Analysis of a Mouse Monoclonal Antibody That Reacts with a Specific Region of the Human Proximal Tubule and Subsets Renal Cell Carcinomas


ABSTRACT

Murine monoclonal antibody (mAb) F31 detects a heat-stable antigen (URO-8) found in the acidic lipid fraction of renal cancer cell extracts. Serological analysis of mAb F31 reactivity was assayed on 176 human cell lines. mAb F31 reacted with 35 of 45 renal cancers, a subpopulation of cells in primary cultures of normal renal epithelia, and two of 13 colon, two of 15 lung, and four of five ovarian cancers. No other epithelial, hematopoietic, or neuroectodermal cell lines tested were reactive.

Immunofluorescence and immunoperoxidase analyses of fresh-frozen tissue sections revealed mAb F31 reactivity in kidney, gastrointestinal tract, biliary canaliculi, bronchial epithelium, and skin. Within the kidney, mAb F31 immunoreactivity was confined to the straight portion of the proximal tubule. A panel composed of previously characterized mAbs as well as mAb F31 defines the antigenic phenotype of proximal convoluted tubular cells as URO-2+/URO-3+/URO-4+/URO-10+/URO-8+; proximal straight tubular cells as URO-2+/URO-3+/URO-4+/URO-10+/URO-8+/URO-5++; and cells of the descending thin limb of Henle as URO-2+/URO-3+/URO-4+/URO-10+/URO-8+/URO-5++. While adult proximal tubular cells demonstrated reciprocal expression of URO-8 and URO-10, fetal kidney proximal tubule progenitor cells coexpressed both antigens (URO-10+/URO-8+).

Fifty renal cancer specimens were typed with these antibodies. Fourteen cases were URO-10+/URO-8+, ten cases were URO-10+/URO-8−, and 25 cases expressed both antigens (URO-10+/URO-8+). These phenotypes are consistent with derivation of these subset cells from the proximal convoluted tubule, the pars recta, or a proximal tubule progenitor cell, respectively. Only one specimen failed to express either URO-8 or URO-10.

INTRODUCTION

Historically, the histogenesis of renal cancer has been controversial (1). Grawitz (2), in 1883, designated these cancers as "hypernephromas" as he believed they derived from the adrenal gland. However, progressive technological advances in the last 35 years have provided increasing precision in determining the actual site of origin of these cancers. Studies utilizing electron microscopy (3–5), conventional heteroimmune sera (6), and, more recently, monoclonal antibodies (7–9) have provided evidence that renal cancers originate from cells of the proximal tubule. In previous studies we described a panel of murine mAbs (4) which identified differentiation antigens of the normal human kidney and used these mAbs to analyze the antigenic phenotypes of renal cancer (7, 10–13). This work demonstrated that kidney cancers express proximal tubular differentiation antigens consistent with their derivation from the proximal tubule. In this paper, we describe a new antibody, mAb F31, which defines the "URO-8" antigen. When studied in conjunction with the previously characterized mAbs, mAb F31 identifies a specific segment of the proximal tubule and defines major subsets of renal cell carcinomas.

MATERIALS AND METHODS

Generation and Production of mAbs. mAbs S4, F23, S27, T43, and T16 have been previously characterized (7, 10–13). mAb F31 was raised by immunizing a BALB/c × C57BL/6 F1 mouse with a cell suspension of SK-RC-1, an established human renal cancer cell line. The hybridoma producing mAb F31 was subcloned 4 times and transplanted to Pristane-primed syngeneic mice for the production of ascitic fluid.

Serological Analysis of Cultured Cell Types and Tissue Specimens. The specificity of mAb F31 was defined using established human cancer cell lines and primary cultures of normal human kidney epithelium and skin fibroblasts. The rabbit anti-mouse immunoglobulin MHA (10) and the indirect immunofluorescence assay (7) were used for the analysis of cultured cells. Normal tissues and tumors were obtained from surgical pathology and/or postmortem specimens. Each tissue specimen was snap-frozen in an isopentane-dry ice mixture and stored at —80°C as described (12). Cryostat tissue sections (6 μm) were fixed in cold acetone or 3.7% formaldehyde and tested by indirect immunofluorescence and immunoperoxidase (indirect and avidin-biotin complex) assays as reported (12–15).

In order to define areas of mutually exclusive or overlapping expression of antigens, two double-immunofluorescence staining techniques were used. As mAb F31 is an IgM and the other mAbs in Table 1 are of the IgG class, secondary antibodies specific for either IgM or IgG and conjugated with different fluorochromes could be used. mAb F31 was detected with rhodamine-conjugated goat anti-mouse IgM, α-chain specific (Cappel Laboratories, Cochranville, PA), and the other antibodies were detected with fluorescein-conjugated goat anti-mouse IgG, Fc fragment specific (Cappel). Briefly, two mAbs were incubated simultaneously on tissue sections for 60 min. After washing with 0.01 m phosphate, 0.15 m NaCl, pH 7.2 (PBS), the two conjugated secondary antibodies were mixed (1:40 final dilution of each reagent) and incubated on the sections for 60 min. After another PBS wash, sections were mounted in 90% glycerol in PBS and examined with a microscope equipped with epifluorescence and appropriate filters. A second double-immunofluorescence technique was used to compare staining reactivities of two IgG mAbs on the same section. In this case, mAb F23 was directly fluoresceinlabeled (16). Tissue sections were incubated first with an unlabeled mAb followed by rhodamine-conjugated goat anti-mouse IgG (Cappel). After PBS washes, excess unbound anti-mouse IgG sites were saturated with a second incubation of the same mAb used initially. Sections were washed with PBS and then incubated with fluorescein-conjugated mAb F23 for 60 min. After washing, the sections were mounted and examined. Negative controls consisted of NS-1 culture supernatant or mAbs not reactive with kidney sections but of the appropriate class and subclass. Photographs were produced by either a single or double exposure technique. In cases where a given field demonstrated a mutually exclusive staining pattern, a single film was exposed sequentially through the two respective filters. Where a field demonstrated structure(s) which bound both antibodies and their respective fluorescent labels, two separate single exposure photomicrographs were produced.

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The abbreviations used are: mAb, monoclonal antibody; MHA, mixed hemadsorption assay; PBS, phosphate-buffered saline.

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For the analysis of glycolipid antigens, direct enzyme-linked immunosorbent assay tests were used for purified glycolipids (17) and inhibition tests (18, 19) for crude chloroform-methanol lipid extracts.

### Table 1. Derivation and characterization of mouse monoclonal antibodies detecting cell surface antigens of human renal cancer

<table>
<thead>
<tr>
<th>Designation of antibody* (immunoglobulin subclass)</th>
<th>Designation of antigen</th>
<th>Biochemical characterization of antigen*</th>
<th>Refs.</th>
</tr>
</thead>
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<tr>
<td>mAb F31 (μ)</td>
<td>URO-8</td>
<td>Heat-stable lipid</td>
<td></td>
</tr>
<tr>
<td>mAb S4 (γ2a)</td>
<td>M, 160,000 glycoprotein</td>
<td>7, 10, 12, 13</td>
<td></td>
</tr>
<tr>
<td>mAb F23 (γ2a)</td>
<td>M, 140,000 glycoprotein</td>
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<td>mAb S27 (γ1)</td>
<td>M, 120,000 glycoprotein</td>
<td>7, 10, 12, 13</td>
<td></td>
</tr>
<tr>
<td>mAb T43 (γ1)</td>
<td>M, 125,000 glycoprotein</td>
<td>11-13</td>
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</tr>
<tr>
<td>mAb T16 (γ2b)</td>
<td>M, 48,000 glycoprotein</td>
<td>11-13</td>
<td></td>
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</table>

*Monoclonal antibodies identifying the “URO” antigens are available from Signet Laboratories, Dedham, MA.

*Antigen immunoprecipitated from [3H]g lysine- or [35S]methionine-labeled cell lysate under reducing conditions.

### RESULTS

Serological Analysis of the URO-8 Antigen on Cultured Cell Types. Rabbit anti-mouse immunoglobulin mixed hemadsorption and indirect immunofluorescence assays were used to detect cell surface expression of the antigen detected by mAb F31. Fig. 1 shows the results of these tests on 176 human cell lines. Thirty-eight of 45 (84%) renal cancer lines expressed the URO-8 antigen with titers of $10^{-3}$ to $10^{-5}$. Short-term cultures of normal renal epithelia, consisting predominantly of proximal tubular cells (25, 26), contained a subpopulation of approximately 10 to 15% URO-8+ cells. All other epithelial, hematopoietic and neuroectodermal cell lines were URO-8 by the anti-mouse immunoglobulin MHA. However, a few cell lines of colon, lung, and ovarian origin showed reactivity by the immunofluorescence assay.

Serological Analysis of URO-8 Antigen on Normal Tissue Specimens. A wide range of fetal and normal adult tissues were studied with mAb F31. Reactivity was found in selected areas of the nephron, epithelial cells of the small intestine and colon, luminal surface of biliary canalicular and bronchial epithelium, skin epidermis (including the hair follicle and sweat glands but not sebaceous glands), as well as the zona granulosa of fetal ovary and seminiferous tubules of the testis (Fig. 2). In the normal adult kidney, visualization of immunoperoxidase-stained sections using low magnification reveals a narrow band of staining at the juxta-medullary zone (Fig. 3). Microscopic examination demonstrates that the reactive cells are found in the pars recta of the proximal tubule. In 12- to 18-wk fetal...
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Fig. 3. Immunoperoxidase staining of serial sections of normal human kidney. In a, mAb F23 reactivity is demonstrated throughout the length of the proximal tubule from glomerulus (G) to the medullary border. In b, mAb F31 reactivity is limited to the juxtamedullary area comprising the terminal straight portion of the proximal tubule and a short segment of Henle’s loop. The proximal convoluted tubules in the cortex show no reactivity. v, vascular channel.

kidney sections, epithelial cells of the developing proximal tubule are URO-8-. The thin and ascending limbs of Henle’s loop are nonreactive with mAb F31 in both fetal and adult specimens. In order to define the site of URO-8+ cells in relation to other nephron antigens, double labeling experiments were performed (Figs. 4 and 5). In the adult, these studies showed that the proximal nephron is composed of cells with 3 distinct antigenic phenotypes: proximal convoluted tubular cells are URO-2+/URO-3+/URO-4+/URO-10+/URO-8-/URO-5-; cells of the terminal straight portion of the proximal tubule are URO-2+/URO-3+/URO-4+/URO-10-/URO-8+/URO-5-; and cells of the thin limb of Henle’s loop are URO-2-/URO-3-or-/URO-4+/URO-10-/URO-8+/URO-5+ (Fig. 6). Expression of URO-8 and URO-10 antigens appears to define mutually exclusive segments of the proximal tubule in the adult kidney. Study of fetal kidney sections using the double labeling technique demonstrated that fetal proximal tubular cells coexpress both the URO-8 and URO-10 antigens. To our knowledge, no previously defined nephron marker has demonstrated a distribution similar to the URO-8 antigen.

Serological Analysis of URO-8 Antigen on Human Cancer Specimens. Fifty unselected specimens of primary renal cell carcinoma were examined for URO-8 expression. Seventy % of cases (35 of 50) were URO-8+. Study of these specimens for both URO-8 and URO-10 expression (data not shown) demonstrated that 10 cases were URO-8+/URO-10-, 14 cases were URO-8-/URO-10+, 25 cases coexpressed both antigens, and only one case failed to express either antigen. A homogeneous staining pattern was observed in almost all cases where reciprocal expression was found; only 2 of these cases demonstrated heterogeneity. Conversely, coexpression of both URO-8 and URO-10 antigens was associated with a heterogeneous staining pattern in 19 of the 25 cases studied.

Of the 46 nonrenal cancers studied (Table 2), mAb F31 reactivity was found in 5 of 8 colon cancers, predominantly associated with apparent secretions within the lumen of glan-
Fig. 4. Double immunofluorescence staining of normal adult kidney section. a and b are single exposure photomicrographs of the same field in the juxtamedullary zone double-stained with mAbs F23 (fluorescein isothiocyanate) and F31 (rhodamine isothiocyanate), demonstrating proximal convoluted tubules (right aspect of field) which are F23 (URO-3)/F31 (URO-8)− and a proximal straight tubule which coexpresses both URO-3 and URO-8.

Fig. 5. Double immunofluorescence staining of normal adult (a and b) and 12- to 18-wk fetal (c and d) kidney sections stained with both mAb T43 (fluorescein isothiocyanate) and mAb F31 (rhodamine isothiocyanate). a and b were photographed using a double exposure technique to demonstrate the mutually exclusive immunoreactivity. a, cortical region of the kidney. The proximal convoluted tubules surrounding the glomerulus (G) are T43 (URO-10)/F31 (URO-8)−. b, subcortical/juxta-medullary area. Proximal convoluted tubules at the top of the field are URO-10+/URO-8−. Distal and collecting tubules in the photomicrograph express neither URO-10 nor URO-8. c and d are photomicrographs of the same field of fetal kidney photographed by single-exposure technique. A proximal tubule can be seen as it exits a glomerulus (G). In the fetal kidney, the proximal convoluted tubule can be seen to express both URO-10 and URO-8 unlike in the adult (see a and b). Despite the coexpression of URO-10 and URO-8, however, the pattern is slightly different with URO-10, but not URO-8, being expressed on the parietal epithelial cells of the glomerulus.

dular elements. Two of 10 ovarian cancers were weakly and heterogeneously stained. All other tumor specimens examined were URO-8−.

Comparison of Antigenic Phenotype and Histopathology of Renal Cancers. Twenty renal tumors were classified according to histopathological features (Fig. 7). Twelve tumors were primary renal cell carcinoma lesions, of which 10 cases displayed typical clear, granular, or mixed cell types with either acinar, tubular, or solid histological patterns; one of these tumors showed a papillary pattern (Case 11), and another specimen had a sarcomatoid cell pattern (Case 12). Eight tumors were metastatic lesions; six showed clear and/or granular cell types, while the other two tumors were anaplastic. Fig. 8 illustrates the immunostaining pattern of two primary renal carcinomas of the clear cell type (Cases 1 and 6). Fig. 7 compares the antigenic phenotype of these 20 renal carcinoma specimens when analyzed with a panel of monoclonal antibodies detecting proximal tubular antigens (URO-2, 3, 4, 8, 10) and a distal nephron antigen (URO-5). In general, renal carcinomas from both primary and metastatic sites were URO-2+/URO-3−/
Immunohistological staining pattern*  

<table>
<thead>
<tr>
<th>Tumor specimen</th>
<th>Total</th>
<th>●</th>
<th>○</th>
<th>O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal cell carcinoma</td>
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<td>10</td>
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<td>Breast carcinoma</td>
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<td>Colon cancer</td>
<td>8</td>
<td>0</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Lung cancer</td>
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<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Ovarian cancer</td>
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<tr>
<td>Sarcoma</td>
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</tr>
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</table>

*Staining patterns for each specimen are summarized as: ●, homogeneous positive reactivity; ○, heterogeneous or weak positive reactivity; and O, no reactivity.

URO-4+/URO-10+/URO-8+/URO-5+.

Biochemical Characterization of the URO-8 Antigen. mAb F31 was tested against a panel of blood group-related substances, including A, B, H, X, Y, Le\(^a\), Le\(^b\), and precursor substances. No positive reactions were observed.

mAb F31 identified a heat-stable antigen (100°C for 5 min) in the lipid fraction of chloroform:methanol-extracted SK-RC-37 renal cancer cells. Acidic and neutral lipid fractions were isolated by DEAE-Sephadex chromatography, and each fraction was separated by thin-layer chromatography followed by immunostaining with mAb F31. Fig. 9 illustrates the immunostaining pattern of mAb F31 detected in the acidic glycolipid fraction. Although the URO-8 antigen has many of the properties of an acidic glycolipid, the antigen is resistant to neuraminidase digestion and mild periodate treatment. These treatments affect neither mAb F31 binding nor migration by thin layer chromatography. Additional studies to further characterize the URO-8 antigen are in progress.

**DISCUSSION**

The six antigenic systems presented in this paper provide cell surface probes for studying human nephrogenesis and the histopathology of renal cancer. These URO antigens represent differentiation markers which are expressed by both fetal and adult kidney cells. The URO-8 antigen, identified by mAb F31, was of particular interest to the present analysis because this antigen defined a restricted region of the proximal tubule and because it was found expressed by 70% of renal carcinomas.

The URO-8 antigen distinguishes two different populations of cultured normal kidney epithelial cells: (a) 80 to 90% of the cells express the proximal tubular glycoprotein markers URO-2, URO-3, URO-4, and URO-10 but fail to express the URO-8 antigen; (b) 10 to 15% of cells express all of these markers including URO-8. The predominant population presumably represents cells of the proximal convoluted tubule; the subpopulation of URO-8* cells presumably derives from the straight segment. Among established cell cultures, 38 of 45 renal cancers are URO-8+. Interestingly, the proportion of URO-8* renal cancers is similar when results obtained with tissue sections (70%) and cell lines (84%) are compared. Comparison of the antigenic profiles from various cell types and tissue specimens suggests that the URO-8 antigen is distinct from known glycolipids or glycoproteins found in the kidney and urogenital system (6-11, 27-32).

Immunopathological review of 50 unselected renal cancer specimens revealed that half of the cases maintained the reciprocal relationship in expression of URO-8 and URO-10. Twenty-eight % of cases (14 of 50) were URO-8+/URO-10*, and 20% (10 of 50) were URO-8+/URO-10-. Expression of these phenotypes by renal cancers is consistent with their respective derivations from the proximal convoluted tubule (URO-8+/URO-10*) or the proximal straight tubule (URO-8*/URO-10*). The remaining 50% (25 of 50) of the renal cancers studied were composed of cells expressing both URO-8 and URO-10. We have not found URO-8*/URO-10* cells in normal adult kidney specimens; however, cells with this phenotype are present in fetal kidney proximal tubules. That is, URO-8*/URO-10* renal cancers express the antigenic phenotype of proximal tubule progenitor cells. Nineteen of the 25 URO-8*/URO-10* tumors demonstrated a heterogeneous, patchy pattern of antigen expression. Perhaps the significantly greater incidence of heterogeneity seen in this subset of renal cancers indicates genetic or epigenetic instability, as these cells transcribe an aberrant genetic program or attempt to differentiate along either the URO-8+/URO-10* or URO-8*/URO-10* pathways. Only one of the 50 cases failed to express both the
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Fig. 8. Two renal cancers (Cases 1 and 6) reacted with mAbs F23 and F31 using the indirect immunoperoxidase method. Case 6 (a and b) is F23(URO-3)*/F31(URO-8)*; Case 1 (c and d) is URO-3*/URO-8~.

Fig. 9. Thin-layer chromatograms of acidic (Lanes 1 and 2) and neutral (Lanes 3 and 4) lipid fractions of SK-RC-37. mAb F31 reactivity is demonstrated in the acidic, but not the neutral, fraction. Lanes 1 and 3 are loaded with 5 μg, while lanes 2 and 4 are loaded with 10 μg of material. For reference, the position where Gb3 ganglioside migrates is indicated.

URO-8 and the URO-10 antigens. This renal cancer did, however, express other proximal tubular antigens (URO-2, URO-3, and URO-4).

Our findings are consistent with earlier studies that renal cell carcinoma derives from proximal tubular cells. However, mAb F31 reactivity demonstrates, for the first time, that histologically and ultrastructurally similar cells of the pars convoluta and pars recta differ not merely in their location along the tubule but also at a molecular level. Through this study of normal embryonic and adult kidney and renal cancers, we have defined the antigenic phenotypes of cells from (a) the convoluted proximal tubule, (b) the straight proximal tubule, (c) the precursor cell from which proximal tubular cells are derived, and (d) corresponding subsets of renal cancers. This analysis brings us one step further in defining the histogenesis of renal cancers. The clinical significance of these different subsets is currently under investigation.

REFERENCES

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