Research Article

Cancer Epidemiology, Biomarkers & Prevention

Prognostic Significance of Cytoplasmic p27 Expression in Human Melanoma

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Abstract

Background: The cyclin-dependent kinase inhibitor p27 plays important roles in cell proliferation, cell motility, and apoptosis. Interestingly, the nuclear and cytoplasmic p27 exert opposite biological functions. In this study, we investigated the prognostic impact of subcellular p27 expression.

Methods: We constructed melanoma tissue microarrays in a large series of melanoma patients, including 29 normal nevi, 52 dysplastic nevi, 270 primary melanomas, and 148 metastatic melanomas. The expression level of subcellular p27 in different stages of melanocytic lesions and its prognostic significance were evaluated.

Results: Compared with dysplastic nevi, nuclear p27 expression was remarkably reduced in primary melanomas and further reduced in metastatic melanoma ($P < 0.001$ for both), whereas cytoplasmic p27 expression is significantly increased from dysplastic nevi to primary melanomas ($P = 0.032$) and further increased in melanoma metastases ($P = 0.037$). Although loss of nuclear p27 expression is correlated with a worse 5-year survival of primary melanoma patients in Kaplan–Meier analysis ($P = 0.046$), it is not a prognostic factor by multivariate Cox regression analysis. On the contrary, Kaplan–Meier analysis showed that gain of cytoplasmic p27 was associated with a poor 5-year survival of metastatic melanoma patients ($P < 0.001$). Multivariate Cox regression analysis revealed that positive cytoplasmic p27 expression is an independent prognostic factor to predict metastatic melanoma patient outcome.

Conclusion: Cytoplasmic p27 may serve as a promising prognostic marker for metastatic melanoma.

Impact: Because there is no reliable prognostic marker for metastatic melanoma, our finding may have important clinical implications using cytoplasmic p27 as a prognostic biomarker for advanced melanoma. *Cancer Epidemiol Biomarkers Prev; 20(10); 2212–21. ©2011 AACR.*

Introduction

Human cutaneous malignant melanoma is an aggressive type of skin cancer, and its incidence in individuals of European origin continues to rise worldwide (1). In the United States, the number of estimated new cases of melanoma in 2010 was estimated to be 68,140 and it was predicted to have 8,700 deaths due to melanoma (2). Although melanoma accounts for only 4% of all dermatologic cancers, it is responsible for more than 80% of deaths from skin cancers, and the 10-year survival rate for patients with metastatic melanoma is less than 10% (3, 4). Although most thin primary melanomas are curable with surgery, some thin melanomas ultimately go on to develop metastases, indicating that even relatively small melanomas can readily metastasize. Thus, identifying biomarkers in conjunction with traditional cancer staging and prognosis could improve early diagnosis and patient care (5). However, despite the efforts that have been made, reliable biomarkers, especially for metastatic melanomas, are still lacking. Therefore, there is an urgent need to better understand the regulating factors contributing to melanoma initiation, progression, and metastases (6).

Cell proliferation is tightly controlled by cyclins, cyclin-dependent kinases (CDK), and CDK inhibitors that function sequentially during the cell cycle (7). p27, also known as Kip1, is an atypical tumor suppressor that regulates G_0–S phase transitions (8). In G_0, p27 translation and protein stability are maximal as it binds and inactivates nuclear cyclin E-CDK2 (9, 10). In early G_1, p27 promotes the assembly and nuclear import of cyclin D-CDK4 and cyclin D-CDK6 complexes (10). The progressive decrease of p27 in G_1 permits cyclin E-CDK2 and cyclin A-CDK2 to activate the G_1–S transition (8).

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p27 is regulated at transcriptional, translational, and posttranslational levels (8–11). p27 mRNA levels usually show little cell-cycle periodicity, but p27 protein levels are largely controlled by ubiquitin-dependent proteolysis (8, 9). In early G_1, mitogens promote p27 phosphorylation at serine 10 (S10) to facilitate nuclear export (12, 13); this simultaneously relieves cyclin E–CDK2 inhibition and permits p27 proteolysis through phosphorylation of p27 at T187 which is targeted by SCF^{Skp2} for degradation (14–16). On the contrary, opposed to other tumor suppressor, p27 is rarely mutated in human cancers (17). In addition to reduced nuclear p27 expression, the protein is mislocalized in many cancers and this is associated with a poor prognosis (8, 18). Cytoplasmic p27 seems to acquire a cell-cycle–independent oncogenic function to promote cancer cell invasion and metastasis (8, 18). Loss of nuclear p27 and increased cytoplasmic p27 are regulated by multiple different oncogenic pathways (18). Although the prognostic significance of reduced nuclear p27 expression has been well studied (19, 20), few studies have evaluated the prognostic potential of cytoplasmic p27. Better understanding of prognostic value of cytoplasmic p27 expression awaits further studies because cytoplasmic p27 drives tumor metastasis.

In this study, we used tissue microarray (TMA) containing 499 melanocytic lesions to evaluate the subcellular p27 expression by immunohistochemistry, and analyzed the correlations between subcellular p27 expression and clinicopathologic features and its potential prognostic value for melanoma patients. Our data showed that both loss of nuclear p27 expression and gain of cytoplasmic p27 are significantly associated with melanoma progression and a worse patient survival. We also found that cytoplasmic p27 is an independent prognostic factor for metastatic melanoma.

Materials and Methods

Ethics statement

The use of human skin tissues and the waiver of patient consent in this study were approved by the Clinical Research Ethics Board of the University of British Columbia. The study was conducted in accordance with the Declaration of Helsinki guidelines.

Patient specimens and TMA construction

We recruited 713 formalin-fixed paraffin-embedded nevi and melanoma tissues from the 1990 to 2009 archives of the Department of Pathology at Vancouver General Hospital, including 49 cases of normal nevi, 100 cases of dysplastic nevi, 403 primary melanomas, and 161 cases of metastatic melanomas. Patients who entered the case cohort were prospectively followed up until death or the latest follow-up. The median follow-up time was 60 months to the last follow-up date, May 2010. During the follow-up period, 35 patients were lost to follow-up, 145 died of melanoma, and 17 died from other causes. Those lost to follow-up were considered as censored data. The most representative tumor area was carefully selected and marked on the hematoxylin and eosin–stained slide. The construction of TMAs was described previously (21).

Immunohistochemistry of TMA

Immunohistochemistry was carried out as described previously (22–24). The monoclonal mouse anti-p27 antibody (1:50 dilution; Santa Cruz Biotechnology) was used for primary antibody incubation at 4°C overnight. The slide without primary antibody incubation was used as negative control.

Evaluation of immunostaining

The evaluation of both nuclear and cytoplasmic p27 staining was blindly and independently examined by 2 observers, including a dermatopathologist. In 9.6% cases (48 cases/499 cases) with discrepancy between the 2 observers, the immunostained slides were reviewed in a double viewing microscope so that the discrepancy was settled. The nuclear and cytoplasmic p27 expression was graded as positive when more than 5% of tumor cells showed immunopositivity (21). Biopsies with less than 5% tumor cells showing immunostaining were considered as negative.

Statistical analysis

Differences in demographic and clinical characteristics and expression levels of either nuclear or cytoplasmic p27 were evaluated by χ^2 tests between patient subgroups. Survival time was calculated from the date of melanoma diagnosis to the date of death or last follow-up. The Kaplan–Meier method and log-rank test were used to evaluate the effects of subcellular p27 expression on the overall and disease-specific survival of patients. Univariate or multivariate Cox proportional hazards regression models were preformed to estimate the crude hazard ratios (HRs) or adjusted HRs and their 95% confidence intervals (CIs). $P < 0.05$ was considered significant, and all tests were 2-sided. SPSS version 11.5 (SPSS Inc.) software was used for all analyses.

Results

Clinicopathologic features of TMAs

A total of 713 melanocytic lesions were used for TMA construction. Because of loss of biopsy cores or insufficient tumor cells present in the cores, 418 melanoma (270 cases of primary melanoma and 148 cases of metastatic melanoma), and 81 cases of nevi (29 normal nevi and 52 dysplastic nevi) could be evaluated for p27 staining (see the CONSORT diagram in the Supplementary Fig. S1). The distributions of selected demographic characteristics of melanoma patients are listed in Table 1.

Of the 418 melanoma patients, 251 were men and 167 women, with age ranging from 7 to 95 years (median, 60 years). We also applied the American Joint Committee on Cancer (AJCC) criteria to p27 evaluation in all melanoma

Table 1. Subcellular p27 staining and clinicopathologic characteristics of melanomas

		Nuclear staining			Cytoplasmic staining		
Variables	**Total (%)**	**Negative (%)**	**Positive (%)**	***P*[a]**	**Negative (%)**	**Positive (%)**	***P*[a]**
All melanoma (n = 418)							
Age, y							
≤60	214 (51.2)	122 (57.0)	92 (43.0)	0.633	115 (53.7)	99 (46.3)	0.457
>60	204 (48.8)	121 (59.3)	83 (40.7)		117 (57.4)	87 (42.6)	
Sex							
Male	251 (60.0)	151 (60.2)	100 (39.8)	0.303	140 (55.8)	111 (44.2)	0.890
Female	167 (40.0)	92 (55.1)	75 (44.9		92 (55.1)	75 (44.9)	
AJCC stage							
I	149 (35.6	57 (38.3)	92 (61.7)	<0.001[b]	87 (58.4)	62 (41.6)	0.037[b]
II	121 (28.9)	75 (62.0)	46 (38.0)		73 (60.3)	48 (39.7)	
III	60 (14.4)	46 (76.7)	14 (23.3)		29 (48.3)	31 (51.7)	
IV	88 (21.1)	65 (73.9)	23 (26.1)		43 (48.9)	45 (51.1)	
PM (n = 270)							
Age, y							
≤60	128 (47.4)	57 (44.5)	71 (55.5)	0.174	72 (56.3)	56 (43.7)	0.339
>60	142 (52.6)	75 (52.8)	67 (41.2)		88 (62.0	54 (48.0)	
Sex							
Male	149 (55.2)	73 (49.0)	76 (51.0)	0.975	87 (58.4)	62 (41.6)	0.747
Female	121 (44.8)	59 (48.8	62 (51.2)		73 (60.3)	48 (39.7)	
T stages							
T1	89 (33.0)	30 (33.7)	59 (66.3)	<0.001	55 (61.8)	34 (38.2)	0.843
T2	69 (25.6)	30 (43.5)	39 (56.5)		38 (55.1)	31 (44.9)	
T3	53 (19.6)	28 (52.8)	25 (47.2)		31 (58.5)	22 (41.5)	
T4	59 (21.9)	44 (74.6)	15 (25.4)		36 (61.0)	23 (39.0)	
Subtypes							
LM	47 (17.4)	20 (42.6)	27 (57.4)	0.018	28 (59.6)	19 (40.4)	0.947
Nodular	44 (16.3)	30 (68.2)	14 (31.8)	0.005[c]	27 (61.4)	17 (38.6)	0.757[c]
SS	100 (37.0)	41 (41.0)	59 (59.0)		57 (57.0)	43 (43.0)	
Nonspecified	79 (29.3)	41 (51.9)	38 (48.1)		48 (60.8)	31 (39.2)	
Ulceration							
Absent	217 (80.4)	104 (47.9)	113 (52.1)	0.522	129 (59.4)	88 (40.6)	0.899
Present	53 (19.6)	28 (52.8)	25 (47.2)		31 (58.5)	22 (41.5)	
Site[d]							
Sun protected	206 (76.3)	103 (50.0)	103 (50.0)	0.513	124 (60.2)	82 (39.8)	0.575
Sun exposed	64 (25.7)	29 (45.3)	35 (54.7)		36 (56.3)	28 (43.7)	
MM (n = 148)							
Age, y							
≤60	86 (58.1)	65 (75.6)	21 (24.4)	0.135	43 (50.0)	43 (50.0)	0.699
>60	62 (41.9)	46 (74.2)	16 (25.8)		29 (46.8)	33 (53.2)	
Sex							
Male	102 (68.9)	78 (76.5)	24 (24.5)	0.538	53 (52.0)	49 (48.0)	0.230
Female	46 (31.1)	33 (71.7)	13 (28.3)		19 (41.3)	27 (58.7)	

Abbreviations: PM, primary melanoma; MM, metastatic melanoma; LM, lentigo maligna; SS, superficial spreading.

[a]χ^2 test.

[b]Comparison between AJCC stage I to II and III to IV.

[c]Comparison between nodular melanomas and other melanomas.

[d]Sun-protected sites: trunk, arm, leg, and feet; Sun-exposed sites: head and neck.

patients. Among the 418 cases, 149 tumors were at AJCC stage I, 121 at stage II, 60 at stage III, and 88 at stage IV (Table 1). Of the 270 primary melanoma cases, 149 were men and 121 women, 148 with age less than 60 years and 142 with age more than 60 years. Eighty-nine primary melanomas were in T1 stages with thickness less than 1.00 mm, 69 in T2 (1.01–2.00 mm), 53 in T3 (2.01–4.00 mm), and 59 in T4 stage (>4.00 mm). Ulceration was

observed in 53 cases. For the histologic subtype, 47 tumors were lentigo maligna melanomas, 100 tumors were superficial spreading melanomas, 44 tumors were nodular melanomas, and 79 tumors were nonspecified. Sixty-four melanomas were located in sun-exposed sites (head and neck) and 206 were located in sun-protected sites (other locations). One hundred and forty-eight melanoma metastases were available for p27 staining evaluation, including 102 men and 46 women.

Loss of nuclear p27 expression correlates with melanoma progression

Positive p27 staining was detected in both nucleus and cytoplasm (Fig. 1A–D). Positive nuclear p27 staining decreased from 86% and 81% in normal and dysplastic nevi to 50% in primary melanomas and further decreased to 25% in metastatic melanomas (Fig. 1E). There is no significant difference in nuclear p27 staining between normal nevi and dysplastic nevi ($P = 0.535$, χ^2 test). However, significant differences for positive nuclear p27 staining were observed between dysplastic nevi and primary melanomas ($P < 0.001$, χ^2 test), between dysplastic nevi and melanoma metastases ($P < 0.001$, χ^2 test), and between primary melanoma and melanoma metastases ($P < 0.001$, χ^2 test).

Gain of cytoplasmic p27 expression correlates with melanoma progression

Positive cytoplasmic p27 staining increased from 10% and 25% in normal and dysplastic nevi to 41% in primary melanomas and further increased to 51% in metastatic melanomas (Fig. 1F). There is no significant difference in cytoplasmic p27 staining between normal nevi and dysplastic nevi ($P = 0.112$, χ^2 test). However, significant differences for positive cytoplasmic p27 staining were observed between dysplastic nevi and primary melanomas ($P = 0.032$, χ^2 test), between dysplastic nevi and melanoma metastases ($P = 0.001$, χ^2 test), and between primary melanoma and melanoma metastases ($P = 0.037$, χ^2 test).

Correlation between p27 expression and clinicopathologic parameters

In all 418 melanoma patients, we found that positive nuclear p27 expression significantly decreased from 51% in early stage (AJCC I and II) to 25% in advanced stage (AJCC III and IV; $P < 0.001$, χ^2 test), whereas positive cytoplasmic p27 expression significantly increased from 41% in early stage to 51% in advanced stage ($P = 0.037$, χ^2 test, Table 1). We also found that positive nuclear p27 expression significantly decreased from 62% in AJCC stage I to 38% in stage II ($P < 0.001$, χ^2 test), and further reduced to 23% in stage III (stage II vs. stage III, $P = 0.048$, χ^2 test), but not from stage III to IV ($P = 0.699$, χ^2 test).

In primary melanoma, significant difference in positive nuclear p27 expression was found in tumors with different thickness with 66% at T1 stage, 57% at T2 stage, 47% at T3 stage, and 25% at T4 stage ($P < 0.001$, χ^2 test),

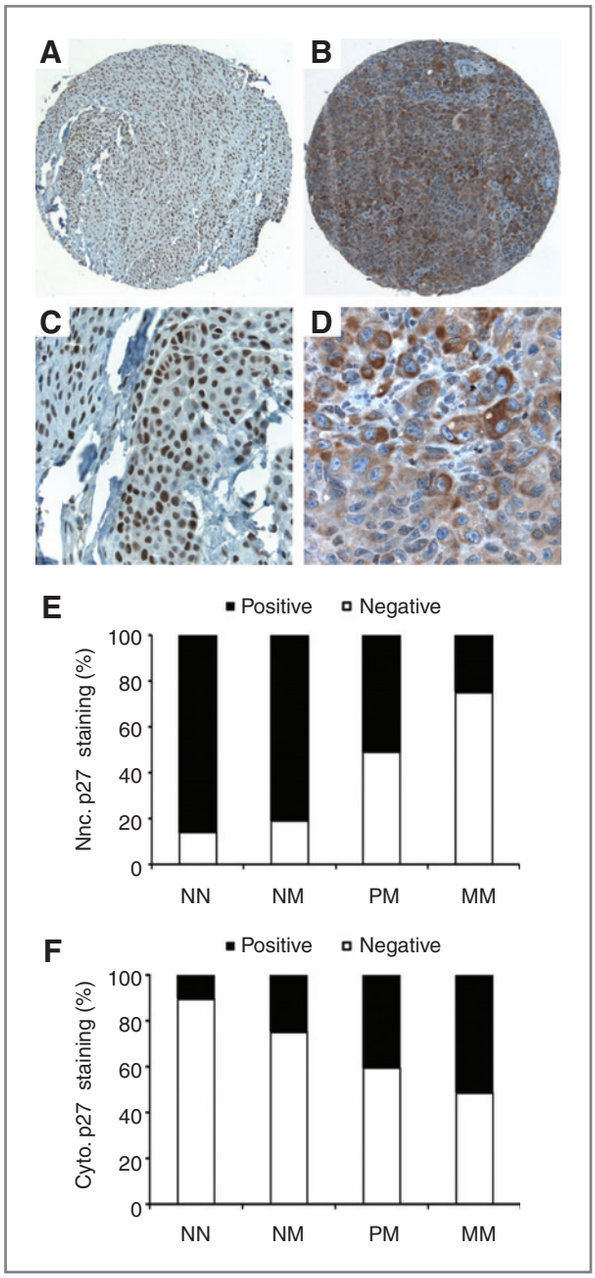

Figure 1. Correlation between subcellular p27 expression and melanoma progression. A–D, representative images of nuclear and cytoplasmic p27 immunohistochemical staining. A and C, positive nuclear p27 staining with negative cytoplasmic p27 in dysplastic nevus; B and D, negative nuclear p27 staining with positive cytoplasmic p27 staining in metastatic melanoma. E, reduced nuclear p27 (Nuc. p27) expression correlates with melanoma progression. F, increased cytoplasmic p27 (Cyto. p27) expression correlates with melanoma progression. NN, normal nevi; DN, dysplastic nevi; PM, primary melanoma; MM, metastatic melanoma. Magnification, 100× for A and B and 400× for C and D.

but there was no significant difference for cytoplasmic p27 expression at different T stages ($P = 0.843$, χ^2 test, Table 1). Nuclear but not cytoplasmic p27 expression was significantly correlated with melanoma subtypes

($P = 0.018$), and positive nuclear p27 expression was found in 32% nodular melanomas compared with 55% in other melanoma ($P = 0.005$). We did not find significant correlations between either nuclear or cytoplasmic p27 expression with other clinicopathologic variables in primary melanoma, including age, gender, ulceration, or location. In addition, both nuclear and cytoplasmic p27 expression were not correlated with age, gender (Table 1), or metastatic sites (data not shown) in metastatic melanomas.

Subcellular p27 expression and patient survival

A total of 383 patients, including 249 primary melanoma and 134 metastatic melanoma patients, had complete follow-up and clinical information (Supplementary Fig. S1). Among the 383 patients, the Kaplan–Meier analyses revealed that loss of nuclear p27 expression was associated with poor overall ($P = 0.005$) and disease-specific 5-year survival ($P = 0.001$, Fig. 2A). In 249 primary melanomas, loss of nuclear p27 staining was correlated with both disease-specific ($P = 0.046$, log-rank test) and overall 5-year patient survival ($P = 0.052$, log-rank test, Fig. 2B) with board-line significance. However, loss of nuclear p27 expression was not associated with either overall or disease-specific 5-year survival ($P > 0.05$ for both) of 134 metastatic melanoma patients (Fig. 2C).

On the contrary, positive cytoplasmic p27 expression was associated with poor overall and disease-specific 5-year survival ($P < 0.001$ for both; Fig. 3A) of all melanoma patients. In primary melanoma, positive cytoplasmic p27 staining did not correlate with either overall or disease-specific ($P > 0.05$ for both; Fig. 3B) 5-year patient survival. However, positive cytoplasmic p27 expression was associated with both overall and disease-specific 5-year survival ($P < 0.001$) of metastatic melanoma patients (Fig. 3C).

The univariate analysis revealed that age, thickness, and ulceration were all significantly associated with overall and disease-specific 5-year survival of primary melanoma patients (Table 2). Cox proportional hazard

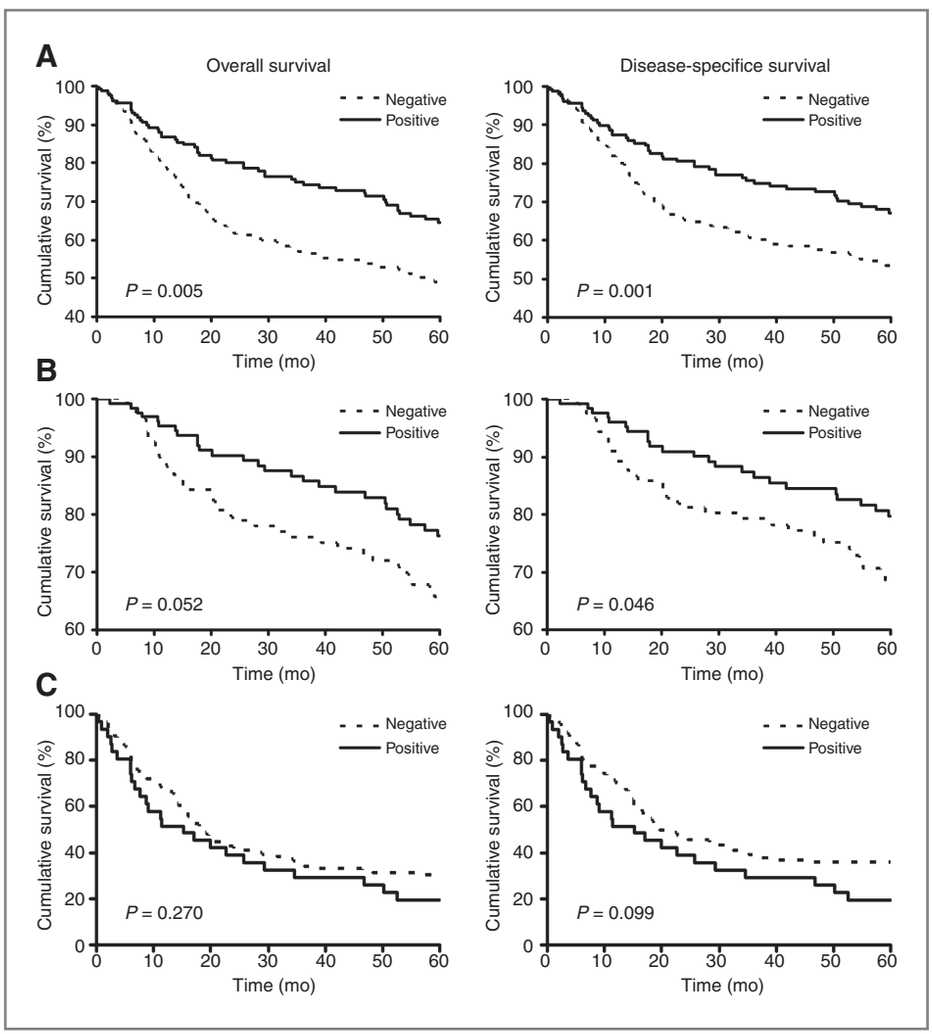

Figure 2. Kaplan–Meier curve analyses of the associations between nuclear p27 expression and melanoma patient survival. A, negative nuclear p27 expression is significantly associated with worse overall ($P = 0.005$) and disease-specific ($P = 0.001$) 5-year survival of 383 melanoma patients. B, loss of nuclear p27 expression is associated with poor disease-specific ($P = 0.046$) but not overall ($P = 0.052$) 5-year survival of 249 primary melanoma patients, C, loss of nuclear p27 is not associated with both overall ($P = 0.270$) and disease-specific ($P = 0.099$) 5-year survival of 134 metastatic melanoma patients.

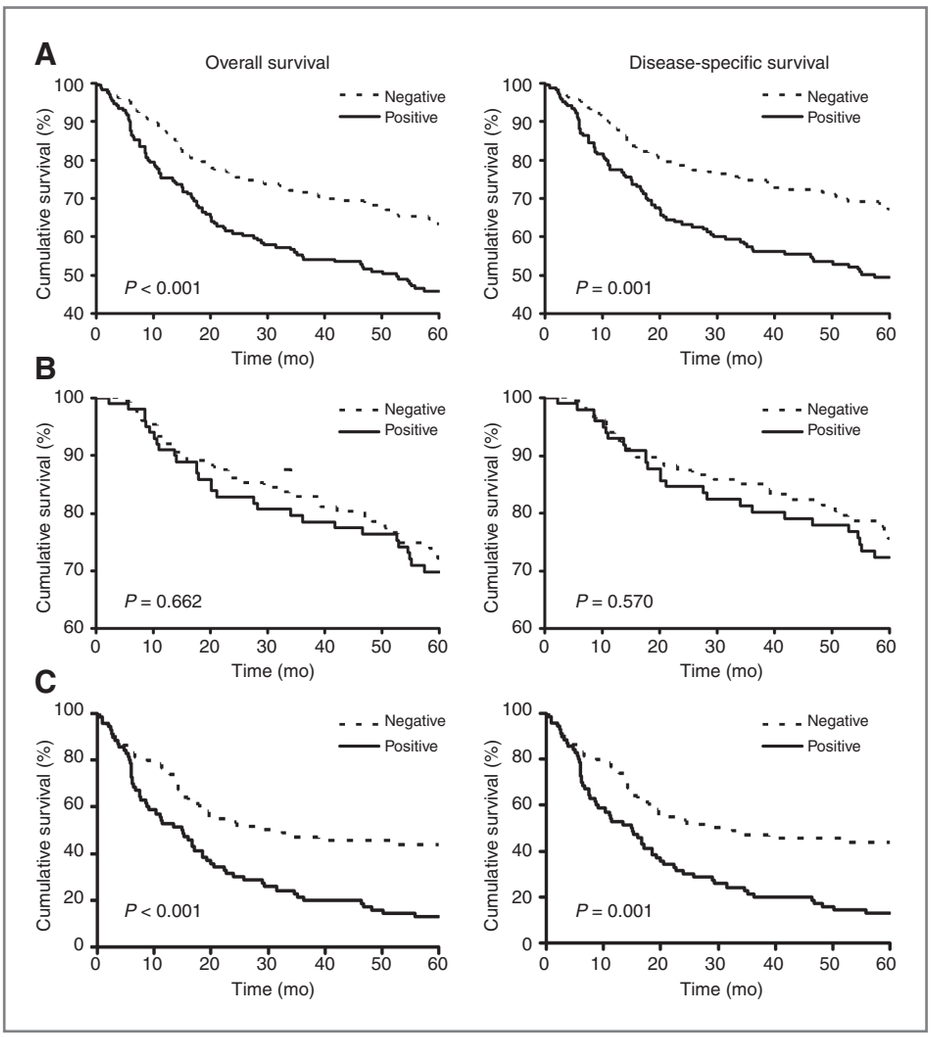

Figure 3. Kaplan–Meier curve analyses of the associations between cytoplasmic p27 expression and melanoma patient survival. A, positive cytoplasmic p27 expression is associated with poor overall and disease-specific 5-year survival of 383 melanoma patients ($P < 0.001$ for both). B, positive cytoplasmic p27 expression is not associated with both overall ($P = 0.662$) and disease-specific ($P = 0.570$) 5-year survival of 249 primary melanoma patients. C, positive cytoplasmic p27 expression is associated with both overall and disease-specific 5-year survival of 134 metastatic melanoma patients ($P < 0.001$ for both).

regression analysis showed that positive nuclear p27 expression was a significantly favorable prognostic factor in primary melanoma (HR = 0.59; 95% CI, 0.35–1.00; $P = 0.048$ for disease-specific survival), whereas positive cytoplasmic p27 expression was a significantly unfavorable prognostic factor for overall (HR = 2.13; 95% CI, 1.41–3.23, $P < 0.001$) and disease-specific survival (HR = 2.18; 95% CI, 1.41–3.38; $P < 0.001$) of metastatic melanoma patients (Table 2).

Next, we examined whether positive subcellular p27 expression is an independent prognostic marker for melanoma patient's survival by multivariate Cox proportional hazard analysis. In all melanomas, our results clearly indicated that similar to AJCC stages, which have been widely accepted as independent prognostic factors for melanoma patient survival, cytoplasmic p27 expression is an independent prognostic factor for both overall (HR = 1.88; 95% CI, 1.36–2.61; $P < 0.001$) and disease-specific 5-year survival (HR = 1.91; 95% CI, 1.31–2.69; $P < 0.001$; Table 3). Furthermore, cytoplasmic p27 expression was also correlated with both overall (HR = 2.20; 95% CI, 1.41–3.45; $P = 0.001$) and disease-specific 5-year survival (HR = 2.12; 95% CI, 1.31–3.41; $P = 0.002$) in metastatic melanoma patients (Table 3). However, both nuclear and cytoplasmic p27 expression were not associated with primary melanoma patient survival by adjusting with patient's age, sex, tumor thickness, presence of ulceration, and location (Table 3).

Discussion

The pattern of p27 expression in melanocytic tumors, as evidenced by immunohistochemistry, is highly heterogeneous (25). Expression of p27 was found to be progressively lost in the transition from benign nevi to primary and metastatic melanomas, and in the transition from thin to thicker primary melanomas (26–31). Although the majority of metastatic melanomas had a

Table 2. Univariate Cox proportional regression analysis on 5-year overall and disease-specific survival of melanoma patients

Variable	Patients, *n* (%)	Overall survival				Disease-specific survival			
		Deaths	Death rate (%)	HR (95% CI)	*P*[a]	Deaths	Death rate (%)	HR (95% CI)	*P*[a]
PM (n = 249)									
Age, y									
≤60	119 (47.8)	19	16.0	2.78 (1.63–4.75)	<0.001	19	16.0	2.29 (1.32–3.98)	0.003
>60	130 (52.2)	46	35.4			38	29.2		
Sex									
Male	135 (54.2)	34	25.2	1.11 (0.68–1.80)	0.682	32	23.7	0.95 (0.56–1.60)	0.847
Female	114 (45.8)	31	27.2			25	21.9		
Thickness, mm									
≤2.00	142 (57.0)	19	13.4	4.60 (2.69–7.88)	<0.001	15	10.6	5.32 (2.94–9.61)	<0.001
>2.00	107 (43.0)	46	43.0			42	39.3		
Ulceration									
Absent	201 (80.7)	38	18.9	4.97 (3.01–8.21)	<0.001	34	16.9	4.69 (2.74–8.04)	<0.001
Present	48 (19.3)	27	56.3			23	47.9		
Site[b]									
Sun protected	189 (75.9)	50	26.5	1.01 (0.56–1.79)	0.987	42	22.2	1.20 (0.66–2.16)	0.549
Sun exposed	60 (24.1)	15	25.0			15	25.0		
Nuclear p27									
Negative	121 (48.6)	38	31.4	0.62 (0.38–1.01)	0.054	34	28.1	0.59 (0.35–1.00)	0.048
Positive	128 (51.4)	27	21.1			23	18.0		
Cytoplasmic p27									
Negative	149 (59.8)	36	24.2	1.12 (0.68–1.82)	0.662	31	20.8	1.16 (0.69–1.96)	0.570
Positive	100 (40.2)	29	29.0			26	26.0		
MM (n = 134)									
Age, y									
≤60	77 (57.5)	59	76.6	0.86 (0.57–1.29)	0.454	57	74.0	0.73 (0.47–1.13)	0.153
>60	57 (43.5)	38	66.7			31	54.4		
Sex									
Male	88 (65.7)	62	70.5	1.28 (0.85–1.92)	0.240	54	61.4	1.43 (0.93–2.20)	0.104
Female	46 (34.1)	35	76.1			34	73.9		
Nuclear p27									
Negative	103 (76.9)	72	70.0	1.29 (0.82–2.04)	0.271	63	61.2	1.47 (0.93–2.34)	0.102
Positive	31 (23.1)	25	80.6			25	80.6		
Cytoplasmic p27									
Negative	64 (47.8)	36	56.3	2.13 (1.41–3.23)	<0.001	32	50.0	2.18 (1.41–3.38)	<0.001
Positive	70 (52.2)	61	87.1			56	80.0		

Abbreviations: PM, primary melanoma; MM, metastatic melanoma.

[a]Log-rank test.

[b]Sun-protected sites: trunk, arm, leg and feet; Sun-exposed sites: head and neck.

loss of p27 expression, some of metastatic melanomas showed an increase in p27 expression (26, 32, 33). However, it remains unclear why these subsets of melanoma cells maintain an ability to upregulate p27 (8). Actually, unlike other cell-cycle proteins, which display distinct nuclear immunoreactivity, some melanomas show additional p27 cytoplasmic positivity (29, 33). In this study, both cytoplasmic and nuclear p27 staining were scored for p27 expression. We found that nuclear p27 expression was remarkably reduced in primary melanomas compared with dysplastic nevi and further reduced in metastatic melanoma. We found that nuclear p27 expression was remarkably reduced, whereas the cytoplasmic p27 was increased, in primary and metastatic melanomas compared with dysplastic nevi. The increase of p27 expression in the cytoplasm may partially be due to the reduction of p27 nuclear expression in melanomas by its translocation into the cytoplasm, and/or increased p27 expression in some metastatic melanomas (27, 29, 32, 33). Our finding supports the

Table 3. Multivariate Cox regression analysis on 5-year overall and disease-specific survival of melanoma patients

	Overall survival				Disease-specific survival			
Variables[a]	**β[b]**	**SE**	**HR (95% CI)**	***P***	**β[b]**	**SE**	**HR (95% CI)**	***P***
All melanoma ($n = 383$)								
Age	0.316	0.159	1.37 (1.00–1.88)	0.047	0.152	0.169	1.16 (0.84–1.62)	0.368
Sex	0.234	0.163	1.26 (0.92–1.74)	0.151	0.216	0.172	1.24 (0.89–1.74)	0.211
AJCC	1.425	0.171	4.16 (2.97–5.82)	<0.001	1.456	0.182	4.29 (3.00–6.13)	<0.001
Nuclear p27	–0.269	0.184	0.76 (0.53–1.10)	0.143	–0.226	0.193	0.80 (0.55–1.16)	0.241
Cytoplasmic p27	0.633	0.166	1.88 (1.36–2.61)	<0.001	0.644	0.176	1.91 (1.35–2.69)	<0.001
PM ($n = 249$)								
Age	0.581	0.292	1.79 (1.01–3.17)	0.046	0.333	0.301	1.40 (0.77–2.52)	0.269
Sex	0.148	0.259	1.16 (0.70–1.93)	0.567	0.053	0.279	1.05 (0.61–1.82)	0.850
Thickness	1.036	0.311	2.82 (1.53–5.19)	0.001	1.240	0.339	3.46 (1.78–6.72)	<0.001
Ulceration	1.011	0.290	2.75 (1.56–4.85)	<0.001	0.975	0.308	2.65 (1.45–4.84)	0.002
Location	–0.063	0.304	0.93 (0.52–1.70)	0.835	0.108	0.313	1.11 (0.60–2.06)	0.729
Nuclear p27	–0.283	0.267	0.75 (0.45–1.27)	0.289	–0.296	0.286	0.74 (0.43–1.30)	0.301
Cytoplasmic p27	0.267	0.257	1.31 (0.79–2.16)	0.299	0.277	0.274	1.32 (0.77–2.26)	0.312
MM ($n = 134$)								
Age	–0.084	0.212	0.92 (0.61–1.39)	0.693	–0.226	0.227	0.80 (0.51–1.24)	0.319
Sex	0.215	0.213	1.24 (0.82–1.88)	0.314	0.304	0.221	1.36 (0.88–2.09)	0.169
Nuclear p27	–0.128	0.251	0.88 (0.54–1.44)	0.611	0.005	0.258	1.01 (0.61–1.67)	0.984
Cytoplasmic p27	0.790	0.229	2.20 (1.41–3.45)	0.001	0.749	0.243	2.12 (1.31–3.41)	0.002

Abbreviations: PM, primary melanoma; MM, metastatic melanoma.

[a]Coding of variables: age was coded as 1 (≤60 y) and 2 (>60 y). Sex was coded as 1 (male) and 2 (female). Both nuclear and cytoplasmic p27 were coded as 1 (negative) and 2 (positive). Thickness was coded as 1 (≤2.00 mm) and 2 (>2.00 mm). Ulceration was coded as 1 (absent) and 2 (present). Location was coded as 1 (sun protected) and 2 (sun exposed).

[b]β, regression coefficient.

hypothesis that sequestration of p27 in the cytoplasm blocks nuclear p27 activity and plays an important role in cancer progression and metastasis (8, 18, 25).

In addition to nuclear p27, recent studies have focused on the role of cytoplasmic p27 (8, 18). In melanoma, Denicourt and colleagues found that targeted cytoplasmic expression of wild-type or non–CDK-binding p27 induced melanoma motility and resulted in numerous metastases to lymph node, lung, and peritoneum (33). This suggests a prominent role of cytoplasmic p27 in melanoma metastasis that is independent of cyclin–CDK regulation. Furthermore, they found that cytoplasmic p27 in 70% of invasive and metastatic melanomas but no cytoplasmic p27 was detected in melanoma *in situ* (33). Although our data did not indicate a correlation between cytoplasmic p27 expression and tumor thickness, we found that positive cytoplasmic p27 expression was significantly increased in invasive primary melanoma comparing with melanoma *in situ* (data not shown). Further studies are required to confirm the association between cytoplasmic p27 expression and melanoma invasion because the case number of melanoma *in situ* is small in either our study or others' (29, 34). In addition, we and other groups found significant correlation between loss of nuclear p27 and melanoma thickness, but it remains unclear whether loss of nuclear p27 contributes to melanoma invasion.

There have been contradictory reports on the association of p27 with clinical outcome in melanoma patients. Previously, Florenes and colleagues found that low p27 expression was correlated with poor disease-free survival in primary nodular melanomas (26). However, others reported that p27 nuclear staining was not associated with patient prognosis (25). This discrepancy may be due to small sample size and low statistical power of these studies (25, 26). On the basis of a large number of melanoma patients, we found that loss of nuclear p27 was significantly associated with poor disease-specific survival of primary melanoma patients although nuclear p27 cannot independently predict primary melanoma patient outcome. More importantly, we for the first time evaluated the association between cytoplasmic p27 expression and survival of a large number of melanoma patients. The data showed that gain of cytoplasmic p27 expression is associated with survival of metastatic but not primary melanoma patients. Multivariate Cox regression analysis also indicated that cytoplasmic p27 can independently predict

outcome of metastatic melanoma patients. Our melanoma cohort represents population-based melanoma patients in Vancouver, British Columbia, Canada. Should our risk model be validated in other different cohort studies, it will be important to incorporate p27 nuclear and cytoplasmic expression as a nonanatomic determinant of risk classification for melanoma patients.

Prognostic studies of tumor biomarkers are valuable as they assist in disease stratification and improve our understandings of tumor progression. However, the most valuable biomarkers are those that reliably indicate response to treatment (5). Because diverse oncogenic signaling cascades regulate p27 proteolysis, subcellular localization, and function, several molecular-targeting drugs impact p27 by inhibiting upstream signaling (8, 18). For example, the inhibitor of tyrosine kinase or the oncogenic kinase Src (upstream factor regulating p27 degradation), restores p27 levels and inhibits cancer cell proliferation (15, 16, 35). In many other preclinical models, pathways driving p27 proteolysis were reversed by targeted therapies, including breast cancer (36), lung cancers (37), pancreatic cancer (38), and melanoma (39). On the contrary, targeting cytoplasmic p27 therapies encounters challenges. Hyperactivation of phosphoinositide 3-kinase (PI3K)/mTOR signaling, through phosphorylation of p27, promotes cytoplasmic p27 mislocalization, increased invasiveness, and may underlie progression in a variety of cancers (10). The limited success of early clinical trials with rapamycin may be due, in part, to incomplete blockade of mTORC1 by rapamycin (40). Furthermore, inhibition of mTORC1 turns on feedback loops leading to PI3K activation, which would promote cytoplasmic p27 sequestration and p27-dependent tumor cell migration and metastasis (41, 42). Recent finding that dual PI3K/mTOR kinase inhibitors have shown great potential in preclinical models and early clinical trials holds tremendous promise for tumor metastases therapy by attenuating this deregulated signaling (43, 44).

In conclusion, our study revealed that positive cytoplasmic p27 expression correlates with a poor 5-year survival of metastatic melanoma patients and may serve as a promising prognostic marker for metastatic melanoma.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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