled TdR-3H incorporation. That the unscheduled incorporation into his cells does, in fact, reflect a normal rate of DNA repair synthesis is supported by independent studies which showed that his UV-irradiated fibroblasts were normal for DNA repair as determined by repair replication studies (7) and also by photolysis of their DNA following postirradiation incubation with bromodeoxyuridine (17, 18) (S. N. Buhl, R. B. Setlow, and J. D. Regan, personal communication). Furthermore, evidence that Patient 4's cells have no defect in any aspect of DNA repair has been obtained by studies (7) that showed that his fibroblasts have a normal survival after UV irradiation in contrast to the deceased survival of fibroblasts from XP patients whose cells have the defective DNA repair typical of the disease. Two additional patients who were siblings have also been reported to have XP with normal DNA repair in their skin fibroblasts (7).

However, as we have noted in our previous studies of unscheduled DNA synthesis in cells from Patient 4 (2, 21), we cannot rule out the possibility that his cells have a small defect in the rate of unscheduled synthesis which we have been unable to detect. Particularly with regard to the radioautographic studies with his intact epidermis as reported in this paper, these studies have significant deficiencies in providing quantitative information concerning the rate of unscheduled synthesis in the basal cells, which are the epidermal cells probably most critically involved in UV carcinogenesis. This deficiency arises not only because of the sparsity of light labeling of the nonreplicating basal cells caused, presumably, by the relatively small amount of UV light which reached such deeply situated cells but also because of the heavy grain accumulation over the replicating cells, which resulted from scheduled synthesis and which completely obscures any UV-induced synthesis which might have simultaneously occurred in those cells. Furthermore, our in vitro studies would not reveal a defect in the rate of unscheduled synthesis in the epidermal cells of Patient 4 if such a defect were uniquely caused by events that exerted their effects only in vivo and, thus, only therein inactivated or inhibited his repair process. Thus, our studies do not rule out the possibility that in our Patient 4 there might still be a relationship between his UV-induced carcinogenesis and the known DNA repair defect of XP.

Since no defect has yet been detected in DNA repair in the epidermal cells, fibroblasts, or lymphocytes of Patient 4, some other mechanism may account for this patient's increased susceptibility to skin cancer and for his other cutaneous manifestations of XP. Moreover, since his clinical manifestations of XP were indistinguishable from those of other typical XP patients, it is possible that any such unknown defect may play an etiological role in some or all of the manifestations of XP in these other patients as well.

ACKNOWLEDGMENTS

We thank Mr. Walter Seewald for taking the photomicrographs, Ms. Rosemary Cuddy and Professor Leonard Ornstein for helpful discussion, and Ms. Betty Sanders of the Pathological Technology Section for processing the histological sections.

REFERENCES


Fig. 9. Patient 1 epidermis, unirradiated.
Fig. 10. Patient 1 epidermis, irradiated.
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