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MOLECULAR EVENTS IN MELANOMA DEVELOPMENT AND PROGRESSION

Friedegund Meier[1], Kapaettu Satyamoorthy[1], Mark Nesbit[1], Mei-Yu Hsu[1], Birgit Schittek[2], Claus Garbe[2], and Meenhard Herlyn[1]

The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104[1], Department of Dermatology, University of Tuebingen, Liebermeisterstr.25, 72076 Tuebingen, Germany[2]

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1. ABSTRACT

Based on clinical and histopathological features, five steps of melanoma progression have been proposed: common acquired and congenital nevi with structurally normal melanocytes, dysplastic nevus with structural and architectural atypia, early radial growth phase (RGP) primary melanoma, advanced vertical growth phase primary melanoma (VGP) with competence for metastasis, and metastatic melanoma. Despite a wealth of research resources (tissues, cell lines, and antibodies), the genetic alterations responsible for the development and stepwise progression of melanoma are still unclear. Cytogenetic analyses have failed to identify consistent gene deletions, mutations, translocations, or amplifications in sporadic cases. However, *in vitro* characterization of melanoma cells has revealed fundamental differences from normal melanocytes. Earlier work using monoclonal antibodies has defined a variety of melanoma-associated antigens that mediate cell-cell or cell-substratum adhesion, growth regulation, proteolysis, and modulation of immune responses. Functional studies of these individual candidate molecules will lead to a better understanding of the pathogenesis of melanoma and of potential targets for rational therapy.

2. INTRODUCTION

Melanoma has been one of the fastest rising malignancies in the last 4 decades, with the incidence increasing from less than 3 per 100,000 individuals to more than 12 today. In the United States, approximately 43,000 new cases and 7,300 deaths occurred in 1997 (1). Among Caucasian females between the ages of 20 and 35 years, melanoma is the main cause of death from malignancy. By the year 2000, 1 in 70 Americans is expected to develop melanoma over his/her lifetime. Despite worldwide efforts in prevention, diagnosis, and treatment, melanoma incidence continues to rise at an alarming rate. Fortunately, the increasing incidence rate exceeds the mortality rate, apparently because of detection of biologically early primary melanomas which are curable through surgery. However, despite significant improvements in diagnosis and surgical, local and systemic therapy, reducing mortality from melanoma metastases remains a major challenge. A better understanding of the underlying mechanisms of melanoma development and progression holds the promise of design of effective interventions for metastatic melanoma.

3. MODEL OF MELANOMA DEVELOPMENT AND PROGRESSION

Figure 1 depicts the five steps of melanoma development and progression (2). A recent refinement divides lesions into three classes: class I represents "precursor" nevi; class II lesions are "intermediates" with melanocytic cells confined to the epidermis or with microinvasion into the dermis and represented by *in situ* and invasive RGP melanomas; and class III are VGP tumorigenic melanomas (3). As in any neoplastic system, individual melanomas can skip steps in their development, appearing without identifiable intermediate lesions. Alternatively, melanoma can arise from malignant transformation of precursor cells.

Figure 2 summarizes the genetic and biological events leading to melanoma development and progression. The dynamic progression from a resting melanocyte to a common acquired nevus is very common and does not appear to accompany genetic changes. Nevus cells isolated from common acquired nevi have a finite life span and generally do not carry cytogenetic abnormalities (4-6). We postulate that melanocytes progress to a nevus by escaping

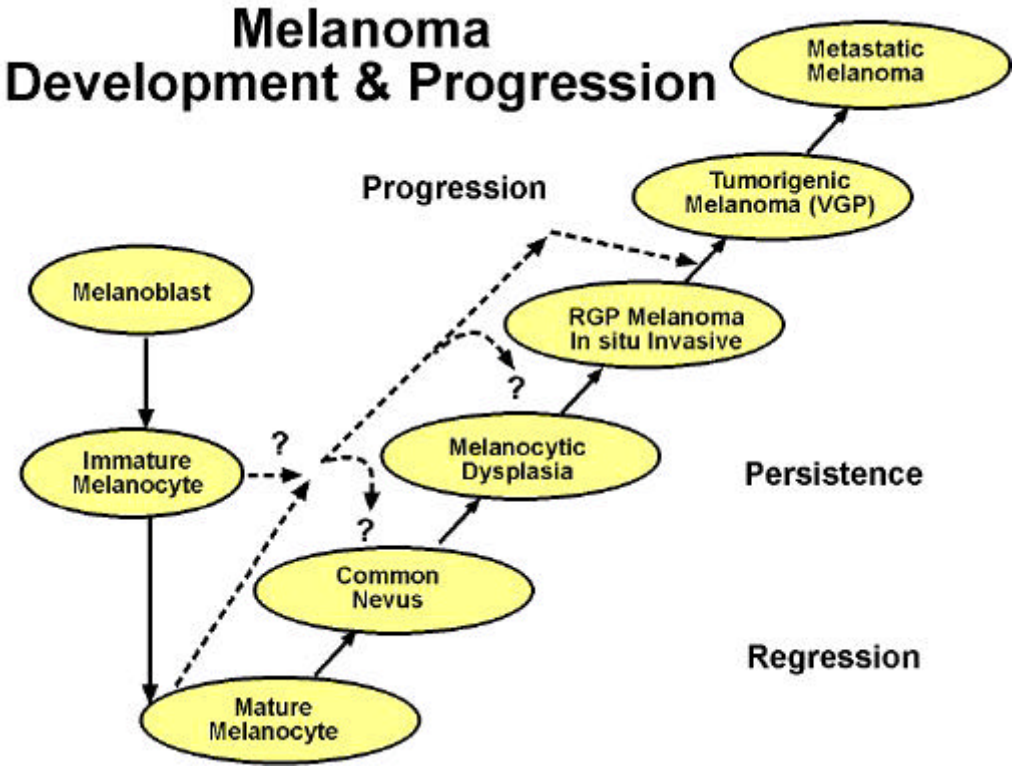

Figure 1. Melanoma development and progression. The model, developed by Clark, Elder, and Guerry (2), implies that melanoma commonly develops and progresses in a sequence of steps from nevic lesions which can be histologically identified in approximately 35% of cases. However, melanoma may also develop directly from normal cells. The role of melanoblasts (immature melanocytes) in melanogenesis remain poorly defined. Cells from lesions persist, but non-tumorigenic lesions tend to disappear through apoptotic or differentiation pathways as yet undefined

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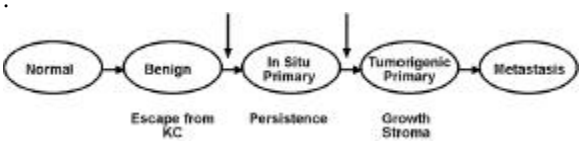

Figure 2. Genetic and biological events leading to tumor progression in the human melanocytic system. The progression from normal melanocyte to nevus may be initiated by loss of contact between melanocytes and keratinocytes, i.e., the melanocytes escape from keratinocyte (KC) control. Genetic changes, which are currently not defined, are expected at the transition from common acquired (benign) nevus to dysplastic nevus/RGP/*in situ* melanoma (left vertical arrow), allowing cells to persist. Additional genetic changes are expected in the progression from RGP/*in situ* melanoma to VGP (right vertical arrow). At the VGP (tumorigenic) step, increased growth and stroma induction occurs.

from the normal contact-mediated controls of keratinocytes, the dominant cellular partner of melanocytes in the epidermis and able to control the growth, morphology, and antigenic phenotype of melanocytes (7, 8) by establishing direct contact through the cell-cell adhesion receptor E-cadherin. This contact, in turn, facilitates formation of gap junctions through connexin 43 (9). It remains unclear whether signals for phenotypic control over melanocytes are relayed through E-cadherin, gap junctions or other accessory mechanisms. Nevertheless, E-cadherin downregulation coincides with melanoma progression. Reduced E-cadherin expression can be observed early in the nevus stage, and the majority of melanomas are E-cadherin-negative (10). In contrast, expression of N-cadherin is upregulated in nevi and melanomas. Such a shift in cadherin profile confers new adhesive properties to the cells. Acquisition of N-cadherin may allow gap junctional communication of nevus and melanoma cells with N-cadherin-expressing fibroblasts and endothelial cells (unpublished data). Genetic changes are anticipated when dysplastic nevi develop, but the nature of these changes is currently unknown. It is possible that mechanisms leading to persistence and proliferation of dysplastic nevi rest in the dysfunction of the physiological cascade of apoptosis. Thus, the cell cycle checkpoint pathways (including p53 and myc) may be involved in the development of melanocytic dysplasia (2). Progression from dysplasia to RGP primary melanoma is gradual and spontaneous, and may not require additional molecular changes.

The transition from RGP to VGP is a biologically and clinically critical step, accompanying additional

Table 1. Biological differences between RGP and VGP melanoma cells

Property	RGP	VGP
Metastatic competence in patients	no	yes
Growth *in vitro*	poor	well
Growth factor dependence	several	only IGF-I
Stimulation by TPA	yes/no (heterogeneity)	No (inhibition)
Growth in soft agar	no	yes
Tumorigenicity	no survival or slow growth	yes

Table 2. Molecular abnormalities in sporadic melanoma

Gene	Mechanisms	Percent
p16	Absent or mutant gene	5-20
N-ras	Overexpression or mutation	5-15
p53	Mutant or absent gene	2-7
beta-catenin	Mutant gene/overexpression	15?
PTEN	Mutant gene	25
myc	Overexpression	25
Other		<1
• PKC-alpha	Mutation	
• c-myb	Mutation	
• CDK-4	Mutation	
• EWS-AFT-1	Translocation	
• NF-1	Mutation	

Figure 3. Antigens on melanoma cells. MAbs have defined a variety of structures on melanoma cells that have functional significance for growth, adhesion, transport, invasion, and interactions with the microenvironment. The patterns of antigen expression of melanoma cells are very different from those in melanocytes. Melanoma cells, but not melanocytes, display melanoma-associated antigens that may be shared by monocytes, fibroblasts, or endothelial cells.

genetic abnormalities. However, the specifics are largely unknown. In sections of lesions and in cultured cells, we have described a variety of changes at the biological level which explain RGP-VGP progression (11, 12). Table 1 summarizes the biological differences between RGP and VGP melanoma cells. Unlike RGP melanomas, VGP cells are metastasis-competent (13) and are easily adapted to growth in culture. In addition, VGP cells are less dependent on exogenous growth factors (14) and have growth characteristics similar to metastatic cells, such as anchorage-independent growth in soft agar and tumorigenesis in immunodeficient mice. VGP primary melanomas display numerous cytogenetic abnormalities, suggesting considerable genomic instability. No major additional genetic changes may be required for further progression to metastatic dissemination since most VGP melanomas can be readily adapted to a metastatic phenotype through selection in growth factor-free medium or induction of invasion through artificial basement membranes (15). This suggests that micro-environmental factors such as cell-matrix and cell-cell signaling are critical for the metastatic phenotype.

4. GENETIC BASIS OF MELANOMA

The genetic changes that lead to melanoma are still poorly understood. Table 2 summarizes the current information on specific gene deletions, mutations, translocations, or overexpression. Less is known about the genetic abnormalities in melanoma than in other cancers such as leukemias, lymphomas, or gliomas and various carcinomas. Unlike many other cancers, melanomas show very few mutations in the p53 tumor suppressor gene. Mutations and deletions in p16 are also less frequent in sporadic melanoma than in other types of cancers such as pancreatic carcinoma (12). Similarly, n-ras mutations are relatively rare and are more frequently seen in sun-exposed areas of the skin. Stabilization of beta-catenin due to mutations has only recently been described in melanoma (16). Since beta-catenin mutations lead to gene activation through complex formation with Lef/Tcf transcription factors (17), it is possible that constitutive expression of this signaling molecule plays a role in melanomagenesis. Although the downstream effectors of the Lef/Tcf pathway are presently unknown, melanoma cells display a variety of antigens (melanoma-associated antigens) that are generally not found on normal melanocytes. These molecules are associated with survival, growth, motility, adhesion, invasion, and inflammatory and immune responses (figure 3), many of which have been defined with monoclonal antibodies (MAbs) that are now extensively used for diagnosis and even therapy.

5. ADHESION MOLECULES IN MELANOMA DEVELOPMENT AND PROGRESSION

Expression of a given adhesion molecule on melanocytes from different stages of tumor progression is a dynamic process. There is upregulation of Mel-CAM/MUC18, chondroitin sulfate proteoglycan (CSPG), gangliosides GD_3 and acetylated GD_3 during the transition from normal melanocytes to nevi (figure 4). Our laboratory has defined Mel-CAM/MUC18 as an adhesion receptor that is involved in cell-cell interactions (18). Its expression is upregulated in a step-wise fashion (figure 4) and coincides with the separation of nevus cells from keratinocytes (18).

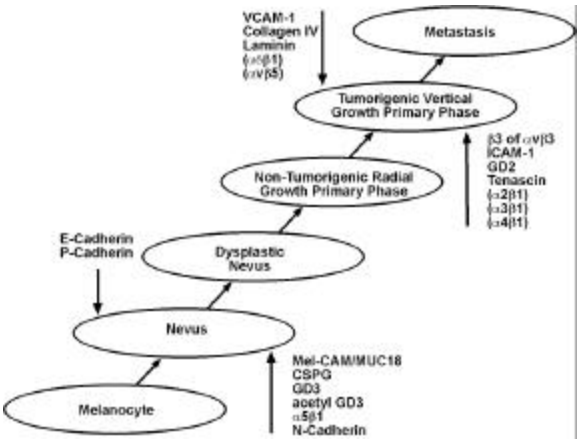

Figure 4. Dynamic changes in expression of adhesion receptors and ECM proteins in melanoma progression. Decreased expression (downward arrow) is seen for some cadherins, CAMs, integrins and ECM proteins and is relatively uncommon. A strong increase (upward arrow) of a variety adhesion-related molecules, first in nevi, then in VGP primary melanomas, is more common. Parentheses indicate molecules exhibiting a gradual increase between steps.

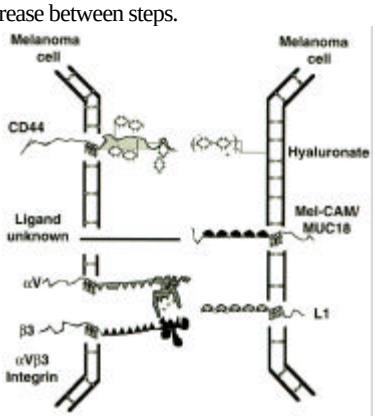

Figure 5. Adhesion receptors in melanoma-melanoma cell interactions. The dominant adhesion systems appears to be Mel-CAM and its unidentified ligand. Vitronectin receptor-mediated adhesion may require prior activation. The functional significance of CD44 and its different isoforms in melanoma adhesion remains unclear.

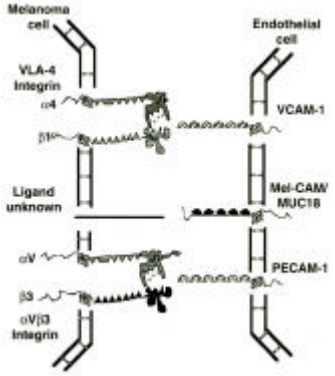

Figure 6. Adhesion receptors in melanoma-endothelial cell interactions. Melanoma cells adhere to endothelial cells apparently in a sequence of steps, starting with Mel-CAM/ligand, followed by integrin-mediated adhesion since proper activation is required for the latter.

Mel-CAM binds to an as yet unidentified ligand (19), and may play a major role in metastasis by mediating not only melanoma-melanoma cell interactions (figure 5), but also melanoma-endothelial cell adhesion (figure 6). Mel-CAM/MUC18 appears to act in concert with $alpha_v beta_3$, the vitronectin receptor, in promoting metastasis. As the cells progress from RGP to VGP, expression of $alpha_v beta_3$, $alpha_2 beta_1$, $alpha_3 beta_1$, $alpha_4 beta_1$, ICAM-1, and GD_2 ganglioside is increased. However, the most notable marker is the beta3 subunit of $alpha_v beta_3$ integrin. In our hands, $alpha_v beta_3$ is the most specific melanoma-associated marker that distinguishes RGP from VGP melanomas (figure 4) (20). It is also a prime candidate for prognostic studies (21). In addition, a decrease in adhesion molecule expression with melanoma progression has been described. The most notable example is E-cadherin which is found on normal melanocytes, to a lesser degree on nevi and little on melanomas (10). The loss of E-cadherin expression has significant biological consequences in melanocytic cells. Forced reexpression of E-cadherin in melanoma cells leads to growth retardation, inhibition of invasion and induction of apoptotic death in a three-dimensional skin reconstruct, and decreased tumorigenicity in mice (22). Thus, E-cadherin may act as a tumor suppressor in the melanoma system.

6. GROWTH FACTORS, CYTOKINES AND THEIR RECEPTORS IN MELANOMA DEVELOPMENT AND PROGRESSION

Melanoma cells express a variety of growth factors and cytokines (figure 7) and their receptors (figure 8) (11). For several years, we have delineated their functions and have distinguished between autocrine growth factors, those involved in stroma induction (including angiogenesis), and those interacting with the host defense system [i.e., with specific T cells and non-specific inflammatory cells (23, 24)]. In a series of experiments, we have evaluated the role of these molecules in angiogenesis, matrix induction, and monocyte and granulocyte attraction (25-27). Growth factors and cytokines are also suitable as progression markers (figure 9). Tumor cells produce growth factors not only for autocrine growth stimulation, but also for paracrine stimulation of the stromal cells (figure 10). Fibroblasts are activated by melanoma-derived PDGF, whereas endothelial cells are activated by VEGF.

7. SUMMARY AND PERSPECTIVES

Melanomas can develop in a sequence of steps which have been clinically and histologically defined. The very first step from a normal cell to a benign lesion appears to be an event not dictated by genetic alterations. Instead melanocytes escape from keratinocytes through loss of E-cadherin expression. At that time, cells must receive a genetic "hit" in order to progress, and then cytological and architectural atypia may follow. The nature of these changes remains to be elucidated. The dysplastic cells can persist for years before they begin to proliferate to form RGP. At the transition from RGP to VGP, one or more genetic hits must occur. Cells then begin to proliferate more rapidly and develop stroma and undergo angiogenesis. Changes in adhesion receptor expression and

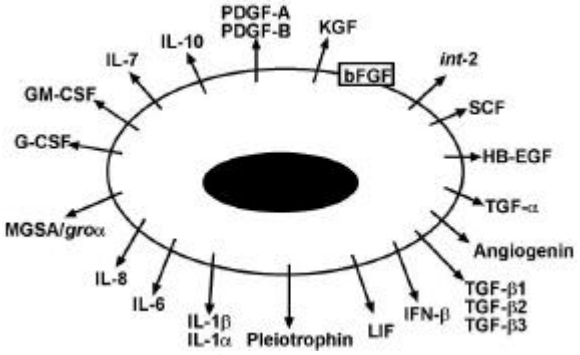

Figure 7. Growth factors and cytokines expressed by melanoma cells. Unlike normal melanocytes, melanoma cells express most of the ligands constitutively, i.e., without prior stimulation. Differences in expression between melanoma cells in culture and *in situ* are only marginal. However, expression *in situ* may be more heterogeneous. Individual cell lines or specimens may express several but not necessarily all factors listed.

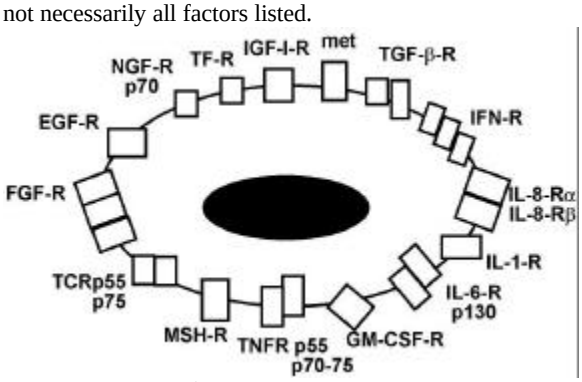

Figure 8. Growth factor receptors expressed by melanoma cells. Melanoma cells express receptors at levels from a few hundred sites (MSH-R), to ten-thousand (EGF-R) or hundred-thousand (NGF-R) per cell.

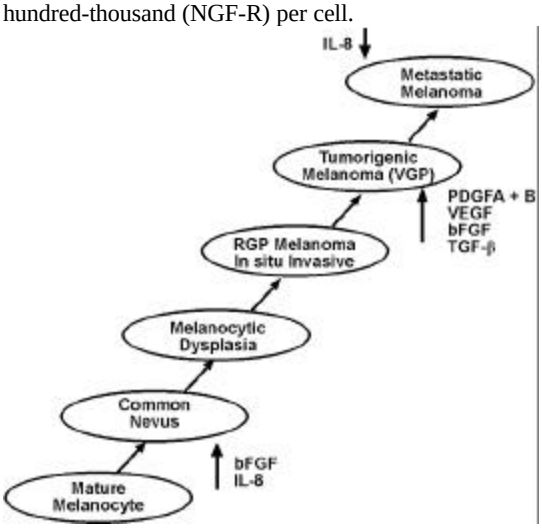

Figure 9. Dynamics of growth factor and cytokine expression during progression. bFGF and IL-8 are apparently upregulated early in progression, but IL-8 decreases in metastases. Other factors show an increase particularly at the transition from RGP to VGP melanoma.

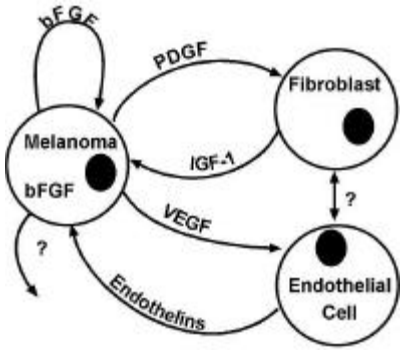

Figure 10. Hypothetical tumor-stroma interactions. Melanoma cells produce bFGF for autocrine growth stimulation. Melanoma-derived PDGF and VEGF activate fibroblasts and stimulate angiogenesis, respectively. The activated stromal cells then provide feedback to the tumor cells by producing their own growth factors.

function further accelerate progression. The final step, metastasis, is dictated by endogenous oncogenes and tumor suppressor genes, and by exogenous interactions with the host. Metastatic cells have a highly unstable phenotype and can rapidly adapt to selective pressure, allowing the cells to survive even under the most unfavorable circumstances. Future studies to further dissect each step of melanoma progression should lead to the development of more specific and effective therapies.

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Send correspondence to: Dr. Meenhard Herlyn, The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104. Tel: (215)-898-3950, Fax: (215)-898-0980, E-mail: herlynm@wista.wistar.upenn.edu