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Mouse Model for the DNA Repair/Basal Transcription Disorder Trichothiodystrophy Reveals Cancer Predisposition[1]

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ABSTRACT

Patients with the nucleotide excision repair (NER) disorder xeroderma pigmentosum (XP) are highly predisposed to develop sunlight-induced skin cancer, in remarkable contrast to photosensitive NER-deficient trichothiodystrophy (TTD) patients carrying mutations in the same *XPD* gene. *XPD* encodes a helicase subunit of the dually functional DNA repair/basal transcription complex TFIIH. The pleiotropic disease phenotype is hypothesized to be, in part, derived from a repair defect causing UV sensitivity and, in part, from a subtle, viable basal transcription deficiency accounting for the cutaneous, developmental, and the typical brittle hair features of TTD. To understand the relationship between deficient NER and tumor susceptibility, we used a mouse model for TTD that mimics an XPD point mutation of a TTD patient in the mouse germline. Like the fibroblasts from the patient, mouse cells exhibit a partial NER defect, evident from the reduced UV-induced DNA repair synthesis (residual repair capacity ~25%), limited recovery of RNA synthesis after UV exposure, and a relatively mild hypersensitivity to cell killing by UV or 7,12-dimethylbenz[a]anthracene. In accordance with the cellular studies, TTD mice exhibit a modestly increased sensitivity to UV-induced inflammation and hyperplasia of the skin. In striking contrast to the human syndrome, TTD mice manifest a clear susceptibility to UV- and 7,12-dimethylbenz[a]anthracene-induced skin carcinogenesis, albeit not as pronounced as the totally NER-deficient XPA mice. These findings open up the possibility that TTD is associated with a so far unnoticed cancer predisposition and support the notion that a NER deficiency enhances cancer susceptibility. These findings have important implications for the etiology of the human disorder and for the impact of NER on carcinogenesis.

INTRODUCTION

Genomic instability is an eminent feature in the progression of a normal somatic cell into a transformed cancer cell. To preserve DNA integrity, a network of genome "caretaking" mechanisms has evolved, including DNA repair processes. The NER[3] system eliminates a wide diversity of DNA lesions, such as cyclobutane pyrimidine dimers and (6-4) photoproducts (main DNA damage induced by UV light), intrastrand cross-links, bulky chemical adducts, and some forms of oxidative damage (1), in a complex multistep "cut-and-paste" type of reaction (1, 2). The importance of NER is illustrated by three rare, autosomal recessive photosensitive human NER-deficiency syndromes: XP, CS, and TTD (3).

XP patients, with a defect in one of the NER components (XPA-XPG), are very sensitive to sunlight and have a ~1000-fold increased risk of developing skin cancer. The age of onset of nonmelanoma skin tumors is reduced from 60 years to 8 years of age (4). Additionally, pigmentation abnormalities in sunlight-exposed areas are a hallmark, and, frequently, accelerated neurodegeneration occurs (reviewed in Ref. 3).

CS is characterized by photosensitivity and several additional symptoms that are difficult to rationalize via a defect in NER, such as severe growth retardation (referred to as cachectic dwarfism), neurodysmyelination, and skeletal abnormalities. A mutation in the *CSA* or *CSB* gene is associated with a selective defect in transcription-coupled repair. This NER subpathway accomplishes very efficient removal of lesions that block transcription and that are less efficiently repaired by the complementary NER process, GGR (5). Remarkably, CS patients seem not cancer-prone. Moreover, patients with combined features of XP and CS were identified with defects in the *XPB*, *XPD*, or *XPG* genes (6–8). Adding to the clinical complexity, *XPB* and *XPD* are also involved in the photosensitive form of the third NER syndrome, TTD (9, 10).

TTD shares many features with CS, including (neuro)developmental and skeletal abnormalities. In addition, TTD patients display ichthyosis (scaling of the skin) and striking brittle hairs and nails (11), the hallmark of the disease. TTD patients have a reduced life expectancy, but extensive clinical heterogeneity exists, ranging from mild growth retardation to life-threatening cachexia. Like CS, TTD seems to be not associated with skin cancer predisposition. Moreover, considerable heterogeneity in severity of the NER defect is seen (12, 13), but no clear correlation exists with the severeness of many TTD features. In fact, a subgroup of nonphotosensitive, NER-proficient TTD patients is also known, suggesting that the NER impairment and the typical TTD phenotypes are clinically, and perhaps molecularly, unrelated. In support of this idea, it was discovered that *XPB* and *XPD* are essential DNA helicase subunits of the dually functional DNA repair/basal transcription initiation factor TFIIH (14–18). Previously, we proposed that mutations in those genes may not only affect NER, causing XP and the photosensitivity in CS and TTD, but, depending on the mutation, may also subtly impair basal transcription explaining the typical CS and TTD features (19, 20). Consistent with this hypothesis, mutation analysis of *XPD* in different patients indicated that each causative mutation is syndrome-specific (21–25). Mostly subtle point mutations are found, consistent with the essential role of *XPD* in basal transcription initiation. Moreover, by gene targeting, we showed that a *XPD* null allele is lethal in mice (26).

To study the complex clinical symptoms and the paradoxical absence of skin cancer in NER-deficient TTD patients, we generated a mouse model for TTD by mimicking the XPD^{R722W} allele in the mouse germline as found in five TTD patients (27). TTD mice reflect, to a remarkable extent, the pleiotropic features of the human disorder,

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[3] The abbreviations used are: NER, nucleotide excision repair; XP, xeroderma pigmentosum; CS, Cockayne's syndrome; TTD, trichothiodystrophy; GGR, global genome repair; MEF, mouse embryonic fibroblast; MED, minimal erythema dose; UDS, unscheduled DNA synthesis; DMBA, 7,12-dimethylbenz[a]anthracene; TCR, transcription-coupled repair; SCC, squamous cell carcinoma.

Table 1 *DNA repair characteristics of NER-deficient mice and MEFs*

Strain	UDS (%)[a]	RNA synthesis recovery (%)	D10 (J/m^2)[b]	MED (J/m^2)	Hyperplasia[c]	Cancer-prone[c]
Wild type	100	100	8	1500	−	NA[d]
XPA	<5	<5	0.9	150	++	++
CSB	>95	<5	1.3	150	++	+
XPC	30	>95	1.4	1500	+[e]	++
TTD	25	~20	4.9	1200	+/−	+

[a] The UDS values represent the mean of several independent experiments. For proper comparison, the data of all cell lines in the text are from one experiment.
[b] UV dose at 10% survival, deduced from Fig. 1.
[c] Epidermal hyperplasia and cancer development after genotoxic treatment. Data are complied (Refs. 28, 29, and 30 and this study). The relative severeness of the symptoms are indicated as far as differences in experimental setup between the studies allow.
[d] NA, not applicable.
[e] Data complied from the study by Sands *et al.* (29) and our unpublished results.

including growth delay, reduced fertility and life span, cutaneous abnormalities, and UV sensitivity of cultured fibroblasts. Like in patients, TTD mice displayed the remarkable brittle hair phenotype due to a reduction of hair-specific cysteine-rich matrix proteins. Having established a valid mouse model for TTD, this study presents further characterization of the repair defect of TTD mice and examines the crucial issue of cancer predisposition.

MATERIALS AND METHODS

NER-deficient Mice and Cell Lines. The term TTD mouse is used for mice homozygous for the *XPD*^{R722W} allele (27). Similarly, XPA, CSB, and XPC refer to mice homozygous for the targeted allele in the respective genes (28–30). Because no heterozygous effect was observed in any experiment, both heterozygous mutant and homozygous wild-type mice are referred to as wild-type. Mice used for generation of MEFs and determination of UV-induced hyperplasia were in mixed 129-C57Bl/6 background. Mice in the MED assay were mixed 129-C57Bl/6 (XPC and TTD) and pure C57Bl/6 (XPA and CSB). No effect of genetic background on MED in wild-type mice was noted.[4]

Human TTD fibroblast cell lines TTD7PV and TTD12PV, TTD1VI, and TTD1BEL were kindly provided by Drs. M. Stefanini (Istituto di Genetica Biochimica ed Evoluzionistica CNR, Pavia, Italy), A. Sarasin (Laboratory of Molecular Genetics, CNRS, Vuilejuij, France), A. R. Lehmann (MRC Cell Mutation Unit, Sussex University, Falmer, Brighton, United Kingdom), respectively.

DNA Repair Characteristics of MEFs. MEFs for all genotypes were obtained in our laboratory, as described before (27).

For UDS testing MEFs were seeded onto coverslips. The next day, cells were washed with PBS and irradiated at 16 J/m^2 UV-C (TUV lamp; Philips). Subsequently, cells were incubated for 2.5 h in culture medium containing 10 μCi/ml [^{3}H]-thymidine, fixed, and subjected to autoradiography as described before (31).

RNA synthesis recovery was measured according to the protocol of Mayne and Lehmann (32). In short, coverslip-grown cells were exposed to 10 J/m^2 of 254-nm UV light, labeled with [^{3}H]-uridine, and processed for autoradiography. The relative rate of RNA synthesis was expressed as the ratio of grains over UV-exposed to unexposed nuclei. In general, UDS values are very well comparable within an experiment, but show variation between experiments.

For UV survival assays, cell cultures were exposed to UV and allowed to grow for another 4–5 days before reaching confluency. Cells were labeled with [^{3}H]-thymidine, as described above, rinsed with PBS, and lysed. The number of proliferating cells in each dish was estimated by scintillation counting of radioactivity during a 3-h pulse-labeling. Cell survival was expressed as the ratio of irradiated over unirradiated cells.

Quantitation of UV-induced Inflammation. To determine the MED, mice were exposed to broadband UVB radiation from a filtered (Schott-WG305 filter) Hanovia Kromayer Lamp Model 10S (Slough, United Kingdom). This is a hand-held lamp that allows short exposures to limited skin areas (such as the ears) by placing the circular port (approximately 2 cm^2) in close contact to the skin (33). The dose rate was 150 J/m^2/s (280–400 nm), and each strain of mice was examined at least at five different doses in triplicate.

Besides macroscopic evaluation of edema and erythema reactions, the increase in skin thickness was determined as a value for acute UVB effects. Ear skin was exposed to the Kromayer Lamp because ears do not contain a fur (shaving was not necessary). Ear thickness was measured before and 24 h after UVB exposure using an engineer's micrometer (Mitutoyo model 193-10; Veenendaal, the Netherlands). The lowest dose that was able to induce a significant swelling response (*i.e.*, edema reaction) was denoted to be the MED for that strain of mice.

UV- and DMBA-induced Skin Effects and Carcinogenesis. Acute effects in the skin of shaven wild-type CSB and TTD mice were assessed by exposure to 100 J/m^2/day UV-B light (250–400 nm; American Philips F40 sun lamps) during 4 consecutive days. Skin samples were obtained from two mice per genotype, 24 h after the last exposure and were routinely processed (H&E staining) for histopathology.

UV-induced carcinogenesis was studied by chronically exposing the shaven back of 8 TTD and 13 wild-type mice (starting age, 8 weeks) to UVB light using an incremental-dose protocol starting at 80 J/m^2/day and gradually increasing to 670 J/m^2/day (250–400 nm). Timer-controlled American Philips F40 sunlamps were positioned 33 cm above the cage and yielded a dose rate of 13.3 J/m^2/min (250–400 nm). Chemically induced carcinogenesis in the mouse skin was tested with DMBA in the complete carcinogenesis protocol (30). Shaven TTD mice and wild-type litter mates (15 per genotype, 8–12 weeks of age) received 20 weekly applications of 10 μg of DMBA dissolved in 100 μl of acetone.

Mice were checked for tumor appearance once a week. Skin tumors were routinely processed for histopathological examination.

RESULTS

Repair Characteristics and Genotoxic Sensitivity of NER-deficient Cells. We first systematically compared various DNA repair parameters in primary MEFs established from wild-type mice, XPA mice with a complete NER deficiency (30), XPC mice carrying a selective defect in GGR (29), CSB mice with a specific TCR defect (28), and TTD mice (27). The UV-induced UDS level is considered a parameter mainly for GGR activity because TCR-defective CSB cells have UDS levels in the wild-type range (Table 1), whereas GGR-deficient XPC MEFs display ~30% residual repair synthesis. TTD MEFs exhibit only ~20–40% UDS similar to cells of the corresponding patients. A significant proportion of this is derived from TCR because inactivating this NER subpathway in CSB/TTD double mutant MEFs reduces residual UDS to less than half (data not shown). TCR activity was assessed indirectly by analysis of the cellular capacity to perform RNA synthesis 16 h after UV irradiation (32). As expected, persistence of lesions in transcriptionally active DNA reduces RNA synthesis in TCR-deficient XPA and CSB MEFs (Table 1), whereas XPC MEFs exhibit a response in the wild-type range. In TTD MEFs, RNA synthesis recovery is only ~20% of wild type, comparable with what we found in TTD1VI cells (data not shown). This indicates that a small but significant level of residual TCR is present in TTD. Thus, both GGR and TCR are clearly affected in TTD MEFs, but substantial residual repair activity for both subpathways persists.

[4] J. Garssen, unpublished data.

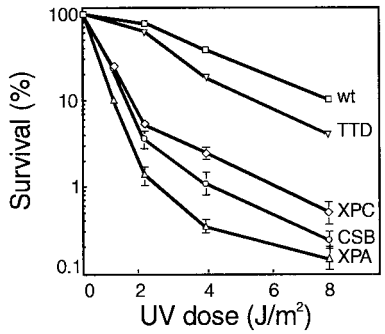

Fig. 1. Comparison of genotoxic sensitivity of various NER-deficient cells. UV survival of primary MEFs of NER-deficient mice is indicated. For proper comparison, all survivals were performed in one experiment. For each genotype, identical results were obtained with several other lines (data not shown). UV sensitivity has been described before for all cells used (27–30). *Bars*, the SE of the mean, omitted when smaller than the symbol.

To study the toxic consequences of defective repair, UV survival of TTD MEFs was compared with that of XPA, XPC, CSB, and wild-type MEFs (Fig. 1). The relative sensitivities of the different cell lines (XPA > CSB > XPC > TTD > wt) correlates very well with literature on the corresponding NER-deficient human cells (1, 3) and our own unpublished data. It is worth noting that the sensitivity of TTD MEFs to UV-induced cell killing is very mild (see Fig. 1 and Table 1). The modest hypersensitivity of TTD MEFs was confirmed using DMBA (a compound that induces bulky DNA adducts that are also substrates for NER; data not shown). To confirm that the XPD^{R722W} allele is associated with mild genotoxic sensitivity, both in mouse and man, we performed UV survival experiments with four human TTD fibroblast lines harboring the XPD^{R722W} allele. Under the conditions used, UV sensitivity was in the same order as for TTD MEFs, again very mild when compared with XPA cells (data not shown).

***In Vivo* Sensitivity of NER-deficient Mice.** UV irradiation has two very distinct effects on the skin. First, acute UV-induced inflammation occurs, macroscopically characterized by erythema (redness) and edema (swelling) of the skin. This effect is predominantly caused by lesions in actively transcribed DNA (34) and, thus, serves as a parameter for TCR. Long-term exposure causes scaling of the skin, histologically characterized by hyperkeratosis and hyperplasia of the epidermis, which is due to persistence of lesions in transcribed genes, as well as the genome overall. Though photosensitivity is reported in TTD patients, the severity of either symptom is not known. TTD mice allowed us to characterize these parameters *in vivo.* UV-induced inflammation is expressed as the minimal UV dose required to induce edema (MED). As shown in Table 1, the TCR defect of XPA and CSB mice is associated with low MED and TCR proficiency in XPC mice with a response in the wild-type range. TTD mice have a slightly reduced MED compared with wild-type and XPC mice (1200 J/m^2 *versus* 1500 J/m^2), but not nearly as outspoken as XPA and CSB mice (150 J/m^2), consistent with the idea that TCR is only partially affected in TTD mice.

Susceptibility to UV-induced hyperplasia of TTD mouse epidermis was examined by irradiating the shaven backs of wild-type, TTD, and CSB mice on 4 consecutive days with 100 J/m^2 UVB. As depicted in Fig. 2, epidermis of irradiated CSB mice displayed hyperplasia as reported before (28), whereas both wild-type and TTD mice were not detectably affected at this low UV dose. Similarly, only mild hyperplasia and inflammation was noted on histological sections of TTD skin, but not wild-type skin after six treatments with 10 μg of DMBA (data not shown).

In conclusion, comparison with NER-deficient mice demonstrates that TTD mice are mildly sensitive to genotoxic agents *in vivo.*

UV Light Induced Skin Cancer Susceptibility. It was of special interest to investigate cancer predisposition under experimental conditions in the TTD mouse model in view of the notion that TTD patients, despite their NER deficiency, do not seem to be cancer-prone. To this end, 8 TTD mice and 13 wild-type mice were chronically exposed on the shaven back to low daily doses of UV light. As the experiment continued, it became apparent that the TTD mice failed to develop clear cutaneous scaling and eye lesions as registered in XPA and CSB mice, despite the fact that the cumulative UV dose for the TTD mice was much higher than that used in the XPA and CSB experiments (103 kJ/m^2 *versus* 25 and 50 kJ/m^2, respectively (28, 30) After 18 weeks, TTD mice started to develop multiple tumors

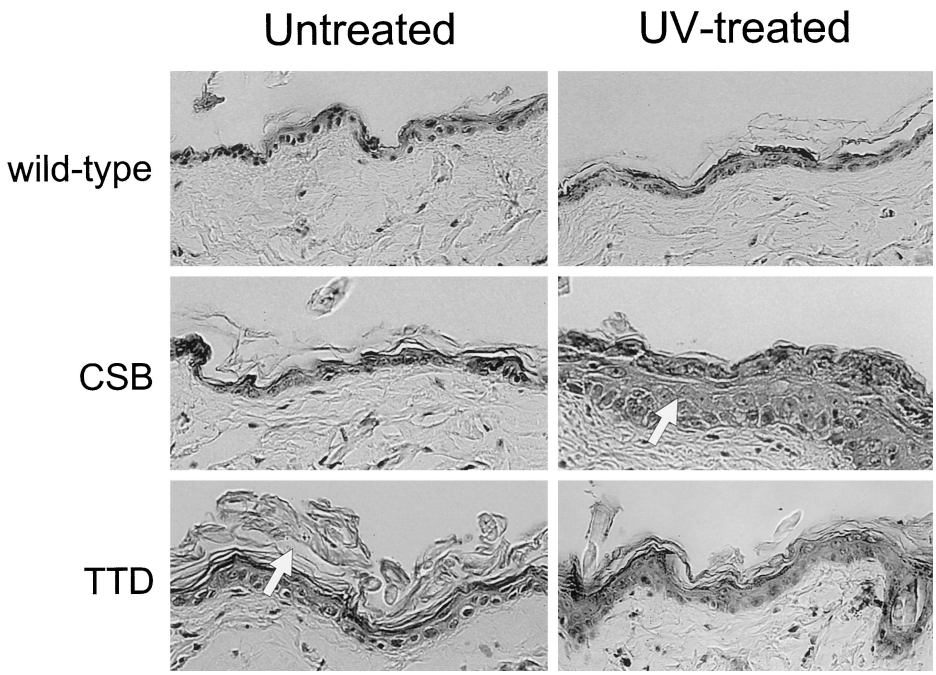

Fig. 2. UV-induced hyperplasia in CSB mice, but not in wild-type and TTD mice. Skin sections of TTD, wild-type, and CSB mice are indicated, untreated or treated with UVB (100 J/m^2/day) for 4 consecutive days on the shaven back. Skin samples were taken 24 h after the final irradiation. Note the thick cornified layer in TTD skin (*arrow*) and hyperplasia in UV-irradiated CSB skin (*arrow*). The difference in thickness between treated and untreated TTD skin lies within the normal range observed.

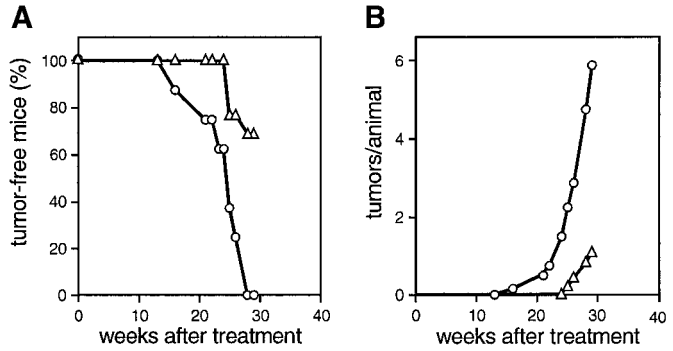

Fig. 3. UV induced skin tumor development in wild-type and TTD mice. Shaven mice were subjected to an UVB carcinogenesis protocol (see "Materials and Methods"). △, wild-type mice; ○, TTD mice. *A* and *B*, incidence, latency, and yield of skin tumor formation after UV treatment. The cumulative dose is 103 kJ/m^2 (250–400 nm).

in UV-exposed areas (Fig. 3, *A* and *B*), which were histopathologically identified as SCCs. From week 27 onward, tumors appeared in wild-type mice. The significantly reduced latency time of developing tumors ($P < 0.01$) together with the overt increased tumor yield demonstrate that TTD mice are more susceptible to UV carcinogenesis than wild-type mice.

DMBA-induced Skin Cancer Susceptibility. To confirm the cancer proneness of TTD mice, we subjected 15 wild-type and 15 TTD mice to a complete DMBA carcinogenesis protocol, by weekly applying 10 μg of DMBA to the shaven back for a period of 20 weeks. After 12 weeks TTD mice started to develop skin tumors, whereas the first tumor in wild-type mice was only observed after 21 weeks (see Fig. 4, *A* and *B*). Despite the very weak cytotoxic effect of DMBA application to the skin, the clear decrease in latency time and the dramatic increase in tumor yield demonstrate that TTD mice are prone to chemical-induced skin cancer.

Tumors on the skin of TTD mice were histopathologically identified as SCCs (Fig. 4*C*) and papillomas (Fig. 4*D*) at a ratio of 2:3 (Table 2), and one tumor was classified as a fibrosarcoma. In contrast, wild-type mice developed predominantly SCCs, whereas, using a similar protocol, XPA-deficient mice only developed papillomas (Ref. 30 and this study).

DISCUSSION

Since the identification of CS and TTD as DNA repair disorders, the paradoxical absence of skin cancer predisposition in these NER-deficient patients has been puzzling. The set of NER-deficient mice generated in our and collaborating laboratories allows a quantitative and *in vivo* approach toward elucidating the role of DNA damage and repair in the multistep process of carcinogenesis. This study describes cellular repair parameters, quantitation of UV-induced inflammation of the skin, and carcinogenic properties of TTD mice compared with other NER-deficient mouse mutants. In contrast to the human syndrome, TTD mice are clearly predisposed to develop skin cancer, although not as cancer-prone as XPA mice.

Repair Defect in TTD Mice. The TTD-specific *XPD*^{R722W} allele, which we mimicked in the mouse genome, is associated with a clear, but partial, DNA repair defect in human fibroblasts. RNA synthesis recovery was severely, but not completely, abolished (Table 1). Moreover UDS, UV-induced mutagenicity levels in a plasmid-based study (35) and UV survival using a protocol with noncycling cells were comparable between TTD/XPD^{R722W} fibroblasts and fibroblasts from XP patients of complementation group D (12, 25).

Despite the repair defect, TTD mouse skin appears not very sensitive to either UV-induced inflammation (only slightly more sensitive than wild-type mice) or hyperplasia induced by UV or DMBA. In TTD patients, photosensitivity of the skin has been reported, but ethics constrain experimental quantitation of this symptom. In addition, mild pigmentation abnormalities on sunlight exposure, one of the hallmark features of XP, have been reported sporadically in TTD patients (25, 36), suggesting that the TTD repair defect resembles XP in this respect, albeit to a mild extent. *In vivo* photosensitivity in TTD mice and patients may be moderated by UV shielding by the thick hyperkeratotic epidermis. However, also the eyes of TTD mice, which

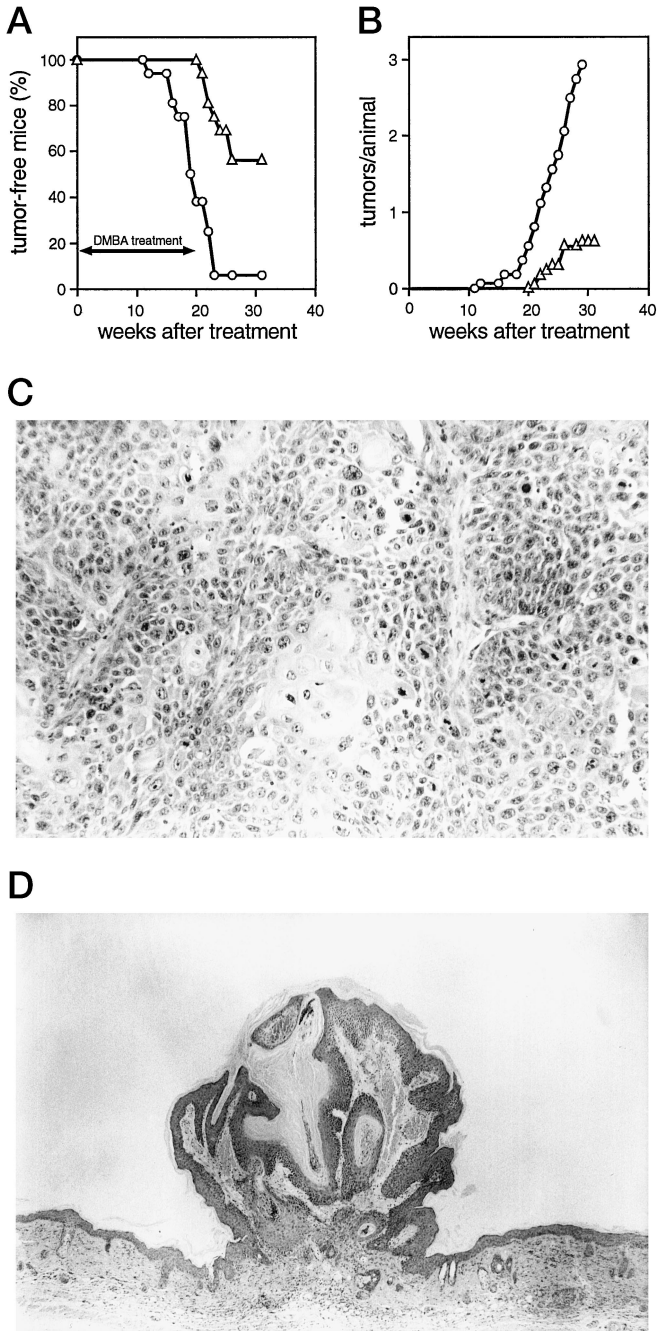

Fig. 4. DMBA induced skin tumor development in wild-type and TTD mice. Shaven mice were subjected to a DMBA carcinogenesis protocol (see "Materials and Methods"). △, wild-type mice, ○, TTD mice. *A* and *B*, incidence, latency, and yield of skin tumor formation after DMBA treatment. Histopathological examination of DMBA-induced skin tumors in TTD mice shows mixture of SCCs (*C*) and papillomas (*D*).

Table 2 *DMBA-induced tumorigenesis in TTD, wild-type, and XPA-deficient mice*

	Papilloma	SCC
XPA[a]	100%	ND[b]
TTD[c]	40% ($n = 18$)	60% ($n = 28$)
Wild type	10% ($n = 1$)	90% ($n = 9$)

[a] de Vries *et al.* (30).
[b] ND, not determined.
[c] Present study.

are histologically normal,[5] fail to display the extreme UV-induced lesions of chronically exposed XPA mice (37). Instead, the mild genotoxic sensitivity in TTD seems to be established at the cellular level for several reasons. First, under the experimental conditions used here, the TTD MEFs and the fibroblasts of four TTD patients carrying the same XPD^{R722W} allele display a comparable mild UV sensitivity. Mild UV sensitivity seems to be a more common feature among TTD fibroblasts, although some heterogeneity is apparent.[6] Second, the mutational spectrum in TTD cells, considered as a fingerprint of the repair defect, resembles more of wild-type than of XP-D cells (35, 38), and TTD cells are less sensitive to UV-induced transcription inhibition of the *ICAM-1* marker gene than XP-D cells (39). A possible rationale for the milder consequences of the TTD repair defect was provided by Eveno *et al.* (40), who showed that photosensitive TTD cells have defective CPD repair but (partially) proficient repair of 6-4PPs. This was confirmed by Marionnet *et al.* (41), who demonstrated that CPDs are the predominant mutagenic lesions in TTD cells. Lesion-dependent efficiency of repair may, thus, underlie the mild sensitivity of NER-deficient TTD cells, but this has not been analyzed in the TTD mouse yet.

TTD Syndrome and Cancer Proneness. An important and intriguing enigma associated with TTD is the observation that repair-deficient TTD patients seem to be free of cancer despite their NER defect, which is in striking contrast to XP and XP/CS cases from the same XP-D complementation group (3). Previously, differences in catalase activity (42), natural killer cell activity (43), and apoptotic response (44) have been reported between TTD and XP-D patients, but the relative importance of each of these parameters has not yet been studied in detail. Obviously, they may be addressed *in vivo* in the TTD mouse mutant. The most significant observation reported here is that TTD mice exhibit enhanced UV- and chemical-induced cancer susceptibility. This shows that TTD does not somehow intrinsically protect against skin cancer and is in agreement with the dogma that a defect in NER predisposes to cancer, but is in apparent contrast with clinical data. Notably, as much as the experimental setup allowed, TTD mice seemed less cancer-prone than XPA mice. The reduction in latency of UV-induced tumors was less pronounced in TTD mice compared with XPA mice (30), and the tumor type induced by DMBA was intermediate: XPA and wild-type mice develop predominantly papillomas and SCCs, respectively, whereas TTD mice develop a mixture of both tumor types. We propose that the molecular characteristics of the TTD repair defect, reflected by low UV sensitivity, imposes less severe predisposition to UV-induced skin cancer in TTD and patients than in XP. Furthermore, possible and established physiological differences between mouse and man (*e.g.*, in metabolic rate, immune system, apoptotic response, spontaneous and induced mutation rates, and certain repair parameters) may influence the difference in cancer proneness differently in the two species. However, additional factors may also explain the paradoxical absence of skin cancer development in TTD patients.

XP and TTD are clinically very different syndromes of which a defect in basal transcription is thought to participate in the clinical outcome of TTD. Discussion of the difference in cancer predisposition between XP and TTD should include possible consequences of repair- and transcription-related phenotypes on tumor development. For instance, the thick cornified layer of TTD patients will shield the underlying proliferating keratinocytes of the basal layer significantly from UV irradiation and, thus, reduce the mutagenic dose. Furthermore, TTD keratinocytes, the target cells for skin tumorigenesis, have a defect in late stages of terminal differentiation (27) that might have an impact on transformation to a tumor cell. At least as relevant is the severity of the disease and the young age at which many TTD patients die, which probably does not allow time to accumulate enough damage to develop skin tumors, as suggested previously for CS (28). This is certainly likely for the five patients with the R722W mutation because they were all severely affected and died very young (at least four before age 5; Ref. 25). In conclusion, the experimental mouse model reveals that TTD syndrome in man may be associated with hitherto unnoticed cancer proneness, although the residual repair activity of TTD cells protects largely against the cytotoxicity and carcinogenicity of UV. This may be particularly relevant under normal conditions when the low damage load does not exceed the limited DNA repair capacity of the TTD cells.

[5] T. Gorgels, unpublished data.
[6] N. G. J. Jaspers and A. Raams, personal communication.

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