CUTANEOUS MALIGNANCY

Global Contributions to the Understanding of DNA Repair and Skin Cancer

Kenneth H. Kraemer and John J. DiGiovanna

Dermatology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, Maryland, USA

Correspondence: Kenneth H. Kraemer, E-mail: kraemerk@nih.gov

doi:10.1038/skinbio.2014.3

Living organisms exposed to sunlight have developed mechanisms to repair the damage that UVR induces in their DNA. These DNA repair systems are present in most life forms including bacteria, yeast, rodents, and mammals. Three rare human genetic diseases have defects in the DNA repair system called nucleotide excision repair (NER). Patients with xeroderma pigmentosum (XP) have defective NER and a more than 10,000-fold increased frequency of skin cancer, whereas patients with Cockayne syndrome (CS) or with trichothiodystrophy (TTD) have NER defects without increase in cancer. These are strikingly different clinical phenotypes (Figure 1). The dramatic differences in their manifestations reflect the complexity of DNA repair and the broad scope and central role of DNA repair on preservation of diverse biological functions. This article will review the major milestones in our understanding of DNA repair and skin cancer (Table 1). These include recognition of the clinical diseases, understanding the types of damage sunlight causes in the DNA, recognition of DNA repair in different organisms, discovery of defective DNA repair in humans, cloning of multiple human DNA repair genes, and identification of UV-type mutations in skin cancer.

THE FIRST HUNDRED YEARS: CLINICAL DISEASE IDENTIFICATION

Moriz Kaposi made many important contributions to dermatology in the late nineteenth century. He moved from Hungary to Vienna, changed his name from Moriz Kohn, married the daughter of Professor Ferdinand Hebra, and co-authored a famous text with him (Hebra and Kaposi, 1874). In this early textbook of dermatology, he presented an 18-year-old girl with ''xeroderma or parchment skin'' (Hebra and Kaposi, 1874). There was an exquisitely detailed description of the pigmentary changes of her skin with abrupt cutoff at sun-shielded sites. She also had eye involvement. The skin was described as ''parchment like and wrinkled.'' This patient died of ''cancer of the peritoneum.'' A second patient was a 10-year-old girl who developed a large ''epithelioma'' of her nose in addition to lesions on her cheek and upper lip that were ''fissured tubercle of the size of a pea,'' thus suggesting the presence of skin cancers. XP with neurological abnormalities was described in 1883 by Albert Neisser from Germany (who also discovered the bacteria that causes gonorrhea, *Neisseria*; Neisser, 1883) and then again in 1932 by the Italians, Carlo deSanctis and Aldo Cacchione (deSanctis–Cacchione syndrome; de Sanctis and Cacchione, 1932). The well-studied groups of XP patients were reported from the US National Institutes of Health (NIH) in 1974 (15 patients) (Robbins *et al.*, 1974) (Figure 1a–c) and in 2011 (106 patients) (Bradford *et al.*, 2011), and from Japan in 1977 (50 patients) (Takebe *et al.*, 1977) and in 1994 (29 patients) (Nishigori *et al.*, 1994). An extensive literature review in 1987 described clinical features of 830 XP patients (Kraemer *et al.*, 1987). XP patients under 20 years of age were found to have a more than 10,000-fold increased risk of development of skin cancer (basal cell carcinoma, squamous cell carcinoma, or melanoma) (Kraemer *et al.*, 1994; Bradford *et al.*, 2011).

Edward Alfred Cockayne, in England, reported two siblings (7-year-old girl and 6-year-old boy) having dwarfism with retinal atrophy and deafness. Their faces were described as ''small with sunken eyes... with short, slender trunks and unduly long legs, and their feet and hands are too large in proportion.'' They ''appear to be a little below the average in intelligence and are far more excitable and laugh much more readily than children of normal mentality…'' (Cockayne, 1936). A follow-up in 1946 reported that the children had ''deeply sunk eyes and partial absorption of the orbital fat.'' Cataracts had been removed and there was sunburning and marked weight loss (Cockayne, 1946). In 1992, Martha Nance and Susan Berry in the United States (Nance and Berry, 1992) published a comprehensive literature review of 140 CS patients. These patients frequently had poor growth and neurologic abnormality, sensorineural hearing loss, cataracts, pigmentary retinopathy, photosensitivity, and dental caries (Figure 1d). The mean age at death was 12.5 years. There were no reports of cancer.

TTD was initially described by Chong Hai Tay (Tay, 1971) as ''ichthyosiform

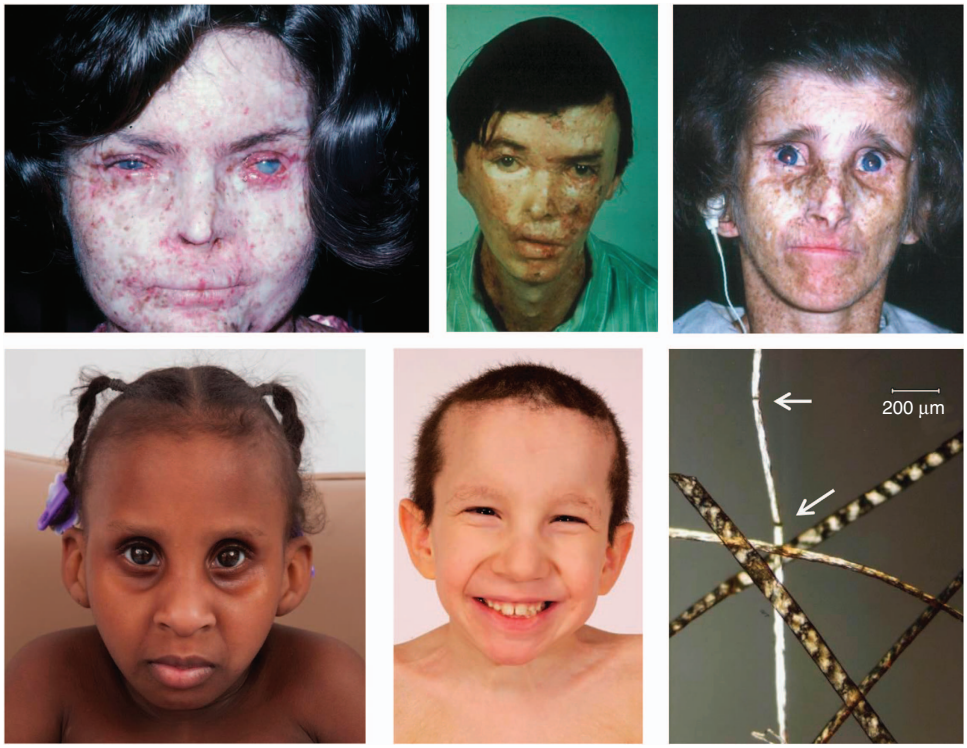

Figure 1. Diverse clinical phenotypes of the DNA repair disorders. (**a**) Xeroderma pigmentosum (XP) patient (XP1BE), age 28 years, has evidence of advanced UV damage including lentiginous hyperpigmentation with hypopigmented areas, clouding of the corneas of the eyes, damage to the eyelids, atrophy and telangiectasias of the lips, and scarring from multiple surgeries for basal cell carcinomas, squamous cell carcinomas, and melanomas. She died at age 49 years of widespread metastatic uterine adenocarcinoma (Lai *et al.,* 2013). Her cells are in XP complementation group C with 15% to 25% of normal unscheduled DNA synthesis (UDS) (see more details in Robbins *et al.,* 1974). (**b**) XP variant (XP4BE), age 27 years, has relatively unpigmented scars at sites of grafts from uninvolved skin used to cover sites of cancer surgeries. He had multiple skin cancers and died at age 27 years from widely metastatic melanoma (Wang *et al.,* 2010). His cells had normal UDS. He was the first XP variant patient described (see more details in Robbins *et al.,* 1974). (**c**) XP/Cockayne syndrome (CS) complex patient (XP11BE), age 28 years, has the sunken eyes, wizened facial features of CS in association with the lentiginous hyperpigmentation, and hypopigmentation of XP. She had multiple skin cancers. She died at age 33 years of possible arteriosclerosis. Her cells are in XP complementation group B with 3% to 7% of normal UDS. She was the first XP/CS complex patient described (see more details in Robbins *et al.,* 1974). (**d**) CS patient (CS507BE), age 7 years, has sunken eyes of CS. She has microcephaly, markedly short stature, developmental delay, sensorineural hearing loss, bilateral optic atrophy with retinal degeneration, and sun sensitivity. She is fed with a g-tube. She died at age 9 years. (**e**) Trichothiodystrophy (TTD) patient (TTD351BE), age 9 years, with short, brittle hair, sparse eyebrows, bright smile, and happy, socially interactive demeanor characteristic of TTD. He died at age 15 years of prolonged complications following hip surgery. His cells are in XP complementation group D with 115% UDS (see more details in Boyle *et al.,* 2008). (**f**) Microscopic appearance of hair from TTD patients. Tiger tail banding of the hair shafts viewed under polarized microscopy is characteristic of TTD. Arrows show areas of sharp breaks (trichoschisis) in the hair shafts (see more details in Liang *et al.,* 2006). All patients, or their parents, provided written permission for publication of their images. Previously published images are used with permission.

erythroderma, hair shaft abnormalities, mental and growth retardation,'' a new recessive disorder, in three children in a Chinese family in Singapore. The hair shafts were easily broken and showed pili torti and trichorrhexis nodosa-like defects and polarized microscopy revealed an alternating pattern of light and dark transverse bands. This ''tiger tail banding'' along with low sulfur content was also described in the United States by Howard Baden (Baden *et al.,* 1976), and by Vera Price (Price *et al.,* 1980) who gave the disorder the name trichothiodystrophy (sulfur-deficient brittle hair). A comprehensive review of the literature from the NIH reported on 112 published TTD cases (Faghri *et al.,* 2007). TTD patients commonly had developmental delay/intellectual impairment, short stature, ichthyosis, abnormal birth characteristics, ocular abnormalities including juvenile cataracts, infections, and photosensitivity (Figure 1e and f). Surprisingly, there was a high frequency of maternal pregnancy complications. TTD patients had a high mortality with a 20-fold increased risk of death under the age of 10 years. There were no reports of cancer in classical TTD patients; however, patients who have both TTD and XP (the XP/TTD complex) had increased risk of skin cancer (Broughton *et al.,* 2001).

THE 1950s–1960s: DISCOVERY THAT UV–DNA PHOTOPRODUCTS CAUSE DNA DAMAGE

In 1953, James Watson and Frances Crick in England published the helical structure of nucleic acids in DNA (Watson and Crick, 1953). After 7 years, Rob Buekers and W Berands in the Netherlands (Beukers and Berends, 1960) reported that UV irradiation (254 nm) of a frozen solution of

Table 1. Timeline for milestones of DNA repair and skin cancer research

Dates	Milestone
	Clinical descriptions of diseases:
1874	Xeroderma pigmentosum (XP)
1936	Cockayne syndrome (CS)
1971	Trichothiodystrophy (TTD)
1950–1969	Identification of UV-induced DNA photoproducts: cyclobutane pyrimidine dimers (CPD), 6-4 pyrimidine-pyrimidone photoproducts (6-4PP)
1960s	Recognition of DNA repair in bacteria, repair replication
1968	Reduced nucleotide excision repair (NER) in XP
1960–1979	Defective DNA repair in humans: XP and CS complementation groups
1971–1975	Description of XP variant: normal NER with defective postreplication repair
1974	Description of XP/CS complex
1985	Identification of transcription coupled repair (TCR): removal of UV-induced CPD from active genes is more efficient than from the genome overall
1980–1999	Cloning of human NER repair genes
1993	Dual role of XPB and XPD helicases in NER and in transcription as part of basal transcription factor IIH (TFIIH)
1928	UV induction of skin cancer in mouse models
1941	Quantitation of UV induction of skin cancer in mice
1977	UV-treated thyroid cells produce tumors in fish, reduced by photoreactivation
1991	UV-type mutations in p53 tumor suppressor gene in squamous cell carcinomas of human skin
1994	Sunburn cells are apoptotic keratinocytes with inactivating mutations in p53. Sunlight-inducted mutations in p53 in skin act as both a tumor initiator and a tumor promoter

thymine in water produced an irradiation product: the thymine dimer with the dimers joined covalently with a cyclobutane bond—the cyclobutane pyrimidine dimer (CPD) (50 years later, the theories and experiments that were used to make this seminal discovery were recollected by one of these scientists (Beukers *et al.,* 2008)). These UV-induced photoproducts could be repaired. Although photoreactivation, which directly reverses the CPD in DNA in the presence of long wavelength (UVA or visible) light, was known since the 1940s (Dulbecco, 1949; Kelner, 1949), other scientists in the United States found that yeast contained photoreactivating enzyme (Wulff and Rupert, 1962); Cook, 1967).

Reginald Deering and Richard Setlow in the United States (Deering and Setlow, 1963) reported that with longer wavelength, UV (280 nm) thymine dimers were formed, whereas with shorter wavelengths (239 nm) the reaction could be reversed (photoreversal). Using this information the husband and wife team of Richard and Jane Setlow performed a series of elegant experiments that showed a biological effect of thymine dimers (Setlow and Setlow, 1962). They demonstrated inactivation of transforming activity of DNA in bacteria and phage by 280 nm UV treatment and its reactivation by treatment with 239 nm UV. In 1963, Setlow *et al.* demonstrated that one thymine dimer was sufficient to inactivate replicating DNA in bacteria (Setlow *et al.,* 1963). Importantly, this same study reported that there were resistant strains that were able to overcome the block. This was an early suggestion that repair of DNA damage might occur (Setlow described this early research in a 2000 lecture in the NIH DNA Repair Interest Group video conference series ''Reflections on how I was led into and onto DNA repair'' http://videocast.nih.gov/launch.asp?10600).

The DNA photoproducts were found to involve pyrimidines and not purines. CPD were found with T = T and T = C. A third thymine containing photoproduct was later reported by the US scientists, Varghese and colleagues—the thymine–cytosine 6-4 photoproduct (6-4PP) (Varghese and Wang, 1967; Varghese and Patrick, 1969).

THE 1960s: DNA REPAIR DISCOVERED IN BACTERIA

In 1964 in the United States, Richard Boyce and Paul Howard-Flanders (Boyce and Howard-Flanders, 1964) and Setlow and William Carrier (Setlow and Carrier, 1964) independently reported that UV-resistant strains of *E. coli* remove thymine dimers from their DNA whereas sensitive strains do not. These papers suggested that a DNA repair process was involved in recovery from UV treatment. This process was called ''dark repair'' as removal occurred without additional light exposure, in contrast to photoreversal that required 240 nm UV and to enzymatic photoreactivation by photoreactivating enzyme that required long wavelength or visible light.

Phillip Hanawalt, after receiving his degree as the first graduate student of Richard Setlow at Yale University (Setlow, 2005), took a postdoctoral

fellowship in Denmark. There, he learned about BrdU—a nucleic acid analog of higher density than thymine. After returning to the United States, at Stanford University, Hanawalt used BrdU to analyze the location of newly synthesized DNA in bacteria following removal of thymine dimers. In cesium chloride density gradients, newly synthesized DNA containing BrdU would migrate at a different density than the parental DNA containing thymine. They performed BrdU pulse labeling experiments on UV-treated bacterial cells. A statement in the classic 1964 paper contains the basis of our current knowledge of DNA repair: ''Our findings are consistent with the view that in ultraviolet-resistant organisms a mechanism for repair replication exists in which damaged single-strand regions of the chromosome can be excised and replaced, using the undamaged DNA strand as template'' (Pettijohn and Hanawalt, 1964) (see also the 2002 video of Hanawalt's description of early DNA repair studies ''Half a Century of DNA Repair: an Historical Perspective; http://videocast.nih.gov/launch.asp?10587).

The search for the enzymes that carried out DNA repair yielded interesting results. Setlow reported an endonuclease in T4 phage that cut DNA at cyclobutane dimers (Setlow *et al.,* 1970). Intensive research by many labs found that removal of UV photoproducts in bacteria was primarily accomplished by the multiprotein UvrABC system (reviewed by a pioneer scientist, Larry Grossman in the United states (Grossman *et al.,* 1988) (see the 1999 video of Grossman ''Four decades of DNA repair: from populations of molecules to populations of people'' http://videocast.nih.gov/launch.asp?10595). However, this is a different DNA repair system from that in higher organisms.

THE 1960s–1970s: DNA REPAIR DEFECTS IN HUMAN CELLS

In 1964, Donald Rasmussen and Robert Painter in the United States reported evidence of repair replication in mammalian cells by finding increased incorporation of radioactive thymidine following UV exposure to cultured hamster and human (HeLa) cells (Rasmussen and Painter, 1964). By use of autoradiography, they also showed that, unlike the scheduled S-phase DNA synthesis, this post-UV thymine incorporation occurs in nearly all cells in all phases of the cell cycle and thus is ''unscheduled DNA synthesis'' (UDS).

James Cleaver, working as a postdoc in the Painter laboratory, read a newspaper article about patients with sun sensitive disorder, XP, who had increased skin cancer risk. In 1968, he reported that cultured skin cells from XP patients had reduced post-UV repair replication (measured by use of H^3-BrdU in cesium chloride gradients) and reduced UDS (measured by autoradiography) compared cells from normal donors (Cleaver, 1968). This seminal result thus linked defective DNA repair to increased cancer risk (see 2001 video of Cleaver ''Mending human genes'' http://videocast.nih.gov/launch.asp?10561).

Setlow *et al.* wrote in 1969 that they based their work on previous studies in bacteria that found that the mechanism of excision of UV induced dimers is a multistep process involving ''(1) a single-strand break on one side of a dimer; (2) dimer excision as a result of a second break on the same strand; (3) repair replication which fills in the resulting gap; and (4) closure of the gap by ligase action'' (Setlow *et al.,* 1969). In an analogous manner, they reported that cultured skin cells from an XP patient did not remove UV-induced thymine dimers from their DNA and did not perform the first step involving DNA strand breaks required for excising dimers. This finding pointed to dimers as playing an important role in UV-induced skin cancer in humans.

In Rotterdam, The Netherlands, the group led by Dirk Bootsma fused cultured fibroblasts from XP patients with neurological abnormalities with cultured fibroblasts from XP patients without these abnormalities. They then performed post-UV UDS assay with autoradiography. They found that certain combinations of cells had increased UDS, indicating that each cell provided what the other was lacking—they complemented the repair defect (De Weerd-Kastelein *et al.,* 1972). This implied that each complementation group had a different genetic defect.

In the United States, Jay Robbins began studying patients with XP at the NIH by examining patients with different clinical features (Robbins *et al.,* 1974) (Figure 1a–c). There were eight XP patients with abnormalities confined to their skin and eyes. Five XP patients, in addition, had progressive neurological degeneration. One XP patient had skin and eye abnormalities plus neurological degeneration with extremely short stature and immature sexual development—features of a second disorder, CS—the first patient described with the XP/CS complex (Figure 1c). All 14 of these XP patients had reduced UDS. Cell fusion studies revealed that they represented four XP complementation groups (Kraemer *et al.,* 1975a). As the cells assigned to each complementation group had a similar range rate of residual DNA repair, the XP complementation groups were named in order of increasing residual repair (XP-A to XP-D) (Kraemer *et al.,* 1975a). An arrangement between the NIH scientists and the group in Rotterdam set the model for collaboration in the early stages of these investigations that has followed to the present day. These groups exchanged cell lines and determined that there were five XP DNA repair complementation groups (XP-A to XP-E) (Kraemer *et al.,* 1975b). In 1979, a Japanese group reported a patient with low UDS and mild disease, forming complementation group XP-F (Arase *et al.,* 1979). A Dutch group reported another patient with low UDS and no skin cancers forming complementation group XP-G (Keijzer *et al.,* 1979) (see also the 2010 video of Kenneth Kraemer and Vilhelm Bohr ''History of DNA repair: Four decades of DNA repair at NIH and the first twenty-five years of the DNA Repair Interest Group'' http://videocast.nih.gov/launch.asp?15954).

One of the NIH patients (XP4BE) had severe disease but normal UDS in skin and blood cells (Burk *et al.,* 1971a, b; Robbins and Burk, 1973; Robbins

et al., 1974) (Figure 1b). These patients have been called XP variants (Cleaver, 1972). In 1975, Alan Lehmann and colleagues in the United Kingdom reported that XP variant cells had normal NER but defective post-replication repair, a process of DNA synthesis after UV irradiation (Lehmann *et al.,* 1975). They also showed that, unlike normal cells, this process was inhibited by caffeine. see also the 2013 video of Lehmann ''Human DNA Repair Disorders: A Historical Perspective 1968–2013'' http://videocast.nih.gov/launch.asp?17965).

In 1977, R Schmickel and colleagues in the United States reported that cultured skin fibroblasts from two patients with CS were hypersensitive to killing by UV but had normal survival after X-ray treatments (Schmickel *et al.,* 1977). CS cells had normal rate of removal of thymine dimers (Schmickel *et al.,* 1977) and normal UDS (Andrews *et al.,* 1978). In 1981, a Japanese group led by Kiyoji Tanaka found that CS cells had delayed recovery of DNA synthesis following UV exposure. They found that certain pairs of CS cells when fused would correct this defect forming complementation groups CS-A and CS-B (Tanaka *et al.,* 1981). Lehmann in England reported that certain pairs of CS when fused would correct a defect in post-UV recovery of RNA synthesis (Lehmann, 1982). This pointed to a defect in repair of active genes in CS.

In 1985, Vilhelm Bohr and Philip Hanawalt reported that in hamster cells, removal of UV-induced pyrimidine dimers from an active gene was more efficient than from the genome overall, i.e., preferential repair of actively transcribing genes (transcription coupled repair) (Bohr *et al.,* 1985). The following year, they demonstrated that post-UV survival of human cells correlated with efficient transcription coupled repair (Bohr *et al.,* 1986). Cells from an XP-C patient and hamster cells had similar overall repair levels. However, the XP-C cells had reduced repair of the dihydrofolate reductase (*DHFR*) gene and low survival, whereas the hamster cells had high repair of *DHFR* and normal survival. European researchers reported in 1990 that CS cells had defective post-UV repair transcription coupled repair and normal repair of nontranscribed regions of the genome (Venema *et al.,* 1990).

A letter to the editor of the *Journal of the American Academy of Dermatology* in 1985 by D. van Neste and colleagues from Belgium and France reported that cells from a photosensitive TTD patient had reduced post-UV DNA excision repair (Van Neste *et al.,* 1985). The following year, researchers from Italy reported that cells from four photosensitive TTD patients were in XP complementation group D (Stefanini *et al.,* 1986).

THE 1980s–1990s: CLONING DNA REPAIR GENES

The XP complementation groups that were reported in the 1970s were believed to represent different genes that when mutated would underlie XP clinical features. Identifying these genes turned out to be a difficult task. As working with human cells was very difficult, and DNA repair systems were present in most organisms, in the 1980s scientists in different parts of the world developed other model systems that were more amenable to experimentation.

Researchers in California mutagenized hamster cells with ethyl methane sulfate and were able to isolate >50 UV-sensitive mutant clones out of ~700,000 clones screened utilizing several selection methods (Busch *et al.,* 1980; Thompson *et al.,* 1980) (see the 2004 video of Larry Thompson ''Chinese Hamster Cells and DNA Repair—a Long Lasting Affair'' http://videocast.nih.gov/launch.asp?12877).

In 1984, the first human DNA repair gene was cloned by researchers in the Netherlands. This human gene corrected the defect in hamster group 1 and was named *ERCC1* (*Excision Repair Cross Complementing 1*) (Westerveld *et al.,* 1984). It was not until 2007 that a patient was reported to have a defect in *ERCC1* (Jaspers *et al.,* 2007). This severely affected child had marked developmental delay with features of a CS-like disorder, cerebro-ocular-facial syndrome, and died at age 14 months.

The group in the United States that generated the clones of UV-sensitive hamster cells succeeded in correcting the NER defect in UV-sensitive hamster complementation group 2—this gene was called *ERCC2* (Weber *et al.,* 1988). They then demonstrated that the addition of the *ERCC2* gene to the mutant hamster cells corrected a defect in excision of CPD (Regan *et al.,* 1990). The husband and wife team of Louise and Satya Prakash in the United States studied methyl methanesulfonate–induced mutants in yeast and isolated 22 complementation groups of radiation-sensitive mutants (Prakash and Prakash, 1977). The human *ERCC2* gene was found to have high homology to the yeast NER protein RAD3 (Weber *et al.,* 1990). *ERCC2* was later found to be defective in cells in XP complementation group D from patients with XP (Takayama *et al.,* 1995) or with TTD (Takayama *et al.,* 1996).

In 1989, in Japan, after screening >100,000 colonies, Kiyoji Tanaka succeeded in isolating a clone of mouse DNA that corrected the UV hypersensitivity of an XPA cell line (Tanaka *et al.,* 1989). The following year, he reported cloning of the human gene on chromosome 9 that corrected the XP complementation group A cells (Tanaka *et al.,* 1990) and described a common founder splice mutation that was present in 20 of 21 Japanese XP-A patients (Satokata *et al.,* 1990). The Japanese researchers used embryonic stem cells to establish *XPA*-deficient mice. These mice had defective NER and were highly susceptible to UVB or chemical (9,10-dimethyl-1,2-benz[a] anthracene)-induced skin carcinogenesis (Nakane *et al.,* 1995). This study clearly provided *in vivo* evidence that XPA protein protects mice from carcinogenesis induced by UV or chemical carcinogens.

The human *ERCC3* gene was cloned by its ability to correct UV sensitivity and post-UV UDS defect in a hamster mutant in complementation group 3 (Weeda *et al.,* 1990a). *ERCC3* was localized to human chromosome 2 (Weeda *et al.,* 1990a). *ERCC3* was

reported to correct the NER defect in cells from the only patient in XP complementation group B (Weeda *et al.,* 1990b), a woman with features of both XP and CS (Figure 1c) (Robbins *et al.,* 1974). Analysis of the 782 amino acid sequence of ERCC3 predicted that it would function as a helicase to unwind DNA (Weeda *et al.,* 1990b).

The human *ERCC5* gene that corrected the defect in hamster mutant complementation group 5 was isolated by a group from Los Alamos (Mudgett and Macinnes, 1990) and later reported to have homology to the yeast RAD proteins (*Saccharomyces cerevisiae* RAD2 and *Schizosaccharomyces pombe* rad13) (Macinnes *et al.,* 1993). In Japan, the group led by Masaru Yamaizumi reported that the *ERCC5* gene corrected the post-UV UDS defect and increased post-UV cell survival in human XP complementation group G cells (Shiomi *et al.,* 1994).

Researchers from the Netherlands isolated the human *ERCC6* gene that corrected the defect in hamster complementation group 6 (Troelstra *et al.,* 1990). Interestingly, the mutant hamster cells have deficient repair of CPD but normal repair of 6-4PP. In 1992, the same group reported that *ERCC6* is a putative helicase that is defective in CS-B cells and is involved in preferential repair of active genes (Troelstra *et al.,* 1992). The gene that is defective in CS-A cells was identified by a group in the United States (Henning *et al.,* 1995). This gene encodes a tryptophan-aspartic acid (WD) repeat that interacts with CS-B protein and also with the basal transcription factor IIH, TFIIH. By 2010, there were reports of mutations in 84 CS kindreds with 62% having mutations in the *CSB (ERCC6)* gene (Laugel *et al.,* 2010).

Randy Legerski in the United States used a library of human DNA that was cloned in an Epstein–Barr virus expression vector to isolate the *XPC* gene in 1992 (Legerski and Peterson, 1992). This group then reported different mutations in the *XPC* gene in five XP-C patients (Li *et al.,* 1993). The XPC gene product, complexed with HR23B, was shown to initiate global genome repair of UV damage (Sugasawa *et al.,* 1998).

Correction of hamster complementation group 4 was accomplished by use of a human cosmid library and isolation of the *ERCC4* gene that was localized to human chromosome 16p13.13–p13.2. The transformed cells had increased ability to excise thymine dimers from a plasmid in an *in vitro* assay (Thompson *et al.,* 1994). In human cells this protein was shown to correct the defect in XP-F cells and to be in close association with ERCC1, the protein that corrects the defect in hamster complementation group 1 (Sijbers *et al.,* 1996). The complex serves as a structure-specific endonuclease that makes the 5′ incision in NER (Sijbers *et al.,* 1996).

Using a UV-treated 8-nucleotide poly-dT fragment as bait, in 1988 Gilbert Chu in the United States isolated two factors that bind to damaged DNA (DDBF1 and DDBF2) but were absent from cells in XP complementation group E (Chu and Chang, 1988). The complementary DNA for the genes encoding these factors (*DDB1* and *DDB2*) was cloned by Stuart Linn's group in the United States and localized to human chromosome 11 (Dualan *et al.,* 1995). The protein products of these genes are a heterodimer of 127-kDa and 48-kDa subunits. The Linn group identified missense mutations in the p48 subunit on patients in XP complementation group E (Nichols *et al.,* 1996) (see the 2006 video Linn '' Life in the serendipitous lane: excitement and gratification in studying DNA repair'' http://videocast.nih.gov/launch.asp?13303). Examination of cell lines that had been classified as XP-E revealed that not all had mutations in *DDB2*. Detailed investigations by scientists in the United States and Italy revealed that the *DDB2*-negative cells had defects in other genes and that true XP group E patients had defects in *DDB2* (Rapic-Otrin *et al.,* 2003). Interestingly, wild-type rodent cells are defective in *DDB2* and have reduced global genome repair, causing some to question their use as models for carcinogenesis in humans (Tang *et al.,* 2000). *DDB2* was reported to be involved in global genomic DNA repair and its expression was dependent on p53, thereby linking the post-UV p53 responses such as cell cycle arrest and apoptosis to DNA repair (Hwang *et al.,* 1999). DDB2 is a ubiquitin ligase that is regulated in response to DNA damage (Groisman *et al.,* 2003).

In 1999, two groups using different approaches independently came to the same conclusions about the defective gene in the XP variants with clinical XP but normal NER (see above and Figure 1b) (Robbins *et al.,* 1974). The Prakash group in the United States working with *S. cerevisiae* yeast found that the *RAD30* gene functions in error-free bypass of UV lesions. They found that RAD30 encodes a DNA polymerase, Pol eta, that can efficiently replicate past a thymine dimer. They reported that the human homolog (*hRAD30*) is defective in XP variant patients (Johnson *et al.,* 1999). (see the 2003 video of Prakash ''Translesion synthesis DNA polymerases of yeast and humans'' http://videocast.nih.gov/launch.asp?10602). In Japan, a group led by Fumio Hanaoka used a cell-free assay system to isolate a DNA polymerase from human cells that continues replication on damaged DNA. They found that this polymerase, the human homolog of yeast Rad30 protein (Pol eta), was defective in XP variant cells and that recombinant Pol eta could correct the defect in DNA replication (Masutani *et al.,* 1999). Subsequent research discovered that there are several similar DNA polymerases (Y-family polymerases) that bypass lesions rather than removing them (reviewed in Sale *et al.,* 2012) (see also the 1999 video by Roger Woodgate ''Translesion DNA synthesis: From mutations in bacteria to the xeroderma pigmentosum variant phenotype in humans'' http://videocast.nih.gov/launch.asp?10601).

Following the identification of the genes involved in NER, the next challenge was to determine how they worked together to excise DNA damage. An early surprise was that the *ERCC3* gene (XP-B complementation group) was an adenosine triphosphate–dependent DNA helicase and a carboxy-terminal domain kinase that was involved in NER and also as part of the basal transcription factor IIH,

TFIIH, that is required for polymerase II class transcription (Schaeffer *et al.,* 1993). This finding provided a strong link between DNA repair and transcription. One additional feature of this link relates to the fact that transcription is necessary for survival. Therefore, mutations in the genes involved in TFIIH (*ERCC3* (XP-B) and *ERCC2* (XP-D)) leading to complete loss of activity would be lethal. This finding might also provide an explanation for the different phenotypes among patients with mutations in *ERCC3* and *ERCC2*. Some mutations would primarily affect NER leading to XP with increased cancer risk, whereas others would affect transcription leading to developmental defects such as short stature and developmental delay in XP/CS complex and in TTD (reviewed in Compe and Egly, 2012).

How the NER proteins interact was shown in elegant experiments by a group from the Netherlands in 2001. They performed local UV irradiation of cultured fibroblasts through isopore polycarbonate filters with pore diameters of 3 μm that are smaller than the size a cell nucleus. This was followed by serial immunostaining of normal or XP cells with different defects at different times after UV exposure. They used antibodies to CPD, 6-4PP, or to different NER proteins (Volker *et al.,* 2001). They found that in normal cells the damage recognition complex of XPC-hHR23B was essential for recruitment of all other NER factors including TFIIH. XPA was associated relatively later and was required for anchoring of ERCC1-XPF endonuclease and for activation of XPG endonuclease. In 2008 at NIH, this technique was used to show differences between XP cells and TTD cells with different defects in the *XPD* (*ERCC2*) gene (Boyle *et al.,* 2008). The XPC protein was rapidly localized in all cells. In normal cells, the other NER proteins (XPB, XPD, XPG, XPA, and XPF) were rapidly recruited and redistributed by 24 hours. In the XP cells, recruitment of the other NER proteins was delayed and persisted for 24 hours, suggesting their failure to be removed may function as a block to bypass repair. In TTD cells, recruitment of NER proteins was reduced and remained low at 24 hours. The TTD cells removed 6-4PP but not CPD, whereas the XP cells removed neither. These differences in NER protein recruitment and removal may be related to the differences in cancer susceptibility of the XP and TTD patients.

MECHANISMS OF UV-INDUCED CANCER

According to a detailed history of photobiology of the skin by Frederick Urbach, P Donald Forbes, Ronald Davies, and Daniel Berger from Philadelphia published in 1976, UV was discovered in 1801 by F Ritter in Germany (Urbach *et al.,* 1976). They attribute the discovery that UV causes skin erythema to P Charcot in France in 1858 who used electric arcs to generate UV. They credit the linkage of UV exposure to increased pigmentation and to skin cancer, as seen sailors and in patients with XP to P Unna in Germany in 1894. An early dermatoepidemiologic study by W Dubreuilh (1896) reported that in the Bordeaux region of France, keratoses and skin cancers were frequently observed in the workers in the vineyards, but only occasionally in the nearby city dwellers. A seminal paper by Karl Hausser and Wilhelm Vahle that presented most of the data that form current understanding of action spectrum of erythema and tanning was published in 1927. Hausser noted that hikes in the afternoon under the sun had almost no effect, whereas ''a brief sojourn on snow at noontime resulted in severe sunburn.'' This paper showed that skin erythema and pigmentation depended on the wavelength of UV radiation. The effect was limited to wavelengths shorter than 320 nm (UVB 280–320 nm) and variations in the amount of this UV that passed through the atmosphere were related to the season and time of day and explain the differences in sunburning (an English translation of this important paper by Urbach appears in the 1969 classic book ''The Biologic Effects of Ultraviolet Radiation – with Emphasis on the Skin'' (Hauser and Vahle, 1969)). Advances in instrumentation and measurement of the spectral composition of light resulted in better understanding of the factors influencing the radiation reaching the surface of the earth including the absorption characteristics of ozone, the angle of the sun, and scattering of UVB radiation by particles such as dust, haze, aerosols, and clouds (Schulze and Grafe, 1969).

The demonstration that UV irradiation of mouse skin produced tumors was published by GM Findlay in England in 1928 (Findlay, 1928). He reported that daily exposure of epilated back skin of albino mice to UV from a mercury arc lamp (output from 200 nm UV through 1,014 nm infrared) produced papillomas and carcinomas following 34 to 58 weeks of exposure. He found that this exposure period could be shortened by application of tar before the UV exposure.

The development of stable sources of UV and instrumentation enabled long-term studies of the quantitative measurement of induction of tumors on the ears of albino mice by Harold Blum and associates at the US National Cancer Institute in 1941 (Blum *et al.,* 1941). These studies indicated that a single dose of UV was not sufficient to induce tumors but there was a ''development time'' before the first tumors appeared that varied with the dose used. There was a constant dose–time relation and reciprocity held until the dose became too small to produce tumors during the life of the mice (Blum, 1969).

Using the Amazon molly fish that grows in clones, the Setlow group in New York injected UV irradiated molly thyroid cells into isogenic recipients. These cells produced tumors. The frequency of tumors was reduced by use of photoreactivation to remove CPD, thereby providing evidence that dimers in DNA can give rise to tumors (Hart *et al.,* 1977). In 1989, using a platyfish–swordtail hybrid fish model system, the Setlow group used different wavelengths of radiation to induce melanomas. They found that multiple exposures of >304 nm resulted in increased melanoma frequency over the background level (Setlow, *et al.,* 1989). These experiments included

UVA as well as UVB exposure. A recent review summarized the evidence that UVA induces CPDs that are mutagenic and alter cell cycle regulation (Runger *et al.,* 2012).

Douglas Brash and colleagues in the United States and Scandinavia examined squamous cell carcinomas of the skin from patients from New England and Sweden (Brash *et al.,* 1991). They performed DNA sequencing of the p53 tumor suppressor gene and found that a high frequency (58%) of invasive squamous cell carcinomas contain p53 mutations that alter the amino acid sequence. These mutations had features of UV mutagenesis including, occurring exclusively at dipyrimidine sites, a high frequency of C to T base substitutions, and having CC to TT double-base changes. These changes were different from those found in p53 mutations from internal cancers. In a follow-up study, this group examined precancerous actinic keratoses in the sun-exposed skin of patients from New England (Ziegler *et al.,* 1994). They found that p53 mutations were present in 60% of the actinic keratoses and 29% also had loss of a p53 allele. The mutations had UV-type features as in the earlier squamous cell carcinoma study. Adjacent actinic keratoses showed clones with different p53 mutations, indicating that they arose from independent photon events. Irradiation of mice resulted in the appearance of ''sunburn cells'' (apoptotic keratinocytes) that had mutations in p53. The sunburn cells were greatly reduced in p53-negative mice. They conclude that skin appears to possess a p53-dependent ''guardian of the tissue'' response to DNA damage that aborts precancerous cells. ''If this response is reduced in a single cell by a prior p53 mutation, sunburn can select for clonal expansion of the p53 mutated cell into an AK. Sunlight can act twice: as a tumor initiator and a tumor promoter'' (Brash describes this work in the 2001 video lecture presented to the Royal College of Pathologists in London http://videocast.nih.gov/launch.asp?10621).

Since Kaposi's 1874 description of patients with XP, it has been a long road to understanding the role of UV damage in causing skin cancer and of DNA repair in skin cancer prevention. Many questions remain. The precise clinical genotype/phenotype correlations within the NER-related clinical syndromes are not well defined. For some patients with clinical features diagnostic of XP and TTD, mutations in known genes have not been found, suggesting additional genes for discovery. Successful therapeutic interventions targeting correction of the underlying gene defects have not been achieved. But the pace of discovery is quickening, and we are confident that we will reach these future milestones faster than the time it has taken us to arrive at this point from Kaposi's observation 140 years ago.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGEMENTS

This research was supported by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research. We thank our research nurse, Debby Tamura, for assistance with the patient-related information. We want to express our appreciation to our XP and TTD patients and their families for their willingness to participate in our studies and to assist in education about these rare diseases.

TO CITE THIS ARTICLE

Kraemer KH, DiGiovanna JJ (2014) Global contributions to the understanding of DNA repair and skin cancer. *J Invest Dermatol* 134: E8–E17.

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