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p-Glycoprotein expression in malignant melanoma *

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Summary. In some human malignancies resistance to chemotherapy is caused by an energy-dependent efflux system, responsible for the removal of chemotherapeutics out of the resistant tumor cells. A major component of this efflux system is the permeability glycoprotein (p-glycoprotein), which depends on the multidrug-resistance gene *MDR1*.

We have tested p-glycoprotein in primary and metastatic human melanoma by use of the monoclonal antibody C219; a substantial expression was only observed in 1/37 primary melanomas and in 1/27 melanoma metastases. None of the patients with negative metastases responded to chemotherapy. Moreover a complete remission of metastasic growth was observed in the patient with the metastasis significantly expressing the p-glycoprotein. Sequential studies revealed no significant increase of p-glycoprotein-positive cells during and after chemotherapy. We conclude that drug resistance in human melanoma does not usually depend on the p-glycoprotein-related efflux system. Other mechanisms are obviously responsible for drug resistance in this human malignancy.

Key words: Melanoma – Multidrug resistance – Permeability glycoprotein

Introduction

Many human cancers are intrinsically resistant to chemotherapy, others acquire cytotoxic resistance during the treatment (Mattern and Volm 1989).

Studies on resistant cell-culture lines revealed a multidrug resistance to several unrelated drugs (Kartner et al. 1985; Mattern and Volm 1989). Some drug-resistant tumor cells exhibit a plasma-membrane-related permeability glycoprotein (p-glycoprotein), the gene product of the *MDR1* gene, which is absent in drug-sensitive cells of the same histological type. Apparently p-glycoprotein is involved in the transport of chemotherapeutics out of the cells either by serving as an efflux pump or by changing the permeability of the lipid bilayer (Roninson 1987).

p-Glycoprotein is highly expressed in normal tissues of the pancreas, liver, colon, adrenal glands and kidneys, and in tumors derived from these tissues (Thiebaut et al. 1987; Mattern and Volm 1989).

Only two groups have so far studied the *MDR1* gene or its products in human melanoma cell lines: Goldstein et al. (1989) have found low *MDR1* RNA levels in three human melanomas; Lemontt et al. (1988) observed increased *MDR* gene expression and decreased drug accumulation in multidrug-resistant human melanoma cells.

Is the *MDR1*-related p-glycoprotein at least occasionally expressed in human melanoma? Do melanoma metastases acquire the *MDR1* phenotype during or after chemotherapy, a possible explanation for the drug resistance often observed in this malignancy? To answer these questions we have analysed 37 primary melanomas and 27 melanoma metastases.

Materials and methods

Antibody. The monoclonal antibody C219 detecting p-glycoprotein was obtained from Isotopen Diagnostik CIS GmbH, 6072 Dreieich, FRG.

Tumors. We have studied 37 primary melanomas of the skin and 27 cutaneous, subcutaneous and lymph-node metastases. Thickness classes, levels and subtypes of primary tumors are listed in Table 1. Melanoma metastases were excised before, during, and up to 3 months after chemotherapy. The specimens were snap-frozen either immediately in liquid nitrogen or after freezing in a cryostat, and stored at −85° C until use. The cryostat sections (4–6 mm thick) were fixed in acetone for 10 min.

Test procedure. Cryostat sections were incubated with mAb C219, diluted 1:4 in tris-(hydroxymethyl)aminomethane-buffered saline ph 7.6, for 1 h. Bound antibody was detected with the alkaline phosphatase anti alkaline phosphatase (APAAP) method as de-

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Table 1. Subtypes, thickness classes and levels of the 37 primary melanomas in this study

Parameter	Number of tumors
Subtype[a]	
SSM	17
NM	10
LMM	1
ALM	4
UCM	5
Thickness class	
⩽0.75 mm	8
0.76–1.5 mm	9
1.51–3 mm	12
>3 mm	8
Level	
II	5
III	8
IV	21
V	3

[a] SSM, superficial spreading melanoma; NM, nodular melanoma; LMM, lentigo maligna melanoma; ALM, acral lentiginous melanoma; UCM, unclassified melanoma

scribed previously (Ostmeier and Suter 1989). Slides were counterstained with haemalaun and 1% methyl green.

Evaluation. The percentage of positive tumor cells was estimated by two authors. When results conflicted, the case was reassessed jointly. Basal cell epitheliomas were tested as negative controls. The reaction with inflammatory cells, sweat glands and fibroblasts served as an internal positive control.

Chemotherapy. Chemotherapy protocols used for melanoma patients in this study are listed in Table 2.

Table 2. Chemotherapy protocols for melanoma patients in this study

Chemotherapy applied	No. of patients
BHD[a]	10
BHD, interferon	2
Dacarbazine, cisplatin	1
Cooper protocol[b]	1

[a] BHD, *N,N*-bis(2-chloroethyl)-*N*-nitrosourea, hydroxyurea, dacarbazine

[b] Cyclophosphamide, methotrexate, prednisone

Results

In 36/37 primary melanomas and in 26/27 metastases p-glycoprotein was either absent or could be detected only in a minority (1%–5%) of the cells. The few positive cells were tumor cells, although we could not discriminate them unequivocally from inflammatory cells containing the p-glycoprotein. Moreover sweat glands (Fig. 2) and fibroblasts reacted with mAb C219 (Fig. 1B).

Sequential studies of metastases revealed no significant change of the p-glycoprotein expression during or after chemotherapy (Table 3).

Contrary to the general trend we found two melanomas with a significant percentage of tumor cells containing the *MDR1*-related p-glycoprotein: in a primary nodular melanoma with a thickness of 2.81 mm, level IV 50%, and in a subcutaneous metastasis before chemotherapy 40% of the tumor cells were positive for this antigen (Table 3, Fig. 1A). Remission of metastatic growth could only be achieved in the patient with the subcutaneous metastasis significantly expressing the p-glycoprotein.

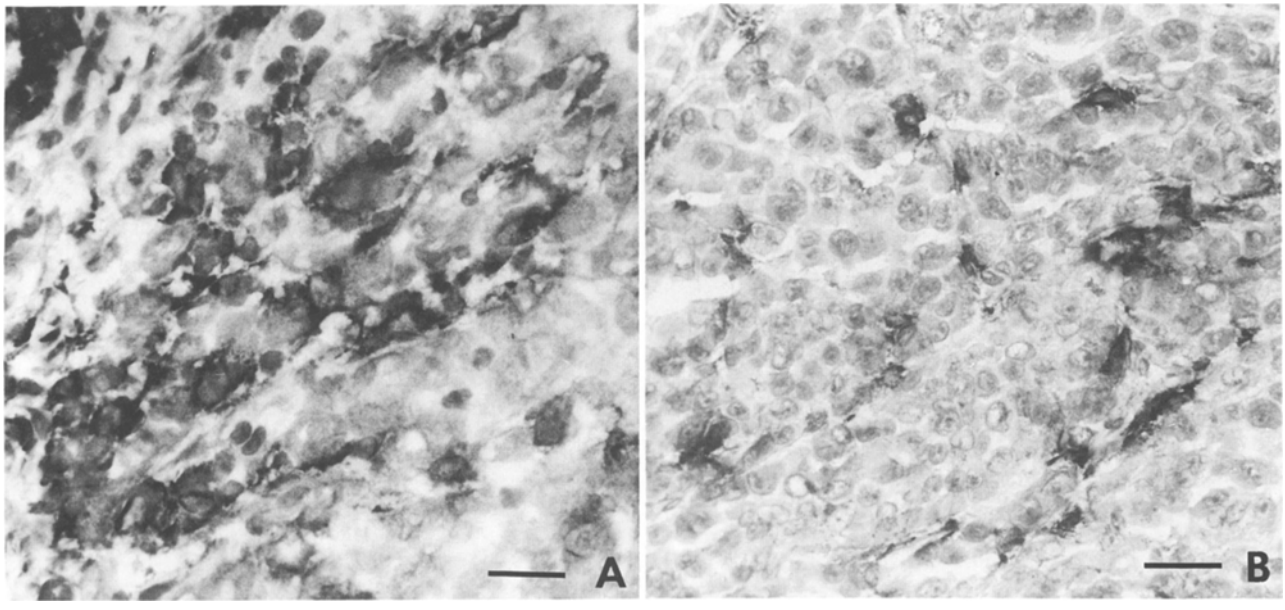

Fig. 1 A, B. p-Glycoprotein expression in human melanoma detected by use of mAb C219: positive cells stain red-brown on the histological slide and appear black on the photomicrograph by use of a green Leitz VG-9 filter (*scale bars*=0.02 mm). **A** 40% of the tumor cells positive in a melanoma metastasis. **B** All tumor cells negative in a melanoma metastasis. Only macrophages and fibroblasts were positive

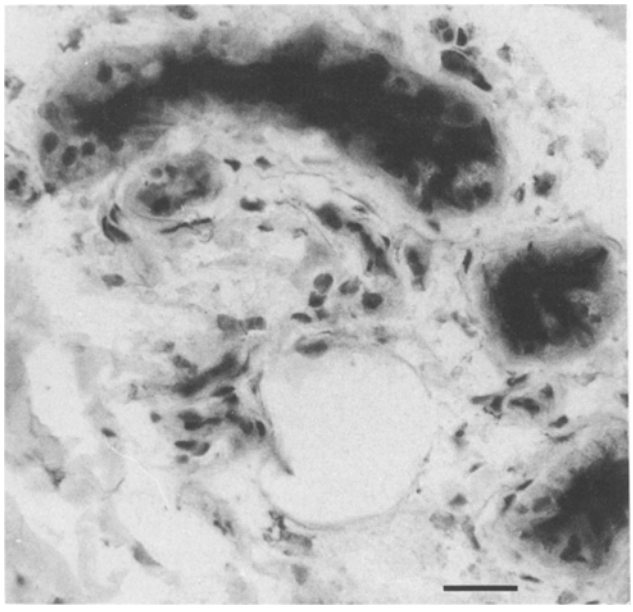

Fig. 2. p-Glycoprotein expression in human sweat glands detected by use of mAb C219. The histological slide was photographed as in Fig. 1 (*scale bar* = 0.02 mm)

Discussion

Malignant melanoma is virtually unaffected by chemotherapy in most cases. The overall response rate to single cytotoxic agents (dacarbazine, hydroxyurea) is between 10% and 20% (De Vita 1985). Is this relative resistance based on drug efflux caused by p-glycoprotein, as described by Juliano and Ling (Roninson 1987) for other tumors? We have observed the absence or low expression of p-glycoprotein in most primary and metastatic melanomas. None of the patients with negative metastases responded to chemotherapy. Sequential studies revealed no significant increase of p-glycoprotein-positive cells during or after chemotherapy. Chemotherapy obviously did not select tumor cells expressing p-glycoprotein. Moreover, remission of metastatic growth was achieved in a patient with a subcutaneous metastasis significantly expressing the p-glycoprotein.

Our results demonstrate that p-glycoprotein is usually not responsible for drug resistance in human melanoma. Obviously drug resistance is caused by other mechanisms in this malignancy, e.g. enzymatic change or consumption of the drug by other substances, repair mechanisms

Table 3. Expression of p-glycoprotein in patients with melanoma metastases in correlation to chemotherapy and clinical course

Patient	Metastases	Type of metastases	Tumor cells reacting with mAb C219			Cytotoxic agents applied	Clinical course
			Before chemotherapy	During chemotherapy	After chemotherapy		
1	a	Skin	0				
	b	Skin	5				
	c	Skin	3				PD[c], died
	d	Skin		0		BHD[a]	
2	a	Skin	0				
	b	Skin	0				
	c	Skin			0	BHD + interferon	PD, died
	d	Skin			5	dacarbazine + cisplatin	
3	a	Skin	40			BHD	CR[d]
4	a	Lymph node	0				
	b	Skin			0	BHD + interferon	PD, died
5	a	Skin	0				
	b	Skin		0		Cooper protocol[b]	PD, died
6	a	Lymph node			0	BHD	PD, died
7	a	Skin		0		BHD	PD, died
	b	Skin		0			
8	a	Skin		0		BHD	PD, died
9	a	Skin	0			BHD	PD, died
10	a	Skin	0			BHD	PD, died
11	a	Skin	0			BHD	PD, died
12	a	Skin		0		BHD	PD, died
13	a	Skin	0				PD, died
	b	Skin		5		BHD	
14	a	Skin			0	BHD	PD, died
15	a	Skin			0	BHD	PD, died
16	a	Lymph node		0		BHD	PD, died
	b	Lymph node		0		BHD	

[a] BHD, *N,N*-bis(2-chloroethyl)-*N*-nitrosourea, hydroxyurea, dacarbazine
[b] Cooper protocol, cyclophosphamide, methotrexate, prednisone
[c] PD, progressive disease
[d] CR, complete remission

in the toxically damaged tumor cells, mutation or selection of resistant tumor cell clones (Stacher and Moser 1986).

At present we have no explanation for the uniquely high expression of p-glycoprotein in one of the 37 tested primary melanomas and in one of the 27 studied melanoma metastases. Immunohistologically such a high expression has not been observed by other authors, who only tested a few melanoma cell lines (Goldstein et al. 1989; Lemontt et al. 1988).

Is the unexpected presence of p-glycoprotein in a minority of human melanomas caused by modulation known to occur in this human malignancy (Suter et al. 1989)?

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