Correlative Study on Expression of CA 19-9 and DU-PAN-2 in Tumor Tissue and in Serum of Pancreatic Cancer Patients

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ABSTRACT

The serum levels of CA 19-9 and DU-PAN-2 antigens and their expression in tumor tissue were examined in 22 pancreatic cancer patients and the results were correlated with the Lewis (Le) blood group phenotypes of the individuals. In tumor tissue, CA 19-9 was expressed in 17 of 22 (77%) specimens. The negative cases included three patients with Le"-, one with Le"+ and the other with Le" phenotype. DU-PAN-2 antigen was expressed in 20 of 22 (91%) cancer tissues. The two DU-PAN-2-negative cases were CA 19-9-positive. The combination of two markers increased the sensitivity to 100%.

In the serum, CA 19-9 level was elevated (>37 U/ml) in 16 of 21 (76%) cases. All Le" patients had values <37 U/ml. An elevated level of DU-PAN-2 (>300 U/ml) was detected in 14 of 21 (67%) patients including three cases with Le" type. In only one patient were both antigens below the cutoff level so that the combination of these two markers elevated the sensitivity to 95%. The study indicated that the cocktail of 19-9 and DU-PAN-2 antibodies might increase the sensitivity and specificity for clinical diagnosis of pancreatic cancer.

In 19 of 21 (90%) cases, the serum CA 19-9 level correlated with the expression of the antigen in the cancer tissue. Discrepancy was seen in two cases; one patient had an elevated level of CA 19-9 in the serum, but lacked this antigen in the cancer cells. In the second case, the situation was reversed. For DU-PAN-2, positive correlation was seen in 14 of 21 (67%) cases. Six of seven patients with low DU-PAN-2 levels expressed the antigens in their tumor cells, and one patient with DU-PAN-2-negative cancer tissue had an elevated level of this marker in the serum. Thus, CA 19-9 expression in serum corresponded more closely to expression in tissue than did that of DU-PAN-2 antigen. The serum levels of these antigens, however, is likely due to multiple factors, only one of which is the qualitative and quantitative expression of the antigens in tumors.

INTRODUCTION

MoAbs 19-9 and DU-PAN-2 have been generated by hybridoma technology by immunizing mice with a colon cancer cell line (1) and a pancreatic cancer cell line (2), respectively. The antigens detected by these MoAbs, CA 19-9 and DU-PAN-2, have been found frequently in the sera and tumor cells of patients with pancreatic cancer (3-17). Both antigens have been detected in patients' sera, saliva, or ascites as a mucin molecule (18-21). The antigenic determinant of CA 19-9 has been found to be a sialylated Le^ blood group substance (22-24), whereas the epitope of DU-PAN-2 has yet to be defined.

In a previous study, we examined the expression of these two antigens in pancreatic cancer specimens and found that the combination of these two antigens elevated the sensitivity of detection in these cells to 97% (25). To examine whether or not a similar situation exists for the circulating antigens, we compared both the serum levels of these two markers and their expression in tumors of the same pancreatic cancer patients. The findings were correlated with Le phenotypes of the individuals, because in patients of Le" phenotype, CA 19-9 has been found not to be expressed either in their serum or cancer cells (19, 26).

MATERIALS AND METHODS

Specimen. Twenty-two clinically proven pancreatic cancer cases were examined. There were 11 males and 11 females with an average age of 61 years (range, 44-77) for males and 65 years (range, 38-82) for females. Blood samples were obtained by peripheral venipuncture after overnight fasting and centrifuged at 1000 x g for 30 min. Serum was pipetted into separate aliquots and stored at -70°C for assay of CA 19-9 and DU-PAN-2 antigens. Seventeen of 22 cancer tissues were from surgical biopsy material, four from fine-needle biopsy, and one from autopsy. When possible, several sections were taken from each tumor. The size of each piece of surgical and autopsy material was at least 10 mm in diameter. Tissues were fixed in Bouin's solution or 10% buffered formalin and processed for histology by conventional methods and by indirect immunoperoxidase procedures, as described below.

Lewis phenotype was determined from the saliva (17 patients) or the red blood cells of the patients (five patients). Fasting saliva samples were collected early in the morning, inactivated by heating for 10 min in a boiling water bath, and stored at -70°C until assay (see below).

Monoclonal Antibodies (MoAbs). MoAb 19-9 (1116 NS 19-9, IgG1 isotype) and MoAbs against Lewis blood group antigens were a gift of Dr. Steplewski, the Wistar Institute (Philadelphia, PA). 151-6-A7-9 (CO 51.4), an IgG3 isotype, is directed against Le^; 143-2-A6-11 (CO 43.1), an IgM isotype, is directed against Le^; 151-5-G3-5 (CO 51.3), an IgG3 isotype, is directed against Le^Le^ (27). MoAb DU-PAN-2 (lgM isotype) was generated by hybridoma technology as described (2). Immunochemistry Procedure. The indirect immunoperoxidase staining procedure was the same as described (25). In brief, reacted sections were incubated in 0.3% hydrogen peroxide in methanol for 30 min, washed in PBS, incubated with a 1:20 dilution of normal goat serum for 10 min, and subsequently incubated for 2 h at room temperature with each of the antibodies at a dilution of 1:5 (this concentration was found to be optimal for demonstration of either antigen with no background staining). After removing the unbound antibody with PBS washes, the sections were incubated with a 1:30 dilution of peroxidase-conjugated, affinity-purified goat anti-mouse IgG+M+A (Cappel Laboratories, Cochranville, PA) for 1 h at room temperature, and washed with PBS. The sections were then treated with 0.05% diaminobenzidine and 0.01% hydrogen peroxide in 0.05 M Tris buffer, pH 7.6, for 10 min, counterstained with hematoxylin, dehydrated, and mounted in Permount.

Scoring of Slides. The reactivity (%) of tumor cells within a given section with the antibody was scored as described (25) in the following categories: 0%, −; 5%, +; 10-30%, ++; 40-70%, +++; >70%, ++++. At least five different areas of each tumor were evaluated by 40× magnification and the average score was considered the representative value.

Radioimmunoassay. Serum CA 19-9 levels were determined by using a CA 19-9 radioimmunoassay kit (Centocor Co., Malvern, PA). A cutoff value of 37 U/ml was used for CA 19-9 (5). The DU-PAN-2 assay has been described (15). Briefly, a standardized amount of partially purified DU-PAN-2 antigen was bound to 96-well microtiter
plates. The serum sample to be tested was incubated with a predetermined amount of purified DU-PAN-2 MoAb. This mixture was then added to the DU-PAN-2 antigen-coated plates and further incubated. Free antibody was bound to the immobilized fixed antigen, while the DU-PAN-2 antigen antibody complex did not bind. The radiolabeled goat anti-mouse antibody was added to the wells, then incubated, and washed. The radioactivity of the mixture was determined in a \( \gamma \)-spectrophotometer. Inhibition due to DU-PAN-2 antigen in the patients’ sera was then compared with a standard antigen inhibition curve and the values of DU-PAN-2 antigen were expressed in arbitrary units. The cutoff value of 300 U/ml was chosen for DU-PAN-2 (15).

Determination of Lewis Phenotypes in Saliva. Le phenotypes expressed in saliva were examined by enzyme-linked immunosorbent assay with a slight modification of the method reported by Steplewski et al. (27).

Statistical Evaluation. The statistical evaluation of the results was carried out using the \( \chi^2 \) test. Linear regression analysis of the log value was used to correlate the data on serum antigen levels. The correlation coefficient was evaluated by Student’s \( t \) test.

RESULTS

Immunohistochemical staining patterns of pancreatic cancer tissues with MoAbs CA 19-9 and DU-PAN-2 have already been reported (25).

Table 1 summarizes the number of patients, their Le phenotype, and the immunohistochemical and serological results.

CA 19-9. Immunohistochemically, 17 out of 22 (77%) patients with pancreatic cancer expressed the antigen in the tumor tissues. In four out of five Le\(^ {a,b} \) patients, CA 19-9 was strongly expressed in the cancer cells, whereas in one case, the antigen could not be found. Thirteen out of 14 Le\(^ {a,b} \) patients expressed the antigen, whereas none of the Le\(^ {a} \) patients expressed the antigen in over 70% of the tumor cells and had a high concentration of the antigen in their sera; in the fourth case, tumor cells lacked the antigen but had a high concentration of the antigen in the serum.

A positive correlation between antigen expression in tissue and in serum was seen in 13 of 14 (93%) Le\(^ {a,b} \) cases. Taking all the patients into consideration, a positive correlation was seen in 100%.

Table 1 The relationship between the Lewis phenotypes and the expression of CA 19-9 and DU-PAN-2 in the tumor and in the sera of pancreatic cancer patients

<table>
<thead>
<tr>
<th>Lewis blood group types</th>
<th>CA 19-9</th>
<th>DU-PAN-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>Serum no. (%)</td>
</tr>
<tr>
<td>Le (a+, b-)</td>
<td>5</td>
<td>4 (80%)</td>
</tr>
<tr>
<td>Le (a-, b+)</td>
<td>14</td>
<td>13 (93)</td>
</tr>
<tr>
<td>Le (a+, b+)</td>
<td>3</td>
<td>0 (0)%</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>17 (77)</td>
</tr>
</tbody>
</table>

\(^ * \) In one patient, sample was not available.

DU-PAN-2. Twenty out of 22 (91%) cases expressed the antigen in the cancer tissues (Table 1). The intensity of the staining was slightly weaker than that of CA 19-9. There were two negative cases; one Le\(^ {a,b} \) and one Le\(^ {b} \). All immunohistochemically CA 19-9-negative cases expressed DU-PAN-2, so that testing for both antigens increased the sensitivity of detection in pancreatic tumors to 100%.

In 14 out of 21 (67%) patients, increased levels of DU-PAN-2 (>300 U/ml) were found in their sera. All Le\(^ {a,b} \), four out of five Le\(^ {a,b} \) and seven out of 13 Le\(^ {a} \) cases were serologically positive for DU-PAN-2. In Le\(^ {a} \) cases, the sensitivity seemed to be lower than that of other Le phenotypes; however, the difference was not statistically significant.

The relationship between the tissue distribution and serum level of DU-PAN-2 is shown in Fig. 2. A positive correlation was seen in 14 out of 21 (67%) cases. A discrepancy was observed in seven cases; one Le\(^ {a,b} \) and five Le\(^ {a,b} \) patients expressed the antigen in cancer tissues but had low serum levels, and one Le\(^ {a,b} \) subject exhibited the reverse situation.

The relationship between the serum levels of the two antigens is shown in Fig. 3. Four CA 19-9-negative cases were DU-PAN-2 positive and six DU-PAN-2-negative cases were CA 19-9-positive. Only one Le\(^ {a} \) patient was serologically negative for both antigens. Overall results demonstrated that 95% of the patients were serologically positive when both CA 19-9 and DU-PAN-2 are considered. The correlation coefficient of serum levels of these two antigens was 0.479 and \( P < 0.05 \).

DISCUSSION

CA 19-9 has been found in the sera and in the tumor tissues of a large number of pancreatic cancer patients (3–14). However, patients with Le\(^ {a,b} \) phenotype have been reported not to express this antigen (19, 26). DU-PAN-2, a mucin-like antigen, has been found in the sera of 68 to 72% of patients with pancreatic cancer (15, 17) and the relationship between the expression of this marker and the blood group type of the patients is not known.

As reported earlier (28), none of the Le\(^ {a,b} \) cases expressed Ca 19-9 neither in the sera nor in the tumor tissues, a finding...
which is in line with the results of others (15, 17). Since all the Le\(^{a-b}\) patients were positive for DU-PAN-2, both immunohistochemically and serologically, it follows that each antibody recognizes different epitopes of the antigen expressed in cancer cells. On the other hand, the correlation coefficient between the serum levels of DU-PAN-2 and CA 19-9 in the same patient showed a fairly good correlation (90%); a discrepancy was seen in only two cases. For DU-PAN-2, 91% of patients expressed the antigen in the tumor tissues, whereas only 67% of the patients had elevated serum antigen levels; a discrepancy was seen in seven cases. In this study, the cutoff value of the serum DU-PAN-2 level was set at 300 U/ml. If the value is set at 400 U/ml (15), the sensitivity of DU-PAN-2 for patients with pancreatic cancer will decrease to 52%.

DU-PAN-2 antigen was expressed more frequently in tumor tissue than in serum. This may be due to differences in the cellular localization of CA 19-9 and DU-PAN-2. CA 19-9 has been shown to be mainly localized on the luminal cell surface and luminal contents of malignant glands whereas DU-PAN-2 was often found within the cell cytoplasm (25). Another explanation for differing results could be that, in serum, the CA 19-9 epitope is expressed as a mucin but in cells it can be expressed as a glycolipid or a mucin (24). DU-PAN-2 has never been detected on a glycolipid (21).

The observed discrepancies in antigen expression in tissue and in serum could be due to the heterogeneity in the expression of these two antigens in tissues (25) and to the relatively small size of some of the sections examined. In fact, in two cases, one negative for CA 19-9 in the tissue, but positive in the serum and the second negative for DU-PAN-2 in the tissue, but positive in the serum, only fine-needle biopsy material was examined. Moreover, serum levels of the antigens could relate to the stage of disease or total amount of tumor cells (30). In fact, a negative correlation can exist when tumor antigens are released in a rhythmic or intermittent pattern.

Since both antigens are also expressed on nonmalignant ductal epithelial cells of the pancreatic and biliary systems (13, 14, 16, 25), these cells may also contribute to elevated serum levels. This normal cell source of the antigens is likely to be increased in patients with obstruction of these duct systems. Moreover, as with carcinoembryonic antigen, the serum levels of these large glycosylated molecules are also likely to be influenced by the liver function of the patient. Other factors which probably contribute to the serum levels of these markers are the extent of the blood and lymphatic supply to the individual tumors. Much of the antigen detected immunohistologically in closed malignant glands may fail to reach the lymphatics or the circulation. Therefore, immunohistochemical studies may reveal only a part of information relative to the antigen expression in the serum.

REFERENCES

6. Ritts, R. E., Jr., Del Villano, B. C., Go, V. L. W., Herberman, R. B., Klug, T. L., and Zurawski, V. R., Jr. Initial clinical evaluation of an immunoradi-
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