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REACTIVITY OF MONOCLONAL ANTIBODIES DIRECTED AGAINST LUNG CANCER ANTIGENS WITH HUMAN LUNG, BREAST AND COLON CANCER CELL LINES

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SUMMARY

A panel of monoclonal antibodies (n=72 including controls) directed against lung cancer antigens was screened immunohistochemically against a panel of seven human lung cancer cell lines (including small cell carcinoma, squamous cell carcinoma, adenocarcinoma and mesothelioma), six human breast cancer cell lines and one human colon cancer cell line. The majority of the antibodies (n=42) reacted also with antigens present on breast and colon cancer cell lines. This cross reactivity especially between lung and breast cancer cell lines is not altogether unexpected since antigens common to breast and lung tissue including their neoplasms such as MUC1 antigen have been described. Our results indicate that epitopes shared by lung and breast cancers are probably more common than previously thought. The relevance for prognosis and therapy of these shared antigens, especially as disease markers in breast cancer, has to be investigated.

KEY WORDS Lung cancer Monoclonal antibodies Reactivity

INTRODUCTION

Some antigens common to lung and breast epithelia can be classified as oncodevelopmental. One of these, the MUC1 antigen, detected during development in lung, mammary gland and other tissues of epithelial origin (Braga *et al.*, 1992), is conserved during evolution (Welsch *et al.*, 1990, Spicer *et al.*, 1991, Pemberton *et al.*, 1992) and the antigen can be recovered from the human broncho-alveolar-lavage (Schumacher *et al.*, 1989).

Since mucin antigens which are expressed in lung and mammary gland have been described, the monoclonal antibody panel (submitted to the panel of the third international association for the study of lung cancer [IASLC] workshop on lung tumour and differentiation antigens) was tested for cross reactivity with human breast cancer cell lines. In addition serving as a further control, a human colon cancer cell line was included in the test panel of cell lines.

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MATERIALS AND METHODS

Human breast cancer cell lines (MCF7, BT-549, T47D, HBL100, MDA-MB-157, HS578T) and the human colon cancer cell line HT29 were obtained from the European Cell Culture Collection (Porton Down, Salisbury, Wiltshire, UK) and maintained under the standard culture conditions supplied with the data sheets for each cell line. The human lung cancer cell lines N-417 (derived from small cell carcinoma, variant, kindly provided by Dr. D. N. Carney, Mater Misericordiae Hospital, Dublin, Ireland), SW2 (classic, kindly provided by Dr. S. Bernal, Boston, USA), ZL5 and ZL34 (from mesothelioma, generated in Zürich), U1752 (from squamous cell carcinoma, kindly provided by Dr. J. Berh, Uppsala, Sweden) and A125 (from adenocarcinoma, obtained from American Type Culture Collection, Rockville, Maryland) were cultured in RPMI-1640 medium supplemented with 2 mMol/l L-glutamine and 10% fetal calf serum. The immortalised human bronchial epithelial-derived cell line (BEAS-2B, kindly provided by Dr. C. Harris, Bethesda, Maryland) was cultured in Hams F12 based medium supplemented with 5mg/L insulin, 5mg/L transferrin, 70ug/L hydrocortisone, 0.1mg/L vitamin A, 650ug/L triiodo-L-thyronine, 2mg/L epinephrine, 30mg/L bovine pituitary extract, 50mg/L bovine serum albumin, 5ug/L purified mouse epidermal growth factor, 5uM/L ethanolamine and 50mg/L gentamycin.

For the immunofluorescence assay the breast and colon cancer cell lines were grown to confluence on coverslips and briefly fixed in cold methanol. The cells were incubated with the antibody panel (Table 1) including the positive and negative control reagents and the anti mouse or rat FITC-labelled antibodies were used for visualisation. The fluorescence intensity was evaluated semi-quantitatively: - = no fluorescence, (+) = very weak fluorescence, + = weak fluorescence, ++ = modest fluorescence, +++ = intense fluorescence, ++++ = very intense fluorescence. The intensity measurements of the lung cancer cell lines were obtained by FACS; the results of the FACS analyses were transformed into the above scale.

RESULTS

The details of the results of this study are summarised in Table 2 and can be classified into four groups: 1) those antibodies which did not react with any of the cell lines (n = 14, including the negative controls), 2) those which reacted with breast and colon cancer cell lines only (n = 11), 3) those which reacted with lung cancer cell lines only (n = 17) and 4) those which reacted with breast, colon and lung cancer cell lines (n = 30). Amongst groups two to four the numbers of reactive antibodies and the intensity of fluorescence varied considerably. In some cases antibodies reacted only with one cell line (e. g. the antibody number 16 reacted with the cell line T47D only), while other antibodies showed a broader reactivity (antibody number 50 reacted with all the cell lines except two breast cancer cell lines MDA-MB-157 and HBL 100). Differences in fluorescence intensity were detected not only between the different cell lines but also between cells of the same cell line (e. g. only 40% of the cells from the cell line BT 549 showed strong reactivity with antibodies number 35 & 36). In other cases some antibodies reacted with all cells of a particular cell line (Fig. 1) while other antibodies reacted only with some cells of that cell line (Fig. 2).

Table 1. List of antibodies used in the present study

No	Name	Isotype	Submitter	Antigen	Publications
1	44-3A6	IgG1	Radosevich	40kd cell surface	*Tumor Biol.*, **9** (2–3), 116–22 (1988) and **11** (4), 181–188 (1990)
2	EMD6087	IgG1	E. Merck	45 + 85kd gp	*Cancer Res.*, **46**, 6369–6373 (1986)
3	EMD5590	IgG2a	E.Merck	EGF receptor	Arch. Biochem. Biophys., **252**, 549–560 (1987)
4	ITK2	IgG1	Kawase	NCAM	*Cancer Immunol. Immunother.*, **33**, 165–170 (1991)
5	KM195	IgG1	Hanai	n/a	
6	RS7-3G11	IgG1	Stein	EGP-1/GA733-1	*Antibody, Immunoconjugates and Radiopharm.*, **4**, 703-712(1991), *Mol. cell. Biol.*, **13**, 1507–1515 (1993), see also No. 7.
7	RS5-4H6	IgG1	Stein	>300kd, mucinlike	*Cancer Res.*,**50**, 1330-1336 (1990) and *Hybridoma*, **7**, 555–567 (1988)
8	A42	IgG2b	Mattes	57kd gp	*J. Exp. Med.*, **164**, 1581–1599 (1986)
9	MR54	IgG2a	Mattes	n/a	n/a
10	MT179	IgG1	Mattes	n/a	*Cancer Res.*, **47**, 6741–6750 (1987)
11	MU78	IgG2b	Mattes	2-5kd	*J. Histochem. Cytochem.*, **33**, 1095–1102 (1985)
12	MW207	IgG1	Mattes	n/a	see No 10
13	MX35	IgG1	Mattes	n/a	see No 10
14	MAB735	IgG2a	Behring-werke	embryonic NCAM	*Proc. Natl. Acad. Sci. U.S.A.*, **82**, 1194–1198 (1985) and *J. Pathol.*, **159**, 23–28 (1989)
15	RCK–107	IgG1	Broers	keratin 14	*Am. J. Pathol.*, **138**, 751–763 (1991)
16	RCK-105	IgG1	Broers	keratin 7	see No 15 and *Experimental Cell Res.*, **170**, 235–249 (1987)
17	RNL-2	IgG1	Broers	25 + 45kd, intracell.	*Cancer*, **67**, 619–633 (1991)
18	RNL-3	IgG1	Broers	25 + 45kd, intracell.	see No 17
19	RNL-1	IgG1	Broers	NCAM	see No 17

20	MLuC-6	IgG1	Menard	67kd Laminin receptor	*Clin. Exp. Metastasis*, **10**, 379-386 (1992)
21	MAR-4	IgG1	Menard	$_1$Integrin	*Tumori*, **78**, 1-4 (1992)
22	MAR-6	IgG1	Menard	$_6{}_4$ Integrin	*Int. J. Cancer*, **54**, 261–267 (1993)
23	MLuC-1	IgG2o.3	Menard	Le^y hapten	*Int. J. Cancer*, **51**, 225–231 (1992)
24	MB-2	IgG1	Gerardy-Schahn	NCAM	submitted
25	KD-11	IgG1	Gerardy-Schahn	NCAM, C-term., intracellular	submitted
26	MG-5	IgG1	Gerardy-Schahn	NCAM, exon 18, intracellular	submitted
27	MOC-31	IgG1	De Leij	cluster 2 control, EGP-2/GA733-2	*Br. J. Cancer*, **67**, 1242–1247 (1993)
28	ME-1	IgG1	Stahel	mesothelial membrane antigen	*Int. J. Cancer*, **41**, 218–223 (1988) and *Am. J. Pathol.*, **136**, 421–428 (1990)
29	SEN36	IgG1	Stahel	NCAM	*Br. J. Cancer*, **63**, Suppl. XIV, 24–28 (1991)
30	SWA11	IgG2a	Stahel	cluster w4 control, CD24	*Cancer Res.*, **52**, 5264-5270 (1992), *Int. J. Cancer*, **53**, 521–528 (1993) and *Clin. Exp. Immunol.*, **93**, 279–285 (1993)
31	SEN31	IgG1	Stahel	cluster 5a control	*Br. J. Cancer*, **63**, Suppl. XIV, 29–32 and 67–70 (1991)
32	SEN7	IgG1	Stahel	NCAM	*Cancer Res.*, **53**, 2840–2845 (1993)
33	MON-114	IgG1	Van de Ven	n/a	n/a
34	MON-150	IgG1	Van de Ven	n/a	n/a
35	KL-6	IgG1	Kohno	mucin-like sial.gp	*Jpn. J. Clin. Oncol.*, **18**, 203–216, (1988) and *Chest*, **96**, 68–73 (1989)
36	OE-130	IgG1	Hida	130kd	*Cancer Res.*, **48**, 2544–2549 (1988)
37	FBP146	IgG1	Franklin	folate binding protein	*Am. J. Pathol.*, (1993), submitted
38	FBP343	IgG1	Franklin	folate binding protein	see No 37
39	FBP458	IgG1	Franklin	folate binding protein	see No 37
40	FBP741	IgG1	Franklin	folate binding protein	see No 37
41	ME-2	IgG2b	Stahel	mesothlial membrane protein	see No 28
42	2.54	IgG2a	Cole	22.5 + 25kd cellsurface	*Cancer Res.*, **49**, 5719–5724 (1989) and *Br. J. Canc.*, **64**, 15–22 (1991)

43	BrE-3	IgG1	Coulter Immunology	mucin, MUC1	*Int. J. Cancer*, **52**, 624–630 (1992)
44	KM432	IgG1	Hanai		
45	CC49	IgG1	Schlom	mucinlike, TAG-72	*Cancer Res.*, **48**, 4588–4596 and 4597–03 (1988)
46	B72.3	IgG1	Schlom	mucinlike, TAG-72	see No 45
47	CC83	IgG1	Schlom	mucinlike, TAG-72	see No 45
48	L6	IgG1	Bristol-Myers Squipp	24kd surfaceprotein	*Proc. Natl. Acad. Sci. U.S.A.*, **89**, 3503–3507 (1992)
49	not submitted				
50	CTM01	IgG1	Celltech Ltd.	MUC 1 gene, mucin	n/a
51	ABL364	IgG1	Sandoz	cluster w6 control	*Ann. Oncol.*, **3**, 319–370 (1992)
52	MOPC21	IgG	Sigma	mouse IgG control	n/a
53	PBS/azide			neg. control	
54	1.291	IgM	Manderino	n/a	n/a
55	2.304	IgM	Manderino	n/a	n/a
56	A-80	IgM	Manderino	cluster w8 control	n/a
57	KM227	IgM	Hanai	n/a	n/a
58	MG-6	IgM	Koubek	n/a	*Folia Haematol.*, **155**, 913–926 (1988)
59	MLuC-5	IgM	Menard	laminin receptor	see No 20
60	NCC-ST-439	IgM	Shimosato	cluster w7 control	n/a
61	NCC-CO-450	IgM	Shimosato	cluster w7 control	n/a
62	SCCL175	IgM	Ball	115 + 155kd	*Cancer Res.*, **48**, 7319–7322 (1988)
63	KM132	IgM	Hanai	n/a	n/a
64	KM93	IgM‹	Hanai	sial. glycoprotein	*Cancer Res.*, **46**, 4438–4443 (1986) and **47**, 1267–1272 (1987)
65	CD57	IgM	Serotec	NK cell antigen	n/a
66	TEPC183	IgM	Sigma	mouse IgM control	n/a
67	PBS/azide			neg. control	
68	KM966	human IgG1	Hanai	n/a	n/a
69	AH41	human IgG3	Boehringer Mannheim	n/a	n/a
70	KUB10	human IgG1	Boehringer Mannheim	n/a	n/a
71	ICR2	rat IgG2a	Wawrzyncak/ Dean	mucin	*Histopathol.*, **16**, 573–581 (1990)
72	ICR12	rat IgG2a	Wawrzyncak/ Dean	erbB-2 gene, EGF receptor	*Int. J. Cancer*, **45**, 320–324 (1990)

as provided by the submitter of the antibody
n/a = not available

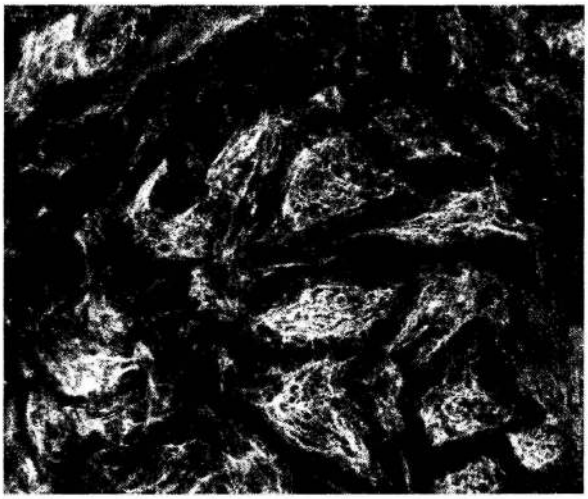

Fig. 1a

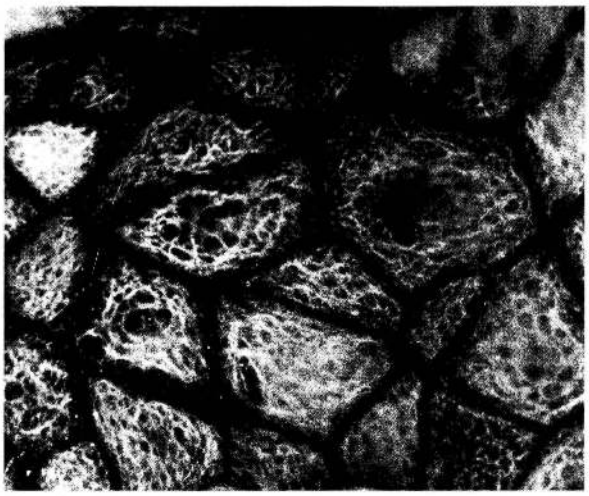

Fig. 1b

Figure 1. Photomicrographs showing FITC labelled cultured (a) HT29 colon cancer cell line and (b) MCF7 breast cancer cell line after reactivity with the antibody number 5 (KM195). X650.
The primary as well as the secondary anti-rat and anti-mouse antibodies were used at 1:50 dilutions and incubated with the cell preparations for 1 hour, both at room temperature.

Table 2. Details of immunofluorescence reactivity.

	BT549	T47D	MCF7	HT29	MDAMB157	HBL100	HS578T	N417	SW2	BEAS2B	ZL5	ZL34	U1752	A125
1	-	-	(+)	(+)	-	-	-	-	-	-	-	-	-	-
2	-	+ clones	-	-	-	-	-	++++	++++	++++	++++	++++	++++	(+)
3	-	-	-	-	-	-	-	(+)	-	-	++++	++++	++	+
4	+ c.m.	-	-	-	-	-	-	++++	++++	-	-	(+)	-	-
5	-	+++	++++	+++	-	+ c.m.	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	++++	-	-	-	-
7	-	+ clones	+ rim	(+)	-	-	-	-	+	+	-	++	+	+
8	(+30%)	-	-	(+)	-	-	-	-	-	++++	++++	++++	-	++++
9	-	-	-	(+)	-	-	-	-	-	+++	-	-	-	-
10	+/++	-	-	(+)	-	-	-	-	-	(+)	-	-	-	-
11	-	-	-	(+)	-	-	-	-	-	++++	++++	++++	(+)	+
12	-	(+)	-	-	-	-	-	(+)	-	-				
13	-	-	-	-	-	-	-	++	-	-				
14	-	-	+ grn	-	-	-	-	++++	++++	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	-	+	-	-	-	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	80% (+)	-	-	-	-	-	-	++++	++++	-	-	-	-	-
20	-	+	-	-	-	-	-	-	++++	++++	-	+	-	+++

	BT549	T47D	MCF7	HT29	MDAMB157	HBL100	HS578T	N417	SW2	BEAS2B	ZL5	ZL34	U1752	A125
21	-	-	-	(+)	-	-	(+)	+++	++	++++	++++	++++	++++	++++
22	-	-	-	-	-	-	-	-	+++	++++	-	++++	+	+
23	-	(+)	+++	++/+++ s.c.	-	-	-	-	++++	++++				
24	-	-	-	-	-	-	-	++/+++	++++	-				
25	-	-	-	-	-	-	-	(+)	++++	-				
26	-	-	-	-	-	-	-	-	(+)	-				
27	-	(+)	+	-	-	-	-	-	++++	++++	-	-	(+)	-
28	-	-	-	-	-	-	-	-	-	+++	++++	++++	-	-
29	-	-	-	-	-	-	-	++++	++++	-				
30	-	-	+++	++	-	-	-	++++	++++	++++	(+)	+++	-	-
31	-	+++	+	-	-	-	-	-	++++	-	-	-	-	-
32	-	-	-	-	-	-	-	++++	++++	-	-	-	-	-
33	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	(+)	-	-	-	-	-	-	-	-	-	-	-	-	-
35	40%+ rest (+)	+++	+++	(+)/++	-	-	-	-	-	+	-	+++	++	++
36	40%+ rest (+)	+	+	-	-	-	-	-	-	++++	-	-	-	-
37	-	-	-	-	-	-	-	(+)	-	-	-	-	-	-
38	-	(+)	-	-	-	-	-	-	-	-				
39	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	BT549	T47D	MCF7	HT29	MDAMB157	HBL100	HS578T	N417	SW2	BEAS2B	ZL5	ZL34	U1752	A125
40	-	-	-	-	-	-	-	(+)	-	-	-	-	-	-
41	40%+ 60% (+)	-	-	-	-	-	-		-	++	++++	++++		
42	-	(+)	+	++	-	-	-	+	(+)	++++	+++	++++	++++	++++
43	-	(+)	+ rim	-	-	-	-	-	++++	-	-	-	-	-
44	10%+ 90% (+)	+	+++	some ++/+++	-	-	-	(+)	++++	+	(+)	+++	+++	+++
45	20%+ 80%(+)	+++	up to +++	-	-	-	-	-	-	-	-	-	-	-
46	-	-	+	-	-	-	-	-	-	-	-	-	-	-
47	-	-	+	-	-	-	-	-	-	-	-	-	-	-
48	-	-	-	-	-	-	+ grn	-	++++	-	++++	++++	-	++++
49	-	-	-	-	-	-	-							
50	80%++ 20%+	+++	+	some +	-	-	(+)	(+)	++++	++	(+)	+++	+++	++++
51	-	-	+	-	-	-	-	-	++++	++++	-	-	-	+++
52	-	-	-	-	-	-	-	-	-	-	-	-	-	-
53	-	-	-	-	-	-	-	-	-	-	-	-	-	-
54	-	-	++ grn	+ grn	-	-	-	-	-	-	-	-	-	-
55	-	-	-	+	-	-	-	(+)	-	-	-	-	-	-
56	-	-	+	-	-	-	-	-	-	-	-	-	-	-
57	-	-	-	-	-	-	-	-	-	++++	-	-	-	(+)

	BT549	T47D	MCF7	HT29	MDAMB157	HBL100	HS578T	N417	SW2	BEAS2B	ZL5	ZL34	U1752	A125
58	-	-	-	-	-	-	-	(+)	-	-				
59	+	-	-	-	-	+ grn	-	(+)	++++	++++	-	+++	+++	++++
60	-	-	-	(+)	-	-	-	-	-	-	-	-	-	-
61	-	-	-	-	-	-	-	-	-	-	-	-	-	-
62	-	+++	(+)	5% +++	+ s.c.	+	-	++++	+++	(+)	-	+	+++	++++
63	-	-	-	-	-	-	-	(+)	+++	++++	-	+++	++	++++
64	-	-	-	-	-	-	-	-	-	-				
65	-	-	-	-	-	-	-	++++	+	-				
66	-	+	-	-	-	(+)	-	++++	+/++	+	-	-		-
67	-	-	-	-	-	-	-	-	-	-	-	-	-	-
68	-	-	-	-	-	-	-	-	-	-	-	-	-	-
69	-	-	-	-	-	-	-							
70	-	(+)	-	-	-	-	-							
71	+	+	++	-	-	-	-	+/++	++++	+++	-	++++	++++	++++
72	-	(+)	-	-	-	-	-	-	(+)	+	+	++++	+++	++++

c.m. cell membrane reactivity.

gm granular fluorescence.

s.c. single cells reacted.

Note: The term clones indicates the reactivity of multiple groups of cells reacting with the antibody; these groups are generally surrounded by non-reacting cells. Single cell reactivity indicates the reactivity of few individual cells reacting with the antibodies, granular fluorescence indicates the reactivity of presumably intracellular granules with the antibodies while the terms rim and cell membranes indicate a reactivity which is confined to the cell membrane and its immediate surroundings such as the extracellular matrix. The differentiation between cell membrane and extracellular membrane reactivity cannot be resolved at the light microscopical level for epithelial cells as the extracellular space is so small.

Fig. 2a

Fig. 2b

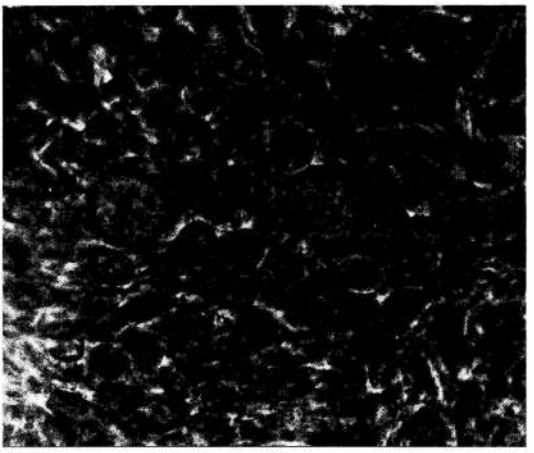

Fig. 2c

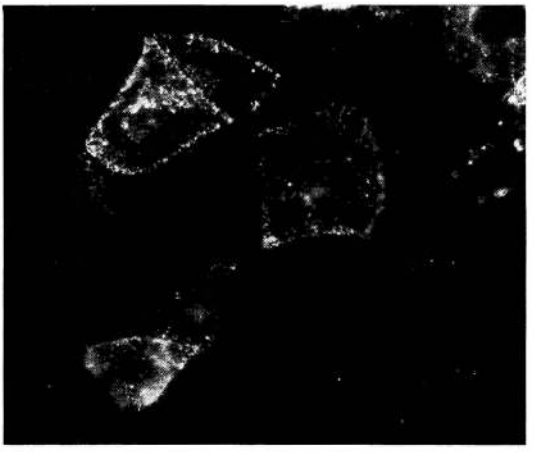

Figure 2. Photomicrographs of cultured (a) HT29 colon cancer cell line and (c) MC7 breast cancer cell line showing some FITC labelled cells after reactivity with the antibody number 23 (MLuC-1) and the antibody number 54 (1.291) respectively. b) is a phase contrast photomicrograph of the same labelled cells in Fig. (a). X415.

DISCUSSION

The present study has shown that monoclonal antibodies designed for detecting oncodevelopmental antigens expressed in lung cancer can also share epitopes of breast and colon cancer cell lines. Furthermore a heterogeneity of the antibody binding towards the different breast cancer cell lines has been observed indicating a phenotypical diversity of these cell lines. Some of the many cross reactivities of the antibodies with the breast and colon cancer cell lines seem to be of functional interest which might have implications concerning the prognosis of both breast and lung cancer:

1) The antibodies no 24-26 directed against the neuronal cell adhesion molecule (NCAM) immunoreacted only with the two cell lines derived from small cell carcinoma of the lung, while other antibodies with NCAM specificity showed a broader reactivity: antibody 4 reacted with the mesothelioma derived cell line ZL 34 and with the breast cancer cell line BT 549, antibody no 14 with the breast cancer cell line MCF7 and antibody no 19 with the breast cancer cell line BT549. At the moment it is not clear whether these are indeed different epitopes of the NCAM molecule recognised by the different antibodies, or whether they are cross reactivities with epitopes other than NCAM. Since neuroendocrine markers can be expressed by breast cancer (for review see Nesland *et al.*, 1988) NCAM expression on these breast cancer derived cells is likely.

2) The antibody no 72 directed against a mucin antigen cross reacted with all lung cancer cell lines except the mesothelioma derived cell line ZL5 and with the breast cancer derived cell lines BT549, T47D and MCF7. Common expression of mucin antigens in normal and pathological conditions of lung and breast tissue is well known (Welsch *et al.*, 1990, Spicer *et al.*, 1991, Pemberton *et al.*, 1992) and this reactivity is therefore not unexpected.

3) The mesothelioma specific antibody no 41 showed its strongest reactivity with the mesothelioma derived cell lines ZL5 and ZL34; in addition it reacted with the immortalised human bronchial-derived cell line BEAS-2B and the breast cancer cell line BT549, the nature of this binding pattern being obscure at present.

4) The distribution of cell surface receptors which react with the extracellular matrix differs. In addition to reactivity with various lung cancer derived cell lines, laminin receptor immunoreactivity was detected on the breast derived cell lines T47D (antibody no 20) and BT549 and HBL100 (antibody no 59). The immunoreactivity of this receptor seems to be more widespread than that for beta-1-integrin antibodies detected in HS578T and HT29 (antibody 21). Alpha-6-integrin was detected on lung derived cell lines only (antibody no 22). The biological implications of these findings are not clear at present, but the interaction of these receptors with their ligand(s) in the extracellular matrix seems to play a crucial role within the metastatic cascade (Hart and Saini, 1992).

Our findings therefore indicate that several antigens thought to be lung cancer specific can also be expressed on breast and colon cancer cell lines. Any claims towards specificity of many of those antibodies therefore have to be treated with great care.

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