Prognostic Value of Concanavalin A Reactivity of Primary Human Breast Cancer Cells

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ABSTRACT

A prospective, double-blind study was carried out to determine whether reactivity with concanavalin A (Con A) of human breast cancer cells was related to early disease recurrence. Mammary epithelial cells were isolated from 138 primary human breast cancers. The cells were placed in culture, and their reactivity with Con A was determined as the concentration of Con A at which approximately 50% of the test cells adsorbed erythrocytes. Con A reactivity of the tumors was classified as high or low (half-maximum value <30 or >30 μg/ml, respectively). Patients were followed for 2 to 60 months after primary surgery (median, 22 months). Those patients having tumors that were highly reactive with Con A were at significantly greater risk of developing early recurrence of their cancers than were those patients with low-reactivity tumors. No correlation was found between Con A reactivity and the age of the patients, their menopausal status, the number of axillary lymph nodes infiltrated with tumor, the estrogen receptor content of the tumor, or the clinical stage of the disease. These data show that Con A reactivity is an independent discriminator for identifying those breast cancer patients who are at high risk of developing early recurrent disease.

INTRODUCTION

Primary, operable breast cancer does not usually pose an immediate threat to the life of patients. Rather, it is the probability of metastatic spread and recurrence which determines the survival of the breast cancer patient.

Recent evidence suggests that adjuvant chemotherapy may delay the clinical onset of recurrent cancer (2, 7). There are, however, significant adverse side effects associated with such treatment, and aggressive therapy should be restricted to those patients who are at high risk for developing recurrent disease. The problem has been accurate identification of these high-risk patients.

With the active participation of our clinical community, we have organized a major study aimed at the detailed biological characterization of large numbers of primary human breast tumors and the identification of those host factors which are correlated with early tumor recurrence and metastasis. A locus of potentially useful prognostic markers is the cell membrane. The outer cell surface is intimately involved in regulating many normal cell functions. It is at this interface that regulatory signals are initially received by cells. Any alteration in surface properties could alter the social behavior of a cell, resulting in neoplastic disease and determining the propensity to metastasize (for review, see Ref. 17).

Plant lectins, because of their specific carbohydrate-binding properties, have been used extensively as probes to study the surface architecture of normal and transformed cells. Some of these lectins induce agglutination of many types of tumor cells, whereas most normal cells are only agglutinated by much higher concentrations of the lectin, if at all (5, 16, 22). Although exceptions can be found, there are reports which suggest that the degree to which cells are agglutinated by lectins such as Con A or wheat germ agglutinin is quantitatively related to their tumorigenicity and in some instances also to the metastatic potential of the cells (12, 19, 24, 26). The molecular basis for these differences in agglutinability and their relationship with tumorigenicity are still controversial (1, 17, 20).

Surface characteristics of normal and malignant human mammary epithelial cells as reflected by lectin reactivity have not been extensively studied. With the use of a Con A-mediated hemadsorption assay, we reported that these cells differ in their reactivity with Con A and that this property is an in vitro marker for malignant transformation of human breast cells (27). In the present study, we investigated whether Con A reactivity of cells isolated from primary breast tumors also provides a means of identifying tumors that differ in metastatic potential. We report here that Con A reactivity appears to be a reliable discriminant for identifying those breast cancer patients who are at high risk of developing early recurrent disease.

A portion of this work has appeared in abstract form (13).

MATERIALS AND METHODS

Source and Isolation of Cells from Solid Tumors. Participating local hospitals were provided tumor collection kits containing a sterile specimen container for collection of the tumor in the operating room, sterile instruments, and vials containing sterile culture medium. Tumor specimens were trimmed and divided into a number of slices (1 to 2 mm thick, dependent upon tumor size) by a resident pathologist and placed into the vials provided. These were held on ice and received from the hospitals generally within 1 hr of surgery. Detailed pathological characterization of the tumors was provided by a panel of 5 clinical pathologists. Primary cultures of malignant human mammary epithelial cells were prepared by collagenase dissociation as described previously (14).

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Briefly, 3 slices of each tumor (average total wet weight, 0.5 g) were finely minced with sterile scalpels, and cells were released by incubating the finely minced tissues for 18 hr without agitation at 37°C in culture medium supplemented with 0.5 mg collagenase per ml (CLS III; Worthington Biochemical Corp., Freehold, N. J.) and 5% (v/v) fetal calf serum. Released cellular aggregates were collected by low-speed centrifugation and resuspended in 10 to 15 volumes of growth medium. Cultures were established in individual 35-mm dishes or 16-mm cluster dishes (Costar, Cambride, Mass.). No attempt was made to determine the exact number of cells plated because of the variability in the number of cells comprising the epithelial cell aggregates. Cells were routinely plated in Waymouth’s MD 705/1 medium (Grand Island Biological Co.) supplemented with 15% (v/v) fetal calf serum, insulin (10 μg/ml), and gentamicin (0.05 mg/ml). The medium was changed at 2- to 3-day intervals, and the dishes were maintained in a humidified 95% air-5% CO2 atmosphere. The growth characteristics of these cells have been described in detail elsewhere (14).

Con A-mediated Hemadsorption Assay. The Con A reactivity of malignant human mammary epithelial cells in primary culture was determined using the Con A-mediated hemadsorption assay as described previously (27). Briefly, human type O erythrocytes were washed 3 times in PBS, resuspended to a 2% hematocrit in various concentrations of Con A, and incubated for 10 min at 37°C. The indicator RBC were washed 3 times in PBS to remove unbound Con A and were suspended to a 2% hematocrit.

Reactivity with Con A was determined by using the cultures prepared from human breast tumors as described above. The cultures consisted, at the time of assay, of islands of proliferating epithelial cells (14, 27). The breast tumor cells attached to the growth surface (test cells) were washed 3 times with PBS and then incubated without agitation for 10 min at 37°C with indicator RBC. Unbound RBC were removed by gently washing with PBS, and the percentage of test cells adsorbing indicator RBC was determined by phase-contrast microscopy. The half-maximum reactivity (the Con A concentration at which approximately 50% of the test cells adsorbed erythrocytes) was determined as described previously (27). Generally, the concentrations of Con A used to prepare the indicator RBC were 5 to 30 μg/ml in 5-μg increments and 50, 100, 200, and 500 μg/ml. Half-maximum values in replicate assays were within one Con A concentration step.

The specificity of the hemadsorption assays was determined by adding 0.05 M α-methyl-D-mannose to the suspension of indicator RBC prior to testing with the tumor cells. This resulted in at least 90% inhibition of the hemadsorption reaction. Uncoated erythrocytes did not adsorb to the surface of the tumor cells.

Clinical Characterization of Patients. A complete clinical history and diagnostic evaluation was obtained for each patient entered. A uniform protocol for patient follow-up at 3 month intervals is included as an integral component of the study. Patients were followed for 2 to 60 months after surgery (median follow-up, 22 months). Clinical suspicion of recurrent disease was usually verified by either X-ray or isotopic scan.

Statistical Analysis of Data. Recurrence curves graphically representing the disease status of patients were calculated by using the life table actuarial method (10). Statistical significance of a difference between the recurrence curves for 2 subgroups of patients was tested using the Mantel test (generalized Savage) (15) and the Breslow test (generalized Wilcoxon) (11). Statistical significance between groups was otherwise determined by the χ² test.

RESULTS

The Con A reactivity of 138 of the primary human breast tumors which were entered into our study was tested using the hemadsorption assay. Adsorption of indicator RBC to malignant human mammary epithelial cells isolated from the primary tumors of 2 different patients is illustrated in Figs. 1 and 2. In Fig. 1, aggregates of RBC are adsorbed to the surface of individual tumor cells attached to the growth surface and many of the tumor cells are no longer clearly visible. These highly reactive cells were incubated with indicator RBC coated with 25 μg Con A per ml. In contrast, almost no RBC are adsorbed to the surfaces of the tumor-derived cells shown in Fig. 2, although they were incubated with indicator RBC coated with 500 μg Con A per ml.

The distribution of Con A reactivities for all patients tested is given in Chart 1. The concentration of lectin at which approximately half-maximum reactivity was observed for individual tumors ranged from 5 μg Con A per ml to greater than 500 μg/ml (median, 50 μg/ml).

Differences in reactivity with Con A were not related to any apparent differences in the culture characteristics of the different tumors tested. All cultures used consisted of islands of proliferating epithelial cells surrounded by occasional fibroblasts and individual, nonproliferating macrophages (14, 27). No significant differences were detected in Con A reactivity of replicate cultures from the same tumors grown for different lengths of time.

For purposes of analysis of the relationship between Con A reactivity and tumor recurrence, tumors were classified into 2 groups containing approximately equal numbers of patients: those with a Con A half-maximum value ≤30 μg/ml (termed...
Fig. 2. Colony of human mammary epithelial tumor cells with low Con A reactivity. Con A-coated indicator RBC (500 μg/ml) were not adsorbed to the surfaces of these cells. x 100.

high-reactivity tumors); and those with a Con A half-maximum reactivity >30 μg/ml (termed low-reactivity tumors). Time to recurrence curves were constructed for these 2 subgroups of patients using the life table actuarial method (10). Chart 2 shows that those patients with highly reactive primary tumors developed recurrent cancer at a higher rate than did those patients with low-reactivity tumors. The difference between these curves was determined to be significant (p = 0.018) using the Mantel (15) and Breslow (11).

A total of 11 recurrences were detected in the low-Con A-reactivity group within the period of observation. Of these, 7 were regional and local recurrences (chest wall, skin, or remaining axillary nodes) and 4 were distant metastases. Twenty-three disease recurrences were observed in the high-Con A-reactivity group which were composed of 6 regional and local recurrences, 16 distant metastases, and one patient in whom both local and distant recurrent disease was detected.

The major clinical characteristics of the patients with high- and low-Con A-reactivity tumors were compared and are shown in Table 1. The median age of the 2 groups of patients was similar. The majority of patients in both groups was postmeno-

Chart 1. Distribution of Con A reactivity of primary human breast tumors. Con A-mediated hemadsorption assays were performed as described in "Materials and Methods."

Chart 2. Rate of recurrence as a function of Con A reactivity. , patients with low-reactivity tumors (>30 μg/ml; n = 74); , patients with highly reactive tumors (≤30 μg/ml; n = 64).
prolonged axillary lymph nodes, which have been shown to be related to a decreased survival of cancer patients (11). The authors highlight the importance of evaluating the reactivity of primary tumor cells with lectins, such as Con A and wheat germ agglutinin, as a significant diagnostic indicator.

The authors describe their method for evaluating the reactivity of cells in vitro and in vivo. They use the Con A-mediated hemadsorption assay to assess the reactivity of cells. This assay allows for the visualization of agglutination reaction to be scored with reference to only the Con A reactivity of primary tumor cells.

The study involves a total of 138 patients, with 34 having developed recurrent disease at various intervals following primary surgery. The majority of these patients with recurrent cancer had a primary tumor with a Con A half-maximum value \( \leq 30 \mu g/ml \). For statistical analysis of these data, the population was arbitrarily divided into patients considered to have high- and low-reactivity tumors. The authors conclude that the degree of reactivity of cells in vitro with lectins, such as Con A and wheat germ agglutinin, and the malignancy of the cells in vivo (12, 19, 24, 26). Most of these studies have used fibroblast-sarcoma systems. The extension of this approach to experimental animal carcinomas has been more limited (1, 3, 21, 23, 28), and studies of the interaction of lectins with carcinomas, which comprise the vast majority of human cancers, are even less common.

In human mammary tissues, cultures established from solid tumors and from normal cells derived from breast fluids consist of a heterogeneous mixture of epithelial cells and stromal fibroblasts and epithelial cells and macrophages, respectively (4, 8, 14). The heterogeneity of these populations would confound evaluation of standard lectin-mediated agglutination assays. However, this problem can be circumvented by using the Con A-mediated hemadsorption assay, which allows the agglutination reaction to be visualized on cells attached to their growth substrate (9). Since this assay can be evaluated on a single-cell basis, it can be scored with reference to only the relevant epithelial cells in the cultures. Moreover, this method obviates the need for large numbers of single cells in suspension, which is difficult to obtain from human mammary epithelial tissues.

With the use of this assay, we demonstrated previously that normal and malignant human mammary epithelial cells differ in reactivity with Con A and that this represents a suitable in vitro marker for malignant transformation of human breast cells (27). The present report extends our initial studies and demonstrates that the degree to which primary human breast tumor cells are reactive with Con A also reflects the metastatic potential of these tumors.

Of a total of 138 patients tested, 34 have developed recurrent disease at various intervals following primary surgery. The majority of these patients with recurrent cancer had a primary tumor with a Con A half-maximum value \( \leq 30 \mu g/ml \). For statistical analysis of these data, the population was arbitrarily divided into patients considered to have high- and low-reactivity tumors. Life table analysis of time to recurrence clearly demonstrated that those patients with tumors that were highly reactive with Con A were at a significantly greater risk of developing early recurrent cancer than were those patients with low-reactivity tumors.

It should be emphasized that a Con A value of 30 \( \mu g/ml \) probably does not represent a biologically meaningful discriminator of subcategories of breast cancer patients. Rather, it serves to operationally define the degree of reactivity for purposes of statistical analysis. Further study may reveal a more efficient cut-off value or one more specifically applicable to the properties of breast cancer.
Con A Reactivity and Human Breast Cancer Prognosis

Con A Reactivity and Human Breast Cancer Prognosis provides more accurate prognostic markers and a greater understanding of the metastatic process.

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