

Review

Cellular and Molecular Biology of Human Melanoma

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ABSTRACT

Melanoma develops from a series of architectural and phenotypically distinct stages and becomes progressively aggressive culminating in metastasis. Over the years, considerable progress has been made in understanding the biological, pathological and immunological aspects of human melanoma progression. Epidemiological and experimental studies have suggested that intense exposures during early childhood to UV radiation may lead to melanoma in adults, but molecular and genetic studies have revealed few autosomal abnormalities, infrequent mutational spectra and very little epistatic and epigenetic mechanisms. At the cellular level it has become clear that deregulated homeostatic control in the skin microenvironment occurs through alterations in the expression of specific proteins. These include growth factors and their receptors, adhesion molecules and their ligands, proteases and their substrates, and transcription factors and their target genes. Like in most other human tumors, there are alterations in the regulatory networks involving signal transduction in human melanoma. Appropriate models mimicking the human disease have been developed. However, these have not yet led to major advances in delineating the precise molecular determinants responsible for melanoma progression. Results from recent studies have put more impetus on identification of new molecules that promise to become better therapeutic targets. This review focuses on the most recent progress in understanding the molecular determinants of tumor progression with a particular emphasis on melanoma as a biological responder to altered homeostasis.

INTRODUCTION

The melanocytes in human skin represent an intriguing and intricate group of cells that primarily impart skin color. Maturation, migration and maintenance of melanocytes which originate from the neural crest cells have been the subject of considerable research in animal models and have provided invaluable insight into the biological and pathological states in humans¹. Unlike the mouse epidermis, melanocytes in human skin are distributed as single cells within the basal layer of epidermis. These cells do not divide rapidly, but can be stimulated by environmental factors, such as ultraviolet light (UV) enabling them to divide and produce cytoprotective pigments. The occurrence of severe sunburn during childhood leading to melanoma in adults is quite frequent.^{1,2}

The American Cancer Society estimates that over 51,000 patients will be diagnosed with melanoma in the year 2001 and 7,800 will die from it. In fact melanoma is increasing at a startling rate of 2.5 to 4% per year and metastatic melanoma is lethal in 86% of the cases. It is also recognized that 95% of melanoma cases are curable if diagnosed early and surgically excised. The treatment options for metastatic melanoma are dismal as the tumors are radioresistant, chemoresistant and hence resistant to apoptosis. The pathways and molecules that are responsible and utilized for conventional therapeutic manipulations do not appear to be involved. For example, oncogenes such as *myc* and *ras* have been implicated in radioresistance but activation of these genes does not correlate with the response to therapy. The search for overexpression of drug resistant genes has also had limited success.³

Etiological and biological factors such as UV or growth factors responsible for melanoma show a strong association with the programmed cell death phenomenon but do not appear to influence apoptosis. Melanoma cells appear to have intact apoptotic pathways but still escape apoptosis apparently due to the multicellular nature of the disease in which melanoma cells (and melanocytes) are embedded in a tissue environment of multiple cell types including keratinocytes, fibroblasts, endothelial cells, and immuno-regulatory cells (Fig. 1).

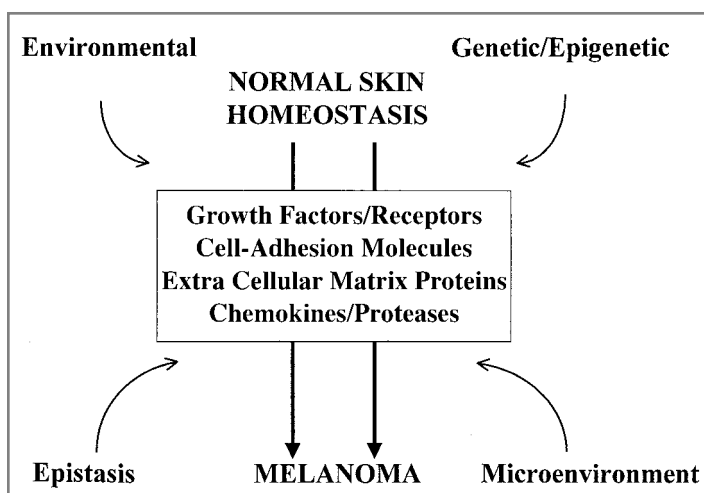


Figure 1. Factors influencing human melanoma progression. The model suggests that environmental, genetic and epigenetic alterations in the target tissue lead to epistatic changes. This gradually develops into disturbances in the normal homeostatic phenomenon at a rate that is related to the site and severity of the damage. Melanoma progression occurs when it is not static and irreversible, and is further aided by cumulative and new tissue micro-environmental changes. Some of the factors affecting homeostatic balance are highlighted.

HOMEOSTASIS AND CADHERINS

It is important to consider melanoma as a disease of homeostatic imbalance in the skin. Biologically, there are a number of components in the skin that influence tumor development. These include epidermal keratinocytes, dermal fibroblasts, endothelial and inflammatory cells. In normal skin, a fine-tuned balance is maintained between these components and the keratinocytes keep melanocytes under control from continuous proliferation.¹ A well-defined basement membrane complex prevents melanocytes from migrating into the dermis. Mature isolated melanocytes will not survive in the dermis because of the foreign environment. In a dermal nevus, melanocytes remain in clusters and may even break the basement membrane barrier, but are prevented from aggressive cell division due to lack of complete transformation. On the other hand, once the melanocytes are in culture and are stimulated to grow, for example, by protein kinase C activators and cAMP enhancers, they rapidly divide and temporarily lose their replicative senescence. For example, the protein kinase activator, tetradecanoyl phorbol 13-acetate (TPA), stimulates the cells to divide and in the process enhances the expression of Bcl-2,⁴ which may prevent cells from undergoing programmed cell death.

Melanocytes in culture acquire expression of a number of cell surface molecules that allow them to adhere, migrate, survive and grow in plastic dishes,⁵ and they turn off genes such as the “invasion suppressor” E-cadherin that are normally expressed when present in the skin microenvironment.⁶ Cadherins are a family of cell surface glycoproteins that function in promoting calcium-dependent homotypic and heterotypic cell-adhesion, promote intercellular communication and are an integral part of cell-cell adherens junctions.^{7,8} The classical cadherins, E-, N-, P-cadherins are expressed during various stages of melanoma progression. In the skin environment, E-cadherin expressed by melanocytes and keratinocytes facilitates homophilic interaction and prevents melanocytes from division without killing the cells, whereas N-cadherin is expressed by fibroblasts and endothelial cells.^{9,10} During melanoma development, expression of

E-cadherin is progressively lost, becomes heterogeneous, gets diffusely distributed in the cytoplasm of the nevus cells and is predominantly absent in melanoma cells.¹¹ This may impart loss of terminal differentiation to melanocytes by disrupting the complex formation between cadherin, catenin and cytoskeleton required for strong cell-cell adhesion. Despite the loss of E-cadherin in melanoma cells, they now acquire expression of N-cadherin and this switching of the cadherin profile is thought to promote their interactions with fibroblasts and endothelial cells (Fig. 2). The switch subsequently leads to tumor-host cell interactions, tumor cell invasion, migration and altered gene expression.¹² Overexpression of E-cadherin in melanoma cells and co-culture with keratinocytes prevents their growth, invasion into the dermis and induces cell death.¹³ How expression of E-cadherin is turned off during melanoma progression remains to be examined. It has often been demonstrated in other cancers that E-cadherin is subjected to silencing through methylation.¹⁴ However, in human melanoma cells, deletions or mutations within the E-cadherin gene and epigenetic mechanisms such as methylation of the E-cadherin promoter are apparently not involved. Transcriptional silencing of E-cadherin promoter and its expression by at least two transcription factors, snail and SIP1, that belong to zinc finger family has been reported^{15,16} suggesting that strategies can be explored to upregulate expression to control melanoma cell growth in the skin.

N-cadherin mediates homotypic aggregation among melanoma cells and heterotypic interactions of melanoma cells to fibroblasts and vascular endothelial cells.¹⁷ This may facilitate melanoma cells to migrate into the dermis and enter the vasculature. Despite the wide disparity in functional effects and similarity in intracellular signaling cascades of N- and E-cadherins, it is obvious that there are subtle differences, which remain to be clearly elucidated. N-cadherin can physically interact with the fibroblast growth factor receptor, FGFR-1, to promote growth and neurite formation.¹⁸ The ligand basic FGF is instrumental in promoting autocrine and paracrine growth effects in melanoma cells and inhibition of FGFR-1, which is widely expressed in melanoma cells, leads to cell death and inhibition of tumor growth.^{19,20} However, this only forms part of the network wherein other molecules can be recruited. These include activation of matrix metalloproteinases, expression of specific cell-adhesion molecules such as $\beta 3$ integrin,²¹ and inhibition of suppressor proteins such as NM23, melastatin (Fig. 2).^{22,23} It is clear that the expression of cell surface adhesion molecule $\beta 3$ integrin increases with progression from radial to vertical growth phase primary and to metastatic melanoma.²⁴ A large survey of genes involved in melanoma has been examined by microarray analysis. Bittner et al²⁵ and Clark et al²⁶ have analyzed molecular profiling of melanoma through microarray technology with two different aims. While molecular profiling by Bittner et al²⁵ attempted to classify melanoma based on tumor-derived tubular network formation, Clark et al²⁶ examined the genes expressed during melanoma progression towards metastasis. Although, the two groups examined two different tumor spectra and identified different sets of genes, new avenues for melanoma diagnosis and therapy were opened. Both studies have identified genes coding and modulating ECM proteins and the cytoskeleton. These findings suggest a role for altered tissue homeostasis in melanoma progression.

GENETIC ABERRATIONS

Despite continuous exposure of melanocytes to environmental hazards such as sunlight and chemicals in the skin, melanomas do

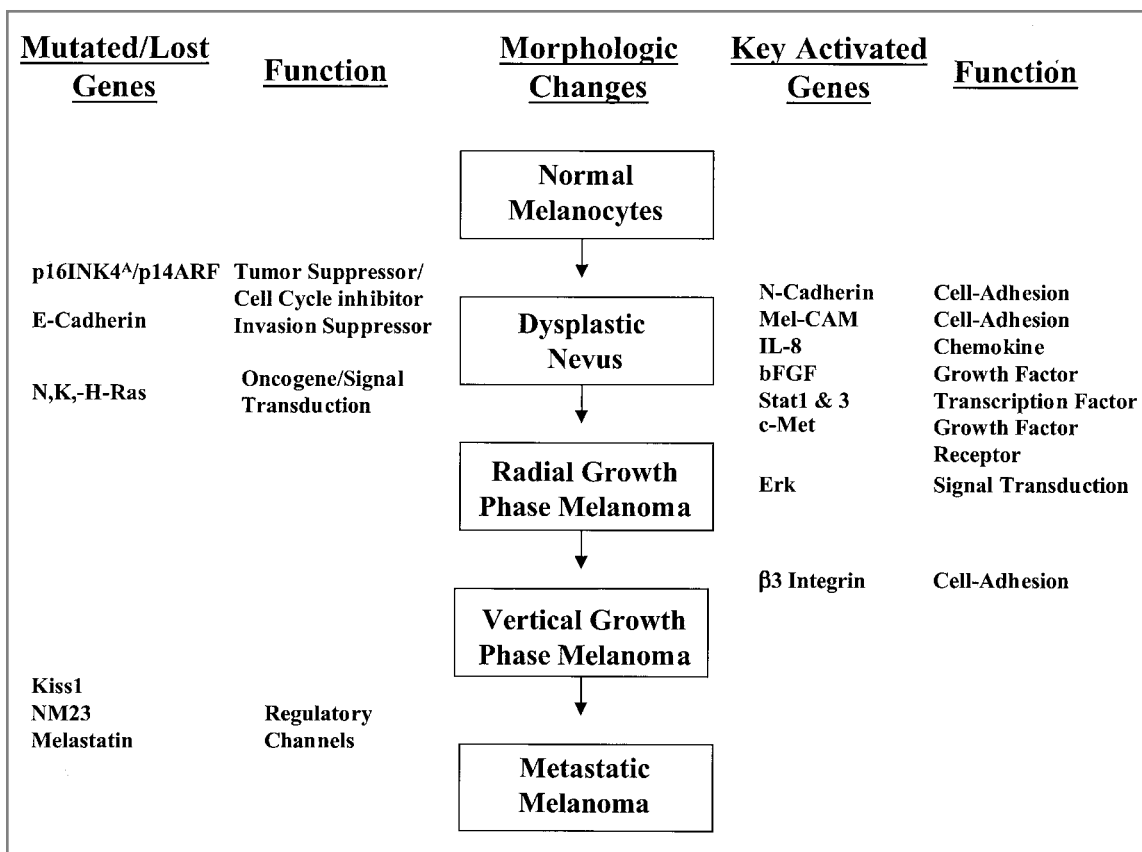


Figure 2. Experimental steps in human melanoma progression and alterations in some of the molecules identified during the progression. Environmental, genetic and epigenetic effects may influence expression of molecules that initiate tumor progression. Dysplastic nevus (multiple atypical nevi) has a higher propensity to progress into melanoma than melanocytic nevi. Radial growth phase melanoma (RGP), is less aggressive and non-tumorigenic in SCID mice. The vertical growth phase (VGP) and metastatic melanomas are accompanied by several well-defined biological and molecular changes. Some of the genes extensively characterized during these experimental stages of melanoma progression are indicated.

not solely demonstrate UV-signature mutations. Mutations in the tumor suppressor gene p53 are very rare in melanoma, less than 5%.¹ Mutation in RAS oncogene have been observed in up to 21% of cases exposed to sunlight.²⁷ Overall mutations or deletions in the tumor suppressor p16^{INK4A} have not exceeded more than 25%.¹ Genetic predisposition of familial melanoma accounts for about 10% of all cases and germ line mutations or deletions in chromosome 9p21 in about 20 to 40%. This region harbors cell-cycle inhibitors and tumor suppressors such as p15^{INK4B}, p16^{INK4A} and an alternatively coded gene product of p16^{INK4A} – p14/ARF.²⁸ The latter is responsible for stabilizing p53 by interfering with MDM2 in a p53/MDM protein complex.²⁹ Although the majority of the mutations are seen in the p16^{INK4A} locus, nearly 40% affect both p16^{INK4A} and p14/ARF.³⁰ The search for mutations in other genes such as β-catenin, CDK4 and PTEN did not yield significant occurrences of mutations in melanoma. The intriguing question remains why p53, despite its wild-type status, is not able to modulate melanoma cells and why melanoma remains chemo- and radiation-resistant. Tumor-derived p53 localizes to the nucleus in melanoma cells; however, its transcriptional activity is weak.³¹ This suggests that p53 is kept in an inactive state either by other factors or through post-transcriptional mechanism(s) thereby functionally inactivating its pathways. In this respect, it has been reported that, like in sarcomas, melanoma cells overexpress the MDM2³² protein. MDM2 is implicated in inactivating p53 by targeting it for degradation.²⁹ If the p16^{INK4A} locus is inoperative either by mutation, methylation or deletion, p14/ARF derived from

the same locus would probably be less functional and hence MDM2 can exert its dominant effect over p53. In addition, expression of anti-apoptotic protein Bcl-2 can inhibit both p53-dependent and independent apoptotic mechanisms.³³

One of the downstream targets of p53 is Apaf-1 (apoptotic protease activating factor-1), which activates caspase-9 and initiates the protease cascade. Apaf-1 has been shown to be inactivated in melanoma cells in several ways (for example through LOH or DNA methylation) and reversal of its inactivation by a demethylating agent increases sensitivity of the malignant cells to anticancer drugs such as adriamycin.³⁴ In a scenario where anti-apoptotic proteins are expressed in melanoma, it is only logical that proapoptotic Apaf-1 is deregulated in a reversible manner in vitro. It also appears that cells have developed several mechanisms to control self-destruction. Activation of caspase-9 is stimulated by Apaf-1/cytochrome c and inhibited by signaling from the serine/threonine kinase Akt/PKB.^{35,36} However, the anti-apoptotic proteins Bcl-2 and Bcl-xL are not completely cytoprotective against Apaf-1/caspase-9 mediated cell death,³⁷ and caspase-9 is phosphorylated by Akt leading to its reduced protease activity.³⁸ A number of substrates for Akt/PKB have been identified suggesting that it participates in cell survival, cellular metabolism and anti-apoptotic pathways. Since growth factors secreted by melanoma cells (HGF/SF) or surrounding stroma (IGF-I) for autocrine and paracrine growth induce phosphorylation and activation of Akt and its signaling pathways,³⁹⁻⁴¹ it is not surprising that the cells are kept under control by the discrete regulatory pathways in the tissue environment.

Kirkwood et al⁴² have demonstrated the constitutive activation of transcription factors Stat1 and Stat3 in precursor lesions such as multiple atypical nevi and this together with other molecules involved in constitutive activation of MAP kinase pathways may lead to exciting new avenues for therapy. In summary, we are only beginning to understand the key regulatory molecules and cofactors required for melanoma progression.

CONCLUSION

It has become clear that melanoma develops as a consequence of multifactorial perturbations. The ability of melanoma cells to invade and metastasize to distant organs in the body suggests its adaptability to varying microenvironments. Cross-talk between several signal transduction pathways and their redundancies suggests the importance of understanding the precise role of multiple factors in a multicellular environment. Clearly, there is a need for potent and specific therapeutic intervention for melanoma progression. This task could be tackled by considering the various components of tissues surrounding the tumor. A combined strategy for restoration of normal homeostasis in the skin with targeted delivery of apoptosis-inducing agents does not seem to be far fetched. Recent knowledge acquired from advances in deciphering the human genome will add exciting new targets and avenues for melanoma diagnosis, prognosis and therapy.

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