Immunophenotyping and Metastatic Localization of Breast Cancer

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ABSTRACT

Monoclonal antibody SM 92 is involved in the immunophenotype of gastrointestinal and liver cells, SM 43 in ovarian cells, and SM 13 in lung cells. Based on a study of 61 breast adenocarcinoma patients, we found that tumors reacting with SM 92 appear associated with liver metastases, SM 43 with ovarian metastases, and SM 13 with lung metastases. These associations are highly significant. They lend some support to the concept that tumor cells that metastasize tend to go to sites where cells normally have the same surface antigens.

INTRODUCTION

The process of tumor metastasis is far from being fully fathomed. This has led to the formulation of many hypotheses concerning the modification of cellular properties needed for metastasis. Analysis of the respective roles, for example, of cellular mobility factors, oncogenes, adhesion molecules, and proteases has shed some light upon aspects of the metastatic process. However, clinical evidence has not permitted one or another of these hypotheses to be confirmed (1–4). Evidence bearing on the phenotypic expression of cellular adhesion molecules may allow a better understanding of the formation of metastases. As a matter of fact, it seems that cellular proteins are involved in the invasion process without any clinical proof thereof, until now.

In our laboratory, the development of numerous antibodies against tumor cells has permitted the study of epithelial tumor specificities. We have catalogued membrane antigens in diverse histological types of carcinomas and proposed profiles adapted to different primary cancer locations (5). Our research on a specific immunological profile of breast cancer proved to be in vain. However, results obtained from the study of 61 patients show the existence of relationships between the immunophenotypic profile and the secondary localization of a mammary cancer cell in a preferred site.

MATERIALS AND METHODS

Materials

The 61 samples under study came from patients at the Centre Antoine Lacassagne and the University Hospital Center of Nice and included all types and grades of breast cancer. There were 14 primary tumors, 33 pleural effusions, 7 ascitic effusions, 3 cutaneous nodules, 1 pericardic effusion, 1 lymphangitis, 1 lymph node, and 1 uterine metastasis. Erythrocytes and granulocytes were removed by Ficoll-gradient centrifugation. Solid tumors bodies from mice were used: SM 92, SM 43, and SM 13. SM 92 is in the immunophenotypic profile of gastrointestinal and hepatic carcinomas, SM 43 is in the profile of ovarian cancer, and SM 13 is in the profile of lung cancer (5, 6). Principal features of these antibodies are given in Table 1. A tumor was considered to have a positive phenotype for an antibody if the tumor contained cells reactive with that monoclonal antibody. Each tumor was classified in this way phenotypically as SM 92 + or −, SM 43 + or −, and SM 13 + or −.

Methods

For each tumor, seven cytocentrifugations were done. Slides were air dried (2 h). They could then be kept at 4°C for 48 h before scoring. One of the slides was stained by the Papanicolaou method for a classic cytological study.

Indirect Immunofluorescence. Immunoscoring was done with six new mouse monoclonal antibodies (Calam 27, 3D7, SM 27, SM 92, SM 43, and SM 13) produced in our laboratory (5–7). The source of antibody was the undiluted supernatant from the corresponding hybridoma. Cell preparations were stained by an indirect immunological method using F(ab') 2 fragment of goat anti-mouse IgG with fluorescein (Immunotech, Marseille, France) as the second-stage reagent. Slides were placed in a Sequenza apparatus (Shandon, Cergy-Pontoise, France). For each type of scoring, cells were in contact with 100 μl hybridoma supernatant for 30 min at room temperature. After washing with PBS 3, 100 μl of fluorescent conjugate were added for 30 min. After another PBS rinse, cells were fixed in absolute ethanol for 5 min; then the cell nuclei were stained with propidium iodide diluted 1/10 in PBS for 2 min. The slides were examined under a fluorescent microscope (Zeiss, Oberkochen, Germany). Green membranes staining served as a positive control for antibody reactivity. All 61 samples in this study had an epithelial phenotype (5), as evidenced by their reactivity to one or more of three antibodies (Calam 27, 3D7, and SM 27) to epithelial membrane antigens (Table 1).

RESULTS

The immunophenotypes and sites of metastasis are presented in Table 2. The numbers of tumors reacting positively or negatively to antibodies SM 92, SM 43, and SM 13 and metastasizing or not to liver, ovary, and lung are tabulated. The 61 tumors consisted of 38 that reacted with antibody and 23 that did not. Twenty-five of the antibody-reactive versus only 3 unreactive tumors metastasized to liver, ovary, or lung. The difference is highly significant (χ 2 16.06; d.f.; 3; P < 0.01), indicating an association between antibody reactivity and metastasis.

Antibody Reactions. Eleven (65%) of 17 tumors reactive to SM 92 metastasized to liver, ovary, or lung versus 11 (58%) of 19 tumors reactive to SM 43 and 3 (25%) of 12 tumors reactive to SM 13. Reactivity to SM 92 and SM 43 was highly significant (P < 0.01) indicating an association between antibody reactivity and metastasis.

Reactivity to SM 92 was positive in 11 tumors: 6 tumors metastasized to liver, 4 to liver plus lung, and 1 to liver plus ovary. Of these 11 cases, 1 involved ovary, 4 lung, and all 11 liver. The key relationship between SM 92 positivity and metastatic site appears to be with liver (Tables 2 and 3).

Reactivity to SM 43 was positive in 11 tumors: 4 tumors metastasized to liver, 2 to liver plus ovary, 4 to ovary, and 1 to ovary plus lung. Of the 11 cases, 1 involved lung, 6 liver, and 7 ovary. Given the overall numbers of tumors reactive and unreactive to SM and tumors metastasizing to liver or not, one would expect 5.3 SM 43-reactive...
tumors metastasized to liver; compared to the observation of six such cases, there is no significant difference. The main relationship between SM 43 and metastasis appears to be with ovary (Tables 2 and 4).

Reactivity to SM 13 was positive in 9 tumors: 1 tumor metastasized to liver, 3 to liver plus lung, and 5 to lung. Of the nine cases, none involved ovary, four involved liver, and eight involved lung. Again, there is no significant excess of antibody-reactive tumors metastatic to liver. The principal relationship between SM 13 and metastatic site would appear to be with lung (Tables 2 and 5).

**Metastatic Locations.** Twenty-eight (46%) of the 61 patients developed metastases: 11 to liver, 5 each to ovary and lung, 4 to liver plus lung, 2 to liver plus ovary, and 1 to ovary plus lung (Table 2). Thus, of 28 cases with metastases, 17 involved liver, 8 ovary, and 10 lung. As regards specifically the 17 cases with liver metastases, 11 were positive for SM 92, 6 were positive for SM 43, and 4 were positive for SM 13. Of the eight cases with ovarian metastases, one was positive for SM 92, seven were positive for SM 43, and none were positive for SM 13. Of the 10 cases with lung metastases, 4 were positive for SM 92, 1 was positive for SM 43, and 8 were positive for SM 13.

The relationships between SM 92 status and liver metastases are set out numerically in Table 3. Of the tumors reactive with SM 92, 6 metastasized to liver, whereas 6 did not. By contrast, of the tumors unreactive with SM 92, only 6 metastasized to liver, whereas 38 did not. The difference is highly significant ($\chi^2$ with Yates' correction 14.63; d.f. 3; $P < 0.01$), indicating that SM 92 positivity appears associated with a tendency to liver metastases.

The numerical relationships between SM 43 status and ovarian metastases are set forth in Table 4. Seven tumors reacting positively to SM 43 metastasized to ovary, whereas 12 did not. By comparison, 1 tumor unreactive with SM 43 metastasized to ovary, whereas 41 did not. The difference is very highly significant ($\chi^2$ with Yates' correction 18.34; d.f. 3; $P < 0.001$), indicating that SM 43 positivity appears associated with a tendency to ovarian metastases.

SM 13 status and lung metastases are considered in Table 5. Eight tumors reacting positively to SM 13 metastasized to lung, whereas 3 did not. By contrast, only 2 of 50 tumors not reacting to SM 13 metastasized to lung. The difference is very highly significant ($\chi^2$ with Yates' correction 26.52; d.f. 3; $P < 0.001$), indicating that SM 13 positivity appears associated with a tendency to lung metastases.

**Relationships.** To sum up the results, it would seem that there are relationships between SM 92 positivity and a tendency to liver metastases, between SM 43 positivity and a tendency to ovarian metastases, and between SM 13 positivity and a tendency to lung metastases. The converse would also hold with an association between SM 92 negativity and a tendency not to metastasize to liver, etc.

**DISCUSSION**

The monoclonal antibody SM 92 is reactive not only with gastrointestinal cells but also with liver (Table 1). Therefore, it is of interest that breast cancer patients whose tumor cells react with SM 92 appear to have an increased chance of hepatic metastases (Table 3).

The monoclonal antibody SM 43 is highly reactive with ovarian cells (Table 1). It is thus of some interest that breast cancer patients with SM 43+ tumor cells seem to have a greater risk of ovarian metastasis than do patients with SM– tumors (Table 4).

The monoclonal antibody SM 13 is reactive not only with head and neck but also lung (Table 1). Similarly, it is intriguing that breast cancer patients with SM13+ tumors look to have an elevated risk of...
lung metastases compared to those patients with SM 13– tumors (Table 5).

The results presented in Tables 3–5 show the correlation between antigens recognized by monoclonal antibodies SM 92, SM 43, and SM 13 and metastases of breast carcinoma to liver, ovary and lung, respectively. To reiterate, if cells from the tumor carry the SM 92 immunophenotype, the patient seems to run a risk of developing hepatic metastases. If there are only cells of the SM 92– phenotype, the patient will tend not to develop hepatic metastases.

Likewise, there appears to be a correlation between the presence or absence of cells with SM 43+ or SM 43– phenotype and the development of ovarian metastases and between the SM 13+ or SM 13– phenotype and the development of lung metastases. All these results are important in showing the ties between the surface antigens of tumor cells and their propensity to metastatic dissemination to specific sites with these antigens.

The ability of a tumor to metastasize has not been completely explained. It has been assumed that the secretion of proteases permits tumor cells to travel across basal membranes and disseminate via blood vessels or the lymphatic system. This line of thought does not explain preferred sites of metastases, even if conjuring up the presence of protease inhibitors would seem to lend weight to this hypothesis. In fact, only the analogies between tumorigenesis and embryogenesis can provide a base for the results reported here.

Thiery and coworkers (8–10) have shown that cells in the embryo that take a particular pathway in differentiation present the analogous surface molecules. Among surface molecules, the cadherins are interesting antigens. For example, all the cells that leave the neural crest to constitute the neuroendocrine system express N-cadherin. Thiery and coworkers (8–10) show also that, while acting on the environment of embryonic cells, one acts on the expression of these surface antigens and the development of the differentiation profile of these cells.

Cells in the primary tumor, through genetic changes, seem to rediscover the embryonal expression of certain surface antigens. These cells coming from a differentiated tissue become capable of expressing part of their potential for acceptance by another tissue. During tumor development, cells that have acquired antigens compatible with a privileged location leave the primary tumor to travel to another site, where a metastasis forms. The work of Fidler and Kripke (11), for example, has suggested that metastases arise from preexisting variant cells within the tumor.

The phenomenon of "homing" of hematopoietic stem cells and lymphocytes also fits in with the same hypothesis, i.e., an attraction of cells to specific sites via surface molecules. For example, spleen cells (both B and T cells) injected into an animal redistribute themselves in the diverse peripheral B- and T-cell compartments; furthermore, when bone marrow is injected, certain undifferentiated stem cells go back directly to the marrow as cells of the myeloid line (12).

The present work relating surface antigens present on breast tumor cells to preferential sites of metastasis may provide some clinical evidence for the hypotheses suggested by research. It can also help foresee, to some extent, the occurrence of hepatic, ovarian, or pulmonary metastases, thanks to a cellular immunophenotype in the primary tumor.

REFERENCES

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