CANCER GENOMICS & PROTEOMICS 5: 293-300 (2008)

The Protein Expression of TRP-1 and Galectin-1 in Cutaneous Malignant Melanomas

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Abstract. *Background: Patients with metastazing malignant melanoma have a poor outcome and determination of thickness of the primary tumor remains as the most important prognostic predictor. The aim of this study was to use an antibody-based proteomics strategy to search for new molecular markers associated with melanoma progression. Two proteins, TRP-1 and galectin-1, were identified as proteins with enhanced expression in cells from the melanocytic lineage. Patients and Methods: Protein profiling of TRP-1 and galectin-1 together with proliferation marker Ki-67 and melanocyte marker Melan-A was performed in normal tissues from 144 individuals and in 216 different tumors using tissue microarrays and immunohistochemistry. The protein expression pattern was further analyzed in a defined cohort of 157 patients diagnosed with invasive cutaneous malignant melanoma. Results: Both TRP-1 and galectin-1 were highly expressed in normal melanocytes and melanoma. The expression of TRP-1 was inversely correlated with tumor stage ($p=0.002$, ($R=-0.28$)). Neither TRP-1 or galectin-1 was associated with overall or disease free survival ($p>0.14$, $p>0.46$ respectively). Ki-67 was associated with tumor stage and survival ($p<0.001$). Conclusion: TRP-1 and galectin-1 protein expression patterns were determined in normal and cancer tissues and both proteins were expressed in the majority of the malignant melanomas. There was no correlation between TRP-1 or galectin-1 expression and survival.*

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Key Words: human Protein Atlas Program, malignant melanoma, TRP-1, galectin-1.

Cutaneous malignant melanoma is a disease with an increasing incidence (1). Melanoma affects the younger patient population and each death related to melanoma corresponds to approximately 19 years of life lost, one of the highest for any cancer (2-5). Melanoma cells are derived from melanocytes but transformation of melanocytes and tumor progression involves multiple changes in biological behavior, accompanied by specific alterations in gene expression (6). These differentially expressed genes, which distinguish the different tumor stages, may comprise new targets for diagnosis, prognosis and therapy.

Using an antibody-based proteomics strategy (7), the Swedish Human Protein Atlas Program (HPA) has developed and published a freely available web-based protein atlas (8) (www.proteinatlas.org). This effort includes a large-scale design and production of recombinant fragments of human proteins, *i.e.* protein epitope signature tags (PrESTs) based on the human genome sequence and subsequent generation of mono-specific antibodies (affinity-purified polyclonal antibodies) (9). These antibodies are used for immunohistochemical staining on a multitude of human tissues, both normal and cancer tissues, assembled together in a tissue microarray (TMA) format (10, 11).

Twelve cases of malignant melanoma are included in the HPA program with duplicates from each case. Two candidate proteins, TRP-1 and galectin-1 were identified as highly expressed in malignant melanomas. The aim of this study was to further investigate the protein expression levels of these markers in a large cohort including 157 patients diagnosed with invasive cutaneous malignant melanoma.

galectin-1 is encoded by the LSGALS1 gene, located on chromosome 22q12 (12). galectin-1 has been shown to be involved in the regulation of immune responses (13) and has further been postulated to be involved in angiogenesis (14). galectin-1 has also been shown to be over expressed in tumors as well as in their surrounding stroma, the latter as a consequence of malignant progression (15).

TRP-1, also known as mesosomal membrane protein gp75 or brown locus protein, is a tyrosinase related protein, involved in the complex synthesis of melanin. TRP-1 is one of the most abundant membrane proteins in melanocytes and pigmented melanoma cells (16). TRP-1 is encoded by the Tyrp1/brown locus on chromosome 9p23 (17) and has been shown to be involved in melanogenesis as well as being a preventive factor in inhibiting melanocytic cell death (18).

galectin-1 and TRP-1 are thus of interest as potential biomarkers and in the present descriptive study the role of these proteins has been investigated in relation to Melan-A and Ki-67 expression in patients with cutaneous malignant melanoma using immunohistochemistry and tissue microarrays (11).

Patients and Methods

Patients. All tissues used as donor blocks were acquired from the archives at the Department of Pathology, Uppsala University Hospital, in agreement with approval from the Research Ethics Committee at Uppsala University, Uppsala, Sweden. TMAs containing duplicate cores (1 mm) from 216 different tumors corresponding to 20 of the most common cancer types and triplicate cores from 48 different normal tissues were generated for initial screening of protein expression (8, 11).

The patients included in the extended study of melanoma were collected from a cohort of 352 patients diagnosed with primary cutaneous malignant melanoma in the Uppsala region during the period 1982-2004. All patients were followed at the Department of Oncology, Uppsala University Hospital, Uppsala, Sweden. Patients diagnosed with melanoma *in situ* were excluded as well as patients where only autopsy material was available. Patients where clinical information or a signed consent was missing were also excluded. In total, 157 patients fulfilled the inclusion criteria and became subjects of the present study. During follow-up, 60 patients relapsed. Relapse was defined as a local recurrence or a metastasis. Metastatic tumor material from 36 of these was available and included in the study. The patients were diagnosed with superficial spreading melanoma (SSM), nodular malignant melanoma (NMM), acral lentiginous melanoma (ALM), lentigo malignant melanoma (LMM) and malignant melanoma not otherwise classified. The median follow-up period was 77 months (range: 1.4-241 months). All patients included were staged according to UICC 2002 (TNM system).

For every patient the following clinical parameters were registered: gender, age (<65 years, >65 years), T-stage and histology.

Generation of tissue microarrays – malignant melanoma. TMAs were created essentially as previously described (10, 11). For all the patients haematoxylin-eosin or van Gieson stained slides were available for comparison with the paraffin tissue blocks and in that way the tumor area was marked and identified. From each paraffin donor block 0.6 mm cylinders were taken and placed into recipient blocks using an automated tissue microarrayer (Beecher Instruments, Silver Springs, MD, USA). Where possible, three cylinders were taken from the tumor and placed in the recipient block but when the tumors were thin and little material was available, only one or two cylinders were taken. In small tumors the majority of the infiltrative part was included but for larger lesions the most suitable area was chosen.

Immunohistochemistry. An in-house generated antibody validated within the HPA project (www.proteinatlas.org) was used to analyze the expression of galectin-1 (HPA000646). For TRP-1 (Novocastra Laboratories Ltd., United Kingdom), Melan-A (Novocastra) and Ki-67 (DAKO) commercially available antibodies were used. For galectin-1 and TRP-1 the immunodetection was achieved by using products from DAKO, Glostrup, Denmark, but for the other markers products from LabVision AB, Värmdö, Sweden, were used. From each TMA block 4 μm thick tissue sections were cut and applied to slides. The slides were deparaffinized in xylene and rehydrated through graded alcohols. For the DAKO immunodetection, HIER (heat induced epitope retrieval) was performed using a Decloaking chamber®. The primary antibody for galectin-1 was applied diluted 1:400 and for TRP-1 (Novocastra) 1:200. Immunodetection was achieved by using secondary reagent goat-anti mouse/rabbit HRP-conjugated Envision™ detection kit (DAKO) before developing with the chromogen diaminobenzidine (DAB). The slides were counterstained with Harris haematoxylin, dehydrated through graded alcohols and xylene and finally mounted with Pertex (Histolab AB, Gothenburg, Sweden). For the Labvision AB immunodetection, heat induced epitope retrieval (HIER) was performed using a Decloaking chamber® (Biocare Medical, Walnut Creek, CA, USA). All the primary antibodies were applied diluted 1:100. Immunodetection was achieved by applying Primary Antibody Enhancer (LabVision AB) and thereafter Large Volume HRP Polymer (LabVision AB) before developing with Large Volume DAB Plus Substrate System (LabVision AB). The slides were counterstained with Mayers haematoxylin, dipped in lithiumchloride and dehydrated through graded alcohols and xylene. Finally, the slides were mounted with Pertex (Histolab AB, Gothenburg, Sweden).

Scoring of immunostainings. For each TMA, immunostained tissue spots were scored according to the amount of positively stained tumor cells using a four-graded scale: >75% of tumor cells staining positively (3 points), 25-75% (2), <25% (1), negative tumor cells (0). The intensity of the staining was evaluated using a three-graded scale: strong (2), weak (1) or negative (0).

From these scoring data three categories were defined based on the amount and the staining intensity of immunoreactive tumor cells: Grade 3: Strong immunoreactivity in >25% of tumor cells. Grade 2: Weak immunoreactivity in >25% of tumor cells or strong immunoreactivity in <25% of tumor cells. Grade 1: Negative immunostaining or weak immunostaining in <25% of tumor cells.

For the evaluation of the proliferation marker Ki-67, tumors were divided into two groups: ≥20% of tumor cells positive and <20% tumor cells positive as previously suggested (19,20). The evaluation of the immunostaining was done by the same pathologist (MA).

Statistics. Patient survival (overall survival and disease free survival) was estimated using the Kaplan–Meier method, in which univariate analysis was performed using the log-rank test. For the overall survival analysis the end-point was death. For the disease free survival the end-point was defined as relapse in the form of a metastases or a recurrent tumor. Spearman rank correlation was used to test for associations between investigated markers and T-stage. Kruskal-Wallis test was used to test for associations between patients diagnosed with SSM or NMM and tumor stage (T-stage). For all these afore mentioned analyses tumors with grade 1, 2 and 3 expression of TRP-1 were included but for galectin-1 and Melan-A only tumors with grade 2 and 3 were included (Table I). Patients

Table I. *Patients' characteristics and the grades of investigated proteins.*

	Number of patients	Median survival in years	galectin-1				TRP-1				Ki-67		
			1	2	3	¶	1	2	3	¶	<20%	≥20%	¶
Gender													
Male	84	6.1	-	38	39	7	17	25	33	9	51	26	7
Female	73	6.9	-	37	29	7	22	18	24	9	52	10	11
Clark													
II	31	7.3	-	12	11	8	1	4	16	10	20	2	9
III	54	7.5	-	29	20	5	15	18	15	6	39	10	5
IV	55	5.3	-	27	27	1	18	18	17	2	35	16	4
V	8	1.2	-	4	4	-	3	1	4	-	3	5	-
¶	9	5.3	-	3	6	-	2	2	5	-	6	3	-
T-stage													
I	63	7.3	-	30	21	13	6	16	26	16	43	5	16
II	34	6.6	-	19	15	-	11	12	10	1	26	8	-
III	31	5.3	-	18	12	1	14	8	8	1	18	11	2
IV	17	4.1	-	6	11	-	7	4	6	-	10	7	-
M1	5	0.6	-	-	5	-	-	2	3	-	3	2	-
¶	7	4.6	-	2	4	-	1	1	4	-	3	3	-
Histology													
SSM	96	7.2	-	48	36	12	15	31	35	15	73	10	13
NMM	47	5.4	-	20	26	1	20	11	15	1	24	22	1
ALM	6	6.6	-	3	3	-	3	-	3	-	3	2	1
LMM	4	5.1	-	2	1	1	-	1	1	2	1	1	2
Others	4	-	-	2	2	-	1	-	3	-	2	1	1

¶: Information/results lacking.

Table II. *Spearman rank and Kruskal-Wallis test (histology only) correlation analysis (p-values).*

	TRP-1	galectin-1	Melan-A	Ki-67
T-stage	0.002 (R=-0.28)	0.29	0.54	<0.001
Histology	0.02	0.14	0.21	<0.001
TRP-1	-	0.03 (R=0.18)	0.02 (R=0.21)	0.47
galectin-1	-	-	0.24	0.01 (R=0.21)
Melan-A	-	-	-	0.67

lacking immunohistochemical staining results were excluded. For the Spearman rank correlation, Kruskal-Wallis test and the disease free survival analysis patients with metastatic disease at diagnosis were excluded (n=5). Throughout the study, a 5% two-sided significance level was used. The patients were followed until time of death or until 1st of August 2007.

Results

Expression patterns in normal and cancer tissues. Protein profiling in normal and cancer tissues showed moderate to strong cytoplasmic expression of TRP-1 in normal epidermal melanocytes and in 6/10 malignant melanomas. In addition, cytoplasmic positivity was observed in subsets of bone marrow and lymphoid cells. All other normal and malignant cells were essentially negative. galectin-1 protein expression was also found in the cytoplasm of normal melanocytes and in 9/9 malignant melanomas. Furthermore, galectin-1 was expressed in various mesenchymal cells, including nerves, heart and smooth muscle fibres as well as extracellular deposits in connective tissue and tumor stroma. In addition, strong staining was found in kidney glomeruli , alveolar cells in the lung, cells from adrenal cortex and parathyroid gland as well as germinal and Leydig cells in the testis. With the exception of melanoma, a subset of breast cancers, thyroid cancers and occasional other tumors, most malignant tumors were either negative or showed weak staining in the cytoplasm.

Expression patterns in malignant melanoma cohort. The present study included 157 primary cutaneous melanomas and 36 metastatic melanomas. Ninety-six patients (61%)

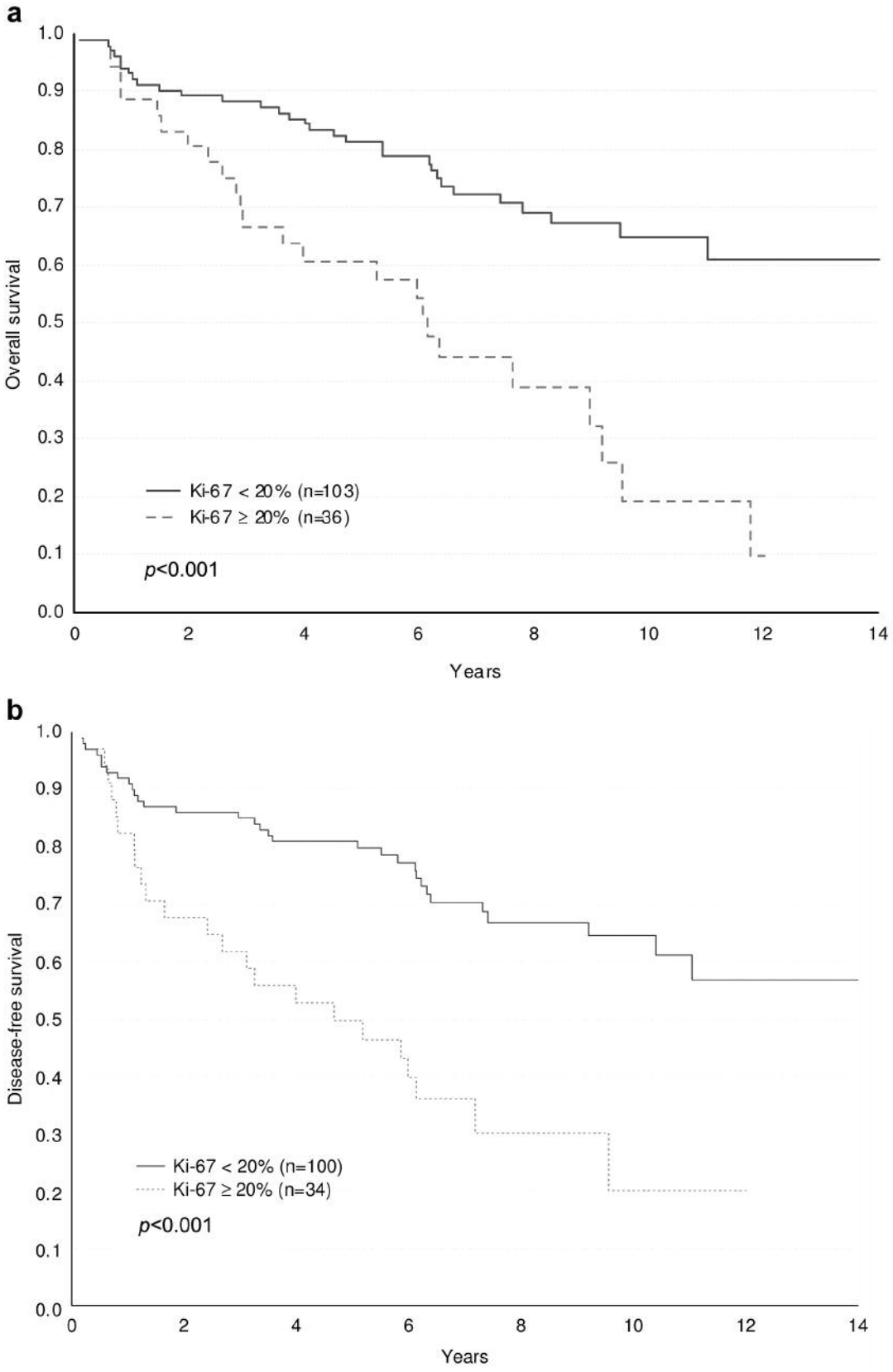

Figure 1. *Overall survival (a) and disease-free survival (b) based on the expression of Ki-67.*

Table III. *Patients who relapsed during follow-up and the grades of the investigated proteins in the metastases.*

T-stage	Number of patients that relapse	Number of evaluated metastases	Metastatic TRP-1				Metastatic galectin-1			Metastatic Melan-A				Metastatic Ki-67		
			1	2	3	¶	2	3	¶	1	2	3	¶	<20%	≥20%	¶
T1	11/63 (17%)	7/11	4	1	1	1	4	2	1	-	-	6	1	1	5	1
T2	8/34 (24%)	2/8	1	-	1	-	1	1	-	-	1	1	-	-	2	-
T3	21/31 (68%)	11/21	4	2	2	3	7	2	2	1	1	7	2	3	7	1
T4	15/17 (88%)	12/15	5	5	2	-	3	9	-	2	2	8	-	7	5	-
¶	5/7 (71%)	4/5	1	1	1	1	2	1	1	1	-	2	1	3	-	1

¶: Information/results lacking.

had SSM, forty-seven (30%) had NMM, six patients (4%) had ALM, four patients (2.5%) were diagnosed with LMM and four patients (2.5%) had other subtypes or were unclassified (Table I).

For TRP-1, 36% of the primary tumors were grade 3, 28% grade 2 and 24% grade 1 (Table I). For galectin-1, 44% of the primary tumors were grade 3, 47% were grade 2, none were grade 1.

The overall survival and disease-free survival was investigated with respect to expression levels of the studied proteins in primary tumors. A total of 60 patients (38%) out of 157 included in the study relapsed during the follow-up period. For TRP-1 no association was found for overall survival ($p>0.63$) or for disease-free survival ($p>0.14$). The same applied for galectin-1 with overall survival ($p=0.46$) and disease free survival ($p=0.63$). A statistically significant association was, however, found between Ki-67 expression and both overall and disease-free survival ($p<0.001$) (Figure 1).

TRP-1 ($p=0.002$, (R=–0.28)) and Ki-67 ($p<0.001$, R=0.3)) were associated with T-stage, where TRP-1 was inversely correlated. This was however not found for galectin-1 ($p=0.29$). Further correlation analyses are shown in Table II. A significant correlation was found between the expression of TRP-1 and galectin-1 ($p=0.03$).

For the metastases included in the study 19% were grade 3 for TRP-1, 25% were grade 2 and 42% were grade 1. For galectin-1, 42% of the metastases were grade 3, 47% grade 2 but none were grade 1. For Ki-67, 39% had low proliferative activity (<20% positive tumor cells) and 53% had high proliferative activity (≥20% positive tumor cells) (Table III).

Discussion

The incidence of malignant melanoma is increasing and with the exception of tumor excision, there is a lack of effective treatment. Further, melanoma is a disease with a very poor prognosis for the patients with advanced stages. A difficult task and a challenge is to select patients in the curable stages where extra efforts could provide the difference between life and death. It is thus of importance to identify genes/proteins that may function as new potential targets for therapy as well as new molecular markers for diagnosis and/or prognosis.

galectin-1 has already been indicated as a potential prognostic marker in other malignancies (15), also, both TRP-1 (21) and galectin-1 (15) have been suggested as potential cancer therapy targets.

The results from the current study show a statistically significant correlation between TRP-1 and galectin-1 ($p=0.03$) (Table II). TRP-1 is activated by wild-type p53 and has promotors with p53 responsive elements (22). galectin-1 expression has also been attributed to involvement by p53 (23) and therefore a hypothetical explanation to their correlation might be that both these proteins' expression is initiated in a p53 dependent manner. However, this explanation is purely hypothetical and the current correlation might simply be due to sequential events in the malignant progression of malignant melanoma. Further studies are needed to fully explore this hypothesis.

galectin-1 expression in tumor samples, or neighbouring tumor tissue, has generally been considered as a sign of malignant progression (15). galectin-1 was in the present study expressed as grade 2 or grade 3 (thus intensely expressed) in all investigated tumor samples. This pattern of expression has previously been found in pancreatic ductal adenocarcinomas (24, 25) and bladder transitional-cell carcinomas (26).

A significant correlation between galectin-1 ($p=0.01$) and the proliferation index marker Ki-67 was found. From preclinical studies of colon cancer cell lines, endogenously expressed galectin-1 has been reported to induce apoptosis and is thus negatively correlated with proliferation (27). Conversely, clinical studies in neuroblastomas and small cell lung carcinomas (28) has implied a correlation with proliferation and are thus in concordance with the present study. In the present study, galectin-1 was not correlated with

disease free survival (p=0.63) or overall survival (p=0.46). In previous studies of prostate cancer (29), gliomas (30, 31), head and neck squamous cell carcinomas (32) as well as in colon cancer (33), the expression of galectin-1 is of prognostic relevance. The lack of correlation with survival, despite the correlation between galectin-1 and high proliferation rate might be due to the limited number of patients in the present study. Perhaps a positive correlation will be found when galectin-1 is investigated in larger patient populations.

The melanogenic pathway occurs within the melanosome (possibly due to the inherent cytotoxicity of melanin intermediates) and melanin pigments are produced by the action of the melanin-forming enzyme tyrosinase and its related proteins. TRP-1, also known as mesosomal membrane protein gp75 or brown locus protein, is a tyrosinase related protein, involved in the complex synthesis of melanin. TRP-1 has been found in breast and prostate cancer cell lines which were derived from non-metastatic tumors, indicating that TRP-1 is not exclusively expressed in cells of melanocytic lineage (34).

TRP-1 (p=0.002, (R=–0.28)) as well as Ki-67 (p<0.001, R=0.3)) were statistically correlated with T-stage but for TRP-1 this correlation was inverse. In general, the more advanced tumor (thus the higher T-stage) the more aggressive biological behaviour and the described correlation above suggests that TRP-1 might be a marker for biological aggressiveness in malignant melanoma. Further, survival analysis did not show that TRP-1 over expression was correlated with disease free survival (p>0.14) or overall survival (p>0.63). These data are in accordance with a study by De Wit *et al.* (35) in which semiquantitative RT-PCR of melanocytic tumor lesions showed that pooled primary malignant melanoma samples express TRP-1 at considerable higher levels than samples from metastases from malignant melanoma. Further, in a study by De Vries *et al.* (36), TRP-1 seemed to be less expressed in advanced primary lesions as well as in metastases of human cutaneous melanoma. Fang *et al.* (37) attributed these findings to the fact that the invasive front in malignant melanoma is in general TRP-1 negative. In the present study, the expression of TRP-1 seemed to be stronger in the superficial parts of the tumor and as described by Fang *et al.* (37), to be lesser expressed in the invasive front of the primary malignant melanoma.

The expression of Ki-67 that was subdivided into two groups (<20% and ≥ 20%) showed a statistical significant correlation with overall survival (p<0.001) and disease free survival (p<0.001), (Figure 1). Patients with the more proliferative active tumors (high Ki-67 labeling index) showed a higher risk of relapse and poorer outcome compared with patients with low Ki-67 labeling index. In addition, the expression of Ki-67 was statistically significant correlated with T-stage and histology (p<0.001) (Tables I and II). High levels of proliferating tumor cells (≥ 20% Ki-67 positive melanoma cells) were associated with thicker tumors and also with the morphologically defined nodular sub-type (NMM). This was clearly evident when compared with the SSM sub-type, which more often showed low proliferative activity (<20%) (Table I). The role of a proliferation index as a marker in diagnostics of established melanoma tumors has been debated and results from different studies have not rendered Ki-67 staining as an independent marker with prognostic significance. Studies have been published describing the importance of the proliferative activity in both thin (<1 mm) (38) and thick melanomas (>4 mm) (39). However, other studies have not been able to verify these results (19, 40).

In conclusion, the present study describes the protein expression of galectin-1 and TRP-1 in cutaneous malignant melanomas, demonstrating the usefulness of protein expression profiling using the protein atlas approach.

Acknowledgements

This work was supported by grants from the Knut and Alice Wallenberg Foundation and the Research Fund "Stiftelsen för onkologiska klinikens forskningsfond" at the Department of Oncology, Uppsala University Hospital.

References

1 Armstrong BK and Kricker A: Cutaneous melanoma. Cancer Surv *19-20*: 219-240, 1994.

2 Hamre MR, Chuba P, Bakhshi S, Thomas R and Severson RK: Cutaneous melanoma in childhood and adolescence. Pediatr Hematol Oncol *19*: 309-317, 2002.

3 Karlsson P, Boeryd B, Sander B, Westermark P and Rosdahl I: Increasing incidence of cutaneous malignant melanoma in children and adolescents 12-19 years of age in Sweden 1973-92. Acta Derm Venereol *78*: 289-292, 1998.

4 Lange JR and Balch CM: Melanoma in children: heightened awareness of an uncommon but often curable malignancy. Pediatrics 115: 802-803, 2005.

5 Strouse JJ, Fears TR, Tucker MA and Wayne AS: Pediatric melanoma: risk factor and survival analysis of the surveillance, epidemiology and end results database. J Clin Oncol *23*: 4735-4741, 2005.

6 Wong AK and Chin L: An inducible melanoma model implicates a role for RAS in tumor maintenance and angiogenesis. Cancer Metastasis Rev *19*: 121-129, 2000.

7 Uhlen M and Ponten F: Antibody-based proteomics for human tissue profiling. Mol Cell Proteomics *4*: 384-393, 2005.

8 Uhlen M, Bjorling E, Agaton C, Szigyarto CA, Amini B, Andersen E, Andersson AC, Angelidou P, Asplund A, Asplund C, Berglund L, Bergstrom K, Brumer H, Cerjan D, Ekstrom M, Elobeid A, Eriksson C, Fagerberg L, Falk R, Fall J, Forsberg M, Bjorklund MG, Gumbel K, Halimi A, Hallin I, Hamsten C, Hansson M, Hedhammar M, Hercules G, Kampf C, Larsson K, Lindskog M, Lodewyckx W, Lund J, Lundeberg J, Magnusson K, Malm E, Nilsson P, Odling J, Oksvold P, Olsson I, Oster E,

Ottosson J, Paavilainen L, Persson A, Rimini R, Rockberg J, Runeson M, Sivertsson A, Skollermo A, Steen J, Stenvall M, Sterky F, Stromberg S, Sundberg M, Tegel H, Tourle S, Wahlund E, Walden A, Wan J, Wernerus H, Westberg J, Wester K, Wrethagen U, Xu LL, Hober S and Ponten F: A human protein atlas for normal and cancer tissues based on antibody proteomics. Mol Cell Proteomics *4*: 1920-1932, 2005.

9 Nilsson P, Paavilainen L, Larsson K, Odling J, Sundberg M, Andersson AC, Kampf C, Persson A, Al-Khalili Szigyarto C, Ottosson J, Bjorling E, Hober S, Wernerus H, Wester K, Ponten F and Uhlen M: Towards a human proteome atlas: high-throughput generation of mono-specific antibodies for tissue profiling. Proteomics *5*: 4327-4337, 2005.

10 Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G and Kallioniemi OP: Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med *4*: 844-847, 1998.

11 Kampf C, Wester K, Björling E, Uhlen M, and Ponten F.: Antibody-based tissue profiling as a tool for clinical proteomics. Clin Proteomics *1*: 285-299, 2004.

12 Barondes SH, Castronovo V, Cooper DN, Cummings RD, Drickamer K, Feizi T, Gitt MA, Hirabayashi J, Hughes C, Kasai K, Leffler H, Liu FT, Lotan R, Mercurio AM, Monsigny M, Pillai S, Poirer F, Raz A, Rigby P, Rini J and Wang JL Galectins: a family of animal beta-galactoside-binding lectins. Cell *76*: 597-598, 1994.

13 Chung CD, Patel VP, Moran M, Lewis LA and Miceli MC: galectin-1 induces partial TCR zeta-chain phosphorylation and antagonizes processive TCR signal transduction. J Immunol *165*: 3722-3729, 2000.

14 Moiseeva EP, Javed Q, Spring EL and de Bono DP: Galectin 1 is involved in vascular smooth muscle cell proliferation. Cardiovasc Res *45*: 493-502, 2000.

15 Camby I, Mercier ML, Lefranc F and Kiss R: galectin-1: A Small Protein with Major Functions. Glycobiology *16*: 137-157, 2006.

16 Vijayasaradhi S, Doskoch PM and Houghton AN: Biosynthesis and intracellular movement of the melanosomal membrane glycoprotein gp75, the human b (brown) locus product. Exp Cell Res *196*: 233-240, 1991.

17 Vijayasaradhi S, Bouchard B and Houghton AN: The melanoma antigen gp75 is the human homologue of the mouse b (brown) locus gene product. J Exp Med *171*: 1375-1380, 1990.

18 Jimbow K, Gomez PF, Toyofuku K, Chang D, Miura S, Tsujiya H and Park JS: Biological role of tyrosinase related protein and its biosynthesis and transport from TGN to stage I melanosome, late endosome, through gene transfection study. Pigment Cell Res *10*: 206-213, 1997.

19 Hazan C, Melzer K, Panageas KS, Li E, Kamino H, Kopf A, Cordon-Cardo C, Osman I and Polsky D: Evaluation of the proliferation marker MIB-1 in the prognosis of cutaneous malignant melanoma. Cancer *95*: 634-640, 2002.

20 Alonso SR, Ortiz P, Pollan M, Perez-Gomez B, Sanchez L, Acuna MJ, Pajares R, Martinez-Tello FJ, Hortelano CM, Piris MA and Rodriguez-Peralto JL: Progression in cutaneous malignant melanoma is associated with distinct expression profiles: a tissue microarray-based study. Am J Pathol *164*: 193-203, 2004.

21 Li CY, Gao TW, Wang G, Han ZY, Shen Z, Li TH and Liu YF: The effect of antisense tyrosinase-related protein 1 in melanocytes and malignant melanoma cells. Br J of Dermatology *150*: 1081-1090, 2004.

22 Nylander K, Bourdon JC, Bray SE, Gibbs NK, Kay R, Hart I and Hall PA: Transcriptional activation of tyrosinase and TRP-1 by p53 links UV irradiation to the protective tanning response. J Pathol *190*: 39-46, 2000.

23 Paron I, D'Ambrosio C, Scaloni A, Berlingieri MT, Pallante PL, Fusco A, Bivi N, Tell G and Damante G: A differential proteomic approach to identify proteins associated with thyroid cell transformation. J Mol Endocrinol *34*: 199-207, 2005.

24 Berberat PO, Friess H, Wang L, Zhu Z, Bley T, Frigeri L, Zimmermann A and Buchler MW: Comparative analysis of galectins in primary tumors and tumor metastasis in human pancreatic cancer. J Histochem Cytochem *49*: 539-549, 2001.

25 Fitzner B, Walzel H, Sparmann G, Emmrich J, Liebe S and Jaster R: galectin-1 is an inductor of pancreatic stellate cell activation. Cell Signal *17*: 1240-1247, 2005.

26 Cindolo L, Benvenuto G, Salvatore P, Pero R, Salvatore G, Mirone V, Prezioso D, Altieri V, Bruni CB and Chiariotti L: galectin-1 and galectin-3 expression in human bladder transitional-cell carcinomas. Int J Cancer *84*: 39-43, 1999.

27 Horiguchi N, Arimoto K, Mizutani A, Endo-Ichikawa Y, Nakada H and Taketani S: galectin-1 induces cell adhesion to the extracellular matrix and apoptosis of non-adherent human colon cancer Colo201 cells. J Biochem (Tokyo) *134*: 869-874, 2003.

28 Gabius HJ, Andre S, Gunsenhauser I, Kaltner H, Kayser G, Kopitz J, Lahm H, Harms D, Szymas J and Kayser K: Association of galectin-1- but not galectin-3-dependent parameters with proliferation activity in human neuroblastomas and small cell lung carcinomas. Anticancer Res *22*: 405-410, 2002.

29 van den Brule FA, Waltregny D and Castronovo V: Increased expression of galectin-1 in carcinoma-associated stroma predicts poor outcome in prostate carcinoma patients. J Pathol *193*: 80-87, 2001.

30 Camby I, Belot N, Lefranc F, Sadeghi N, de Launoit Y, Kaltner H, Musette S, Darro F, Danguy A, Salmon I, Gabius HJ and Kiss R: galectin-1 modulates human glioblastoma cell migration into the brain through modifications to the actin cytoskeleton and levels of expression of small GTPases. J Neuropathol Exp Neurol *61*: 585-596, 2002.

31 Camby I, Belot N, Rorive S, Lefranc F, Maurage CA, Lahm H, Kaltner H, Hadari Y, Ruchoux MM, Brotchi J, Zick Y, Salmon I, Gabius HJ and Kiss R: Galectins are differentially expressed in supratentorial pilocytic astrocytomas, astrocytomas, anaplastic astrocytomas and glioblastomas, and significantly modulate tumor astrocyte migration. Brain Pathol *11*: 12-26, 2001.

32 Le QT, Shi G, Cao H, Nelson DW, Wang Y, Chen EY, Zhao S, Kong C, Richardson D, O'Byrne KJ, Giaccia AJ and Koong AC: galectin-1: a link between tumor hypoxia and tumor immune privilege. J Clin Oncol *23*: 8932-8941, 2005.

33 Nagy N, Legendre H, Engels O, Andre S, Kaltner H, Wasano K, Zick Y, Pector JC, Decaestecker C, Gabius HJ, Salmon I and Kiss R: Refined prognostic evaluation in colon carcinoma using immunohistochemical galectin fingerprinting. Cancer *97*: 1849-1858, 2003.

34 Agarwal D, Goodison S, Nicholson B, Tarin D and Urquidi V: Expression of matrix metalloproteinase 8 (MMP-8) and tyrosinase-related protein-1 (TYRP-1) correlates with the absence of metastasis in an isogenic human breast cancer model. Differentiation *71*: 114-125, 2003.

35 de Wit NJ, Burtscher HJ, Weidle UH, Ruiter DJ and van Muijen GN: Differentially expressed genes identified in human melanoma cell lines with different metastatic behaviour using high density oligonucleotide arrays. Melanoma Res *12*: 57-69, 2002.

36 de Vries TJ, Trancikova D, Ruiter DJ and van Muijen GN: High expression of immunotherapy candidate proteins gp100, MART-1, tyrosinase and TRP-1 in uveal melanoma. Br J Cancer *78*: 1156-1161, 1998.

37 Fang D, Hallman J, Sangha N, Kute TE, Hammarback JA, White WL and Setaluri V: Expression of microtubule-associated protein 2 in benign and malignant melanocytes: implications for differentiation and progression of cutaneous melanoma. Am J Pathol *158*: 2107-2115, 2001.

38 Gimotty PA, Van Belle P, Elder DE, Murry T, Montone KT, Xu X, Hotz S, Raines S, Ming ME, Wahl P and Guerry D: Biologic and prognostic significance of dermal Ki67 expression, mitoses, and tumorigenicity in thin invasive cutaneous melanoma. J Clin Oncol *23*: 8048-8056, 2005.

39 Ramsay JA, From L, Iscoe NA and Kahn HJ: MIB-1 proliferative activity is a significant prognostic factor in primary thick cutaneous melanomas. J Invest Dermatol *105*: 22-26, 1995.

40 Ilmonen S, Hernberg M, Pyrhonen S, Tarkkanen J and Asko-Seljavaara S: Ki-67, Bcl-2 and p53 expression in primary and metastatic melanoma. Melanoma Res *15*: 375-381, 2005.

Received September 29, 2008
Accepted October 13, 2008