

# Towards a Better Understanding of the Molecular Mechanisms Involved in Sunlight-Induced Melanoma

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Although much less prevalent than its nonmelanoma skin cancer counterparts, cutaneous malignant melanoma (CMM) is the most lethal human skin cancer. Epidemiological and biological studies have established a strong link between lifetime exposure to ultraviolet (UV) light, particularly sunburn in childhood, and the development of melanoma. However, the specific molecular targets of this environmental carcinogen are not known. Data obtained from genetic and molecular studies over the last few years have identified the INK4a/ARF locus as the “gatekeeper” melanoma suppressor, encoding two tumour suppressor proteins in human, p16<sup>INK4a</sup> and p14<sup>ARF</sup>. Recent developments in molecular biotechnology and research using laboratory animals have made a significant gene breakthrough identifying the components of the p16<sup>INK4a</sup>/Rb pathway as the principal and rate-limiting targets of UV radiation actions in melanoma formation. This review summarizes the current knowledge of the molecular mechanisms involved in melanoma development and its relationship to sunlight UV radiation.

## INTRODUCTION

Cutaneous malignant melanoma (CMM) is a neoplasm affecting melanocytes, pigmented cells found predominantly in the epidermal layer of the skin [1]. Whilst once a rarity, this type of skin cancer has become a common cancer in the United Kingdom and has increased in incidence faster than any other cancer [2]. Although much less prevalent than its nonmelanoma skin cancer (NMSC) counterparts, it affects a younger population and mortality rates are high for thicker lesions (5 years survival for lesions > 3.5 mm is 48%) [3]. CMM constitutes only 2% of all cancers but it is the seventh commonest cause of cancer death in the UK. In the USA, melanoma will claim 7000 lives per year with a predicted lifetime risk of 1 in 90 [2]. The alarming increase of CMM incidence and its resistance to currently available therapies has emphasized the need to understand the molecular mechanisms involved in its development. Accumulating evidence indicates that the risks of CMM include both genetic and environmental factors [4]. Using well-characterized murine ultraviolet (UV) radiation melanoma model, recent study has provided evidence identifying the components of the retinoblastoma (Rb) pathway as the principal targets of UV mutagenesis in melanoma development [5]. In addition to the Rb pathway, activation of the Ras-Raf-MAPK signalling pathway is linked to CMM development [6]. This review will focus on examining the relationship between UV and the recent findings in the molecular mechanisms of melanoma development.

## CYTOGENETIC STUDIES

Cytogenetic studies on melanoma families have been meticulously carried out to find chromosome band regions of 1p, 1q, 6q, 9p, and 11q as being involved at a significantly increased frequency (reviewed in [7, 8]). 1p has been reported to be structurally abnormal in 82% of analyzed cases, the largest proportion for any chromosome, and anomalies of chromosome 9 were detected in 46% of the cases [9]. However, the chromosome band region 9p21 was disrupted in premalignant atypical nevi and early primary melanomas, raising the possibility that the genes encoded at 9p21 are important in the pathogenesis and progression of early malignant melanomas. The melanocyte initially becomes dysplastic then has a superficial spreading phase or the radial growth phase, followed by the vertical growth phase when tumour cells invade the dermis [1]. Studies of melanoma families have identified two genes predisposing to melanoma, *CDKN2A* (INK4a/ARF) and *CDK4* located at 9p21 and 12q13, respectively (reviewed in [10]). The loss of the INK4a/ARF locus (seen in 50% of melanomas), along with activation of the BRAF mutation, is considered the most common genetic lesion in human melanoma. This gene is mutated in a large majority of melanoma cell lines, as well as in many uncultured melanoma cells and in the germline of melanoma kindreds [10].

The *CDKN2A* (INK4a/ARF) locus mapping to chromosome 9p21 encodes for tumour suppressor genes which are strongly associated with familial melanoma and

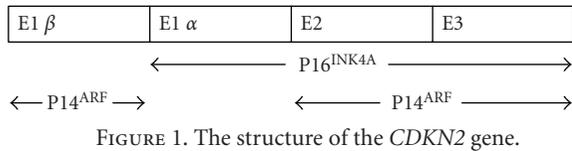


FIGURE 1. The structure of the *CDKN2* gene.

altered in 10–60% of sporadic melanomas [1, 10, 11]. In human, *INK4A/ARF* encodes two distinct tumour suppressor proteins, the inhibitor of kinase 4A,  $p16^{INK4a}$  and  $p14^{ARF}$  ( $p19^{ARF}$  arising in major part from an alternative reading frame of the mouse *INK4a* gene) [1, 12, 13, 14]. The locus has three regions named 1( $\alpha$  and  $\beta$ ), 2, and 3.  $P16^{INK4A}$  is composed of exons 1 $\alpha$ , 2, and 3, whereas  $P14^{ARF}$  is composed of exons 1 $\beta$ , 2, and 3 (Figure 1). The  $p16^{INK4A}$  and  $p14^{ARF}$  genes are interesting as they are both from the same locus, *CDKN2A*, but from different promoters.

### UV-MELANOMAGENESIS: THE ROLE OF THE RB PATHWAY

Melanoma is a multifactorial disease, where both genetic and environmental sun exposure factors are involved [15]. The role of UV light in inducing CMM is a long-held belief although there is more evidence for this in the NMSC whose distribution mirrors the areas of high exposure to UV [16, 17]. Epidemiological studies that suggested a link between sun exposure and melanoma were first published in 1956 in Australia [18]. These studies noticed that mortality from the disease increased closer to the equator. This research was extensively reviewed in 1992 by the International Agency for Research on Cancer, who definitively concluded that “there is sufficient evidence in humans for the carcinogenicity of solar radiation in causing melanoma” [19]. Retrospective epidemiological data currently suggest that unlike other skin cancers that are associated with cumulative lifetime UV exposure, CMM is provoked by intense intermittent exposure to UV, particularly during childhood [5, 17]. In addition to UVB, exposure to UVA is now thought to play a part in the development of melanoma [20]. However, the functional relationship between genes and sunlight in melanoma pathogenesis is not well understood. Recently, prospects for elucidating this relationship have brightened considerably through the development of UV-responsive experimental animal models of melanoma [21, 22]. In the skin, absorption of UV photons by the DNA of epidermal cells and the rearrangement of electrons lead to the formation of photoproducts at adjacent pyrimidine sites and unrepaired damage can lead to specific gene mutations, which are usually C to T or CC to TT, considered as “UV molecular signature” [23]. The most significant mutations induced by UV in the skin occur in tumour suppressor genes, *p53* and  $p16^{INK4a}$  genes being the major targets of UV [23, 24]. Recent studies using laboratory animals have identified the components of the Rb pathway (divided

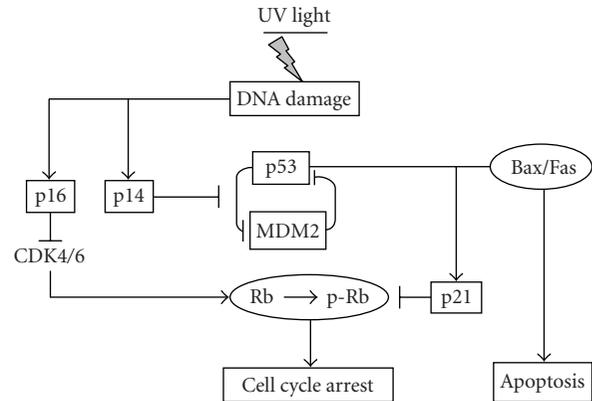


FIGURE 2. UV radiation and the components of the Rb pathway in the skin.

into two genetically distinct pathways: “ $p16^{INK4A}/Rb$ ” and “ $p14^{ARF}/p53$ ”) as the main UV target(s) disrupted in the early stages of melanoma genesis [5]. However, a definitive UV- $p16$ -melanoma relationship, and the nature of this interaction, is yet to be clarified. UV light is known to initiate a series of molecular events in the skin, acting via two distinct biochemical pathways; the  $p16$  and  $p53$  pathways (Figure 2).

#### UV radiation and the $P16^{INK4A}$ pathway in the skin

$P16^{INK4a}$  is a cell cycle regulator that inhibits specifically *CDK4/6* and consequently cyclin D-dependent phosphorylation of the Rb, leading to less transcription of *E2F*-responsive genes necessary for S phase entry (Figure 2) [25]. When  $p16$  is induced, the Rb protein is maintained in its nonphosphorylated (active) form, *E2F* is not activated, and replication is halted. The relationship between  $p16$  and melanoma has been explained by the observation that UV light can induce  $p16$  expression in human skin [26, 27], thereby implying a role for  $p16^{INK4a}$  in the repair of UV-induced DNA damage. In cells irradiated with low doses of UVB,  $P16^{INK4A}$  is upregulated within 12–24 hours leading to a cell cycle arrest at G1 [28]. This is thought to allow DNA repair before allowing resumption of the cell cycle (Figure 2). If  $P16^{INK4A}$  is inactivated via missense mutation, deletion, or methylation [29, 30, 31], the Rb protein is no longer maintained in its active form and cell replication is unchecked.

The *CDK4* gene, situated on chromosome 12q13 and coding for cyclin-dependent kinase 4 which binds to  $p16$  (Figure 2), has also been identified as a melanoma-susceptibility gene [32]. The *CDK4* (Arg24Cys) germline mutation has been identified in melanoma-prone families [33, 34] and produced a mutated protein that interferes with the binding of the *CDK4* protein to  $p16$  and so prevents the inhibition of its enzymatic activity [35]. As a result, the *CDK4* protein is constantly activated promoting the Rb pathway and subsequent cellular division. Therefore, unlike *CDKN2A*, the *CDK4* gene functions as an oncogene [35] and not as a tumour suppressor gene.

### UV radiation and the p53 pathway in the skin

The effect of UV radiation on p53 expression in the skin has been well documented (Figure 2). Using hairless mouse model, we have characterized the temporal events implicated in the immediate and adaptive responses in the skin [36, 37]. Following acute UV irradiation of mouse skin, p53 expression increased 12 hours post-UV followed by using p21 protein to arrest the cell cycle and permit the repair of UV DNA damage [36]. If the damage is severe, apoptosis is induced in the skin (in keratinocytes and melanocytes) via p53-dependent or independent pathways [36, 38, 39, 40]. To date, no direct link has been established between p53 and melanomagenesis. However, ARF (also known as p14, or p19 in mice), also a regulator of the cell cycle, has been shown to be a principal regulator of HDM2 (MDM2 in mice), an E3 ubiquitin ligase regulating p53 degradation and stability [41]. The ARF protein prevents the interaction between p53 and MDM2 and consequently p53 levels rise (Figure 2). This activates p21 which then inhibits phosphorylation of Rb, leading to cell cycle arrest in both G1 and G2. ARF is ubiquitously expressed and is elevated in cells lacking functional p53. Somatic mutations have been observed which affect the ARF-coding sequence exclusively [42, 43]. If the ARF gene is mutated, the events described above will be disrupted disabling ARF's tumour suppressor function that might promote melanoma development. In UV skin carcinogenesis, p53 and p16 pathways work in tandem (Figure 2), and mutations in either p16 or p53 could cause unregulated cell proliferation leading to tumour development in the skin.

### OTHER GENES ASSOCIATED WITH MELANOMA

The Ras-Raf-MAPK/extracellular signal-regulated kinase signalling pathway is activated in the vast majority of melanomas (reviewed in [44]). Activation occurs through either NRAS or BRAF mutations, both of which arise early during melanoma pathogenesis and are preserved throughout tumour progression. This cascade is activated by sequential phosphorylation of a number of kinases in order to alter cellular behaviour in response to different environmental factors. The extracellular-signal-regulated kinases (ERK1 and ERK2) belong to one branch of this cascade responsible for sensing extracellular stimuli, including UV light. This stimulus then activates the Ras family of proteins which then activates the RAF family of serine/threonine kinases (c-RAF 1, BRAF, and ARAF). This activation subsequently phosphorylates and activates ERK1 and ERK2. ERK phosphorylation has been linked to G-protein-coupled receptor (GPCR) signalling, as well as activation by upstream receptor tyrosine kinases (RTKs). N-Ras mutations are found in melanomas from chronically UV-exposed sites in 26% of melanomas [45]. These proteins are stimulated at the cell membranes by epidermal growth factors causing senescence with the cells remaining in G1 phase of the cell cycle. Ras proteins

also upregulate p53 via ARF [46] and p16<sup>INK4A</sup> protein [47]. However, although Ink4a/Arf<sup>-/-</sup> mice did not exhibit melanoma tumours [48], expression of activated H-Ras on an Ink4a/Arf-deficient background produced a high incidence of melanoma [49].

The BRAF gene encoding a serine/threonine kinase is a key component of the MAPK signalling pathway highly mutated in CMM [50]. However, although BRAF somatic missense mutations have been detected at a very high frequency in melanoma tumours, and at a lower frequency in many human cancers, this gene may not be a melanoma-susceptibility gene [51]. Thus, other genes in the Ras-Raf-MAP kinase pathway might play a significant role in melanoma susceptibility.

### CONCLUSION

Although a number of different genes have been associated with melanoma development and progression, the CDKN2A remains the candidate gene in UV radiation-induced melanoma. The two products of this gene form the main backbone of the Rb pathway acting via two distinct pathways (Figure 2): one involving loss of the tumour suppressor gene p16<sup>INK4A</sup> which acts through the Rb protein and the other involving ARF regulating p53 degradation and stability. Both can lead to a loss of the cell cycle control following UV-induced DNA damage. Ongoing work is aiming to further elucidate the mechanisms of action of UV light on the regulation of the components of the Rb pathway and identification of novel UV-target genes involved in melanomagenesis.

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