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Melanocyte Pygmentation – Friend or Foe on the Route to Melanoma

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1. Introduction

The epidermis forms the top protective covering of normal human skin and is itself composed of multiple layers from the stratum corneum at the top, to proliferating cells in the deep/basal layer (Liu & Fisher, 2010).

The epidermal cell population is mainly constituted of two cell types: keratinocytes and melanocytes. Keratinocytes constitute the majority of the epidermis; they have a "supporting" and regulatory role for the melanocytes. Keratinocytes are linked through tight desmosomal intercellular junctions and also anchored into the basal membrane through hemidesmosomes, but melanocytes remain as singly scattered, unattached cells.

Each single, well-differentiated, melanocyte interacts with 36 viable keratinocytes at various stages of progression to the upper cornified layer of the epidermis (Fitzpatrick & Breathnach, 1963) to form epidermal units. These structural and functional cellular units exhibit complex, life-long, cellular interactions originally laid down during embryonic life.

There are considerable interindividual and intraindividual variations in melanocyte population densities, with more than twice as many melanocytes located in head and forearm skin compared with elsewhere on the body, as well as darker skin in the folded areas of axillae and perineum, traits that remain remarkably consistent between races (Szabo, 1967).

Despite significant variation in skin pigmentation, the density of melanocytes at the epidermal-dermal junction is very similar across different skin types (Yamaguchi & Hearing, 2009). Thus the main contributor to racial differences in skin pigmentation is cellular activity rather than absolute melanocyte numbers (Szabo, 1967).

Melanocytes play a central role in the response of skin to sunlight exposure. They are directly involved in UV-induced pigmentation as a defense mechanism. People with different skin color possess varied sensitivity to ultraviolet (UV) exposure, with darker skinned individuals being less susceptible to sun-induced skin alterations, including cancer, than fair skinned ones (Elwood & Diffey 1993).

Such a difference can be explained in terms of protective UV filtering by epidermal pigmentation, because the skin color is also related to the type of melanin, the number, size, type, distribution and degradation of melanosomes, and the tyrosinase activity of melanocytes (Nordlund & Ortonne 1998, Yamaguchi & Hearing 2009).

The decreased photocarcinogenesis seen in individuals with darker skin may also be attributed to the more efficient removal of UV-damaged cells (Yamaguchi & Hearing 2009, Alonso & Fuchs, 2003, Fuchs, 2008).

The melanocytes, when differentiated, assume the highly dendritic phenotype that facilitates closer contact and transfer of the melanosomes to nearby keratinocytes where they shield nuclei from ultraviolet (UV) irradiation.

At least four theories have been proposed to be involved in the transfer of the melanosomes (Slominski et al., 2004): 1) the "cytophagic" theory, where the keratinocyte, as active partner, phagocytoses the tips of dendrites that contain stage IV mature melanosomes (Garcia, 1979); 2) the "discharge" theory, where mature melanosomes are released into the intercellular space to be internalized by adjacent keratinocytes; 3) the "fusion" theory, where mature melanosomes pass from melanocyte to keratinocyte via fusion of their respective plasma membranes (Okazaki et al., 1976.); and 4) the "cytocrine" theory, whereby melanocytes would inject melanin into recipient keratinocytes (Masson, 1948).

Epidermal melanocytes rarely collect mature melanosomes intracytoplasmically; instead, they translocate them to keratinocytes. This is in contrast to bulbar follicular melanocytes, which are commonly heavily laden with fully mature stage IV melanosomes. When melanocytes were cocultured with keratinocytes, a highly dendritic phenotype was induced through filopodia, many of which contained melanosomes (Scott et al., 2002).

Keratinocytes in coculture with melanocytes can also suppress melanogenic proteins such as the TRP1 (Phillips et al., 2001). Besides this, melanocyte growth, dendricity, spreading, cell-cell contacts, and melanization can all be regulated by keratinocyte-secreted factors (Tenchini et al., 1995). Interestingly, the regulatory role exercised by keratinocytes is restored in melanoma cells if expression of E-cadherin is induced, permitting their adhesion to keratinocytes (Hsu et al., 2000).

An increase in melanogenically active melanocytes is seen following UV irradiation (Rosdahl et al., 1978), but it is not clear if these additional cells are truly derived from division of already functioning melanocytes.

In contrast, melanocytes in the hair follicle divide during the hair cycle. Melanocyte loss (mostly probably via apoptosis) occurs in both sun-exposed and covered skin with an 10% reduction per decade after 30 years of age until 80 years, followed by more dramatic cell loss thereafter (Nordlund, 1989).

Melanocyte alteration can lead to melanoma, a tumor that has become one of the most rapidly increasing malignancies in the Caucasian population with 2.5-3% more cases each year in the US. It shows a relatively high incidence among young people compared to most other cancer types. (Wang et al., 2001).

2. Melanin and melanogenesis

Cutaneous melanin pigment plays a critical role in camouflage, mimicry, social communication, and protection against harmful effects of solar radiation. In the epidermis, melanin is synthesized by melanocytes. Besides skin, melanin can also be found in hair, retinal pigment epithelium, iris, and certain parts of the central nervous system.

The primary function of skin melanin has not yet been established. A number of roles have been proposed that include photoprotection, thermoregulation, antibiotic, cation chelator, free radical sink, and by-product of the scavenging of the superoxide radical in the skin by tyrosinase (Giacomoni, 1995; Hill & Hill, 2000; Morisson, 1985).

Melanins are synthesized, matured, and stored within melanosomes. Melanogenesis includes: melanosome biogenesis, melanin synthesis and transfer of the melanosomes to the surrounding keratinocytes (Chen et al., 2009).

Melanosome biogenesis can be classified into four distinct stages of maturation (I, II, III, IV) based on melanosomal morphologies (Hearing, 2005; Raposo et al., 2002). Stage I and II melanosomes are termed premelanosomes, as melanin synthesis has not yet begun. Stage I melanosomes are closely related to lysosomes because they share a lysosomal lineage (Hearing, 2005; Orlow, 1995; Raposo et al., 2001, 2002; Valencia et al., 2006). Typical stage II melanosomes are ovoid in shape, containing elongated and highly organized fibrillar - matrics. Active melanin synthesis occurs in stage III melanosomes and results in the deposition of black electron-dense pigment on the fibrillar matrix. When the internal matrix of a melanosome is completely filled with melanin, it no longer has discernable internal filaments at the electron microscope level, and the melanosome reaches a mature stage (i.e., stage IV). More detailed staging of melanosomes can be obtained from electron microscope images when melanosomes undergo inter-stage transitions from stage I to IV. These additional stages are I-II, II-III, and III-IV (Chen et al., 2006, 2009a, 2009b).

Late-stage melanosomes (III and IV) are transferred to keratinocytes in normal skin. However, that process may be impaired in melanoma cells. Stage IV melanosomes frequently have damaged membranes and leak melanin into the cytoplasm, a morphological indicator of endogenous melanogenic cytotoxicity (Chen et al., 2006, 2009 a, 2009b).

There are two categories of melanin: black-brown eumelanin and yellow-reddish pheomelanin.

The availability of L-tyrosine for enzymatic oxidation is a central component for the initiation of melanogenesis. In the melanosomes L-tyrosine comes either from the hydroxylation of L-phenylalanine to L-tyrosine by phenylalanine hydroxylase (PAH) or is actively transported inside the organnells from cytoplasm (Schallreuter et al., 2008).

Tyrosine uptake is largely through system L -transport, which supplies the tyrosine for both protein synthesis and melanogenesis, as shown in a melanoma cell line (SK-mel 23) (Jara et al., 1991). Tyrosine uptake by cells was inhibited by the analog substrate 4-S-cysteinylphenol and by tryptophan as well as the specific system L -inhibitor 2-amino-bicyclo-2,2,1-heptane-2-carboxylic acid. (Pankovich & Jimbow 1991) Although this mechanism is responsible for the cellular uptake of tyrosine, there may be separate permeases to regulate the access of tyrosine to the melanosome because the process is analogous to amino acid export (Land et al., 2006).

L-tyrosine is then hydroxylated to L-dihydroxyphenylalanine (L-DOPA), a reaction catalyzed by either the tyrosine hydroxylase activity of tyrosinase (TYR) or tyrosine hydroxylase (THI) (Simon et al., 2009). Thus, the three enzymes, i.e. phenylalanine hydroxilase, tyrosine hydrolase and tyrosinase are crucial for the initiation of melanogenesis, supporting the concept of a "three enzyme theory" (Schallreuter et al., 2008).

The next step, oxidation of L-DOPA to dopaquinone (DQ) is common to both eu and pheomelanin synthesis. Orthoquinines such as dopaquinone (DQ), are extremely reactive molecules (Ito & Wakamatsu, 2008) and can be formed directly during the initial stage of melanogenesis (Cooksey et al., 1997).

The first step in eumelanogenesis is a relatively slow process involving the intramolecular addition of the amino group to produce cyclodopa (Land & Riley, 2000; Land et al., 2003). However, as cyclodopa is formed, it is rapidly oxidized to dopachrome through a redox exchange (Land et al., 2003).

Production of dopachrome, an orange red pigment is faster than the production of cyclodopa, when the cyclodopa concentration is above 0.7μM, leading to dopachrome accumulation during the early phase of eumelanogenesis. Dopachrome spontaneously

decomposes by decarboxylation at neutral pH to give di-hydro indol (DHI) and di-hydro indol carboxylic acid (DHICA) in a 70: 1 ratio (Palumbo et al., 1987). However, in the presence of dopachrome tautomerase (DCT), also termed tyrosinase-related protein-2 (TYRP2), dopachrome undergoes tautomerization to preferentially produce DHICA (Palumbo et al., 1991). The ratio of DHICA to DHI in natural eumelanins is thus determined by the activity of DCT (Tsukamoto et al., 1992). DHICA synthesis seems to protect melanocytes against cytotoxic effects of melanogenesis, thus DCT was reported to be essential for melanocyte survival (Hearing, 2000).

Metal cations, especially Cu^{2+} also accelerate the dopachrome rearrangement and affect the DHICA/DHI ratio, but DCT seems to be more effective in catalyzing the tautomerization (Palumbo et al., 1987, 1991). During eumelanogenesis, DHI oxidation takes place by redox exchange with DQ (Edge et al., 2006), although such a reaction is likely to be less efficient for DHICA. Thus, DHICA may require its oxidation to the quinone form by a direct action of tyrosinase in humans (Olivares et al., 2001) or by TYRP1 in mice (Jimenez-Cervantes et al., 1994; Kobayashi et al., 1994). Human TYRP1 is unable to catalyze the DHICA oxidation (Boissy et al., 1998).

The initial step of pheomelanogenesis is the conjugation of dopaquinone to cysteine or glutathione to yield cysteinyldopa (CD) and glutathionyldopa (Simon, 2009). The reaction of DQ and cysteine produces the 5-5 and 2-5-CD isomers in a ratio of 5.3:1 (Ito and Prota, 1977). Cysteinyldopas are further transformed into dihydrobenzothiazine-3-carboxylic acid (DHBTCA). (Greco et al., 2009). In the later stages of pheomelanogenesis, benzothiazine groups are slowly converted to the benzothiazole (BZ) and thus the polymeric structure of the pheomelanin pigment contains both benzothiazine and benzothiazole units (Wakamatsu et al., 2009).

Trichochromes are dimeric and trimeric intermediates that have a bi (l,4) benzothiazine chromophore. The close similarity in structural features of trichochromes and pheomelanin and their coexistence in pigmented tissues suggest that they are formed oxidatively from the same monomer units and differ only in their mode of polymerization (Simon, 2009).

In vivo melanogenesis produces mixtures of eumelanin and pheomelanin. The total amount of melanin produced is proportional to DQ production, which is in turn proportional to tyrosinase activity.

A mixed melanogenesis three-step pathway has been proposed (Ito & Wakamatsu, 2008; Simon et al., 2009). The initial stage is the production of CD isomers, which continues as long as the cysteine concentration is above 0.13 μM. The second stage is the oxidation of CDs to produce pheomelanin as long as CDs concentrations are above 9 μM. The last stage is the production of eumelanin, which begins only after most CDs and cysteine are depleted. Therefore, the ratio of eumelanin to pheomelanin is determined by tyrosinase activity and the availability of tyrosine and cysteine in melanosomes (Land et al., 2003).

Human epidermal and uveal melanocytes in culture produce pheomelanin at rather constant levels regardless of the degree of pigmentation while they produce eumelanin at levels proportional to pigmentation (Ito & Wakamatsu, 2008; Wakamatsu et al., 2006, 2008).

The most important enzyme which regulates the velocity and specificity of the melanogenesis is tyrosinase (Slominski et al., 2004). Tyrosinase catalyzes three distinct reactions in the melanogenic pathway: hydroxylation of monophenol (L-tyrosine), dehydrogenation of L-DOPA, and dehydrogenation of DHI; L-DOPA serves as cofactor in the first and third reactions (Hearing & Tsukamoto, 1991; Korner & Pawelek, 1982; Pawelek & Korner, 1982; Wood & Schallreuter, 1991; Ros et al., 1993). L-DOPA is the most efficient electron donor

necessary to start tyrosine hydroxylation, although ascorbic acid, dopamine, and superoxide anion radicals can potentially activate the enzyme (Wood & Schallreuter, 1991).

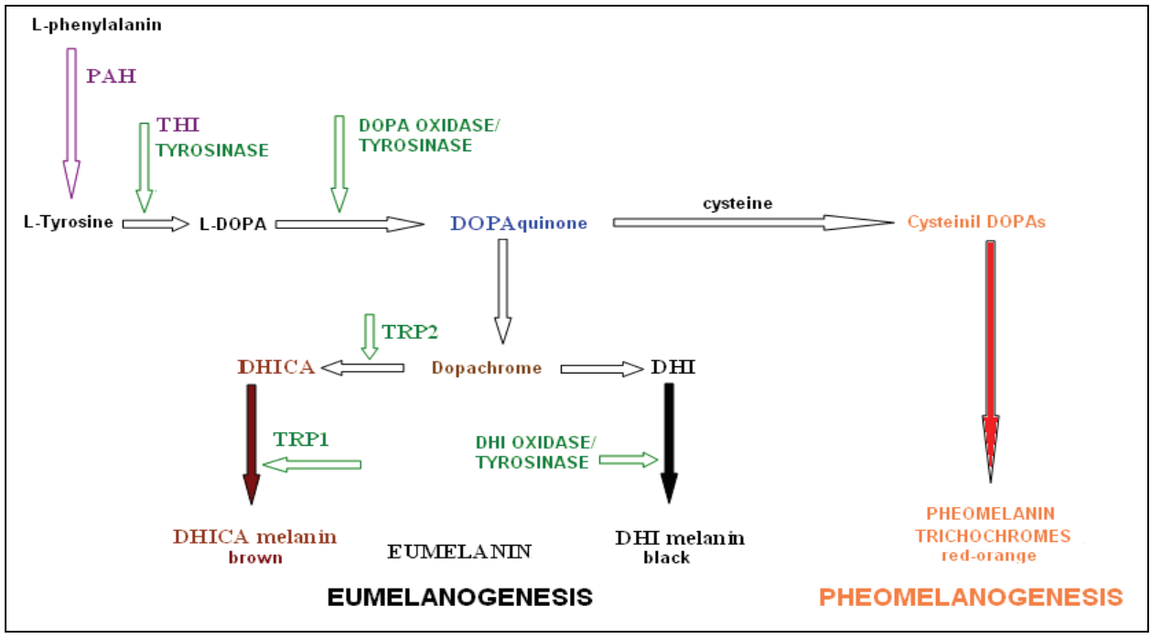

Fig. 1. Biochemical reactions of melanin synthesis; Abbreviations: DHI- di hydro indol, DHICA- di hydro carboxylic acid, DOPA -dihydroxyphenylalanine, PAH- phenylalanine hydroxilase, THI- tyrosine hydroxilase, TRP- tyrosinase related proteins. The initial step in the melanin biosynthesis is the hydroxylation of tyrosine to DOPA, which is then converted to DOPAquinone. Pheomelanogenesis takes place in the presence of cysteine and leads to the red-orange pigments, pheomelanins and trichochromes, while eumelanogenesis pathway needs the participation of tyrosinase and related proteins to produce either brown DHICA melanin of black DHI melanin

The effect of ascorbic acid on the monophenolase activity of tyrosinase has been explained by its reducing action on enzymatically generated quinines, thus inducing accumulation of L-DOPA, the main electron donor to the Cu^{2+} enzyme active site (Ros et al., 1993).
Tyrosine related protein TRP1/TYRP1 share structural similarities with tyrosinase and originated by the duplication of the ancestral tyrosinase gene (Olivares et al., 2001, 2009). The activities of these tyrosinase-related proteins, TYRP1 and TYRP2, greatly affect the quantity and quality, the ratio of DHI to DHICA and the degree of polymerization of eumelanins produced (Lamoreux et al., 2001; Ozeki et al., 1995, 1997a, 1997b).
Both of them are important for the eumelanogenic pathway; they also act as stabilizers and regulators of the tyrosinase activity. Tyrp1 appears to control the molecular size of eumelanin produced in mice. Brown mice that lack Tyrp1 activity produce eumelanin with lower molecular weights than wild type, black mice (Ozeki et al., 1997a, 1997b). In humans, TYRP1 has tyrosine hydroxylase activity (Jackson et al., 1991; Jimenez-Cervantes et al., 1994; Kobayashi et al., 1994) and TYRP2 acts as dopachrome tautomerase (Jackson et al, 1992; Tsukamoto et al., 1992; Yokoyama et al., 1994). An additional function of TYRP1 may be the securing of appropriate processing of tyrosinase and stabilization of its enzymatic activity and, possibly, maintenance of melanosomal structure integrity (Hearing, 2000; Le Poole et

al., 2000; Sarangarajan & Boissy, 2001; Sarangarajan et al., 2000). TYRP2 is important for melanocyte survival by implication in endogenous melanogenesis cytotoxicity homeostasis (Hearing, 2000).

3. Regulation of melanogenesis – internal factors

Cutaneous pigmentation plays a crucial role in defending the body against harmful UV rays and other environmental challenges (Costin & Hearing, 2007). This process is under a complex genetic control: in mammals, over 350 loci associated with pigmentation have been cloned or mapped (Olivares & Solano, 2009; Hearing, 2000; Montoliu et al., 2009).

Protein products of these loci acting as enzymes, structural proteins, transcriptional regulators, transporters, receptors and growth factors have a wide array of functions and cellular targets (Hearing, 1999; Nordlund et al., 2006). Among them are the important enzymatic melanosomal proteins coded by the albino(c)/TYR (Kwon et al., 1991, 1993), brown (b)/TYRP1 (Jackson et al., 1991) and slaty(slt)/TYRP2/DCT (Jackson et al., 1992) silver (slt)/SILV (Kwon et al., 1991, 1993), pink-eyed dilute (p)/P/OCA2 (Box et al., 1998; Oetting & King, 1999; Silvers, 1979; Simoncini et al., 2000), underwhite (uw)/LOC51151, MART1 (Carlson et al., 2003; Slominski, 2002; Slominski et al., 2001; Wankowicz-Kalinska et al., 2003) and OA1 (Orlow & Brilliant, 1999) loci.

The behavior of the melanocytes in vitro is different than in vivo, as a part of the epidermal melanin unit. The pheomelanin / eumelanin ratio is regulated by keratinocytes (Duval et al., 2002). Key elements in the regulation of melanogenesis are represented by tyrosinase and TYRPs. Their activities are also involved in the type of melanin pigment produced in melanosomes and also in controlling the endogenous citotoxicity of the melanogenesis process to the melanocytes.

The most important positive regulator of melanogenesis is the melanocortin (MC1) receptor with its ligands, melanocortins, other proopiomelanocortin (POMC) products, adrenocorticotropic hormone (ACTH) (Eberle et al., 1988; Lerner, 1993; Pawelek, 1976, 1985; Pawelek et al., 1988, 1992), whereas among the negative regulators, agouti protein stands out, determining the intensity of melanogenesis and the type of melanin synthesized.

α-MSH, which binds the MC1 receptor is an important regulator of skin pigmentation and the UV response of melanocytes and stimulates eumelanin synthesis (Abdel-Malek et al., 2009).

In vivo α-MSH injected to pubertal viable yellow mice producing a mixed-type melanin increased tyrosinase activity twofold with a concomitant increase in total melanin and more eumelanic hair was produced (Burchill et al., 1986).

Decreased α-MSH secretion following bromocriptine led to reduced tyrosinase activity and pheomelanic hair production along with a total melanin decrease. In vitro treatment of human melanocytes with the synthetic α-MSH resulted in an increase of eumelanin content in all the seven cell lines of different ethnic origins examined (Hunt et al., 1995).

Treatment with phenylthiourea (a tyrosinase inhibitor) resulted in a reduction of eumelanin content to a half with a concomitant twofold increase of pheomelanin content in melanocytes (Le Pape et al., 2008).

The main antagonist for the MC1R is the agouti signaling protein (ASP) (Lu et al., 1994). ASP acts within the microenvironment of the hair follicle during hair growth, switching eumelanin synthesis into pheomelanin synthesis.

The human agouti gene is expressed in adipose tissue, testis, ovary, and heart and at lower levels in liver, kidney, and foreskin (Wilson et al., 1995). Expression in transgenic mice of the human agouti protein produced a yellow coat (Wilson et al., 1995), although human hair does not show the agouti pattern.

When treated with agouti protein combined with phenylthiourea and extra cysteine to induce tyrosinase inhibition, non agouti melan-a (ala) mouse melanocytes (mainly eumelanic) produced over 200-fold increases in the pheomelanin to eumelanin ratio (Hida et al., 2009). Expression of ASP in cell culture blocks the α-MSH-stimulated accumulation of cAMP in mouse melanoma cells (Wilson et al., 1995).

In lower vertebrates, melanin concentrating hormone (MCH) induces melanosome aggregation within melanophores (whitening of the color). In mammals, MCH expression was detected in cultured human endothelial cells but not in human keratinocytes, melanocytes, and fibroblasts (Hoogduijn et al., 2002).

MCHR1, but not MCHR2, expression was detected in human melanocytes and melanoma cells (Hoogduijn et al. 2001, 2002; Saito et al., 2001). Stimulation of cultured human melanocytes with MCH reduced the α-MSH-induced increase in cAMP production (Hoogduijn et al., 2002). Furthermore, the melanogenic actions of α-MSH were inhibited by MCH. MCHR1 has also been identified as a novel autoantigen in patients affected with vitiligo (Kemp et al., 2002).

MC1 and MC2 receptors are coupled to pathways that have the cAMP as a second messenger (Coneet al., 1996; Nordlund et al., 1998). Their main ligand, α-MSH stimulates the processing of tyrosinase and tyrosinase-related proteins and the formation of melanosomes (Hearing, 1999, 2000; Nordlund et al., 1998).

Regulation by α-MSH of transcription and translation of tyrosinase and melanin related proteins (MRPs) could be mediated indirectly through microphthalmia-associated transcription factor (MITF) or directly through activation of PKA-or PKC-dependent pathways. Nevertheless, there is a consensus that MSH stimulates production and activity of MRPs at the transcriptional, translational, and posttranslational levels. Furthermore, MSH stimulates delivery of tyrosine to melanosomes (Potterf & Hearing, 1998).

Besides circulating MSH and other POMC products, the final POMC peptides can potentially be produced in all cutaneous compartments (epidermis, dermis, and adnexa) by epithelial and melanocytic cells and cells of mesenchymal origin such as immune cells, fibroblasts, and endothelial cells and also by release from sensory nerve endings (Slominski et al., 2000). Local POMC gene expression and production of POMC peptides can be modulated with UVR, cytokines, growth factors and cAMP and varies according to phase of the hair cycle (Slominski et al., 2000). Thus locally produced melanocortins and adrenocorticotropin could regulate melanogenesis though para-, auto-, or intracrine mechanisms (Slominski et al., 2000). Melanocytes produce, process POMC and express intracellular MSH receptors (Slominski et al., 2000), and a POMC processing system has been identified in human melanosomes (Peters et al., 2000). Cultured normal epidermal melanocytes treated with β-endorphin show increased melanogenesis, dendricity and proliferation and β-endorphin and μ-opiate antigens have been colocalized in melanosomes (Kauser et al., 2003).

Factors known to raise intracellular cAMP levels such as MSH itself, ACTH, dibutyryl cAMP, cholera toxin, forskolin (Simon et al., 2009) and phosphodiesterase inhibitors also stimulate MC receptors expression and activity (Slominski et al., 2004), leading to an activation of melanogenesis.

Topical application of forskolin to K14-stem cell factor transgenic mice with Mclr$^{e/e}$ background (producing mostly pheomelanin) resulted in a dramatic shift to eumelanogenesis (Spry et al., 2009).

In normal and malignant melanocytes, interleukin (IL-1α, IL-1β, endothelin-1, adult T-cell leukemia-derived factor/thioredoxin (ADF/TRX), interferons (IFN-α, IFN-β, IFN-γ), dibutyryl cAMP, and the hormones MSH and ACTH can stimulate expression of the MC-1 gene and of functional cell surface MSH receptors (Slominski et al., 2004).

Endothelin (ET) is secreted by keratinocytes and stimulated by UVB radiation (Imokawa et al., 1992; Yada et al., 1991); it is a potent stimulator of proliferation and differentiation of human melanocytes (Yada et al., 1991), it increases tyrosinase activity and TYRP1 mRNA expression (Imokawa et al., 1995), also it increases melanocyte dendricity (Hara et al., 1995).

IL-1 can also stimulate MC-1 receptor expression in normal and malignant human keratinocytes (Birchall et al., 1991). A similar effect was described for thymidine dimers and small single-stranded DNA fragments (ssDNA) that are produced intracellulary after UV-induced damage (Eller & Gilchrest 2002; Gilchrest & Eller, 1999).

Tumor necrosis factor (TNF-α) inhibits MC1 expression in melanocytes (Funasaka et al., 1998).

The c-kit/SCF interaction is critical for melanocyte survival (Steel et al., 1992) as shown by the induction of apoptosis in murine melanocytes after injection of a c-kit-blocking antibody (ACK2) (Ito et al., 1999). C-kit is also required for melanocyte activation during the murine hair cycle (Botchkareva et al., 2001; Peters et al., 2002). Epithelial-derived SCF may be the physiological regulator in the c-kit-expressing melanoblasts and melanocyte of mammalian skin by modulating migration and melanocyte cytoskeleton (Botchkareva et al., 2001), differentiation (Lahav et al., 1994; Luo et al., 1995), melanogenesis (Costa et al., 1996; Luo et al., 1995), and cell survival/apoptosis (Ito et al., 1999).

Studies performed on cultured melanoma cells have shown that epinephrine or norepinephrine as well as other adrenergic agonists can stimulate moderately tyrosinase activity and melanin production (Slominski et al., 2004).

Other positive intrinsic regulators of skin pigmentation are endocrine factors (estrogens, androgens), vitamin D, growth factors (fibroblast growth factor - β-FGF), inflammation related factors (eicosanoids, histamine), bone morphogenic proteins; while amongst negative regulators are endocrine factors (glucocorticoids, melatonin), melanocortin antagonists, neural factors (acetylcholine, serotonin, dopamine), cytokines (IL-1, IL-6, IFN-α, IFN-γ, TNF-α, TNF-β), growth factors (TGF-β1), retinoids (Slominski et al., 2004).

Many of the factors described previously that increase melanogenesis are upregulated upon UV irradiation (Yamaguchi & Hearing, 2009).

The aminoacids L-tyrosine and L-DOPA increase melanogenesis through increasing MC1R expression and activity, besides being the substrate for tyrosinase activity (Slominski et al., 2004). L-Tyrosine and L-DOPA act through related but distinct mechanisms (Slominski et al., 2004), with L-tyrosine inducing both melanosomes synthesis and tyrosinase translocation to melanosomes, while L-DOPA primarily increased tyrosinase. Their effects on tyrosinase gene transcription differ, e.g., L-tyrosine has no effect on tyrosinase mRNA, while L-DOPA produces an initial increase in tyrosinase mRNA followed by a decrease below control levels (Slominski & Costantino, 1991). The latter effect could be due to tyrosinase mediated oxidation of L-DOPA generating toxic intermediates of melanogenesis,

which would in turn shut-off tyrosinase gene expression as protective mechanism against self-destruction (Slominski & Costantino, 1991).

The ratio of tyrosine to cysteine have also been involved in controlling melanogenesis. By decreasing the extracellular concentration of cystine in cultures of human melanoma cells, a shift to more eumelanic cells was obtained (del Marmol et al., 1996). When cells were cultured at a higher concentration of tyrosine it was found a twofold increase in melanin production in human melanocytes with a decreased ratio of pheomelanin to total melanin (Smit et al. 1997).

High levels of tyrosine are known to reduce the proliferative effect of α-MSH and forskolin (Schwan, 2001) and also alter melanocytes morphology (Schwan, 2001); tyrosine also stimulates the activity of tyrosinase and melanogenesis. (Baldea et al., 2009; Smit et al., 1997, 1998, 2008; Wenczl et al., 1998)

The concentration of cysteine in melanosomes is genetically regulated. The subtle gray *(sut)* pigment mutation in mice arose due to a mutation in the Slc7a 11 gene that encodes the plasma membrane cystine/glutamate exchanger xCT. The resulting low rate of extracellular cystine transport into sut melanocytes reduced pheomelanin production with minimal or no effect on eumelanin production (Chintala et al., 2005).

Melanosomal pH in melanosomes is involved in switching between eu and pheomelanogenesis (Simon et al., 2009). Melanosomes in melanocytes from white/fair skin are acidic while those from black/dark skin are near neutral (Smith et al., 2004). Furthermore, the great diversity in normal human skin pigmentation appears to stem from mutations in only several genes, including P, MATP and SLC24A5 (Lamason et al., 2005; Norton et al., 2007).

Mutations in those genes may result in changing the melanosomes ph (Ancans et al. 2001). The effects of more acidic ph on mixed melanogenesis are twofold: a lower activity of tyrosinase and a slower rate of dopaquinone cyclization (the first step in eumelanogenesis) while the CD-quinone cyclization (yielding the first bicyclic intermediate in pheomelanogenesis) proceeds faster (Thompson et al., 1985). Thus, pheomelanogenesis is kinetically favored under more acidic environment in melanosomes.

Effects of metal ions on mixed melanogenesis might have a role in regulating melanogenesis because some metal ions are present at certain levels in melanosomes (Liu et al., 2005).

In eumelanogenesis, DCT plays an important role in promoting the production of DHICA in tautomerization of dopachrome, a reaction also catalyzed by Cu^{2+}.

Pheomelanosomes isolated from red human hair contain Fe^{3+}, Zn^{2+}, and Cu^{2+} at 98, 25, and 20 μmol/g melanin, respectively, in addition to 141 μmol/g of Mg^{2+} (Liu et al., 2005). In pheomelanogenesis, these metal ions have been shown to modify the course of melanogenesis at the monomer level. During oxidation of 5SCD by a chemical oxidant, the presence of Zn^{2+} protects the carboxyl group of QI through chelate formation to preferentially form BTCA in place of BT (Di Donato et al., 2002; Napolitano et al., 2001). Under the same conditions, Fe^{3+} ions appear to form chelates with intermediates to accelerate the ring contraction leading to BZ (Di Donato et al., 2002). Cu^{2+} ions are also involved in modification of the reaction pathway, with a greater yield of the 3-oxo-derivative ODHBT.

At the intracellular level, the major regulatory pathway is cyclic adenosine monophosphate (cAMP) through the activation of protein kinase A (PKA) (reviewed in Slominski et al., 2004) and involves phosphorylation of cAMP responsive element binding protein (CREB) and CREB binding protein (CBP). At the intracellular level, the major regulatory pathway is cyclic adenosine monophosphate (cAMP) through the activation of protein kinase A (PKA)

and involves phosphorylation of cAMP responsive element binding protein (CREB) and CREB binding protein (CBP). Phosphorylated CREB interacts with CBP to activate the expression of MITF throughout the CRE in the promoter region of the gene. MITF in turn regulates transcription of genes coding MRPs. Because CRE is absent from the promoter of tyrosinase and TYRP1 genes, the transcriptional control of melanogenesis by cAMP is coordinated predominantly by MITF. However, transcription of TYRP2 can potentially be activated through direct activation of CRE by CREB (reviewed in Slominski et al., 2004).

MITF is one of the most critical factors for the regulation of melanocyte function. In addition to serving as an essential regulator for expression of enzymes and structural proteins involved in melanin production (such as tyrosinase, TYRP2/DCT), MITF is also an essential regulator of genes involved in melanocyte development, proliferation, replenishment during feather and hair cycles, survival (such as BCL2, p21, p16, CDK2, TBX2) and malignant transformation. (Arnheiter, 2010; Liu & Fisher, 2010)

In addition, PH and TH hydroxylation phenylalanine to tyrosine and tyrosine to DOPA, respectively, are controlled by the PKA-dependent phosphorylation of regulatory serine residues (Stryer, 1988).

cAMP also modifies other pathways controlling melanocyte differentiation and proliferation, for example, the phosphatidylinositol (PI) 3-kinase pathway with its downstream regulatory element p70S6 kinase (Busca & Ballotti, 2000; Haddad et al., 1999). Inhibition of this pathway stimulates melanogenesis, and the pathway can be partially inhibited by cAMP (Busca & Ballotti, 2000).

cAMP may also regulate dendritogenesis and possibly melanogenesis through activation of the Rho family of small GTP-binding proteins (Busca & Ballotti, 2000).

cAMP can also inhibit melanogenesis through PKA-independent p21Ras activation (Busca & Ballotti, 2000). Ras would activate Braf kinase and consequently mitogen-activated protein (MAP) kinases ERK1 and ERK2. MAP kinases phosphorylate MITF leading to its ubiquitination and degradation, thus removing a major transcriptional regulator of MRP genes expression (Busca & Ballotti, 2000; Englaro et al., 1998; Jordan & Jackson, 2000). In addition, activation of ras oncogene inhibits melanogenesis in normal and malignant melanocytes (Englaro et al., 1998).

Another signal transduction pathway important in the regulation of melanogenesis is represented by protein kinase C (PKC). Thus diacylglycerol (endogenous activator of PKC) can stimulate melanin synthesis both in cell culture and in vivo, while melanogenesis is blocked by PKC inhibitors or cellular depletion of PKC (Slominski et al., 2004).

Additional pathways that have been involved in the positive regulation of melanogenesis include those activated by nitric oxide (NO) and cGMP (Romero-Graillet et al., 1996) as well as thymidine dimers (Romero-Graillet et al., 1996; Eller et al., 2002) and small single-stranded DNA fragments (ssDNA) (Eller et al., 2002).

These pigmentary effects of small oligonucleotides could follow a pathway functionally similar to the SOS response system of bacteria (Eller et al., 2002).

4. Biologic roles of ultraviolet light in melanocytes

The detrimental effects of solar UVR (295-400nm) on the skin are well established and are usually categorized as: acute or chronic. Acute effects include DNA and oxidative damage, mutation, immunosuppression, erythema (sunburn) and tanning. The chronic effects include skin cancers, which are thought to be a consequence of mutation,

immunosuppression and photoaging, which is thought to be a consequence of the induction of matrix metalloproteinases (MMPs) (Young, 2006).

The emission spectrum of the sun is rich in UVA radiation with UVB radiation accounting for less than 5% of total UVR content. However, because most skin chromophores are primarily UVB absorbers, it is that part of the solar spectrum that causes most of the biological effects.

UV radiation from sunlight increases the risk of developing three types of skin cancer: basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and malignant melanoma, in order of frequency (Liu & Fisher, 2010). While melanoma arises from the pigment-producing melanocytes in skin, BCC and SCC arise from keratinocytes.

UV is a powerful extrinsic regulator of skin pigmentation. As a first line of defense, melanin produced by melanocytes within the epidermis filters UV light, preventing UV-induced DNA damage upon sun exposure. Hence, individuals with fairer/lighter skin suffer more frequently from skin cancers, as darker skin prevents photocarcinogenesis more efficiently (Miyamura et al., 2007; Yamaguchi, 2006).

Although direct evidence is lacking, it is assumed that solar ultraviolet A (UVA) radiation (320–400 nm) may play a significant role relative to ultraviolet B (UVB) radiation (290–320 nm) in melanoma etiology (Wang et al., 2001). When UV radiation damages a cell, it mutates the cellular DNA with distinctive mutational patterns.

The major UVB lesions produced are cyclobutane-type pyrimidine dimers (CPDs) due to direct absorption of UVB radiation in DNA. CPDs are quite mutagenic, especially in mammalian cells, initiating primary base substitutions in DNA. UVB induces cytosine to thymine transitions at the dipyrimidine sites, creating a UV-specific mutation signature that is ubiquitously observed in multiple organisms (Liu & Fisher, 2010).

In contrast, UVA radiation is very weakly absorbed by DNA. Most of the studied biologic effects of UVA radiation, like lipid peroxidation and membrane damage are mediated by reactive oxygen species (ROS) and they are probably the major contributors to UVA-induced cell death (Tyrell, 1994; Girotti et al., 2001; Schmitz et al., 1995). Failure to eliminate UV-damaged cells through control led apoptosis may result in disease states such as skin cancer or lead to faster aging of the skin (Brash et al., 1991).

Repair of DNA photolesions requires cell cycle arest prior to replication and mitosis (Murray, 1992). It has been hypothesized that the DNA photodamage to the telomere 3' overhang (TTAGGG) may be a specific trigger for the cellular defense responses to UVR (Eller et al., 2003) and that this is the reason why oligonucleotides with homology (i.e. TT) to this sequence are able to induce such responses as p53 activation.

Skin darkening in response to solar UVR occurs via two distinct mechanisms: immediate pigment darkening (IPD) and delayed tanning (DT). Both processes are influenced by genetic factors and are more pronounced with darker constitutive pigmentation. They are mechanistically different processes, and their exact biological role remains to be discovered. IPD starts during UV irradiation as a grayish coloration that gradually fades to a brown color over a period of minutes to days depending on UVR dose and individual complexion. These changes are due to oxidation of pre-existing melanin and redistribution of melanosomes from a perinuclear to a peripheral dendritic location (Routaboul et al., 1999). The color change may be so subtle as to be almost undetectable in fair-skinned individuals but is easily observed in skin types IV (or darker). IPD was not showed so far to have a photoprotective effect; hence, its biological function remains unknown.

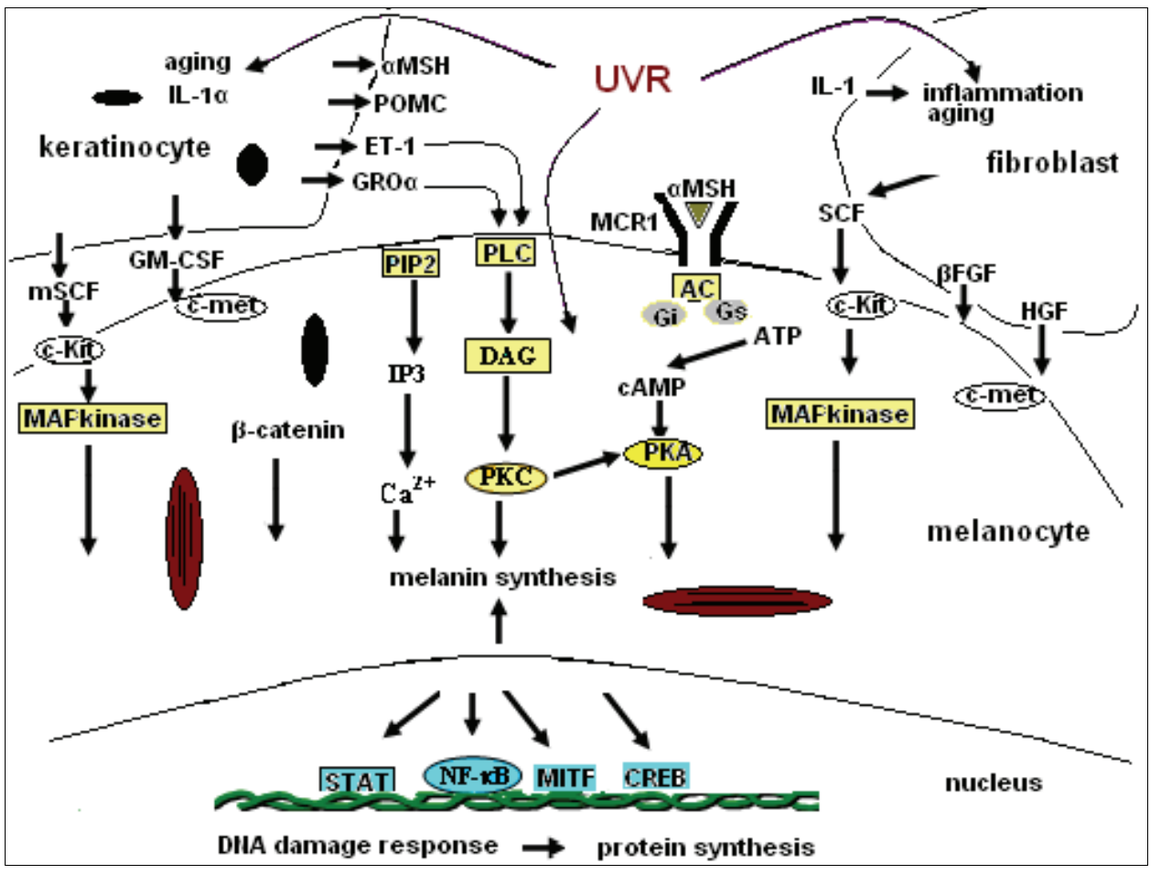

Fig. 2. Mechanisms of ultraviolet radiation - induced pigmentation in the skin cells; Abbreviations: AC- adenilate cyclase, αMSH- α melanocyte stimulating hormone, cAMP- cyclic adenosine monophosphate, βFGF-βfibroblast growth factor, DAG- diacilglycerol, ET-1- endothelin-1, GROα- growth related oncogene α, GM-CSF- granulocyte-macrophage colony-stimulating factor, HGF- hepatocyte growth factor, IL-1- interleukin -1, IP3- inositol-tri-phosphate, MAPkinase- mitogen activated phosphokinase, MC1R- melanocortin receptor 1, mSCF- membrane bound stem cell factor, PIP2-phospho inositol di-phosphate, PKA, PKC- protein kinase A & C, PLC- phospholipase C, POMC- proopiomelanocortin, SCF -m stem cell factor, transcription factors (STAT, MITF, CREB, NF-kβ), UVR- ultraviolet radiation. The keratinocytes and fibroblasts stimulate the melanocyte melanogenesis through paracrine secretion of the above factors and activation of their subsequent receptors. The most important mechanisms rely on PLC-DAG-PKC, respective AC-cAMP-PKA activation of the transcription factors. Also Ca^{2+}increase in cytoplasm through the PIP2-IP3 pathway, stimulates the synthesis of the enzymes involved in melanogenesis (e.g. tyrosinase and related proteins)

DT, which results from melanogenesis, is associated with increased melanocyte activity and proliferation. It is evident 3-4 days after UVR exposure and is maximal from 10 days to 3-4 weeks depending on complexion and UVR dose. It may take several weeks for the skin to return to its base constitutive color. UVA-induced DT is two or three orders of magnitude less efficient per unit than the UVB induced, has an earlier onset and is oxygen dependent (Eller & Gilchrest; 2000).

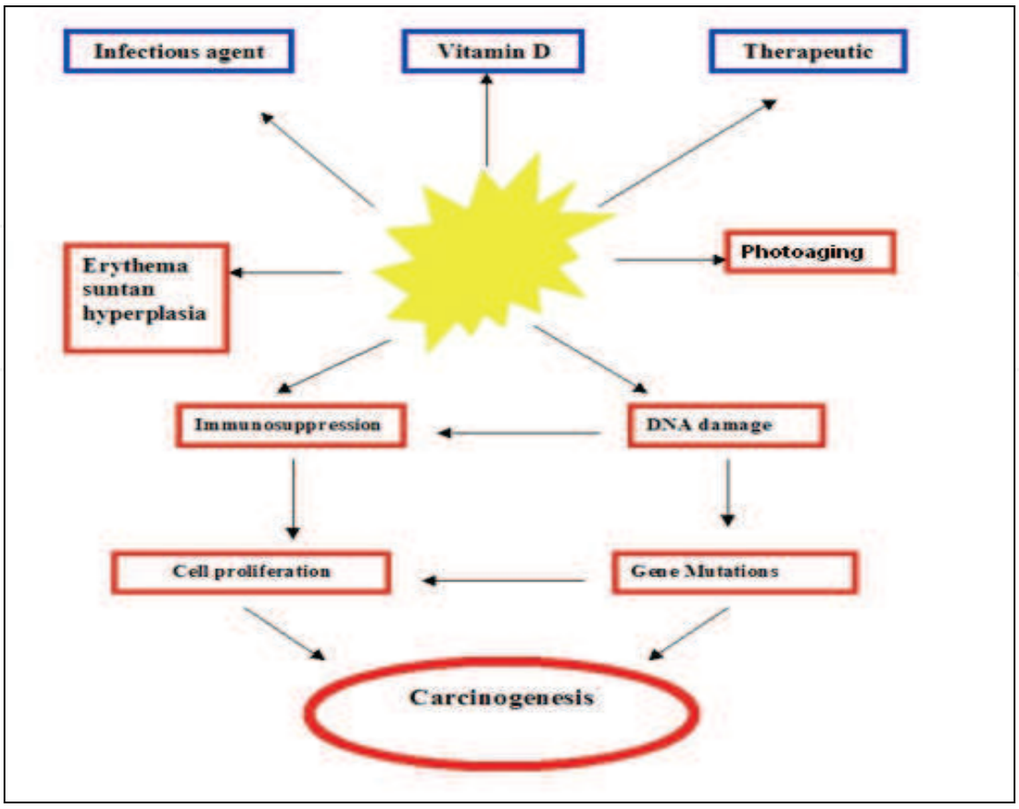

Fig. 3. Biological effects of solar irradiation on the skin (source WHO, 2006)

In response to UV induced DNA damage, the p53 expression in keratinocytes is activated, initiating the pigment synthesis in melanocytes via the p53-dependent cAMP- MITF tanning pathway (Cui et al., 2007), although this protection is probably not as effective as constitutive epidermal pigmentation. P53 binds directly to the POMC promoter, initiating POMC transcription and α-MSH production; α-MSH binds with its receptor MC1 R, activating the cAMP pathway in melanocytes. MITF activation leads to activation of melanogenesis through enhanced transcription of the pigment enzyme genes TYR, TYRP1, and DCT/TYRP2. Besides melanin synthesis, the expression of the genes necessary for melanosome biogenesis, melanosome transport and dendritogenesis is control led coordinately by MITF (Cheii et al., 2010).

UVR modulates the process of melanosome transfer from the melanocytes to the keratinocytes through upregulated expression of PAR-2 and lectin-binding receptors and increase phagocytic activity of cultured keratinocytes (Boissy, 2003). UVR also decreases the cytoplasmic dynein levels resulting in augmented melanosomal anterograde transport (Randolph et al., 2000). The melanosomes are redistributed to supranuclear areas, thus shielding the nuclei of skin cells and eventually transported to the superficial epidermis during epidermal keratinocyte maturation.

UV radiation has also been implicated in modulating the proliferation and differentiation state of melanocytes (Kawaguchi et al., 2001). Normal epidermis contains TYRP1-positive mature melanocytes, KIT-positive precursors, and few MITF-positive cells. In addition, UV appears to contribute to melanocyte maturation and development through a MITF-activation-dependent pathway (Kawaguchi et al., 2001). Upon UV irradiation, SCF has been

observed to be released by keratinocytes and activates KIT on melanocytes (or their precursors), which first differentiate into MITF-positive and TYRP2 (DCT)-positive melanoblasts, and then mature into TYRP1-positive melanocytes. Pre-existing TYRP1-positive mature melanocytes may be incapable of proliferating and differentiating further and thus may enter alternative pathways upon UV irradiation (Kawaguchi et al., 2001).

Another biologic role for UV in skin is stimulating the production of vitamin D from cholesterol-derived precursors, but it destroys folate through photolysis. Maximum production of vitamin D can be achieved after exposure to suberythemal doses of UVB.

Many factors that increase melanogenesis (POMC, α-MSH, β-FGF, endothelins, and inflammation mediators) are upregulated upon UV irradiation (Yamaguchi & Hearing, 2009). α-MSH is an important regulator of the response of melanocytes to UV and stimulates eumelanin synthesis (Abdel-Malek et al., 2009).

UVR also upregulates the expression of MSH receptors, amplifying the melanogenic effect of exogenous MSH in a dose-dependent manner in vivo and in cell culture systems (Slominski et al., 2004). In murine melanoma, UVR action appeared to involve arrest of the cell cycle at the G2 phase, when cultured melanocytes express maximal MSH receptor activity and responsiveness to MSH (Pawelek et al., 1992). The G2 phase coupling of increased MSH receptor expression was associated with increased cellular responsiveness to the ligand (Pawelek et al., 1992). Nevertheless, G2 restricted expression and activity of MSH receptors appears to be specific for rodent melanocytes, since it has not been observed in a human model.

UV also activates DNA damage response pathways within minutes of a single exposure, regulating damaged cells via UV-induced apoptosis, cell cycle arrest, DNA repair, or pathways linked to oxidative stress (Liu & Fisher, 2010).

There is considerable variation between the ability of human populations to efficiently tan. This variation has been clinically classified by Fitzpatrick in six skin phototypes (Fitzpatrick, 1988).

Phototype I has pale, white skin, blue eyes, blond/red hair, it never tans, phototype II has fair skin, light colored eyes, it tans poorly, both of them have a high risk of sunburn; phototype III has darker white skin, it has a good ability to tan and a moderate risk of sunburn; phototype IV has olive skin, it tans easily and has a low risk of sunburn; phototype V has brown skin, and phototype VI has dark brown or black skin, they both have an excellent ability to tan and a very low risk of sunburn (Fitzpatrick, 1988). The variations in human skin inducible pigmentation by UVR are partially due to the existence of the redhead/MC1 R allele (Liu & Fisher, 2010). Epidemiological studies found a strong association between MC1R loss-of-function allele and the risk for malignant transformation of epidermal melanocytes (Abdel-Malek et al., 2009), thus implying that MC1R functions as a melanoma susceptibility gene (Cui et al., 2007).

Exposure of cultured melanocytes to T-oligonucleotides, which activated the DNA damage response, resulted in increased melanogenesis via increasing MClR expression. Moreover, forskolin activation of the cAMP pathway, had the same effects as α-MSH on the UV response of human melanocytes, and reduced the extent of DNA photoproducts in UV-irradiated mouse skin as well as human skin substitutes (D'Orazio et al., 2006; Passeron et al., 2009). Besides increasing pigmentation, α-MSH reduces UV-induced oxidative DNA damage by inhibiting the generation of hydrogen peroxide and enhances the repair of DNA photoproducts (Bohm et al., 2005; Kadekaro et al., 2005). Activation of MC1R upregulates the expression of DNA repair genes (Smith et al., 2008).

Skin phototype	Constitutive skin color	Ability to tan (facultative skin color)	Susceptibility to sunburn	Susceptibility to skin cancer
I	White	Very poor/none	High	High
II	White	Poor	High	High
III	White	Moderate/Good	Moderate	Moderate
IV	Olive	Very good	Low	Low
V	Brown	Very good	Very low	Very low
VI	Black	Very good	Very low	Very low

Table 1. Classification of human skin types with respect to their ability to tan and UV-induced carcinogenesis (adapted after Fitzpatrick, 1988)

5. Cytotoxicity of melanins and their precursors

The transformation process whereby UV damage may result in melanoma initiation is poorly understood, especially in terms of UV-induced genotoxicity in pigmented cells, where melanin can act either as a sunscreen or as a photosensitizer (Picardo et al., 1999). Patients with OCA1, (who do not present tyrosinase activity) develop nonmelanoma skin cancers but not melanoma (Streutker et al., 2000).

It seems that melanogenesis, especially pheomelanogenesis, is more potent in inducing oxidative damage in the melanocytes and surrounding cells than melanin itself. Also, the synthesis of pheomelanin, consumes cysteine and this may further limit the capacity of the cellular antioxidative defense (Smith et al., 2008). In fact, in cultured normal melanocytes, stimulation of melanogenesis leads to the suppression of proliferation, cell senescence and eventually to cell death (Hirobe et al., 2003). Melanogenesis process generates highly toxic melanin intermediates and byproducts. Those intermediates are usually small molecules, such as 5,6-dihydroxyindole, 5,6-dihydroxyindole-2-carboxylic acid, quinones, indole-quinones, 5-S-cysteinyldopa and hydrogen peroxide (Hearing, 2005). Amongst them, some are the result of tyrosinase activity: ortho-quinones including dopaquinone, dopachrome, DHI-quinone, and DHICA-quinone (Hearing, 2005). Orthoquinines can also be formed directly during the initial stage of melanogenesis (Cooksey et al., 1997).

Ectopic expression of tyrosinase in the absence of TYRP1 or DCT may cause severe cytotoxicity to nonmelanocytic cells in which no melanosomal compartmentalization is present (Singh & Jimbow, 1998). The mechanism of cytotoxicity of DHI and DHICA, and also of 5-S-cysteinyldopa, involves the production of reactive oxygen species, but their toxicity seems to be less important in melanocytes due to melanosomal containment (Singh & Jimbow, 1998).

The escape of those toxic substances from melanosomes into the cytoplasm, nucleus, and mitochondria induces cytotoxic effects that are deleterious to melanocytes and melanoma cells (Chen et al., 2009a, 2009b). One of the roles of melanosomes in normal melanocytes is to provide an environment where these chemical reactions can occur and to permit the formation of melanin biopolymers, which in turn inactivate the process and keep the cytotoxic effects under control (Chen et al., 2009a).

Dopachrome, although less reactive, is able to inactivate important sulfhydryl enzymes, eventually leading to cell death. Thus, TYRP2/DCT who catalysis the transformation of dopachrome to DHICA is considered to be a "rescue" enzyme, essential for melanocyte survival (Hearing, 2005). DHICA sensitizes DNA SSB with 313 nm exposure, especially in

the presence of oxygen (Routaboul et al., 1995). Mutations in DCT that decrease catalytic function affect DHICA production and are generally quite cytotoxic to melanocytes. Melanocytes typically express DCT before any of the other melanogenic enzymes, presumably to minimize such toxicity (Steel et al., 1992).

Melanin precursors may have protective roles in melanocytes. DHI and to a less extent, DHICA can contribute significantly to the skin protection from damaging UV radiation by quenching oxygen species and providing an additional amount of photoprotective pigment. Upon photoexcitation, DHI can react with oxygen and related species, giving rise to hydroxylated oligomer species that can polymerize to eumelanic pigments (Ito & Wakamatsu, 2008).

Hence, detoxification of endogenous melanogenic cytotoxicity appears to be a double edged sword. In the case of melanomas, the detoxifying functions of melanosomes might render cells resistant to anticancer drugs. It has been shown that the melanogenic system is involved in the regulation of drug sensitivity. Melanosomes are involved in drug trapping and export (Chen et al., 2006) and in the regulation of drug sensitivity through the melanosome biogenesis pathway (Chen et al., 2009a, 2009b).

The cytotoxicity of ortho-quinones could potentially lead to chemotherapeutic approaches to treat melanoma; 4-S-cysteaminylphenol and its derivatives appear to be the most promising antimelanoma agents (Ito & Wakamatsu, 2008).

The end product of eumelanogenesis, eumelanin acts as a redox pigment with both reducing and oxidizing capabilities towards oxygen radicals and other chemical redox systems, it mainly functions like a pseudosuperoxidedismutase. Also, the pigment has free radical scavenger activity since it binds redox active metals and initiates photon/phonon conversion (Ito & Wakamatsu, 2008).

In contrast to eumelanin, pheomelanin is hypothesized to play a role in melanomas. Some hypothesize that there is a link between the increased cancer risk in pacients with lower phototype and the increased phototoxicity of pheomelanin (Simon, 2009). The pheomelanin pigment is synthesized starting from a combination of 5-5 and 2-5-CD isomers in different ratio. The pigment derived from 5-5-CD is more photoreactive than that derived from 2-5-CD, the later being resistant to photochemical damage by natural sunlight (Greco, 2009).

Studies of UVB-induced cyclobutane dimerization and apoptosis of keratinocytes in congenic black, yellow and albino mice showed that pheomelanin sensitizes apoptosis (via caspase-3 activation) (Brash et al., 1991; Takeuchi et al., 2004).

There remains the need to understand the molecular composition of pheomelanins to determine what chromophores activate oxygen (Simon, 2009).

In vitro studies showed that 5-SCD photobinds to native DNA after exposure to UVB radiation and also induced single-strand breaks (SSB) in DNA (Chedekel and Zeize, 1988). 5-SCD is photochemically unstable in the presence of UVA radiation and oxygen (Costantini et al., 1994), leading to superoxide anion production (Chedekel and Zeize, 1988). UV irradiation of pheomelanin can also lead to the formation of hydroxyl radicals, and hydrogen peroxide capable of affecting important biological targets such as DNA (Chedekel and Zeize, 1988) or membrane lipids (Schmitz et al., 1995), acting as UVB and UVA photosensitizer in mammalian skin in vivo (Takeuchi et al. 2004).

Melanogenesis, but not melanin itself, was associated with oxidative based damage in human melanoma cells (Kvam & Tyrrell 1999, Kvam & Dahle, 2003). Melanocyte "autodestruction" by intermediates of melanin metabolism has been implicated in the etiology of vitiligo (Le Poole et al., 1994).

Tyrosine-induced melanogenesis in melanocytes was accompanied by increased production of ROS (Baldea et al., 2009) and decreased concentration of intracellular glutathione (Smit et al., 2008); it also increased early induction of Heme Oxygenase 1 gene, a typical response to oxidative stress, after UVA irradiation (Marrot et al., 2005). The ratio of pheomelanin to total melanin in melanocyte cultures from skin type 1 and skin type IV, after tyrosine stimulation remained the same in skin type IV, but relatively more pheomelanin was induced in skin type 1. This was associated with an increase in UVA-induced SSB in DNA (Wenczl et al., 1998).
The hypothesis of melanocyte carcinogenesis states that an essential part of melanocytes' malignant transformation is a change in the redox state of melanin from a mostly antioxidant state to a prooxidant state (Meyskens et al., 2001). This is supported by data that show that melanoma cells have a remarkably abnormal content of antioxidants, including vitamin E, polyunsaturated fatty acids, and catalase (Picardo et al, 1996; Briganti & Picardo, 2003; Kwan & Dahle, 2003). Also, clinical displastic nevi, recognized precursors of melanoma, suffer from chronic oxidative stress, even without the influence of UV radiation, due to increased pheomelanin synthesis. (Smit et al., 2008)
Melanin precursors have genotoxic and mutagenic effects, which may be amplified by the free radicals and reactive oxygen species generated during melanogenesis. This mutagenic environment in melanoma cells may lead to genetic instability and appearance of new, more aggressive cell populations resistant to therapy (Slominski et al., 1998).
Diffusion of potentially cytotoxic products of melanogenesis from melanosomes is thought to be minimal in normal melanocytes. There are several cytosolic processes that are invoked as cytoprotective mechanisms (Smith et al., 2008). The intermediates that may reach the cytosol consist essentially of quinones liberated directly or generated by oxidation from hydroquinones that leak through the melanosomal membrane. Quinone detoxification can be done by quenching by glutathione with the formation of S-glutathionyl adducts, by quinone reductases (DT-diaphorase), inactivation of dihydroxyindoles by O-methylation catalyzed by the enzyme catechol-O-methyl transferase (Axelrod & Lerner, 1963), glucuronidation and sulfonation mainly due to hepatic metabolism of the methylated derivatives formed in mellanogenic cells (Pavel et al., 1986).

6. Oxidative stress defence in melanocytes

Melanogenesis and stratum corneum thickening occur concurrently during the normal tanning response. Although photoprotection may be considered to be a passive physical process, e.g. the attenuation of UVR by melanin and/or stratum corneum thickening, it may also be considered as an active enzymatic process, e.g. as a means by which DNA repair is enhanced or ROS are inactivated. Chimeric epidermal reconstructs with melanocytes from one skin type added to keratinocyte cultures of a different skin type suggest keratinocyte/melanocyte interaction with both cell types regulating antioxidant defense in a skin type-dependent way (Bessou-Touya et al., *1998).*
Melanocytes seem to be extremely susceptible to free radicals, either in the activation of their physiologic role or in deleterious effects (Shindo et al., 1993; Picardo et al., 1991; Romero-Graillet et al., 1996). One reason is low catalase levels in these cells (Maresca et al., 2006). Therefore, antioxidants are considered to be among the physiologic photoprotective compounds of the skin (Applegate & Frenck 1995; Briganti & Picardo, 2003).
The initial free radical scavenging machinery involves superoxide dismutase (SOD), which catalyzes the dismutation of two molecules of the superoxide radical anion into hydrogen

peroxide and diatomic oxygen. Then, hydrogen peroxide is converted by catalase (Cat) and peroxidases into water (Steenvoorden & van Henegouwen, 1997).

Thioredoxin reductase together with its electron acceptor thioredoxin, thioredoxin peroxidases, glutathione reductase/glutathione coupled to glutathione peroxidase - present in small amounts in melanocytes (Yohn et al., 1991) are involved in the removal of H_2O_2 deriving from enzymatic dismutation of superoxide anion (O^{2-}) catalyzed by SOD (Nordberg & Arner, 2001; Schallreuter & Wood, 2001). The high SOD/Cat ratio, can lead to an increased intracellular production of hydrogen peroxide, thus is considered as a parameter of the cells susceptibility to external oxidative stress (Maresca et al., 2006).

UVA irradiation is depleting the skin of antioxidants (Sanders et al., 2004), among which Cat is the most sensitive. Low levels of Cat activity were previously observed in different cutaneous experimental models (which contained lightly pigmented melanocytes) and they were always associated with a stress-prone status (Maresca et al., 2006; Bessou-Touya et al., 1998; Gramatico et al., 1998; Picardo et al., 1999, Kadekaro et al., 2003; Kvam E & Dahle, 2004). In melanocytes, the role of Cat is critical because it is the first enzyme devoted to the neutralization of H_2O_2 (Yohn et al., 1991) a byproduct of the melanogenic pathway (Nappi & Vass, 1996). Cat oxidative damage is detrimental, because when damaged it recovers slowly (Shindo et al., 1994; Shindo & Hashimoto, 1997). This results in accumulation of H_2O_2 in the cell and damaged several structures, including Cat (Shindo et al., 1994; Shindo & Hashimoto, 1997) and tyrosinase (Schallreuter et al., 2008).

Overall, UVA was also more effective than UVB in inducing impairment in Cat activity (Zigman et al., 1996; Rhie et al., 2001; Hellemans et al., 2003) .

In the melanocytes, the dominant skin pigment melanin and its precursors are complex redox systems, the resultant properties of which are modified by ph, temperature, illumination with ultraviolet and visible light. Melanins act as a filter absorbing UV photons as well as a quencher of free radicals generated in the skin after UV exposure.

Eumelanin is capable of scavenging the superoxide anion and hydrogen peroxide, whereas pheomelanin acts as a photosensitizing agent (Prota, 1997), amplifying ROS production and increasing DNA damage after UVA (Menon et al., 1983; Ranadive et al., 1986; Prota, 1997; Kvam & Dahle, 2004).

The overall scheme proposed for auto-oxidation of melanin consists of one electron reduction of molecular oxygen to superoxide anion, followed by SOD reduction of superoxide to H_2O_2, degraded by catalase and oxidation of superoxide to O_2, and spontaneous dismutation of superoxide to equimolar H_2O_2 and O_2. Autooxidation of melanin may be important, rate limiting process in coupled reactions where melanin functions as an electron transfer agent (Sarna & Swartz, 2006).

Oxygen radicals, and in particular hydrogen peroxide, are considered as intracellular second messengers since they have major roles in cell survival and integrity (Schalreuter et al, 2008). Their level is increased by extracellular ligands such as cytokines (Schreck & Bauerle 1991), ROS act as biologic mediators of UV-induced phosphorylation of membrane receptors (Tyrell, 1994; Schmitz et al, 1995, Peus et al., 1999; Girotti, 2001, Kvam & Dahle, 2003); H_2O_2 is a normal byproduct of the melanogenic pathway (Nappi & Vass, 1996); in several cellular systems, H_2O_2 acts as an intracellular second messenger for TNFα and TGFβ (Thannickal & Fanburg, 1995; Chen et al., 1995; Lo et al., 1996); it activates growth factor receptors and in particular those of epidermal growth factor (EGFR) and initiates multiple signaling responses associated with mitogenesis and cell growth regulation (Ulrich & Schlessinger 1990).

H_2O_2, in micromolar concentrations can be deleterious to many proteins and peptides leading to deactivation / disruption of many important proteins and peptides involved in melanogenesis including tyrosinase (deactivation of the enzyme active site due to a methionine residue in position 374) (Schweikardt et al., 2007), POMC derived peptides (α-MSH, β-endorphin) (Spencer et al., 2007), 6R-l-erythro 5,6,7,8-tetrahydrobiopterin (6BH4), acetylcholinesterase (Schallreuter, 2005), the prohormone convertases PC1, PC2, Furin, PACE4 and, even the antioxidant mechanism including catalase, thioredoxin reductase and the methionine sulfoxide reductases A&B (Schallreuter, 2005; Spencer et al., 2007; Gibbons et al, 2006). However, H_2O_2 is upregulating multiple transcription factors including p53, MITF and NFkB (nuclear factor kappa B), epidermal growth factor (EGFR), and the antioxidant enzymes catalase, thioredoxin reductase, glutathione reductase and the methionine sulfoxide reductases A & B (Schallreuter et al, 2001; Schallreuter, 2005; Gibbons et al, 2006). Enzyme activities are directly controlled by H_2O_2 in a concentration dependent manner. This is also the case for PAH, THI and tyrosinase, (Wood et al., 2004; Schallreuter et al, 2008) thus being involved in melanocyte mitogenesis, melanogenesis and cell growth regulation. Regulation and protection of tyrosinase against a ROS burst is also provided by both tyrosinase related proteins TYRP1 and TRP2. TYRP1 has been recognised as a peroxidase (Halaban et al., 1990), while TRP2 has an additional function as dopachrome tautomerase (Prota, 1992). In addition to TYRP1 and TRP2 the calcium binding protein calnexin is present in the melanosomal membrane adding another force for redox homeostasis in the melanosome (Jimbow et al., 2001).

7. Melanocyte senescence – defence against carcinogenesis

Senescence is a checkpoint response due to DNA activation by the disfunctional telomeres after exposure to oncogenic stress (such as UV radiation). Senescence blocks proliferation (and the resultant oncogenic threat), but allows the cell to live on and perform its physiologic function (Mooi & Peeper, 2006).

As activation of BRAF, or NRAS, alone induces senescence in melanocytes, melanoma progression must be accompanied by compensating events, for example inactivation of the CDKN2A locus encoding p161NK4a via genetic lesions (Bennett, 2008), epigenetic silencing (Richards and Medrano, 2009; Rothhammer and Bosserhoff, 2007), or repression of p161NK4a expression through activation of Wnt/β-catenin signalling (Delmas et al., 2007). Consistent with senescence representing a major barrier to melanoma initiation is the observation that benign nevi, as well as carrying frequent activating mutation of BRAF or NRAS, include a mass of senescent melanocytes (Gray-Schopfer et al., 2006; Michaloglou et al., 2005). If the initial senescence barrier is overcome, melanomas can progress to a radial growth phase. For the majority of melanomas that do not arise from a pre-existing nevus, senescence bypass via bi-allelic loss of p161NK4a would occur prior to activation of BRAF/ NRAS. By contrast, nevi may be generated by monoallelic loss of p161NK4a prior to BRAF/NRAS mutation, with subsequent inactivation of the second p16INK4a allele leading to melanoma. Thus, the order in which mutations occur will determine whether melanoma arises de novo or from a pre-existing nevus (Hoek & Goding, 2010).

Normal, adult skin melanocytes are long lived cells with a very low, if any, proliferation rate in vivo that produce abundant levels of the antiapoptotic protein Bcl2 (McGill et al, 2002) and Slug (Gupta, 2005). These factors protect cells from p53-dependent apoptosis and promote melanocyte survival, even at the cost of entering senescence after mutagenic stimuli

induced damage (Hoek & Goding, 2010). It is possible that melanoma originates from differentiated melanocytes by a process of de-differentiation arising from "phenotype-switching", sustained by the observation that differentiated melanocytes can be induced to proliferate in culture (Hoek & Goding, 2010).

The most important factor that regulates the choice between a proliferation-type response in melanocytes or a differentiation, pigment production and senescence/apoptosis response seems to be MITF. MITF is implicated in differentiation through the activation of pigmentation genes and is the key regulator of cell division, driving a differentiation-associated cell cycle arrest via up-regulation of p16 and p21 (Goding, 2010). MITF can also promote cell division by suppressing p27 expression and senescence and inhibit proliferation via up-regulation of p21 Cipl and p 161NK4a. Thus in addition to its role in survival and differentiation, MITF is also charged with suppressing senescence and coordinating cell cycle entry and exit depending on its levels and activity (Hoek & Goding, 2010).

MITF was also identified as a lineage-addiction oncogene, being amplified to varying degrees in about one-sixth of melanomas (Garraway et al., 2005).

8. Conclusions

UV exposure alone or through induced melanin synthesis, generates cytotoxic compounds that might trigger oxidative damage of important biological targets such as DNA and membranes, but it also activates the defense mechanisms of the cell. However, how much these phototoxic and photoprotective events are functionally important in vivo requires further studies.

There is an intimate relationship between pigmentation, senescence, apoptosis and cell division, and the key role in regulating these mechanisms is attributed to MITF. A better understanding of the skin responses to oncogenic threats, and especially UV radiation could be beneficial in improving the prevention of skin carcinogenesis and photo-aging.

9. References

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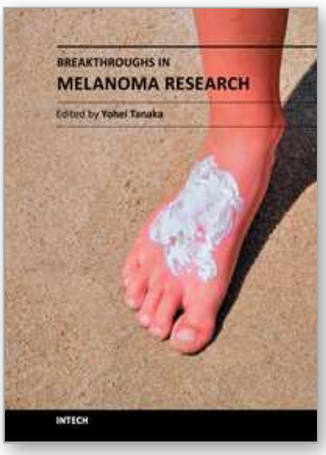

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Melanoma is considered to be one of the most aggressive forms of skin neoplasms. Despite aggressive researches towards finding treatments, no effective therapy exists to inhibit the metastatic spread of malignant melanoma. The 5-year survival rate of metastatic melanoma is still significantly low, and there has been an earnest need to develop more effective therapies with greater anti-melanoma activity. Through the accomplishment of over 100 distinguished and respected researchers from 19 different countries, this book covers a wide range of aspects from various standpoints and issues related to melanoma. These include the biology of melanoma, pigmentations, pathways, receptors and diagnosis, and the latest treatments and therapies to make potential new therapies. Not only will this be beneficial for readers, but it will also contribute to scientists making further breakthroughs in melanoma research.

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