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Ultraviolet Light as a Modulator of Melanoma Development

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1. Introduction

Epidemiological evidence is overwhelming that exposure of the skin to ultraviolet radiation (UVR) can increase one's risk of developing malignant melanoma. However the situation is complex, as melanoma development is associated with "intermittent" sun exposure, whereas epidermal keratinocyte-based skin cancers like squamous cell carcinoma (SCC) are associated with chronic UVR exposure. Thus it is difficult to talk in terms of a classical UVR carcinogenic mechanism for melanoma in general. Melanoma risk seems intricately associated with pigmentation characteristics. Genome wide association studies identify variants in genes involved in pigmentation as risk factors, generally the strongest signal being for the melanocortin receptor 1 gene (*MC1R*). One reason postulated to explain the odd relationship between UVR exposure and tumorigenesis is that there may be a unique carcinogenic mechanisms at play involving UVA, that is a weak carcinogen for skin cancer in general. Evidence for UVA causality in melanoma comes from some epidemiological studies, and to some extent from work with animal models. On the other hand, one can argue that there may not be a unique carcinogenic mechanism for melanoma, and that there are several factors that may help explain the apparent difference from typical mechanism of UVR mutagenesis involving classical UVR mutations. Firstly, in terms of normal cellular function, melanocytes principal function is to produce melanin pigment while epidermal keratinocytes are programmed to proliferate and then die as they generate and maintain the epidermis, a barrier for internal tissues and organs. Secondly, there may be genetic differences between individuals developing particular subtypes of melanoma and/or form of sun exposure. Thus particularly relates to susceptibility to naevus development, a critical factor associated melanoma development on the trunk, a site presumably receiving mainly "intermittent" sun exposure.

2. Epidemiology of melanoma

Major risk factors for cutaneous melanoma are shown below - approximate relative risk (RR) and 95% confidence intervals (CI) are shown in brackets (Gandini et al., 2005a; Gandini et al., 2005b):

1. One atypical naevus (RR 1.60, CI 1.4-1.8)

2. Five or more atypical naevus (RR 10.5, CI 5.1-21.5).
3. Multiple banal melanocytic naevi - 100 vs <15 (RR-6.9, CI 4.6-10.3).
4. Red versus dark hair (RR 3.6, CI 2.5-5.4).
5. Sunburns in childhood (RR 2.2, CI 1.73-2.89
6. Sunburns in adulthood (RR 1.9, CI 1.6-2.7)
7. Chronic sun exposure (RR 1.0, CI 0.8-1.1)

Apart from familial predisposition, the strongest risk factor for the development of cancer generally, the presence of naevi, especially dysplastic naevi, is the innate, or phenotypic factor that most increases the probability of developing a melanoma. Sunlight is the only environmental factor that has been consistently implicated as a cause of melanoma, leading to a melanoma incidence 10- to 20- fold higher among fair-skinned than dark-skinned peoples (Armstrong & Kricker, 1993). Among fair-skinned people, melanoma incidence increases with proximity to the equator and several studies have shown that fair-skinned migrants moving from high (e.g. UK) to low latitude countries (e.g. Australia, South Africa) have lower melanoma rates than native-born residents (Whiteman and Green, 1999; Khlat et al., 1992; McCredie et al., 1990; McMichael and Giles, 1988). Individuals with xeroderma pigmentosum (XP), a disorder in which sufferers have a gene mutation that diminishes their ability to repair UVR-induced DNA damage, have much higher risk of melanoma than the population average (Kraemer et al., 1994; Cleaver, 2006). Those with a past history of non-melanoma skin cancer (caused by high exposures to solar UVR) have a 3-fold higher risk of melanoma than the general population (Green et al., 1993).

3. Genetic basis of melanoma

3.1 Genes involved in familial melanoma susceptibility

Although many different genes can be somatically mutated in melanoma, as yet there are only two confirmed familial melanoma susceptibility loci, *CDKN2A* and *CDK4*. *CDKN2A* encodes INK4A and ARF, which regulate cell cycle progression via the INK4A/CDK4/pRB and ARF/MDM2/p53 pathways respectively, although undoubtedly there is significant cross talk between these two pathways, and with other pathways. The overwhelming majority of *CDKN2A* mutations in melanoma-prone kindreds affect only the *INK4A* transcript, or both transcripts, but *ARF*-specific mutations also predispose to melanoma (e.g. Randerson-Moor et al., 2001; Rizos et al., 2001). As *INK4A* mutations generally prevent CDK4 from being bound and inhibited by p16INK4A, the mechanism of tumorigenesis with *INK4A* or *CDK4* mutations is presumed to be equivalent (via pRB deregulation). Families carrying *CDKN2A* mutations usually, although not always, exhibit a naevus-prone phenotype (Goldstein et al., 2000) indicating that relaxation of melanocyte proliferation control induced by INK4A (or ARF) loss may be important in naevogenesis. However a recent study comparing the influence of sun exposure on melanoma risk in *CDKN2A* mutations carriers in Australia and the United Kingdom (Cust et al., 2011) suggests that they have to have the same cumulative risk of melanoma irrespective of the ambient UV irradiance in the region in which they live.

3.2 Genes associated with melanoma in genome wide association studies

Genome wide association (GWA) studies have been used to discover genes that confer risk for skin cancer development (Table 1). Some genes are associated both melanoma and non-melanoma skin cancer, especially basal cell carcinoma (BCC). Five genes associated with

melanoma, *SLC45A2*, *TYRP1*, *TYR*, *MC1R,* and *ASIP*, encode proteins that are involved in various ways in regulating pigmentation. Little is known about how most of these may effect melanoma genesis and we are left to assume that the risk alleles may encode variants in these genes that simply result in lower levels of protective pigmentation. Notably, in respect to *MC1R* and *ASIP* (agouti signaling protein, an MC1R antagonist), there may be other explanations, which will be discussed below. The genes that confer the strongest risk for the development of naevi, *MTAP*/*CDKN2A* and *PLA2G6*, are not involved in the regulation of pigmentation.

Chromosome	Candidate gene	Pigmentation	Naevus count	Melanoma	BCC	SCC
1p36	*PAD15*	-		-	++	-
1q42	*RHOU*	-		-	++	-
5p13.3	*SLC45A2*	++		++	++	++
5p13.33	*TERT*	-		++	++	-
6q25	*IRF4*	++	+?	+?	-	-
7q32	*KLF14*	-		-	++	-
9p21	*CDKN2A*	-		-	++	-
9p21	*MTAP*		++	++		
9q23	*TYRP1*	++		++	-	-
11q13.2	*TPCN2*	++		-	-	
11q14	*TYR*	++		++	++	-
12q13	*KRT5*	-		-	++	-
12q21	*KITG*	++		-	-	
14q23	*SLC2A4*	++		-	-	
15q11	*OCA2*	++		-	-	-
15q13.1	*HERC2*	++		-	-	
15q21	*SLC24A5*	+		-	-	-
16q24.3	*MC1R*	++		++	++	
20q11	*ASIP*	++		++	++	-
22q13	*PLA2G6*		++	++		

Table 1. Adapted from Gerstenblith et al. (2010). A double plus sign (++) indicates a significant association ($P < 10^{-7}$) in GWAS. A single plus sign (+) indicates an association (P between 0.01 and 10^{-7}). A minus sign (-) indicates a null association ($P > 0.01$). A blank cell indicates that the locus in the left column has not been not tested. BCC=basal cell carcinoma. SCC =squamous cell carcinoma.

3.3 *MC1R*, melanoma risk and sun exposure

MC1R, the receptor for α–melanocyte stimulating hormone, is the most thoroughly studied melanoma risk gene. It functions largely to control the switch between red/yellow pheomelanin and black/brown eumelanin, hence it is sometimes referred to as the "red haired" gene. This gene is highly polymorphic in human populations with >65 variants documented. Variants have been classed into two groups based on the strength of their association with red hair (Sturm et al., 2003). The R variants (i.e. Asp84Glu, Arg151Cys, Arg160Trp, and Asp294His) are most highly correlated with red hair (mean OR 63.3, range 50.5-118.3) although the r variants are still associated to a lesser degree (mean OR 5.1, range

2.4-6.4). *MC1R* variants are not the sole determinant of hair colour, twin studies have observed discordant hair colour but identical *MC1R* haplotypes (Box et al., 1997). The molecular consequences of UVR upon melanocytes with variant melanocortin-1 receptors are variable. This has lead to debate over MC1R classification and which variants to include in assessing the impact of impaired *MC1R* function in melanoma (Hacker & Hayward, 2008). Beaumont et al. (2007) used *in vitro* studies to examine the functional impact of nine common *MC1R* variants and found that the V60L, D84E, R151C, I155T, R160W and R163Q variants showed impairment in cAMP coupling. Normal receptor expression was found for R142H and D294H variants, but reduced functional responses were observed, indicating that altered G-protein coupling may be responsible for this loss of function. The V92M isoform shows similar activity to the wild-type receptor, and along with V60L, is not associated with melanoma (Raimondi et al., 2008). Interestingly, melanoma risk due to the carriage of *MC1R* variants is stronger in individuals with dark hair and eyes, who do not have freckles, and tan well (Kanetsky et al., 2010). Thus the risk due to *MC1R* variation is certainly not limited to red heads.

The mechanism by which the carriage of *MC1R* variants increases melanoma risk is an area of intense investigation. The simplest explanation is simply the lower photo-protection afforded by red/yellow pheomelanin than brown/black eumelanin. Notably, *MC1R* variants are also associated with increased risk of non-melanoma skin cancer (table 1). In addition, pheomelanin is more likely than eumelanin to generate potentially damaging reactive oxygen species following UVR exposure (Hill, 1992; Takeuchi et al., 2004; Baldea et al., 2009). A popular explanation for the protective role of MC1R comes from cell culture experiments showing that melanocytes carrying melanoma-associated *MC1R* variants have less effective repair of both UVR-induced pyrimidine dimers and oxidative damage than wild-type cells and are more sensitive to UVR-induced cell death (Kadekaro et al., 2005; Bohm et al., 2005; Hauser et al., 2006; Song et al., 2009). Functional MC1R appears to be necessary to prevent UV-induced genomic instability within melanocytes.

4. The epidemiological association between sunlight and melanoma is complex

Despite the persuasive descriptive evidence linking sunlight with melanoma, several observations make clear that the association is complex and does not accord with a simple model in which the risk of melanoma increases directly with increasing levels of exposure to the sun. Melanoma occurs more commonly among indoor than outdoor workers (Beral & Robinson, 1981). Even in sunny countries most melanomas develop on sites that are habitually covered by clothing (such as the back), as opposed to sites more frequently exposed to the sun such as the face (Green et al., 1993). Many case-control studies of melanoma incidence report stronger associations with intermittent (short periods of intense sun exposure to untanned skin) rather than chronic patterns of sun exposure (Elwood & Jopson, 1997). Recreational sun exposure is a risk factor for melanoma on the trunk and limbs but not on the head and neck (Chang et al., 2009).

Chronic sun exposure and a "classical" UVR carcinogenic mechanism involving UVB-induced DNA damage is accepted to be responsible for the development of SCC. One reason frequently proposed for the lack of association of melanoma with chronic sun exposure is that there may be a different carcinogenic mechanism for melanoma, possibly involving UVA exposure. The potential role of UVA in the induction of melanoma has been reviewed elsewhere (e.g. Wang et al., 2001; Moan et al., 2008; Godar et al. 2009). Sunlight at different

latitudes contains vastly different rations of UVA/UVB, with a greater proportion of UVB nearer the equator, and less closer to the poles. Because the change in melanoma incidence with latitude is much smaller than that for SCC (which is dependent upon cumulative UVB exposure) it is hypothesized that UVA play a role at least in exacerbating the development of melanoma (Godar et al., 2009; Wang et al., 2010). Other ideas revolve around the notion that office workers are at higher relative risk possibly due excessive UVA that can penetrate glass (Godar et al., 2009). Further, recreational exposure, generally agreed to increase melanoma risk, can include solarium use. Depending on the lamp type used, artificial tanning devices (sunbeds or solariums) emit higher UVA/UVB ratios and possibly higher UVA doses than found in sunlight (Miller et al., 1998; Gerber et al., 2002). A meta-analysis of nineteen studies has shown that exposure to sunbeds at a young age is the most damaging, with a relative risk for "first exposure under the age of 35" of 1.75 (95% CI, 1.35, 2.26) (International Agency for Research on Cancer Working group on artificial UV light and skin cancer, 2007). A large prospective cohort study of 106,366 women in Sweden and Norway showed that solarium use at ages 30-39 linked to a relative risk of 1.49 (95% CI, 1.11-2.00)(Veierod et al., 2011). Thus epidemiological evidence suggests that sunbeds are health hazards in terms of melanoma risk and that UVA has a plausible role in the development of this neoplasm. Hence epidemiological data is somewhat supportive of the view that the full UVR spectrum may be carcinogenic in melanoma. It should be noted that at any point on the earth it is difficult to precisely predict the UVA/UVB ratio in sunlight as it can greatly vary with time of day, altitude, latitude and climate factors (De Fabo et al., 2004).

5. Naevus and melanoma subtypes

From the point of view of basic biology differences between melanoma and non-melanoma skin cancer in terms of their relationship with UVR exposure is not surprising. Melanocytes are long living cells, resistant to apoptosis, whose principal function is to produce melanin. In contrast, the primary function of keratinocytes is to provide a protective barrier, the epidermis, which is in a continual state of regeneration, supplied by proliferation of epidermal basal layer keratinocytes that initiates a programmed process of differentiation and apoptosis as needed. Melanocytes can undergo a form of proliferation, where they form senescent groups, or nests, which are termed naevi. Such lesions are negative for proliferation markers, but can progress to malignancy, albeit at an extremely low frequency (Grichnik, 2008). There are multiple subtypes of naevi. These include dermal (blue naevi), compound (common acquired, spitz and congenital naevi) and epidermal (e.g. reed naevi) lesions (Grichnik, 2008). These subtypes may be influenced differently by UVR exposure, and there may be differences in their propensities for transformation (e.g. which is probably much less for dermal naevi). Hence the subtype of naevus can be a confounding factor when studying environmental and genetic factors influencing naevo genesis. For instance the positive association between naevus count and *IRF4* gene variation (Duffy et al., 2010) varies greatly for different subtypes (dermal versus compound naevi).

Likewise there are several major melanoma subtypes, and then subtle forms within each group. Superficial spreading melanoma (SSM) is the most common form in Caucasians (around 70% of all melanomas). It follows a radial growth phase with atypical melanocytes, either as single cells or nests at all levels of the epidermis (Smoller, 2006), followed by an invasive vertical growth phase. Nodular melanoma (NM) are primary dermal lesions characterized by growth through the dermis, generally lack epidermal involvement, and a

very sharply circumscribed with virtual lack of radial spread (Smoller, 2006). Lentigo maligna melanoma (LMM) is the only subtype unequivocally associated with chronic sun exposure. Lesions display confluent spread of melanocytes along the epidermal basal layer and in the upper portion of the hair follicle and are invariably associated with solar elastosis in adjacent skin (Smoller, 2006). SMM and NM, but not LMM, sometimes have naeval remnants present on histopathology. Acral lentiginous and mucosal melanomas are epidermal lesions that occur on palmoplantar and mucosal surfaces respectively, and are assumed not to be influenced by UVR exposure. Clearly, any discussion of the effects of chronic versus intermittent sun exposure has to consider melanoma subtype.

6. The divergent pathway model of melanoma

To assess the effects of chronic versus intermittent sun exposure melanomas have been stratified into chronic sun damage (CSD) or non-chronic sun damage (non-CSD) melanomas, either histologically by assessing solar elastosis, a measure of chronic exposure (e.g. Curtin et al., 2005), or by comparing melanomas developing on the head and neck (an anatomical region of high cumulative sun exposure), and the trunk (a region of intermittent exposure). While these two methods of classification may create some confounding differences, overall the use of either system supports the conclusion of a complex relationship between melanoma and sun exposure that has lead to the proposal of a "divergent pathway" model (Whiteman et al., 2003). According to this model (Figure 1) the pathways diverge after an initial insult that stabilizes the melanocyte. This may be early life exposure to UVR given that childhood sunburns are a risk factor for melanoma (Whiteman et al., 2001). What happens thereafter depends upon a combination of host characteristics and subsequent patterns and doses of UVR exposure. Melanomas that develop on the head and neck are associated with solar elastosis (a marker of CSD), low naevus count, and relatively late age of onset. In contrast, melanomas developing on the trunk via the intermittent UVR (non-CSD) pathway tend to have relatively earlier age of onset and are associated with higher naevus count.

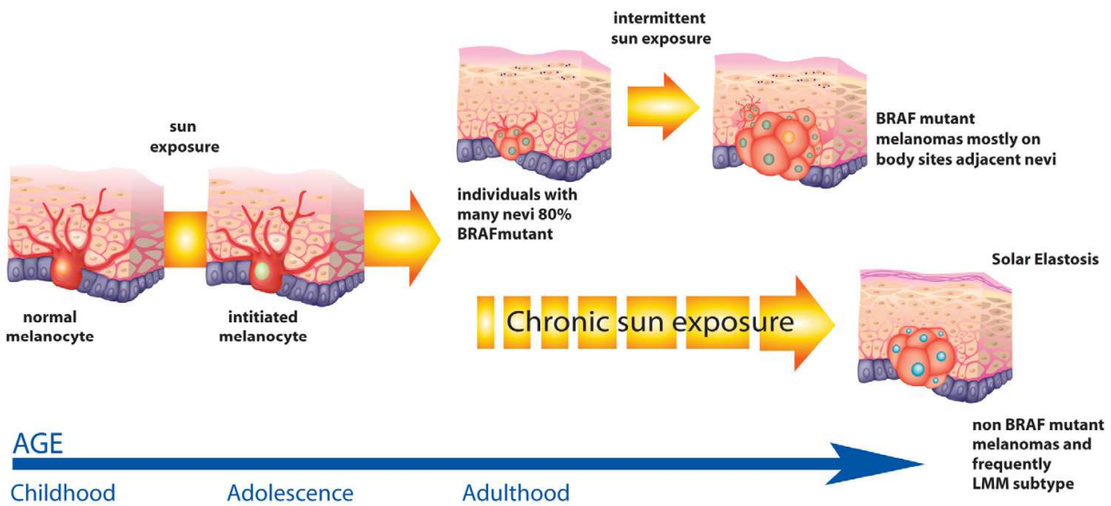

Fig. 1. Schematic depiction of the divergent pathways model for melanoma development.

Several studies have published findings concordant with the divergent pathway hypothesis (Carli and Palli, 2003; Chang et al., 2009; Bataille et al., 1998). Given the strong association between truncal (non-CSD) melanomas and naevus development, and the fact that most naevi carry *BRAF* mutations (Pollock et al., 2003), it is not surprising that the non-CSD melanomas also tend to carry *BRAF* mutations (reviewed in Platz et al., 2008). A greater tendency for melanocytes to proliferate in this branch of the model has been proposed (Whiteman et al., 2001). This may manifest as innate propensity for "proliferation" (a tendency to form nests, i.e. naevi), or proliferation in response to UVR exposure. To our knowledge inter-individual variation in the latter has only been reported once. Stierner et al. (1989) exposed buttock skin (seven UVB exposures with dose gradually increased to give slight erythema), and collected biopsies three weeks after last exposure. They found variation in the melanocyte proliferative response to UVR. Individuals that showed the biggest increase had the lowest pre-existing density. There was no association between melanocyte number increase, minimal erythemal dose, or skin type (the presence, or not, of naevi was not mentioned). Hence, although the sample size was small, this study does indicate different proliferative potential of melanocytes between individuals. We cannot know how these responses influence melanoma development except in prospective studies, but we can begin to look at genes and other phenotypic measures that may stratify the two pathways and allow better predication of risk. There are some suggestions that not only naevus risk may be important, but also the propensity of individuals with less naevi to be prone to developing solar elastosis (Thomas et al., 2010). Arguing against this, individuals with DNA repair defects (e.g. XP patients) frequently develop lentigo melanomas without solar elastosis (Spatz et al., 2001), indicating that repeated or unrepaired UVR-induced DNA damage in the skin may be more important than the presence of solar elastosis *per se*. To sum up, the divergent pathway hypothesis provides some basis for explaining why melanoma as a whole is most associated with intermittent sun exposure.

7. Stratification of CSD and non-CSD melanomas by innate phenotypic and genetic variation

7.1 MC1R variants

Landi et al. (2006) reported that individuals in Italian and U.S. cohorts that developed *BRAF*-mutant melanomas via the "naevus" (non-CSD) pathway tended to carry *MC1R* variants more frequently than those developing CSD melanomas. However studies undertaken in cohorts from Australia and North Carolina found no association between germline *MC1R* status and somatic *BRAF* mutations in melanomas (Thomas et al., 2007; Hacker et al., 2010a). More recently, the Italian sample population originally reported in Landi et al. (2006) has been expanded to include another 92 melanomas and they reported that germline *MC1R* variants were associated with melanomas carrying *BRAF*-mutations independent of solar elastosis measures (Fargnoli et al., 2008). Conflicting data has continued to appear, with results from a German sample of 173 melanoma patients showing the opposite effect, with individuals carrying *MC1R* variants less likely to acquire somatic *BRAF* mutations in tumours (Scherer et al., 2010). *MC1R* is considered the most important of the "moderate" risk genes for melanoma. However its relationship to CSD versus non-CSD associated melanoma is a matter of debate. It is possible that the discordant study findings reflect that fact that *MC1R* is extremely polymorphic within and between ethnic populations, and that the small sample sizes for each study means that chance association with the non-CSD melanoma pathway cannot be excluded.

7.2 Genes controlling the development of naevi

Given that the "intermittent" exposure arm of the divergent pathway is associated with the presence of naevi, we should be able to obtain clues about how to differentiate the pathways based on genes that confer naevus risk. Naevi are benign proliferations of melanocytes, and the number of naevi individuals tend to develop is under strong genetic control (English & Armstrong, 1994; Harrison et al., 1994). Monozygotic, or identical twin pairs, share all genes and have extremely highly correlated naevus counts (twin1 vs twin2, r=0.94), whereas dizygotic twin pairs share on average only half of their genes, and their naevus counts are considerably less correlated (r=0.60) (Zhu et al., 1999). The great majority naevi carry the $BRAF^{V600E}$ which seems to be an early event in melanoma development but not sufficient to transform naevocytes (Pollock et al., 2003). Instead, the expression of the mutant form in melanocytes leads to growth arrest characteristic of senescence (Michaloglou et al., 2005). However the presence of $BRAF^{V600E}$ does not inform in terms of how naevi might develop. Here we must look to genetic studies that might provide some hints to the molecular mechanisms involved. GWAS have also identified variants associated with development of naevi at chromosomal regions 9p21 and 22q13 (Falchi et al., 2009; Zhu et al., 2007). The strongest signal on 9p21 was located in the *MTAP* gene, which encodes methylthioadenosine phosphorylase, an enzyme involved in nucleoside metabolism. On 22q13 the SNP with the highest association lies within *PLA2G6*, a gene belonging to the phospholipase A2 superfamily. Notably, the 9p21 locus accounted for 3.0% of nevus count variance, whereas the 22q13 locus accounted for only 0.7%. Thus the *MTAP/CDKN2A* locus is the strongest candidate region for naevus susceptibility. Clearly *MTAP* is an excellent candidate, but so is *CDKN2A* given its historical involvement in melanoma and the fact that individuals in families carrying *CDKN2A* mutations commonly have many naevi. It is thought that SNPs in the *MTAP* gene may confer long-range regulation of the *CDKN2A* locus. The various lines of evidence for long distance regulation of the *CDKN2A* locus are reviewed in Peters (2008). This would be an analogous situation to the *OCA2* gene, whose influence on eye colour is not due to *OCA2* coding variants, but to remote regulation by a SNP in the adjacent gene (Sturm et al., 2008). Another study on twins (Duffy et al., 2010) has revealed another association, this time with *IRF4* (Interferon regulatory factor-4). Here the effect is somewhat weaker, and associated only with naevus development in an age-specific context (stronger effect in younger individuals). It will be very important to understand the mechanisms by which these genes confer naevus susceptibility given that the potential to develop naevi is the critical stratifying factor for the divergent pathways.

The number of naevi an individual develops does not appear to just an innate trait, it may also be associated with levels of sun exposure, especially in children (reviewed in Gallagher et al., 1995; Bauer et al., 2003). Recent studies examining the association of holidays overseas among young white English women found an increased in naevus count, particularly on anatomical sites intermittently exposed to sunlight, supporting the hypothesis that intermittent sun exposure is of relevance in the aetiology of naevi (Silva Idos, et al., 2009).

7.3 UVR-induced proliferation of melanocytes

Of possible relevance is how the branches of the divergent pathways differ in terms of the propensity of melanocytes in the skin to proliferate after UVR. Early studies in humans demonstrated that melanocyte density was correlated with sun exposure (Mitchell, 1963; Staricco & Pinkus, 1957; Stierner et al., 1989). Work by Quevedo and Colleagues (1965) reported that in mice melanocyte density increased up to 4-fold following repeated UVR

exposure, possibly due to increased mitotic activity of melanocytes (Rosdahl, 1978). More recent experiments have shown that melanocyte proliferation is greater following exposure to UVB than UVA, and that a single dose has a substantially greater effect than the same dose fractionated over several days (An et al., 2001; van Schanke et al., 2005). The generation of melanoma in mouse models using neonatal UVR is usually, although not always, accompanied by a strong proliferative response of melanocytes and their migration to the burnt area of the skin (Walker et al., 2009; Ferguson et al., 2010). Melanocyte proliferation would seem to be linked to the tanning response, which increases the amount of pigment in the skin, and is driven by UVB-induced damage to the skin. This is akin to "delayed tanning", that can occur 1-5 d after exposure, and is primarily due to increased melanin production, although multiple exposures induce proliferation of melanocytes resulting in increased numbers in human skin (Yamaguchi et al., 2008). This is a long lasting protective pigmentation, unlike UVA-induced intermediate pigment darkening (IPD), which results from oxidation of pre-existing melanin, fades quickly, and is not protective against subsequent exposures. Interestingly, the induction of active melanocytes in mouse skin is also produced by chemical carcinogens, and the more carcinogenic the compound the greater the tanning response (Iwata et al., 1981). Liver carcinogens that are not metabolically activated in skin are ineffective. The compound most effective in inducing melanocyte proliferation was 7,12-dimethylbenz[a]anthracene (DMBA), a very potent skin carcinogen. Thus the response of melanocytes is driven either by UVR or compounds that induce adducts within the DNA of skin cells.

Melanocyte proliferation after UVR is thought to be driven by cytokines released by the microenvironment (Figure 2). UVR exposure modulates the production by keratinocytes (and probably other cells) of endothelins, Kit ligand (KITL), fibroblast growth factors (FGFs), and many others, which are all regulate melanocyte function (Hirobe, 2005; Lin & Fisher, 2007; Imokawa, 2004). These include α-MSH (alpha melanocyte stimulating hormone), ACTH and a range of other growth factors. α-MSH and ACTH both bind to the MC1R on the surface of melanocytes, which activates the cyclic-AMP dependent kinase pathway, and the production of melanin pigments and possibly melanocyte proliferation. Most of these signaling molecules are known to enhance pigmentation, but little is known about how they might influence melanocyte proliferation *in vivo*. Mutations in mice that cause disruption keratinocyte function (resulting in epidermal thickening), for instance by germline activating keratin 4a or epidermal growth factor mutation (Fitch et al., 2003), or keratinocyte-specific ablation of β1-Integrin (López-Rovira et al., 2005), result in increased melanocyte numbers in the epidermis. This can also occur without epidermal hyperplasia by keratinocyte-specific overexpression of p53 (McGowan et al., 2008), Kit ligand (Kunisada et al., 1998), or, surprisingly, deletion of Fgf2 (Weiner et al., 2007). Treating human skin xenografted on to mice with exogenous FGF2, endothelin 3, and KITL resulted in the development of pigmented lesions, which only required UVB exposures repeatedly for one month for progression to melanoma (Berking et al., 2004). Hence signals from DNA damaged keratinocytes may play a role in inducing melanocytes to proliferate. To quote Lin and Fisher (2007), "*could it be that keratinocytes are the primary UV responding population, and melanoma formation is largely a consequence of reactive secondary stimulation?*" In fact the injection of highly active αMSH analogues may be naevis promoting (Cardones et al., 2009; Langan et al., 2009). However the notion of UVR-induced melanocyte proliferation being melanomagenic is at odds with findings that stimulation of melanocytes with factor such as a-MSH (e.g. Bohm et al., 2005; Hauser et al., 2006; Abdel-Malek et al., 2009), Endothelin 1, (Kadekaro et al., 2005) and KITL

(Serre et al., 2011) improve DNA repair efficiency after UVR and are thus proposed to be protective for melanoma. Clearly we are only at the beginning of understanding how melanocyte UVR responses influence melanoma development. Whether genetic variation in humans that augment the proliferative response of an individual's melanocytes to UVR could increase susceptibility to a particular pathway of melanoma development (as suggested by Whiteman et al., 2003, and Rivers, 2004) remains to be determined.

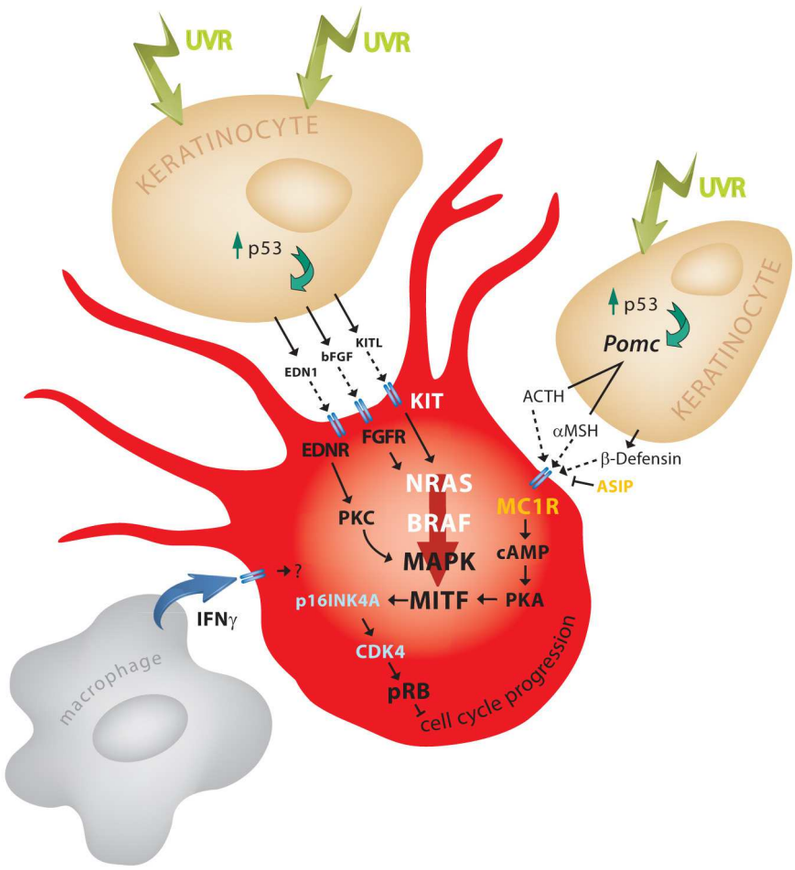

Fig. 2. Pathways regulating melanocyte function.
Keratinocytes expresses various growth factors that bind to melanocyte receptors that regulate critical intracellular pathways. Expression of these factors is increased after damage to the skin such as after UVR exposure. Germline mutations in *p16INK4A* and *CDK4* (light blue) confer susceptibility to familial melanoma. *KIT*, *NRAS* and *BRAF* (white) are mutated somatically in melanomas. *MC1R* and *ASIP* variants (yellow) confer increased risk for melanoma development. In addition, macrophages that infiltrate the skin after UVR may stimulate melanocytes. Activation of the pathways depicted result in increased pigment production and distribution to adjacent keratinocytes, and increased survival, DNA repair, and proliferation of the melanocyte.

7.4 Stratification of CSD and non-CSD by somatic mutations signatures

Examination of melanomas of various subtypes by array comparative genome hybridization (CGH) has detected significant differences at specific genomic locations such that DNA copy

number differences could stratify melanomas into CSD, non-CSD, acral and mucosal melanomas (the latter two assumed not associated with sun exposure)(Curtin et al., 2005). Subsequently the same group detected activating mutations of KIT in 28% (n=18) of CSD melanomas versus 0% (n=18). This raised hopes that KIT mutation status may differentiate CSD and non-CSD melanomas, but a subsequent Australian publication (Handolias et al., 2010) showed that the frequency of *KIT* receptor gene mutations in CSD melanomas is very low. Thus *KIT* mutation may not a good discriminator of CSD and non-CSD melanomas. In contrast, it has consistently been shown that mutation of *KIT* is much more common in acral and mucosal lesions (Curtin et al., 2005; Smalley et al., 2009). As described above we are left with fact that *BRAF* mutations are more common in melanomas arising in the non-CSD group, and a tendency for *NRAS* mutations to be more often present CSD melanomas. Notably the frequency of *NRAS* mutants in this group is relatively low, hence it would be only a signature for a small proportion of CSD melanomas. Nonetheless a recent meta-analysis of all published studies showed that *NRAS* mutation is found in 24% of CSD melanomas and 17% of non-CSD melanomas and calculated that *NRAS* mutation is significantly associated with CSD melanoma (OR 1.9, 95% CI 1.11-3.20)(Lee et al., 2010). Despite the significant difference, *NRAS* mutation appears to be a weak discriminator of the CSD and non-CSD pathways. *BRAF* mutation is a better discriminator, having been found in 30% of CSD melanomas and 49% of non-CSD melanoma. *BRAF* mutation is significantly associated with non-CSD melanoma (OR 2.4, 95% CI 1.35-3.10). Unlike SSM and NM, few LMs (10-20%) harbor $BRAF^{V600E}$, (Hocker & Tsao, 2007). Hence there does not seem to be a strong mutations signature for the non-CSD pathway except for *BRAF*, which is mutated in over 80% of naevi. The stratification of CSD and non-CSD pathways in terms of *BRAF* mutation may have more to do with their differential association with naevus development than the forms of UVR exposure.

8. Vitamin D and potential protective effects of chronic sun exposure on melanoma?

Vitamin D has been shown to inhibit proliferation and induce differentiation in some melanoma cells, although melanoma cell lines have demonstrated resistance to vitamin D growth arrest (Danielsson et al., 1998,1999; Reichrath et al., 2007). A population-based study of 528 melanoma cases found that the presence of solar elastosis (dermal sun damage) was associated with a better prognosis for melanoma patients (Berwick et al., 2005). These findings have provoked speculation that as chronic sun damage induces a less aggressive form of melanoma (LMM), perhaps vitamin D levels might somehow slow melanoma growth and/or improve prognosis. To further determine if the anti-proliferative effect of vitamin D is modifying outcome for melanoma patients, Downing and colleagues, (2008) carried out a study to compare two populations with similar ethnic background but potentially different environmental influences. Patients diagnosed with invasive melanoma between 1993 and 2003 in Yorkshire (n= 4170) and New South Wales (NSW, n= 30,520) were identified from cancer registry databases and prognostic information (age, sex, socioeconomic background, tumour site and Breslow thickness) was examined. Five-year relative survival was 86.9% (95% CI, 85.2-88.5) in Yorkshire and 88.6% (95% CI, 88.1-89.1) in NSW. There was a suggestion of reduced risk for death in Australia, but differences in tumour thickness appeared to be the most important factor. The difference in survival may be due to the strong health promotion message for screening of skin cancer in Australia

resulting in increased detection of early thin lesions with better outcomes. A recent follow-up study of 872 patients from the Leeds cohort (median follow-up, 4.7 years) has shown that higher 25-hydroxyvitamin D3 levels, at diagnosis, were associated with both thinner tumours and better survival from melanoma, independent of Breslow thickness (Newton-Bishop et al., 2009). This data needs to be validated in additional sample sets and the level of vitamin D in the follow up period examined. Understanding the balance between optimal sun exposure to limit skin cancer risk while maintaining adequate vitamin D levels has been further complicated by work from Damian et al. (2010), which found that vitamin D had a presumably undesirable immunosuppressive effect when vitamin D analogues were applied topically to irradiated skin. On the other hand, Mason et al. (2010) reported that increased vitamin D levels reduced DNA damage *in vitro* following UVR and subsequently reduced UVR-induced immunosuppression in mouse and human skin. Currently data do not allow us to predict with any accuracy whether there may be a true causal influence of low Vitamin D levels on melanoma outcome. Although there is no solid evidence as such, we cannot discount that CSD melanomas may have an innately better prognosis because they are not associated with naevus susceptibility (certainly this would be the case for LMM which are well known to have better outcomes than SSM and NM).

9. Animals as model systems for melanoma

To mechanistically link sun exposure and melanoma is very difficult because individual sun exposure, especially based on recall, is difficult to assess, and the ratio of UVB/UVA varies greatly with geographical location, season, and time of day. This leads to great uncertainty in inferences about how different wavelengths influence melanoma development, hence sometimes model experimental systems can be useful, and animal models for carcinogenesis can provide complementary information when epidemiological studies have difficulty avoiding confounding factors. Grey horses and certain strains of pig are models for genetic susceptibility to melanoma, although there is no evidence for any effects of UVR exposure (Rosengren Pielberg et al., 2008; Seltenhammer et al., 2004). Opossom, guinea pigs and Angora goats have also been used as models for melanocytic lesion development (Chan et al., 2001; Menzies et al., 2004; Green et al., 1996). However except for goats, UVR exposure is not known to play any role in melanoma development in these animals. All of these species are very expensive to maintain, and generally limited in terms of the availability of reagents such as antibodies, and resources for genetic analyses. Various strains of fish including zebrafish (reviewed by Patton et al., 2010) and other fish species such as *Xiphophorus* (discussed in more detail below) are tractable models where UVR exposure can exacerbate the development of melanoma.

9.1 Modeling chronic-induced melanoma in mice

In contrast to the ability to induce SCC in wild type mice using chronic treatment regimens, a pre-existing genetically engineered mutation, and exposure of neonates, is necessary for inducing murine melanoma (Noonan et al., 2001). There are three reasons proposed to explain why mice develop melanoma after neonatal UVR, but not after chronic exposures to adult animals. First, neonatal mice have epidermal melanocytes that are likely to be damaged by UVR, whereas adult mice do not. Second, the heightened sensitivity of neonatal melanocytes to proliferation following UVR may be destabilizing (Walker et al., 2009), and third, the lack of inflammatory response to UVR in neonates may create a tolerant

environment for melanocyte transformation (Wolnicka-Glubisz et al., 2007; McGee et al., 2011). It is thought that murine neonatal UVR may be somewhat analogous to childhood sunburn (Noonan et al., 2001). Despite it being a somewhat specialized system, there is much we can learn using the neonatal UVR about how UVR results in melanocyte transformation. For instance using the *Mt-Hgf* model (with overexpression hepatocyte growth factor throughout the skin) it has been shown that UVB, and not UVA, induces melanoma (de Fabo et al., 2004), and that use of sunscreen can attenuate its development (Klug et al., 2010).

The skin of hairless mice contains some epidermal melanocytes, hence the animals represent a murine system amenable to chronic UVR exposures. Van Schanke et al. (2006) have carried out extensive UVR carcinogenesis studies on such animals carrying *Ink4a/Arf* deletion, with some cohorts also with co-deletion of the nucleotide excision repair gene *Xpa*. The mice developed naevi at a low rate spontaneously which was greatly increased by UVB treatment (how much depended upon the protocol and genotype). The naevi occasionally progressed to melanoma. Naevus development was dramatically increased by *Xpa* deletion, implicating UVB-induced pyrimidine dimer-type mutations in the pathogenesis of the lesions. Consistent with human melanoma, where intermittent exposures are most important, a single high dose erythemal exposure was much more effective at inducing naevi than the same dose delivered daily in a fractionated regimen (van Schanke et al., 2006).

In terms of using adult mice for UVR studies, a major problem is that they do not have epidermal melanocytes (and murine melanomas that develop are mostly dermal). Mice overexpressing Kitl in their keratinocytes (*K14-Kitl*) have epidermal melanocytes throughout life (Kunisada et al., 1998). They do not develop melanoma after chronic UVR exposures (Yamazaki et al., 2005). Even when crossed onto a DNA repair defective background (Xpa-null) no lesions were detected using a standard chronic UVB exposure protocol. But when the total UVB dose was increased over one half of the *K14-Kitlg::Xpa*$^{-/-}$ animals developed epidermal lesions reminiscent of lentigo and nodular melanomas (Yamazaki et al., 2005). Thus very high (almost physiological irrelevant) doses of UVB, plus a DNA repair defect, is need to induce transformation of the epidermal melanocytes in these mice. Interestingly, the animals developed very few SCCs, which both the high and low dose regimens do very effectively in wild type mice, hence it is thought that the extreme hyperpigmentation may be somewhat protective for both forms of skin cancer in this model. Nonetheless, murine epidermal melanocytes are apparently not totally resistant to transformation by UVR *per se*. It is possible that albino versions of the *K14-Kitl* model may have potential as a mouse model for chronic UVR-induced melanoma. Chronic UVR is somewhat effective in inducing melanomas in *Tyr-Hras*^{G12V} transgenic mice on an albino, but not pigmented strain background (Broome Powell, et al., 1999). However in *Mt-Hgf* transgenics chronic adult exposures do not exacerbate the development of melanoma (Noonan et al., 2001).

10. Mechanisms of UVR carcinogenesis in melanoma

10.1 Evidence of UVB causality in melanoma

The ultraviolet spectrum that plays a physiological role in skin cancer development is arbitrarily divided into UVB (280-315 nm) and UVA 315-400 nm). Non-melanoma skin cancer (especially SCC) is undeniably associated with chronic UVR exposure, and tumours carry "classical" UVB signature mutations resulting from mis-repaired cyclobutane pyrimidine dimer (CPD) or pyrimidine (6-4) pyrimidone photoproducts (6-4 PPs) adducts. The action spectrum for SCC induction in mice, and the inferred action spectrum for SCC in

humans, peaks at 293 nm, firmly within the UVB range (de Gruijl et al., 1993). This overlaps with the action spectrum for CPD formation and sunburn. UVB, but not UVA, very effectively induces non-melanoma and melanoma skin cancer in mice (De Gruijl et al., 1993; De Fabo et al., 2004). Evidence of a critical role for UVB in melanoma induction comes from humans (van Steeg & Kramer, 1999) and mice (Yang et al., 2007) carrying nucleotide excision repair (NER) enzyme mutations (in *XP* genes) where melanoma incidence is dramatically increased after UVR exposure. XP patients have extreme sun sensitivity and burn very easily (van Steeg & Kramer, 1999). Melanomas from these individuals frequently carry *TP53* and *PTEN* gene mutations that show classic C-T or CC-TT UVB signatures and lesions are similar in body site distribution associated with chronic UVR exposure (Spatz et al., 2001; Wang et al., 2009). Further, the majority of melanomas in these individuals appear to be of the lentigo type, but they do not exhibit the solar elastosis that is invariably present in the same melanoma subtypes developing in DNA repair-proficient individuals (Spatz et al., 2001).

More information about the melanoma UVR mutation signature comes from the first melanoma genome sequence (Pleasance et al., 2010). Of 33,000 single point mutations detected, nearly 70% were C-T transitions. The only other nucleotide change above levels expected by chance were G-T transitions (9%) that can be a marker for UVA-induced damage (Agar et al., 2004). Notwithstanding the fact that only one melanoma, a secondary with undetected primary, was sequenced (Pleasance et al., 2010), and that some mutations could have been acquired by sun exposure during tumour development, these results suggest that CPD adducts may be critical driver of melanoma genesis. This is remarkably similar to the results of a recent review of all known *CDKN2A* and *TP53* point mutations in melanoma (Hocker & Tsao, 2007) which found that the frequency of UVB-signature mutations (65 and 55 % respectively) in these two genes in melanoma is similar to that found in SCC, a skin cancer with well-characterized UVB causality. Despite the presence of these UVR signature mutations, the overall mutation rate of these two genes in primary cutaneous melanomas is very low (7.9% and 11.8% respectively) (Hocker & Tsao, 2007), accounting for only about 10% of all melanomas. By comparison, *BRAF* or *NRAS* are mutated in more than 70% of all cutaneous melanomas (Hocker & Tsao, 2007).

Although we usually concentrate on CPDs as the mutagenic adduct, UVB also induces 6-4PPs, which are larger the CPDs, hence recognized and removed much more rapidly by nucleotide excision repair (NER). It has been hypothesized that 6-4PPs may be involved in melanoma induction, not via a mutagenic mechanism, rather via their deregulation of genome surveillance and transcription mechanisms leading to downstream changes that may deregulate the melanocyte (Mitchell et al., 2010). It is known that the two forms of UVB-induced photoproduct induce differential effects within cells (Lo et al., 2005). 6-4PP lesions are much more important in triggering cell death, whereas the response of the cell to CPD lesions mainly involves cell cycle arrest. An important role for 6-4PPs in melanoma is a speculative but interesting potential alternate aetiogy. Of note, 6-4PPs play no role in the generation of SCC in mice, CPD adducts are necessary and sufficient (Jans et al., 2005).

10.2 Evidence of UVA causality in melanoma

In contrast to UVB, UVA is generally extremely inefficient at inducing CPDs, oxidative damage, erythema, and non-melanoma skin cancer in mice (De Gruijl et al., 1993; Besaratinia & Pfeifer, 2008; Runger & Kappes, 2008). However UVA can induce 8-oxo-guanine (8-oxoG) oxidative adducts that can results in the formation of G-T transversions

(Agar et al., 2004). Results of another study suggest that T-G transversion is a UVA "signature" (Drobetsky et al., 1995). UVA-specific lesions in the p53 gene have been detected in skin constructs and squamous tumours (Agar et al., 2004; Huang et al., 2009). In contrast, *in vivo* studies using "Big Blue" mice, and *in vitro* data, suggests that UVA-induced mutations are mainly of the pyrimidine dimer type (Mouret et al., 2006; Besaratinia & Pfeifer, 2008; Runger & Kappes, 2008). An interesting idea regarding the role of UVA in melanoma is that UVA and UVB generate a similar DNA mutation spectrum (although UVA is much less effective at inducing CPDs), but that UVA-induced cellular stress and repair response is not as great, thus lesions may not be as effectively removed (Runger & Kappes, 2008). Possibly this would only apply after relatively pure UVA exposures, for instance from solaria, or through glass.

Notably, most of the studies mentioned above have used keratinocytes and fibroblasts, and not melanocytes to assess the mutagenicity of UVA. A recent study suggests that UVA is much more effective than UVB in inducing reactive oxygen species in melanocytes than in the other cell types (Wang et al., 2010). In addition, melanocytes are less efficient in removing CPDs and oxidative DNA damage. As discussed by Runger, (2011), these findings are at odds with some other studies, but nonetheless are indicative of potential differences between the responses of melanocytes and other skin cells to UVR. Runger, (2011) also raises the question of why if there is so much oxidative damage induced by UVA why are lesions typical for such stress vastly underrepresented in melanoma (e.g. Pleasance et al., 2010)? He suggests that this could relate to the low mutagenicity of 8-oxoG adducts.

The ability of UVA to generate melanoma in *Xiphophorus* backcross fish is suggestive of a role for UVA in melanoma development (Setlow et al., 1989; Setlow, 1999). This has long been interpreted to infer UVA causality for melanoma, probably based on melanin photosensitization and subsequent oxidative damage to DNA. UVA is about 1000-fold less effective than UVB in inducing erythema and SCC, whereas in fish melanoma induction there is only about a 10-fold difference between the effects of UVB and UVA. Given the overwhelming preponderance of UVA in natural sunlight, if the fish action spectrum held up in humans, UVA would dominate melanoma causality. *Xiphophorus* are a complicated model. They carry photolyase, a light-inducible system that very rapidly and specifically repairs specific DNA adducts (e.g.. CPD-photolyase removes CDPs). Such repair systems are present in most of the plant and animal kingdom except for rodents and primates. Thus the fish experiments are carried out in the dark, and activation (by light) of the photolyase reduces melanoma incidence to background levels. Recently Timmins and colleagues used electron paramagnetic resonance assays to show that the action spectrum for melanoma and melanin radical production overlap (Wood et al., 2006), further evidence for melanin radical causation. However the notion that UVA is more effective than UVB in inducing melanoma in fish has been questioned by Mitchell et al., (2010), after similar experiments using apparently the same strain of fish. This conflicting result from the original study (Setlow et al., 1989) may be largely explained by the fact that latest study used more animals to make the results more statistically significant, and followed the fish for a longer time, allowing for later age of onset of some melanomas. Further, the fish also carry nucleotide excision repair activity, and melanoma development is exacerbated in fish with defective NER (Mitchell et al., 2007). The authors point out that UVA exposure is still potentially very important in the induction of melanoma in humans, but it may not be via a melanin radical-based mechanism.

One would expect that if melanin sensitization were an important mechanism, we would not see the huge increase in melanoma risk for patients with XP, unless they also lacked a

defence against the melanin radicals.However there remains an anomaly that Africans with albinism (i.e. no melanin, or low levels), who practice poor sun protection, have been consistently shown to only very rarely develop melanoma (reviewed in Wood et al., 2006). In 164 such patients in Tanzania actinic keratoses were found in 100%, and SCC in 34%, of albino individuals over 30 years old, but no melanomas were found (Lookingbill et al., 1995). In these cases childhood sunburns do not seem to drive subsequent melanoma development.

The only other model used as evidence for UVA causality in melanoma is the South American opossum, *Monodelphis Domestica*, although the effect is weak (Mitchell et al., 2007). A study on focal pigmented hyperplasia developing in the opossum after UVA (Ley, 2001) showed that the action spectrum for the development of these lesions was much closer to the SCC action spectrum rather than the fish action spectrum. Nonetheless pure UVA does seem able to induce melanocyte proliferations in these animals, albeit not melanomas. Another interesting model is the guinea pig. These animals develop naevi after chronic UVB but not after chronic UVA exposures (Menzies et al., 2004). Neonatal UVB and not UVA induces melanoma in albino *Mt-Hgf* mice (De Fabo et al., 2004). In short, the fish is the only published model for UVA-induced melanoma and the conclusions have been questioned. However there is some evidence using pigmented *Mt-Hgf* mice that UVA can increase melanoma penetrance after neonatal exposure (Fisher et al., 2009, meeting report from the 6th international melanoma congress), but it does not induce melanoma in albino *Mt-Hgf* mice. UVB effectively induces melanoma on both pigmented and non-pigmented backgrounds. Because the UVA effect is only seen in pigmented mice, the carcinogenic mechanism may involve increased oxidative stress induced by photosensitized melanin. Application of inhibitors of melanin synthesis before and after UVR exposure of appropriate animal models may provide an avenue to test the melanin radical hypothesis. It appears that the debate about the role of UVA in melanoma induction is not over.

It must be pointed out that the murine and fish studies cannot model cumulative lifetime exposure to UVA in sunlight. We cannot rule out a role for UVA given that although the genotoxicity (i.e. frequency of dimers) is much higher in the UVB, UVA is far more abundant in sunlight (at least 20-fold). Not only are there debates about the role of UVA, there are even studies suggesting a protective role for UVA. Here, with UVB dose kept constant, increasing UVA dose protects against epidermal apoptosis (Ibuki et al., 2007) and SCC induction in mice (Forbes et al., 1978). Which wavelengths are critical for melanoma formation? In some ways this is irrelevant, and the real question is what type of adducts are needed? The balance of evidence to date suggests that the susceptibility of a melanocytes to UVR-induced transformation depends mostly upon the presence of classical CPD type adducts that if not properly removed result in C-T or CC-TT mutations. However this has not been formally proven.

10.3 Role of UVR in generating *BRAF* and *NRAS* mutations

The DNA base changes causing activating mutations in *BRAF* and *NRAS* do not represent classical UVB signatures, thus other mechanisms have been proposed for their causation. $BRAF^{V600E}$ is found in several internal malignancies, arguing against a specific role for UVR, and more suggestive of a role for generalized oxidative damage, or another mechanism (Dhomen et al., 2007). The $BRAF^{V600E}$ mutation is generally caused by a T>A transversion, and one theory regarding the possibly role of UVR in the generation of this change relates to error prone repair at the V600 mutation site in *BRAF* caused by adjacent pyrimidine dimers (Thomas et al., 2006). On the other hand, Besaratinia, & Pfeifer, (2008) show that there are

many types of lesions that can be induced by solar UVR, which although uncommon, could explain some of the mutations in detected in *BRAF*, particularly as there is selection pressure for the "required" the amino acid change. As discussed by Lund & Timmins, (2007) bulky adducts formed by reactive melanin species may be involved. None of these theories have been functionally tested. However in many melanomas the base change resulting in the *NRAS* codon 61 mutations is a G>T transversion (Hocker & Tsao, 2007). It has been experimentally confirmed *in vitro* using murine fibroblasts, that an 8-oxoG-mediated transcriptional mutagenesis mechanism greatly enhances the acquisition of such mutations (Saxowskya et al., 2008). Using a system that selected for clones carrying mutant $HRAS^{Q61}$ mutations they showed that these were very rare in wild type murine fibroblasts but common in cells lacking the enzyme 8-oxoguanine DNA-glycosylase 1 (Ogg1), which repairs 8-oxoG lesions. The mutations were induced by G-T changes in the transcribed strand of the *HRAS* transcript. Thus while *NRAS* mutations may be induced in melanomas following UVB exposure, this mechanistic data best supports a role for oxidative adducts in their formation rather then CPDs. There is little mechanistic data to support the genesis of the $BRAF^{V600E}$ mutation from oxidative stress, only the observation that it is sometimes found in mucosal and acral melanomas, not associated with sun exposure, and in internal cancers (thyroid and colorectal) (Dhomen et al., 2007).

It is difficult to glean much from murine melanoma models regarding the potential role of UVR in inducing *BRAF* or *NRAS* mutations. *Braf* mutations have not been detected in murine melanomas. One interesting finding comes from work with $Ink4a/Arf^{-/-}/Xpc^{+/-}$ mice. These mice, essentially the only example of mice developing melanoma due to UVR exposure without carrying an engineered oncogenic mutation, resulted in development of melanomas that frequently carried $Kras^{Q61}$ mutations. Similarly, melanomas induced by UVB in $Ink4a/Arf^{-/-}/Xpa^{+/-}$ adult hairless mice occasionally carried an $Nras^{Q61}$ mutation (van Schanke et al., 2006). The Ras^{Q61} mutations in the NER-deficient mice were mainly G-T changes, again reflective of mis-repaired 8-oxoG adducts rather than mis-repaired CPDs.

As suggested by Runger, (2011), oncogenes can only function as such due to very specific gain-of-function mutations that can only occur as certain amino acid changes, "*thus the DNA base change may rather indicate a constraint on the amino acid change than the identity of the mutagen*".

11. UVR, melanoma, and the immune system

11.1 Immunosuppression

There is undoubtedly an interplay between damaged melanocytes and immunocytes, whether just after UVR exposure or during tumour progression. It has long been known that the UVR exposure can suppress the immune system and create an environment tolerant to the growth of tumour cells that should be targeted for immunological destruction. Margaret Kripke and colleagues (Donawho et al., 1996) described how the growth of implanted tumours in mice is enhanced by local photoimmunosuppression. How much of a role it plays in melanoma development is unknown. Transplant patients taking immunosuppressive drugs are at a particularly heightened risk of skin cancer, particularly SCC, but it is a matter of debate whether they are at increased risk of melanoma. Out of nine studies recently reviewed (Bastiaannet et al., 2007), five reported between 2 and 4-fold increased risk, and four reported no increased risk. If immunosuppressed patients are at increased melanoma risk, it is low, and much less than the risk of developing SCC. Despite

this, individuals taking immunosuppressive drugs sometimes develop eruptive naevi, and this form of melanocyte proliferation is proposed to be due to the effects of immunosuppression rather than the drugs that induce it (Zattra et al., 2009).
An important factor in the initiation of melanoma in mice by neonatal and not adult exposure is that neonates exhibit a defective inflammatory response to UVR compared to adults (Wolnicka-Glubisz et al., 2007; McGee et al., 2011). There may be ways to investigate the role that photoimmunosuppression plays in UVR-induced tumorigenesis. For example, (Jans et al. 2005) used mice carrying an inducible photolyase system that very rapidly removes CPDs from the skin after UVR exposure. Removal of CPDs from the whole skin significantly reduces both SCC development and immunosuppression. However, removal of CPDs specifically from the epidermal basal layer (using *K14-Photolyase* transgenics) similarly reduced tumorigenesis, but did not prevent photoimmunosuppression (Jans et al., 2006). Thus immunosuppression seems to depend upon damage throughout the epidermis and dermis, whereas SCC is driven largely by UVR damage to basal layer keratinocytes, suggesting that immunosuppression may be important but not essential for the initiation of this skin cancer in mice (at least in terms of UVR damage-driven SCCs). The relative contribution of direct DNA damage to melanocytes, and photoimmunosuppression, in melanoma needs to be clarified.

11.2 UVR-induced inflammation

One of the difficulties in studying UVR causality in melanoma is not only that multiple UVR response mechanisms such as DNA repair, proliferation and immune response play a role, but they are often not independent of each other. We can look at the normal response of melanocytes to UVR (Figure 2), in particular the multiple effects of cytokines that are released in the skin to activate melanocytes. Upon UVR-induced damage keratinocytes upregulate their expression of the pro-opiomelanocortin (Pomc) gene. Pomc encodes a pro-peptide that is cleaved to generate α-MSH, ACTH and β-endorphin (Cui et al., 2007). Together, these peptides have pleiotropic effects on endocrine and neuroendocrine signaling, and the immune system (Brzoska et al., 2008), in addition to the melanotropic function of αMSH. Another protein upregulated in the epidermis after UVR exposure, KITL, can drive proliferation and migration of both pro-inflammatory mast cells as well as melanocytes (Kunisada et al., 1998). Pro-inflammatory cytokines like interleukin 12 (Schwarz et al., 2002) and interleukin-18 (Schwarz et al., 2006) can increase DNA repair capability of melanocytes after UVR exposure. In the case of interleukin-18, this may be via upregulation of KITL (Hue et al., 2005). Another secreted protein, previously known only for its role in immune responses to infectious agents, β-Defensin, is upregulated over 50-fold in human epidermis after UVR exposure (Enk et al., 2006) and is possibly involved in melanocye response to UVR as another ligand for the MC1R (Candille et al., 2007). Thus the release of cytokines within the skin not only activates the immune system, but also induces protective responses in the melanocye itself (e.g. increased pigmentation, proliferation, and DNA repair).
The proliferative burst of melanocytes emanating from the upper portion of the hair follicle in neonatal mice presents an excellent opportunity to investigate how melanocytes are activated by UVR exposure. Zaidi et al. (2011) have cleverly utilized the power of the genetically modified mice to look into the mechanism of this melanocyte response. They used a genetically engineered mouse model inducibly expressing green fluorescent protein (GFP) in melanocytes. GFP was induced immediately after UVB exposure and melanocytes

were isolated via fluorescence activated cell sorting at various time-points after neonatal UVR. Gene expression array analysis on these cells detected a strong signature of interferon-gamma (IFNγ)-induced genes that coincided with the appearance of melanocytes in the epidermis. It was subsequently shown that that the melanocyte response is largely driven by IFNγ released from infiltrating macrophages. Further experiments indicated that not only can macrophages influence melanocyte proliferation in the context of UVR exposure, but that they also contribute to the pro-tumorigenic inflammatory microenvironment of melanomas. This is possibly the first study to establish a direct link between the immune and melanocytic systems during the immediate skin response to UVR.

12. Conclusion

Because, measured on a population basis, melanoma induction by UVR appears to be via a very different mechanism (i.e. via intermittent exposure) than for keratinocyte-derived cancers (i.e. via chronic exposure), it has been postulated that there are different carcinogenic mechanisms at play (Setlow, 1999). Different mutagenic DNA adducts are proposed to be involved, including UVA-induced oxidative lesions, UVB-induced pyrimidine dimers and 6-4PPs. In studies of human populations individual sun exposure level, based on recall, can be difficult to assess, but also the ratio of UVB/UVA varies greatly with geographical location, season, and time of day. This leads to uncertainty in inferences about how much exposure and which wavelengths most influence melanoma development. However epidemiological work has lead to the proposal of the divergent pathway model for melanoma, where some melanomas develop as a result of intermittent exposure, others after chronic exposures (Whiteman et al., 2003). The major difference between the chronic and intermittent branches of the model is the presence of naevi, the great majority of which carry *BRAF* mutations, in the former. Naevi can develop spontaneously. Hence the presence of or propensity to develop naevi increases melanoma risk. Even limited sun exposure appears to increases this risk, whereas for individuals not prone to develop naevi a relatively high cumulative lifetime UVR damage may often be necessary. Nevus cells proliferate strongly, and move to a suprabasal (malignant-like) location if subjected to only a single UVB exposure *in vivo* (Carrera et al., 2008). Thus naevus cells, unlike normal melanocytes within the skin, are extremely sensitive to UVB-induced damage. It is not known if there is a mechanistic difference in UVR mutagenesis between the two CSD and non-CSD pathways. However, judging from the mutation spectrum in human melanoma, dominated by pyrimidine dimer type mutations, the most parsimonious conclusion may be that it is not necessary to invoke a different mutagenic mechanism *per se* to explain apparent differences in UVR causality between melanoma and non-melanoma skin cancer. In both cases pyrimidine dimer type DNA lesions are involved.

If multiple carcinogenic mechanisms are at play, this can be tested in a number of ways. The stratification of melanomas into CSD and non-CSD has been critical in enhancing our understanding of divergent mechanisms of melanoma genesis, but since the two major forms of melanoma, SSM and NM can be associated with either forms of exposure, further stratification may be necessary (Of note, LMM clearly has a different aetiology from NM and SSM). This may be in the form of the discovery of better somatic mutation signatures, as well as further innate genetic differences between the two groups. The development of further tests to differentiate between the two groups could help in terms of targeting particularly susceptible groups within the population for health education campaigns and

more frequent screening. High throughput genome sequencing of large numbers of melanomas of various subtypes and association with CSD or non-CSD should clarify which type of DNA adducts are driving melanoma development, and in doing so might go some way towards clarifying the role of UVB versus UVA in the genesis of melanoma. Improved animals models should also be informative.

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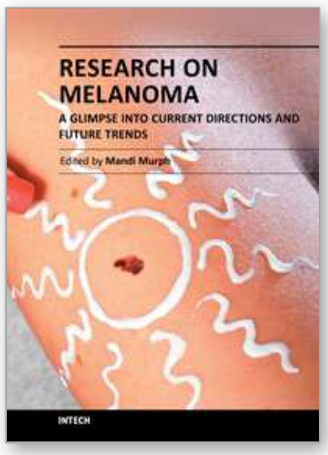

Research on Melanoma - A Glimpse into Current Directions and Future Trends

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The book Research on Melanoma: A Glimpse into Current Directions and Future Trends, is divided into sections to represent the most cutting-edge topics in melanoma from around the world. The emerging epigenetics of disease, novel therapeutics under development and the molecular signaling aberrations are explained in detail. Since there are a number of areas in which unknowns exist surrounding the complex development of melanoma and its response to therapy, this book illuminates and comprehensively discusses such aspects. It is relevant for teaching the novice researcher who wants to initiate projects in melanoma and the more senior researcher seeking to polish their existing knowledge in this area. Many chapters include visuals and illustrations designed to easily guide the reader through the ideas presented.

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