ORIGINAL ARTICLE

Prognostic relevance of P-cadherin expression in melanocytic skin tumours analysed by high-throughput tissue microarrays

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Aim: To investigate whether protein expression or cellular localisation of P-cadherin is associated with clinicopathological characteristics in benign and malignant melanocytic skin tumours.

Experimental design: P-cadherin expression and the Ki-67 labelling index were analysed immunohistochemically by using tissue microarrays (TMAs). Membranous and cytoplasmic expression was scored semiquantitatively (0 to 2+).

Results: P-cadherin protein expression of any intensity (1+ to 2+) was detected in the membrane in 41.5% (132/318) and in the cytoplasm in 64.2% (204/318) of patients. In general, P-cadherin expression was significantly reduced in malignant melanomas ($p < 0.001$) and melanoma metastases ($p < 0.001$), compared with benign nevi. Additionally, loss of membranous P-cadherin was associated with Clark level ($p = 0.011$) and tumour thickness ($p < 0.001$). Interestingly, a significantly lower P-cadherin expression was shown by dermal nevi than by compound and junctional nevi ($p = 0.005$; $p = 0.025$). In primary melanomas, a Ki-67 labelling index $< 5\%$ was not associated with P-cadherin protein expression, suggesting that loss of P-cadherin expression was not associated with proliferation. None of the other clinical and histological factors analysed was significantly related to P-cadherin expression. Low cytoplasmic P-cadherin expression was associated with tumour recurrence ($p = 0.03$) in all the patients who were analysed. After testing various multivariate Cox regression models, loss of cytoplasmic P-cadherin expression remained a highly significant adverse risk factor for tumour recurrence in patients with tumours < 2 mm.

Conclusions: Loss of cytoplasmic P-cadherin expression is common in advanced melanomas and can be a prognostic marker of progression in patients with melanoma, most useful in patients with primary tumours < 2 mm in thickness.

Cadherins are an important group of cell–cell adhesion molecules that mediate intercellular adhesion by calcium-dependent homophilic interactions.¹ So far, more than 80 members of the cadherin superfamily have been identified, including classic cadherins, desmogleins, desmocollins, protocadherins, fats, seven-pass transmembrane cadherins and Ret tyrosine kinase. Dynamic rearrangement of cell–cell adhesion has an important role in many physiological and pathological processes such as embryonic and tissue development, cell scattering, wound healing, tumour metastasis and cell migration.¹

Migration and invasion of human melanoma cells is an essential pathogenic process in the development of systemic metastasis. This event is characterised by several molecular changes leading to proliferation, migration and invasion. Migration of melanoma cells is mediated by dynamic reorganisation of the actin cytoskeleton, changes in gene transcription, with up regulation of new membrane components (integrins and junctional adhesion molecules), and controlled cell–matrix and cell–cell interactions.^{2–3}

It is well known that loss of E-cadherin expression is an important step in the development of malignant melanoma.^{4–7} Owing to its critical function in intercellular adhesion, E-cadherin is assumed to act as a tumour suppressor, negatively regulating several crucial steps of invasion and metastasis. The transfection of E-cadherin cDNA into invasive carcinoma cells leads to a marked reduction of their invasive capacity *in vitro*^{8–9} and activation of E-cadherin expression results in

growth retardation of tumour cell lines.¹⁰ Cadherins, other than E-cadherin, were scarcely analysed in melanoma before now.

We have previously shown the expression of a short truncated 50-kDa form of the N-terminal part of P-cadherin in seven melanoma cell lines compared with melanocytes and keratinocytes.¹³ Protein analyses of melanoma tissue samples and immunohistochemistry of a small group of primary and metastatic melanoma tissue verified the expression of this short form of P-cadherin *in situ* as well. Furthermore, analysis showed that this short 50-kDa form of P-cadherin is secreted by melanoma cells in contrast with the membrane-bound form in melanocytes. Functionally, the secreted form of P-cadherin has the role of a regulator of the homophilic interaction between P-cadherin molecules by antagonising their biological role, acting as a dominant negative form to interrupt cell–cell attachment. In addition to the shift from the membranous to the secreted form, expression of P-cadherin is down regulated in malignant melanoma.¹¹

Tissue microarrays (TMAs) are highly efficient tools for investigating a large series of tumour cases with defined clinical characteristics, including disease outcome. This study aims at investigating whether P-cadherin expression or localisation in the cell is associated with clinicopathological

Abbreviations: IHC, immunohistochemistry; RFS, recurrence-free survival; TMA, tissue microarray

Table 1 Analysis of P-cadherin expression in melanocytic skin tumours by tissue microarrays

Melanocytic lesions (n = 368)	Cytoplasmic P-cadherin immunohistochemistry					Membranous P-cadherin immunohistochemistry				
	Analysable (n)	0 (n)	1+ (n)	2+ (n)	p*	Analysable (n)	0 (n)	1+ (n)	2+ (n)	p*
Total	318	114	161	43		318	186	59	73	
Primary malignant melanoma†		44	35	2			56	9	16	
Melanoma metastases		47	42	8	<0.001		73	11	13	<0.001
Benign nevi		23	84	33			57	39	44	

*Fisher's exact test (two-sided), bold type representing significant data.

†Only initial and unifocal malignant melanomas were included.

parameters in benign and malignant melanocytic skin tumours.

MATERIALS AND METHODS

TMA_s

TMA_s were constructed as described previously,¹² and contained a total of 350 formalin-fixed, paraffin-wax-embedded human tissues: 88 (25.1%) primary malignant melanomas, 101 (28.9%) metastases and 161 (46.0%) benign nevi. In patients with multiple subsequent neoplasms, only initial and unifocal malignant melanomas were included. Slides of all tumours stained with haematoxylin and eosin were evaluated by two pathologists, PJW and FB. Clinical follow-up data, provided by the Central Tumor Registry, Regensburg, Germany, were available for all patients with primary malignant melanomas. The median follow-up for all patients was 54 (range 0–135) months.

To prevent structural imbalance between patients with and without recurrence in the course of time, a staggered matching algorithm based on tumour thickness was used. Initial matching criteria were as follows: tumour depth ≤ 2.0 mm, match 1:2—that is, one patient with recorded relapse was matched to two patients without recorded relapse but identical tumour thickness; tumour depth >2.0 mm and ≤ 4.2 mm, match 1:1. In the group of tumours >4.2 mm, 12 patients with tumour recurrence were compared with 9 patients without recurrence. In the group with tumours >4.2 mm, no exact match was possible, as advanced tumour stages almost always had a history of melanoma recurrence. According to recent follow-up data (1 March 2005), there was no noticeable difference in melanoma thickness between patients with and without recurrence with the Mann–Whitney U test. Tables 1 and 2 summarise the characteristic parameters of the TMA.

Table 2 Clinicopathological parameters in relation to P-cadherin immunohistochemistry (IHC)

		Cytoplasmic P-cadherin IHC					Membranous P-cadherin IHC				
Variable	Categorisation	Analysable (n)	0 (n)	1+ (n)	2+ (n)	p*	Analysable (n)	0 (n)	1+ (n)	2+ (n)	p*
Primary malignant melanoma†											
Age at diagnosis											
	≤60 years	45	26	17	2	0.394	45	29	6	10	0.607
	>60 years	36	18	18	0		36	27	3	6	
Sex											
	Female	46	29	17	0	0.072	46	32	4	10	0.646
	Male	35	15	18	2		35	24	5	6	
Clark level‡											
	II	4	1	3	0	0.108	4	1	0	3	<0.001
	III	12	4	8	0		12	5	6	1	
	IV	52	29	22	1		52	39	2	11	
	V	12	9	2	1		12	10	1	1	
Tumour thickness											
	≤2.0 mm	33	11	21	1	0.003	33	17	7	9	0.011
	>2.0 mm	48	33	14	1		48	39	2	7	
Growth pattern											
	NOS	8	4	3	1	0.214	8	6	1	1	0.298
	SSM	37	18	18	1		37	22	7	8	
	ALM	5	1	4	0		5	3	0	2	
	NMM	29	20	9	0		29	24	1	4	
	LMM	2	1	1	0		2	1	0	1	
Ki-67 labelling index											
	<5%	30	14	15	1	0.114	30	17	4	9	0.116
	≥5%	36	25	10	1		36	28	4	4	
Ulceration											
	Without ulceration	63	33	29	1	0.302	63	41	8	14	0.401
	With ulceration	18	11	6	1		18	15	1	2	
Melanoma metastases											
	Lymph node	41	18	20	3	0.673	41	33	3	5	0.512
	Skin	56	29	22	5		56	40	8	8	
Benign nevi											
	Compound and junctional	52	2	33	17	0.005	52	13	21	18	0.025
	Dermal	37	12	19	6		37	21	7	9	
	Congenital	51	9	32	10		51	23	11	17	

ALM, acral lentiginous melanoma; LMM, lentigo malignant melanoma; NMM, nodular malignant melanoma; NOS, not otherwise specified; SSM, superficial spreading melanoma.

*Fisher's exact test (two-sided), bold type representing significant data.

†Only initial and unifocal malignant melanomas were included.

‡According to UICC. In: Sobin LH, Wittekind CH, eds. *TNM classification of malignant tumours*. 6th edn. New York: Wiley, 2002.

Figure 1 (A–F) Immunohistochemical P-cadherin staining of nevi, malignant melanomas and metastasis on the tissue microarrays. Semiquantitative scoring (0 to 2+) on the basis of cytoplasmic and membranous staining intensity. Squamous epithelium of the epidermis serving as internal positive control (star, D). (A, B) Representative example of nevi with strong immunoreactivity for P-cadherin (score 2 (cytoplasmic), 1 (membrane); A, 50 $\times$; B, 400 $\times$). (C, D) Representative sections of malignant melanoma showing no P-cadherin staining (score 0; C, 50 $\times$; D, 400 $\times$). (E, F) Representative example of melanoma metastasis with no immunoreactivity for P-cadherin (score 0; E, 50 $\times$; F, 400 $\times$).

Immunohistochemistry (IHC)

Paraffin-wax-embedded preparations of tissues from patients with benign melanocytic nevi, malignant melanomas and melanoma metastases were screened for P-cadherin protein expression by IHC as described previously.¹³ In brief, tissues were deparaffinised, rehydrated and subsequently incubated with primary polyclonal P-cadherin-antibody (1:100; BD Bioscience Franklin Lakes, USA) or anti-Ki-67 (rabbit monoclonal, clone MIB1; Dako Cytomation GmbH, Hamburg, Germany; 1:10, final concentration 5 μ g/ml) overnight at 4°C. The secondary antibody supplied with the kit was incubated for 30 min at room temperature. Antibody binding was visualised with 3-amino-9-ethylcarbazole solution (LSAB2-Kit, Dako). Finally, the tissues were counterstained by hemalaun.

A surgical pathologist, FB, carried out a blinded evaluation of the stained slides. According to Behrmann *et al*,¹⁴ the staining intensity was estimated by using a semiquantitative three-step scoring system (0 to 2+): 0, negative; 1+, weak positive; and 2+, strong positive. Cytoplasmic and membranous immunoreactivity were scored independently. Because of the small amount of tissue of each individual tumour on

the TMA, even weak P-cadherin immunoreactivity (1+) was considered to be positive. The percentage of Ki-67-positive cells of each specimen was determined as described previously.¹² A high Ki-67 labelling index was defined as at least 5% of the tumour cells being positive.

Statistics

Values of $p < 0.05$ were considered to be significant. Statistical analyses were completed with SPSS V.10.0. All specimens on the TMAs were considered independently. Contingency table analysis and two-sided Fisher's exact tests were used to study the statistical association between clinicopathological and immunohistochemical parameters. Recurrence-free survival (RFS) curves comparing patients with or without any of the factors were calculated by the Kaplan–Meier method, with significance evaluated by two-sided log rank statistics. For the analysis of RFS, patients were censored at the time of their last tumour-free clinical follow-up appointment. For multiple testing, the closed test principle was used. A stepwise multivariate Cox regression model was adjusted, testing for the independent prognostic relevance of cytoplasmic P-cadherin expression. The limit for reverse selection

Number of melanoma patients at risk:

Time (months)	0	20	40	60	80	100	120
Score 0	44	25	15	12	4	2	1
Score 1+ 2+	37	28	21	15	7	2	1

Figure 2 Distribution of time (months) to melanoma recurrence among patients with negative (0) and positive (1+ to 2+) cytoplasmic P-cadherin immunoreactivity as estimated by the Kaplan–Meier method. HR, hazards ratio; IHC, immunohistochemistry; RFS, recurrence-free survival.

procedures was $p = 0.1$. The proportionality assumption for all variables was assessed with log-negative-log-survival distribution functions.

RESULTS

Investigation of P-cadherin protein expression in a large series of nevi and malignant melanomas by TMA technology was informative in 90.9% (318/350) of cases. Membranous and cytoplasmic P-cadherin protein expression of any intensity (1+ to 2+) was detected in 41.5% (132/318) and 64.2% (204/318) of informative cases, respectively. Figures 1A–F show representative P-cadherin immunostaining patterns. Table 1 summarises the IHC results for each tumour entity on the TMA. Expressions of both membranous and cytoplasmic P-cadherin were significantly reduced in malignant melanomas ($p < 0.001$) and melanoma metastases ($p < 0.001$) compared with benign nevi.

For descriptive data analysis, all relevant variables were compared with P-cadherin IHC (table 2). In primary melanomas, loss of cytoplasmic expression was associated only with tumour size > 2 mm ($p = 0.003$), whereas loss of membranous expression was associated with tumour size > 2 mm ($p = 0.011$) and higher Clark levels ($p < 0.001$; table 2). A high Ki-67 labelling index, however, was not associated with loss of P-cadherin immunoreactivity. Additionally, none of the other factors such as age, sex and growth pattern was markedly related to P-cadherin IHC.

Interestingly, a difference in P-cadherin expression was observed between the various types of benign nevi on the TMA (table 2); a significant reduction of membranous ($p = 0.025$) and cytoplasmic ($p = 0.005$) P-cadherin expression was observed in dermal nevi compared with junctional and compound nevi.

Figure 3 Distribution of time (months) to melanoma recurrence among patients with negative (0) and positive (1+ to 2+) cytoplasmic P-cadherin immunoreactivity in a subgroup analysis as estimated by the Kaplan–Meier method. (A) Subgroup of tumours with thickness ≤ 2 mm; and (B) subgroup of tumours with thickness > 2 mm. IHC, immunohistochemistry; RFS, recurrence-free survival.

Prognostic relevance

RFS was compared between patients with negative and positive (1+ to 2+) P-cadherin expression by univariate log rank statistics. The effect of cytoplasmic and membranous immunoreactivity was investigated independently. Cytoplasmic P-cadherin staining ($p = 0.03$; fig 2) and tumour thickness ≤ 2 mm ($p = 0.03$) were the only factors that were associated with better RFS (table 3). The univariate hazard ratio for positive cytoplasmic P-cadherin expression was 0.0469 (95% confidence interval (CI) 0.235 to 0.936); accordingly, in patients with negative P-cadherin IHC, the probability of melanoma recurrence was twice as high as that in patients with positive IHC (1+ to 2+).

Table 3 Univariate analysis of factors associated with tumour recurrence and tumour-related death

Variable	Categorisation	Tumour recurrence (RFS)			Tumour-related death (OS)		
		n*	Events	p†	n*	Events	p†
Age at diagnosis (years)	≤60	48	25	0.7	48	7	0.6
	>60	40	18		40	7	
Sex	Female	39	15	0.06	39	5	0.4
	Male	49	28		49	9	
Clark level‡	II	5	0	0.4	5	0	0.3
	III	15	8		15	2	
	IV	54	28		54	8	
	V	13	7		13	4	
Tumour thickness	≤2.0 mm	38	14	0.03	38	4	0.2
	>2.0 mm	50	29		50	10	
Ki-67 labelling index	<5%	33	17	0.7	33	7	0.9
	≥5%	36	16		36	7	
Ulceration	No	68	32	0.4	68	10	0.5
	Yes	20	11		20	4	
Membranous P-cadherin immunohistochemistry	Score 0	56	30	0.2	56	9	0.7
	Score 1+ to 2+	25	9		25	5	
Cytoplasmic P-cadherin immunohistochemistry	Score 0	44	26	0.03	44	7	0.8
	Score 1+ to 2+	37	13		34	7	

OS, overall survival; RFS, recurrence-free survival.

*Only initial and unifocal malignant melanomas were included.

†Log rank test (two-sided), bold type representing significant data.

‡According to UICC. In: Sobin LH, Wittekind CH, eds. *TNM classification of malignant tumours*. 6th edn. New York: Wiley, 2002.

In a multivariate analysis, two different Cox regression models were developed for assessing the RFS rate. Table 4 shows the characteristics of the variables. Only tumour thickness, Clark level, ulceration and cytoplasmic P-cadherin expression were considered. In the raw model, only tumour thickness >2 mm was correlated with a higher relapse rate ($p = 0.014$). With reverse selection, the hazard ratio for melanoma recurrence was 2.476 (95% CI, 1.197 to 5.124); accordingly, in patients with tumours >2 mm, the probability for recurrence was at least twice as high as that in patients with tumours ≤2 mm in thickness. Finally, multiplicative terms of interaction (interactions 1–3) were considered, representing interactions between cytoplasmic P-cadherin expression and dichotomous covariables. After reverse selection, a model containing cytoplasmic P-cadherin expression and interaction 2 (tumour thickness × cytoplasmic P-cadherin expression) was found ($p < 0.1$). For graphical visualisation, we carried out a subgroup analysis with Kaplan–Meier plots (figs 3A,B); in the subgroup of tumours with tumour thickness ≤2 mm, strong cytoplasmic P-cadherin expression was associated with longer RFS ($p = 0.0292$), whereas cytoplasmic P-cadherin IHC had no prognostic effect in patients with tumours >2 mm.

Assuming different model constructs, loss of cytoplasmic P-cadherin expression can be an independent adverse risk factor for tumour recurrence in patients with tumours <2 mm.

DISCUSSION

This is the first study showing loss of P-cadherin protein expression in primary melanomas to be predictive of tumour recurrence.

We recently showed in a small study an inverse association between P-cadherin protein expression and progression of melanocytic tumours by immunohistochemical staining of tissue samples of benign melanocytic nevi, melanomas and melanoma metastasis, which yielded a decreasing amount of P-cadherin protein staining from benign melanocytic nevi to metastatic melanomas.¹³ Accordingly, loss of membranous P-cadherin expression was thought to play an important part in the progression of melanoma. Further, a quantification of P-cadherin expression in melanomas was of particular clinical interest as the secreted form of P-cadherin or loss of P-cadherin could force tumour dissemination. Consistent with this hypothesis, a reduced expression of membranous P-cadherin was associated with tumour thickness and Clark level. Even more important, a reduced expression of P-cadherin correlated significantly with a reduced RFS time ($p = 0.03$). A recent study by Pacifico *et al*¹⁵ also showed that loss of P-cadherin expression correlated with poorer outcomes; however, no association with tumour thickness or Clark level was observed.

Our data, supporting the previously suggested association between P-cadherin expression and metastasis,¹³ may be of great clinical importance because new biological markers predictive of RFS are still needed. In this study, expression of P-cadherin was shown to be a predictive marker for the subgroup of patients with tumours <2 mm in thickness. Especially in this group, it is of high relevance to determine patients with high risk of developing metastasis. In the past, several proteins were suggested to be predictive markers, such as matrix metalloproteinase-2, cyclins, heat shock protein 70, P-AKT, HNK-1, CXCR4, although none of these have yet been implemented in routine diagnosis.^{16–22}

Table 4 Multivariate Cox regression analysis of possible recurrence-free survival factors

Variables	Categorisation	Global	Stepwise reverse selection*		
		p	Hazard ratio	95% CI	p†
Raw model					
Clark level‡	0 II–III 1 IV–V	0.927	—		
Tumour thickness	0 ≤2.0 mm 1 >2.0 mm	0.158	2.476	1.197 to 5.124	0.014
Ulceration	0 No 1 Yes	0.998	—		
Cytoplasmic P-cadherin IHC	0 Score 0 1 Score 1+ to 2+	0.214	—		
Model with multiplicative terms of interaction					
Clark level‡	0 II–III 1 IV–V	0.671	—		
Tumour thickness	0 ≤2.0 mm 1 >2.0 mm	0.861	—		
Ulceration	0 No 1 Yes	0.722	—		
Cytoplasmic P-cadherin IHC	0 Score 0 1 Score 1+ to 2+	0.418	0.278	0.206 to 0.729	0.009
Interaction 1 between Clark level and cytoplasmic P-cadherin IHC		0.717	—		
Interaction 2 between tumour thickness and cytoplasmic P-cadherin IHC		0.202	3.150	0.998 to 9.942	0.050
Interaction 3 between ulceration and cytoplasmic P-cadherin IHC		0.505	—		

IHC, immunohistochemistry.

*Limit for stepwise reverse selection procedures $p=0.1$; —, excluded from the model.

†Log rank test (two-sided), bold type represents significant data.

Two phenomena are presented here: early in melanoma development, membranous P-cadherin expression is lost, resulting in the expression of the secreted variant, as recently published by our group.¹³ Later, during progression, expression of P-cadherin seems to be down regulated. Interestingly, down regulation of P-cadherin expression seems to be associated with localisation of the melanocytic cells. Down regulation of P-cadherin occurred more often in dermal nevi and in deep primary melanoma than in junctional nevi or thin primary tumours, respectively. Reports on phenomena such as this have been published before—for example, for IL8, where the expression level was influenced by the microenvironment.²³ Factors produced by fibroblasts are known to influence gene expression in melanoma cells and add to down regulation of P-cadherin expression. The regulation of P-cadherin expression, however, needs to be analysed in more detail in studies on the transcriptional control of the P-cadherin promoter.

The data presented here suggest that P-cadherin represents a potential immunohistochemical marker to predict metastasis in patients with primary melanomas <2 mm in thickness. This may provide a new basis for a decision on whether to treat patients with such primary melanomas in the future.

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Take-home messages

- Migration and invasion of human melanoma cells is an essential pathogenic process in the development of systemic metastasis.
- Dynamic rearrangement of cell–cell adhesion by modulation of cadherin expression has an important role in tumour metastasis and cell migration.
- P-cadherin expression is markedly reduced in malignant melanomas and melanoma metastases compared with benign nevi.
- Loss of membranous P-cadherin was associated with Clark level and tumour thickness.
- Loss of cytoplasmic P-cadherin expression is a highly salient adverse risk factor for tumour recurrence in patients with tumours <2 mm.

REFERENCES

- 1 **Wheelock MJ**, Johnson KR. Cadherins as modulators of cellular phenotype. *Annu Rev Cell Dev Biol* 2003;**19**:207–35.
- 2 **McGary EC**, Lev DC, Bar-Eli M. Cellular adhesion pathways and metastatic potential of human melanoma. *Cancer Biol Ther* 2002;**1**:459–65.
- 3 **Bogenrieder T**, Herlyn M. Cell-surface proteolysis, growth factor activation and intercellular communication in the progression of melanoma. *Crit Rev Oncol Hematol* 2002;**44**:1–15.
- 4 **Poser I**, Dominguez D, de Herreros AG, et al. Loss of E-cadherin expression in melanoma cells involves up-regulation of the transcriptional repressor Snail. *J Biol Chem* 2001;**276**:24661–6.
- 5 **Berx G**, Staes K, van Hengel J, et al. Cloning and characterization of the human invasion suppressor gene E-cadherin (CDH1). *Genomics* 1995;**26**:281–9.
- 6 **Silye R**, Karayiannakis AJ, Syrigos KN, et al. E-cadherin/catenin complex in benign and malignant melanocytic lesions. *J Pathol* 1998;**186**:350–5.
- 7 **Sanders DS**, Blessing K, Hassan GA, et al. Alterations in cadherin and catenin expression during the biological progression of melanocytic tumours. *Mol Pathol* 1999;**52**:151–7.
- 8 **Frixen UH**, Behrens J, Sachs M, et al. E-cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. *J Cell Biol* 1991;**113**:173–85.
- 9 **Vleminckx K**, Vakaet L Jr, Mareel M, et al. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. *Cell* 1991;**66**:107–19.

- 10 **Watabe M**, Nagafuchi A, Tsukita S, *et al*. Induction of polarized cell-cell association and retardation of growth by activation of the E-cadherin-catenin adhesion system in a dispersed carcinoma line. *J Cell Biol* 1994;**127**:247–56.
- 11 **Hoek K**, Rimm DL, Williams KR, *et al*. Expression profiling reveals novel pathways in the transformation of melanocytes to melanomas. *Cancer Res* 2004;**64**:5270–82.
- 12 **Wild PJ**, Herr A, Wissmann C, *et al*. Gene expression profiling of progressive papillary noninvasive carcinomas of the urinary bladder. *Clin Cancer Res* 2005;**11**:4415–29.
- 13 **Bauer R**, Hein R, Bosserhoff AK. A secreted form of P-cadherin is expressed in malignant melanoma. *Exp Cell Res* 2005;**305**:418–26.
- 14 **Behrmann I**, Wallner S, Komyod W, *et al*. Characterization of methylthioadenosin phosphorylase (MTAP) expression in malignant melanoma. *Am J Pathol* 2003;**163**:683–90.
- 15 **Pacifico MD**, Grover R, Richman PI, *et al*. Identification of P-cadherin in primary melanoma using a tissue microarray: prognostic implications in a patient cohort with long-term follow up. *Ann Plast Surg* 2005;**55**:316–20.
- 16 **Vaisanen A**, Tuominen H, Kallioinen M, *et al*. Matrix metalloproteinase-2 (72 kD type IV collagenase) expression occurs in the early stage of human melanocytic tumour progression and may have prognostic value. *J Pathol* 1996;**180**:283–9.
- 17 **Alonso SR**, Ortiz P, Pollan M, *et al*. Progression in cutaneous malignant melanoma is associated with distinct expression profiles: a tissue microarray-based study. *Am J Pathol* 2004;**164**:193–203.
- 18 **Farkas B**, Hantschel M, Magyarlaci M, *et al*. Heat shock protein 70 membrane expression and melanoma-associated marker phenotype in primary and metastatic melanoma. *Melanoma Res* 2003;**13**:147–52.
- 19 **Carlson JA**, Slominski A, Linette GP, *et al*. Biomarkers in melanoma: staging, prognosis and detection of early metastases. *Expert Rev Mol Diagn* 2003;**3**:303–30.
- 20 **Scala S**, Ottaviano A, Ascierto PA, *et al*. Expression of CXCR4 predicts poor prognosis in patients with malignant melanoma. *Clin Cancer Res* 2005;**11**:1835–41.
- 21 **Dai DL**, Martinka M, Li G. Prognostic significance of activated Akt expression in melanoma: a clinicopathologic study of 292 cases. *J Clin Oncol* 2005;**23**:1473–82.
- 22 **Thies A**, Schachner M, Berger J, *et al*. The developmentally regulated neural crest-associated glycotape HNK-1 predicts metastasis in cutaneous malignant melanoma. *J Pathol* 2004;**203**:933–9.
- 23 **Gutman M**, Singh RK, Xie K, *et al*. Regulation of interleukin-8 expression in human melanoma cells by the organ environment. *Cancer Res* 1995;**55**:2470–5.