Tumor Progression, Early Diagnosis and Prognosis of Melanoma

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Primary melanomas evolve from melanocytes or from precursor lesions through two stages: the radial and vertical growth phases. The radial growth phase may be in situ or microinvasive, but is a non-tumorigenic neoplastic process, while the vertical growth phase is tumorigenic. The prognosis in radial growth phase is excellent irrespective of thickness or other variables. Curable radial growth phase melanomas can be recognized by surveillance of patients identified by screening for risk markers which include dysplastic nevi, common nevi, freckles, and other indicators of chronic or acute sun exposure or sun sensitivity. The prognosis in vertical growth phase depends on attributes of the tumor and of the host. The tumor mitotic rate, the presence of host tumor-infiltrating lymphocytes (TIL) within the vertical growth phase, and tumor thickness are the most powerful predictors of survival. New prognostic attributes are needed not only to provide for more accurate prognosis and diagnosis, but also to test the relevance of in vitro or animal studies in a human neoplastic system. Such attributes will be developed in the future based on markers that are associated with tumor progression. Candidate markers include growth factors and cytokines and their receptors, adhesion molecules and their ligands, chemotactic and motility factors, immune response-related molecules, and tumor-associated proteases. Some of these markers that are represented in the transition from radial to vertical growth phase will be reviewed. The tumor progression model presented here has been of value in the development of more accurate prognostic models, and in the elucidation of mechanisms of the malignant phenotype in melanoma.

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TUMOR PROGRESSION IN THE MELANOCYTIC SYSTEM

Malignant melanoma is a neoplasm that occurs for the most part on the skin, where its origins and evolution are susceptible to direct clinical observation supplemented if necessary by microscopic evaluation. Neoplasms of melanocytes constitute a model of tumor progression, a process whereby malignancy evolves in a stepwise fashion from benign or low-grade antecedent 'precursor' neoplasms, to intermediate lesions, to early malignant neoplasms, to advanced primary and to metastatic cancers. This process can be observed in most epithelial neoplastic systems (1). Melanoma can serve as a useful model for those neoplasms that are less accessible to direct observation. This manuscript will briefly discuss the morphology and some of the biology of stepwise tumor progression in the melanocytic system. The material has been expanded from a talk given at the Karolinska Institute, Stockholm, in July 1998, and has been abstracted and updated from several recent reviews (1–4).

The concept of tumor progression has been attributed to Rous, who discussed the 'process whereby tumors go from bad to worse' in 1935 (5, 6). In 1969 Foulds defined tumor progression as 'the development of a tumor by way of permanent irreversible changes in one or more of the characters of its cells'. He emphasized that the process was 'a revolutionary change in a portion of the old lesion establishing a new tumor having properties not formerly manifest' (5, 6). This focality of tumor progression is consistent with a model of clonal evolution, marked by the appearance of new sub-populations of cells with properties that confer a 'selective growth advantage' compared to normal cells (7). Early lesions in a tumor progression sequence are usually benign. Malignant neoplasms may arise within some of these benign initial lesions, which therefore are commonly characterized as 'precancerous' or 'precursor' lesions. Foulds emphasized, however, that progression does not always reach the endpoint of metastasis within the lifetime of the host. Animal studies cited by Foulds showed that most 'precancerous' lesions produced by a carcinogenic stimulus are stable, do not progress, and may commonly regress or involute. While in 'precursor lesions', the statistical chance of neoplastic development is greater than in normal tissue, they are best viewed in

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clinical context as markers of individuals at increased risk of 'cancer'.

Clark classified the steps of primary neoplasia as Classes I through III (8). Class I or 'precursor' lesions are characterized by stability, indolence, or regression, except for those rare lesions that progress to the next step. The Class I human melanocytic lesions include common acquired melanocytic nevi (Class IA); melanocytic nevi with an abnormal pattern of intraepidermal melanocytic growth ('aberrant differentiation', Class IB); and melanocytic nevi with dysplasia (Class IC). The Class I lesions in the Clark model are limited in their ability to invade tissue and their capacity for cell proliferation is self-limited. Class I lesions rarely progress to Class II lesions, which are limited in their ability to invade tissue, but their lesional cells tend to proliferate inexorably in the epidermal compartment of their origin. In the melanocytic system, Class II lesions include radial growth phase melanoma in situ (Class IIA), and invasive radial growth phase or 'microinvasive' melanoma (Class IIB). Class III lesions tend to proliferate inexorably, may invade and form tumor masses in stromal tissue compartments, and may have competence for metastasis. Class III melanocytic tumors include primary malignant melanomas with growth in the mesenchyme as well as in the epithelium of the primary site, lesions termed vertical growth phase (VGP) or 'tumorigenic' melanoma in this model. Because it is the first step in tumor progression that is associated with potential for metastasis, VGP is a pivotal lesion in melanocytic tumor biology.

The concepts of stepwise tumor progression originally based on morphological and experimental observations are supported by mathematical and molecular models. Several mathematical modeling studies have concluded that most cancers arise after multiple finite steps (9–13). Molecular genetic analysis of human and animal neoplasms also provides compelling evidence for stepwise lesional tumor progression driven by a series of mutational events. Vogelstein et al. have demonstrated that colorectal cancers arise as a result of mutational changes that activate oncogenes and cause the functional loss of tumor suppresser genes (14). The studies of Fearon et al. in colorectal carcinogenesis have established directly that mutations of at least four to five genes are necessary to generate a malignant tumor (carcinoma), while fewer changes are required to form a benign tumor (adenoma) (14). In this model, lesions termed early, intermediate and late adenomas represent tumors of 'increasing size, dysplasia and villous content'. The risk of carcinoma presumably increases within this progression sequence.

Risk markers for cancer are phenotypic or behavioral attributes that are statistically associated with increased incidence of cancer. Risk may be assessed in populations either in case-control studies or in cohort studies where individuals with particular attributes are followed and their cancer risk is determined by direct observation. Both of these methods have been used to determine the major risk markers for melanoma. The single most important risk marker is the presence on the skin of clinically dysplastic nevi. These may be regarded as 'intermediate lesions' of tumor progression, in that approximately 30% of melanomas arise in association with a precursor nevus, which is most commonly dysplastic (1, 15). In addition, family and personal history of melanoma are important markers of risk for subsequent melanoma (16). A third major category of risk markers includes indicators of acute and chronic exposure to the sun, including freckles, actinic skin damage, and a history of sunburn (17). Attention to these markers in oncologic patients and their first-degree relatives can identify a population of individuals whose risk for melanoma ranges from several-fold to more than a hundred-fold greater than that of random population members (16). Efforts directed at early diagnosis in these individuals can result in recognition of melanomas in their early, curable stages (18).

Most melanomas evolve through an initial stage termed the radial growth phase (in situ or 'microinvasive' melanoma) in which the probability of cure approaches 100% (19, 20). Despite recent improvements in the earlier recognition of cutaneous melanoma, by the time of diagnosis about 70% of melanomas currently have evolved to the next stage of tumor progression, VGP or 'tumorigenic' melanoma. In tumorigenic melanomas, the probability of cure is related to certain attributes of the tumor and of the host. These attributes can be used to develop prognostic models that may be useful in estimating the probability of cure for individuals and for groups of patients. Attributes that have been identified as strong independent predictors of survival in VGP melanoma patients include tumor thickness, mitotic rate, and tumor-infiltrating lymphocytes (TIL) (21). Other biologically relevant prognostic variables will likely be developed based on tumor progression markers that may have functional significance in mechanisms of metastasis.

One of the most important candidate prognostic markers is the $\beta 3$ integrin subunit which when complexed with an appropriate α subunit such as αv is a receptor for vitronectin and other matrix molecules. This cell surface adhesion receptor has been demonstrated to play a role in adhesion of melanoma cells to matrix molecules, migration of melanoma cells in vitro, proliferation of melanoma cells in response to ligand, activation of the collagenase MMP-2, and protection against apoptosis (22–29). Routine utilization of this and other candidate markers will increase the precision of diagnosis and prognostication for melanomas in the near future.

EARLY TO INTERMEDIATE LESIONS OF MELANOCYTIC TUMOR PROGRESSION: NEVI AND DYSPLASTIC NEVI

Melanocytic nevi in tumor progression

Melanocytic nevi are focal tumor-like lesions that may be regarded as benign neoplasms of melanocytes. Except for cosmetic significance, these nevi are important only for their relation to melanoma. We shall review evidence that nevi and especially dysplastic nevi are important simulants of melanoma, potential precursors of melanoma, and markers of increased risk for melanoma

Common acquired nevi

Nevi are almost ubiquitous in humans, appearing first in early childhood, and reaching a maximum in young adults (30). There is evidence that sunlight is a major etiologic factor for nevi. From migrant studies it appears that sun exposure in the first 10 years of life is especially important (31). The epidemiological risk factors for nevi are similar to those for melanoma, including sun exposure and sun susceptibility factors (30), and genetic factors (32). Abundant clinical and anecdotal evidence (reviewed previously (33)) indicates that nevi may be potential precursors of melanoma, though the frequency of such progression is very low. The total number of nevi is also one of the strongest risk factors for malignant melanoma (34), and nevi are also common potential simulants of melanoma.

At any stage of their evolution, common acquired nevi are usually smaller than 5 mm in diameter, and rarely larger than 10 mm. They may be flat pigmented junctional nevi, raised pigmented compound nevi, or raised non-pigmented dermal nevi, but in any case they are symmetrical, with regular well-defined borders. There may be slight nuclear size and shape variation, but there is no high-grade atypia histologically (35).

Dysplastic nevi

Dysplastic nevi, first described in hereditary melanoma kindreds in 1978 (36, 37) and in non-familial ('sporadic' or 'random') melanoma patients in 1980 (38), are also known as 'familial atypical multiple mole melanoma moles (FAMMM moles)' (39) or as 'clinically atypical nevi' (40–43). Numerous case-control studies have also demonstrated that similar lesions occur in individuals without a family or personal history of melanoma, and that they are markers of individuals at increased risk for melanoma (34, 40, 44–48). In a classification based on family history of melanoma and dysplastic nevi (49), it was proposed that the relative risk for melanoma ranged from a relatively low risk in patients with dysplastic nevi without a family or personal history of melanoma (type A) to a highest risk in patients with dysplastic nevi and a history of melanoma in two family members (type D-2). Clinically, dysplastic nevi are defined as lesions greater than 5 mm in diameter, with a macular component always present by definition. A papule, if present, is usually located in the center of an ovoid macule, and the lesions are generally symmetrical. The border is usually fairly regular, but it is characteristically ill-defined or 'fuzzy' about all or a part of its circumference. The lesions are usually brown or tan, with some variegation of hue and depth often including salmon-pink shades, but without black or blue in most cases (36, 38, 50–53). Substantial asymmetry, excessive pigmentary variegation, focal black areas or gray areas suggestive of partial regression should prompt biopsy to rule out melanoma

The three salient histologic features of dysplastic nevi are the presence of an immature or disordered pattern of growth, of focal ('random') cytologic atypia in melanocytes, and of a lymphocytic host response (36–38, 53–55). These parallel the attributes of dysplasia in other neoplastic systems. The criteria for diagnosis and studies of diagnostic reproducibility under different circumstances have been reviewed (1).

Nevi and dysplastic nevi as melanoma risk factors

The significance of nevi as markers of individuals at increased risk for melanoma has been evaluated by two epidemiological methods: cohort and case-control studies. Cohort studies have been published for hereditary melanoma kindreds and for populations followed in speciality clinics. Case-control studies have compared the prevalence of nevi in melanoma patients and in the community at large. The various studies have considered unselected nevi, typical nevi, and/or dysplastic nevi. The cases have usually been drawn from speciality clinics but occasionally from population-based registries, as have the control groups. Here, we briefly discuss important studies in sporadic melanoma.

Case-control studies in 'random' melanoma

Case-control studies, by comparing prevalences in patients with melanoma (cases) with those in controls, have established that nevi and clinically dysplastic nevi are associated with increased risk for melanoma in people who are not members of hereditary melanoma kindreds (Table 1). These studies have shown that dysplastic nevi are relatively common in the community, their incidence ranging from 5 to 20% (median 13%). Other risk markers identified in case-control studies include total number of nevi (17, 34, 46, 56), the presence and number of freckles (17, 40), the number of large nevi, a derivative of nevus number and size called total nevus density (57, 58), quantitative history of sunburn (17, 59), and sometimes other markers of sun exposure or susceptibility. In every case-control study reported, the relative risk for melanoma is increased in patients who have clinically dysplastic nevi, and this risk persists after adjustment for the total number of nevi and other risk factors. The relative risk estimates for common

nevi and dysplastic nevi are presented in Table 1. The estimates (from different continents and time periods) are remarkably consistent, in the four to eight-fold range for both common nevi and dysplastic nevi as independent traits. Most studies have demonstrated a dose-response relationship, with risk for melanoma increasing as the number of nevi or dysplastic nevi increases (Table 1). The studies reviewed above have been based on clinically-defined 'dysplastic or 'atypical' nevi. The only formal case-control study that has reported a relative risk estimate for histologic dysplasia as a risk marker for melanoma is that of Augustsson et al. (Sweden) (60). These authors reported a relative risk of 4.6, which is in the same range as that from the clinical studies reviewed above.

Cohort studies in 'random' and hereditary melanoma

These findings have been confirmed by cohort studies that have included patients from both hereditary and random kindreds. In Green's and Tucker's long-term study of familial melanoma kindreds, 47 melanomas occurred in follow-up, all in family members with dysplastic nevi. The cumulative risk for melanoma by age 50 among family members with dysplastic nevi was 48.9%, and the risk of developing melanoma was increased 85-fold in family members with dysplastic nevi (62). In Halpern's follow-up study of patients with dysplastic nevi with and without a family or personal history of melanoma, the age-adjusted incidence rate of development of melanoma was strongly correlated with prior history of melanoma (Table 2) (16). The incidence of melanoma in patients with dysplastic nevi but no history of melanoma was about 15 times above population rates. This estimate is probably biased upward somewhat because of a tendency toward a more 'florid' dysplastic nevus phenotype in the clinic-based follow-up cohort. Even so, it is similar to the estimates of two to twelve-fold excess risk in the case-control studies cited above, and the seven-fold risk estimated by Kraemer et al. from theoretical considerations (63). The risk in patients with dysplastic nevi who have both a prior personal and a family history of melanoma ranges up to an astounding 1269-fold observed to expected incidence ratio in Halpern's study (16). This and other studies demonstrating that dysplastic nevi are a major independent risk factor for subsequent primary melanoma in individuals who have had prior melanoma are indicative of the need for lifelong cutaneous surveillance of these patients, who are not uncommon in oncologic, dermatologic, and surgical clinics (16, 64–69).

The cohort studies, as well as other studies of familial melanoma kindreds have demonstrated that most melanomas detected in surveillance studies are early lesions, for which cure can be confidently expected by simple therapy (18, 70). Thus, the recognition of clinically dysplastic nevi permits focused efforts in patient education and surveillance in a high-risk group, especially for individuals who have a personal or family history of melanoma, large numbers of dysplastic lesions, or a complex phenotype with multiple risk factors.

INTERMEDIATE TO ADVANCED LESIONS OF MELANOCYTIC TUMOR PROGRESSION: RADIAL AND VGP MELANOMAS

Clinical and histologic features of radial and VGP

The term 'radial growth phase' (RGP) refers to a clinical metaphor of melanoma growth as a pigmented patch or plaque that expands more or less inexorably along the radii of an imperfect circle. A useful diagnostic mnemonic, 'ABCD', has been widely used (71). These criteria include lesional Asymmetry (one half of a lesion does not match

Table 1

Relative risk estimates for common and dysplastic nevi

	Study	Adjusted relative risks
	Common nevi	Dysplastic nevi
Nordlund et al., 1985 (Australia (45))		7.7 (any atypical nevi)
Holly et al., 1987 (San Francisco (34))	4.4 (26–50 nevi)	3.8 (1–5 dysplastic nevi)
	6.2 (51–100 nevi)	3.9 6.3 (6+ dysplastic nevi)
Garbe et al., 1989 (Germany (48))	7.3 (41–60 nevi)	11.4 (1–2 atypical nevi)
	14.7 (>60 nevi)	6.0 (>2 atypical nevi)
MacKie et al., 1989 (Glasgow (61))	6.7 (>20 nevi)	2.1 (1–2 atypical nevi)
		4.4 (3+atypical nevi)
Halpern et al., 1990 (Pennsylvania (46))	6.5 (>25 nevi)	6.8 (any dysplastic nevi)
Augustsson et al., 1991 (Stockholm (60))	1.4 (75–149 nevi)	2.5 (1–2 atypical nevi)
	(1–2 atypical nevi)	15.6 (>2 atypical nevi)
	3.9 (>149 nevi)	4.6 (histologic dysplasia)
Tucker et al., 1997 San Francisco, Philadelphia (17)	1.6 (25–49 nevi)	7.3 (2–4 dysplastic nevi)
	2.5 (50–99 nevi)	4.9 (5–9 dysplastic nevi)
	3.1 (>100 nevi)	12 (>9 dyplastic nevi)

Table 2

Incidence rates of melanoma in follow-up of dysplastic nevus cases. (Rates per 100 000 patient-years, from Halpern et al. (16))

Unselected Pennsylvania residents Personal history of melanoma	Total melanomas Total melanomas in family	Crude incidence Crude incidence rate	incidence rate * Age-adjusted incidence rate *	1 269 O/E ratio **
Unselected Pennsylvania residents		9.7	9.7	1
Negative	0 or 1	295	154	16
Positive	1	1 479	968	100
Negative	≥2	2 838	1 955	201
Positive	≥2	2 966	12 313	1 269

Rates were standardized to the 1985 Pennsylvania population using the direct method. 1985. Pennsylvania (white) overall melanoma incidence = 9.7/100 000.
O/E > ratio of observed to expected incidence rates.

the other in shape or in color distribution); lesional Border irregularity (lesions tend to have an indented coastline like the map of a small island); lesional Color variegation (the surface is multicolored including shades of tan, brown, blue-black, gray-white and other variations); and lesional Diameter generally > 6 mm (though some melanomas may be excised when their diameter is less than this value (72), the discovery of such small lesions is often serendipitous).

Clinically, RGP lesions form patches or plaques, and histologically this morphology is explained by the fact that most of the lesional cells in the RGP are located in the epidermis, which is the compartment in which lesional cell proliferation occurs. By definition, the cells are entirely within the epidermal compartment when the melanoma is in situ. In many melanomas, however, the in situ phase is relatively short-lived, and in these cases a few lesional cells extend into the papillary dermis to constitute 'microinvasive' melanoma. If there is no evidence of tumorigenic proliferation in the dermis (defined below), a microinvasive melanoma is still considered to be within the RGP, and is not specifically distinguishable from an in situ melanoma on clinical grounds. Whether microinvasive or not, RGP melanomas lack competence for metastasis; in our large database of clinical stage I melanoma cases, the eight-year survival rate from invasive or in situ RGP melanomas was 100 > 1% (20, 73).

Clinically and histologically, the VGP is qualitatively different from the plaque-like RGP (Fig. 1). Often within the confines of a previously indolent plaque lesion, the VGP makes its appearance as an expanding papule, which grows in three dimensions in a balloon-like fashion to form a nodule. The critical biologic feature that distinguishes VGP from RGP is the capacity for proliferation of melanoma cells in the extracellular matrix of the dermis to form an expansile mass (3). In contrast, RGP melanoma cells have the capacity to proliferate inexorably in the epidermal but not in the dermal compartment. In the epidermis, the RGP melanoma cells are located in the normal microenvironment of the melanocyte, which is a labile cell that can proliferate in response to a stimulus such as sunburn (74). The importance of VGP may be explained in relation to the concept of tumorigenicity. Tumor formation as a result of lesional cell proliferation in the matrix of a distant site is essential to the development of a clinical metastasis. A neoplasm whose cells lack the capacity to proliferate in the matrix at its local site of origin is unlikely to have the capacity to do so in a metastatic site. Thus, tumorigenic VGP is considered to be present in a melanoma when any mitoses are present in the dermis, or when a mass of melanoma cells is present in the dermis that contains at least one cluster (nest) that is larger than the largest intra-epidermal cluster. Either of these findings is presumptive evidence of a neoplasm with capacity for tumorigenic expansile growth in the dermis (Fig. 1).

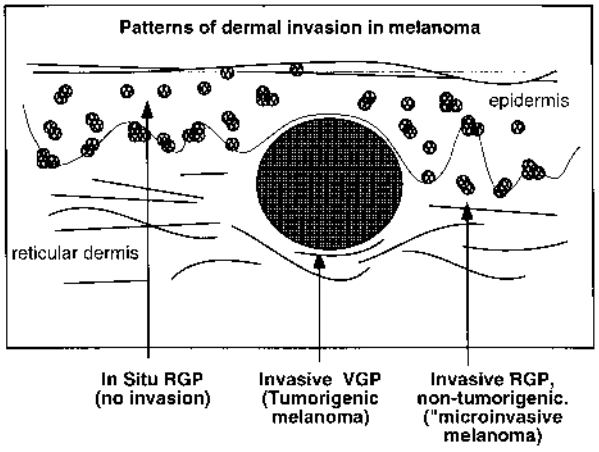

Fig. 1. Schematic diagram showing radial growth phase melanoma, in situ on the left, and invasive on the right, adjacent to a vertical growth phase papular or nodular component. The key feature of the vertical growth phase is tumorigenic proliferation in the dermis. A melanoma that is entirely in the radial growth phase may be termed non-tumorigenic. In many complex primary melanomas, all three components can be seen together. Other melanomas may be entirely in the radial growth phase, either in situ (no invasion), or invasive but non-tumorigenic ('microinvasive'). When tumorigenic vertical growth phase is absent, the prognosis is excellent, regardless of the presence or absence of invasion.

Table 3

Survival rates in Stage I melanoma by level of invasion or thickness

Level of invasion (Clark 1967)	Survival %	Thickness (Breslow) 1970	Survival %
I. Intraepidermal	100	0 mm	100
II. Papillary dermis, non-tumorigenic	98	<0.76 mm	98
III. Papillary dermis, tumorigenic	83	0.76–1.69 mm	86
IV. Reticular dermis invasion	76	1.70–3.60 mm	69
V. Subcutaneous invasion	<15	>3.60 mm	33

From Clark, 1967 (76) and 1989 (21).

Reproducibility of diagnosis of VGP

Interobserver agreement for diagnosis of VGP has been studied by a pathology panel established by the British Cancer Research Campaign (75). It was concluded that the findings 'emphasized the possibility of recognizing more threatening melanomas not only by thickness, but also by the VGP'. This panel also supported the use of the term microinvasion, as discussed above.

Prognostic modeling for melanoma based on tumor progression

Early prognostic models for melanoma were introduced about 25 years ago as single-variable models. Clark's levels of invasion relate to the anatomical extent of tumor in the skin, while Breslow's thickness relates to the measurement of thickness from the top of the granular layer to the deepest invasive tumor cell (76, 77). Either of these variables is related to survival, as shown from our data in Table 3.

Prognosis of RGP

Considerations reviewed above suggest that the cells of the RGP of primary melanoma do not have the capacity for tumorigenic proliferation, even though they are neoplastic and may have the capacity for invasion of the dermis. Since tumor formation may be regarded as an essential attribute of a metastasis, this consideration suggests that RGP lesions might not have the capacity to establish clinical metastatic disease. Accordingly, we reviewed survival data for invasive melanoma cases (Clark's level II or greater) that had presented in clinical stage I, had been accessioned prospectively, and had been treated by standard wide local excision to a disease-free state. The survival was 100% (with a 1% confidence limit) (19, 20). This important finding indicates that invasion, though it may be necessary for metastasis, is not sufficient, and that metastasis does not occur in the absence of the capacity for tumorigenic growth at the primary site. Fig. 2 shows cumulative survival rates for patients with microinvasive RGP and for patients with tumorigenic VGP melanomas thinner than 0.76 mm. As shown above in Table 3, the overall survival for all patients with such 'thin' VGP melanomas is 98%. Fig. 2 shows that the survival for patients with pure RGP melanomas (most of which are 'thin' by the above definition) is 100%. In striking contrast, the survival for patients with thin tumorigenic melanomas is 88% at 10 years and beyond. These data indicate that metastasis in 'thin' melanomas is confined to those thin lesions that also are tumorigenic (in VGP). Put another way, the presence of VGP explains metastasis from thin melanoma (20), with only a few anecdotal exceptions in our experience.

Barnhill et al. have analyzed prognostic factors in 548 melanoma patients from the Connecticut tumor registry (78). The survival of patients with VGP negative (RGP only) primary melanomas was 98.2%, similar to that for 'thin' (<0.76 mm, 97.9%) and for Clark level II melanoma (98.8%). However, only thickness and mitotic rate proved to be independent risk factors in a multivariable model. Taken together, these data are consistent with the hypothesis that invasive RGP lacks capacity for metastasis, with possible rare exceptions, some of which could be related to sampling error.

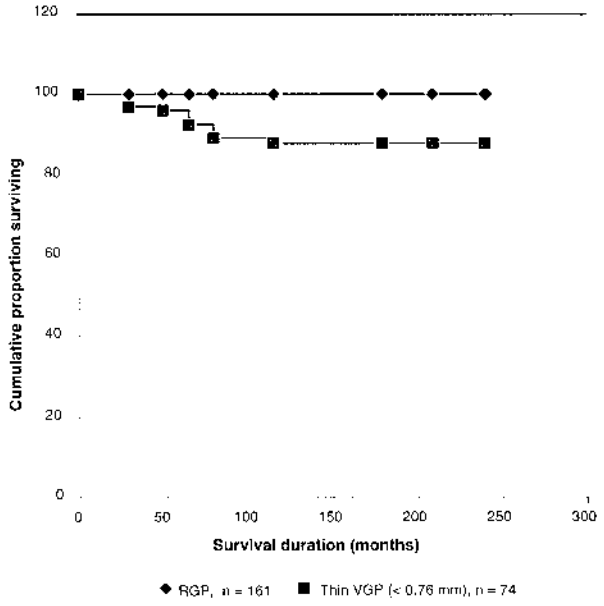

Fig. 2. Survival curves for 161 patients (diamonds) with non-tumorigenic invasive ('microinvasive') melanomas (radial growth phase, RGP), and 74 patients (squares) with 'thin' (Breslow thickness <0.76 mm) tumorigenic melanomas (vertical growth phase, VGP). All patients were followed until death or at least 10 years. Note that metastases in thin melanomas all occurred in lesions with VGP.

Table 4

Survival odds ratios for primary Stage I malignant melanoma

Attribute	Value	Survival odds *
Mitotic rate	>6.0 per square mm	1.0
	<6.0 per square mm	3.5
	0.0 per square mm	11.7
Tumor-infiltrating lymphocytes absent	1.0	
	Non-brisk	3.5
	Brisk	11.3
Tumor thickness	<1.7 mm	4.0
Anatomic site	Extremities v axial/subvol **	3.8
Patient gender	Female	2.9
Regression in radial growth phase	Absent	2.8

From Clark et al., 1989 (21).

* Survival odds ratio is an expression of the likelihood of survival of patients with different values of the attributes listed, compared to the baseline value. For example, a patient with a mitotic rate of 0.0 is 11.7 times more likely to survive than a patient with >6.0 mitoses per square millimeter. Conversely, the patient with the highest rate is 11.7 times more likely to die (mortality odds ratio).

** Subungual, palmar and plantar (volar) sites are included with the high-risk 'axial' group.

Prognostic model for VGP

The findings reviewed above indicate that survival is 100% for RGP melanomas and biological considerations suggest that the VGP is the source of metastatic cells. These considerations suggest that separate prognostic models should be developed based on attributes of the VGP compartment of melanomas.

Accordingly, we developed a multivariable prognostic model based on logistic regression analysis of a series of prospectively followed, clinical stage I invasive melanoma cases (21). As the survival of invasive pure RGP cases was 100%, these were not used for model building. We studied multiple clinical and histological variables, including treatment modalities. The attributes that entered the final model are shown in Table 4 with their associated survival odds ratios (approximating relative risk) for eight-year survival. For example, an individual with a mitotic rate of zero is almost 11-fold more likely to survive than an individual in whose melanoma the mitotic rate is >6 per square millimeter, other attributes being held constant (Table 4).

The regression model provides an equation that allows for the estimation of prognosis for a patient with a particular set of attributes. Since the process of logistic regression analysis involves the use of categorical variables, there is a limited set of possible outcomes. These possible outcomes have been published in tabular form (3, 21, 79). The VGP prognostic model includes attributes of the tumor as well as some attributes of the patient. The strongest prognostic attributes are mitotic rate, TIL, and thickness. The analysis of mitotic rate, like that of thickness, is a relatively simple quantitative determination that can be done by any pathologist using simple equipment. The prognostic significance of mitotic rate has also been recognized by others (80, 81). We characterize the lymphocytic host response to the VGP as either infiltrative (TIL), or non-infiltrative. In the infiltrative pattern, lymphocytes extend among tumor cells, often rosetting around individual cells and sometimes associated with observable cellular degeneration (21). The TIL response in the VGP is a strongly favorable finding, which has been confirmed in a study conducted by the WHO Melanoma Study Program based in Milan (82).

The tumor progression-based prognostic model reviewed above has been validated in one independent study in which 55 patients were followed for eight years or more and categorized as to the presence or absence of VGP. The survival of the RGP cases was 100%. Using an arbitrary 50% cutoff (probability of survival >50% is taken to predict survival) the model for VGP was 85.1% accurate in predicting eight-year survival (83). This accuracy is virtually identical to the 83.1% accuracy achieved by the original model in a 'hold-back' sample of 71 cases that had not been used for model-building. The overall accuracy of the original model, including the RGP cases in which accuracy was 100%, was 89%.

Need for new prognostic and diagnostic markers

Although the 89% accuracy of the VGP multivariable model presented above is superior to that of predictions based on thickness alone, the reliability of predictions in the middle of the probability range is insufficient to encourage their confident use. Additional prognostic variables are needed to improve the specificity and predictive value of this and other extant models for Stage I melanoma in the selection of therapy or in stratification for therapeutic trials. A search for such variables should begin with the rapidly expanding body of knowledge having to do with mechanisms of tumor progression in melanocytic and other neoplasms. Candidate markers for use in addition to traditional histopathology should have the following attributes.

Methods for their demonstration should be accessible to routine pathology laboratories. At the present time, for example, immunohistochemistry is universally available to pathologists, but in situ hybridization for RNA or DNA (fluorescence in situ hybridization, FISH) is not. Although image analysis is available in many laboratories, there is no standardization as to methodology.

The methods should be applicable to routine formalin-fixed, paraffin-embedded material. The routine collection of melanomas as frozen material is not practicable because

of several logistic considerations. For example, most melanomas are excised in physician's offices, where facilities for snap-freezing are not available. Systems for transport of frozen material are not routinely provided by laboratories. Many melanomas are not recognized as such until they have been examined in paraffin sections by pathologists. Routine use of frozen sections for diagnosis could create serious diagnostic difficulties and potential errors because of the greatly inferior quality of frozen sections compared to paraffin.

The reading of results should be specific and reproducible and the time required to generate the data should be reasonable and consistent with the demands of busy practitioners.

Molecular tumor progression markers as prognostic variables

As has been demonstrated in the colon adenoma-carcinoma progression models of Vogelstein's group (14), the tumor progression 'steps' of Foulds and Clark likely represent mutational events that affect attributes of the neoplastic phenotype, such as cell proliferation, invasion, migration, and metastasis. Factors involved in mechanisms of progression can potentially be identified by comparing 'early' lesions of progression (common nevi, dysplastic nevi, microinvasive melanomas) with later lesions (tumorigenic primary and metastatic melanomas). Some of these factors may be identifiable immunologically as 'tumor progression antigens' (or by molecular methods as mRNA species). These factors may therefore be known generally as 'tumor progression markers'. Study of these markers may illuminate mechanisms of acquisition of the malignant phenotype (invasion, tumorigenicity in the extracellular matrix of the dermis or of distant sites, metastasis and so on), and may also provide the basis for new diagnostic and prognostic tests for malignancy. An example of one mechanism that appears to be of particular relevance to the important transition between non-tumorigenic and tumorigenic melanoma (RGP to VGP) will be discussed below.

Cell culture, cytogenetics, oncogenes and tumor suppressor genes

The biology of tumor progression in melanoma has been recently reviewed (4, 84–86). Several lines of evidence are consistent with the hypothesis that the cells of radial and VGP lesions differ in several important properties that relate to the malignant phenotype. First, despite considerable effort, only a few permanent cell lines have been established from RGP cells (87). Most initial cultures have tended to proliferate slowly for a time, but never reach confluence and ultimately senesce, taking on a spindled fibroblast-like morphology. In contrast, cell lines are readily established from VGP primaries and from metastases. Furthermore, cells from short-term culture of RGP lesions are weakly if at all tumorigenic in immunodeficient mice, in contrast to VGP and metastatic melanoma cells, which often readily form tumors in these animals (87).

When studied by traditional cytogenetics, the cells of RGP primaries tend to exhibit minor random chromosomal abnormalities, while cells from VGP primaries, like those from metastases, exhibit more extensive abnormalities that tend to be distributed non-randomly to particular chromosomal locations (88, 89). The existence of these non-random lesions has provided clues to the location of genes relevant to melanoma susceptibility and progression, as has also been the case in other cancers. For example, the common finding of extra copies of chromosome 7 has been linked to the over-expression of the EGF receptor (90). More recent studies have screened DNA from fresh tumors and cell lines for loss of heterozygosity (deletion of all or part of one member of a chromosomal pair). Non-random chromosomal abnormalities and loss of heterozygosity are commonly observed in the short arm of chromosome 9 (91–93), and these findings coupled with linkage studies have recently led to the discovery of a tumor suppressor gene whose protein product (p16) is an inhibitor of the cell cycle (93, 94). Mutations of this gene have been associated with both progression (94), and familial susceptibility (94, 95), for melanoma. Other regions in which non-random chromosomal abnormalities have been identified are located on chromosomes 1, 3, 6, 10, 11, and 19, suggesting that these are sites of relevant oncogenes or tumor suppressor genes (96–99). Mutations or other regulatory disturbances have been described in other well-known oncogenes and tumor suppressor genes or their products, including p53, ras, myb, Bcl-2, and others (100–106). Most of these latter changes have been observed in late stages of melanoma progression (advanced primaries and metastases). Taken together, the findings reviewed above are consistent with the hypothesis that melanomas evolve through a series of tumor progression steps that may be related to the sequential acquisition of mutations in particular oncogenes. However, the genes critically involved in this progression remain to be elucidated (Table 5).

Genetic abnormalities may lead to altered expression of proteins that control manifestations of the malignant phenotype such as invasion, tumorigenicity, or metastasis. For example, some such gene products have been demonstrated to be growth factors such as fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), (107–110), or cytokines such as IL-8 (111). Such factors may stimulate growth of the tumor cells themselves by an 'autocrine loop' mechanism, or alternatively may stimulate proliferation of stromal cells such as fibroblasts or endothelial cells that may be necessary for continued tumor growth (a 'paracrine' function). Those products that are over-expressed in more advanced compared to earlier stages of tumor progression may be defined as tumor progression

markers (112). Some of these may be detectable in situ by immunohistochemistry or by other means. These progression markers may be of utility in the diagnosis of malignancy, or in the estimation of prognosis. Since VGP is the stage of tumor progression in melanoma at which competence for metastasis may be established, we are especially interested in markers of the important progression step from RGP to VGP. One such marker, the $\beta 3$ integrin subunit, is reviewed below.

The integrin family of cell-matrix adhesion molecules

Integrins are cell-matrix adhesion receptors that are heterodimers of an α subunit that is non-covalently associated with a β subunit (113). The major integrin families are defined by a common β subunit. We and others have shown that melanoma cells express $\alpha 1$ through $\alpha 7$ of the $\beta 1$ family, and αv which can associate with several β subunits including $\beta 3$, $\beta 5$, $\beta 6$, and $\beta 8$ (4, 114). The $\alpha 2\beta 1$ and $\alpha 4\beta 1$ integrins are overexpressed in advanced melanomas (114, 115). Expression of the former has been demonstrated in highly metastatic melanoma cell lines (116, 117). The $\beta 3$ integrin subunit was first identified on human metastatic melanomas but not on melanocytes using an antibody raised against platelet glycoprotein IIb/IIIa, which shares the same $\beta 3$ subunit (118).

The integrin $\beta 3$ subunit associated with the αv chain has affinity for vitronectin, fibronectin, von Willebrand factor, fibrinogen, collagen, and thrombospondin (119). Human melanoma cells derived from lymphatic metastases use integrin $\alpha v\beta 3$ to adhere to lymph node vitronectin and this adherence correlates with metastatic ability in nude mice (23). Vitronectin or antibody binding to the $\alpha v\beta 3$ receptor results in growth stimulation by a mechanism that involves internal signaling and may require activation by PDGF (24). Differential expression of the $\alpha v\beta 3$ and $\alpha 5\beta 3$ integrins variably modulates the 72 kDa type IV collagenase and results in increased invasion of gelatin (25, 26). The $\alpha v\beta 3$ and $\alpha 5\beta 31$ integrins are also expressed on endothelium; this expression is enhanced by IL-1 stimulation, and mediates increased adhesion to endothelium (27). The $\alpha v\beta 3$ receptor has been shown to prevent apoptosis of melanoma cells in dermal collagen (28). Hsu et al. demonstrated that the adenoviral gene transfer of $\beta 3$ integrin subunit induced conversion from RGP to VGP in primary human melanoma. In an artificial skin reconstruct model, untransfected RGP cells remained confined to the epidermis and underwent apoptosis on descent into the artificial dermis (a matrix of collagen fibers within which fibroblasts are suspended). After transfection, the cells survived and proliferated to form tumors in the dermis. In this model, forced expression of functional $\alpha v\beta 3$ integrin in the RGP primary melanoma cells (i) promoted both anchorage-dependent and anchorage -independent growth; (ii) initiated invasive growth from the epidermis into the dermis in the three-dimensional skin reconstructs; (iii) prevented apoptosis of invading cells; and (iv) increased tumor growth in vivo (29). Thus, $\beta 3$ integrin expression is involved in five mechanisms of importance to the metastatic phenotype: growth, migration, adhesion, invasion, and protection from apoptosis in the dermis. All of these mechanisms are likely to be of importance in the transition from non-tumorigenic RGP to tumorigenic VGP melanoma.

We have demonstrated in frozen and in paraffin sections that the $\beta 3$ subunit is expressed in the VGP of primary melanomas and in metastases, but not in earlier lesions of tumor progression, including RGP primary melanomas (114, 120, 121). Using two different antibodies, we studied formalin-fixed, paraffin-embedded melanocytic lesions for expression of the $\beta 3$ integrin subunit (Fig. 3).

Expression of $\beta 3$ integrin is homogeneously negative (with rare exceptions) in melanocytes, common acquired nevi, and RGP, heterogeneously positive in VGP, and homogeneously positive in metastases. This heterogeneity in VGP suggests that this molecule may be a very useful marker of prognosis. The significance of $\beta 3$ integrin expression in relation to prognosis in primary melanoma has recently been studied (122). Expression of $\beta 3$ integrin was detected in 107/160 primary melanomas (69%). The $\beta 3-$ integrin-positive ($\beta 3+$) tumors were thicker (mean 2.98 ± 0.3 mm) than the $\beta 3-$ integrin-negative ($\beta 3-$) melanomas (mean 1.64 ± 0.2 mm) ($p = 0.002$). Patients with $\beta 3+$ melanomas were more likely to relapse (57/107, 53%) and to die from disease (45/107, 42%) than those with $\beta 3$ tumors (6/53, 11%; and 4/53, 8%, respectively)

Table 5

Summary of tumor progression biology in melanoma (105, 106)

	RGP	VGP	Metastases
Growth in culture	slow	Rapid	Rapid
Permanent lines	No	Yes	Yes
Tumorigenicity in immunodeficient mice	No	Yes	Yes
Cytogenetic abnormalities	Few, random	More, some non-random	Many, some non-random
Growth factor production	No	Yes	Yes
Growth factor independence	No	Infrequent	Often
Progression antigens	Low	High	High

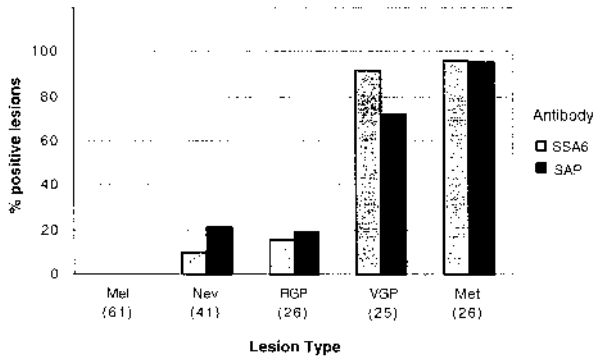

Fig. 3. Reactivity of melanocytic lesions, using two monoclonal antibodies, SAP and SSA6, directed against the β3 integrin subunit. The columns show the percentage of lesions or lesional compartments positive for expression of the marker in $> 5\%$ of the lesional cells. Mel = melanocytes (n = 62), nev = nevus cells (n = 41), RGP = radial growth phase compartments (n = 31), VGP = vertical growth phase compartments (n = 25), met = metastatic melanoma lesions (n = 26).

($p < 0.001$). Overall survival was greater for $\beta 3-$ than for $\beta 3+$ patients (mean 102 ± 9 vs. 69 ± 6 months) ($p = 0.001$). These data show that $\beta 3$ integrin expression in primary cutaneous melanoma predicts subsequent metastatic progression.

CONCLUSION

Tumor progression, the process whereby tumors may evolve in stepwise fashion from precursor lesions, has clinical and biological implications. In the melanocytic system, the primary significance of the major potential precursor lesions, dysplastic nevi, is as markers of individuals at increased risk for melanoma. In addition, these lesions are important simulants of melanoma, and precursors of about one-third of melanomas. The development of a melanoma, whether de novo or within an antecedent nevus, is associated with acquisition of the property of clinically more or less inexorable growth. However, early lesions of melanoma, termed the RGP, do not form clinical or microscopic mass lesions (tumors). Whether in situ or 'microinvasive', these non-tumorigenic RGP melanomas are not associated with competence for metastasis, with only anecdotal exceptions. The onset of the next phase of progression, the tumorigenic or VGP, is of key importance because it is associated with potential competence for metastasis. The risk of metastasis can be assessed using clinicopathologic attributes, such as the mitotic rate, the presence of a TIL response, and the tumor thickness. It may be expected that additional prognostic attributes will be developed, based on expression patterns of molecules that are functionally involved in mechanisms of progression. One such molecule, the $\beta 3$ integrin subunit, has been shown in vitro and in animal models to be involved in adhesion to the matrix and in migration, in growth stimulation, in activation of proteases, and in protection against apoptosis (reviewed in (29)). As reviewed above, expression of this marker increases dramatically with the development of tumorigenicity in a primary melanoma. Another marker, Ki-67, which is a cell cycle-dependent nuclear proliferation marker (123), appears in preliminary studies to be expressed in a similar fashion (124). The biological properties potentially mediated by these and similar markers are among those which might be involved in progression from non-tumorigenic to tumorigenic melanoma. Based on these considerations, expression of these markers is likely to be useful in diagnosis, and also to be predictive of survival. Other prognostic and diagnostic markers will likely be discovered in the future through screening of expression profiles of candidate markers recognized as protein products or mRNA precursors.

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