Blood Group-related Antigens in Human Kidney: Modulation of Lewis Determinants in Renal Cell Carcinoma


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ABSTRACT

Seven mouse monoclonal antibodies and the lectin Ulex europaeus, detecting blood group related antigens of the ABH and Lewis systems, have been used to determine the immunophenotype of human renal cell carcinomas. Immunohistochemical analyses have demonstrated that these antigenic systems are differentially expressed by distinct normal cell types and domains of the human nephron. In the present study we analyzed the immunophenotype of 29 primary and 15 metastatic renal carcinomas by the immunoperoxidase method. Blood type was known in all of the cases and secretor status in nine cases. ABH specificities were not detected in tumor cells of the primary tumors studied, although two of the metastases showed heterogeneous expression of H and A antigens, respectively. Lewis\(^{b}\) (Le\(^{b}\)\) determinant was detected in 76% of primary renal cell carcinomas; however, Le\(^{b}\) was only expressed by occasional cells in 20% of the metastatic tumors analyzed. Lewis\(^{a}\) (Le\(^{a}\)\) was detected with a heterogeneous pattern of expression in 31% of the primary and 26% of the metastatic renal tumors studied. Lewis\(^{b}\) (Le\(^{b}\)\) antigen expression was found in 17% of the primary and 20% of the metastatic tumors analyzed. Detection of precursor type 1 structure was observed in 28% of primary and 20% of metastatic renal cell carcinomas.

The present study suggests the histogenesis of renal cell carcinoma in the proximal nephron, based on the expression of Le\(^{b}\) and Le\(^{c}\) antigens. It also shows: (a) an apparent deletion, downregulation or structural modification of Le\(^{b}\) determinant in most of the metastatic tumors; (b) undetectable levels of ABH specificities in tumor cells of primary renal cell carcinoma; and (c) enhanced expression and/or neosynthesis of precursor type 1 structure and Le\(^{b}\) determinant in some renal cell carcinomas.

INTRODUCTION

Antigenic determinants of the A, B, H, and Lewis blood group-related molecules are carbohydrate structures carried on both lipids and proteins (1-3). Initially found on erythrocytes (4-6), they have also been described on certain epithelial cells and in body fluids, and have been mapped in normal fetal and adult tissues (7-10). Using antibodies detecting blood group specificities, distinct immunophenotypes were assigned to normal cell types and domains within the nephron (11-16). Secretor status has also been shown to influence the expression of these blood group related antigens in tissues (3, 17). Interest in these antigens as differentiation markers has increased in recent years because of modifications observed during transformation, including simplification of mature structures, accumulation of precursor molecules, deletion of A/B/H antigens, abnormal A/B specificities, and enhanced or modified expression of Lewis determinants in certain tumors (18-25). We now report on the expression of these antigens in a series of 29 primary and 15 metastatic renal cortical carcinomas using a well characterized panel of antibodies and the lectin Ulex europaeus detecting precursor type 1 chain, H, A, B, Le\(^{a}\), Le\(^{b}\), Le\(^{c}\), and Le\(^{d}\) blood group related antigens.

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2 The abbreviations used are: PBS, phosphate buffered saline; mAb, monoclonal antibody; SSEA-1, stage-specific embryonic antigen-1.

MATERIALS AND METHODS

Tissues

Normal and neoplastic tissues were obtained from surgical specimens within 1-2 h of resection. Fresh tissues were fixed overnight in 10% formaldehyde in phosphate buffered saline, pH 7.5 (PBS,\(^{2}\) Difco Laboratories, Detroit, MI), and embedded in paraffin. Additional tissue from some cases was snap-frozen in isopentane precooled in liquid nitrogen, embedded in OCT compound (Miles Laboratories Inc., Naperville, IL), and stored at -70°C until needed.

Radical nephrectomy specimens from 29 patients with primary renal cortical carcinoma, and metastatic renal carcinomas from 15 patients were obtained for the present study (39 different individuals representing 44 specimens). In the majority of primary tumors we were able to examine adjacent normal kidney. Primary and metastatic tumors from the same individual were available for study in four cases. In seven cases we were able to compare immunoreactivities on frozen and paraffin-embedded tissue sections of the same tumor. Selection of cases was based on excellence of tissue preservation. All tumors were classified according to histologic pattern(s) and cell type(s). The patients included 13 group O, 18 group A, seven group B, and one group AB individuals. Secretor status was determined in nine cases by either presence of Lewis antigens in saliva and/or on red blood cells (10, 24). The blood group and secretor status of the patients from whom the specimens were derived were correlated with the immunohistologic patterns of reactivity.

Reagents

Purified agglutinin I from Ulex europaeus at 4 \(\mu g/mL\) (Vector Laboratories, Burlingame, CA) served to identify the H-antigen. Mouse mAb HT 29-36 (T-36) recognizes A antigen (all variants), mAb S8 detects B-antigen, mAbs T-174, T-218, P-12 and F-3 have specificities for Le\(^{a}\), Le\(^{b}\), Le\(^{c}\), and Le\(^{d}\) antigens, respectively. Finally, mAb K-21 detects precursor type 1 chain antigen (Table 1). The antibodies were used as undiluted culture supernatants, or purified immunoglobulin preparation at an approximate concentration of 25-40 \(\mu g/mL\) (17, 24).

Immunohistochemistry

The method chosen for the present analysis was the avidin-biotin complex immunoperoxidase technique (17, 24, 26). Formalin-fixed and paraffin-embedded tissue sections were deparaffinized, then treated for 30 min in 1% hydrogen peroxide in PBS to remove endogenous peroxidase activity. Frozen tissue sections were fixed in cold acetone for 10 min and treated with 0.3% hydrogen peroxide for 15 min. Sections were washed in PBS and then incubated with the blocking serum for 20 min. Blocking serum was drained off and deparaffinized sections were incubated with mAb overnight at 4°C, whereas frozen sections were incubated for 1 h at room temperature. The secondary antibodies, biotinylated horse anti-mouse IgG or goat anti-mouse IgM (Vector Laboratories, Burlingame, CA), were incubated on both deparaffinized and frozen sections for 1 h and then washed and incubated with the avidin-biotin complex for 30 min. The peroxidase reaction was performed by incubating tissue sections for 6 to 12 min with 5 mg of diaminobenzidine tetrahydrochloride (Sigma Chemicals, St. Louis, MO) in 100 mL of tris buffer containing 100 \(\mu L\) of 0.3% hydrogen peroxide. Sections were washed with distilled water, counterstained with hematoxylin, and mounted with permount.

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RESULTS

Table 1 summarizes the derivation of the panel of antibodies, their immunoglobulin subtype, and their specificity for blood group related antigens. Tables 2 and 3 summarize the clinical information and immunoreactivities of these antibodies on sections of renal tumors, correlating blood type and secretor status of the individuals as well as histological pattern(s) and cell type(s) of the tumors. In seven cases we were able to compare the immunostaining patterns in formalin-fixed paraffin-embedded and frozen tissue sections of the same tumor. In nine cases secretor status was determined and correlated with tumor immunophenotype.

Blood Group Antigen Expression in Normal Adult Kidney. As previously reported, antibodies detecting blood group specificities were differently expressed in the human nephron of secretor and nonsecretor individuals (17).

Glomerular tufts were immunoreactive for A/B/H antigens in endothelial cells, according to the blood group type of the individuals, regardless of secretor status. Parietal and visceral epithelial cells and mesangial cells of glomeruli were unreactive for all antibodies tested.

Epithelial cells of proximal convoluted tubules in secretor individuals expressed the Le" determinant. Epithelial cells of Henle's loop showed marked reactivity for Le' determinant, with patchy expression of precursor type 1 chain, Le" and Le' antigens in some secretors. Nonsecretors expressed only Le" determinant, while precursor type 1 and other Lewis determinants were not detected in the proximal domain of the nephron.

Collecting ducts in secretor individuals showed a gradient of increasing staining of the epithelial cells towards the deep medulla for A/B/H antigens, as well as for Le" and Le' determinants. However, epithelial cells within collecting ducts in nonsecretors showed complete lack of immunoreactivity for these determinants. Precursor type 1 antigen was heterogeneously expressed on epithelial cells of collecting ducts, regardless of secretor status.

Blood Group Antigen Expression in Primary Renal Carcinoma. Precursor type 1 chain was detected in eight of 29 cases with a heterogeneous or focal staining pattern of reactivity (Fig. 2, D and F), showing strong immunostaining in only one case.

A, B, and/or H specificities were not detected in any of the primary tumors studied, regardless of histological pattern of the tumor or secretor status of the individual (Fig. 1, A, D, G). Anomalous expression of A and/or B antigens was not observed in the present study. Endothelial cells and erythrocytes in the tumors were immunostained with the appropriate antibodies, regardless of secretor status.

Lewis was found in nine of 29 cases, with a heterogeneous pattern of staining. These tumors were all acinotubular and papillary. The pattern of reactivity was patchy with strong cytoplasmic staining and marked luminal positivity (Fig. 1, B and E). Secretor status did not influence the results. The two sarcomatoid tumors were unreactive for Le" (Fig. 1H). Expression of Le" was only found in one primary tumor, with heterogeneous immunoreactivity (20-30% positive tumor cells) (Table 2, Case 15). None of the metastases studied showed positive reactivity for Le" determinant.

Lewis was determined was expressed in 22 of 29 cases. 14 showed strong and homogeneous immunoreactivity (Fig. 1, C and F); the others showed either heterogeneous staining (four cases) (Fig. 2E) or focal staining with 5-10% tumor cells being immunoreactive (four cases) (Fig. 2C). Both acinotubular and papillary tumors showed variable reactivities; however, sarcomatoid tumors were unreactive for Le" antigen (Fig. 1I). Secretor status did not influence the expression of Le" in tumor cells. In some cases where patchy staining was noted clear cells showed diffuse staining (Fig. 2A) whereas granular cells were unreactive (Fig. 2B) with P12 antibody (Table 2, Case 6). Le" determinant was absent in the majority of the cases analyzed. It was detected in four of 29 cases with patchy staining; two of these cases showed only a few immunoreactive tumor cells, with punctate, cytoplasmic staining pattern.

Blood Group Antigen Expression in Metastatic Renal Carcinomas. 15 metastatic renal carcinomas were analyzed for the present study. In five cases we were able to compare the primary and metastatic tumors from the same patients; one of them (Table 2, Case 15) had two metastases at different sites (Table 3, Cases 7 and 8).

Precursor type 1 chain was found in three of 15 metastatic tumors, but with only occasional cells staining variably. In general, A, B, and/or H specificities were not detected (13 of 15 cases). However, one of these metastatic tumors (Table 3, Case 11) showed heterogeneous expression of both A and H antigens in approximately 10-20% of tumor cells, while another tumor (Table 3, case 9) showed patchy staining for H specificity in approximately 50% of tumor cells (Fig. 1J).

Lewis antigen was expressed in four of 15 metastases with patchy immunostaining; the remaining 11 cases were unreactive (Fig. 1K). Lewis" was undetected in all metastatic tumors studied. Lewis" determinant was expressed in occasional cells of three metastatic tumors. The remaining cases were unreactive with anti-X antibody, regardless of histological pattern and cell type of the tumor (Figs. 1L and 2G). Lewis" determinant was detected in three of 15 cases. One of these showed diffuse

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Table 1: Derivation and specificity of mouse monoclonal antibodies identifying blood group-related antigens

| Antibody (immunoglobulin subclass) | Immunizing cell type | Blood group specificity* | CRL designation#
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>K21 (IgM)</td>
<td>Tera-1</td>
<td>Precursor 1</td>
<td>BG1</td>
</tr>
<tr>
<td>T36 (IgG)</td>
<td>HT-29</td>
<td>A</td>
<td>BG2</td>
</tr>
<tr>
<td>S8 (IgM)</td>
<td>SK-RC-7</td>
<td>B</td>
<td>BG3</td>
</tr>
<tr>
<td>T174 (IgG)</td>
<td>SK-CO-10</td>
<td>Le&quot;</td>
<td>BG5</td>
</tr>
<tr>
<td>T218 (IgM)</td>
<td>SK-CO-10</td>
<td>Le&quot;</td>
<td>BG6</td>
</tr>
<tr>
<td>P12 (IgM)</td>
<td>Human placenta</td>
<td>Le&quot;</td>
<td>BG7</td>
</tr>
<tr>
<td>F3 (IgM)</td>
<td>SK-LU-3</td>
<td>Le&quot;</td>
<td>BG8</td>
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* The antigenic determinant recognized by each monoclonal antibody is cited elsewhere (22, 25, 31).
# Antibodies are available from Cambridge Research Laboratories (CRL, Cambridge, MA). Purified antiglobulin I from Ulex europaeus (Vector Laboratories, Burlingame, CA) served to identify the H antigen.

**Table 2**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Immunizing cell source</th>
<th>Blood group specificity</th>
<th>CRL designation</th>
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<tr>
<td>SK-LU-3</td>
<td>Human placenta</td>
<td>Le&quot;</td>
<td>BG1</td>
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<td>Le&quot;</td>
<td>BG2</td>
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<td>SK-RC-7</td>
<td>Human placenta</td>
<td>Le&quot;</td>
<td>BG3</td>
</tr>
<tr>
<td>Tera-1</td>
<td>Human placenta</td>
<td>Le&quot;</td>
<td>BG4</td>
</tr>
<tr>
<td>HT-29</td>
<td>Human placenta</td>
<td>Le&quot;</td>
<td>BG5</td>
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<td>SK-CO-10</td>
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<td>BG6</td>
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<tr>
<td>SK-LU-3</td>
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<td>Le&quot;</td>
<td>BG7</td>
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<tr>
<td>SK-RC-7</td>
<td>Human placenta</td>
<td>Le&quot;</td>
<td>BG8</td>
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**Table 3**

<table>
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<tr>
<th>Antibody</th>
<th>Immunizing cell source</th>
<th>Blood group specificity</th>
<th>CRL designation</th>
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<tbody>
<tr>
<td>SK-LU-3</td>
<td>Human placenta</td>
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<td>BG7</td>
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<tr>
<td>SK-RC-7</td>
<td>Human placenta</td>
<td>Le&quot;</td>
<td>BG8</td>
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immunoreactivity (Fig. 2H), while the other two contained only occasional tumor cells that were reactive.

In five cases we studied the primary and metastatic lesions from the same patient. Four of five primary renal carcinomas had clear and/or granular cell features and expressed Le⁺ antigen with strong and homogeneous immunoreactivity. However, three metastases were unreactive and two showed staining in only a few tumor cells (less than 5%) with mAb P12. The fifth case had spindle cell features and showed no immunostaining in both primary and metastatic tumor cells.

Lewis⁺ Antigen Expression in Normal Kidney and Renal Carcinomas after Neuraminidase Treatment. Expression of Le⁺ antigen on normal kidney specimens showed a heterogeneous immunoreactivity with variable staining intensity on the proximal tubules, as well as on the descending and thin loop of Henle. After neuraminidase treatment, enhanced immunoreactivity and a more uniform, homogeneous staining pattern was observed on the proximal tubules. Lewis⁺ immunoreactivity detected along the thin loop was not altered after enzyme digestion; distal tubules and collecting ducts remained negative.

Neuraminidase treatment of primary and metastatic lesions, either unreactive or heterogeneously expressing Le⁺ determinant, revealed no qualitative changes in immunostaining pattern; however, increased staining intensity in heterogeneously Le⁺-positive areas was observed following enzyme digestion. These results cannot distinguish between sialylation of the Le⁺ antigen or masking of the Le⁺ determinant by other sialylated carbohydrate structures in the heterogeneous areas. Unreactive lesions remained negative after neuraminidase digestion.

Comparative Analysis of Formalin-fixed, Paraffin-embedded, and Frozen Tissue Sections. Determinants of the A, B, H, and Lewis blood group related antigens are carbohydrate structures carried on both glycoproteins and glycolipids. Since these determinants may reside on lipid moieties, processing of tissues for paraffin sections could alter the antigenic determinants by: solubilization through ethanol immersion, masking due to for-
Fig. 1. Localization of blood group related H, Lewis1, and Lewis* antigens in primary renal cortical carcinomas (acinar, A–C; papillary, D–F; sarcomatoid, G–I) and a metastatic renal carcinoma (J–L). A, H blood group antigen expressed in normal endothelial cells but not detected in tumor cells; B, Lewis1 antigen heterogeneously expressed in tumor cells; C, Lewis1 antigen homogeneously expressed in tumor cells; D, detection of H antigen in endothelial cells and absence in tumor cells; E, Lewis1 antigen heterogeneously expressed in tumor cells; F, localization of Lewis1 antigen in tumor cells; G, H antigen expressed in endothelial cells but not detected in tumor cells of a sarcomatoid renal cortical carcinoma; H, Lewis1 antigen not detected in a sarcomatoid renal cortical carcinoma; I, Lewis1 antigen not detected in a sarcomatoid renal cortical carcinoma; J, H antigen heterogeneously expressed by the majority of tumor cells in a metastatic renal carcinoma; K, Lewis1 antigen not detected in a metastatic renal carcinoma; L, Lewis1 antigen not detected in a metastatic renal carcinoma. (x 200)

We compared frozen and deparaffinized tissue sections from the same tumors in seven cases (Tables 2 and 3: primary tumors, 4, 7, 11, 13, 15; metastatic tumors, 8, 15). Both Le1-positive and Le1-negative tumors were examined and no differences were observed. Since paraffin embedding and deparaffinization involve use of ethanol, it is likely that the blood group-related determinants that remain to be stained by this panel of antibodies are on glycoproteins (mucins). These structures are probably the dominant forms of blood group-related antigens in normal kidney and renal cell carcinoma.
DISCUSSION

The expression and/or modulation of blood group-related antigens in the human urinary tract has been extensively discussed (11–17). However, most of the studies concerned either normal kidney development or changes in blood group antigens in neoplastic urothelium. These studies revealed deletion of A, B and/or H antigens in transitional cell carcinomas (18–20, 25). They also showed correlation of abnormal patterns of Lewis determinants with histological grade of urothelial carcinomas (21, 23).

Previous studies on the expression of A, B, and H antigens in kidney tumors produced conflicting results. Sarodsky et al. (32) analyzed the expression of A, B, and H specificities in 42 renal carcinomas by the red cell adherence test on tissue sections. They reported that agglutination was observed in 39 of the 42 cases studied, with only three tumors being negative. More recently, Ghazizadeh et al. (33) analyzed the expression of A, B, and H specificities, as well as T-antigen and carcinoembryonic antigen, in 30 cases of renal carcinoma by immunohistochemical methods. They reported that none of the 30 tumors studied showed immunoreactivity for A, B and/or H. Our data agree with that of Ghazizadeh and collaborators since none of the 29 primary tumors showed positive staining for either A, B, or H antigens. Differences in results may be due to the enhanced specificity of immunohistochemistry over the red cell adherence test (34, 35), or to the difficulty in interpreting the site of red cell agglutination in highly vascularized renal tumors.
Expression of Le\textsuperscript{a} and Le\textsuperscript{b} determinants was analyzed in eight renal carcinomas by Ernst \textit{et al.} (12) using immunoperoxidase techniques on paraffin-embedded tissue sections. They reported detection of Le\textsuperscript{a} in six of eight cases; Le\textsuperscript{b} was undetected in all their cases. Similarly, we have identified Le\textsuperscript{a} expression in a subset of our cases (nine of 29 primary tumors), and lack of expression of Le\textsuperscript{b} determinant in the majority of tumors. Only one of the primary renal cell carcinomas showed heterogeneous immunoreactivity for Le\textsuperscript{b} determinant in our study. In this particular case, we were able to analyze frozen and deparaffinized tissue sections, observing similar patterns of staining and the same immunophenotype in both situations.

Expression of Le\textsuperscript{b} was demonstrated in 12 of 19 primary renal carcinomas by Liebert \textit{et al.} (36) using an antibody detecting SSEA-1. Anti-SSEA-1 antibody is directed against the carbohydrate determinant as anti-Le\textsuperscript{a} antibodies (lacto-N-fucopentaose III) (37, 38). Expression of 3-fucosylated-N-acetyllactosamine carbohydrate determinants was reported in 12 of 30 renal carcinomas by Fleming \textit{et al.} (39) in a recent study. The two antibodies used, AGF 4.36 and AGF 4.48, detect epitopes on the Le\textsuperscript{a} hapten. Our data on renal tumors showed expression of Le\textsuperscript{a} in 22 of 29 primary (76%) and in only three metastatic tumors. We have observed a similar degree of tumor heterogeneity in the staining pattern to that reported for anti-SSEA-1. In addition, in our study sarcomatoid tumors were found to be unreactive for mAb P12. Variations observed among these studies could be explained by differences in the fine specificities of the antibodies used for the Le\textsuperscript{a} structure.

Lewis\textsuperscript{a} determinant was observed in normal human kidney of both secretor and nonsecretor individuals in epithelial cells of proximal tubules and segments of Henle's loop (17). We observed that the majority of primary renal carcinomas express Le\textsuperscript{a} and to a lesser degree Le\textsuperscript{b} determinants, regardless of secretor status. Moreover, ABH specificities, which are known markers of the distal nephron and absent in the proximal tubules, were not detected in tumor cells of renal carcinomas. These findings are consistent with suggestions that renal carcinoma arises from the proximal nephron (40–43). However, most of the metastatic tumors showed low expression or absence of Le\textsuperscript{a} determinant. Consequently, deletion, down-regulation, or structural modifications of Le\textsuperscript{a} determinant (e.g., sialylation) may be regarded as possible markers for poor prognosis in renal cell carcinoma (2). Intriguing as these observations may be, more cases with clinicopathological correlation must be analyzed in order to draw firm conclusions.

Even though most of the antigenic markers of the distal nephron were undetected in renal carcinoma, we have identified precursor type 1 chain and/or Le\textsuperscript{a} determinant in a subset of renal tumors. This is not surprising since increased expression of blood group precursor structures have been previously reported in other types of epithelial tumors and their derived cell lines, especially gastrointestinal tumors (24, 31). Since these molecules are the backbone structure for the synthesis of other blood group-related antigens, accumulation of precursor may be due to inactivation of glycosyltransferases and/or down-regulation of fucosyltransferases. Enhanced expression and neosynthesis of Le\textsuperscript{a} determinant has also been reported in studies of gastrointestinal tumors (31, 44), but it is not a significant occurrence in kidney tumors. Modulation and neosynthesis of Lewis antigens, including Le\textsuperscript{a} and Le\textsuperscript{b} determinants, in tumor cells may be due to activation of suppressed genes (i.e., \textit{Hh} and \textit{Se}) and/or upregulation of fucosyltransferases. Activation of these enzymes has been previously reported in other epithelial tumors (44, 45), and their modulation related to possible oncogene-coded products (2).

Differentiation in renal carcinoma may be assessed by histological pattern, cell type, or nuclear grading (46, 47). Immunophenotypic characteristics which are indicators of malignant transformation (19, 20, 25, 41–43, 48, 49) may also reflect tumor cell behavior. In a recent study of renal tumors, for example, Ulrich and coinvestigators correlated histological growth pattern, nuclear grade, and lectin-binding. They showed that nine of 10 low grade renal carcinomas bind \textit{Lotus tetragonolobus} agglutinin, but none of eight poorly differentiated tumors showed binding activity for \textit{Lotus tetragonolobus} agglutinin (43). Similarly, detection of SSEA-1 (Le\textsuperscript{a}) antigen was enhanced in renal cell carcinomas of the clear cell type, but a sarcomatoid tumor was negative (36). In the present analysis, we have examined histological pattern and predominant cell types, and observed the expression of Le\textsuperscript{a} determinant in 76% of the primary renal carcinomas, with a heterogeneous pattern in some cases. However, sarcomatoid tumors and 80% of the metastatic tumors studied were found to be unreactive.

The variable pattern of Le\textsuperscript{a}-antigen reactivity observed between primary tumors of the same morphological type and within lesions of some individual tumor specimens could result from structural modifications or masking of the Le\textsuperscript{a} epitope. For example, Fukushi \textit{et al.} (50) showed that well differentiated renal carcinomas had a higher incidence of positive staining for polyfucosylated and sialylated Le\textsuperscript{a}-determinants than did undifferentiated tumor cells. In the present study, primary and metastatic lesions from the same patient were examined in five cases. Lewis\textsuperscript{a} antigen was not expressed in the metastatic lesions from three cases. One case, an acinotubular lesion with predominantly clear cell features, showed Le\textsuperscript{a} antigen reactivity in the primary tumor and in one of two metastases with a heterogeneous staining pattern. The remaining case, a sarcomatoid tumor, failed to express Le\textsuperscript{a} antigen in both primary and metastatic tumors. Neurominidase treatment of primary and metastatic lesions, either unreactive or heterogeneously expressing Le\textsuperscript{a} determinant, revealed minor changes in some immunostaining patterns; these were, mainly, increased staining intensity and homogeneous staining in heterogeneous Le\textsuperscript{a}-positive areas after neurominidase digestion. However, unreactive lesions remained negative after neurominidase treatment. These preliminary results cannot distinguish between sialylation of the Le\textsuperscript{a} antigen or masking of the Le\textsuperscript{a}-determinant by other sialylated carbohydrate structures in the heterogeneous staining areas.

This study yields additional evidence favoring the origin of renal cell carcinoma from the proximal nephron. The apparent loss of Le\textsuperscript{a} antigen reactivities found between primary and metastatic tumors is significant (24% primary versus 80% metastatic) and may be clinically relevant. The absence of Le\textsuperscript{a} antigen in metastatic and some primary tumors may represent an obstacle to clinical applications using monoclonal antibodies for immunolocalization. However, our data points toward the possibility of an immunophenotypic grading system potentially capable of predicting tumor cell behavior or response to therapy. Further analyses to correlate the immunophenotype and clinicopathological data in a large series of patients with renal tumors are presently underway.

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REFERENCES


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