Advances in Brief

Micropthalmia Transcription Factor: A New Prognostic Marker in Intermediate-thickness Cutaneous Malignant Melanoma[1]

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Abstract

Microphthalmia transcription factor (Mitf) is involved in melanocyte development and differentiation. The current study was undertaken to determine whether there is a relationship between Mitf expression and survival in patients with intermediate-thickness (1.0–4.0 mm) melanoma. Original paraffin blocks or slides of the primary tumor were accessible in 63 such patients. Mitf expression was evaluated by immunocytochemistry and analyzed visually. Slides were graded as follows according to the percentage of cells whose nuclei stained positive for Mitf: (*a*) 0, 0%; (*b*) +1, 1–25%; (*c*) +2, 26–50%; (*d*) +3, 51–75%; and (*e*) +4, >75%. Median follow-up was 50 months. Mean thickness was 2.2 ± 0.7 mm. Mean overall survival was 171.90 ± 13.12 months. Mean disease-free survival was 168.53 ± 13.96 months. Fifty-two melanomas (82.5%) stained positive for Mitf. By univariate analysis, mean overall survival and disease-free survival in patients whose melanomas did not express Mitf were 80.89 ± 17.98 months (median, 51 months) and 71.36 ± 19.87 months (median, 40 months), respectively. This compares with 187.90 ± 13.41 months (median, not reached) and 186.78 ± 13.84 months (median, not reached), respectively, for patients whose melanomas expressed Mitf ($P = 0.0086$ and $P = 0.0054$). These findings persisted in multivariate analysis. In addition, patients with >50% Mitf expression had significantly fewer nodal metastases after node dissection than patients with ≤50% Mitf expression ($P = 0.04$). Our data suggest that Mitf may be a new molecular prognostic marker in patients with intermediate-thickness melanoma.

Introduction

The incidence of cutaneous malignant melanoma continues to rise. It is estimated that in the year 2000, 47,700 new cases will be diagnosed, with 7,700 deaths (1). The prognosis of primary cutaneous melanoma is usually estimated by several histopathological criteria, such as tumor thickness, ulceration, levels of invasion, and mitoses/mm^2 (2). However, limitations in the accuracy of these prognostic markers have led investigators to search for additional clues in the microenvironment in primary cutaneous melanomas. Notable cellular and molecular markers include expression of various types of integrins (3, 4). Although these markers have helped define several subsets of patients with different natural histories, the search continues for additional prognostic markers.

A number of genetic and cell biological models indicate that pigment cell development and function depend on both cell-extrinsic and -intrinsic factors. Although how these factors are functionally integrated is poorly understood, it is generally agreed that the basic helix-loop-helix-zipper Mitf[3] relates to the downstream targets responsible for cell proliferation, fate determinism, and melanin biosynthesis (5, 6). Mutations in Mitf are found from fishes to man. In fact, Mitf is critical not only for neural crest-derived melanocytes of the integument and inner ear (7) but also for the cells of the retinal pigment layer, which develop from the neural epithelium of the optic cup.

Mouse mutations at the microphthalmia locus (mi) are characterized by small nonpigmented eyes, lack of integumentary melanocytes, and deficiencies in the inner ears. Also, these mutations are associated with a deficiency in mast cells and osteoclasts, resulting in a condition similar to osteopetrosis in humans. In humans, Mitf mutation is characterized by hearing deficiencies, white forelock, and ocular anomalies (Waardenburg syndrome IIA; Ref. 8). It is believed that Mitf can induce pigment cell-specific transactivation of tyrosinase and tyrosinase-related protein 1 (9–11). In addition, α-MSH has been shown to up-regulate the pigment gene expression via a cyclic AMP-mediated signal transduction pathway that stimulates the expression of Mitf (12). Moreover, it was reported that the c-Kit signaling pathway in melanocytes targets transcription factor for microphthalmia simultaneously for short-lived activation and net degradation (13). Recently, King *et al.* (14) reported that Mitf is a sensitive and specific melanocyte marker for melanoma diagnosis.

Because Mitf appears to be involved in melanocyte development and differentiation, we hypothesized that its expression correlates with survival in patients with intermediate-thickness (1–4 mm) melanoma. Our findings suggest that Mitf expression in the primary tumor correlates with patient survival.

Materials and Methods

Patients and Tumor Specimens. We analyzed the primary lesions of 63 patients with intermediate-thickness (1.0–4.0 mm) malignant melanomas treated in the Department of Surgical Oncology at the University of Illinois at Chicago. The slides and blocks were obtained from our melanoma tissue and sera bank. All patients have been managed by the Department of Surgical Oncology at the University of Illinois at Chicago. The clinical and follow-up data of these patients are maintained in the melanoma research registry.

Immunohistochemistry. Four-μm-thick sections were used for immunocytochemical studies. Standard avidin-biotin-peroxidase immunohistochemistry was performed with the mouse monoclonal IgG1 microphthalmia antibody Ab-2 (Neomarkers, Union City, CA). Antigen retrieval was accomplished with 1 mM EDTA buffer. We used 1% dried skim milk to block nonspecific binding before incubation with the primary antibody for 2 h (1:25 dilution). Aminoethylcarbazine was used as a chromagen.

Evaluation of Staining. The stained slides were graded as follows according to the percentage of cells whose nuclei stained positive for Mitf: (*a*) 0, 0%; (*b*) +1, 1–25%; (*c*) +2, 26–50%; (*d*) +3, 51–75%; and (*e*) +4, >75%. Cytoplasmic staining was excluded from the analysis. Representative immunohistochemical staining of two different primary melanomas is shown in Fig. 1.

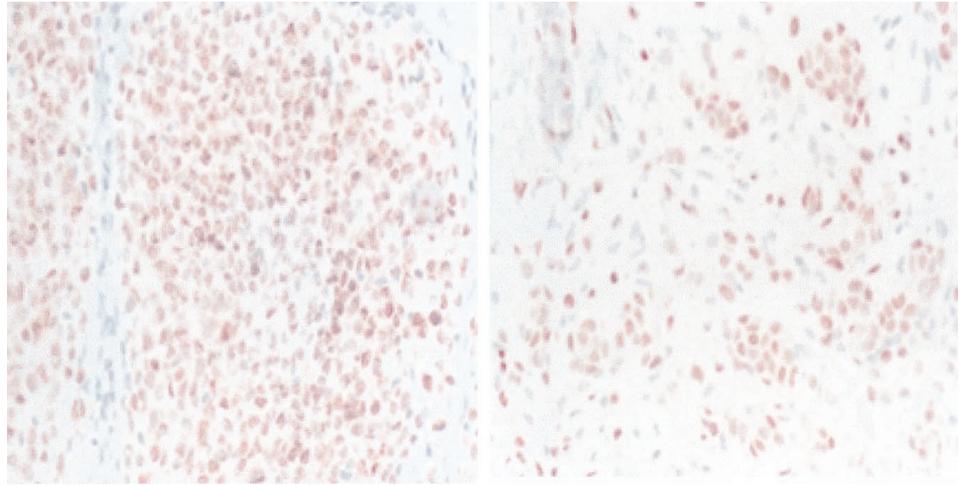[3] The abbreviations used are: Mitf, microphthalmia transcription factor; OS, overall survival; DFS, disease-free survival.

Fig. 1. Representative immunostaining of different levels of Mitf expression in two primary cutaneous malignant melanomas.

Statistical Analysis. Survival curves were generated by the Kaplan-Meier product limit estimate. Statistical comparisons were performed using the log-rank test. Outcomes evaluated were DFS and OS. We evaluated the effect of Mitf staining (graded from 0 to +4) on both DFS and OS. In addition, we arbitrarily evaluated the effect of three different cutoff levels of Mitf staining on survival: (*a*) 0 (negative) *versus* +1 to +4 (positive); (*b*) 0 to +1 ($\leq$25%) *versus* +2 to +4 (>25%); and (*c*) 0 to +2 ($\leq$50%) *versus* +3 to +4 (>50%). Pairwise association between Mitf staining and known prognostic variables such as thickness, histology, location of primary tumor, number of mitoses/mm^2, ulceration, and nodal status was also evaluated. Differences in distribution of variables were assessed by the Mann-Whitney and Kruskal-Wallis tests. Multivariate analysis was performed by using the Cox proportional hazards model. $P <$0.05 was considered statistically significant. Death from melanoma was considered the only event for OS. DFS events included regional recurrences and distant metastases.

Results

There were 36 males and 27 females. The mean age at diagnosis was 53.25 $\pm$ 15.49 years (age range, 17–81 years). The primary cutaneous melanomas were located on the trunk in 23 patients (36.5%), the lower extremities in 23 patients (36.5%), the upper extremities in 10 patients (15.9%), and the head and neck in 7 patients (11.1%). Thirty-one (49.2%) melanomas were superficial spreading melanomas, 18 (28.5%) were nodular melanomas, 9 (14.3%) were acral lentiginous melanomas, and 5 (7.9%) were lentigo maligna melanomas. Mean thickness was 2.2 $\pm$ 0.7 mm (range, 1–4 mm). Twenty-one melanomas were ulcerated (33.3%), and 42 (66.7%) were nonulcerated. The mean number of mitoses/mm^2 per high-power field was 4.3 $\pm$ 2.62 (range, 1–12). The majority of melanomas had Clark's level IV invasion (52 of 63 melanomas, 82.5%). Of 63 patients, 11 (17.5%) did not stain for

Mitf, 17 (27%) had +1 staining, 13 (20.6%) had +2 staining, 13 (20.6%) had +3 staining, and 9 (14.3%) had +4 staining. Complete node dissection was performed in 56 of 63 (88.8%) patients. Twenty-six (46.4%) of these patients were node positive, and 37 (58%) were node negative.

The median follow-up was 50 months. The mean DFS (Fig. 2*A*) was 168.53 $\pm$ 13.96 months (5-year DFS, 74.1%). Mean OS (Fig. 2*B*) was 171.9 $\pm$ 13.12 months (5-year OS, 74.5%).

Nodal status significantly affected survival in our cohort of patients. Mean DFS and OS in those patients who had nodal disease were 105.37 months (5-year DFS, 58.4%) and 109.90 months (5-year OS, 58.6%). In contrast, patients without nodal disease had a DFS of 192.55 months (5-year DFS, 82%; $P = 0.04$) and an OS of 193.54 months (5-year OS, 82.2%; $P = 0.05$).

In this small series of 63 patients, only thickness significantly influenced survival when evaluated by univariate analysis. The survival advantage was most marked when patients had primary melanoma thicker than 2.6 mm. In those instances, the DFS and OS were 66.48 months (5-year DFS, 60%) and 67.1 months (5-year OS, 58%), respectively, whereas the DFS and OS were 180.52 months (5-year DFS, 76.5%; $P = 0.067$) and 183.24 months (5-year OS, 78%; $P = 0.039$%), respectively, in patients with primary lesions <2.6 mm thick.

Curiously, when stratified according to the presence or absence of ulceration in the primary tumor, ulceration was significant only in patients whose melanomas were >2 mm thick. In patients with ulceration, the DFS and OS were 119.41 months (5-year DFS, 58.9%) and 123.20 months (5-year OS, 60%), respectively, compared with 160 months (5-year DFS, 80.7%; $P = 0.086$) and
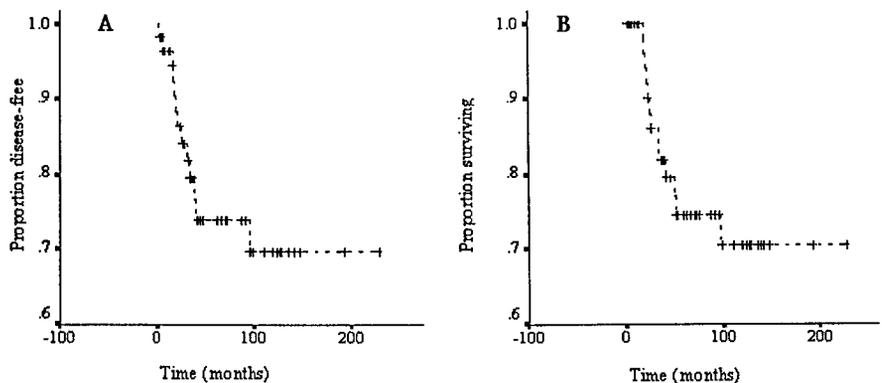

Fig. 2. Kaplan-Meier survival curves of the entire population. *A*, DFS. *B*, OS.

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Table 1 *Influence of Mitf expression on DFS and OS*

Mitf expression	N	DFS[a]	OS[a]
0	11	71.36 (P = 0.048)	80.89 (P = 0.077)
+1	17	162.32	172.73
+2	13	119.62	120.05
+3	13	[b]	[b]
+4	9	105.8	106.8
Absent	11	71.36 (P = 0.0086)	80.89 (P = 0.0054)
Present	52	186.78	187.9
≤25%	28	127.14 (P = 0.023)	136.68 (P = 0.039)
>25%	35	137.84	147.32
≤50%	41	127.27 (P = 0.035)	133.37 (P = 0.054)
>50%	22	212.57	212.64

[a] Mean values in months.
[b] All observations were censored; mean values were not calculated.

165.14 months (5-year OS, 82.7%; $P = 0.05$) in patients with nonulcerated primaries.

Expression of Mitf as determined immunohistochemically in the primary lesion appears to significantly influence both DFS and OS (Table 1; Fig. 3). Moreover, by applying semiquantitative methods, it appears that the amount of Mitf expression could be directly correlated to both DFS and OS. Mean DFS and OS in the 11 patients whose melanomas did not stain positive for Mitf were 71.36 months (median, 40 months; 5-year DFS, 37.6%) and 80.89 months (median, 51 months; 5-year OS, 42.1%), respectively. These figures are significantly lower than those for patients who showed evidence of Mitf expression, for whom the mean DFS and OS were 186.78 months (5-year DFS, 83.1%; $P = 0.0086$) and 187.9 months (5-year OS, 84%; $P = 0.0054$), respectively. Mean DFS and OS with >25% of the melanoma cells staining positive for Mitf expression were 137.84 months (5-year DFS, 92.2%; $P = 0.023$) and 147.32 months (5-year OS, 92%; $P = 0.039$) compared with cells in which <25% of the cells stained positive for Mitf expression, in which the DFS and OS were 127.14 months (5-year DFS, 55.8%) and 136.68 months (5-year OS, 58%), respectively. This observation was most marked in DFS where >50% of the cells expressed Mitf. DFS was 212.57 months (5-year
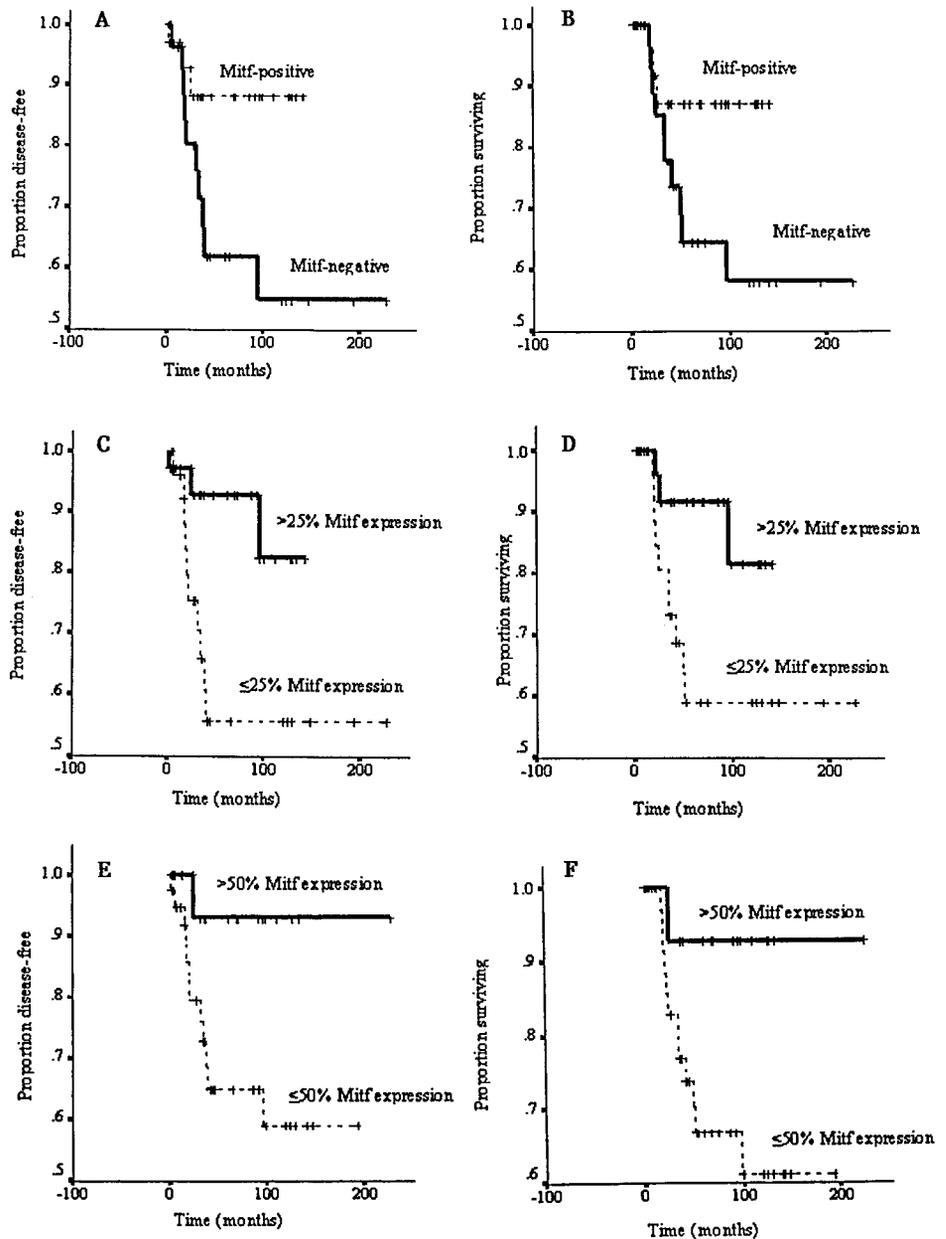

Fig. 3. Kaplan-Meier survival curves that compare patients with different levels of Mitf expression. DFS and OS were significantly better for those patients whose melanomas stained positive for Mitf [*A* (DFS), $P = 0.0086$; *B* (OS), $P = 0.0054$] and for those patients whose melanomas expressed >25% Mitf [*C* (DFS), $P = 0.023$; *D* (OS), $P = 0.039$]. DFS was significantly better for those patients whose melanomas expressed >50% Mitf (*E*, $P = 0.035$). OS tended to be better for those patients whose melanomas expressed >50% Mitf (*F*, $P = 0.054$).

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Table 2 *Multivariate Cox regression analysis of all prognostic variables*

Variable	DFS	OS
Thickness	**0.016**[a]	**0.008**
Ulceration	0.382	0.586
Histology	0.359	0.366
Location of primary tumor	0.562	0.335
Mitoses/mm^2	**0.035**	**0.033**
Nodal status	**0.012**	**0.016**
Mitf expression 25%	**0.008**	**0.011**

[a] Significance shown in bold.

DFS, 92.3%) in these patients as compared with patients in whom <50% of the cells expressed Mitf, where the mean DFS was 127.27 months (5-year DFS, 64.8%). The *P* between these two survival figures was 0.035.

In this series, location of the primary tumor, age, sex, histological subtype, and level of invasion did not play any role in either DFS or OS.

When multivariate analysis was performed on known primary prognostic variables, patients whose melanomas were >2.6 mm thick had worse DFS ($P = 0.005$) and OS ($P = 0.032$). When the nodal status was included as a covariate, both thickness and nodal status significantly influenced survival: (*a*) DFS, $P = 0.078$ and $P = 0.05$, respectively; and (*b*) OS, $P = 0.005$ and $P = 0.048$, respectively. When Mitf expression with a cutoff value of 25% was included with the other primary tumoral prognostic variables, it significantly affected DFS ($P = 0.014$) and OS ($P = 0.018$). When all covariates were analyzed together (Table 2), Mitf expression emerged as the most significant variable influencing DFS ($P = 0.008$). By stepwise Cox regression, the final model consisted of Mitf expression ($P = 0.015$) and thickness ($P = 0.035$) influencing DFS, and regional nodal status ($P = 0.048$) influencing OS.

A significant relationship was noted between quantitative Mitf expression and the presence or absence of metastases in the regional nodes. When ≥50% of melanoma cells expressed Mitf, the incidence of nodal metastases was significantly lower. Of the 26 patients with nodal metastases, 6 (23%) had primary lesions with >50% Mitf expression, whereas 20 (77%) of the primary melanomas had <50% of the cells expressing Mitf ($P = 0.04$).

Discussion

We have evaluated the biological significance of Mitf expression in primary cutaneous melanoma. It appears that Mitf expression is a molecular marker that can be used clinically along with such established markers as thickness of the primary lesion, ulceration, and incidence of regional node metastases. Our data suggest that Mitf expression is a marker for improved survival. This assumption is substantiated by the fact that 6 of 11 patients whose primary tumors did not stain positive for Mitf died of melanoma, and the mean DFS in these patients was 40 months. Furthermore, using a semiquantitative immunocytochemical analysis, it appears that the higher the number of melanoma cells showing Mitf expression in a given primary tumor, the better the prognosis. For example, when 25% of the melanoma cells expressed Mitf, both DFS and OS increased significantly compared with the primary lesions in which no Mitf was expressed. If the cutoff point for positivity of Mitf expression is assumed to be 50% of the cells expressing Mitf, then the DFS of this group of patients increased significantly, and OS tended to increase significantly.

Clinically, nodal status is probably the most important prognostic marker in cutaneous melanoma, and in the primary lesion, thickness and ulceration are considered the most significant markers for survival. In this study, we confirmed that all of these three aspects are clinically reliable markers. However, in this small series, thickness and ulceration became statistically significant when the lesions were ≥2 mm. Mitf expression, in contrast, remains significant in all instances when compared by univariate analysis. In multivariate analysis, using the stepwise Cox regression model, Mitf expression and nodal status emerged as the most important variables influencing DFS and OS.

This prognostic correlation between nodal status and Mitf expression suggests that Mitf expression or lack thereof in primary lesions is an indicator of regional node metastases. This certainly was the case in our study. Patients with Mitf expression of >50% had a significantly lower incidence of nodal disease than those with a Mitf expression of ≤50%. The biological role of Mitf in melanocyte differentiation continues to show new facets. Our findings suggest that increased Mitf expression in patients with intermediate-thickness melanomas may lead to more differentiated tumors. This, in turn, leads to decreased relapse and nodal metastases and improved survival.

In the study reported by King *et al.* (14), all melanomas evaluated stained positive for Mitf, but not for other melanocytic markers evaluated. In our study, 82.5% of melanomas stained positive for Mitf. It should be noted that in addition to tissue blocks, we examined archival specimens only for which slides from outside institutions were sent to us for evaluation. The difference in tissue handling may explain, in part, the difference in staining. In addition, we have used a commercially available antibody for immunostaining. However, it is germane to emphasize that Mitf expression is a sign of differentiation; thus, dedifferentiated tumors will either not express or will barely express Mitf, and therefore there will be some lesions in which no Mitf expression will be detected. Thus, lack of expression should not be attributed merely to random occurrence. Our data also suggest that Mitf expression may not be as highly conserved in melanomas as suggested previously.

In summary, the data presented above suggest for the first time that Mitf expression, as determined by immunocytochemistry, can be used as a new prognostic molecular marker in melanoma. Additional prospective studies are warranted to determine whether or not these findings will be validated.

Acknowledgments

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