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Expression of gp100 in Melanoma Metastases Resected Before or After Treatment with IFNα and IL-2

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Summary: The melanosomal protein gp100 was recently described as an antigen associated with tumor rejection in adoptive immunotherapy using tumor-infiltrating lymphocytes. In this study, we investigated whether the expression of gp100 in melanoma cells correlates with responsiveness to treatment with interferon- α and interleukin-2. Using the monoclonal antibody HMB-45 recognizing gp100, we examined metastatic tissue resected before therapy in 44 patients with melanoma including 9 patients with subsequent complete or partial remission. A very heterogeneous pattern of gp100-expression was found between patients, but the percentage of gp-100 positive cells in different metastases resected from the same patients was rather constant. This suggests that the gp100 expression determined in a single metastasis may be judged as being representative for other metastatic lesions of a patient. We found no correlation between expression of gp100 and responsiveness to subsequent immunotherapy. Our results show that the lack of gp100 before therapy is not associated with decreased responsiveness to subsequent cytokine treatment. Key Words: gp100—Melanoma— Interleukin-2—Interferon- α .

Several melanoma-associated antigens recognized by specific cytotoxic T lymphocytes could be identified in melanoma patients (reviewed in ref. 1). Melanomaspecific T cells grown from metastatic tissue or from peripheral blood frequently recognize two melanocytelineage specific proteins, gp100 and MART-1 (2-4). Gp100 seems to be an important tumor rejection antigen for the adoptive transfer of tumor-infiltrating lymphocytes. Response to therapy was associated with reactivity of the tumor-infiltrating lymphocytes against gp100 but not MART-1 (4).

Little is known so far about which role these melanoma-associated antigens play in cytokine-induced tumor regression. There are several findings suggesting that interleukin-2 (IL-2)-induced tumor regression in

vivo is mediated by specific T cells. Overexpressed Tcell receptor V β chains and clonal expansion of V β families were shown to be present only in responding, but not in progressive tumor sites from patients with melanoma (5–7). Although immunotherapy with interferon- α (IFN- α) and IL-2 can induce profound tumor regression even in patients with advanced metastatic melanoma, many patients show progressive disease despite treatment (8,9). Heterogeneity in expression of tumor antigens could account for differential responsiveness. The expression of gp100 in particular varies considerably within the tumor and from patient to patient. Gp100 expression can be determined using the specific monoclonal antibodies HMB-45 or NKI-beteb, which are widely used as diagnostic markers for human melanoma (10,11). Although >95% of primary melanoma lesions stain with NKI-beteb, only 80% of metastases are positive, and the percentage of positive cells declines with Breslow thickness in primary melanomas (12).

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Considering the variable expression of gp100, we addressed the question of whether the downregulation or loss of gp100 expression in melanoma metastases influences the responsiveness to immunotherapy with IFN α / IL-2. After immunotherapy, we further analyzed the expression of gp100 in regressing and progressing metastases.

MATERIALS AND METHODS

Tissue Samples

RESULTS

Heterogeneity of gp100 Expression Before Treatment

Metastatic tissue resected before treatment with IFN α / IL-2 from 46 patients was available. Melanoma cells from all but two patients showed intense reactivity with S100. The two S100-negative specimens were excluded from further analysis because the integrity of the material was uncertain. Metastatic tissue of the remaining 44 patients originated from skin (n = 6), subcutaneous tissue (n = 9), lymph node (n = 24), liver (n = 2), and gut (n = 2). Three were of unknown organ site that could not be determined histologically. The percentage of stained melanoma cells within a lesion showed a strong heterogeneity between different patients. Only 14 metastases from 13 patients had >50% of gp100-positive melanoma cells (Fig. 1; two patients are represented twice, because metastases from two different sites were available). In nine patients, melanoma cells were completely negative for gp100, and another nine had only 5% or less of melanoma cells expressing gp100. No obvious difference of gp100 expression was seen in metastases originating from different tissue. From eight patients, more than one simultaneously resected pretreatment metastasis was available, all showing corresponding percentages of gp100-positive cells (Table 1).

We made an effort to collect paraffin-embedded tissue of melanoma metastases from 75 melanoma patients from 16 different pathology departments. These patients had been treated with IFN α and high-dose IL-2 between 1988 and 1994 at our institution (9,13). From 46 patients, metastases resected before therapy were available. No samples could be retrieved from the remaining 29 patients for various reasons. Residual melanoma metastases were resected in 4 patients responding to immunotherapy and in 6 patients with stable disease within 12 weeks after the last treatment cycle (14). From two patients with progressive metastatic melanoma after treatment with immunotherapy we obtained metastases resected as palliative treatment. Most of this tissue was frozen and stored in liquid nitrogen. The treatment protocols including the surgical approach were approved by the University of Heidelberg Ethics Committee and informed con-

sent was obtained from all patients. Responses were defined according to standard World Health Organization criteria.

Histology and Detection of gp100 by Monoclonal Antibody HMB-45

Sections of 4 µm thickness were cut from paraffinembedded and from frozen tissue and were stained with hematoxylin and eosin for routine histology. The immunohistochemical stains were performed according to a three-layer APAAP technique. The following primary antibodies were used: the polyclonal rabbit antibody S100, and the monoclonal mouse antibody HMB-45 (both purchased from Dako, Hamburg, Germany). Metastatic lesions were examined by two independent pathologists unaware of the clinical data. HMB-45 staining



FIG. 1. Pattern of gp100 expression in metastatic melanoma lesions according to tissue origin (cut., cutaneous; s.c., subcutaneous; l.n., lymph node; visc., visceral; u.o., unknown origin). Each dot represents one single lesion (n = 46 from 44 patients; from 2 patients, 2 metastases resected from different organ sites were included). The percentage of cells stained per lesion was divided into six intensity groups (negative, 1-5, 6-25, 26-50, 51-75, 76-100% positive melanoma cells).

was expressed as estimated percentage of positive tumor cells and classified as negative, 1-5%, 6-25%, 26-50%, 51-75%, and 76-100% positive.

gp100 EXPRESSION IN MELANOMA METASTASES

TABLE 1.	Expression of gp100 in metastases resected	l
before	interferon-\alpha/interleukin-2: comparison of	
	simultaneously resected metastases	

Patient	Site	gp100
A.B.	5.C.	Neg.
	Liver	Neg.
M.R.	S.C.	Neg.
	S.C.	1-5%
	S.C.	1-5%
W.R.	S.C.	1-5%
	S.C.	1-5%
	s.c.	1-5%

TABLE 2. Expression of gp100 in metastases resected after interferon- α /interleukin-2 and response to treatment

S.S.s.c.PR+Negatives.c. $1-5\%$ S.Sch.s.c.PR+A.R.LungPR+	e of itive
s.c.I-5%S.Sch.s.c.PR+NegativeA.R.LungPR+ $6-25\%^a$	
S.Sch.s.c.PR+NegativeA.R.LungPR+ $6-25\%^a$	
A.R. Lung PR + $6-25\%^a$	
Gut $6-25\%^{a}$	
A.L. Adr. gland PR + $1-5\%^{a}$	
A.B. Pancreas SD + Negative	
V.W. Gut SD + Negative	
P.R. l.n. SD + $1-5\%^{a}$	
H.P. s.c. 3x SD – 1–5%	
s.c. 2x – 6–25%	
F.T. l.n. SD – 51–75%	
M.M. s.c. SD – 51–75%	
H.B. Thyr. gland PD – 26–50%	
G.W. s.c. PD - 51-75%	

C.D.	Lymph node Lymph node	1–5% 1–5%
E.M.	Lymph node Lymph node Lymph node	51–75% 51–75% 51–75%
K.G.	Cutaneous Cutaneous Lymph node	51–75% 51–75% 76–100%
J.F.	Cutaneous Cutaneous	76–100% 76–100%
M.M.	S.C. S.C.	76–100% 76–100%

s.c., subcutaneous.

Expression of gp100 Before Therapy and Response to Treatment

s.c., subcutaneous; l.n., lymph node; PR, partial response; SD, stable disease; PD, progressive disease. " Positive only in necrotic areas.

therapy in 12 patients (4 PR, 6 SD, 2 PD; Table 2). Metastases from three of six patients with SD showed histologically marked regressive changes and fibrosis. In these three patients, the tumors were also found to be encapsulated by a fibrous wall. In six of the seven patients with either clinical PR or SD and marked regressive changes on histology, either few or no gp100positive melanoma cells were found in residual lesions. An interesting observation was made in three of these patients (A.R., A.L., P.R.), all showing widely necrotic areas and in between areas with intact tumor structure

Based on the postulated role of gp100 as a tumor rejection antigen, we wanted to know whether patients with a higher percentage of gp100-expressing melanoma cells were more likely to respond to immunotherapy. We therefore compared the expression of gp100 in responders [complete responders/partial responders (CR/PR) n = 9], patients with stable disease (SD) (n = 12) and nonresponders [progressive disease (PD) n = 23] to subsequent therapy with IFN α /IL-2. With our semiquantitative classification, we found a clear distinction between positive (>25% of cells stained with HMB-45) and mainly negative ($\leq 5\%$) melanoma lesions. Patients with >25% gp100-positive melanoma cells did not respond better than patients with $\leq 5\%$ positive cells (20% CR/ PR, 24% SD, and 56% PD, n = 25 versus 22% CR/PR, 28% SD, and 50% PD, n = 18, respectively; Fig. 2).

Expression of gp100 in Metastases



FIG. 2. Pattern of gp100 expression in metastatic melanoma lesions according to response to subsequent treatment with interferon- α / interleukin-2. Each dot represents a single lesion from 44 patients [complete responders (CR) n = 2; partial responders (PR) n = 7; stable disease (SD) n = 12; PD n = 23]. The percentage of cells stained per lesion was divided into six intensity groups (negative, 1-5, 6-25, 26-50, 51–75, 76–100% positive melanoma cells).

Resected Postimmunotherapy

We analyzed the expression of gp100 in melanoma metastases resected within 3 months after immuno-

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TABLE 3. Expression of gp100 in metastases resected before and after interferon-α and interleukin-2: comparison in individual patients

Patient	Clinical response	Regressive changes on histology	Site	Percentage of gp100-positive cells
S.S.			s.c. (pre)	26-50%
	PR	+	l.n. (post)	Neg.
	PR	+	s.c. (post)	1-5%
P.R.			l.n. (pre)	76-100%
	SD	+	I.n. (post)	1-5%
S.Sch.			s.c. (pre)	Neg.
	PR	+	s.c. (post)	Neg.
A.B.			Liver (pre)	Neg.
	PR	+	Pancr. (post)	Neg.
V.W.			Gut (pre)	Neg.
	SD	+	Gut (post)	Neg.
F.T.			l.n. (pre)	26-50%
	SD		l.n. (post)	51-75%
M.M.			s.c. (pre)	76-100%
	SD		s.c. (post)	51-75%
H.B.			l.n. (pre)	26-50%
	PD		Thyr. (post)	26-50%

positive melanoma cells was seen in the metastases before therapy.

DISCUSSION

We found a very heterogeneous pattern in the percentage of gp100-expressing melanoma cells in the metastases from 44 patients resected before immunotherapy. This is in accordance with the findings reported in an earlier study from the EORTC Melanoma Cooperative Group, in which a similar distribution in the percentage of gp100-positive melanoma cells was found in metastases (12). In this former study, the monoclonal antibody NKI-beteb was used, which recognizes a different epitope of gp100, but shows the same reactivity as HMB-45 in melanoma metastases (11,15). In contrast to this interindividual heterogeneity, we found that different melanoma metastases originating from the same patient showed a corresponding pattern in the percentage of gp100-positive tumor cells. We also could not observe a different pattern in cutaneous, subcutaneous, or lymph node metastases. This suggests that the gp100 expression determined in a single metastasis may be judged as being representative for other metastatic lesions of a patient. This cannot be concluded for visceral metastases from our data, because we examined only four metastases, and three of them were gp100 negative. The main question of this study was whether heterogeneity in gp100 expression correlates with responsiveness to immunotherapy with IFN α /IL-2. Patients with gp100-negative or $\leq 5\%$ positive tumor cells, however, responded as well as patients with >25% positive cells. Therefore, additional or other melanoma-associated antigens than gp100 may play a role in cytokine-mediated tumor destruction and could influence the differential responsiveness. A heterogeneity of expression of both MART-1 and tyrosinase-related protein in metastatic melanoma was shown recently (16,17). Also the loss of HLA alleles, of functional β 2-microglobulin and differential expression of adhesion antigens were shown to occur in individual patients during tumor progression, which could lead to escape from T-cell recognition (18-20).

s.c., subcutaneous; I.n., lymph node; thyr., thyroid gland; PR, partial response; SD, stable disease; PD, progressive disease.

(Fig. 3). Some remaining cells in these necrotic areas were positive for gp100, whereas the tumor cells in areas with intact tumor structure were gp100 negative. Three patients with SD and 2 patients with PD showed no signs of tumor regression on histology. In four of these five patients, metastases with >25% positive melanoma cells were found.

Comparison of gp100 Expression Pre- and Postimmunotherapy

From eight patients, metastases resected before and after therapy were available (Table 3). In two patients, S.S. and P.R., who had responded to therapy, in the residual lesions <5% of the remaining cells expressed gp100. The histology in patient P.R., who had clinically a SD, revealed a tumor with extensive necrosis and only some intact tumor cells at the rim encapsulated by fibrous tissue. Only a few remaining cells in the widely necrotic areas were positive for gp100 (Fig. 3). In the metastases resected from these two patients from the same anatomic area before therapy, >25 and >75% of melanoma cells, respectively, were gp100 positive. In three responding patients, metastases resected before and after immunotherapy were negative for gp100. The metastases of two patients (F.T. and M.M.) with clinical SD

Our findings in the metastases resected after immunotherapy are only descriptive because of the low number of patients with metastases available before and after immunotherapy. We found that the melanoma cells in most of the regressing lesions after therapy expressed either little or no gp100. In two of the patients pretreat-

ment metastases were available, in which at least 25% of tumor cells were gp100-positive. It is unclear whether this loss of gp100 is a consequence of dedifferentiation

and no evidence of tumor regression on histology and of one patient (H.B.) with PD all contained >25% gp100positive melanoma cells, and a similar percentage of

SPIOD EXPRESSION IN MELANOMA METASTASES



FIG. 3. Immunohistochemical detection of gp100 using the monoclonal antibody HMB-45 (red staining) in two melanoma metastases from one patient (P.R., see also Table 3). Both slides are counterstained with hematoxylin (blue staining) to visualize gp100-negative tumor cells. A: Iliac lymph node metastases resected 1 year before immunotherapy shows 76–100% reactivity. B: Iliac lymph node metastasis resected 6 weeks after treatment with interferon- α and interleukin-2. Most of the melanoma cells are necrotic (left side of the picture); some of the remaining cells in the necrotic area react gp100-positive. In an area with intact tumor structure, all melanoma cells are gp100-negative (right side of B).

of the turnor or a result of preferential destruction of gp100-positive tumor cells. A loss of gp100 is also observed independent of therapy during tumor progression: The percentage of NKI-beteb-positive cells decreases with Breslow thickness in primary melanoma and is lower in metastases (12). In accordance with these data, we had seen that the disease duration inversely correlates with the percentage of gp100-positive melanoma cells in metastases (C. Scheibenbogen et al., unpublished observations). Two observations support the hypothesis of an immunoselection of gp100-positive tumor cells: (a) gp100-reactive T lymphocytes are frequently found in melanoma metastases (3,4,21), and (b) in three of our patients with regressing metastases we found necrotic tumor areas with some remaining gp100-positive melanoma cells, whereas intact tumor areas were gp100negative.

gp100 is not associated with decreased responsiveness to immunotherapy with IFN α and IL-2. Further studies of sequential biopsies would be necessary to clarify whether gp100 is a preferential target for cytokine-mediated tumor rejection in gp100-positive metastases.

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Taken together, our findings show that the lack of

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