HUMAN OVARIAN CANCER XENOGRAPHS IN NUDE MICE: CHARACTERIZATION AND ANALYSIS OF ANTIGEN EXPRESSION

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We have characterized 13 different human ovarian cancer xenografts grown subcutaneously in nude mice. The tumor lines represented 5 histological subtypes: serous (4), mucinous (4), clear-cell carcinoma (1), carcinosarcoma (1) and undifferentiated (3). The specific histology and the degree of differentiation resembled those of the original patients' tumors and were maintained upon serial transfer. Volume doubling times of the xenografts ranged from 3.5 to 15 days. The xenografts were also analyzed for their antigen expression using 20 monoclonal antibodies (MAbs) reactive with 15 tumor-associated antigens. Immunohistochemical examination of tissue sections showed a positive reaction pattern with MAbs I15D8, I40C1, I39H2, I75C5, HMFG1 and HMFG2, each recognizing episialin, as well as with MAbs AUA1, 358.4.32 and 199-157 in xenografts of the serous, mucinous and clear-cell carcinoma subtype. MAb OC125 was reactive with xenografts of the serous subtypes. Other antibodies, such as 494 and OV-TL3 infrequently demonstrated positive reactions. Reactivity of all MAbs was low in the carcinosarcoma and undifferentiated tumor lines. With the exception of AUA1, 495 and I26E5, all MAbs revealed a heterogeneous staining pattern. MAbs against episialin and OC125 predominantly stained the apical site of the tumor cells. Strongest reactivity with almost all histological subtypes was observed with MAbs I15D8, I40C1, I39H2 and AUA1. In cases where we were able to compare the patients' tumor tissue with the respective xenografts, retention of antigen expression was demonstrated in each instance. Release of tumor-associated antigens was shown for CA125 in 2 serous-tumor lines, for CA15.3 in 1 serous-tumor line, and for CEA in 3 lines of the mucinous subtype. This panel of human tumor xenografts could be a valuable tool to determine the potential usefulness of MAb-guided therapy in ovarian cancer.

A series of well-characterized human ovarian cancer xenografts may provide a clinically relevant model to investigate new therapies in this disease. Human tumor xenografts retain the histological pattern and several biological properties of the original tumor of the patient (Winograd *et al.*, 1987). Over the past years, we have established several human ovarian cancer lines in nude mice, grown either from fresh tumor tissue (patients) or from *in vitro* cell lines. In a preliminary report we have shown the retention of histological characteristics in a series of ovarian cancer xenografts (Boven, 1988a).

Monoclonal antibodies (MAbs) conjugated to toxins, cytostatic drugs or radionuclides have offered the possibility of a new therapeutic approach. For ovarian cancer, several radio-labelled MAbs have been employed with encouraging results, but none of the conjugated MAbs was fully effective (Epenetos *et al.*, 1987; Stewart *et al.*, 1989). Therefore, an *in vivo* model representing various characteristics of human ovarian cancer may add to knowledge of the optimal application of conjugated MAbs in these patients.

In the present study we have characterized a large panel of human ovarian cancer lines according to their histological subtype, growth rate, and the ability to release tumor-associated antigens. Furthermore, we have tested a number of MAbs known to react with various carcinomas. Apart from the retention of the histological subtypes, we could demonstrate that the antigen pattern of the individual xenografts reflected that of the original patients' tumor tissue.

MATERIAL AND METHODS

Animals

Female B10/Cpb or NMRI/Cpb nude (nu/nu) mice (Harlan, Zeist, NL) were housed in filter-top cages. Cages, covers, bedding, food and water were sterilized and changed weekly. Animal handling was done in a laminar downflow hood.

Xenografts

Xenografts were established from fresh tumor material or cell lines grown in tissue culture. Details of the patients' stage and tumor histology are given in Table I. The extent of the disease at the time of operation was classified according to the guidelines of the FIGO committee (FIGO, 1971). Fragments from solid tumor tissue with a diameter of 2-3 mm were implanted subcutaneously (s.c.) through a small skin incision into both flanks of 8- to 10-week-old mice. All cell lines in tissue culture were maintained in Dulbecco's modified Eagle's medium supplemented with 10% FCS. To establish xenografts from cell lines, 10⁷ cells were injected s.c. in both flanks of the animals.

Tumor lines FKO, FCo, and FMa were kindly provided by Dr. W. Kleine (Albert-Ludwigs University, Freiburg, Germany), and MRI-H-207 by Dr. A.E. Bogden (Mason Research Institute, Worcester, MA). The *in vitro* cell line NIH:OVCAR-3 (OVCAR-3), kindly provided by Dr. T.C. Hamilton (Fox Chase Cancer Centre, Philadelphia, PA), was originally established from the malignant ascites of a patient with poorly differentiated papillary adenocarcinoma of the ovary. A2780 was also kindly provided by Dr. Hamilton. Cell line H134 was grown from the ascitic fluid of a patient with serous adenocarcinoma and established in our laboratory.

Solid tumors arising at the inoculation site were transferred as described for fresh tumor tissue. Tumor growth was measured weekly in 3 dimensions with slide calipers by the same observer. Tumor volume was expressed by the equation length × width × height × 0.5 in mm³. Volume doubling time was calculated as the number of days for the tumor to grow from 100 mm³ to 200 mm³.

LDH analysis

Tumors were analyzed for lactate dehydrogenase (LDH) isoenzymes with the Paragon LDH Isoenzyme Electrophoresis Kit (Beckman, Fullerton, CA). Briefly, 500 mg of tumor tissue was minced mechanically in 2.5 ml 0.05 M sodium barbital buffer (pH 8.6) and centrifuged at 100 g to remove debris. Seven microliters of this tumor cell solution was prepared for electrophoresis. Human serum and serum from non-tumor-bearing mice were used as controls.

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TABLE I - CHARACTERISTICS OF OVARIAN CANCER PATIENTS AND XENOGRAPHS

Patient			Xenograft		
Tumor line	Stage ¹	Histology	Histology	TD ²	Number of passages ³
Ov.Ri(C)	III	Moderately differentiated serous	Moderately differentiated serous	11	15
FKo	III	Poorly differentiated serous	Moderately differentiated serous	12	47
Ov.Gl	IV	Poorly differentiated serous	Poorly differentiated serous	10	17
OVCAR-3 ⁴	n.d.	Poorly differentiated papillary	Poorly differentiated serous	8	4
Ov.Pe	III	Moderately differentiated mucinous	Moderately differentiated mucinous	8	33
Ov.He	III	Moderately differentiated mucinous	Moderately differentiated mucinous	9	23
Ov.Gr	IV	Well differentiated mucinous	Moderately differentiated mucinous	15	11
FMa	I	Moderately differentiated mucinous (recurrence)	Poorly differentiated mucinous	5.5	29
FCo	III	Clear-cell carcinoma	Clear-cell carcinoma	6.5	18
Ov.Me	III	Carcinosarcoma	Carcinosarcoma	6	18
MRI-H-207	III	Undifferentiated	Undifferentiated	3.5	74
A2780 ⁴	n.a.		Undifferentiated	3.5	2
H134 ⁴	III	Serous adenocarcinoma	Undifferentiated	11	6

¹International Federation of Gynecology and Obstetrics Classification. -²Volume doubling time in days. -³Number of passages analyzed for histology and histochemistry. -⁴Xenografts established from cell line. -n.d., not determined; n.a., not available.

Histology and cytology

Xenografts from each passage were routinely examined by light microscopy. Tissues were fixed in 10% neutral buffered formalin solution and embedded in paraffin. Sections of 4-6 µm were stained with hematoxylin/eosin, periodic acid Schiff (PAS), PAS/diastase or Alcian blue and the intensity was expressed as negative (-) to strongly positive (++). The original tumor tissue of the patients had been processed similarly.

Antigen expression

Twenty MABs (Table II), kindly provided by various insti-

tutes, were used for immunohistochemical staining of frozen and formalin fixed paraffin-embedded tissue sections. An indirect immunoperoxidase assay according to Taylor and Chir (1978) was performed using ascites fluid containing MAB, hybridoma supernatant or purified MAB, and horse-radish-peroxidase-labelled goat anti-mouse Ig (Dakopatts, Glostrup, Denmark) as secondary antibody. Dilutions up to 1:1000 of the various ascites fluids and up to 1:50 of the hybridoma supernatants gave identical staining intensities. For this reason, 1:100 dilutions of the ascites fluids, undiluted hybridoma supernatants and a concentration of 10 µg/ml of purified antibody

TABLE II - MONOCLONAL ANTIBODIES AND IMMUNOPEROXIDASE SETUP

MAB	Specificity	Isotype	Reference	p/c ¹	a/s/p ²
115D8	episialin (MAM-6), a carcinoma- associated sialomucin	IgG _{2b}	(Hilkens <i>et al.</i> , 1984)	p + c	a
140C1		IgG ₁	(Hilkens <i>et al.</i> , 1985)	p + c	a
139H2		IgG ₁		p + c	a
175C5		IgG ₁	(Hilkens <i>et al.</i> , p.c.)	c	a
HMFG1		IgG ₁	(Taylor-Papadimitriou <i>et al.</i> , 1981)	p + c	p
HMFG2		IgG ₁		p + c	p
AUA1	a 35-kDa gp	IgG ₁	(Epenetos <i>et al.</i> , 1982)	c	p
H17E2	PLAP	IgG ₁	(Travers and Bodmer, 1984)	p + c	p
126G5	n.d.	IgG ₃	(Gerö <i>et al.</i> , 1987)	p + c	a
175F11	n.d.	IgG _{2a}	(Hilkens <i>et al.</i> , p.c.)	c	a
495	"pancarcinoma antigen"	IgG ₃ /IgG ₁	(Bosslet <i>et al.</i> , 1986a)	c	p
494	n.d.	IgG ₁	(Bosslet <i>et al.</i> , 1986b)	p + c	p
431	CEA	IgG ₁	(Bosslet <i>et al.</i> , 1985)	p + c	p
358.24.6	n.d.	IgM	(M. Herlyn <i>et al.</i> , p.c.)	c	s
358.24.5	n.d.	IgM		c	s
358.4.32	n.d.	IgG ₁		c	s
199-157	n.d.	IgG ₁		c	s
OC125	CA125	IgG ₁	(Bast <i>et al.</i> , 1981)	p + c	p
OV-TL3	20/40-kDa gp	IgG ₁	(Poels <i>et al.</i> , 1986)	c	a/s
OV632	n.d.	IgG _{2b}	(Fleuren <i>et al.</i> , 1987)	c	s

¹Formalin-fixed, paraffin-embedded (p) and/or cryostat (c) tissue sections used. -²Ascites (a), hybridoma-supernatant (s) and/or purified MAB (p) used. -p.c., personal communication; n.d., not determined.

were used to ensure antibody excess. A non-relevant MAb (ascites 1:100) was used as a control. Freshly prepared diaminobenzidine hydrochloride (50 mg in 100 ml of 0.02% H₂O₂ in PBS) was used as a substrate and sections were counterstained with hematoxylin. Intensity of staining was scored as strong (++), moderate (+), weak (±), and negative (-). The site of antigen expression in a particular cell compartment and the degree of heterogeneity were also described.

Measurement of CA125, CA15.3 and CEA

The presence of circulating tumor-associated antigens was analyzed in the sera of tumor-bearing mice. At the time of blood sampling, tumors measured between 100 and 1000 mm³ in size. Sera were frozen at -20°C until analysis. The CA125 antigen, which is associated with serous epithelial tumors of the ovary (Kabawat *et al.*, 1983), was measured with the Abbott CA125-EIA monoclonal assay and the carcinoembryonic antigen (CEA) with the Abbott CEA-EIA monoclonal one-step assay (Abbott, North Chicago, IL). For CA15.3, a carcinoma-associated antigen, analysis was carried out using the CIS enzyme immunoassay (CIS, ORIS, Gif-sur-Yvette, France). In this assay, MAb 115D8 and DF3 (Kufe *et al.*, 1984) are used as tracer and catcher antibody respectively.

RESULTS

Tumor lines and patients' tumor tissue

We have investigated 13 human ovarian cancer xenografts. Ten lines were established from fresh tumor tissue and 3, namely OVCAR-3, A2780 and H134, from *in vitro* cell lines. The human nature of the xenografts used in this study was demonstrated by the analysis of LDH isoenzymes in tumor tissue extracts (Fig. 1).

The extent of the disease and the histological subtype of the original tumor in the patients are indicated in Table I. Most histological subtypes occurring in ovarian cancer were represented. The histological subtype and the degree of differentiation have been retained in the xenografts to a large extent, even after several passages, which confirmed our earlier findings in a smaller panel of xenografts. Representative examples are illustrated in Figure 2 for tumor lines Ov.He, Ov.Pe and Ov.Ri(C). While the original tumor tissue usually contained large zones of connective tissue, the stroma in the xenografts was constituted by thin layers of mouse cells. Doubling times of the xenografts in the nude mice varied between 3.5 and 15 days and were maintained upon serial transplantation. No clear relationship was observed between the growth rate and the histological subtype or the degree of differentiation, although undifferentiated and poorly differentiated tumor lines showed a tendency to faster growth (Table I).

We have extended these studies by comparing the histochemical data of the original tumor tissue and the respective xenografts obtained with PAS (glycogen and mucopolysaccharides), PAS-diastase (neutral mucopolysaccharides) and Alcian-blue (acid mucopolysaccharides) staining in each passage. The patterns visualized in the xenografts were similar to the original human tumor tissue in most instances (Table III).

Antigen expression

We have investigated whether the tumor lines retained the antigen characteristics of the original patients' tumor tissue. Also, different passages (range: 2-15, with a median of 5) of all tumor lines were analyzed. For this purpose, the indirect immunoperoxidase assay was carried out using a panel of 20 MAbs known to react with adenocarcinomas (Table II). MAbs recognizing antigens which were sensitive to formalin fixation were tested on frozen sections fixed with acetone. Since only formalin-fixed material was available from patients, it was not

FIGURE 1 - LDH isoenzyme pattern in extracts of 13 human ovarian cancer xenografts. First 2 lanes: mouse and human LDH serum controls. Human LDH V can be detected clearly in all xenografts.

TABLE III - HISTOCHEMICAL STAINING OF FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUE SECTIONS

Tumor line	PAS		PAS-diastase		Alcian blue	
	x ¹	o ²	x	o	x	o
Ov. Ri(C)	± ³	-	-	-	±	±
FKo	+	+	+	±	+	±
Ov. Gl	-	±	-	-	-	-
OVCAR-3	+	n.a.	-	n.a.	±	n.a.
Ov. Pe	++	++	++	++	++	++
Ov. He	++	++	++	++	++	++
Ov. Gr	++	++	++	++	++	++
FMa	+	++	+	++	+	++
FCo	++	++	-	-	+ ⁴	+ ⁴
Ov. Me	±	+ ⁵	-	+ ⁵	± ⁴	+ ⁴
MRI-H-207	±	n.a.	-	n.a.	±	n.a.
A2780	±	n.a.	-	n.a.	±	n.a.
H134	±	n.a.	-	n.a.	±	n.a.

¹Xenograft. - ²Original patients' tumor. - ³+, +, ± and -, strong, moderate, weak, and no staining. - ⁴Extracellular matrix. - ⁵Heterogeneous reactivity. - n.a., not available.

possible to analyze all antibodies on the original tumor tissue. Still, 10 MAbs could be tested on 6 different tumor lines and retention of antigen expression was proven in all instances. The reactivity of the MAbs with the various tumor lines is listed in Table IV and examples are shown in Figures 3 and 4.

The 6 MAbs reactive with different epitopes of an epithelial sialomucin present on carcinomas and known as episialin

FIGURE 2 – Sections of formalin-fixed, paraffin-embedded patients' tumors and corresponding xenografts stained with hematoxylin/eosin. Serous and mucinous subtypes: (a) tissue of patient He; (b) Ov.He xenograft, passage 26; (c) tissue of patient Pe; (d) Ov.Pe xenograft, passage 36; (e) tissue of patient Ri; (f) Ov.Ri(C) xenograft, passage 16. Bar, 0.27 μ m.

(Hilkens, 1988) showed large differences in intensity and staining pattern. MAbs 115D8, 140C1 and 139H2 bound strongly to all serous and mucinous tumor lines, but MAbs HMFG1, HMFG2 and 175C5 showed a weaker reaction (Fig. 3, *b,d*). Interestingly, some MAbs recognizing episialin reacted poorly with tumor lines Ov.Me, MRI-H-207 and A2780 and staining was always restricted to the more differentiated parts within these xenografts.

None of the xenografts expressed the placental alkaline

phosphatase antigen recognized by MAb H17E2, an antibody regarded as a marker for differentiation (Travers and Bodmer, 1984). On the contrary, AUA1, recognizing a 40-kDa epithelial membrane antigen, showed good and homogeneous staining of tumor sections, especially those from the serous (Fig. 3, *c*) and mucinous subtypes. MAb 495, directed against a 200-kDa pancarcinoma glycoprotein, showed homogeneous but weak staining reaction on sections from most of the serous, mucinous and clear-cell carcinoma lines. MAb 431, recogniz-

FIGURE 3 – Sections of formalin-fixed, paraffin-embedded FKo xenograft (passage 12). The control section and the sections stained with the specific MAbs, were counterstained with hematoxylin for 2 min and 30 sec respectively. (a) Control; (b) MAb 115D8; (c) MAb AUA1; (d) MAb HMFG2. Bar, 0.27 μ m.

TABLE IV – IMMUNOPEROXIDASE STAINING ON FROZEN OR FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUE SECTIONS

MAb	Serous types						Mucinous types						Other types						
	Ov.Ri(C)		FKo	Ov.Gl		OVCAR-3	Ov.Pe		Ov.He		Ov.Gr		FMa	FCo	Ov.Me		MRI	A2780	H134
	x ¹	o ²		x	o		x	o	x	o	x	o			x	o			
115D8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-*	-*	-*	±	+
140C1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-*	-*	-*	±	+
139H2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-*	-*	-*	±	+
175C5	+		+	+		+	+		±		+		-		-		±	-	-
HMFG1	+	+	±	±	±	±	+		+		±	±	-	±	-	-*	-	-	±
HMFG2	±	+	±	±	+	+	+	+	+	+	±	±	-	±	-*	-*	-*	±	±
AUA1	±		+	+		+	+		+		+		+	+	-		+	-	-
H17E2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
495	±		-	±		+	±		±		±		±	±			-*	-	-
494	±	±	+	-	-	-	±	±	+	+	+	+	-	-	-	-	-	-	-
431	±	±	-	-	-	-	±	±	-	-	-	-	-	-	-	-	-	-	-
358.24.6	+		-	-		-	-		-		-		-	-	-		-	-	-
358.24.5	+		-	-		-	-		-		-		-	-	-		-	-	-
358.4.32	+		+	+		+	+		+		+		+	+	-		+	+	+
199-157	+		+	+		+	+		+		+		+	+	-		+		+
OC125	+	+	-	+	±	+	-	-	-	-	-	-	-	-	-	-	-	-	-
126G5	-	-	-	-	-	-	±	+	-	-	+		±	-	-	-	-	-	-
175F11	-		-	±		-	+		-		+		-	-	-		-	-	-
OV-TL3	-		±	±		+	-		-		±		±	±	-		-	-	-
OV632	-		-	±		+	+		-		-		-	-			-	-	-

¹Xenograft. ²Original patients' tumor. ³+, +, ± and -: strong, moderate, weak, and no staining. -*, differentiated parts.

FIGURE 4 – Sections of formalin-fixed, paraffin-embedded xenografts. Control sections (*a, c, e*) and sections stained with specific MABs (*b, d, f*) were counterstained with hematoxylin for 2 min and 30 sec respectively. (*a*) FCo xenograft (passage 20) control; (*b*) FCo stained with MAB 139H2; (*c*) OVCAR-3 xenograft (passage 19) control; (*d*) OVCAR-3 stained with MAB OC125; (*e*) Ov.Gr xenograft (passage 12) control; (*f*) Ov.Gr stained with MAB 494. Bar, 0.27 μ m.

ing CEA, reacted weakly or did not react with our xenografts. MAB 494, reported to bind to an antigen expressed in the majority of well differentiated adenocarcinomas, reacted only with the more differentiated tumor lines of the serous and mucinous types (Fig. 4, *f*).

Of the MABs 358.24.6, 358.24.5, 358.4.32 and 199-157, raised against tumor cells obtained from ascitic fluid of ovarian

cancer patients, only MABs 358.4.32 and 199-157 showed good staining with most tumor lines, but they reacted also with connective tissue.

As expected, MAB OC125 recognizing the CA125 antigen reacted with all serous-tumor tissue sections (Fig. 4, *d*). MABs 126G5 and 175F11, raised against human breast carcinomas, showed a positive reaction with most of the mucinous-tumor

TABLE V - TUMOR MARKERS IN SERUM OF HUMAN-OVARIAN-CANCER-BEARING NUDE MICE

Tumor line	CA125 (U/ml)	CA15.3 (U/ml)	CEA (ng/ml)
Ov.Ri(C)	374 (5->650) ¹	16 (8 -33)	— ²
FKo	—	0.8 (0.2- 1.1)	—
Ov.Gl	27 (0- 133)	0.6 (0.2- 1.4)	—
OVCAR-3	16 (0- 18)	2 (0.4- 3.2)	—
Ov.Pe	—	—	112 (10-455)
Ov.He	—	—	27 (5- 69)
Ov.Gr	—	—	33 (2- 64)
FMa	—	—	—
FCo	—	—	—
Ov.Me	—	—	—
MRI-H-207	—	—	—
A2780	—	—	—
H134	—	0.6	—
Control mouse serum	—	0.1 (0 -0.3)	—

¹Mean (range).—²Not detectable.

lines. With MAb OV-TL3, originally developed against an antigen on ovarian endometrioid carcinoma cells, OVCAR-3 was the only tumor line showing a clearly positive staining reaction. MAb OV632, reactive with over 80% of non-mucinous ovarian cancers, showed a positive staining with the mucinous line Ov.Pe and the serous lines OVCAR-3 and Ov.Gl.

The localization of the staining reaction in the tumor cells was also studied. Most of the antibodies investigated are directed against membrane-associated antigens. MAbs reactive with episialin and OC125 predominantly stained the apical site of the cells, while the other antibodies usually showed more diffuse cytoplasmic staining. With the exception of MAbs AUA1, 495 and 126E5, all antibodies revealed a heterogeneous staining pattern of the xenografts.

Release of antigen

The presence of tumor-associated antigens CA125, CA15.3 and CEA was investigated in the sera of tumor-bearing mice and control animals (Table V). Mice bearing the mucinous xenografts Ov.Pe, Ov.He and Ov.Gr expressed high levels of CEA in serum. In serum of mice with tumors of the serous subtypes OVCAR-3, and especially Ov.Ri(C), CA125 levels were elevated. In tissue sections from these xenografts, CA125 expression could also be detected immunohistochemically with MAb OC125. CA15.3 was elevated in the serum of Ov.Ri(C) tumor-bearing mice. As shown above, MAb 115D8, which antibody is used in the CA15.3 assay, clearly stained tissue sections from this xenograft.

DISCUSSION

The 13 ovarian cancer xenografts characterized in our study have retained the specific histology and antigen expression, the degree of differentiation and the human nature of the patients' tumors. Based on the microscopical patterns, the established tumor lines could be categorized into serous, mucinous and clear-cell carcinoma, carcinosarcoma and undifferentiated types, representing the main histological subtypes of ovarian cancer.

Retention of the original antigen expression is a prerequisite for a xenograft model to adequately study MAbs for therapy. In this respect, inconsistency has been described previously by several investigators. Ward *et al.* (1987) have shown that xenografts established from 2 different cystadenocarcinoma cell lines lacked the expression of the ovarian-cancer-associated antigens HMFG2 and F 36/22, which were present in the orig-

inal patients' tumor tissue. Ripamonti *et al.* (1987) have demonstrated loss or variation in antigen expression in xenografts established from cell lines of different origin (breast, ovarian and colon cancer). For the colorectal tumor markers CEA, CA19-9, TAG-72, CSAp and CMA, discrepancies in antigen expression between cell lines or patients' tumor tissue and established xenografts have also been reported (Gold *et al.*, 1983; Park *et al.*, 1987). However, in our tumor lines and their subsequent passages we could demonstrate the retention of a large number of tumor-associated antigens. The difference between the observation may be explained by the fact that we have established xenografts from tissue fragments in most instances, while certain cell populations may be lost in xenografts grown from *in vitro* cell lines.

Reactivity of the MAbs with the various human ovarian cancer lines was variable in intensity and heterogeneity. In general, the more differentiated xenografts showed better staining and this correlated well with the patterns observed in the patients' tumors. Best reactivity was observed with the 6 MAbs reactive with episialin, as well as with AUA1, 358.4.32, 199-157 and OC125. With the exception of MAbs 126G5, AUA1 and 495, marked heterogeneity in antigen expression within the xenografts was observed. A reason for this may be a variation in cellular differentiation resulting in the loss of certain antigens in the undifferentiated subtypes. In the use of this model for experimental MAb-guided therapy, we consider the following criteria important with respect to the reactivity of the MAbs: (1) strong membrane-bound staining reaction with different ovarian cancer subtypes; (2) good correlation between reactivity of MAbs with the xenograft and the corresponding patients' tumor tissue; (3) retention of the antigen expression upon serial transfer of the xenografts; and (4) low cross-reactivity with non-malignant tissue. MAbs 115D8, 140C1, 139H2 and AUA1 fulfilled most of these conditions.

Radioimmunolocalization may be negatively affected by circulating antigens. Antigen-releasing xenografts being used for studies on the *in vivo* behavior of MAbs could add an extra element to the optimal clinical application of such antibodies. Therefore, we have determined the presence of the carcinoma-associated antigens CA125, CA15.3, and CEA in the serum of tumor-bearing mice. The CA125 antigen is expressed by the majority of patients with non-mucinous ovarian cancer and serum CA125 levels correlate with the course of the disease in 80% of cases (Bast *et al.*, 1983). Mice with the serous subtypes Ov.Ri(C) and OVCAR-3 showed elevated levels of CA125 in their serum. In 78% of patients with advanced ovarian cancer, elevated levels of episialin could be determined with a serum assay described by Hilkens *et al.* (1986). The mouse sera were analyzed with a commercially available assay used for breast cancer (CA15.3 assay), in which MAb DF3 (Kufe *et al.*, 1984) is used as a catcher antibody and 115D8 as a tracer antibody. Since MAb DF3 reacts with a lower percentage of ovarian cancer types than 115D8, this may explain why elevated levels of episialin were detected only in the serum of Ov.Ri(C)-bearing mice. Mice bearing mucinous-tumor lines Ov.Pe, Ov.Gr, and Ov.He were the only ones to show elevated CEA levels, a finding which is in agreement with clinical observations (Bast *et al.*, 1987). With MAb 431, known to react with an epitope on the CEA molecule, we found only weak reactivity with Ov.Pe tumor tissue. This discrepancy may be based on the different MAbs used in both methods. Also, MAb-defined epitopes on circulating CEA can differ from those on tumor-associated CEA (Bosslet *et al.*, 1988).

The xenograft model presented here has already proven its predictive value in analysis of the therapeutic potential of cytostatic agents (Boven, 1988b). Since the model reflects the biological characteristics of the original patient's tumor to a great extent, it offers a way to study the efficacy of MAb-

guided therapy before introducing such antibodies for treatment in ovarian cancer patients.

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