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Transcription coupled repair deficiency protects against human mutagenesis and carcinogenesis
Personal Reflections on the 50th anniversary of the discovery of xeroderma pigmentosum

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ABSTRACT

Xeroderma pigmentosum (XP) patients who lack the main damage recognition protein for global genome repair (GGR), XPC, have greatly increased skin cancer rates and elevated mutation frequencies originating from unrepaired ultraviolet photoproducts in the nontranscribed regions of the genome and in nontranscribed strands of expressed genes. But they show no increased mutations in transcribed strands. In contrast, cancer is absent from Cockayne syndrome (CS) patients that have defective transcription coupled repair (TCR) despite severe photosensitivity, CS patients remarkably show no elevation of UV induced mutagenesis implying that defective TCR may be protective against mutagenesis and carcinogenesis. Mutation avoidance in CS is postulated to occur through arrested transcription that generates a tripled stranded R loop consisting of DNA double strands and a nascent mRNA strand. R loops result in S phase apoptosis or activation of ATM kinase that causes a delay in DNA replication until TCR, or transcript cleavage by TFIIS or RNAaseH, relieves the transcription block. Resumption of replication then occurs on repaired DNA without concomitant mutagenesis.

1. Introduction

Progression in scientific research opens up new vistas through novel ideas and revolutionary technology. Along the way many observations are abandoned either through loss of interest, new generations of investigators, or lack of relevance to emerging areas of research. Some of these early observations may actually be forerunners of later ideas and deserve renewed attention.

As we near the 50th anniversary of the discovery of the human DNA repair deficient disease xeroderma pigmentosum (XP) first reported to a Radiation Research Society meeting in 1967 and published subsequently [1,2], it is appropriate to take a look at some of the unsolved issues in the DNA repair field. The discovery of XP as a defect in nucleotide excision repair (NER) of DNA damage caused by solar ultraviolet light (UV) was remarked as the first demonstration that cancer could be a genetic disease [3]: a concept that is rarely questioned today but was not generally understood at that time.

The decades after discovery of XP saw other diseases associated with DNA repair deficiencies including Cockayne syndrome (CS) [4,5], trichothiodystrophy (TTD, [5], Cerebro-Oculo-Facio-Skeletal syndrome (COFS) [6], and the UV sensitive syndrome (UV^S) [7]. A version of XP that lacks a low fidelity polymerase, Pol H, named the XP variant (XPV) was also discovered with symptoms that were not easily distinguished from other XP patients [8–10]. A disease associated with signaling from DNA double strand breaks and oxidative stress, ataxia telangiectasia (AT) was also identified [11]. Deficiencies in mismatch repair were identified with nonpolyposis colon cancer [12]. Fanconi's anemia and hereditary breast cancer were found associated with mutations in overlapping functions involving DNA–DNA crosslinks, oxidative damage repair and homologous recombination [13,14]. Not all genes associated with DNA damage and repair have been assigned to human diseases either due to embryo lethality, rarity, subclinical expression, or difficulty in recognition, so there may be others yet to be recognized.

I will focus on some long outstanding issues with the two NER deficient disorders: XP and CS. The difference between them lies in the respective branches of NER that are involved. XP is mainly associated

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with defects in global genome repair (GGR) by which both strands of expressed genes and nontranscribed regions of the genome are repaired. In normal cells an increased rate of repair of the transcribed strand of expressed genes is superimposed on GGR, which represents the transcription coupled repair (TCR) pathway [15–18]. Increased repair of the transcribed strand is missing from CS cells due to mutations in cofactors of RNA polymerase II: CSA. CSB or UVSSA, while GGR is normal. CSA (ERCC8) is a WD40 protein that is a component of the COP9 signalosome that ubiquitylates CSB targeting it for degradation releasing blocked forks [19](Shah and He, 2015). CSB facilitates the progress of RNA Pol II through damaged sites, and natural transcription pause sites [17,18]. UVSSA is a scaffold protein that interacts with CSA and CSB and recruits the deubiquitylation factor USP7 [19].

1.1. This theme

The NER diseases display a large range of clinical symptoms and organ involvement. Some of the genes have secondary functions in pathways of gene expression [20], chromatin remodeling [21] and mitochondria [22,23]. The range of symptoms and their underlying mechanisms involve major disruptions in transcription and spliceosome function that impact many cellular functions [20,24]. It remains difficult therefore to relate clinical symptoms to individual mutations since functional changes in both proteins themselves and protein–protein interactions play important clinical roles [25]. The ultraviolet sensitive syndrome (UV^S) is a case in point. Although this disorder of TCR is mainly caused by mutations in *UVSSA*, patients have also been reported with mutations in either *CSA* or *CSB* [26]. The latter most likely arise from mutations in protein sites that interact with UVSSA or have minimal impact on protein functions.

The causes of the major clinical symptoms are not, however, the subjects of this review. Rather it is the question of what is missing: specifically the absence of cancer in CS despite strong solar sensitivity [27] in stark contrast to the high levels of skin cancer, both melanoma and nonmelanoma, in XP [28,29].

Approximately half of all CS patients are severely photosensitive often with blistering sunburns [27,30,31]. In the general population such sunburns correlate with later cancers [32]. CS patients, however, have never been reported with skin cancer despite surveys covering over 350 patients in US, UK, Japan and elsewhere [27,30,31,33]. An open question is whether this absence of skin cancer is due simply to the patients' short life-span [27,30,31,33] or whether it represents a fundamental issue of the relationship between TCR deficiency, mutagenesis and carcinogenesis [34].

1.2. Mutations & cancer in xeroderma pigmentosum

The association between defective NER, mutagenesis and cancer in XP was evident early and continues to provide insights into mutational mechanism of carcinogenesis.

(a) Mutagenesis in XP

The discovery of DNA repair deficiency in XP led to the expectation that UV induced mutagenesis in XP cells should be increased. This expectation was soon fulfilled. Maher & McCormick, in a series of technically difficult experiments, measured mutation rates at the *HPRT* locus in primary XP fibroblasts [35–37]. These experiments demonstrated increased mutagenesis in several of the NER and polymerase defective XP fibroblasts. Sequencing studies of mutant colonies confirmed that mutations occurred at dipyrimidine sites, as expected.

Maher & McCormick also showed that holding cells in a non-proliferative state permitted NER to remove potentially mutagenic lesions in normal and XPC cells that retain a low level of repair but not XPA cells defective in TCR and GGR [35]. This important observation highlighted the need for DNA replication to fix damage into changed DNA sequence. This now seems obvious, but its relevance has resurfaced in understanding the absence of cancer and mutations CS, as we shall discuss later [34].

Another approach to studying mutagenesis was the use of the episomal plasmid pZ189 [38,39]. Extracellular irradiation of the plasmid and its passage through XP cells produced increased mutations in the plasmid that were sequenced and shown due to deficient repair of both cyclobutane dimers and non-dimer photoproducts.

(b) Cancer incidence in XP patients

In XP both nonmelanoma skin cancer (NMSC) and melanoma are increased, by factors of 10,000 and 2000 respectively. In XP SCCs occur earlier in life than melanomas, a reversal of that seen in non-XP patients [28,29]. These clinical observations indicate significantly different mechanisms in the two kinds of skin cancer, suggesting that the solar exposure and repair deficiency is a more important etiological factor for NMSC than melanoma.

A recent longitudinal survey of XP patients in Britain examined patients at one location minimizing variations in clinical evaluations and possibly solar exposure. The age at first diagnosis (median estimated at 12–14 yrs from [28]) was slightly later than in the US population (9 yrs [29]). It is tempting to ascribe this small difference to differences in climate in the two countries, though there may be other reasons such as mutated genes, behavioral and ethnic variations. Two main clinical presentations were identified [28] (Fig. 1). Patients with increased pigmentation were mainly linked to mutations in GGR or Pol H (*XPC, XPE, XPV*); patients with increased erythema and sunburns were mainly linked to mutations that affected both GGR and TCR (*XPA, XPD, XPF, XPG*). The former developed cancer earlier than the latter by

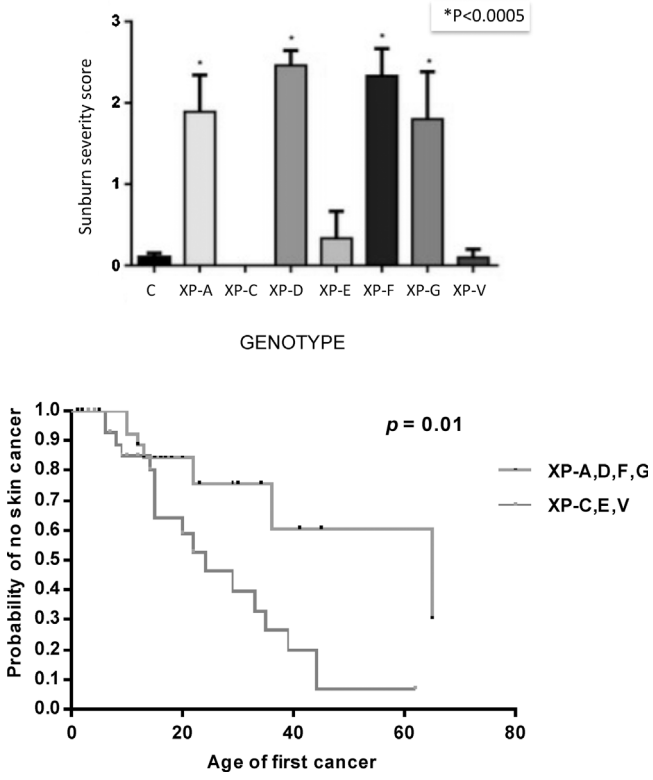

Fig. 1. Sunburn reactions and cancer incidence in different XP complementation groups. Top: Histogram of mean sunburn scores for each complementation group. There are very few abnormal sunburn reactions in patients with XP-C, XP-E and XP-V, with no significant difference compared with the control group ($P > 0.05$). In contrast, sunburn responses were grossly exaggerated in patients with XP-A, XP-D, XP-F and XP-G and significantly different from controls ($P < 0.0005$). Bottom: Kaplan–Meier curves showing probability of the absence of skin cancer stratified by sunburn severity scores. Patients with XP in complementation groups with normal sunburn reactions (XP-C, XP-E, XP-V) developed skin cancer at a significantly earlier age than those with high sunburn severity scores (XP-A, XP-D, XP-F and XP-G) ($P = 0.01$). (Reproduced from [28] in grey scale from the Brit J Dermat with permission from Wiley Inc.).