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Cover Page Footnote

I give thanks to Dr. Rachel Patton-McCord and Dr. Mariano Labrador for their assistance and helpful discussions.

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Mechanisms of Familial and UV-Induced Melanoma

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Melanoma is the most common form of skin cancer in the United States. Melanoma is a disease where cancerous cells derive from melanocytes. Common melanoma susceptibility genes induce dysregulation in the mitogen-activated protein kinase (*MAPK*) pathway, phosphatase and tensin homolog (*PTEN),* and *AKT* expression. Melanoma can be induced through prolonged UV-radiation or familial diagnosis. This article aims to discuss the mechanisms of both familial and UV-induced melanomagenesis.

Introduction

Skin cancer is the most common form of cancer, representing 40-50% of all cancers diagnosed in the United States. There are approximately 3.5 million cases of skin cancer diagnosed within the United States each year alone [1]. Melanoma is a highly dangerous form of skin cancer that consists of 4-5% of all skin cancers but contributes to 70-80% of skin cancer deaths [2]. Melanoma prevalence has increased throughout the world through environmental and genetic origins, mainly induced by ultraviolet radiation or through genetic mutations inherited from family risk factors. Hereditary cancer predisposition factors are responsible for approximately 5-10% of all diagnosed cancer cases. Likewise, 5-10% of all cutaneous melanoma cases are familial [3]. In these families, patterns of heritability are consistent with an autosomal dominant inheritance with incomplete penetrance, where individuals that possess risk factor genes may or may not express associated cancer traits [3]. There is an estimated 60-70% of cutaneous malignant melanoma cases that are thought to be caused by ultraviolet radiation exposure [1]. However, in the past decade (2012-2022) the number of new invasive melanoma cases diagnosed annually increased by 31%, accounting for more than 90% of skin cancers due to prolonged sun exposure [4].

Melanoma Biology

Melanocytes are specialized pigmented cells that are found predominantly in the skin and eyes, where they produce melanin [5]. Melanin is produced within the cytoplasmic melanosomes, in which tyrosinase acts upon tyrosine to yield dihydroxyphenylalanine (DOPA) which is converted into dopaquinone to serve as a precursor for melanin formation. The melanin pigment is then transported to adjacent keratinocytes that possess external protease-activated receptors (PAR-2) [6] (Figure 1). Melanocytes originate from the neural crest cells with pluripotent cells that gradually become lineage-specific during development [6]. The lifecycle of a melanocyte consists of several steps including lineage specification from embryonic neural crest cells (melanoblasts), migration and proliferation of melanoblasts, differentiation of the melanoblasts into melanocytes, maturation of melanocytes, and transport of mature melanosomes to keratinocytes [7]. Melanocyte development, function, migration, and survival is mainly dependent on the expression of tyrosine kinase receptor *c-kit* gene (CD117) [6]. CD117 has been shown in previous studies that its activation mainly initiates melanocyte migration rather than proliferation [8].

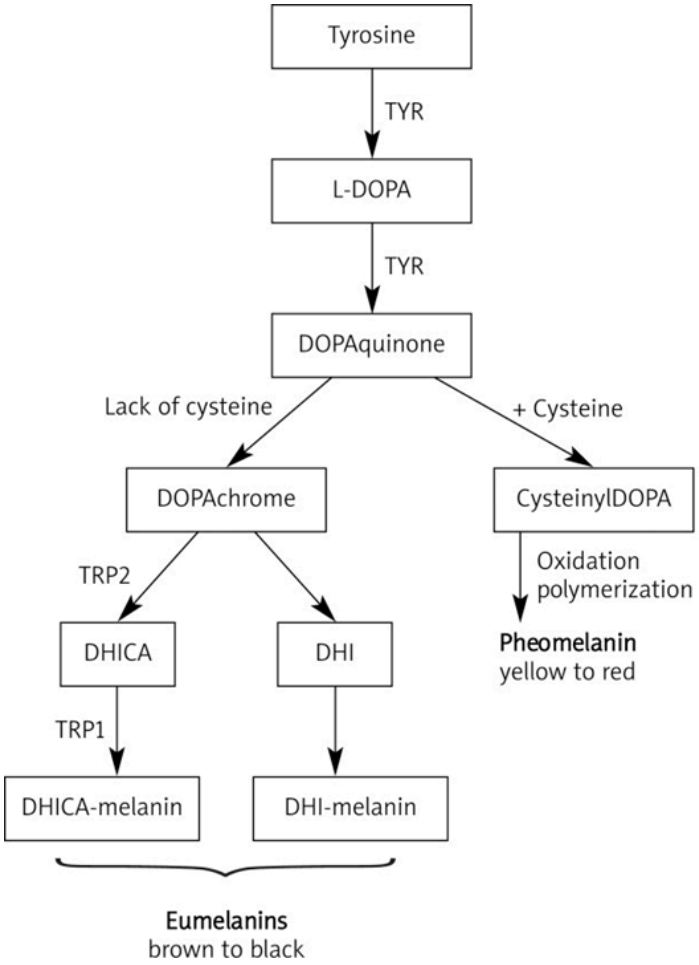

Figure 1. Melanin Synthesis. During melanogenesis in melanocytes, tyrosine is under the influence of enzymes such as tyrosinase (TYR), tyrosine-related protein 1 (TYRP1), and TYRP2 that influence its change into a polymer of melanin. This results in a mixture of pigments known as eumelanin (brown-black) and pheomelanin (yellow-red) [7].

In malignant melanoma, these melanocytes can possess a variety of different histological features that can mimic other tumors such as lymphomas, poorly differentiated carcinomas, neuroendocrine tumors, and germ cell tumors. Melanocytes can also possess complex cytoplasmic morphologies such as a clear cell, signet ringing shape, rhabdoid, ballooning and plasmacytoid appearance [2]. Melanoma can be characterized by a general appearance of a nevus, skin mole, with an irregular border or increased diameter and dark skin coloration. Melanocytic nevi are abnormal tissue masses that result from melanocyte proliferation and spreading, leading to the early stages of melanoma. Melanocyte proliferation can also be restricted within the epidermis (junctional nevus), dermis (dermal nevus), or as an overlap of both skin regions (compound nevus) [9]. Nevi can progress into a radial-growth phase (RGP) melanoma which involves local micro-invasion of the dermis. RGP melanoma can then progress into vertical-growth phase (VGP) melanoma, giving rise to cells with metastatic potential and nests of

cells that can invade the dermis. These phases can lead to the formation of malignant melanoma [6] (Figure 2). However, this sequence of progression can be histologically documented on about a third of all melanoma cases, and malignant melanoma can potentially arise in the absence of visually obvious pathological progenitor lesions [10].

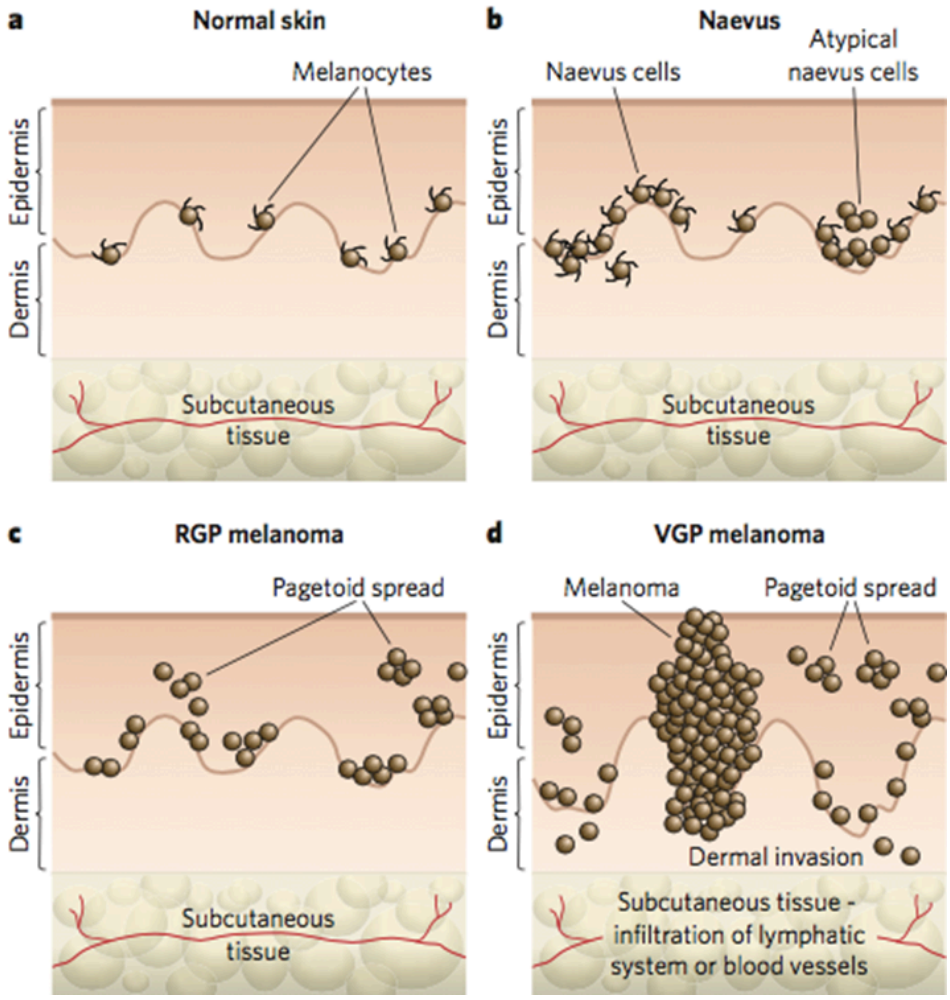

Figure 2. Melanocyte transformations. There are various stages of melanocytic lesion, each of which is marked by a new clone of cells with growth advantages over the surrounding tissues. A) In normal skin, an even distribution of melanocytes is present within the basal epidermis. B) In early stages, benign melanocytic naevi occur with increased numbers of dendritic melanocytes. Some naevi are dysplastic, with morphologically atypical melanocytes. C) Radial-growth phase (RGP) melanoma is considered to be the primary malignant stage. D) Vertical-growth phase (VGP) melanoma is considered to have malignant potential and leads directly to metastatic malignant melanoma via infiltration of the vascular and lymphatic systems. Pagetoid spread refers to the upward migration or vertical stacking which is a histological characteristic of melanoma [5].

Melanoma-Susceptible Genes

Melanoma affects various genes rather than one specific gene. Three key molecular pathways have been found to be highly deregulated in melanoma: mitogen-activated protein kinase (*MAPK*), as a result of mutations in *RAS*, *RAF*, and *KIT*; *PI3K/AKT*, due to mutations in *RAS*, mutations or loss of *PTEN* (phosphatase and tensin homolog); and dysregulated expression of *AKT* and *p16INK4A* due to mutations in *CDKN2A*, *ARF*, and *p53* [11] (Figure 3).

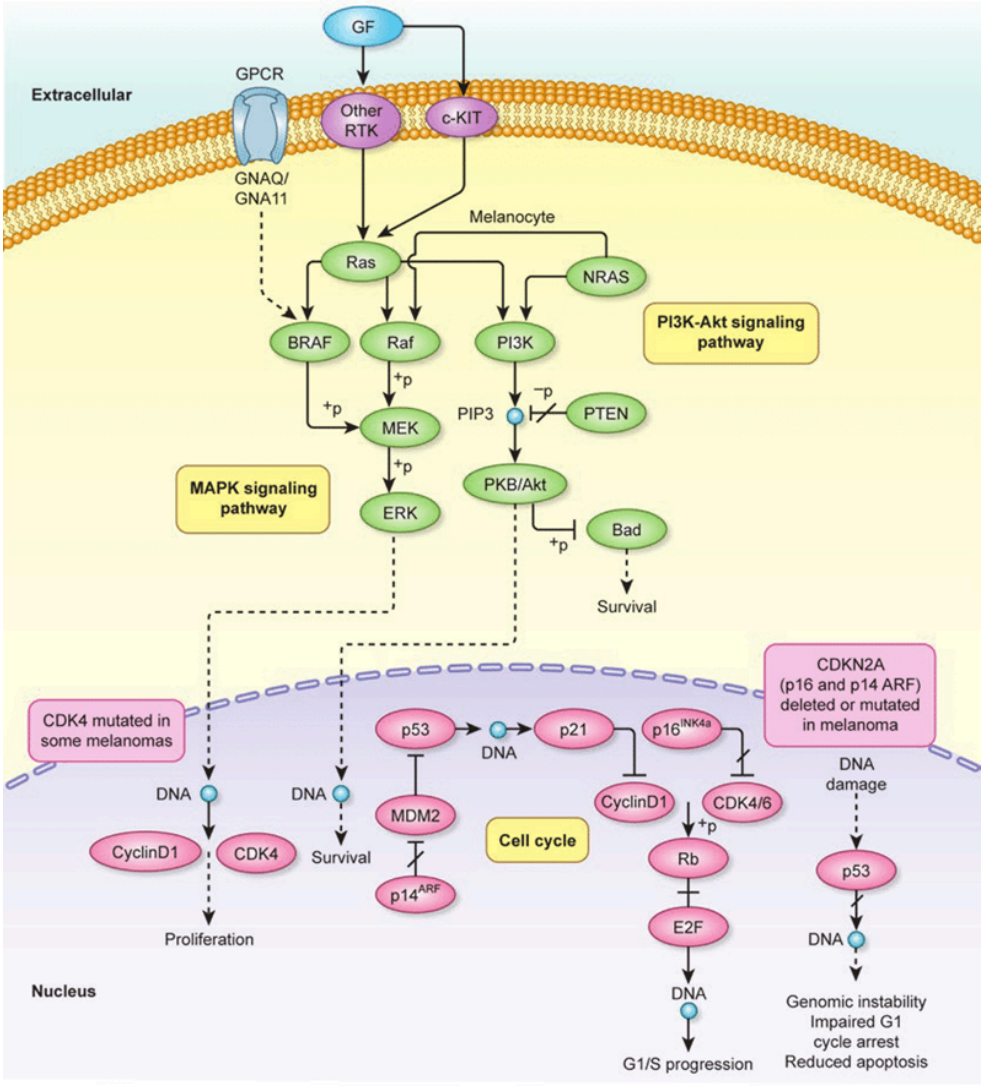

Figure 3. Genetic alterations within the development of melanoma. Melanoma can develop by mutations within the MAPK, PI3K-AKT, and CDKN2A pathways [12].

The MAPK pathway functions to transport and amplify extracellular signals into and among other cells. The *KIT* gene encodes for a tyrosine-kinase membrane receptor that stimulates MAPK and PI3K pathway. Previous studies have shown that *KIT* is involved in melanocyte development due its lack of migration and disappearance in *KIT*-deficient melanoblasts, thus stimulating melanocyte migration rather than proliferation [13].

The MAPK pathway contains the proteins RAS, RAF, MEK, and ERK and it is a part of a signaling cascade that uses a chain of phosphorylation to transfer extracellular signals from the cell membrane to the nucleus, where the pathway activation signals genes within the nucleus to promote cell proliferation [13]. The RAS proteins include HRAS, KRAS, and NRAS which contain GTPase activity and are located within the plasma membrane to serve as mediators of cell growth, development, and differentiation. RAS bound with GTP activates effector molecules such as RAF and PI3K, and through the activation of these effectors, RAS can regulate cellular development, proliferation, and processes linked to tumorigenic cell transformation. Mutations in the *RAS* genes can lead to abolishing GTPase activity and decrease sensitivity to GTPase-activating proteins (GAPs), thus, preventing the dissociation of GTP during GDP-GTP cycling [11]. The RAF proteins consist of ARAF, BRAF, and CRAF that function as protein kinases. BRAF is located downstream of RAS and induces phosphorylation of MEK when activated. The most common BRAF mutation is known as $BRAF^{V600E}$. The mutation consists of a single-base missense trans version of a T to an A at nucleotide 1,799 and a change in amino acids from valine to glutamic acid in codon 600 (V600E) of exon 15. This results in constitutive activation of RAF kinase as well as increased MEK and ERK phosphorylation [11]. A frequent event within cancerous cells is when the MAPK pathway with extracellular signal-regulated kinase (ERK) acting as a terminal kinase is aberrantly activated, often the result of activating mutations in the oncogenes BRAF and RAS. Mutations of RAF and RAS are mutually exclusive in associated malignancies including melanoma [11] (Figure 4 & 5).

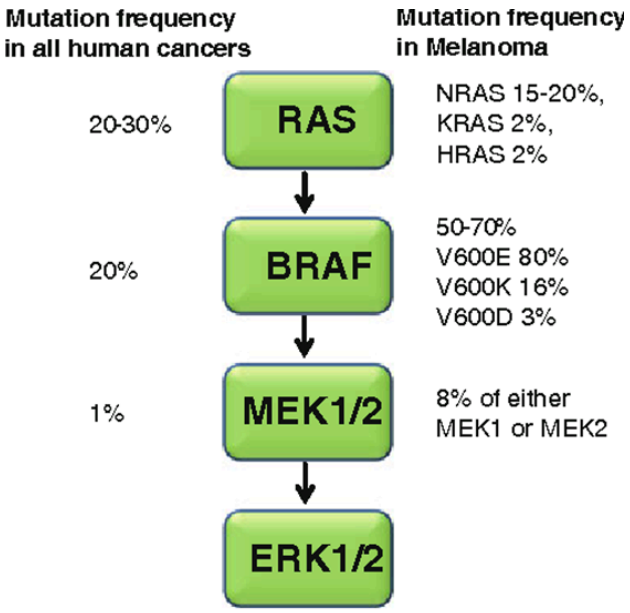

Figure 4. Mutation rates of MAPK pathway components. Mutations for RAS are the most common within human cancers ranging from 20-30% and 15-20% in melanoma specifically. BRAF mutations occur at the highest rates in melanoma when compared to all human cancers. MEK1/2 and ERK1/2 have relatively low to none rates of mutations in all human cancers and melanoma [14].

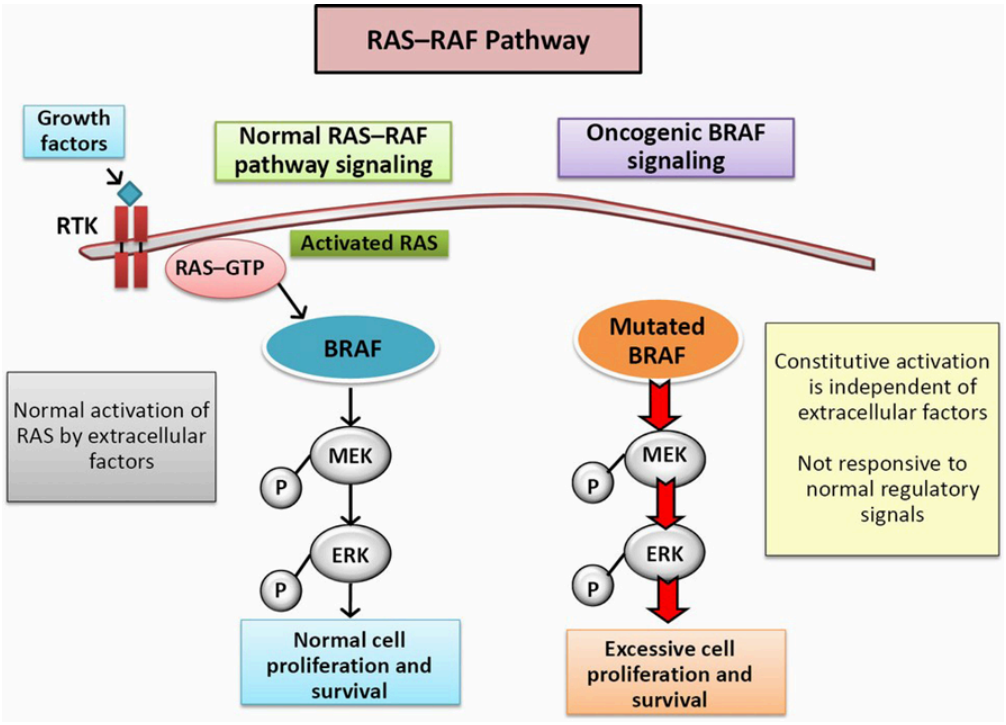

Figure 5. MAPK pathway under normal and mutated BRAF conditions. Under normal conditions, a growth factor ligand binds to its respective RTK receptor to stimulate the RAS-GTPase activity to activate the RAF proteins to function as effector molecules. RAF proteins relay cellular signals through phosphorylation of MEK which will in turn phosphorylate ERK to overall mediate normal cellular proliferation and survival. In oncogenic conditions, the mutation in BRAF will induce increased levels of phosphorylation of MEK and ERK, resulting in excessive cellular proliferation and survival [15].

The PI3K/AKT pathway is a secondary pathway alongside MAPK that is also activated by RAS-GTPase activity. The class of IA PI3K enzymes mainly function to induce the formation of a second messenger known as phosphatidylinositol 3,4,5-triphosphate (PIP3) that functions in activating a signaling cascade that regulates other effector molecules such as AKT and mTOR that are located downstream (Figure 6). The activation of mTOR has been shown to be involved in regulation of glucose availability in cell and tumorigenesis [11].

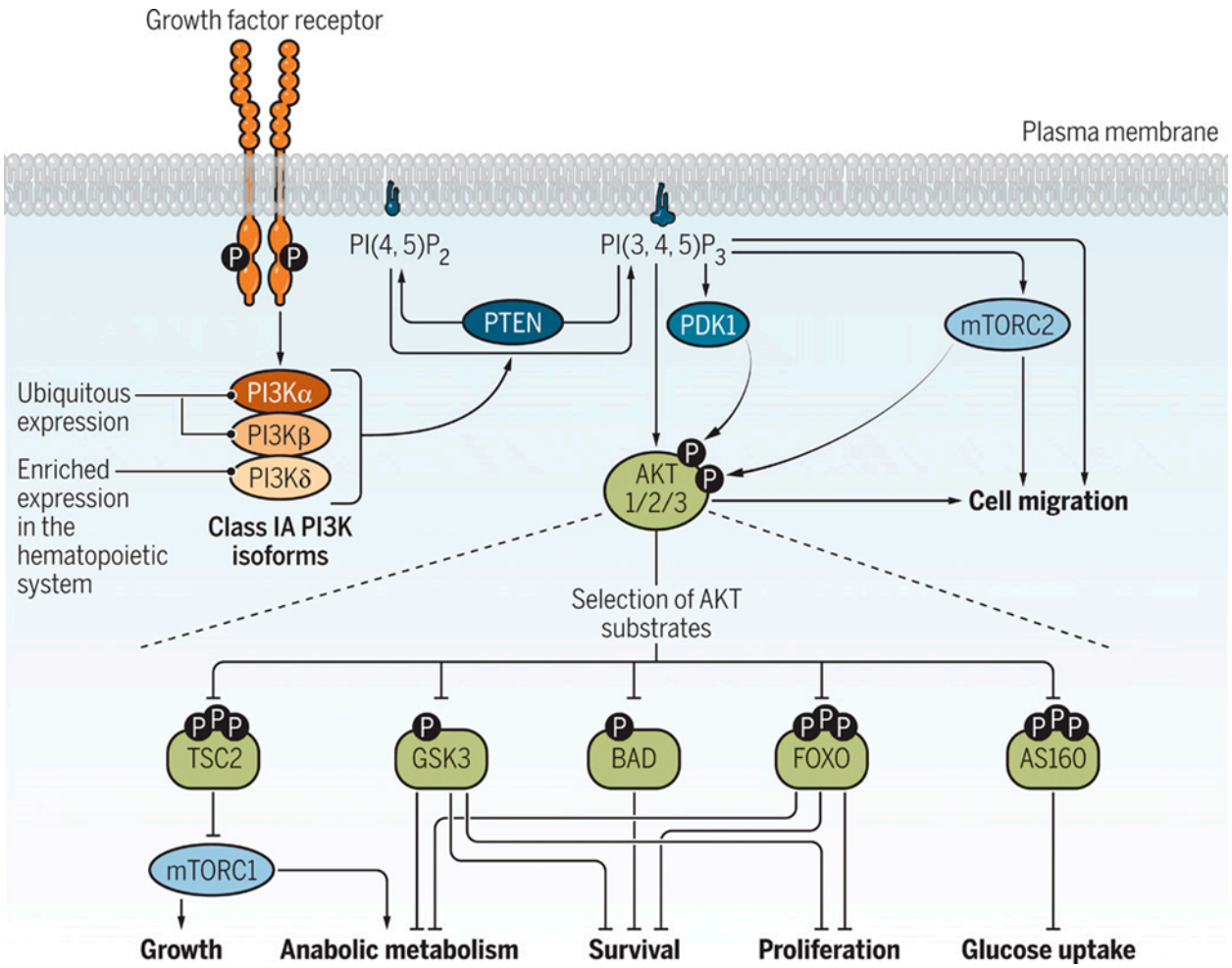

Figure 6. PI3K signaling and cellular output pathways. The class IA PI3K contains three isoform subunits (PI3Ka, PI3Kß, and PI3K that are activated downstream from the tyrosine kinase receptors when regulatory regions bind to the phosphorylated tyrosine residues. Activation of PI3K isoform subunits can be enhanced further by the activation of RAS. PI3K activation leads to the formation of the PIP3 second messenger that signals by binding and recruiting effector proteins containing pleckstrin homology (PH) domains. An effector known as AKT is involved in the major PI3K-dependent cellular phenotypes by acting on various cellular substrates such as TSC2, GSK3, BAD, FOXO, and AS16O to mediate other cellular processes [16].

This pathway is critical for physiological processes along with tumorigenic development through positive regulation of G1/S phase progression, inhibition of apoptosis, and increased survival [11]. Mutations in the PI3K pathway can lead to disruptions in PTEN, which functions as a tumor suppressor and a negative regulator of AKT [11] [13]. In a previous study, analysis of frozen melanoma clinical specimens and cell lines demonstrated that phosphorylation (activation) of AKT correlated inversely with PTEN protein expression [17]. This study also concluded that cells with baseline or compensatory activation of PI3K/ATK pathway were generally resistant to apoptosis following BRAF or MEK inhibition in the MAPK pathway [17]. Another study provided evidence that oncogenic PIK3 mutations can reduce the dynamic range over which FOXO3 can respond to growth factors in human cancer cell lines [16] [18]. mTOR regulation in melanoma induced cells is over-expressed, resulting in increased cell proliferation. Inhibition of mTOR by rapamycin can potentially inhibit tumorous cell growth that contain activated PI3K/AKT signaling resulting from PTEN loss, AKT overexpression, or growth factor activation [19].

The cyclin dependent kinase inhibitor 2A (*CDKN2A*) gene encodes for two tumor suppressors known as p16INK4a and p14ARF. p16INK4A functions as the main tumor suppressor by functioning as an inhibitor of cyclin-dependent kinase 4 (CDK4) to prevent the S phase in cellular cycling. p14ARF functions in regulating p53, a tumor suppressing protein encoded by the *TP53* gene that is involved in apoptosis. p16INK4a inhibits CDK4 by binding cyclin D to arrest the cell cycle within the G1 phase [20] (Figure 7&8).

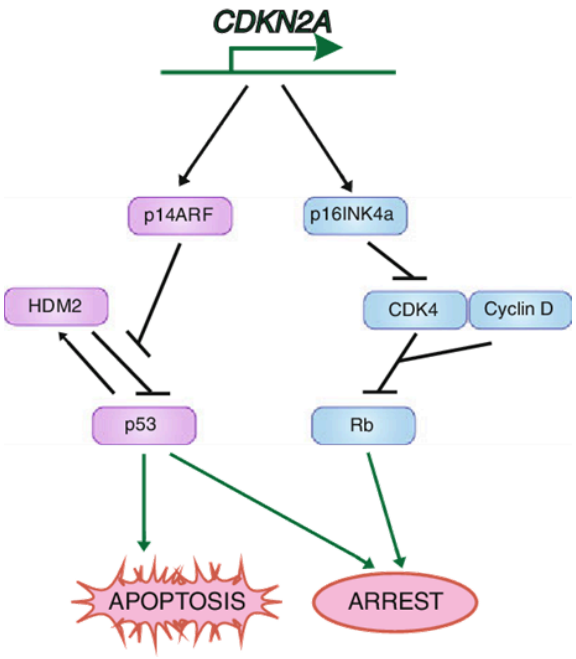

Figure 7. Protein products and signaling pathways of the CDKN2A gene. The production of p14ARF and p16INK4a are involved in cellular apoptosis and arrest, respectively [21].

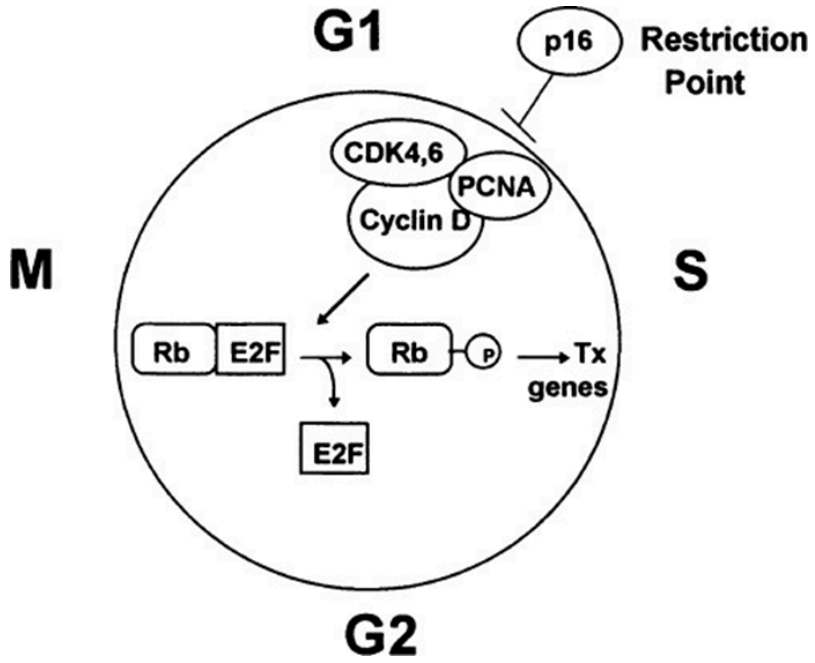

Figure 8. Involvement of p16 in cell cycle regulation. The production of p16 induces the formation of stable complexes with CDK4, inhibiting the development of oncogenic cells [20].

UV Radiation

Ultraviolet radiation (UVR) can induce mutations and changes within gene expression to affect the function and structure of cells and tissues. UVR exposure that primarily induces carcinogenesis can be distinguished into three types of radiation: UVA (315-400nm), UVB (280-315nm), and UVC (200-280nm). UVA is more abundant than UVB in sunlight, accounting for 95% of solar UV radiation, and penetrates more deeply into the dermis. However, UVB can induce direct damage within DNA due its higher efficiency in being absorbed by intracellular molecules, resulting in the formation of pyrimidine dimers between adjacent pyrimidine sites that affects the spatial structure of DNA. Common mutations that can be induced by UVB are C to T and CC to TT at pyrimidine sites. UVB can also generate photochemical reactions resulting in cross-linked DNA and proteins, pyrimidine-purine base pairing of unknown roles [2]. Although UVB initiates cell damage at lower levels than UVA, UVB can induce cell cycle changes and mutations along with the formation of cyclopyrimidine dimers and 6-4 photoproducts (Figure 9). Although UVA is low in energy and is weakly absorbed by DNA, it can be absorbed by other cellular chromophores and induce oxidative changes within the cell. Photons within the UVA range excite chromophores, contributing to the generation of reactive oxygen species (ROS) that indirectly leads to DNA damage [2] (Figure 10). Oxidation of nucleotide bases promotes mispairing outside of the normal Watson-Crick parameters. For example, the trans version of G to C is a well-characterized mutation induced by ROS that oxidizes guanine at the 8th position to

produce 8-hydroxy-2'-deoxyguanosine (8-OHdG). 8-OHdG tends to pair with an adenine instead of cytosine, mutating the G/C pair to an A/T pair [22]. In previous studies, it has been shown that UVA combined with environmental pollutants, such as cigarette smoke, significantly increases the risk of skin cancer and when ozone exposure proceeds UV exposure, there is an enhancement of UV-induced depletion of protective vitamin E from the skin's stratum corneum [23]. More generally, there are several reports that UVA is mutagenic to cultured cells at physiological reflexes and at high physiological doses [24]. UVA can induce DNA single and double-stranded breaks in human keratinocytes [24]. The UVC spectrum is highly mutagenic but does not reach the earth's surface due to being absorbed by the stratospheric ozone layer, leaving only a small group of people exposed to this UV spectrum range [2].

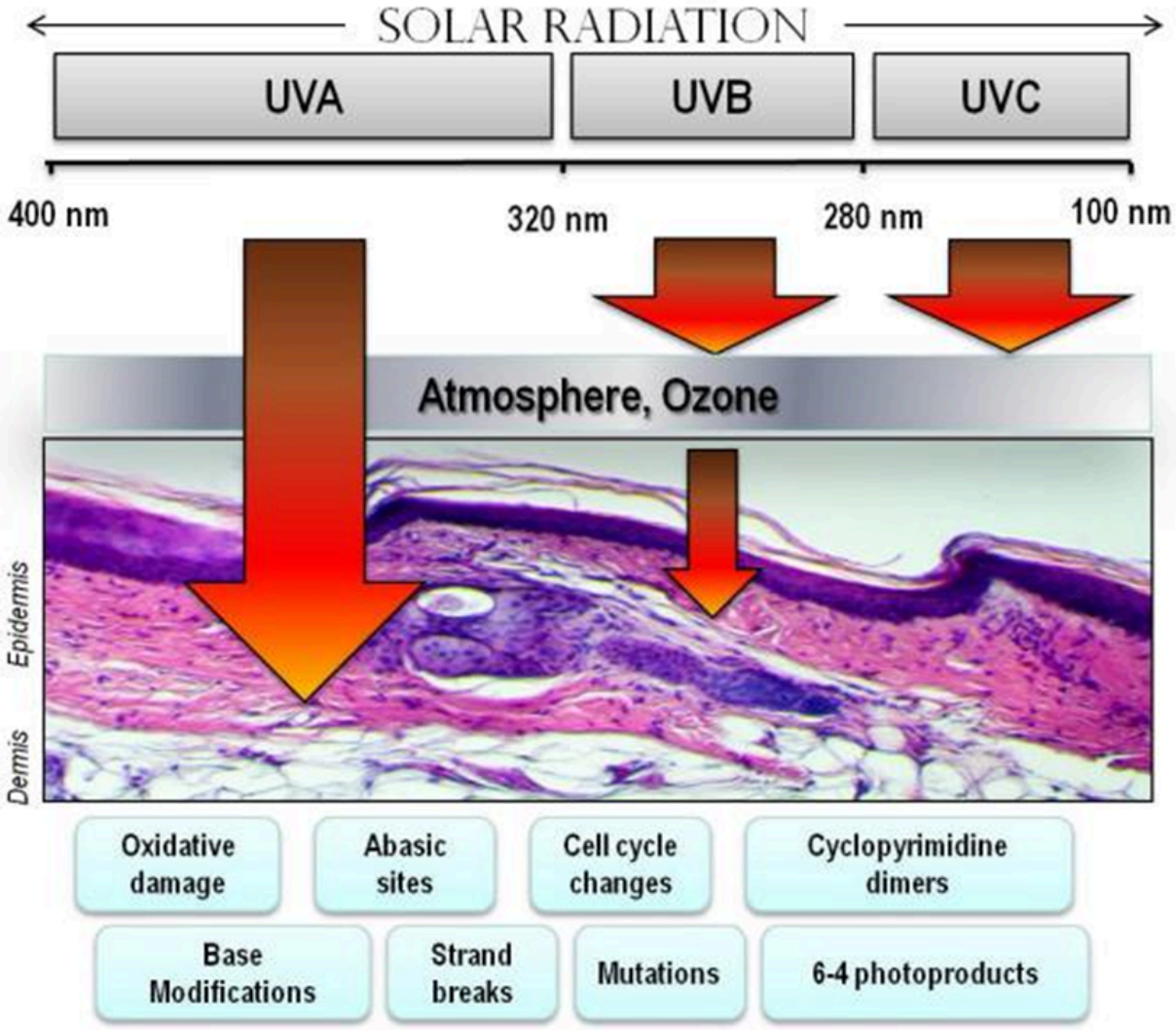

Figure 9. Electromagnetic spectrum of visible and UV radiation and biological effects on the skin. UV penetrates the skin in a wavelength-dependent manner. Longer wavelength UVA penetrates deeply into the dermis whereas UVB is almost completely absorbed by the epidermis, with comparatively little penetrance in the dermis. UVA is efficient at creating oxidations that can damage DNA via indirect photosensitizing reactions. UVB is directly absorbed by DNA which causes molecular rearrangements and the formation of specific photoproducts [22].

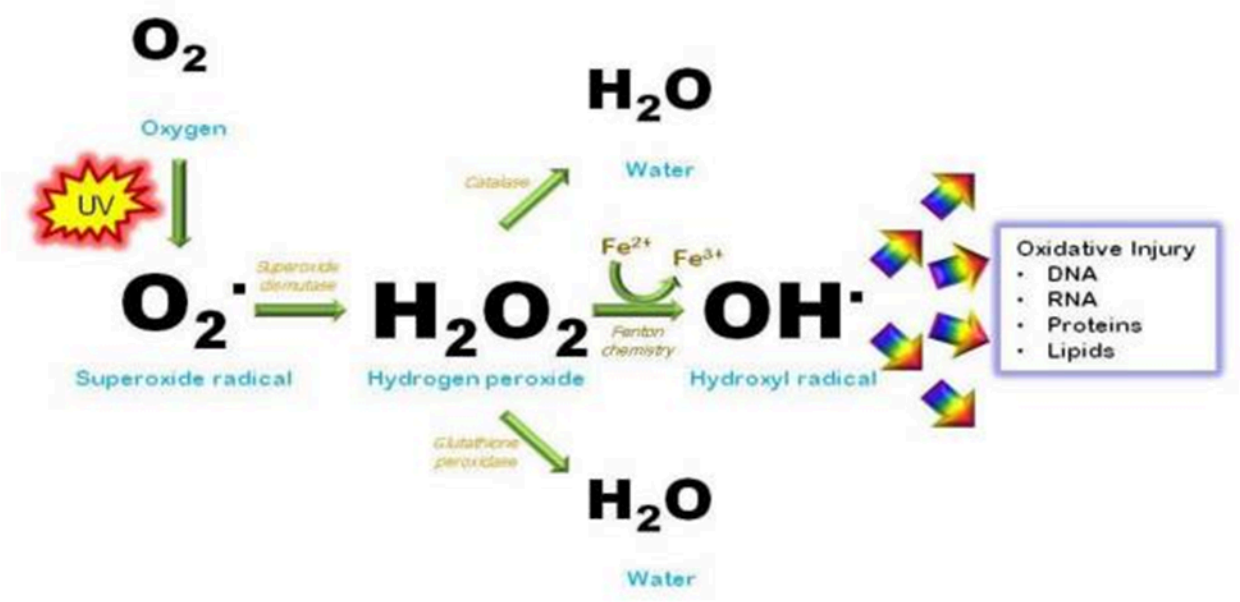

Figure 10. Generation of oxidative free radicals via UV radiation. UV photons interact with atomic oxygen to promote formation of free radical derivatives such as superoxide, hydrogen peroxide, and the high-reactive hydroxyl radical. Free radicals can actively attack macromolecules, such as DNA, RNA, proteins, and lipids, altering their structure and interfering with their function. Detoxifying and protective enzymes such as dismutase, catalase and glutathione peroxidase detoxify and reduce levels of oxidative species in the cell [22].

Previous studies have shown that tanning devices emit both UVA and UVB radiation and individuals that use tanning devices regularly obtained melanoma more frequently than those who did not [25]. There is also a dosage-response relationship between melanoma risk that can be measured by the total amount of time spent using tanning devices [25]. UV dosages in tanning devices exceed the requirements for adequate vitamin D production, resulting in hypervitaminosis D, derived from an increase in 1,25-dihydroxyvitamin D concentrations. An increase in 25(OH)-D concentrations with hypervitaminosis D is due to few mechanisms that prevent 25(OH)-D accumulations [22] [26].

Familial Melanoma

Although familial inheritance of genes susceptible to mutated melanoma does not have as significant of a role as UV radiation, they allow assessments to be made about whether familial clusters of melanoma cases are due to the carriage of multiple low-penetrance risk variants rather

than a single dominant mutation [27]. Several high-and-intermediate penetrance melanoma susceptibility genes have been identified [3] (Table 1).

Table 1

Overview of High- and Intermediate-Penetrance Genes Involved in Melanoma Susceptibility

Gene Penetrance	Gene	Encoded Protein	Role	Mutation Prevalence	References
High-penetrance	*CDKN2A*	$p16^{INK4a}$	Cell cycle regulator	~20%–40% of families	10–13
		$p14^{ARF}$	Cell cycle regulator	~1% of families	9,14,15
	CDK4	CDK4	Cell cycle regulator	17 families	16,17
	TERT	Catalytic subunit of telomerase	Telomere elongation	2 families	18,19
	POT1	POT1	Telomere maintenance	14 families	20–22
Intermediate-penetrance	*MC1R*	MC1R	Melanin synthesis and melanocyte proliferation	NA	23,24
	MITF	MITF	Melanocyte development and differentiation	NA	25,26

CDK4 = cyclin-dependent kinase 4; *CDKN2A* = cyclin-dependent kinase 2A; *MC1R* = melanocortin 1 receptor; *MITF* = microphthalmia- associated transcription factor; NA = not applicable; *POT1* = protection of telomeres 1; *TERT* = telomerase reverse transcriptase.

Table 1. Familial melanoma-susceptible genes. Germline susceptibility has been associated highly in *CDKN2A* and less frequently in *CDK4*, *TERT*, and *POT1*. Intermediate penetrance can be associated with *MCIR* and *MITF*. Penetrance relates to a lifetime risk for a mutations carrier of developing melanoma and reflects the overall contribution of a specific gene alteration to the risk of melanoma [3].

Mutations that occur in *CDKN2A* are mainly associated with 20-40% of familial melanoma via autosomal dominant inheritance of the germ line [28]. A positive *CDKN2A* mutation has been associated with a high number of effected family members, multiple primary melanomas, pancreatic cancer, and early age at melanoma onset [3]. Mutations in *CDKN2A* have been proven to encode proteins that are unable to inhibit the activity of *CDK4* along with mutations in the tumor suppressors p16, p14, and p53 (Figure 11). The loss of p53 impairs the mechanism that normally targets damaged cells for degradation, apoptosis, to instead promote proliferation of damaged cells. Thus, the overall damage or loss to *CDKN2A* contributes to the disruption of the p53 and pRb pathway within p16 and p14, respectively [29]. The inactivation of *CDKN2A* also involves histone modifications as well as DNA methylation, where the promotor region is hyper-methylated [30]. Other independent variants are proposed to contribute to the complex association signals in the *CDKN2A* region, and the association with melanoma risk is likely to involve multiple single nucleotide polymorphisms (SNPs) such as rs869330 and rs7023329 within the *MTAP* gene and rs1101970 in *CDKN2B-AS1* [28]. A previous study has shown that there are various mutations that can occur within *CDKN2A* and the corresponding proteins within patients that contain multiple primary melanomas. The primary mutation that occurred within these families was the Arg24Pro, eighth-amino acid insertion, and the Ser56Ile mutations that are usually prevalent in melanoma-prone families [31] (Figure 12&13).

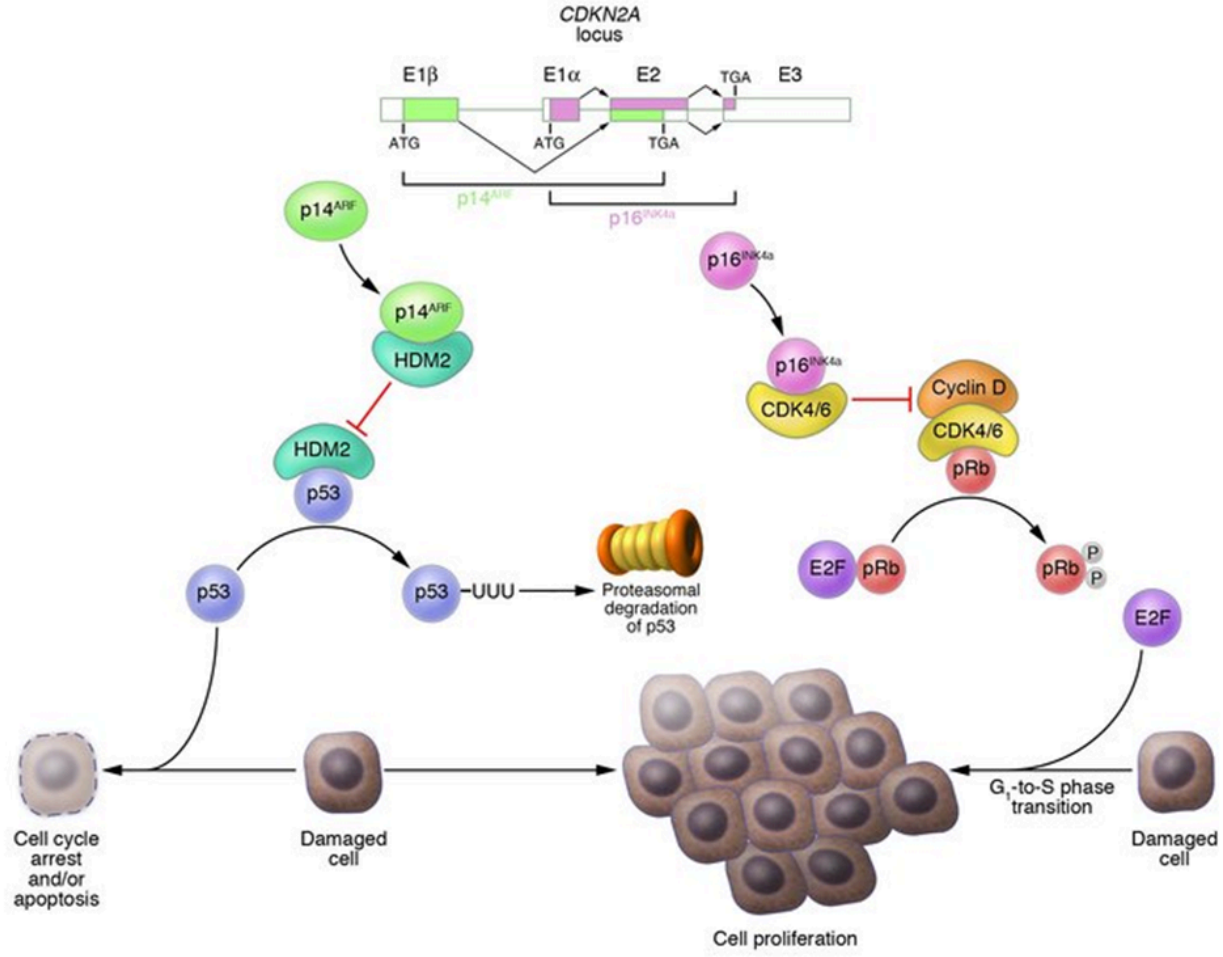

Figure 11. Genetic encoding mechanism of *CDNK2A* in melanoma. In the absence of p16, *CDK4* and *CDK6* phosphorylate Rb to release transcription factor E2F to promote the G1-S phase transition of the damaged cells. In the absence of p14, HDM2 targets p53 for ubiquitination (UUU) and proteasomal degradation, resulting in a loss of p53 and accumulation of damaged cells [29].

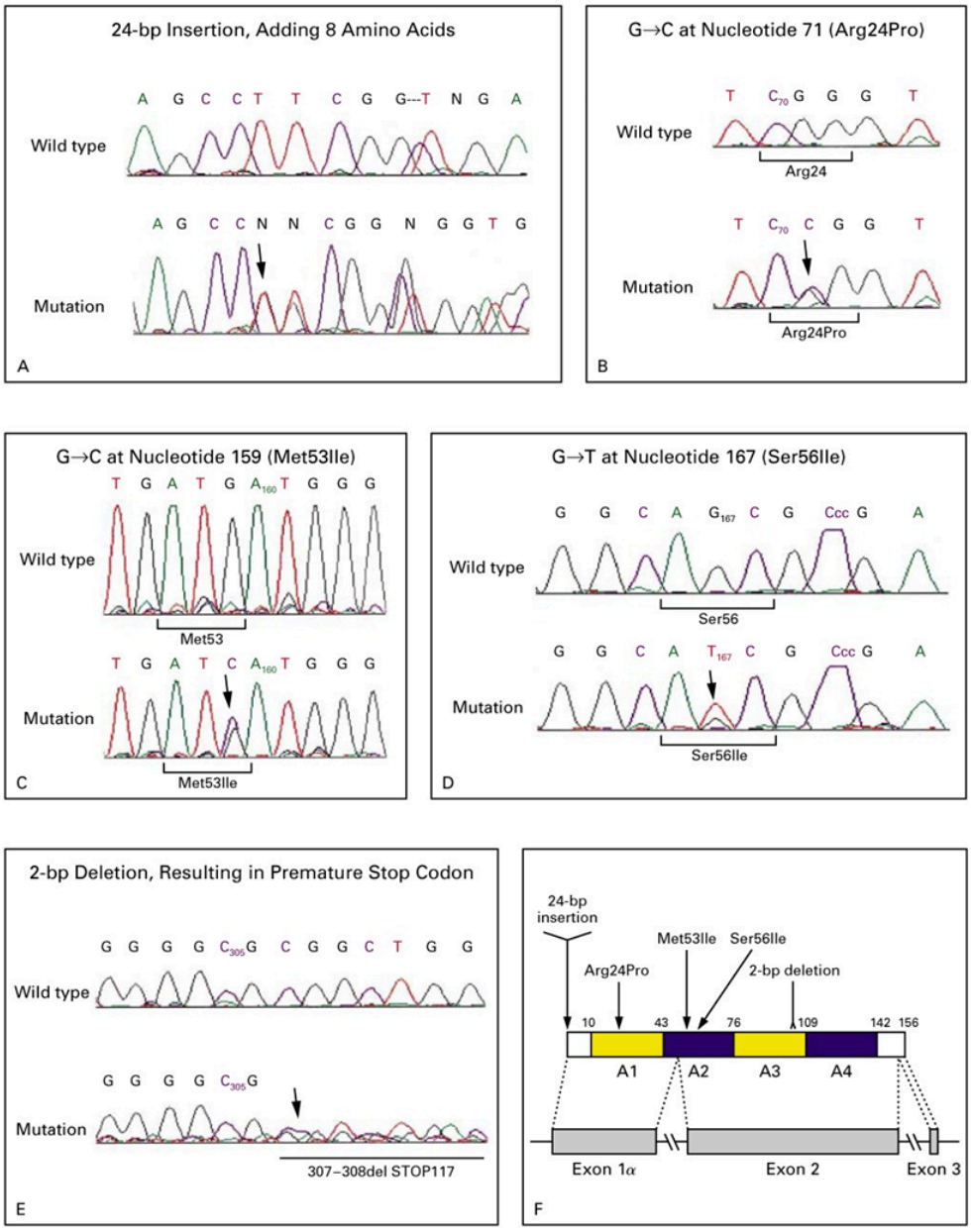

Figure 12. Heterozygous mutations within *CDKN2A* and corresponding proteins.

Panel A displays an insertion of 24bp, resulting in the addition of eight amino acids on the amino terminal of *CDKN2A*. Panel B displays the substitution of C to G at nucleotide 71 (Arg24Pro). In panel C, there is a substitution of C for G at nucleotide 159 (Met53Ile) Panel D displays the substitution of T for G at nucleotide 167 (Ser56Ile). Panel E displays the deletion of 2bp at nucleotides 307 and 308, resulting in a premature stop codon at codon 117. Panel F shows the locations of the mutations on the *CDKN2A* gene and protein as well as the Ankyrin-like repeats (A1, A2, A3, and A4) [31].

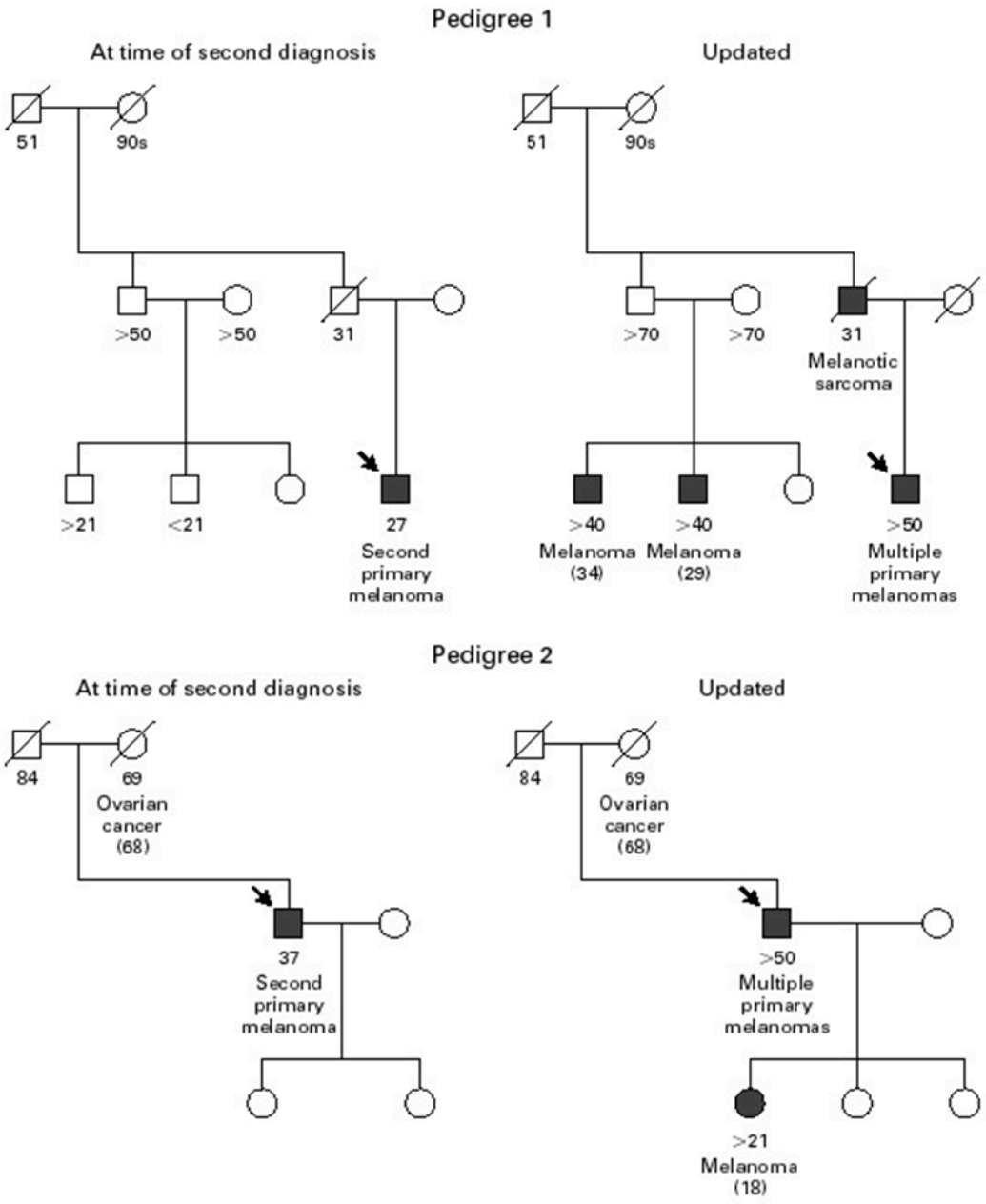

Figure 13. Pedigrees of two patients with germ-line *CDKN2A* mutations and multiple primary melanomas. The circles and squares represent the female and male family members respectively. Solid symbols represent family members with melanoma, clear symbols represent family members without melanoma, and slashes represent deceased family members. The number below indicate the age of the family member at the time of death for deceased members or history taking. The numbers in parentheses represent the age of diagnosis [31].

Other high penetrance genes include *CDK4*, *TERT*, and *POT1* which have also been extensively studied (Table 1). *CDK4* germline mutations have been characterized to lack a systemic investigation of their phenotype and they have been observed to be phenotypically indistinguishable from *CDKN2A* melanoma families in terms of age of melanoma diagnosis, tumor localization, histological type, and increased incidence of multiple primary melanomas and clinically atypical nevi. Thus, within clinical settings, the *CDK4* gene should be examined alongside *CDKN2A* mutations [32].

Conclusion

Melanoma is strongly associated with mutations and disruptions in cellular signaling pathways that promote the proliferation of oncogenic cell types. Early-onset melanoma can often be cured surgically whereas later stages can be treated with a form of therapy. Melanoma therapeutics include chemotherapy, immunotherapy, bio-chemotherapies, and signal transduction inhibition with the utilization of specific inhibitors for associated signaling pathways. A key prevention of melanoma induction can be done by protection from UV radiation. There are still many unknown high-susceptible genes that can be mutated in melanoma development. In the future, the identification of unknown high-risk genes as well as the design of specific drugs and therapeutics that target more specific aspects in tumor-specific mutations of a given cellular pathway will be the focus within melanoma genetics.

Acknowledgements

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