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ARTICLE

Differential Diagnostic Significance of The Paucity of HLA-I Antigens on Metastatic Breast Carcinoma Cells in Effusions

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Distinction between benign reactive mesothelial cells and metastatic breast adenocarcinoma cells in effusions from patients with a known prior history of breast cancer is not the easiest task in diagnostic pathology. Here, we report the usefulness of testing the expression of class I HLA antigens (HLA A, B, C) in this respect. Cytospins were prepared from effusions of patients without the history of breast cancer (5 cases) and from effusions of patients with infiltrating ductal carcinoma (11 cases). Three effusions from cancerous patients were not malignant cytologically. The expression of HLA-A, B, C, HLA-DR and β2-microglobulin as well as the macrophage antigen, CD14, was evaluated by immunocytochemistry. In 10 of 11 effusions the cytologically malignant cells expressed very weak or undetectable HLA-A,B,C as compared to the mesothelial cells and macrophages. The paucity of expression of HLA-A, B, C was detectable in those 3 cases where a definitive cytological diagnosis of malignancy could not be established. In contrast, mesothelial cells and macrophages from all samples were uniformly and strongly positive for both HLA-A, B, C and β2-microglobulin. We conclude that the paucity of HLA-I antigens provides a marker helpful in distinguishing metastatic breast carcinoma cells from reactive mesothelial cells in effusions. (Pathology Oncology Research Vol 5, No 1, 32–35, 1999)

Key words: HLA-A, B, C antigens, breast cancer, metastasis, cytology

Introduction

It is a frequent task for cytopathologists to evaluate pleural or peritoneal effusions from patients with history of breast cancer. Highly accurate diagnosis is expected due to the therapeutic and prognostic consequences. However, frequently the reactive mesothelial cells assume many features associated to malignancy (enlarged nuclei, multiple prominent nucleoli, nuclear irregularities and vacuolated cytoplasm, mitosis), and breast cancer cells can also have very bland cytological features giving rise to false negative or positive diagnosis.

A possible way to identify malignant cells in body cavity effusions is to take advantage of an antigen present selectively on epithelial cells, which is absent in mesothelial cells, or vice versa. Antigens, characteristically expressed by epithelial cells are cytokeratins[1,2] and epithelial membrane antigen[3] but these are also expressed on mesothelial cells, therefore have limited usefulness in differential diagnosis. CEA expression was found in 50% of peritoneal carcinomatous effusions,[4] however, reactive macrophages could also be positive in certain cases.[4] It is now well established that HLA-I antigens are frequently decreased or completely lost in breast cancer cells[5,6] and the degree of loss of this antigen has also prognostic significance since it is associated to a more malignant behaviour of the tumor.[5,6,7] Therefore, our aim was to compare HLA-I expression on breast cancer cells and on reactive cells in effusions in order to evaluate its capability in distinguishing normal and malignant cells.

Materials and Methods

Specimens

The effusions were obtained from patients admitted to the Warner Grant Clinical Center, NIH and were submitted to routine cytology. Aliquots of each sample were used

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Table 1. **Cytology and immunocytochemistry of reactive effusions from patients without a history of breast carcinoma**

					Cytology		*Immunocytochemistry*			
Case No	*Age*	*Sex*	*Source*	*Clin. dg.*	*Mesothel*	*Macrophage*	*CD14*	*HLA-DR*	*HLA-A, B, C*	*β-$_2$m*
1	28	F	pericardium	SLE	1	99	99	99	100	–
2	28	F	pleura	SLE	2	98	98	98	100	–
3	35	F	pericardium	HCMP	52	48	70	70	100	100
4	72	M	pleura	Cirrhosis	12	88	86	87	100	–
5	26	M	pleura	Fever	12	88	87	85	100	–

Only non-lymphoid cells were evaluated. Data are expressed in % of positive cells. Cases 1 and 2 are separate specimens from the same patient. F = female, M = male, SLE = systemic lupus erythematosus, HCMP = hypertrophic cardiomyopathy, Cirrhosis = liver cirrhosis, β_2m = β_2-microglobulin

for marker studies where cytospins were prepared on a Shandon instrument. Samples were fixed in acetone and were stored on 4°C.

Antibodies

Mouse anti-HLA monoclonal antibody recognizing class I HLA antigens (A, B, C) was purchased from Bethesda Research Laboratories, Gaithersburg, MD. Other antibodies – mouse monoclonal anti-HLA-DR recognizing class II HLA antigens, anti-β-$_2$microglobulin, anti-Leu-M3 (CD14) recognizing CR3 – were from Beckton-Dickinson (Sunnyvale, CA). For specificity control murine myeloma proteins MOPC21, 173, 37 (Sigma) were used.

Immunocytochemistry

For blocking 1% non-immune horse serum diluted in Tris buffer was used (5 min). Primary antibodies were applied at 10 μg/ml concentration for 30 min. The secondary reagent was biotinylated anti-IgG diluted 1:200 followed by avidin-peroxidase complex. The enzymatic reaction was developed by DAB/Ni. The sections were counterstained by methyl green, dehydrated, cleared and coverslipped. Specificity controls included incubation with isotype-matched IgG or buffer alone instead of the primary antibody. The % of positive cells was counted by evaluating minimum 100 nucleated cells. In case of HLA and β_2-microglobulin, besides the positive (+) and negative (–) cells, weakly positives were also determined.

Results

16 cytological specimens from 13 patients were studied including 11 pleural-, 3 pericardial- and 2 peritoneal effusions. The 5 reactive effusions were composed of neutrophil leukocytes, mesothelial cells and macrophages. Since neutrophils and lymphocytes were cytologically distinctive they were not included in cytological and immunocytochemical evaluation. In the reactive effusions *(Table 1)* all of the cells examined stained strongly positive for HLA and β_2-microglobulin. Furthermore, the % of cells stained positive for HLA-DR and CD14 correlated well with the percent of cells judged cytologically to be macrophages. Positive staining for HLA and β_2-microglobulin of a reactive effusion is shown on *Figure 1 b, c*, while CD14 labeling of macrophages on *Figure 1d*.

Eight of the 11 effusions from patients breast cancer patients (cases 6–16) contained variable percentage of cells with suspicion of malignancy *(Table 2)* where the rest of the cell population was composed of mesothelial cells or macrophages. Out of the 11 effusions, in seven cases HLA became negative in a significant proportion of the cells (≥10%) and the decrease in the percentage of strongly positive cells was also significant in 6 cases (≥25%). *Figure 2 b, c* illustrates malignant effusion with negative tumor cells for HLA and β_2-microglobulin. In three cases

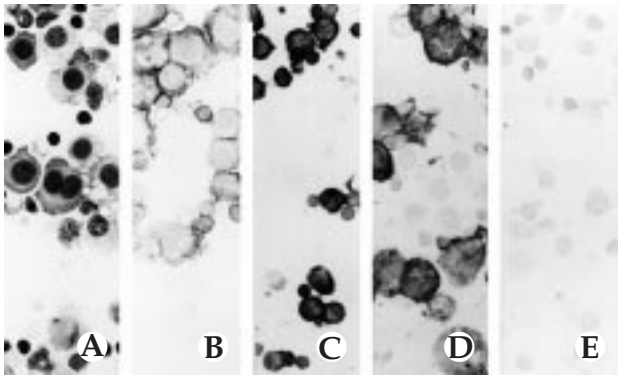

Figure 1. *Cytology and immuncytochemistry of a reactive effusion (case 5). A) Diff-quick stain of cytospin containing mesothelial cells, macrophages and small lymphocytes. B) HLA-A, B, C reaction. Note the reaction at the cell surface of the cells. C) β_2-microglobulin reaction. Strong and uniform reaction at the cells surface. D) Detection of CD14 by Leu-M3 on the reactive cells. Note the intense surface reaction on the large macrophages. E) Negative control using mAb MOPC37. (x400)*

Table 2. **Immunocytochemistry of malignant effusions from patients with a history of breast cancer**

Clinical data				*Cytology*			*Immunocytochemistry*							
									HLA			*β_2-m*		
Case	*Age*	*Sex*	*Source*	*Tumor cells*	*Meso-thel*	*Macro-phages*	*CD 14*	*HLA-DR*	+	+/–	–	+	+/–	–
6	56	F	pericardium	90	0	10	0	0	100	0	0	100	0	0
7	47	F	Pleura	85	9	6	5	6	11	39	50	25	43	32
8	55	F	Peritoneum	83	0	17	20	43	11	89	0	11	88	1
9	68	F	Pleura	44	21	35	47	54	71	28	1	99	0	1
10	40	F	Pleura	12	2	86	86	86	88	2	10	96	4	0
11	59	F	Pleura	10	5	85	81	85	84	12	4	98	2	0
12	53	F	Pleura	9	88	3	4	6	8	6	85	7	32	61
13	53	F	Pleura	6	89	5	5	4	5	12	83	5	26	69
14	35	F	Pleura	2	92	6	5	9	5	1	94	0	100	0
15	55	F	Peritoneum	?	40	60	61	63	64	14	22	n.t.	n.t.	n.t.
16	44	F	Pleura	?	98	2	3	6	11	0	89	8	86	5

Only non-lymphoid cells were evaluated. Data are expressed in %. Cases 8.15 as well as 12.13 are separate specimens from the same patient. F = female, M = male, n.t. = not tested, β_2m = β_2-microglobulin

(14–16) presence of malignant cells in the effusion was not evident by cytology, however, decreased HLA expression was found in a significant proportion of cells *(Table 2)*. At the death and the subsequent autopsy of these patients disseminating tumors in the body cavities were found. On the other hand, β_2-microglobulin staining was negative or minimal (0–1%) in 6 out of the 11 cases. In the malignant effusions, similarly to the reactive ones, the % of CD14 and HLA-DR positive cells correlated well to the % of macrophages evaluated by cytology.

Discussion

Identification of malignant cells in effusions is often a serious differential diagnostic problem. This is due to the fact that the antigenic profile of the mesothelial cells is similar to cancer cells including common cytokeratin, but accidental EMA or CEA expressions.[1,2,3,4] To improve the diagnostic accuracy, DNA ploidity analysis,[8,9] and highly specific antibody to mesothelial cells[9] can be included in the diagnostic protocol.

HLA antigens are expressed by virtually all tissues except CNS and endocrine ones.[10] Neoplastic transformation affects the expression of HLA; the HLA class-I antigens are frequently decreased (SCLC, NBL, CHCC,[11,12] NHL[13]) while HLA-DR can be upregulated on tumor cells.[5,14] It has been shown that in breast cancers HLA-I expression is decreased in tumor cells and this phenotype is associated with a poorer prognosis.[5,6,7] On the other hand, it has been also reported that β_2-microglobulin expression follows the pattern of HLA-I in breast cancer cells.[15] Based on these observations we have postulated that the loss of HLA-I expression in disseminating breast carcinoma cells could have diagnostic significance in certain conditions, e.g. in the cytological evaluation of effusions from body cavities.

Our study indicated that in reactive effusions macrophages and mesothelial cells are highly positive for HLA-I while CD14 and HLA-DR positivity characterize macro-

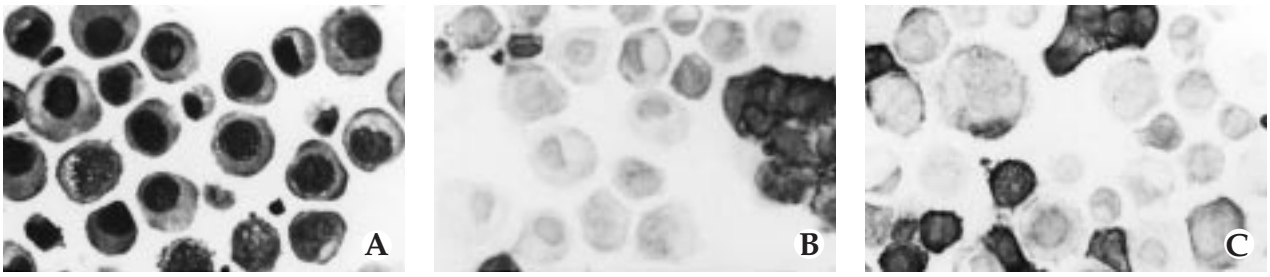

Figure 2. *Cytology and immuncytochemistry of a malignant effusion (case 12). A) Diff-quick stain of cytospin containing mesothelial cells, macrophages as well as single cells with hyperchromatic nuclei and increased nuclear to cytoplasmic ratio. B) HLA-A, B, detection. Note the large number of negative cells. C) β_2-microglobulin labeling. The majority of the cell population is negative. (x400)*

phages. In the 11 malignant effusions a significant decrease was observed in the expression of HLA-I as well as that of β_2-microglobulin. Seven of the 11 cases showed complete loss of HLA-I in >10% of the cells and in 2 other cases a decrease was found. Only 1 of the 11 malignant effusions exhibited an unchanged HLA-I pattern. A further indication for the usefulness of the HLA-I loss in the differential diagnosis of effusions was observed in 3 cases where the cytological diagnosis was uncertain (cases 14,15,16) due to the absence of recognizable malignant cells. In these cases HLA-I expression decreased in the cells of the effusion and the presence of disseminated cancer cells was proved at the autopsy in the previously studied cavities. Thus, while the presence of HLA-I does not indicate that the cells are surely reactive, the lack of the expression on metastatic breast carcinoma cells in effusions clearly distinguishes malignant cells from reactive mesothelial cells.

It is not common to propose the use the loss of a normal antigen as a marker for malignancy, however, since normal cells are rather consistent in their antigenic profile, the lack of a common antigen in tumor cells (in this case in breast cancer cells) could have differential diagnostic significance.

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