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Ultraviolet Radiation and Skin Carcinogenesis

by

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BCC: basal cell carcinoma
Cdk: cyclin-dependent kinase
NMSC: non-melanoma skin cancer
$^1O_2$: singlet oxygen
ROS: reactive oxygen species
UV: ultraviolet

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Abstract

Ultraviolet (UV) radiation is the main cause of skin cancer. Sun exposure in children increases the risk of skin cancer development in their later life. The identification of UV specific mutations in oncogenes and tumor suppressor genes in skin cancers provides confirmation at the molecular level of the importance of UV mutagenesis in skin carcinogenesis. Ultraviolet-induced skin immune supression may contribute to the carcinogenic effects of UV radiation. Ultraviolet-induced reactive oxygen species can cause permanent genetic changes in photooncogenes and

tumor suppressor genes. This paper will review the recent developments in the understanding of the epidemiology, immunology, molecular and free radical biology of skin carcinogenesis.

Introduction

Ultraviolet (UV) radiation comprises a broad band of energy extending from 200 nm to the 400 nm range [1]. The UV part of the solar electromagnetic spectrum can be subdivided into three regions: UVC (200-280 nm), UVB (280-315 nm) and UVA (315-400 nm). UVC radiation, although widely used in *in vitro* studies of the effects of UV radiation, is not relevant to human skin cancer as it is efficiently attenuated by the earth's atmosphere. UVB radiation is the main waveband responsible for sunburn, skin aging and skin cancer induction. UVA differs from UVB in a number of ways. UVA is the main component of terrestrial sunlight and accounts for more than 90% of the spectral energy in sunlight. UVA is carcinogenic and less biologically active than UVB. Both UVA and UVB are largely contributed to the increasing skin cancer [1].

Skin cancer epidemiology and UV carcinigenesis

The incidence of skin cancer increased dramatically over the last 30-40 years [2]. The evidence implicating sun exposure as the main cause of skin cancer is overwhelming. Studies of skin cancer incidence and mortality rates in people who have emigrated from areas of low to high ambient sun exposure suggest that sun exposure in childhood is especially important and the sharp change in relative risk for arrival in Australia before and after the age of 10 years suggests that the effects of UV radiation on the skin may be qualitatively different in early life [2]. The American service personnel who had brief periods of high intensity sun exposure in early life had

a increased incidence of skin cancer [3]. These findings imply that UV radiation can produce an irreversible change in melanocytes and kerotinocytes many years before the development of skin cancer. Also, the differences in body site distribution of skin cancer suggest that intermittent sun exposure is even more important than cumulative sun exposure as a risk factor [3].

Molecular biology and UV carcinogenesis

Exposure to both UVB and UVA can cause genetic changes in many biological systems [4]. Although UVB is a much more efficient mutagen than UVA, there is evidence that UVA alone can induce mutations. For UVB the action spectra for DNA damage closely coincides with the absorption spectra of DNA and exposure produces pyrimidine photoproducts. Misrepair of these photoproducts is associated with a high frequency of C to T transitions at dypyrimidine sites which is a signature of UVB-induced DNA damage [5]. For VUA the spectra for DNA damage diverges from that for DNA absorption. Pyrimidine photoproducts are rare and do not correlate with mutation rates. The types of DNA damage associated with exposure to UVA are DNA strand breaks, and DNA to protein crosslinks [4]. The mutation at GC base pairs induced by UVA suggest that oxidative DNA damage is important.

Critical target genes implicated in skin cancer development can be broadly divided into two types: oncogenes and tumor suppressor genes [6]. C to T type mutations at dypyrimidine dimer sites have been identified in oncogenes and tumor suppressor genes in both melanoma and non-melanoma skin cancer (NMSC), providing evidence at the molecular level of the importance of

UVB as a mutagen. UV specific mutations in members of the Ras oncogen family have been reported in melanoma and NMSC [7].

C to T transitions at dypyrimidine sites in the p53 tumor suppressor are a common finding in human NMSC [8]. p53 mutations have also been detected in actinic keratosis and Bowen's disease of the skin which are though to be precancerous precursors of cutaneous squamous cell carcinoma (SCC) [9]. Also, Jonason *etal* found p53 mutant cells in normal sun exposed skin [10]. These findings indicate that p53 gene is an important target for UV induced mutations in keratinocytes in human skin. UV specific mutations in melanoma have been identified in the p16 tumor suppressor gene on chromosome 9p in some melanoma cell lines [11]. Petrocelli *etal* repoeted that UVB radiation induces p21 and mediates G1 and S phase checkpoints [12]. They found that UVB radiation caused a rise in p53 protein levels, in association with induction of p21 and cyclin G expression. UVB treatment of asynchronous neonatal rat keratinocytes led to a marked inhibition of replicative DNA synthesis and prolonged G1 and S phase arrests, persisting to 18-24h, with recovery of cycling by 36h post-UVB. G1 arrest was accompanied by inhibition of cyclin D-, E- and A-associated kinases. The association of cyclin E with Cdk2 was moderately reduced. An incremental binding of p21 with Cdk4 paralleled the inhibition of cyclin D1/Cdk4 kinase and similar rise in Cdk2 binding to p21 was associated with inhibition of cyclin E and cyclin A dependent kinases. Furthermore, a rise in measurable p21-Cdk2 inhibitory activity paralleled the loss of G1 cyclin-dependent kinase activity, supporting the important role for p21 in the UVB-induced checkpoints [12].

Further evidence for the importance of UV-induced mutations in skin cancer development have emerged from studies of the patched tumor suppressor gene in basal cell carcinoma

(BCC). The patched gene, which is the human homolog of a gene originally identified in *Drosophila*, is mutated in patients with the naevoid BCC syndrome [13]. This syndrome is an autosomal dominant familial cancer syndrome which predisposes affected individuals to the development of multiple BCCs of skin. Mutation analysis of sporadic BCCs has shown that 50% of patched mutations in sporadic BCCs on sunexposed sites are UV specific [14].

UV-induced Immunosuppression and skin cancer

In addition to the direct effects of UV radiation on keratinocytes and melanocytes there has been considerable speculation about the importance of alterations in skin immune function following UV radiation exposure in skin carcinogenesis [15] . The importance of the immune system in human skin carcinogenesis is dramatically illustrated by the marked increase in skin cancer incidence, particularly cutaneous SCCs in immunosuppressed organ transplant recipients. In mice, exposure to low dose UV radiation prevents rejection of highly immunogenic tumor cell lines. Also, UV radiation exposure decreases the ability to mount a delayed type hypersensitivity response when the initial immunization occurs on exposed skin. In humans this effect is seen with doses of UVB which produce little or no reddening of the skin [16]. These findings and the importance of pharmacological inhibition of the immune system as a risk factor for skin cancer indicate that UV-induced immunological injury is likely to contribute to the carcinogenic effects of UV radiation.

UV-induced reactive oxygen species and skin cancer

Reactive oxygen species (ROS) have been shown to be involved in all three stages of carcinogenesis (initiation, promotion and progression), and are produced by both UVA and

UVB component of sunlight [17]. Besides direct absorption of UVB-photons by DNA and subsequent structural changes, generation of ROS following UVA and UVB radiation requires the absorption of photons by endogenous photosensitizer molecules. The absorption of UV-photons by a sensitizer results in its electronically excited state. The excited sensitizer subsequently reacts with another substrate (type I reaction) or with oxygen (type II reaction). The resulting products of type I reactions are radicals or radical ions, where type II reactions produce ROS including superoxide anion ($O_2^{\bullet -}$), and singlet oxygen ($^1O_2$). Superoxide dismutases can convert $O_2^{\bullet -}$ to hydrogen peroxide (H_2O_2). Hydrogen peroxide is able to cross all membranes easily. However, both $O_2^{\bullet -}$ and H_2O_2 can not react directly with DNA [18]. Therefore, both $O_2^{\bullet -}$ and H_2O_2 are thought to participate in the generation of more dangerous species such as hydroxyl radical ($HO^{\bullet}$). This can happen *in vitro* and *in vivo* by two mechanisms. Superoxide can reduce Fe(III) or Cu(II) and the subsequent Fe(II) or Cu(I) can reduce H_2O_2 finally resulting in the generation of the $HO^{\bullet}$ via the Fenton reaction [19]. *In vivo*, $O_2^{\bullet -}$ enhances a release of Fe(II) from [4Fe-4S] clusters of dehydratases and the released Fe(II) subsequently reduces H_2O_2 to OH^- and $HO^{\bullet}$. In addition, $O_2^{\bullet -}$ is able to release Fe(II) from ferritin. This release could also be mediated by UVB-generated $O_2^{\bullet -}$. Using electron spin resonance techniques, $HO^{\bullet}$ has been detected in skin upon UV-irradiation [20].

Increased ROS upon UV radiation cause extensive damage to DNA (e.g. in tumor suppressor genes and oncogenes), proteins and lipids. Furthermore, ROS activate signal transduction pathways and modulate stress genes that regulate effector genes related to growth, cellular senescence and transformation of cells to a malignant matastatic phenotype [21]. DNA oxidation by $^1O_2$ has been shown to give rise to G $\rightarrow$ T transversions . Interestingly, G $\rightarrow$ T

transversions are frequently observed in the hotspot codon 12 of Ha-ras and Ki-ras in non-melanoma skin cancer [22]. These mutations could be generated by misreplication of 8-hydroxy-deoxyguanosine (8-oxo G) lesions induced by ROS. 8-oxo G is the major mutagenic oxidative DNA lesion and most likely involved in the formation of both spontaneous cancers and neoplasias induced by a number of prooxidant agents. In addition, G → A transversions in the p53 tumor suppressor gene of non-melanoma skin cancer may also be due to 8-oxo G induced by ROS. In the multistep carcinogenesis, the second wild-type allele of a mutated tumor suppressor gene or a protooncogene is often inactivated, resulting in the loss of the compensatory effect of the wild-type allele and thus to the fully malignant phenotype.

Summary

The evidence implicating UV radiation as the most important skin carcinogen comes from the diverse aspects including clinical medicine , epidemiology, immunology, molecular and free radical biology. The key aspect over the next few years will be to determine the critical molecular changes in keratinocytes and melanocytes that underlie skin cancer development after UV radiation exposure.

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