



Biology of Melanocytes and Primary Melanoma

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Abstract

Melanocytes make up only a tiny proportion of the skin cellular milieu but have a major impact on skin appearance as well as skin cancer risk by modulating skin pigmentation. Cutaneous melanocytes are derived from precursor cells called melanoblasts that originate from the neural crest of the developing embryo and migrate for long distances to their niches in the epidermis and hair follicles, where they differentiate into melanin pigment-producing mini-factories. Melanin is synthesized and packaged within melanosomes, which are lysosome-related organelles responsible for melanin trafficking through dendrites to interacting keratinocytes. Melanocyte development, migration, proliferation, and differentiation are regulated by a complex network of extrinsic and intrinsic signaling pathways, which are responsive to key signals, such as ultraviolet radiation that stimulates melanin production (tanning). Alterations in components of these pathways may lead to melanomagenesis. Here we present an overview of the genes and pathways that regulate different aspects of the biology of melanocytes as well as their transformation to melanoma.

Keywords

Development of melanoblasts · Regulation of specification · Regulation of migration · Regulation of survival and proliferation · Differentiation of melanocytes · Regulation of differentiation · Regulation of survival · Melanomagenesis · From melanocyte to melanoma: a multistep process · Molecular genetics: early lessons from familial and sporadic melanoma · Melanoma: a consequence of homeostatic disruption · Melanoma: cell of origin · Melanoma and the environment · Sun exposure and epidemiology · Photobiology and melanoma

Introduction

Melanocytes are highly specialized cells that produce and distribute melanins, which are high molecular weight pigmented biopolymers responsible for pigmentation in the skin, hair, eyes, and inner ear. Although melanocytes represent a small proportion of cells in these pigmented tissues, the pigments they produce have several diverse and important functions (Hearing and Leong 2006; Nordlund 2006; Prota 1992). Melanocytes destined for the skin, hair, and choroid of the eye originate from pluripotent neural crest cells during embryonic development. In contrast, melanocytes populating the retinal pigment epithelium are derived from the optic cup (Bharti et al. 2006) and are not considered further in this chapter.

Epidermal melanocytes reside among the basal keratinocytes in an approximate ratio of 1:10 and transfer melanin via elongated dendrites that contact up to 40 overlying suprabasal keratinocytes. The melanin-carrying keratinocytes form a vital barrier against environmental assaults. Consequently, skin color is not defined solely by the melanocytes that produce the pigment but occurs in partnership with the pigment-carrying keratinocytes in the superficial skin layers. Given the intimate relationship between melanocytes and other cell types present in the surrounding microenvironment, the importance of microenvironmental cues in melanocyte development, function, and transformation to melanoma (melanomagenesis) is well established.

In many respects, the process of melanomagenesis is integrally but inversely related to the process of melanocyte development, representing a return to a proliferative, dedifferentiated, and migratory phenotype. In fact, the same essential cascades of genes that regulate development and differentiation of melanocytes are involved in the growth and eventual metastasis of melanoma cells. This chapter provides an

overview of the processes critical to melanocyte development and pigmentation in mammals and describes the disruption of these processes during the transformation of melanocytes into melanoma cells.

Development of Melanoblasts

A recent curation effort by the Loftus group at the National Human Genome Research Institute (USA) has compiled a list of 650 genes that are currently known to be involved in integumentary pigmentation in humans, mice, and/or zebrafish (Baxter et al. 2018). Among these, 128 genes have been validated to have associations with human pigmentary phenotypes, while others have only been shown to exhibit phenotypes in mice and zebrafish to date. Clearly, the mechanistic network involved in the process of pigmentation is highly complex, which is exemplified by the wide spectrum of protein complexes, signaling pathways, and functions represented by the 26 different protein classes featured among the products of the pigmentary genes (Baxter et al. 2018). Disruption of the functions of these genes leads to a variety of pigmentary disorders, which can result from perturbations in melanocyte development, differentiation, and regulation. Although the process of melanocyte development is a continuous one, it is generally considered in four discrete stages: specification, migration, survival, and proliferation.

Regulation of Specification

Specification describes the process early in development when pluripotent neural crest cells, which exhibit all the key features of stem cells, begin to commit to becoming pigment cells (melanocytes) in adult tissues. Before their final specification, neural crest cells (which are specified at an earlier stage of development and originate from the dorsal edge of the neural tube) have the ability to develop into a number of distinct types of cells, including neurons, glial cells, cardiac cells, and pigment cells (Bronner and LeDouarin 2012). As one might expect, transcription factors and

intracellular signaling factors play major roles in determining which pathway of development a given neural crest cell will take. Regarding those destined to become melanocytes, the precursor cells, once specified, are called *melanoblasts*. Early during gestation (approximately 1 month in humans), neural crest cells destined to become melanoblasts begin to express several genes encoding transcription factors (Table 1), including PAX3, LEF1, and FOXD3 (Blake and Ziman 2005; Ignatius et al. 2008; Kos et al. 2001; Silver et al. 2006; Yasumoto et al. 2002). These transcription factors regulate downstream target genes that participate in specifying further development of melanoblasts; some of those downstream genes have been identified (Blake and Ziman 2005; Hornyak et al. 2001; Ignatius et al. 2008; Kos et al. 2001). Concurrently, factors that regulate the WNT signaling pathway (WNT1 and WNT3) are also expressed and thought to trigger melanocytic differentiation through direct transcriptional targeting of the MITF gene (Dorsky et al. 2000). The canonical WNT signaling pathway is activated by extracellular WNT proteins binding to the surface receptor complex which then regulates the redistribution of β -catenin to the nucleus, where it acts to modulate transcription through activities that involve the transcription factors LEF1 and MITF (Dunn et al. 2005; Garcia-Castro et al. 2002; Hari et al. 2012; Lewis et al. 2004; Liu et al. 2014; Takeda et al. 2003). The multiple roles of MITF and WNT signaling, as well as many other intracellular signaling pathways, at virtually all phases of melanocyte development and differentiation will be a recurring theme in the discussion to follow (Kawakami and Fisher 2017; Liu et al. 2014).

At least two other transcription factors are thought to play roles in the early developmental process of specification, SOX9 (Meulemans and Bronner-Fraser 2004) and SNAI2/SLUG (LaBonne and Bronner-Fraser 1998). SOX9 has also been shown to regulate MITF expression (Passeron et al. 2007). While the transcription factor SOX10 is required for the maintenance and differentiation of neural crest cells as they migrate, SOX9 plays an essential role in neural crest generation (Guth and Wegner 2008). SNAI2

Table 1 Partial list of genes involved in determination of melanocyte development, differentiation, and malignancy

Gene	Protein name	Function	Specification	Migration	Proliferation	Survival	Differentiation	Melanoma
<i>PAX3</i>	Paired box 3	Transcription factor	X		X	X		X
<i>LEF1</i>	Lymphoid enhancer-binding factor 1	Transcription factor	X				X	X
<i>FOXD3</i>	Forkhead box D3	Transcription factor	X					
<i>SNAIL2</i>	Slug	Transcription factor	X	X				X
<i>SOX9</i>	SRY-box 9	Transcription factor	X	X			X	
<i>WNT1</i>	Wnt family member 1	Wnt signaling	X		X		X	X
<i>WNT3A</i>	Wnt family member 3A	Wnt signaling	X		X		X	
<i>CTNNB1</i>	Catenin Beta 1	Intracellular signaling, regulation of transcription	X				X	X
<i>BMP2</i>	Bone morphogenetic protein 2	Secreted ligand to TGF- β receptors	X					
<i>BMP4</i>	Bone morphogenetic protein 4	Secreted ligand to TGF- β receptors	X					X
<i>CDH2</i>	N-cadherin	Surface binding protein		X				X
<i>CDH3</i>	P-cadherin	Surface binding protein		X				X
<i>CDH11</i>	OB-cadherin	Surface binding protein		X				X
Integrins, laminin, lectin fibronectin		Extracellular matrix proteins	X					X
<i>ADAMTS20</i>	ADAM metalloproteinase with thrombospondin type I motif 20	Metalloproteinase		X	X			
<i>SOX10</i>	SRY-box 10	Transcription factor		X	X	X	X	X
<i>MITF</i>	Microphthalmia-associated transcription factor	Transcription factor		X	X	X	X	X
<i>KIT</i>	Kit proto-oncogene	Receptor tyrosine kinase		X	X	X	X	X
<i>KITLG</i>	KIT ligand; stem cell factor	Ligand for c-KIT		X	X	X	X	X
<i>EDNRB</i>	Endothelin receptor type B	G protein-coupled receptor		X	X	X	X	X
<i>EDN3</i>	Endothelin 3	Ligand for EDNRB		X	X	X	X	X
<i>DCT</i>	Dopachrome tautomerase	Enzyme		X	X	X	X	

Table 1 (continued)

Gene	Protein name	Function	Specification	Migration	Proliferation	Survival	Differentiation	Melanoma
<i>OCA2</i>	Oculocutaneous albinism II	Solute carrier, transporter, cargo unknown					X	
<i>SLC24A5</i>	Solute carrier family 24 member 5	Na-Ca exchange					X	
<i>SLC7A11</i>	Solute carrier family 7 member 11	Cysteine/glutamate transporter					X	
<i>RAB7A</i>	RAS-associated protein 7A	GTPase, regulates melanosome transport					X	
<i>RAB27</i>	RAS-associated protein 27	GTPase, regulates melanosome transport					X	
<i>RAB38</i>	RAS-associated protein 38	GTPase, regulates TYRPI trafficking					X	
<i>AP3</i>	Adaptor protein 3	Regulates TYR trafficking					X	
<i>MYO5A</i>	Myosin VA	Regulates melanosome transport					X	
<i>ATP7A</i>	ATPase copper transporting alpha	Copper transport, required for TYR activity					X	
<i>IRF4</i>	Interferon regulatory factor 4	Transcription factor					X	
<i>TRPM7</i>	Transient receptor potential M7	Cation channel				X		X
<i>YY1</i>	Yin and Yang 1	Transcription factor			X	X		X
<i>ATF2</i>	Activating transcription factor 2	Transcription factor			X	X		X
<i>PREX1</i>	PIP3 dependent Rac exchange factor 1	Guanine nucleotide exchange factor						X
<i>NEDD9</i>	Neural precursor cell expressed developmentally Down-regulated protein	Focal adhesion protein						X

The order of the genes is based on their predominant function in the different stages of melanocyte development

has been shown, at least in lower organisms, to regulate the expression and function of SOX10 (Jiang et al. 1998; Sanchez-Martin et al. 2002). Bone morphogenetic proteins play critical roles in determining patterning during development, and these are closely regulated by the WNT signaling pathway; in that regard, BMP2 and BMP4 have been shown to be important to melanoblast specification (Jin et al. 2001). The expression of BMP2/4 is not specific to cells destined to become melanoblasts, and other factors that are specific to melanoblasts and regulate their specification will undoubtedly be identified in the future. The majority of studies defining the genes and developmental pathways involved in melanoblast specification have been performed in lower species, including mice, chicks, zebrafish, and amphibians, although results to date suggest that these pathways and genes are highly conserved in humans (as are the defects involved in pigmentary disorders) (Baxter and Pavan 2013; Liu et al. 2014; Pavan and Raible 2012). Cells expressing these developmental markers then undergo an epithelial-mesenchymal transition and begin to migrate dorsolaterally below the ectoderm (called *early migration*). Expression of these same markers (and others, as described in the “Differentiation” section) is found in melanocyte stem cells in adult tissues that serve as reservoirs of cells that can be recruited to become differentiated melanocytes by appropriate environmental cues (Buac and Pavan 2007; Nishimura 2011; Osawa et al. 2005).

Regulation of Migration

As the precursor cells migrate (Fig. 1), they remain multipotent (i.e., they retain the capacity to become pigment cells, smooth muscle cells, neurons, and so on). Those destined to become melanoblasts continue to express SOX9 but now begin to express other genes that regulate their further development, including SOX10, MITF, KIT, and, a bit later, a melanogenic (pigmentation-inducing) enzyme called *dopachrome tautomerase* (DCT), although

expression of DCT is thought to result simply from the increased function of SOX10 and MITF and is not relevant to developmental processes (Guyonneau et al. 2004). Of the newly expressed genes, SOX10 and MITF are transcription factors, whereas KIT is a surface receptor that plays a critical role in regulating intracellular signaling in melanocytes at many levels of their development and differentiation. Although these transcription factors are not specific to the pigment cell precursors, MITF is differentially expressed in different cell lineages using alternative promoters and initial exons, which are alternatively spliced to perform distinct functions and activate distinct genes, with the form of MITF specific to pigment cells designated MITF-M (Harris et al. 2010; Kawakami and Fisher 2017) (Fig. 2), although other spliced isoforms (non-tissue-specific) are typically expressed in melanocytes as well as other cell types. MITF-M (hereafter, MITF) is often referred to as the master regulator of melanocytes, because it is a central focal point through which environmental signaling modulates the behavior of melanocytes at many levels (see the “Differentiation” section). Melanoblasts move in either a ventral or a dorsolateral direction, giving rise to the peripheral nervous system and endocrine cells or to melanocytes, respectively. The full complement of signals that regulate these processes not only involves genes expressed by melanoblasts but also factors produced in the local environment in the developing embryo. For example, KIT is a tyrosine kinase receptor on the plasma membrane that is regulated by the environmentally derived stem cell factor (SCF, encoded by the *KITLG* gene) ligand, which works through the MAP kinase signaling pathway to regulate the expression of genes critical for melanoblast migration and survival (Wehrle-Haller 2003). Most notable among KIT-regulated genes is MITF. Yet another receptor, the endothelin receptor (EDNRB), which works through the phospholipase C (PLC) signaling cascade, is also critical for regulating melanoblast migration and proliferation, and although that receptor is expressed on the surface of melanoblasts, it is activated by endothelins (EDN) produced in the local environment (Lee et al. 2003;

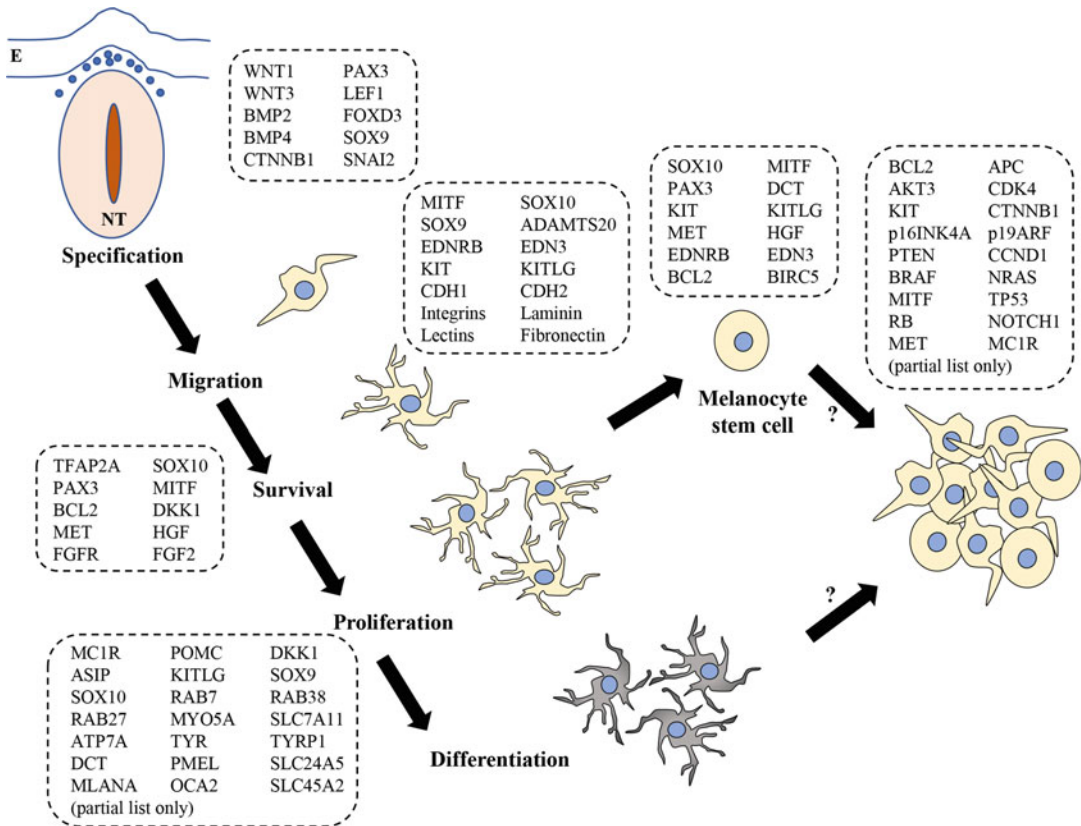


Fig. 1 Stages of melanoblast development, melanocyte differentiation, and transformation to melanoma. Genes important to various stages are identified in the boxes, as

discussed in the text. (Adapted from Yamaguchi et al. 2007; Brenner and Hearing 2008)

Saldana-Caboverde and Kos 2010). One must always bear in mind that for appropriate pigmentation patterns to occur, the signaling for melanoblast migration to begin, and later to stop, must be carefully controlled. Some melanoblasts move only short distances in the developing embryo, whereas others need to migrate long distances to reach the extremities. Defects in many of the genes involved in migration and specification are associated with developmental pigmentary diseases, often eliciting hypopigmented areas in ventral regions. For example, deficiency of the Rac-specific guanine nucleotide exchange factor (GEF) P-Rex1, encoded by the *PREX1* gene, causes a white belly and feet phenotype in *Prex1-knockout* mice, characterized by defective melanoblast migration (Lindsay et al. 2011).

Cell-cell interactions are critically important during migration; thus, it is no surprise that the expression of various proteins regulating such interactions (e.g., cadherins and components of the extracellular matrix [ECM], such as integrins, laminin, lectins, and fibronectin (Bonaventure et al. 2013; Haass et al. 2005; Herlyn et al. 2000; Jouneau et al. 2000; Pinon and Wehrle-Haller 2011)) is also closely involved in the regulation of melanoblast migration. Similarly, proteases associated with penetration of biologic membranes are also important to cell migration, and ADAMTS20 (a metalloproteinase) has been closely associated with melanoblast survival and migration during development, with mutations affecting this gene resulting in abnormal patterning in the skin (Rao et al. 2003; Silver et al. 2008). Studies in mice suggest that melanoblasts undergo

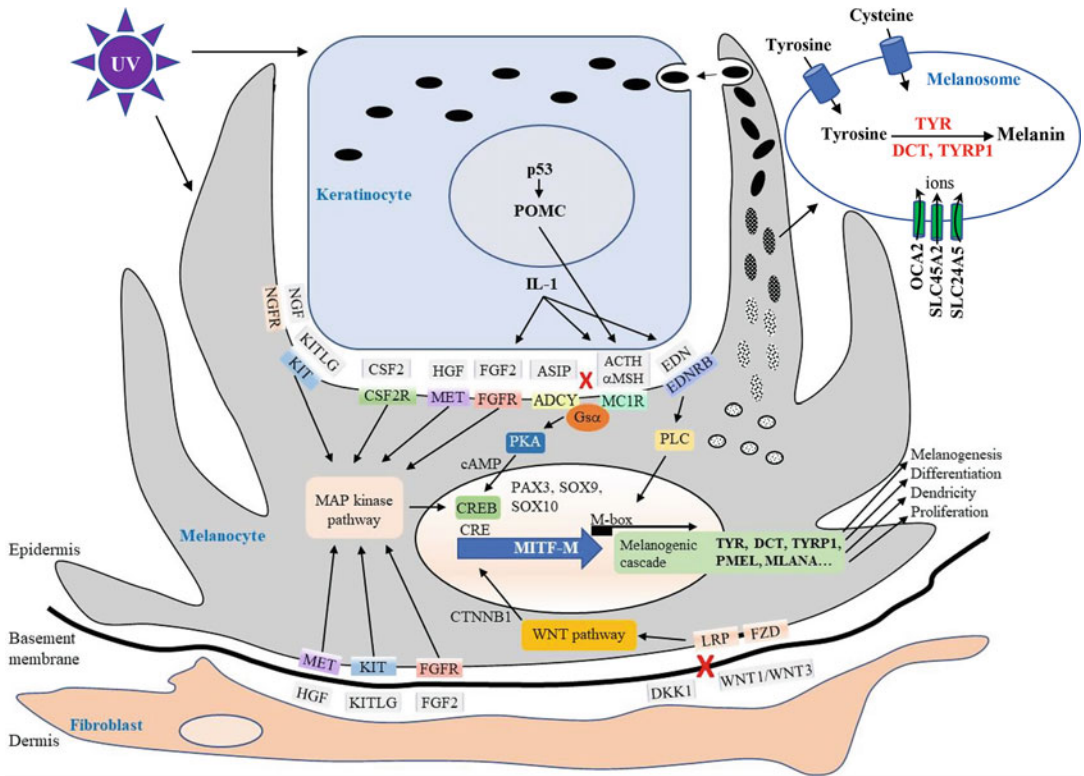


Fig. 2 Factors involved in interactions between melanocytes and neighboring keratinocytes and fibroblasts. (Ligands and receptors are discussed in the text)

proliferation before their initial migration and then cease proliferating while they migrate, after which they may undergo extensive proliferation once they have reached their final destination (Thomas and Erickson 2008; Wilkie et al. 2002). With respect to invasion and metastasis, migrating melanoblasts have the ability to cross the basement membrane and other biologic barriers to tissue penetration during development.

Regulation of Survival and Proliferation

Once melanoblasts reach their intended destination, they must cease migrating, then survive and proliferate, and eventually differentiate. Because survival and proliferation occur concurrently at this time during development, most genes known to be involved play roles in both processes

(or at least, it is very difficult to distinguish their roles in those two processes), and they will be considered together in this section. The survival of differentiated melanocytes in the skin is discussed further on. Many of the genes mentioned above are also involved in regulating survival and/or proliferation (i.e., genes encoding MITF, SOX10, and PAX3), but additional genes act as “supporting actors,” including the transcription factor AP2, as well as additional signaling factors (basic fibroblast growth factor [bFGF, encoded by the *FGF2* gene] and hepatocyte growth factor [HGF]) that join the previous list (EDN3 and KITLG). The KIT receptor and its ligand KITLG play important roles in regulating survival and proliferation, and mutations in either of these two genes are associated with pigimentary disorders of the skin, which can result in hypopigmentation (e.g., vitiligo and piebaldism) as well as hyperpigmentation

(e.g., UVR melanosis and lentigo senilis), depending on whether their function is decreased or increased, respectively (Diwakar et al. 2008; Imokawa 2004).

It is an interesting paradox that melanocytes are relatively fragile cells because of the inherent stresses they undergo, primarily as a result of their production of highly toxic intermediates during melanogenesis, yet they typically have extremely long biologic lives and seldom proliferate in adult tissues outside of the hair follicle. In fact, stimulation of their proliferation is a key to their malignant transformation, as discussed in the “*Melanomagenesis*” section later. Melanoblasts and melanocytes typically express AP2, BCL2, and survivin (BIRC5) (Brauer et al. 2011; McGill et al. 2002; Nishimura et al. 2005; Raj et al. 2008), all of which are antiapoptotic, and thus prevent melanocytic cells from succumbing to the cytotoxicity of their microenvironment.

Three new sets of ligands and receptors come into play at this point. HGF and its receptor MET also work via the MAP kinase pathway (with MITF contributing as a downstream effector) to influence melanoblast survival and proliferation (Hirobe et al. 2004; Kos et al. 1999). The FGF receptor and its ligand FGF2 also work through the MAP kinase pathway to regulate proliferation and survival (Beauvais-Jouneau et al. 1999; Ito et al. 1999). Similarly, DKK1, an inhibitor of WNT signaling, also participates in regulating the survival and proliferation of melanoblasts (and later, their differentiation) (Lin and Fisher 2007; Yamaguchi et al. 2007; Yamaguchi et al. 2008).

Differentiation of Melanocytes

Regulation of Differentiation

Human melanocytes reside at the dermal-epidermal junction of the skin and are interspersed among basal keratinocytes on the basement membrane. Differentiated melanocytes produce specialized lysosome-related organelles, termed melanosomes, which contain the enzymatic and structural components required for the synthesis of melanins (Dell’Angela 2003; Park et al. 2009;

Schiaffino 2010). Melanocytes then distribute those melanosomes to the overlying suprabasal keratinocytes via their elaborate network of dendrites and cell-cell contacts (see Fig. 2). Melanocytes and keratinocytes in the basal layer of the epidermis are highly stable cell populations that proliferate extremely slowly under normal circumstances. In contrast, keratinocytes in the upper layers of the epidermis proliferate relatively rapidly and differentiate further into highly keratinized layers that eventually form the stratum corneum at the surface of the skin. Their upward proliferation carries them toward the surface of the skin along with their ingested melanin where they form a critical barrier against the environment (including ultraviolet radiation [UVR]). In humans, keratinocytes move from the basal layer to the surface of the skin and are lost by desquamation in approximately 4–5 weeks, so it is a relatively rapid process. Because of that, transformation events in proliferating keratinocytes are not thought to pose a long-term risk for skin cancers, but rather it is the transformation of melanocytes or keratinocytes in the basal layer that have long-term consequences concerning skin cancer.

Although the various types of melanins produced by melanocytes (eumelanins and/or pheomelanins) are important for the eventual characteristic color of the skin, it is also the distribution of those melanins in the more superficial layers of the skin that has major effects. Although melanocytes in lower species often produce only one type of melanin at a given time, in humans different mixtures of eumelanins and pheomelanins are typically produced on a constitutive basis (Ito and IFPCS 2003; Micillo et al. 2016). Melanocytes in other tissues (e.g., hair follicles, eyes, and inner ears) interact with their surrounding cells in distinct manners, yet the basic processes of melanin production and melanosome biogenesis are comparable, as are some of the factors that regulate melanogenesis. Factors that influence pigmentation at sites other than the skin are covered in several reviews (Bharti et al. 2006; Hubbard et al. 2010; Lavado and Montoliu 2006; Slominski et al. 2005; Sturm and Larsson 2009; Tobin 2008) and books (Hearing and Leong 2006; Nordlund 2006).

As noted earlier, 650 distinct loci are currently known to be involved in regulating pigmentation either directly or indirectly (Baxter et al. 2018). Many of those loci/genes affect developmental processes, but others regulate either differentiation/survival of melanocytes once *in situ* or regulate physiological factors that affect pigmentation. The remainder of this section will provide an overview of the latter two categories. Many of the pigment regulatory genes affect the biogenesis and/or function of melanosomes, the discrete membrane-bound organelles within which melanins are synthesized. Melanosomes, which are closely related to lysosomes, are within the family of lysosome-related organelles (LROs) and require a number of specific enzymatic and structural proteins to mature and become competent to produce melanin (Chi et al. 2006; Hu et al. 2007; Yamaguchi et al. 2007). Several reviews have elaborated on processes involved in melanosome biogenesis and on the specific functions of melanosomal proteins (Barral and Seabra 2004; Schiaffino 2010). Briefly, the critical enzymes include tyrosinase (TYR), TYR-related protein 1 (TYRP1), and dopachrome tautomerase (DCT), and mutations in their encoding genes dramatically affect the quantity and quality of melanins synthesized. Important structural proteins of melanosomes include PMEL (also known as gp100) and MLANA (MART-1), both of which are required for the structural maturation of melanosomes (Berson et al. 2001; Hoashi et al. 2005). Interestingly, most (perhaps all) of those melanosomal proteins have been identified as targets of the immune system in patients with melanoma (Kawakami et al. 2000; Sakai et al. 1997). Because melanosomes are LROs, there are many proteins involved in the sorting and trafficking of proteins to melanosomes, some of which affect all LROs (such as AP3 and BLOC components), whereas others (such as OCA2 and SLC45A2) affect only melanosomes, and mutations in any of those typically lead to inherited hypopigmentary disorders (Costin and Hearing 2007; Costin et al. 2003; Nordlund 2006; Toyofuku et al. 2002). As melanosomes mature and their constituent proteins are delivered, they become cargos themselves, carried by various molecular motors

(actin-based and myosin-based) from the perinuclear area to the cell periphery (Coudrier 2007; Wu and Hammer 2000), after which they are transferred to neighboring keratinocytes (Scott et al. 2003; Wu and Hammer 2014). Several critical components are involved in linking melanosomes to molecular motors responsible for their transport (e.g., RAB27A, melanophilin, and myosin Va), and mutations in those genes result in a pigmentary disease known as the Griscelli syndrome.

Constitutive skin pigmentation Human skin color ranges from extremely fair and light to extremely dark, depending on racial or ethnic background, among other factors, but the density of melanocytes in a given geographic area of the body is virtually identical in all skin types (Tadokoro et al. 2003; Yamaguchi et al. 2004). Keratinocytes in lighter skin tend to cluster less-pigmented melanosomes above the nuclei (called *capping*), while in darker skin, greater numbers of pigmented melanosomes are distributed individually in keratinocytes, thus maximizing their absorption of light and their photoprotective value. Constitutive melanocyte density in the skin can be affected by the environment, such as by chronic UV radiation (which can increase melanocyte density threefold or fourfold), by secreted paracrine factors in the skin (Hirobe 2011; Scott et al. 2002) (see Fig. 2), and by toxic compounds such as hydroquinone (which antagonizes the melanogenesis enzyme tyrosinase but can also permanently destroy melanocytes in the skin.) Several types of inherited pigmentary disorders also affect melanocyte density (e.g., increased numbers in freckles or decreased numbers in vitiligo (Thong et al. 2003; Whiteman et al. 1999; Yoshida et al. 2007).)

Epidermal melanocytes proliferate slowly, if at all, under normal circumstances, and they are quite resistant to apoptosis because of their expression of BCL2 and survivin (BIRC5) (McGill et al. 2002; Raj et al. 2008). Melanocyte density and differentiation are influenced by the environment, including UVR and factors secreted by neighboring keratinocytes and fibroblasts (see Fig. 2). For example, fibroblasts in the dermis of the palms/soles secrete high levels of DKK1,

which suppresses melanocyte growth and function by inhibiting the WNT/ β -catenin signaling pathway (Yamaguchi et al. 2004; Yamaguchi et al. 2008). DKK1 inhibition of WNT signaling in melanocytes dramatically inhibits the melanogenic pathway, ranging from effects on transcription factors (e.g., MITF, PAX3, SOX9, and SOX10) to downstream melanogenic proteins. DKK1 also affects keratinocytes in overlying epidermis, reducing their uptake of melanin and inducing a thicker less-pigmented skin phenotype (Yamaguchi et al. 2008).

One major determinant of pigment phenotype of the skin is the melanocortin 1 receptor (MC1R), a G protein-coupled receptor which regulates virtually all functional aspects of melanocytes, including dendricity, melanogenesis, and proliferation, among other things (Herraiz et al. 2017; Wolf Horrell et al. 2016). MC1R function (and dysfunction) has critical implications for the risk for skin cancer and is considered in detail in the “Melanomagenesis” section. MC1R function is regulated by peptide agonists encoded by the *POMC* gene (α MSH and ACTH) and by an antagonist, agouti signaling protein (ASIP). Activation of MC1R stimulates the melanogenic cascade leading to the synthesis of eumelanin, while ASIP can reverse those effects and favor the production of pheomelanin. α MSH and ACTH also upregulate expression of the *MC1R* gene, thus acting in a positive feedback loop. Although MC1R is closely involved with regulating skin pigmentation, it is not the only melanocytic receptor that functions in pigmentation. In fact, virtually all of the receptor/ligand pairs discussed above that participate in developmental processes also regulate mammalian pigmentation in adult tissues, including KITLG:KIT, CSF2:CSF2RA, HGF:MET, FGF2:FGFR, and EDN:EDNRB. Those ligands can originate from neighboring cells in the epidermis (keratinocytes) and the dermis (fibroblasts) and even from more distant locations, induced by environmental factors (e.g., UVR). As seen in the schematic outline in Fig. 2, virtually all known intracellular signaling pathways are involved in responses to these factors: receptors, the common effect of many of them being to regulate the function of MITF.

In addition to regulation by a wide variety of receptor:ligands, melanin production is also modulated by multiple other factors within melanocytes. The SLC45A2 and OCA2 proteins are melanocyte-specific proteins with 12 transmembrane domains that play critical roles in the sorting/trafficking of TYR to melanosomes. If TYR is not successfully delivered to melanosomes, no melanin will be produced, even if the TYR is a functional enzyme (TYR can be abnormally degraded by proteasomes or secreted from melanocytes). SLC45A2 is an intracellular pump/transporter that regulates ion transport across intracellular membranes which regulate the sorting of TYR and thus the biosynthesis of melanin. OCA2 acts on multiple levels to influence the melanosome, the melanogenic enzymes, pH, and glutathione metabolism (Ainger et al. 2017). Mutations in any of these proteins result in the abnormal secretion of TYR from melanocytes rather than normal sorting of TYR to melanosomes. Population studies have shown that polymorphisms of the *SLC45A2* or *OCA2* genes (together with polymorphisms of the *MC1R* gene) play major roles in determining the normal range of pigmentation in the hair, skin, and eyes (Ainger et al. 2017; Graf et al. 2005; Shriver et al. 2003). A close association between a polymorphism in a sodium:calcium exchanger (SLC24A5) and melanin content of the skin has also been described (Ginger et al. 2008; Lamason et al. 2005).

Facultative skin pigmentation Facultative skin pigmentation is the term coined for increased skin color due to some type of physiologic regulation, the most obvious being UVR in what is commonly termed the tanning reaction (Eller and Gilchrist 2000; Tadokoro et al. 2005). Several studies have detailed the complex kinetics of responses of the skin to UVR, which result in tanning over the course of several weeks (Chen et al. 2014; Coelho et al. 2009). UVR is the most significant factor that influences human skin pigmentation and elicits several stages of increased skin color. Immediate pigment darkening occurs within minutes and persists for several hours, followed by persistent pigment darkening, which occurs within several hours and lasts for several

days (Young 2006). These rapid increases in skin color do not result from increased melanin synthesis but rather are due to the oxidation and polymerization of existing melanin and the redistribution of existing melanosomes. A slower process, termed *delayed tanning*, occurs several days after UVR exposure but requires more time since it involves the activation of melanocyte function and depends on increased melanin production. As might be expected, UVR induces skin pigmentation due to its effects on melanocytes and also via indirect effects on keratinocytes. Regarding effects on melanocytes, ultimately UVR elicits increased expression of MITF and the downstream melanogenic cascade (Miyamura et al. 2007; Yamaguchi and Hearing 2006).

Keratinocytes in the skin respond to UVR by increasing their secretion of α MSH and ACTH as a result of the stimulation of POMC expression, which was shown to result from increased TP53 expression (Cui et al. 2007). Interleukin-1 (IL1) secretion by keratinocytes is also elicited by UVR and stimulates the autocrine secretion of ACTH, α MSH, EDN1, and FGF2 (Hirobe and Ootaka 2007). Each of those factors functions via receptors expressed on melanocytes, as discussed earlier. UVR can also affect fibroblasts in the dermis, and growth factors secreted from those cells in response to UVR include HGF, FGF2, and KITLG, each of which stimulates pigmentation via distinct receptors expressed by melanocytes (Imokawa 2004). The tanning response also relies on stimulation of secretion of nerve growth factor (NGF) by keratinocytes, which prevents apoptosis following UVR exposure (Botchkarev et al. 2006; Kadokaro et al. 2003). In humans, the red-hair/light-skinned phenotype is associated with an inability to tan after UVR exposure. The gene most commonly implicated in this phenotype is *MC1R*. The so-called “red-hair” alleles of *POMC* are notable for their inability to signal to adenylate-cyclase upon exposure to the ligand (α MSH). These *MC1R* variants thus provide genetic evidence of the rate-limiting role for *MC1R* signaling in the UVR-induced tanning pathway. Correspondingly, it has been demonstrated in animal models of red hair (carrying *MC1R* loss-of-function) that topical

administration of cAMP-inducing compounds can rescue eumelanin (dark) skin pigmentation (D’Orazio et al. 2006).

In sum, skin pigmentation is determined at multiple levels, by (1) the migration of melanoblasts to various tissues during development, (2) the melanoblast survival and differentiation to melanocytes, (3) the density of melanocytes, (4) the expression/function of enzymatic and structural constituents of melanosomes, (5) the synthesis of different types of melanin (eumelanin and pheomelanin), (6) the transport of melanosomes to dendrites, (7) the transfer of melanosomes to keratinocytes, and (8) the distribution of melanin in suprabasal layers of the skin. Skin pigmentation plays a critical role in protecting the underlying skin from UVR damage that can ultimately result in various types of skin cancers, including melanoma.

Regulation of Survival

Survival is not only a critical issue for developing melanoblasts but also for melanocytes in the skin and in hair bulbs. As noted above, this is especially important to melanocytes because of their extremely low rates of proliferation, combined with the high stresses imposed by their environment and also by the potentially toxic by-products of melanin synthesis within them. In this regard, the functions of antiapoptotic proteins, such as BCL2 and survivin, are essential for melanocyte survival, as are transcription factors involved in regulating their expression (e.g., MITF and PAX3) and upstream signaling events (e.g., mediated by the actions of KITLG:KIT, FGF2:FGFR, HGF:MET, and α MSH:MC1R). Dysfunctions of many of those genes/proteins have been associated with premature hypopigmentation of the skin and hair graying, as discussed and cited previously.

Melanocyte stem cells A closely related issue is the presence and maintenance of melanocyte stem cells, i.e., cells that retain the potential to replenish melanocytes in the skin and hair as needed yet remain undifferentiated. This population has potential significance regarding the

process of melanomagenesis, as discussed later in this chapter. Melanocyte stem cells typically reside in what is termed the bulge region, a hair follicle stem cell niche located at the insertion site of the arrector pili muscle (which produces a small “bulge”) along each hair shaft. During the hair growth cycle, active melanocytes are normally present exclusively in the hair bulb and function by synthesizing melanin granules and transferring them to keratinocytes within the bulb, which incorporates the melanin into the growing keratin-containing hair matrix. Hair follicles undergo cyclic patterns of growth, involution, and rest. The growth phase (anagen) has a finite lifetime (measured in years in adult humans), which is followed by the catagen stage, at which time all active keratinocytes and melanocytes within the hair bulb die. New hair bulbs require a fresh supply of functional melanocytes; else the hair will be partially or totally unpigmented. That supply of active melanocytes comes from a pool of melanocyte stem cells that remain localized in the bulge region throughout the follicle stages. Telogen is the resting phase that follows the catagen (involution) phase. During telogen, melanocyte stem cells are in a largely undifferentiated state, but they do not express significant levels of TYR and other proteins required to produce melanin; hence they are unpigmented in the hair bulge region. Although there is some argument about the exact patterns of marker expression, the melanocyte stem cells do express a number of markers that identify them. Typically they are positive for PAX3 and SOX10 and weakly positive for MITF and DCT (which is likely expressed due to PAX3/SOX10 activation); they are negative for other melanosomal proteins (enzymatic and structural) such as TYR, TYRP1, and PMEL (Buac and Pavan 2007; Lang et al. 2005; Nishikawa and Osawa 2007; Nishimura et al. 2005; Osawa et al. 2005). They retain expression of surface receptors such as KIT, MET, and EDNRB, which allows them to respond to environmental cues and start the differentiation/proliferation phase (anagen) to become functional melanocytes that incorporate into the newly formed hair bulb. Although these melanocyte stem cells are typically thought of as reservoirs required to repopulate melanocytes in

hair bulbs, there is evidence to suggest that they also can be recruited to replace melanocytes at the dermal-epidermal border. The self-renewing capacity of bulge melanocyte stem cells is thought to be imperfect as depletion of these hair follicle melanocyte stem cells has been seen to correlate with age-dependent hair graying in both mice and humans (Nishimura et al. 2005).

Melanomagenesis

From Melanocyte to Melanoma: A Multistep Process

Over 20 years ago, Clark et al. (1984) devised a model for the stepwise development of melanoma from normal melanocytes based on both clinical and histopathologic features. According to this model, melanoma progression includes the following stages: (1) common acquired (benign) and congenital nevi consisting of nests of cytologically normal melanocytes, thought to exist in a senescent state; (2) dysplastic nevi characterized by structurally and architecturally atypical features and posing a risk factor for melanoma (Goldstein and Tucker 2013); (3) radial growth phase (RGP) or microinvasive malignant melanoma characterized by intraepidermal proliferation and “pagetoid spreading”; (4) vertical growth phase (VGP) or invasive malignant melanoma associated with the ability to penetrate through the basement membrane into the underlying dermis and metastasize and with a significantly poorer prognosis; and (5) metastatic melanoma, which has spread to other areas of the skin and other more distant sites. However, this sequence of progression can be histologically documented in only about a third of all melanoma cases, and malignant melanoma may also be able to arise in the absence of visually obvious pathologic progenitor lesions.

How does this aggressive, often fatal disease arise from melanocytes? What goes wrong? Based originally on extensive cytogenetic analyses of a variety of melanocytic lesions, and later confirmed by high-resolution genomic analyses (e.g., array-comparative genomic

hybridization [CGH]), the degree of detectable genetic alterations increases rather dramatically as it progresses from nevus to primary melanoma to metastatic melanoma (Chin et al. 2006). There are few consistent changes associated with each of these stages, but years of such analyses have identified a number of genes and pathways that have proved critical to melanoma development and provided important hints about the transformation process (Shain et al. 2015). While numerous reports demonstrate gene expression changes in melanoma (Riker et al. 2008), we begin by examining some of the major early insights that came from extensive classic cytogenetic studies and array-CGH-based genome-wide analyses of non-random chromosomal alterations in melanomas (Bauer and Bastian 2006; Jonsson et al. 2007; Stark and Hayward 2007).

Molecular Genetics: Early Lessons from Familial and Sporadic Melanoma

The *CDKN2A* locus It has long been recognized that melanoma susceptibility can be inherited, as in familial atypical multiple mole melanoma (FAMMM) syndrome (Soura et al. 2016). Subsequent genetic analysis of large melanoma-prone families led to the identification of the melanoma susceptibility locus *CDKN2A* at chromosome band 9p21 (Aoude et al. 2015; Chin et al. 2006; Kamb et al. 1994). The *CDKN2A* locus shows loss of heterozygosity (LOH) and loss of function mutations in 25–40% of melanoma kindreds and less frequently in sporadic melanoma. Subsequent experimental studies using mice genetically engineered to carry inactivated *CDKN2A* have corroborated the importance of this locus in cutaneous malignant melanoma (Perez-Guijarro et al. 2017). The *CDKN2A* locus is quite unique, encoding two distinct proteins within overlapping DNA sequences: p16INK4A and p14ARF (p19Arf in mice). This is accomplished through the initiation of transcription from two distinct promoters upstream of two distinct first exons (α for *p16INK4A* and 1β for *p14ARF*). Thus, the genes encoding p16INK4A and p14ARF share common second and third exons, translated in

different reading frames. Although genetic deletions in this region typically inactivate both *p16INK4A* and *p14ARF* in melanoma, many cases have been documented in which expression of only one is affected, suggesting that each has a key role in melanomagenesis (Chin et al. 2006). Remarkably, both function as tumor suppressor genes, with p16INK4A functioning as a cyclin-dependent kinase inhibitor (CKI) to help regulate transit through the cell cycle and p14ARF stabilizing TP53 by binding and inhibiting the TP53 E3 ubiquitin ligase, HDM2 (Zhang et al. 1998).

p16INK4A binds to cyclin-dependent kinases (CDK) 4 and 6, preventing its association with D-type cyclins and inhibiting its ability to phosphorylate and inactivate RB, which in turn initiates S phase associated DNA synthesis (Fig. 3). This is a critical pathway in melanomagenesis, as evidenced by the fact that in melanoma p16INK4A expression can be suppressed by mutation, methylation, and/or polymorphic variation at both the 5' and 3' untranslated regions (Chin et al. 2006). Moreover, germline mutations in *CDK4* have been documented in melanoma patients that prevent binding of CDK4 to p16INK4A, as have amplifications in *CCND1* (encoding Cyclin D1) and occasional mutations in *RBI* (Tsao et al. 2012). Notably, the various *p16INK4A/CDK4/RB* mutations were largely found to be mutually exclusive and can be considered redundant.

p14ARF acts as a positive regulator of TP53. It targets the degradation of HDM2 (Mdm2 in mice), which destabilizes TP53. TP53 is a sequence-specific transcription factor that triggers either cell cycle arrest or programmed cell death (apoptosis) on DNA damage or other stress (Liebermann et al. 2007), depending on the cell type and the circumstances (see Fig. 3). *TP53* is the most frequently mutated gene in human cancer but is a relatively infrequent target in melanoma, estimated to be mutated in fewer than 10% of melanomas (Bennett 2008; Hussein et al. 2003b; Petitjean et al. 2007). As mentioned above, p14ARF is frequently inactivated in melanoma, and *HDM2* amplification has been documented as well. Genetically engineered mouse models (GEMMs) of human melanoma

analysis, suggesting its contribution to melanomagenesis in at least a subset of cases (Murali et al. 2013).

The PTEN-AKT pathway *PTEN* is localized on chromosome band 10q24, a region long associated with deletions and LOH in melanoma (Chin et al. 2006). *PTEN* is a dual phosphatase, containing both lipid phosphatase and protein phosphatase activities (Lee et al. 2018). Extracellular growth signals are often mediated through the intracellular second messenger lipid phosphatidylinositol-3,4,5-triphosphate (PIP_3), levels of which increase upon signaling through PBK, resulting in the phosphorylation/activation of AKT (also known as protein kinase B) (see Fig. 3). The *PTEN* lipid phosphatase antagonizes PI3K by dephosphorylating PIP_3 and therefore negatively regulates AKT activity, helping to control both cell proliferation and survival. *PTEN* deletions or inactivating mutations can be found in approximately 28% of melanoma cell lines, 7% of primary melanomas, and 15% of metastatic melanoma (Aguissa-Toure and Li 2012), causing sustained phosphorylation/activation of AKT; moreover, *AKT3* overexpression has been reported to be associated with an increased DNA copy number in some melanomas (Robertson 2005). Despite intense study, the regulation and complex roles of *PTEN* are still incompletely understood. For example, *PTEN* has been found to be secreted into the extracellular space to be taken up by recipient cells, intriguingly suggesting a function as a cell nonautonomous tumor suppressor (Hopkins et al. 2013; Putz et al. 2012).

The WNT/ β -catenin pathway β -catenin, encoded by the gene *CTNNB1*, is a key regulator of the WNT signaling pathway, well-documented for its involvement in the progression of many cancer types (Kaur et al. 2016; Zhan et al. 2017) and also in the physiologic differentiation of melanocytes from neural crest progenitors. In the absence of WNT signaling, β -catenin is targeted for degradation in association with a complex that includes adenomatous polyposis coli (*APC*) and glycogen synthase kinase 3 β (*GSK3 β*) (see Fig. 3). Upon WNT signaling, β -catenin is stabilized, accumulates, and is transported to the nucleus

where it binds to LEF1 and acts as a transcriptional coactivator of LEF1 target genes. Downstream targets include the proto-oncogenes *MYC* and *CCND1* and the gene encoding MITF (Zhan et al. 2017). Mutations have been detected in *CTNNB1* that confer a resistance to β -catenin degradation in 2–23% of melanoma tissues and cell lines (Dahl and Guldborg 2007; Larue and Delmas 2006), with a higher frequency *in vitro*. Although infrequently mutated in melanoma, *APC* is more frequently characterized by decreased expression. β -catenin has also been shown to repress *p16INK4A* transcription (Delmas et al. 2007).

NRAS and BRAF In 2002 Davies et al. (2002) published a remarkable paper in which a genome-wide cancer sequencing program was used to discover that *BRAF* was mutated in 66% of melanoma samples, a finding that had significant implications for the treatment of melanoma. This finding was later confirmed and extended to melanocytic nevi, where approximately 80% harbored mutant *BRAF*, including benign nevi (Pollock et al. 2003). Oncogenic *BRAF* mutations in melanoma appear to be somatic, as germline mutations are not associated with enhanced cancer risk (Niihori et al. 2006; Rodriguez-Viciano et al. 2006). The most common *BRAF* mutation (V600E, found in more than 90% of melanoma mutations) introduces a conformational alteration in the *BRAF* activation domain, causing constitutive kinase activation (Wan et al. 2004). *BRAF*, a member of the *RAF* family of genes (*ARAF*, *BRAF*, *CRAF*), is a serine-/threonine-specific protein kinase that activates sequentially MEK1/2 and then ERK1/2 (Garnett and Marais 2004). *RAF* is a downstream target of the *RAS* family of small guanine-nucleotide-binding proteins (*NRAS*, *HRAS*, *KRAS*). It was therefore not surprising to find that a *RAS*, almost always *NRAS*, is mutated in 15–25% of melanoma cell lines and tumors but rarely in melanomas harboring mutations in *BRAF* (Bennett 2008; Davies et al. 2002; Jonsson et al. 2007). These results strongly endorse the importance of the *NRAS*-*BRAF*-*MEK*-*ERK* (*MAP* kinase) pathway in melanomagenesis (see Fig. 3). It has been suggested that cancers may become overly dependent on, or “addicted” to, one or a few specific

genes for maintenance of the malignant phenotype and survival (Weinstein and Joe 2008); in melanoma this may apply to oncogenic members of this pathway. In vivo mouse experiments have confirmed the key role of this pathway in melanoma, as well as the ability of members of this pathway to collaborate with loss of the *CDKN2A* locus in provoking melanoma development (Ackermann et al. 2005; Burd et al. 2014; Chin et al. 1997; Damsky et al. 2015; Dhomen et al. 2009; Goel et al. 2009; Kwong et al. 2012; Perez-Guijarro et al. 2017; Sharpless et al. 2003).

Receptor tyrosine kinases (RTKs) RTKs play crucial roles in regulating virtually all basic cell processes under normal physiological conditions and have been implicated in the development of most cancers. The exact phenotypic response to RTK signaling is extremely complex, depending on the RTK and its signal, the cell type, and the circumstances under which it is stimulated. In melanoma, examples of DNA copy number gains and point mutations have been documented that involve several RTKs. For example, increased copies of regions of chromosome 7, which encodes the epidermal growth factor receptor (EGFR) at 7p12, have been detected in late-stage melanomas, along with enhanced EGFR expression (Chin et al. 2006). The EGFR is an intriguing candidate because melanomas develop in *Xiphophorus* fish in association with aberrant expression of activated *Xmrk*, an EGFR-related gene. Another region of chromosome 7 (7q33-qter) that exhibits increased copy number in late-stage melanoma harbors the *MET* gene, encoding the HGF receptor; moreover, increased MET expression has been detected in metastatic melanoma (Natali et al. 1993). Activating point mutations in EGFR or MET have not been detected to date in melanomas. However, a recurrent L576P mutation in KIT has been reported for a small number of melanomas, with amplification and/or selective loss of the normal allele (Curtin et al. 2006; Willmore-Payne et al. 2005). Interestingly, melanomas with *KIT* mutations do not carry *BRAF* mutations and arise only from chronically sun-damaged skin (see the “[Melanoma and the Environment](#)” section). L576P is a GIST-associated, activating mutation, and as such

represents a promising target for the RTK inhibitor imatinib in this subset of melanoma patients. However, clinical trials of the RTK inhibitors imatinib and sunitinib for KIT-mutated melanomas showed only modest responses (Buchbinder et al. 2015; Carvajal et al. 2011; Guo et al. 2011; Hodi et al. 2013). Some enzymes with appropriately targeted phosphatase activity counter the RTKs and may act as tumor suppressors. For example, the gene encoding protein tyrosine phosphatase receptor type D (*PTPRD*) was found to be homozygously deleted in 9% of melanoma cell lines (Stark and Hayward 2007). Moreover, upregulation of RTKs, e.g., PDGFR and IGF-1R, has been shown to play a key role in determining resistance of melanoma to BRAF inhibitor drugs (Nazarian et al. 2010; Villanueva et al. 2011).

Transcription factors High-density single nucleotide polymorphism (SNP) arrays were used to detect recurrent focal amplifications of the transcription factor *MITF* in 10% of primary melanomas and up to 20% of metastatic melanomas (Garraway et al. 2005). A recurrent activating mutation in *MITF* has also been identified in the germline of kindred from multiple continents with familial melanoma (Yokoyama et al. 2011). The recurrent mutation stimulates *MITF*'s transcriptional activity by disrupting a SUMO-modification consensus sequence that otherwise suppresses *MITF* function. As discussed earlier, *MITF* is a melanocyte lineage-survival factor required for the commitment of immature cells to the melanocyte lineage during development and heavily involved in melanocyte survival, growth, and differentiation (Kawakami and Fisher 2017) (see Fig. 3). Melanomas resistant to BRAF inhibitors have been shown to overexpress *MITF*, and knocking down *MITF* expression reversed this resistance (Smith et al. 2013). The reliance of melanocytes on *MITF* makes it an intriguing candidate target for melanoma patients (Merlino 2005). However, akin to most transcription factors, the lack of a catalytic domain in *MITF* protein makes it a problematic therapeutic target. Consequently, a number of strategies are being employed to inhibit *MITF* indirectly; for example, inhibitors of HDAC, AMP-activated kinase

(AMPK), and HIV1-protease have been shown to suppress MITF expression (Borgdorff et al. 2014; Smith et al. 2016; Yokoyama et al. 2008). A number of additional transcription factors have been implicated in melanoma progression (Poser and Bosserhoff 2004). The transcription factor TBX2, which can repress expression of p14ARF and p21CIP1 (product of *CDKN1A* gene), exhibits an increased gene copy number and over-expression in melanoma (Jacobs et al. 2000; Jonsson et al. 2007; Vance et al. 2005). Gene copy number increases up to 40% have also been reported in melanomas for the transcription factor MYC (Bennett 2008), but conflicting reports (Stark and Hayward 2007) have indicated a complex and yet unresolved role of MYC in melanoma. Recently, MYC overexpression in melanoma has been suggested to be correlated with accelerated tumor metastasis *in vivo* (Lin et al. 2017). The factors TP53 and β -catenin have already been discussed above.

The pro-metastasis gene *NEDD9* Over the years many genes have been implicated in melanoma metastasis (Chin et al. 2006; Crowson et al. 2007; Qiu et al. 2015; Xu et al. 2008). Proceeding on the assumption that overlapping genomic changes in human and mouse melanomas arise through shared selective pressure and represent critical events in melanomagenesis, Kim et al. used genome-wide high-resolution array-CGH to identify the scaffold protein NEDD9 as a pro-metastasis factor (Kim et al. 2006). Genetically engineered mouse models of human melanoma were employed to narrow down candidates in an amplified syntenic region in the human genome to *NEDD9*. Loss-of-function and gain-of-function experiments were employed to confirm that NEDD9 confers enhanced invasiveness and metastatic potential to melanocytic cells. NEDD9 was found to facilitate metastasis through interaction with focal adhesion kinase (Kim et al. 2006).

Melanoma: A Consequence of Homeostatic Disruption

No cell is an island, and melanocytic cells live in dynamic and harmonic equilibrium with

their microenvironment. An intricate network of interactions ensures a state of homeostasis both during development and under normal adult physiological conditions. As discussed earlier, the melanocyte microenvironment includes cellular neighbors such as keratinocytes, fibroblasts, endothelial and immune cells, as well as components of a complex ECM. In particular, melanocytes and keratinocytes (the so-called melanin unit) are intimately associated with each other in the basal layer of the epidermis of the human skin. Each melanocyte makes contact with, and sends pigment-containing melanosomes to, dozens of keratinocytes, a process that can be dramatically altered by exposure to UVR. Keratinocytes are thought to employ the pigment as protection from subsequent UVR damage. Keratinocytes regulate melanocyte growth and behavior through the activity of cell-cell adhesion molecules and paracrine growth factors (Haass et al. 2005). The initiation and progression of melanoma marks a significant disruption in this tranquil homeostasis, involving key changes within the melanocyte genome and epigenome, and in the way these melanocytes grow, survive, and interact with their microenvironment (Haass and Herlyn 2005). Moreover, it is clear that exposure to UVR, known to initiate melanomagenesis, is a powerful homeostatic disruptor.

Dysregulation of melanocyte gene expression Gene expression profiling using multiple technologies has permitted the simultaneous analysis of the entire melanoma genome. Studies have identified multiple significant changes at the level of gene expression as melanocytes are transformed into melanoma cells and further into metastatic melanomas (Haqq et al. 2005; Hoek et al. 2004; Hoek 2007; Kaufmann et al. 2008; Talantov et al. 2005). Moreover, exposure to UVR has been shown to induce significant alterations in gene expression patterns of melanocytes (Yang et al. 2006; Zaidi et al. 2011).

Several mechanisms undoubtedly contribute to the observed global changes in expression. First, the expression or activity of many important transcription factors can be altered in melanoma, including MITF, LEF1/ β -catenin, TBX2, MYC, PAX3, SOX10, and SLUG (Poser

and Bosserhoff 2004) (see Table 1). Another interesting transcriptional regulator, inhibitor of differentiation (ID1), has been shown to repress *p16INK4A* transcription and delay melanocyte senescence (Cummings et al. 2008; Polsky et al. 2001) and may play an early role in melanomagenesis. These transacting factors not only help regulate the expression of a plethora of critical downstream targets but can influence each other's expression/activity as well, further demonstrating the complexity of this regulatory network. In addition, the modification of chromatin is another level of gene regulation that plays a major role in the development of tumors, including melanoma (Moran et al. 2018). Epigenetic changes include DNA methylation and numerous covalent modifications of histones, including methylation, acetylation, phosphorylation, sumoylation, and ubiquitination. In melanoma, promoter methylation has been documented in suppressing the expression of genes such as *CDKN2A*, *PTEN*, *RASSF1A*, *MGMT*, *DAPK*, *RARB2*, and *APC*.

Finally, a relatively newly recognized category of powerful gene regulators is the microRNAs (miRNAs), which has been shown to participate in tumorigenesis, including metastasis (Di Leva et al. 2014). miRNAs are short molecules of RNA of about 22 nucleotides in length and are the most expressed class of noncoding RNAs in eukaryotic cells. Their main gene regulatory function is through silencing or degrading mRNAs, and a single miRNA can potentially regulate several target mRNAs simultaneously. Consequently, miRNAs can have oncogenic or tumor suppressor effects depending on the types of genes they regulate. miRNAs have been extensively studied in melanoma cells and clinical samples, and dysregulation of a relatively large number of melanoma-specific miRNAs has been identified, with regulatory effects on gene expression affecting a variety of pathways (Fattore et al. 2017). Next-generation sequencing studies have identified another category of regulatory RNAs called long noncoding RNAs (lncRNAs), which are defined as transcripts >200 nucleotides in length. Several lncRNAs have been characterized to play key roles in cellular proliferation, survival, migration, and genome stability, and their aberrant

expression is associated with many cancer types, including melanoma (Huarte 2015; Leucci et al. 2016). Together, genetic and epigenetic alterations can cooperate to disrupt normal homeostasis as melanocytes move along the path to malignancy.

Dysregulation of melanocyte proliferation/differentiation Adult melanocytes are built to manufacture pigment, to respond to environmental challenges through enhanced differentiation but controlled growth, and to survive. In contrast, melanoma cells proliferate autonomously and resist differentiation and growth inhibition signals. How does this happen? It is important to appreciate that these critical melanocytic functions are regulated more through highly interactive webs or networks and not simple linear pathways (Palmieri et al. 2015) (see Fig. 3). What sits at the center of this web is the melanocyte master regulator MITF (Kawakami and Fisher 2017; Palmieri et al. 2015). MITF can regulate virtually all known melanocyte-specific genes, including *TYR*, *DCT*, and *TYRP1*. MITF is absolutely required for melanocyte survival, partly by regulating expression of survival factors such as *BCL2*, and a number of MITF-mutant mouse strains have been described that are devoid of melanocytes. Both a positive and a negative influence, MITF is also required for melanocyte proliferation but depending on its level of expression or posttranslational modification can also be growth inhibitory (Kawakami and Fisher 2017). For example, MITF can transactivate stimulators of growth such as *CDK2* and *TBX2* but can also directly transactivate growth inhibitors such as *p16INK4A* and *p21CIP1* and indirectly activate the CDK inhibitor *p27KIP1* (product of *CDKN1B* gene). The expression or activity of MITF can in turn be regulated by numerous key melanocytic factors, including the transcription factors *PAX3*, *SOX9*, *SOX10*, *CREB*, and *LEF1*; the cofactors *CBP*, *RB1*, *p300*, *SWI/SNF*, and β -catenin; and the *MAPK* pathway through *EDN* and *EDNRB*. The network centering on MITF radiates out to myriad pathways regulating virtually every aspect of melanocyte function. Notably, *MITF* amplification or mutation can occur within melanomas, suggesting that under the appropriate

circumstances, *MITF* can function as a lineage-survival oncogene (Garraway and Sellers 2006). The ability of melanocytes to produce pigment is also heavily regulated through MC1R and its ligands, POMC-derived α MSH, and ACTH (Rouzaud et al. 2005). Polymorphic variations and mutations in MC1R in the general population determine the type of pigment that is produced (eumelanin or pheomelanin) and therefore the color of the skin (dark skinned or fair skinned). Notably, fair-skinned individuals have a greater risk for melanoma, linking MC1R to melanomagenesis. A significant association was detected between germline *MC1R* variants, *BRAF* mutations, and a subset of melanomas (Landi et al. 2006).

As in other types of cells, the key to controlling proliferation is through regulation of transit through the cell cycle. Entry into the cell cycle is normally promoted by growth factors, which in melanocytes include FGF2, HGF, KITLG, EDN3, IGF-1, and the WNTs; these factors can of course regulate other melanocyte behaviors as well, such as survival, differentiation, and migration (see Table 1). By triggering cascading signaling pathways leading to the activation of downstream cell cycle-associated transcription factors (e.g., MYC, FOS, JUN, and ATF2), these growth factors help overcome the G1-S cell cycle checkpoint. These factors stimulate CDK4/6-cyclin D phosphorylation/inhibition of RB and thereby the release of E2F to transactivate critical cell cycle control genes, such as those encoding the E and A cyclins, MYC, and CDK1. CKIs normally serve as key regulators of G1 cell cycle progression and include p16INK4A, p15INK4B, p21CIP1, and p27KIP1. An important part of the melanoma story is written in the deregulation of these pathways. We have already noted above that mutations or altered expression characterizes virtually every key cell cycle regulator. Moreover, melanoma cells often become growth factor-independent, constitutively initiating these important pathways through, for example, activating mutations in RTKs such as *KIT*. Another mechanism of deregulated growth frequently described in melanoma is through the creation of autocrine signaling loops, wherein melanoma cells express a

growth factor or cytokine (e.g., HGF, FGF2, TGF α , VEGF, MGSA/GRO, IL-8/CXCL8), as well as its cognate receptor (e.g., MET, FGFR1, EGFR, VEGFR1/2, CXCR1/2).

A critical intermediary between the initial phosphorylation event triggered by the binding of a growth factor to its receptor and the activation of downstream cell cycle-associated transcription factors is the MAPK pathway. *RAS* gene products are 21 kD GTPase proteins that serve as molecular switches converting the kinase activation events at the cell membrane to nuclear events and ultimately to altered melanocyte behavior (Simanshu et al. 2017). There are three different *RAS* genes, *NRAS*, *HRAS*, and *KRAS*. Despite their structural similarity, these three *RAS* genes have distinct functions. In fact, genetically engineered mouse models have confirmed that *NRAS* is significantly more effective than *HRAS* at transforming melanocytes *in vivo* (Perez-Guijarro et al. 2017). Based on the strong preference for *NRAS* mutations in melanomas, it has been shown that *NRAS* is the key regulatory G-protein in melanocytes (Burd et al. 2014). *RAS* activating point mutations cause impaired GTPase activity resulting in a constitutively activated G-protein and deregulated growth control. Similarly, the most common *BRAF* mutation in melanomas, V600E, is also forced into a state of perpetual activation. The obligatory nature of the deregulated MAPK pathway in melanomagenesis is evident from the high percentage of melanomas with either *NRAS* or *BRAF* mutations, which together range from 80% to 90%. Because mutant *BRAF* and *NRAS* can be detected in nevi (Damsky and Bosenberg 2017), it is assumed that these mutations are early events in melanomagenesis and are important in clonal expansion. Although the MAPK pathway is most central to melanocyte growth control, a second key *RAS* effector, PBK, also helps regulate proliferation. Although not as frequently mutated in melanomas, the PTEN-PBK-AKT pathway also plays a critical role in other melanocyte behaviors, including regulation of migration and apoptosis. A mouse model employing grafted human skin tissue containing melanocytes genetically engineered to express specific mutations has confirmed the importance of activated PBK

and NRAS, but notably not that of BRAF in melanomagenesis (Chudnovsky et al. 2005).

In an extreme example of growth control, cells can enter an irreversible state of growth arrest called senescence. This phenomenon was described in cultured cells many years ago and was initially argued to be an *in vitro* artifact; however, a number of impressive examples have been reported demonstrating the relevance of *in vivo* senescence in various types of cancers, including melanoma (Liu et al. 2018; Mooi and Peeper 2006). Early benign pigmented nevi are now thought to represent collections of mostly senescent melanocytes, and senescence is considered a significant barrier that melanoma cells must overcome to become proliferative melanomas (Bennett 2008). Cellular senescence can be induced through two distinct mechanisms. Replicative senescence occurs after extensive proliferation and is associated with telomere exhaustion. The shortening of telomeres is a natural by-product of replication in normal cells with limited telomerase, a heterodimer consisting of the protein TERT and the RNA TERC. After enough replication cycles, chromosome ends become so short that their protective cap structure is compromised, resulting in the activation of TP53-mediated DNA damage signaling, chromosome fusion, and mitotic disruption, termed crisis (Shay 2016). Cancer cells cannot therefore grow indefinitely without employing a mechanism to overcome telomere shortening, as they would become senescent or die. A mechanism that is often exploited by cancer cells, including melanoma cells, to gain the ability to grow indefinitely, or become immortalized, is reactivation of telomerase expression.

Senescence can also be induced by oncogene activation or an equally dramatic cellular stress (Ben-Porath and Weinberg 2005; Lowe et al. 2004; Mooi and Peeper 2006). Ironically, despite the fact that mutant BRAF and NRAS appear to be nearly obligatory for the development of human melanomas, these same activated oncogenes can induce a senescence response in melanocytes (Michaloglou et al. 2005). A number of factors have been implicated in overcoming early oncogene-induced senescence, most notably the

p16INK4A-CDK4/6-RB and ARF-HDM2-TP53 pathways, and the *CDKN2A* locus in particular. *p16INK4A* has long been considered a key melanocyte senescence gene; BRAF^{V600E} induces p16INK4A expression, and p16Ink4a deficiency in genetically engineered mice confers immortality to melanocytes and induces melanomagenesis (Liu and Sharpless 2012; Perez-Guijarro et al. 2017). Evidence in mice indicates that the other member encoded by the *CDKN2A* locus, p19ARF, is also very important in melanocyte senescence, highlighting the importance of the simultaneous loss of p16INK4A and p19ARF function when *CDKN2A* experiences deletions. Notably, p19ARF was found to induce senescence independently of TP53 in melanocytes (Ha et al. 2007). However, the view that human nevi undergo oncogene-induced senescence has been challenged in the light of the observations that the biomarkers widely used to define senescence are not exclusive to the senescence program (e.g., p16INK4A and SA- β -gal). p16INK4A was found to be abundantly expressed in both nevi and primary melanomas, and SA- β -gal, the most widely accepted senescence marker, was detected in both nevi and metastatic melanoma (Tran and Rizos 2013). These observations cast doubt on human nevi being defined as truly senescent (Tran and Rizos 2013).

Dysregulation of melanocyte survival The ability of cancer cells to overcome built-in programming designed to trigger cell death (apoptosis) in response to DNA damage, depletion of survival factors, or disruption of interactions with the microenvironment is a hallmark of successful progression (Hanahan and Weinberg 2011). Apoptosis is activated when a sensor, usually TP53, detects a death-inducing signal, which in turn transmits an execution order to the cell death machinery, often associated with mitochondria. Pro-apoptotic signals, including TP53, NOXA, PUMA, BAX, BAD, tumor necrosis factor (TNF), TNF-related apoptosis-inducing ligand (TRAIL/TNFSF10), and the Fas ligand (FASLG), induce the release of cytochrome c, which in concert with apoptotic protease-activating factor 1 (APAF1) activates caspase-9 and the resulting effector protease cascade (Lowe and Lin 2000).

To achieve balance, these pathways are countered by pro-survival factors, such as BCL2, BCL2L1, nuclear factor- κ B (NF κ B), survivin (BIRC5), and livin (BIRC7) (Hussein et al. 2003a). Melanocytes have evolved to survive the adverse environment created from the production of melanin and exposure to UVR. Therefore, it is not surprising that melanoma cells utilize several effective means to avoid apoptotic destruction (see Fig. 3) (Hussein et al. 2003a). Although *TP53* is infrequently mutated in melanomas, loss of its positive regulator, p14ARF, indirectly hampers the TP53-mediated DNA damage response. Melanoma cells characteristically produce elevated levels of BCL2, limiting cytochrome c-mediated caspase-9 activation. Caspase-9 activity is also inhibited through the influence of the kinase AKT, often highly active in melanomas. Advanced melanomas have been found to overexpress survivin, which binds to and inhibits effector caspase-3 and caspase-7, acting downstream of caspase-9 (Grossman et al. 1999). Similarly, livin is overexpressed in melanomas and can inactivate caspase-3, caspase-7, and caspase-9 (Kasof and Gomes 2001). Loss of APAF1 expression has been associated with melanoma cell survival and chemoresistance (Soengas et al. 2001).

Caspases are also triggered by activation of the death receptors, through binding of TNF, FASLG, and TRAIL (TNFSF10) (Ivanov et al. 2003). For example, TRAIL can induce apoptosis through interactions with the death receptors TRAIL-R1 (TNFRSF10A) and TRAIL-R2 (TNFRSF10B), which are expressed in most normal tissues as well as in melanomas; normal tissues survive by expressing the inhibitory decoy receptors TRAIL-R3/DcR1 and TRAIL-R4/DcR2 (Lowe and Lin 2000). However, these survival pathways are complex, and death receptor activation also triggers NF- κ B, which is frequently upregulated in melanoma and is an essential survival-promoting factor in melanoma. Countering the ability of melanoma cells to evade apoptotic destruction is an attractive therapeutic approach and is currently being actively pursued (Fulda 2015).

Dysregulation of cell-cell and cell-ECM interactions Melanocytic cells, particularly melanoblasts, are highly migratory by nature. It has

therefore been suggested that melanoma cells become metastatic by coopting and enhancing this innate migratory tendency (Gupta et al. 2005). In the initial phase of this cellular relocation, melanocytes must first sever their molecular ties with keratinocytes, ties consisting of cell surface molecules that promote interactions with keratinocytes (Haass et al. 2005). Alterations in expression of cell adhesion molecules such as cadherins, integrins, and immunoglobulin superfamily members can significantly disrupt the homeostatic balance between melanocytes and keratinocytes, facilitating melanoma progression. Cadherins are multifunctional transmembrane proteins that act as both receptor and ligand to maintain appropriate cell-cell contacts. The switch in expression from E-cadherin (encoded by *CDH1*) to N-cadherin (*CDH2*), first observed in RGP melanoma, redirects gap junction formation from E-cadherin-expressing keratinocytes, which inhibit melanocyte proliferation and maintain a differentiated morphology, to N-cadherin-expressing fibroblasts and vascular endothelial cells, encouraging migration from the epidermis to the dermis (McGary et al. 2002). N-cadherin also enhances melanoma survival by stimulating β -catenin signaling, LEF1 activity, and subsequent activation of *MITF* and *CCND1*. E-cadherin is downregulated in part by the transcription factor Snail (SNAIL) (Tsutsumida et al. 2004), whereas N-cadherin expression is upregulated by the Notch signaling pathway (Liu et al. 2006), already implicated in melanomagenesis (Nickoloff et al. 2005). Loss of E-cadherin is often accompanied by changes in other cell adhesion molecules, including integrins.

Integrins are heterodimeric cell surface receptors, consisting of 1 of 20 different α chains and 1 of 9 different β chains. The anchorage of melanocytes to the dermal-epidermal basement membrane can be weakened through changes in melanocytic integrins. Melanomas express specific integrins (e.g., α v β 3 and α 4 β 1) that have been implicated in adhesion to ECM, promoting motility, invasion, and metastasis, which correlates closely with poor clinical outcome in melanoma patients (Kuphal et al. 2005). For example, integrin α v β 3, the vitronectin receptor,

can facilitate melanoma cell growth, survival, and matrix invasion and marks the progression of RGP to VGP melanoma. Expression of integrin $\alpha\beta 3$ has also been shown to correlate with the activity of the matrix metalloproteinases (MMPs) MMP-1 and MMP-2 (Hofmann et al. 2000). Melanomas also overexpress immunoglobulin superfamily members, including MCAM (MUC-18/CD146), ICAM-1 (CD54), and ALCAM (CD166) (Crowson et al. 2007; McGary et al. 2002). MCAM is overexpressed in more than 80% of melanomas and mediates interactions between melanoma cells and both the matrix and other cell types such as endothelial cells. In a three-dimensional epidermal skin model, melanoma cells expressing MCAM exhibited enhanced separation from the epidermis and invasion through the basement membrane and into the dermis (Satyamoorthy et al. 2001).

Melanoma cell invasiveness can be accounted for in part by their ability to induce basement membrane breakdown and other forms of remodeling through secretion of proteolytic enzymes such as hyaluronidase, heparanase, and MMPs (Edward and MacKie 1993). Melanoma cells can also recruit surrounding fibroblasts to aid and abet invasion by encouraging secretion of their proteolytic enzymes. The degraded ECM in turn releases additional factors that can induce melanoma cell and endothelial cell growth, stimulating angiogenesis. The MMPs are broadly acting, zinc-dependent enzymes capable of functioning as collagenases, gelatinases, or stromelysins and are classified accordingly. MMP activity is carefully regulated, partly through their tissue inhibitors (TIMPs). Increased expression of a number of MMPs has been documented in invasive melanomas, including MMP-1, MMP-2, and MMP-9 and MT1-MMP (Crowson et al. 2007). One focal point in melanoma research has been MMP-2, which localizes to the melanomastromal interface in primary and metastatic lesions and correlates with progression. Of interest, MMP-2 is activated in association with a complex that includes MT1-MMP, TIMP-2, and integrin $\alpha\beta 3$ (Hofmann et al. 2000).

Melanoma: Cell of Origin

Pluripotent stem cells are defined by their abilities to indefinitely self-renew and to give rise to an organ-specific differentiated repertoire of cell types. These stem cells tend to be relatively quiescent and to express drug resistance and anti-apoptotic genes. Based mostly on data from hematologic malignancies and a few solid tumors, it has long been suggested that tumors harbor relatively rare cells with similar properties, called *cancer stem cells* or *tumor-initiating cells* (Batlle and Clevers 2017). Several critical questions followed upon acceptance of this hypothesis. One involved the cancer cell of origin: Do cancers arise from stem cells that have become malignant, or do cancers arise from more differentiated cell types, which then acquire the ability to self-renew? A second question concerned the ability of tumors to recur after drug treatment: Would cancer stem cells be intrinsically resistant to conventional therapeutic treatment? Adult melanocyte stem cells have been identified and reside within a specialized niche within the hair follicle called the *bulge* region (Nishimura 2011; Nishimura et al. 2005; Nishimura et al. 2002). During cyclic hair renewal, dormant bulge melanocyte stem cells are activated by specific signaling molecules and then give rise to transiently amplifying progeny, which proliferate, differentiate into pigment-producing melanocytes, and migrate to the base of the hair follicle. Transiently amplifying melanocytes can also migrate from the bulge region to the epidermis where they function as differentiated, pigmented melanocytes. Interestingly, a number of factors implicated in regulating melanocyte stem cell function – MITF, PAX3, NOTCH, and SLUG (SNAI2) – were also linked to melanomagenesis (Garraway and Sellers 2006; Gupta et al. 2005; Hoek et al. 2004; Nickoloff et al. 2005; Plummer et al. 2008), supporting the idea that a cancer stem cell was the melanoma cell of origin. Fang et al. (2005) cultured nonadherent spheroids from human melanoma cells or from metastases in an ES-like medium and identified a CD20-rich subpopulation that could be induced to differentiate into multiple phenotypes, consistent with stem

cell properties. Frank and colleagues (Frank et al. 2005; Schatton et al. 2008) identified a subpopulation of human melanoma cells expressing the chemoresistance mediator ABCB5, which was capable of self-renewal and differentiation as well as serial and limiting-dilution transplantation. It was postulated that the ABCB5-expressing cancer stem cells would represent a population highly resistant to conventional melanoma therapy. Recently, however, it has been reported that differentiated pigment-producing melanocytes located in the interfollicular epidermis can give rise to melanoma (Kohler et al. 2017). Using a *Braf*^{V600E}-driven mouse melanoma model, Kohler et al. showed that the melanocyte stem cells residing in the hair follicle bulge region are refractory to melanomagenesis, and in fact, melanoma originates from the pigment-producing, but not amelanotic, melanocytes located in the interfollicular regions. These pigment-producing mature melanocytes exhibited loss of differentiation markers before dermal invasion, suggesting that a transcriptomic reprogramming had caused a reversion to a dedifferentiated tumor-initiating state (Kohler et al. 2017). It is unclear what micro-environmental cues play a role in dictating this reprogramming.

Melanoma and the Environment

Sun Exposure and Epidemiology

Melanoma rates continue to rise in most populations at a time when the incidence of many other cancers is on the decline (Cronin et al. 2018; Nikolaou and Stratigos 2014). Extensive epidemiologic studies have provided strong support for the notion that UVR is a significant environmental carcinogen for melanoma (Garibyan and Fisher 2010; Moan et al. 2008a; Tran et al. 2008). The relentless rise in skin cancer incidence despite broad knowledge of the carcinogenic activity of UVR is likely a manifestation of recent evidence demonstrating that release of beta-endorphin and consequent opiate-like responses occur as by-products of the UVR-tanning response (Fell et al. 2014). Supporting

experimental evidence for UVR's carcinogenic activity has come from studies of the effects of UVR on a variety of animal models, including the opossum, the fish *Xiphophorus*, the Sinclair swine, and several lines of genetically engineered mice (Jhappan et al. 2003; Zaidi et al. 2008). However, significant questions remain as to how UVR causes melanoma and under what circumstances (Maddodi and Setaluri 2008). For example, unlike other skin cancers, melanoma risk does not appear to be associated with cumulative lifetime exposure to UVR; instead, intermittent burning doses of UVR represent a more significant risk factor. Other epidemiologic data draw a critical association between melanoma and childhood UVR exposure (Green et al. 2011; Whiteman et al. 2001). Immunologic differences between children and adults, or differences in their proportion of melanocytic progenitors, have been suggested as possible explanations for this observation (Zaidi et al. 2012). So, while the association between melanoma and sunlight exposure has long been appreciated, the relationship between exposure and risk is complex, and the underlying mechanisms are largely unknown.

In contrast, great strides have been made in classifying melanomas based on epidemiologic and molecular associations (Bastian 2014; Cancer Genome Atlas 2015). In perhaps the best example, Curtin et al. (2005) demonstrated that melanomas arising from skin sites not subjected to chronic sun damage (e.g., those receiving intermittent sun exposure) were highly likely to carry characteristic BRAF mutations, whereas in melanomas arising from skin with chronic sun damage, or regions that virtually never see sunlight (e.g., acral and mucosal melanomas), such mutations were very rare. In support of this relationship, congenital nevi arising without UVR exposure also lacked BRAF mutations (Bauer et al. 2007). Interestingly, as if to compensate for the lack of BRAF mutations, melanomas from chronically sun-damaged skin frequently harbor activating oncogenic mutations in KIT, a receptor tyrosine kinase upstream of BRAF (Curtin et al. 2006).

Despite the general agreement on the causal role of UVR in melanoma, the efficacy of sunscreens in the prevention of melanoma remained

controversial; in fact, some earlier epidemiologic data suggested that people who used sunscreens were more likely to develop skin cancer, not less (Bastuji-Garin and Diepgen 2002). Part of this controversy can be traced to the fact that the early sunscreens were designed to block UVB, and not UVA, because erythema (the reddening accompanying burning) is caused by UVB. If in fact UVA played a significant role in melanomagenesis, early epidemiologic data would reflect the failure of early sunscreens to effectively filter out this waveband. However, the role of UVA in melanoma was itself controversial. Another interpretation of these data was that they reflected improper use of sunscreens in terms of frequency and thickness of sunscreen application by those spending significant time out in the sun. Recent studies have started to shed light on these controversies. Klug et al. (2010) performed a matched logistic regression analysis of sunscreen use adjusted for average UVB intensity, sun exposure, skin type, number of sunburns, age group, study site, and gender to show that sunscreen use was not associated with melanomagenesis. Moreover, utilizing the HGF transgenic mouse model of UVR-induced melanomagenesis, they showed that SPF-15 sunscreen-treated mice developed significantly fewer melanomas than the vehicle-treated control mice; and this effect was associated with significant reduction in UVB-induced DNA damage, indicating a distinct protective effect of sunscreen (Klug et al. 2010). Using a *Braf*-mutant mouse model, Viros et al. showed evidence that sunscreen at least partially protected against UVR-induced melanomagenesis (Viros et al. 2014). In another *in vivo* study using the UVR-susceptible *NRas*^{G1R} mutant mouse model of melanoma, Hennessey et al. recently showed that aerosol sunscreens effectively blocked UVR-induced DNA damage and delayed melanoma formation (Hennessey et al. 2017).

Yet another controversy surrounded the opposing values of restricting sunlight to prevent the development of melanoma and other skin cancers and of receiving enough sunlight to ensure optimal production of vitamin D, which in adequate amounts prevents a variety of diseases (Moan et

al. 2008b). For example, in a post-hoc analysis of a randomized controlled trial, vitamin D supplementation was not associated with reduced melanoma risk (Tang et al. 2011). On the other hand, vitamin D deficiency has been shown to be associated with a worse prognosis in metastatic melanoma patients (Timerman et al. 2017). Extensive studies in the last two decades have provided strong evidence that vitamin D deficiency is associated with reduced survival of melanoma patients and single-nucleotide polymorphisms (SNPs) in the vitamin D receptor (VDR) gene affect melanomagenesis and/or disease outcome (Slominski et al. 2017). Active forms of vitamin D₃ protect against oxidative DNA damage by upregulating genes such as TP53 and GADD45 (Fleet et al. 2012), and its newly discovered derivatives exhibit VDR-mediated antiproliferative effects on melanoma cell lines (Janjetovic et al. 2011; Slominski et al. 2012). Based on these and other extensive studies, multiple randomized clinical trials are currently underway to evaluate whether vitamin D supplementation improves survival outcome in melanoma patients. Importantly, however, these questions do not lead to the conclusion that exposure to UVR would be an optimal means of maintaining healthy vitamin D levels, as oral vitamin supplements are effective, inexpensive, and unlike UVR, not associated with any known carcinogenic activity.

Photobiology and Melanoma

UVB versus UVA To begin to understand how UVR affects biological systems (photobiology), one needs to appreciate the complexity of UVR itself. UVR from sunlight is subdivided into shorter wavelength UVC (200–280 nm), middle wavelength UVB (280–320 nm), and longer wavelength UVA (320–400 nm). UVC is biologically irrelevant, because it is absorbed by the atmospheric ozone layer. UVB and UVA are very different in their properties and biologic consequences. UVB penetrates poorly into the skin but is efficiently absorbed directly by DNA and proteins (Bruls et al. 1984). The two major types of DNA damage induced by UVB are cyclobutane

pyrimidine dimers (CPDs) and 6–4 photoproducts, which are repaired via the nucleotide excision repair pathway. The UVB-induced CPDs are characterized by signature C to T or CC to TT transitions. However, in melanomas such CPD signature mutations are relatively rare in the oncogenes or tumor suppressors that have been implicated in melanomagenesis. For example, 90% of BRAF mutations contain a T to A transversion (T1799A) (Wan et al. 2004). Only UVB can cause skin erythema. In contrast to shortwave UVB, UVA penetrates much more deeply into the skin but is poorly absorbed by DNA. UVA does not directly damage DNA, instead exerting its effects through photosensitizers. Thus, UVA induces oxidative DNA damage, such as the formation of 8-oxo-7,8-dihydroguanine through the generation of species such as reactive melanin radicals (Hill and Hill 2000).

UVR and ROS In eukaryotic cells, mitochondrial-mediated oxidative phosphorylation produces energy and, because electron transfer is not completely efficient, also generates potentially harmful side products called *reactive oxygen species* (ROS) (Wittgen and van Kempen 2007). Some ROS are required as secondary messengers of signaling cascades and for other normal cell functions, so cells have developed an antioxidant network that allows excess ROS to be scavenged, resulting in a state of homeostasis. However, melanocytes face a daunting ROS burden because of their ability to produce melanin and because they are routinely exposed to UVR, a situation that can have melanomagenic consequences. UVR stimulates the synthesis of melanin that, although designed for protection from further UVR damage, can become a prooxidant under oxidative stress conditions. Specifically, UVR exposure can induce the formation of oxidized melanin, forming a variety of radicals and disrupting ROS homeostasis. UVR, and UVA in particular, can damage DNA through the production of these melanin-derived radical species. Using the HGF transgenic mouse model, Noonan et al. (2012) showed that UVA was indeed melanomagenic but required the presence of melanin pigment and was associated with oxidative DNA damage, whereas UVB-induced melanomagenesis was independent of melanin.

Interestingly, ROS activity is related to the hypoxic response, mediated by the transcription factor hypoxia-inducible factor-1 (HIF-1 α / β) (Wittgen and van Kempen 2007). The epidermis of the normal skin exists in a mildly hypoxic environment, an environment that can promote melanomagenesis. Bedogni et al. (2005) demonstrated that AKT-mediated transformation of melanocytes only occurs under HIF-1 α -inducing hypoxic conditions similar to those found in the normal skin. In addition, AKT hyperactivation can bring about ROS generation by inducing NOX4 and by inhibiting apoptosis, thereby damaging mitochondria (Govindarajan et al. 2007).

UVR and the microenvironment UVR is known to induce an increase in the number of melanocytes and to enhance melanin synthesis, and ultimately UVR stimulates an increased transfer of melanin to those keratinocytes that it contacts (Miyamura et al. 2007; Zaidi et al. 2011). UVR can alter the melanocyte microenvironment, which subsequently influences the melanocytic response. D’Orazio et al. (2006) reported that UVR induces enhanced synthesis and secretion of α MSH by keratinocytes, which in turn triggers an appropriate melanocyte response through MC1R and the cAMP pathway, activating melanin-synthesizing enzymes and melanin production. In fact, UVR affects most of the components of the skin including melanocytes, keratinocytes, fibroblasts, and elements of the vascular and immune systems (Slominski and Pawelek 1998). UVR induces secretion of multiple factors by multiple cellular components, including neuropeptides (POMC-derived α MSH, ACTH, and β -endorphin), growth factors (FGF2, IGF-1, TGF α), and numerous cytokines (TNF- α , CSF2, IL-1, IL-6, IL-8, IL-10) (Slominski and Pawelek 1998).

UVR triggers inflammatory reactions and has an overall immunoinhibitory effect on the skin (Hart and Norval 2017; Kripke 1990). Moreover, the effect of UVR on the immune system in mouse models may be most influential at the neonatal stage, perhaps explaining the elevated risk associated with childhood UVR exposure (Zaidi et al. 2011, 2012). More specifically, UVR suppresses the ability of antigen-presenting Langerhans cells

in the skin to induce immunity by decreasing their number and their expression of MHC. UVR also stimulates an influx of macrophages and a decrease in the viability of T-lymphocytes. It has also been shown that inflammation can have positive influences on tumorigenesis. Together, it is evident that the abilities of UVR to induce mutagenesis, inflammation, and long-term immunosuppression can all profoundly influence melanomagenesis. Importantly, however, there is evidence to suggest some UVR-independent melanoma risk in certain circumstances. In individuals with the red-hair/light-skin phenotype, melanoma risk is elevated in both sun-exposed and non-sun-exposed skin (in contrast to their risk of non-melanoma skin cancers whose incidence is elevated only in sun-exposed skin). Mitra et al. (2012) utilized mouse models of red-hair/Mc1r-deficiency to show that red pigment (pheomelanin) is intrinsically carcinogenic – elevating the spontaneous incidence of BRAF^{V600E}-associated melanomas in the complete absence of UVR exposure. When red-haired mice were crossed to albinos (carrying *Tyr* mutations, thus unable to make any melanins), the melanoma risk was fully blocked. Significant levels of pheomelanin are dominantly present in the skin of not only redheads but also other Caucasians, suggesting that it may contribute chemically to melanomagenesis. However, it is also very likely that UVR amplifies the ROS-inducing capacity of pheomelanin, suggesting that UVR significantly exacerbates skin carcinogenicity in humans with fair skin.

Conclusion

It has been more than 200 years since the Parisian physician René Laennec first reported “melanoma” in Europe, while back in 1857 William Norris (1857) recognized that melanoma had a familial component. It is unlikely that either could have foreseen that by the twenty-first century, cutaneous malignant melanoma would become one of the fastest increasing cancers. In the United States alone, melanoma incidence has more than quadrupled over the past four decades, and it is estimated that more than 90,000 new cases will be diagnosed in 2018 (Cronin et al.

2018). Melanoma remains one of only a few major malignancies demonstrating significant positive rates of increase in the United States. We have come a long way with respect to our understanding of the origins of melanoma and the mechanisms by which it develops. As documented in this chapter, the first decade of the twenty-first century featured tremendous progress in the molecular technologic weaponry with which to address the most outstanding questions about the melanoma disease initiation, progression, and metastasis. Utilizing these impressive tools, researchers generated a treasure trove of data, gathered from both broadly global and exquisitely detailed analyses of melanocytic lesions from patients and from genetically engineered mouse models. In the second decade of this century, these data have advanced our understanding of the fundamental mechanisms of the disease, which has fueled an explosive expansion of therapeutic strategies that have revolutionized the clinical management of melanoma. There is excited anticipation within the melanoma research community that the holy grail of eliminating melanoma-related death is within reach.

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