Chapter 8

Molecular Mechanisms and Biomarkers of Skin Photocarcinogenesis

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Additional information is available at the end of the chapter

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Abstract

Skin cancer is the most commonly diagnosed malignancy in the United States and worldwide. While melanoma is the deadliest form of skin cancer, non-melanoma skin cancers, which include basal cell carcinoma and squamous cell carcinoma, are responsible for significant morbidity in millions of Americans each year. While numerous attempts have been made to reduce skin cancer risk factors related to ultraviolet radiation exposure, skin cancer incidence continues to rise. Improved understanding of the molecular pathways underlying skin cancer pathogenesis has led to the investigation of new approaches to skin cancer prevention. In particular, the search for ultraviolet radiation associated biomarkers of skin cancer has become a rapidly expanding and promising area of research. Advances in genetic sequencing have facilitated the discovery of novel biomarkers, which have the potential to profoundly improve patient care. Here we will review the molecular genetics of skin cancer and analyze the existing literature of proposed biomarkers for potential use in skin cancer diagnosis and prevention.

Keywords: ultraviolet radiation, UV biomarkers, basal cell carcinoma, squamous cell carcinoma, actinic keratosis, UV signature

1. Introduction

Skin cancer is the most common type of cancer in the United States and is estimated to affect one in every five Americans [1, 2]. Skin cancer can be classified as either non-melanoma skin cancer (NMSC), which includes basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), or melanoma skin cancer. The costs associated with the treatment of skin cancer are substantial and has placed a significant burden on the healthcare system. In the United States, it is estimated that the combined medical expenditures for the treatment of all skin cancers cost approximately $8.1 billion dollars per year [3].

Unlike many other malignancies, skin cancer is largely preventable in the majority of cases. Ultraviolet radiation (UVR) is strongly associated with cutaneous malignancies and is a primary environmental risk factor for the development of all types of skin cancer [4]. While numerous public health initiatives have increased skin cancer awareness, guidelines developed to mitigate the risks associated with UV exposure are not regularly practiced by many Americans and sunburn rates remain high [5]. Furthermore, recreational and indoor tanning still remains popular among certain groups within the population, which leads to excess UVR exposure [6]. Although most associate sunburns with UV overexposure, few recognize that the increase in melanin production from tanning is triggered by direct UVR damage to skin cells [7]. Repeated exposure to intense UVR in the form of sunburn or tanning can result in cumulative damage within skin cells, leading to cell dysregulation. These sun damaged, cancer-prone cells may exist in the skin for years or even decades before becoming visibly apparent in the form of cancerous or precancerous lesions.

Various strategies to encourage sun protective practices and interventions to modify sun related behaviors have had limited success. Low compliance with UV protection guidelines, particularly among young adults, has been attributed to various factors including inconvenience of sunscreen application and societal attitudes toward tanned skin as a sign of beauty [5, 8, 9]. In addition, the long delay from the time of UV exposure to carcinogenesis lowers risk perception of UVR as being dangerous [10]. Although primary prevention of skin cancer is ideal and remains core to decreasing disease incidence, patient risk stratification following UV exposure is critical for both early detection of skin cancer and prevention of worsening disease.

Biomarker discovery has come to play an increasingly important role in both disease diagnosis and prevention. The application of biomarker-based tests has led to revolutionary changes in medical screening, diagnosis, and targeted therapies for a variety of cancers [11]. While the use of biomarkers has become incorporated into the standard of care for numerous malignancies, the application of biomarker studies within NMSC has not yet been clearly established. By identifying UV biomarkers of NMSC, patients can be risk stratified to ensure routine skin cancer screening and pointed efforts can be made to encourage timely lifestyle changes.

Here we review the relevant literature regarding skin cancer pathogenesis, molecular genetics, and potential biomarkers for use in NMSC. In addition, we will critically analyze the strengths and limitations of various types of biomarkers and detection methods. Due to the significant differences that exist at the molecular level between the development of melanoma and NMSC, an in-depth discussion of related literature in melanoma research is beyond the scope of this review. As such, this discussion will focus on biomarker discovery and its applicability to NMSC diagnosis and prevention.

2. Non-melanoma skin cancers

Each year in the United States over 5.4 million cases of NMSC are treated in more than 3.3 million people [2]. Actinic keratosis (AK), the most common precancerous lesion, affects

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more than 58 million Americans and represents the large number of individuals at risk for the future development of NMSC [12]. While genetic factors such as skin type and family history contribute to an individual's risk of development of skin cancer, UVR exposure and age are the leading risk factors in skin cancer pathogenesis. It is estimated that approximately 90% of NMSCs are associated with excessive exposure to UVR and incidence increases with age [13, 14]. Other general risk factors include chronic arsenic exposure, radiation therapy, photosensitizing drugs, certain genetic disorders, and prolonged immunosuppression [15].

2.1. Basal cell carcinoma

Basal cell carcinoma is the most common cutaneous malignancy and represents approximately 80% of all NMSCs [16]. BCCs arise secondary to malignant transformation of cells in the basal layer of the epidermis and its appendages. While BCCs can be aggressive and destructive to surrounding tissue, it has low metastatic potential: estimated at only 0.0029–0.55% [17]. The majority of BCCs arise sporadically and only a small number of cases are inherited, which typically arise within the setting of syndromic disorders such as nevoid basal cell carcinoma syndrome. It is estimated that approximately 70% of BCCs occur on the face and 35% of patients with one BCC will go on to develop another BCC within 5 years [18]. Based on histologic examination, BCC can be classified as nodular, infiltrative, micronodular, superficial, sclerosing, or morpheaform subtypes. Analysis of the histologic growth pattern and cell differentiation is critical as it provides a means to further categorize the lesion as a high or low risk BCC [19]. Infiltrative, sclerosing, morpheaform, and micronodular BCCs are considered high-risk subtypes given the higher likelihood of subclinical spread and more frequent local recurrence [20]. However, patient treatment and prognostication are frequently complicated by the fact that more than 30% of BCCs have a mixed pathology that combines both less aggressive and more aggressive subtypes within the same lesion [21].

2.2. Squamous cell carcinoma

Cutaneous squamous cell carcinoma arises due to malignant proliferation of epidermal keratinocytes and is the second most common type of skin cancer. In a 2013 meta-analysis, the number of new SCCs (exclusive of SCC in situ) in the United States was estimated to be between 186,157 and 419,543 cases [22]. While a number of genetic disorders are associated with increased risk of both BCC and SCC, many hereditary syndromes are skewed toward development of cutaneous SCC (**Table 1**) [23]. Actinic keratosis, which results from the proliferation of atypical epidermal keratinocytes, is strongly associated with UV exposure and is considered a precancerous lesion of SCC [24]. Although some have suggested that AKs are also precursors of BCCs, this association is less clear [25]. Most AKs will not progress to SCCs and many will either regress or persist as AKs [26]. While the likelihood of an individual AK progressing to SCC is low, the presence of AKs is a marker of chronic sun damage and indicates an increased risk of NMSC [27, 28]. In general, SCC is considered highly curable and metastasizes in less than 1–5% of cases [29, 30]. Whereas specific subtypes of BCC are associated with higher or lower risk, there is less consensus as to which features best characterize the aggressiveness of cutaneous SCC. Acantholytic, adenosquamous, and desmoplastic

Fanconi anemia
Xeroderma pigmentosum
Ferguson-Smith syndrome
Rothmund-Thomson syndrome
Oculocutaneous albinism
Epidermolysis bullosa
Epidermodysplasia verruciformis
Dyskeratosis congenita
Bloom syndrome
Werner syndrome

Table 1. Hereditary syndromes predominantly associated with increased risk of cutaneous SCC.

subtypes are recognized as high risk variants; however, traditionally low risk variants may achieve high risk status based on tumor features including size, location, and lesion depth, which have been independently correlated with SCC risk [31].

3. Role of ultraviolet radiation in the development of NMSC

The link between UVR exposure and NMSC is well established [32]. Sun light exists on the electromagnetic spectrum and is divided into ultraviolet, visible, and infrared light based on wavelength. UVR is further broken down into three subtypes: type A (UVA), type B (UVB), and type C (UVC), which, again, are distinguished from one another by wavelength [32]. Of the UVR that reaches earth, 97.5% is comprised of UVA (315–400 nm) and 2.5% is UVB (280–315 nm) [33]. UVC is entirely absorbed by the atmosphere; however, UVC (200–290 nm) is emitted by man-made sources such as welding torches and mercury lamps. Thus, by and large, UVA and UVB are the most clinically relevant sources of UVR as it pertains to skin cancer [34].

While the daily dosage of UVB is significantly less when compared to UVA, UVB is considered far more dangerous. Based on the optical properties of skin, UVB is strongly absorbed within the stratum corneum and epidermis, therefore exerting a stronger effect on epidermal keratinocytes [34]. UVB is directly absorbed by proteins and nucleic acids and is responsible for the majority of sunburns. Melanin, found in the basal layer of the epidermis, is an important chromophore within the skin and primarily acts to absorb UVR. Upon stimulation by UVR, melanocytes undergo melanogenesis, which results in the increased production of melanin pigments that cause the skin to visibly tan [35]. Due to this property, melanin's photoprotective nature is believed to protect skin cells from UVB radiation damage. Furthermore, this reasoning has been used as evidence to support the observation that the incidence of skin cancer is much lower in individuals with higher levels of melanin at baseline (i.e. darker skin phenotypes) [36]. However, when effects of UVR exposure outpace the synthesis of melanin or its capacity to absorb harmful rays, skin damage will clinically appear as a sunburn.

The wavelength of UVB radiation primarily corresponds to the absorption spectrum of DNA. Upon skin exposure to UVB radiation, electromagnetic energy is absorbed by biologic molecules and transformed into chemical energy [34]. When UV photons are absorbed by DNA molecules, electrons are excited to a high energy state, which can result in formation of photoproducts [37]. The two major DNA lesions induced by UVB radiation are the formation of cyclobutane pyrimidine dimers (CPDs) and pyrimidine 6-4 pyrimidone photoproducts (6-4PPs). These lesions are ultimately caused by the misbonding of two pyrimidines, either thymine or cytosine, within the same DNA strand. Both lesions can lead to genetic mutations including C→T and CC→TT transitions; however, CPDs are considered more carcinogenic because they are more prevalent and less efficiently repaired than 6-4PPs [34]. If left unrepaired, these dimers become mutagenic, which highlights the equally important role of the DNA repair system in skin carcinogenesis. Pyrimidine dimers are normally removed by nucleotide excision repair (NER) enzymes. In patients with xeroderma pigmentosum (XP), a disease characterized by defective NER enzymes, individuals exhibit a decreased ability to repair DNA mutations, especially those caused by UVR. In individuals with XP, this clinically manifests as the development of NMSC and melanoma skin cancers at a young age [38]. In building on the knowledge learned from patients with XP, studies have subsequently sought to uncover novel defects in NER enzymes within the general population. It has been suggested that polymorphisms within NER enzymes increase susceptibility to the development of NMSC [39]. While polymorphic variants of DNA repair genes may contribute to an individual's risk of developing NMSC following UV exposure, it is unclear at this time to the extent by which one is affected.

In contrast to UVB, the exact role of UVA in skin carcinogenesis is far more nebulous. Although UVA is partially absorbed by the epidermis, it also penetrates to the dermis where collagen fibers function to scatter light [34]. Until relatively recently, UVA has long been considered to play a minor role in the development of skin cancer as photons of UVA are not within the absorbable wavelength of DNA [34]. However, molecular studies have since illuminated the potentially significant function of UVA in photocarcinogenesis. Recently, research has shown that UVA causes indirect DNA damage via the generation of reactive oxygen species, and DNA-protein crosslinks, as well as direct DNA damage by formation of CPDs or single-strand DNA breaks [40, 41]. While the role of UVA radiation in the formation of skin cancer is not yet clarified, epidemiologic studies also seem to support its harmful effects. Indoor tanning beds, which primarily emit UVA radiation, have been linked to the increased incidence of skin cancer among users [42, 43]. It has been reported that just one indoor tanning session can increase a user's risk of developing SCC by 67% and BCC by 29% [44]. There is thus compelling evidence to elevate the role of UVA in the formation of skin cancer by perhaps alternative pathways that lead to carcinogenesis.

4. Molecular mechanisms underlying skin carcinogenesis

Our understanding of cancer biology and the molecular pathology underlying malignant transformation has grown considerably within the past several decades. In particular, the advent of high-throughput sequencing technologies has enabled the detection of various mutational signatures. Dysregulation of proto-oncogenes and tumor suppressor genes, which

are critical for controlling cellular growth, is the mechanistic basis of cancer development [45]. Gain of function mutations convert proto-oncogenes into oncogenes, which lead to unregulated cell growth [46]. Likewise, mutations in tumor suppressor genes, which normally inhibit cell growth, also lead to uncontrolled cellular proliferation due to loss of negative control [47].

Past studies on UV radiation in skin carcinogenesis have identified at least three pathways involved in skin cancer development (**Figure 1**): genetic mutations, epigenetic changes and alterations in gene expression. While these three pathways can act alone to cause cancer, they often interact with each other to trigger cancer development.

4.1. Gene mutations

UVR is recognized to induce a specific pattern of genetic mutations, namely C→T and CC→TT substitutions. To date, this specific mutation is found to be specifically enriched in skin cancers [48]. Since UVR, in particular UVB, is known to induce these types of mutations, C→T transitions are now widely referred to as 'UV signatures' [49]. Our understanding of these specific mutations as being UV dependent has enabled deduction of UV induced genetic mutations by backward inference and pattern recognition. While there have been many pathways implicated in skin carcinogenesis, there is strong evidence to support the impact of selected genetic mutations as being crucial for malignant transformation and tumorigenesis within clonal populations.

4.1.1. P-53 and skin cancer

Often described as the 'guardian of the genome,' the tumor suppressor protein p53 is arguably one of the most important regulatory proteins for its role in maintaining cellular integrity.

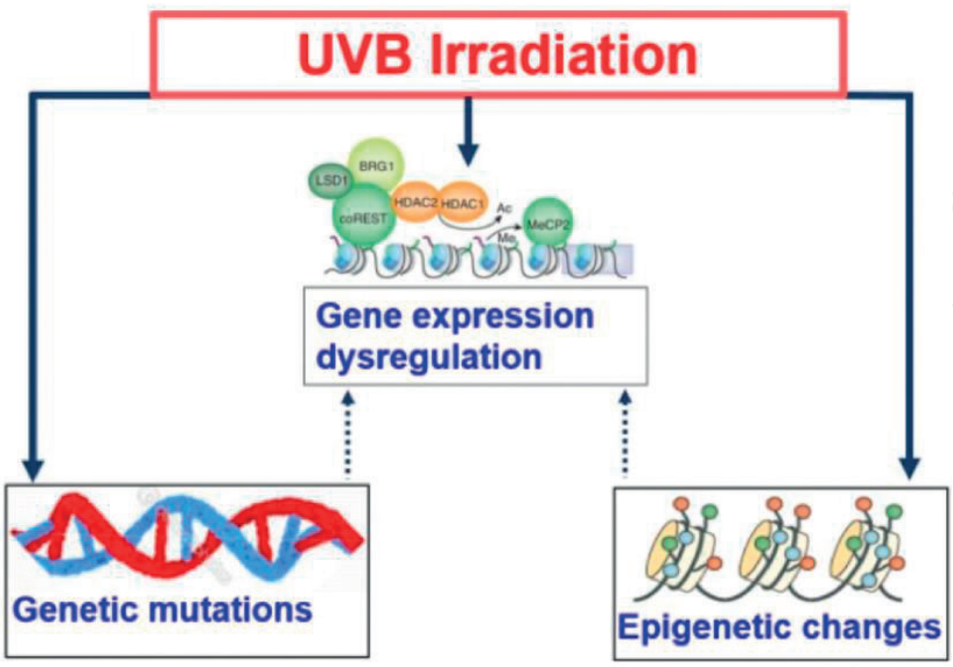

Figure 1. Schematic illustration of major molecular mechanisms underlying UV-induced skin photocarcinogenesis.

p53 is a transcription factor that is responsible for controlling genes involved in cell cycle regulation, apoptosis, and DNA repair [50]. In skin cancer, many mutations in p53 are characterized by the C→T and CC→TT transitions, which are characteristic of the UV mutational signature. It is estimated that mutations in p53 occur in 58% of SCCs and 33% of BCCs [51, 52]. Mutations in p53 are found in many malignancies, which suggest its involvement in aberrant signaling pathways and subsequent DNA damage. However, in skin cancer, each p53 allele often carries different mutations at different locations along the gene. This is opposed to other cancers in which the p53 mutations occur within conserved regions [53]. Although p53 mutations in skin cancer do not consistently occur at the same locus, mutations do not occur randomly. Rather, these UV signature mutations accumulate in 'hot spots,' which, importantly, are different from regions of p53 that are mutated in internal malignancies [54]. This suggests that the proclivity for these mutations to occur within specific loci is perpetuated by a selective advantage. p53 mutations are believed to confer resistance to apoptosis in response to UVR, thereby leading to positive selection of p53 mutant cells and clonal expansion [55].

4.1.2. Basal cell carcinoma and PTCH mutation

Patched (PTCH) is a transmembrane receptor protein that suppresses the hedgehog (HH) signaling pathway. While SCCs are believed to originate in the interfollicular epidermis, histologic evidence suggests that BCCs preferentially arise within stem cells of the hair follicle [56]. HH signaling through PTCH is critical for maintenance of skin stem cell populations, regulation of hair follicle, and sebaceous gland development. Binding of HH protein to the PTCH receptor inhibits the activation of smoothened (SMO) protein to dampen the expression of the HH pathway. Inactivating mutations in the PTCH gene or gain of function mutations in SMO can lead to constitutive expression of the HH pathway [57]. Aberrations in the HH signaling pathway are now recognized as major contributors in BCC tumorigenesis. Mutations in the PTCH gene were initially detected in patients suffering from basal cell nevus syndrome [58]. It was later discovered that a significant proportion of sporadic BCCs and BCCs arising in patients with XP also carried mutations in PTCH [59, 60]. High levels of mutant PTCH transcripts have been found in BCCs but not in normal skin or other types of tumors, making this a relatively specific genetic mutation observed in BCC [61]. The molecular link between PTCH mutations and BCC formation is regarded as a major scientific discovery; however, the exact mechanism by which over expression of the HH pathway leads to unrestricted proliferation of skin basal cells is not known.

4.1.3. Complexity of mutational patterns in squamous cell carcinoma

While mutations in the HH signaling pathway appears to be the most important genetic change leading to BCC oncogenesis, the search for a pathway of similar significance in SCC development is ongoing. Whole exome sequencing has revealed a very high mutational burden in cutaneous SCC with an average of one mutation per 30,000 base pairs [62]. Efforts to identify key driver mutations in SCC have thus been hindered by the high background mutation rate. Furthermore, when compared to BCCs, delineation of the mechanisms underlying SCC formation is somewhat more complex. While BCCs are largely believed to arise 'de novo,' SCCs can arise from clinically apparent precursor lesions, namely actinic keratoses [63].

Histologically characterized by atypical keratinocytes, AKs give rise to approximately 65% of SCCs [25]. In addition to clinical observation, this notion has been further supported by genomic analysis. While AKs and SCCs exhibit similar karyotypes, AKs demonstrate less genotypic complexity, suggestive of an earlier stage of tumor development [64]. While the genetic relationship between AKs and SCCs has not yet been clearly defined, it appears that AKs and SCCs exist on a continuum in which certain mutations drive progression from premalignant to malignant forms [65]. Thus, mutations in both AKs and SCCs have been explored for their potential roles as drivers of carcinogenesis.

Increasing evidence suggests that the underlying pathogenesis of cutaneous SCC involves mutations in several genes and pathways. Besides p53, published research has mainly focused on a handful of key mutations frequently found in cutaneous SCC including NOTCH, RAS, EGFR, TGFB, NF-KB, and most recently, KNSTRN [66]. KNSTRN gene, one of the newest genetic mutations reported in SCC, encodes a kinetochore associated protein that modulates anaphase onset and chromosome segregation during mitosis. Recurrent UV signature point mutations in KNSTRN at codon 24 (p.Ser24Phe) have been observed in 19% of SCCs and 13% of AKs [67]. Functionally, this specific mutation in KNSTRN results in disruption of chromatid cohesion in normal cells, which can lead to aneuploidy and chromosomal aberrations. Since KNSTRN mutations occur rarely in other malignancies, this may represent a previously unrecognized oncogene in skin tumorigenesis; however, studies to clarify its clinical applicability are needed.

4.2. Epigenetic alterations

Apart from the mutagenic effects of UVR on the genome, the role of epigenetic changes induced by UVR in skin cancer remains underappreciated. Epigenetics refers to heritable changes in gene expression that are not due to alterations within the DNA sequence itself. This includes changes in DNA methylation, histone modification, and miRNAs. The human genome encodes a subset of genes that function in epigenetic modifications of the genome and thus regulate the activities of other genes. While the list of such epigenetic regulators continues to grow, DNA methyltransferases (DNMTs) and histone modifying enzymes are among the best studied epigenetic regulators [68]. DNA methylation is a process catalyzed DNMTs by which a methyl group is added to the 5′ carbon of a cytosine ring of DNA. These modified cytosine residues are often adjacent to guanine bases, resulting in the formation of a CpG dinucleotide. These CpGs are often concentrated in short CpG-rich DNA segments, known as CpG islands, the majority of which are found in the promoter region of genes [68]. Methylation events in promoter regions can have profound effects via the secondary effects on transcription.

Both DNA hypomethylation and hypermethylation have been implicated in tumorigenesis of skin cancers. In particular, DNA hypermethylation in CpG islands is an important mechanism by which tumor suppressor genes are silenced. This in turn leads to downstream effects on gene expression that may eventually lead to a neoplastic phenotype [69]. Like other human malignancies, the development of skin cancer involves a complex interplay between environmental factors and alterations in gene expression within skin cells. While the

development of skin cancer is multifactorial, epigenetic alterations are believed to be among the earliest detectable changes in UVR exposed skin [70]. Over exposure of the skin to UVR leads to oxidative stress, inflammation, and DNA damage, factors that are known to significantly alter epigenetic pathways. In particular, chronic inflammation has been shown to accelerate the formation of DNA methylation changes [71]. Thus, environment-induced aberrant expression of epigenetic regulators can trigger widespread epigenetic changes that may subsequently disturb a variety of downstream target genes, potentially increasing the risk of cancer development.

Despite the increasing recognition that abnormal DNA methylation is a crucial factor in skin carcinogenesis, histone modifications serve as another dynamic epigenetic pathway that is frequently altered in cancer. Histone modifying enzymes can be divided into multiple families [72, 73]. Among these, proteins involved in histone acetylation and deacetylation have been shown to play important roles in both normal development and disease states. Limited data suggest that silencing of tumor suppressor genes in UV-induced skin leads to photocarcinogenesis and aberrant epigenetic modifications including alterations in DNA methylation and histone acetylation at specific loci [74, 75]. UV-induced differential gene expression has also been linked to changes in global H3 lysine 27 acetylation (H3K27ac), an epigenetic marker of active promoter and enhancer regions. Using ChIP-seq analysis, one study revealed that UVR induced genome-wide loss of H3K27ac, as well as regional gains in H3K27ac levels. Upon further analysis, UV-induced differential H3K27ac acetylation was functionally correlated with differential gene expression was observed [76]. The genome wide loss of H3K27ac may be attributable to the suppression of histone acetyltransferases activities, whereas the regional gain of H3K27ac may occur secondary to the binding of UV-responsive transcription factors, such as JUN/FOS or TP53, which subsequently recruit HATs to their target regions [76, 77]. Though the epigenetic mechanisms underlying the effects of UVR in promoting skin cancer warrant more extensive studies, the use of histone biomarkers for clinical diagnosis and/or prognosis is an interesting approach that is also being investigated for use in other malignancies [78, 79].

There is a growing body of evidence to suggest that non-coding RNA (ncRNA) is involved in the development of many malignancies, including skin cancer [80]. ncRNAs are RNA transcripts transcribed from DNA that are not translated into protein and classified based on size: small non-coding RNA (<200 nucleotides) and long non-coding RNA (>200 nucleotides). While there are various different subtypes of ncRNA, microRNA (miRNA), a subtype of small non-coding RNA, is perhaps the most widely studied. miRNAs are single stranded ncRNA molecules that modulate gene expression by binding to the 3′ untranslated region of target mRNA. This ultimately causes mRNA instability and eventual degradation, leading to subsequent alterations in cell differentiation, metabolism, apoptosis, and signal transduction [81]. Studies have demonstrated that UV irradiation of human keratinocytes modulates the expression of numerous miRNAs. In addition to a common miRNA response, UVA and UVB were also shown to induce wavelength specific miRNA expression signatures [82]. Although interpretation of changes in UV-responsive miRNA expression is complex, miRNA mediated gene silencing likely has important downstream effects, which contribute to the development of skin cancer.

While less studied than short non-coding RNA, long non-coding RNA (lncRNA) has recently become an area of significant interest within cancer research. It is now known that lncRNA can effect gene expression through interactions with transcription factors and can interact with miRNAs to regulate mRNA stability [83, 84]. Though thousands of lncRNA transcripts have been identified, the majority remain uncharacterized with unknown functions [85]. Nonetheless, a growing number of lncRNAs are being investigated for their role in cancer growth, tumor initiation, and metastasis.

5. Current measures of ultraviolet radiation exposure and skin damage

The current indicator of skin sun damage relies on the use of minimal erythema dose (MED), which refers to the amount of UVR that produces visible skin redness within 24 hours following exposure [86]. As an indicator of UV damage, MED is insensitive and inadequate because UV-induced molecular damage may occur at sub-MED UV doses [87, 88]. Other markers of UV exposure include clinical findings such as solar lentigines and solar elastosis. While these lesions are completely benign, they do have a positive association with NMSC, mainly due to the fact that they arise secondary to photodamage [89]. Although clinical findings of photodamage provide prognostic value, these lesions are neither sensitive nor specific as markers of skin cancer risk as many individuals with solar elastosis and lentigines will never develop skin cancer [89].

While the association between UVR and skin cancer is well established, quantitative assessment of skin UV exposure and its effect on skin cancer development remains unknown. In a small case control study of 58 patients with cutaneous SCC, the risk was greatest in patients who had more than 30,000 hours of cumulative lifetime sun exposure [90]. This is in contrast to BCCs where studies suggest that intense, intermittent sun exposure resulting in sunburns may be more important for the development of BCC [91]. Quantifying the amount of sun exposed hours necessary to induce NMSC is technically challenging and is not practical for implementation as a risk measure at the population level.

6. Biomarkers as a novel tool in monitoring ultraviolet skin damage to improve skin cancer prevention

Biomarkers are defined as measurable cellular, biochemical or molecular alternations in biological media such as blood or tissue. Several FDA-approved multi-gene panel tests are now approved for risk prediction and diagnosis of various cancers; however, no similar biomarker tests exist for patient risk stratification of NMSC [92]. At present, there are no skin cancer screening guidelines in the United States. Despite this, physicians are routinely confronted with the decision of who should receive total body skin exams and at what time interval. The United States Preventive Task Force (USPSTF) has repeatedly stated that in the absence of randomized controlled clinical trials, there is insufficient evidence to recommend skin cancer

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screening in the general population [93]. While the majority of the USPSTF skin cancer screening report focuses on melanoma, one of the main arguments made against screening for NMSC is that there is limited evidence regarding the diagnostic accuracy of primary care physicians to correctly identify BCCs and SCCs. Given the lack of rigorous dermatology training in most medical school and primary care residencies, this argument is not necessarily unfounded [94]. Although there are several other arguments to be made against the USPSTF recommendations and reasoning, the lack of clear skin cancer screening guidelines is problematic.

It is unlikely the USPSTF will obtain a satisfactory level of evidence in the near future to recommend skin cancer screening in the United States given the large number of patients required to adequately power a clinical trial [95]. It thus behooves physicians and scientists to search for alternative measures as a means to quantify skin cancer risk. The presence of an objective UV biomarker test could facilitate patient triage by identifying high risk individuals for dermatology referral. Identification of susceptible patients in the primary care setting via the use of an accessible genetic screening test would thus provide physicians with an evidence based method to make informed decisions regarding which patients should receive regular skin cancer screening.

The development of a UV biomarker panel has the potential to have a profound impact on patient care. Breakthroughs in next generation sequencing technology have provided a powerful tool for identifying biomarkers of a given physiological status or exposure [96]. Many studies have attempted to identify biomarkers that correlate UV exposure and skin damage with variable success; however, no consensus UV biomarkers have been established to date. Various types of biomarkers have been investigated including DNA, RNA, and protein.

7. Candidate biomarkers for assessing UV damage: strengths and weaknesses

7.1. DNA markers

Given the potent mutagenic effect of UVR and its role in skin carcinogenesis, skin cancer research in the past several decades has primarily focused on mapping cancer-related mutations. Such efforts have been greatly enhanced by recent advances in whole-genome and whole-exome sequencing studies, which have identified recurrent genomic aberrations that underlie the development of BCC and SCC [97]. As such, these particular gene mutations have been suggested for use as markers of skin cancer risk. Unfortunately, numerous issues have been encountered with this approach.

Based on our current understanding of cancer development, tumor growth is initiated by the presence of driver mutations, which lead to clonal expansion of mutant cells. This increases the total number of cells that are at risk to develop further mutations and malignant transformation [98]. Given the high prevalence of p53 mutations observed in NMSC, this had previously been suggested as a potential genetic marker of patient risk. Upon further investigation, it was found that mutant clones with p53 UV signature mutations were found in high numbers

within clinically normal skin [99]. As another example, both copies of NOTCH1 are frequently inactivated in SCCs via point mutations and copy number alterations. While studies have demonstrated that up to 60% of SCCs have mutations in NOTCH1, approximately 20% of clinically normal skin cells also carry this mutation [100]. These instances suggest that DNA mutation alone is insufficient to drive skin carcinogenesis. Although this may signal that a significant number of keratinocytes are predisposed to developing skin cancer, the specific combination of events leading to malignant transformation is not well understood.

One of the more promising DNA-based UV biomarkers involves the use of mitochondrial DNA (mtDNA) as a biomarker of cumulative UVR exposure and oxidative stress [101]. Given that mitochondria lack classical NER pathways, UV signature mutations that form within mtDNA have limited capacity for repair [102]. Thus, the entirety of UV induced damage can be observed and correlated quantitatively with UV exposure. Since mutations in nuclear DNA (nDNA) are capable of repair via NER enzymes, the UV mutational burden in nDNA is less representative of lifetime UV exposure. Furthermore, individual variability in NER enzyme activity makes the assessment of nDNA mutations more complicated as repair rates are not uniform [39]. The use of mtDNA as a marker of UV damage is appealing; however, the most important question: whether there is a relationship between mtDNA mutations and actual disease: remains to be answered. Additional studies are required to establish a functional correlation between mtDNA mutation and skin cancer development.

7.2. RNA markers

Fundamental to the central dogma of molecular biology, RNA is a necessary intermediary between DNA and protein. The exploration of mRNA-based UV biomarkers is an exciting area of research that has been facilitated by recent advancements in next-generation sequencing technology [96]. While RNA only exists for a finite period of time before being degraded, it is easy to detect and quantify at very low levels [103]. There are currently numerous methods available by which RNA expression can be analyzed in a reliable and reproducible fashion. Compared to DNA biomarkers, RNA biomarkers provide a snapshot of temporal and spatial changes in regulatory pathways, which cannot be observed with singular DNA changes.

The increasing power of high throughput sequencing has allowed for detection of changes in both protein-coding RNA (i.e. mRNA) and non-coding RNA (i.e. small nuclear RNA, micro RNA, small nucleolar RNA, lncRNA) expression with high sensitivity and specificity [104]. Non-protein coding RNAs are well known to play regulatory roles in gene expression via post-transcriptional modification and there is a growing body of evidence that implicates non-coding RNA as key regulators of tumor pathways [105, 106]. Studies have demonstrated that specific UV induced miRNAs are differentially expressed in NMSCs (**Table 2**). While altered miRNA expression has been found within malignant tumors themselves, various cancers also demonstrate specific miRNA aberrations within serum and plasma [107]. Therefore, analysis of circulating miRNA expression levels can be potentially employed for use in a non-invasive biomarker test for NMSC. Plasma profiling for early detection of NMSC has been explored in one recent study, which showed significant changes in expression of miRNA-19a, miRNA-25, miRNA-30a, miRNA-145, miRNA-186 [108]. While these are interesting findings, extensive validation of the clinical utility of these miRNAs as NMSC biomarkers is required.

microRNA	Expression	NMSC association	Reference
miR-203	Downregulated	BCC	[133]
Let-7a	Downregulated	BCC	[124]
miR-21	Upregulated	BCC, SCC	[124–129]
miR-29c	Downregulated	BCC	[130]
miR-130a	Upregulated	BCC	[126, 130]
miR-124	Downregulated	SCC	131]
miR-203	Downregulated	SCC	[132]
miR-184	Upregulated	SCC	[132]
miR-30a	Downregulated	SCC	[126]
miR-387	Downregulated	SCC	[126]
miR-135b	Upregulated	SCC	[126]
miR-424	Upregulated	SCC	[126]
miR-766	Upregulated	SCC	[126]
miR-145	Downregulated	SCC	[126]
miR-140-3p	Downregulated	SCC	[126]
miR-26a	Downregulated	SCC	[126]
miR-31	Upregulated	SCC	[127, 134]
miR-205	Upregulated	SCC	[135]
miR-365	Upregulated	SCC	[136, 137]
miR-1	Downregulated	SCC	[138, 139]
miR-34a	Downregulated	SCC	[140]
miR-124/214	Downregulated	SCC	[131]
miR-125b	Downregulated	SCC	[127]
miR-193b/265a	Downregulated	SCC	[141]
miR-199a	Downregulated	SCC	[142]
miR-361-5p	Downregulated	SCC	[143]
miR-483-3p	Downregulated	SCC	[144]

Table 2. Aberrantly expressed microRNAs in NMSC tumors.

LncRNA is also being explored for its utility as a biomarker for cancer diagnosis and therapy. Following genome wide association studies, it is now known that 88% of trait associated single nucleotide polymorphisms (SNPs) are located in intergenic regions [109]. Since the majority of lncRNAs are transcribed from these regions, it has been suggested that SNPs of lncRNA may represent differential disease risk. While studies are limited, the potential role of circulating lncRNA for use as a biomedical tool is exciting. Dysregulation of lncRNAs has

been reported in a number of malignancies including colon, prostate, breast, and liver cancer where they act as tumor suppressors and oncogenes [110]. Similar to miRNAs, lncRNA can easily be detected by PCR in bodily fluids. Studies demonstrating the detection of lncRNA PCA3 in urine and lncRNA HULC in blood have been suggested as novel modes of cancer screening and diagnosis for prostate cancer and hepatocellular carcinoma respectively [111–113]. With regard to skin cancer, the aberrant expression of lncRNA appears to be functionally important in skin carcinogenesis. In one study, lncRNA lincRNA-p21—a transcriptional target of p53 and HIF-1α—was found to be highly inducible by UVB radiation and crucial for p-53 mediated apoptosis of damaged keratinocytes [114]. The interaction between the vitamin D receptor and lncRNA also appears to play an important role in maintaining cellular homeostasis prevention of skin tumor formation [115]. In another study, differential expression analysis revealed 1516 lncRNAs were upregulated and 2586 lncRNAs were downregulated in cutaneous SCCs when compared to normal controls [116]. A similar study demonstrated analogous findings in BCCs that showed upregulation of 1851 lncRNAs and downregulation of 2165 lncRNAs when compared to normal skin [117]. While the prognostic, diagnostic, and therapeutic application of lncRNAs within skin cancer is not yet developed, their potential role in the molecular pathogenesis of NMSC warrants further analysis.

In addition to non-coding RNA, mRNA has been explored extensively for its use as a biomarker in numerous studies [118–123]. Similar to miRNA, multiple mRNA transcripts have been found to be differentially regulated in response to UV radiation. Despite the large number of differentially expressed genes identified so far, there is no consensus in terms of which genes are the most sensitive and specific markers of NMSC. Due to frequent inter-individual variations in the expression of many genes, it is unlikely that a singular mRNA will be sufficient as a reliable biomarker. There is thus a growing consensus that multi-gene biomarker panels will be required for the development of a robust and reliable screening test.

Analysis of combined transcriptomic data from previous studies has been complicated by large variations in experimental design including cell type, UV exposure, dose, and time points of analysis [118–123, 145, 146] (**Table 3**). Therefore, development of a validated consensus UV biomarker panel has not yet been achieved. However, a recent transcriptomic profiling study has begun to shed light on the viability of UV biomarker panel for clinical application [147]. In this study, rigorous bioinformatics and statistical analyses were performed to identify UV-responsive genes that are conserved among different donors, in response to various UVR doses, and at different time points after UV exposure. Through this comprehensive transcriptomic analysis, 401 conserved UV-responsive genes were identified out of approximately 4000 U-induced differentially expressed genes detected following each specific UVR condition. Through RNA-seq analysis, this study also generated a SCC-specific signature based on differential gene expression analysis of five pairs of human SCC tumor tissue and adjacent normal skin tissue. Moreover, there is a significant similarity between the conserved UV responsive genes and the SCC signatures genes. The conserved UV-responsive gene set has little similarity to gene sets dysregulated in other human cancers, highlighting their specificity for skin cancer gene expression signatures [147]. Additional validation studies will be needed to select which of these conserved UV-responsive genes may be used to develop a consensus UV biomarker panel.

Author	UV type & dose (mJ/cm²)	Transcriptional analysis: time post UVR (hours)	Cell type	Platform
Rieger and Chu [118]	1 (UVC)	4	Lymphoblastoid cell lines	U95A-v2 chips
Dazard et al. [119]	20–80 (UVB)	0, 0.5, 3, 6, 12, 24	NHEK vs. SCC	U95A-v2 chips
Takao et al. [120]	10 (UVB)	6	NHEK	HuGene FL chips (6800)
Dawes et al. [121]	1000 (UVB)—rat 3 MED (UVB)—human	48	Human vs. rat epidermal cells	RNA-Seq
de la Fuente et al. [122]	300 (UVB) + 3700 (UVA)	6	NHM, MHEK, NHDC	Whole genome chips
Yang et al. [123]	25 (UVB)	24	NHM	U133+-v2 chips
Koch-Paiz et al. [145]	5 (UVA) + 12.5 (UVB) + 1.2 (UVC)	6, 12	Differentiated mammary epithelium	7684 probes cDNA array
Enk et al. [146]	150–250 (UVB)	2, 24, 72	NHEK	U95A-v2 chips

Abbreviations: NHEK, normal human epidermal keratinocytes; NHM, normal human melanocytes; NHDC, normal human dendritic cells.

Table 3. Comparison of study design and methods of previous gene expression profiling studies analyzing differential expression of UV induced mRNA.

7.3. Protein markers

The majority of genes function through their protein products. Therefore, it is generally accepted that gene activity and function are positively correlated with their protein expression level. Characteristics of protein expression such as variation in quantity, timing of synthesis, and interaction with other proteins has been studied as a means to correlate molecular mechanisms with clinical pathology. Although there are benefits to directly measure changes in protein expression, the use of protein biomarkers has several significant challenges. The main limitation with the use of protein markers lies within currently available detection methods. Protein biomarker panels can be expensive because each protein requires a specific antibody for detection. Secondly, it remains technically challenging and laborious to standardize the quality and specificity of antibodies in their research and clinical applications. Synthetically produced protein antibodies often exhibit great variability between manufacturers, increasing the likelihood of inconsistent results [148]. Moreover, there is a significant number of proteins whose activity depends on specific forms of posttranslational modifications, thus making it more complex in interpreting their clinical relevance solely based on their expression levels [149]. For these reasons, no studies have focused on the role of aberrantly expressed proteins in NMSC. Unless improvements in technology are able to overcome the aforementioned issues, the use of protein biomarkers for patient risk stratification of NMSC is unlikely to be practical.

8. Conclusions

Skin cancer is the most commonly diagnosed cancer in the U.S. and has become a major and growing public health problem. Despite numerous public health initiatives to promote sun safety, many Americans do not adhere to recommended guidelines to protect themselves from UV exposure. Given the long lag between UV induced skin damage and clinically apparent skin cancer, this reduces the perceived risk of UVR and does not encourage timely behavior modification.

Our understanding of the pathogenesis of skin cancer at the molecular level has dramatically expanded within the past several years. Although there is still much to be learned about the underlying mechanisms of skin cancer pathobiology, advances in genetic sequencing have provided great insight into the ways in which effective tests may be developed for patient risk stratification of NMSC. This has since paved the way for pursuit of novel applications of this information, which have the potential to profoundly improve patient care.

Clinical biomarker discovery has led to revolutionary changes in medical screening, diagnosis, and target based therapies for a variety of cancers. In the era of precision medicine, individualized patient care is becoming increasingly important in all fields of medicine. While UVR has long been known to be a key risk factor for skin cancer development, increasing evidence has demonstrated that its role in carcinogenesis is likely multifactorial and involves multiple biologic pathways. Despite this, identification of cellular dysregulation in key regulatory pathways has provided insight into potential biomarkers of disease.

Various types of biomarkers including DNA, RNA, and protein have been suggested for use in diagnostic and prognostic testing for various malignancies. Identification of individual biomarkers that produce consistent and reliable information on UV damage has posed a significant clinical challenge. We believe that a successful clinical test consisting of a panel of UV signature genes will provide the most sensitive and specific means for patient risk stratification of UV skin damage. Within NMSC research, RNA-based UV biomarkers currently exhibit the most promise for future clinical application given the multiple, reliable, and cost-effective modalities for RNA detection.

The current lack of skin cancer screening guidelines in the United States has resulted in a non-standardized approach to skin cancer screening and physician risk assessment. Thus, a UV biomarker-based screening test could provide an objective and evidence based method to determine which patients should receive regular skin cancer screening facilitate the identification of high risk individuals for dermatology referral and regular skin cancer screening. By encouraging early risk assessment, we believe that a biomarker-based diagnostic test will greatly improve skin cancer prevention and reduce skin cancer incidence.

Furthermore, translation of UV biomarker expression patterns into a risk score would hopefully offer quantitative and convincing evidence to alert susceptible individuals and encourage UV protective behaviors. Finally, development of a reliable UV biomarker panel could be used for other purposes such as testing the UV-protective effects of sunscreens. We believe this area of research deserves continued attention as the development of UV biomarker based tests has the potential to completely transform the preventative paradigm pertaining to skin cancer.

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