Cell Surface Antigens of Human Bladder Tumors: Definition of Tumor Subsets by Monoclonal Antibodies and Correlation with Growth Characteristics

Yves Fradet, Carlos Cordon-Cardo, Willet F. Whitmore, Jr., Myron R. Melamed, and Lloyd J. Old

ABSTRACT

Normal human urothelium and tumors of urothelial origin were analyzed with a panel of seven mouse monoclonal antibodies that identify surface antigens of cultured bladder cancer cell lines. Three categories of antigens were defined on the basis of differential expression on normal urothelium versus bladder tumors. OmS (a category 1 antigen) is highly restricted, differentiation antigen detected in the normal urothelium of 50-60% individuals. No other normal cell type in OmS- or OmS+ individuals expresses OmS. The incidence of OmS expression in superficial bladder tumors is significantly higher (88%) than in normal urothelium, whereas its expression in invasive or metastatic tumors is far lower (20%), suggesting OmS gain/loss in bladder tumors. Paired biopsies of normal urothelium and bladder tumors from the same individuals have shown OmS induction in the superficial bladder tumors of OmS- individuals and OmS loss in invasive bladder cancers of OmS+ individuals. Category 2 antigens (T43, T138, T23) are not expressed by normal urothelium or most superficial bladder tumors but are detected on a high proportion of invasive or metastatic bladder tumors, indicating that category 2 antigens are associated with late stages of tumor progression. Category 3 antigens (T16, T87, J143) provide lineage markers for normal or neoplastic cells of urothelial origin, being found on normal urothelium and virtually all bladder tumors. Thus, differential expression of category 1 and 2 antigens divide bladder tumors into distinct subsets, and these subsets correlate with pathological and clinical features of the disease.

INTRODUCTION

Monoclonal antibodies have provided a range of new probes to investigate molecular changes at the cell surface during development, differentiation, and malignant transformation. Human cancer cells have been a particular focus of study with intent to define tumor-specific antigens. Although such restricted antigens have not been found, a wide array of differentiation antigens have been defined that are being used to investigate the basis of tumor heterogeneity and tumor subsets and as targets for antibody imaging and therapy (1-4).

Several groups have generated monoclonal antibodies reacting with bladder cancer (5-14). In the study of Fradet et al. (7) antibody specificity was defined by (a) tests on a large panel of cultured normal and malignant cell lines representing a range of differentiated cell types; (b) immunohistological studies of normal fetal and adult tissues and a panel of different types of cancers; and (c) biochemical characterization of the antigens. Seven of the antibodies defining distinct antigenic systems reacted either with all bladder tumors or with a subset of them. To investigate the significance of differential expression of these antigens on tumor subsets, we have typed a panel of bladder tumors and have related antigenic phenotypes to morphological and biological features of this disease.

MATERIALS AND METHODS

Monoclonal Antibodies. For origin and specificity analysis, see Fradet et al. (7).

Tissues. Tissues were obtained at autopsy or from surgical specimens. Specimens were frozen in liquid nitrogen and embedded in cryomolds in O.C.T. compound (Miles Laboratories, Naperville, IL). Frozen sections (4-8 μm) were prepared using a Bright OTF cryostat (Hacker Instruments, Inc., Fairfield, NJ).

Immunohistochemistry. Indirect immunofluorescence and avidin-biotin complex immunoperoxidase tests were performed as described (15, 16). Undiluted hybridoma supernatants or purified immunoglobulins were the source of antibodies. Secondary antibodies were obtained from the following sources: fluoresceinated goat antimouse (1:40 dilution; Cappel Laboratories, Cochranville, PA) and avidin-biotin reagents (Vector, Burlingame, CA). Specificity controls for antibody reactivity and tissue reactivity were included in each test.

RESULTS

Antigenic Phenotype of Normal Adult and Fetal Urothelium. Table 1 summarizes the characteristics and reactivity of seven mouse monoclonal antibodies detecting surface antigens of human bladder tumors. Each antibody recognizes a distinct antigenic system with a characteristic pattern of expression in normal tissues. Three categories of antigens can be defined on the basis of reactivity with normal urothelium (Table 2; Fig. 1).

Category 1. OmS is the most restricted of the antigens. In the original analysis of OmS, no expression of OmS was detected in any normal adult or fetal tissues, including eight specimens of fetal and five adult urothelium (7). Further testing has shown that OmS is expressed in normal urothelium of 12 of 22 adults and 1 of 9 fetuses. No other normal cell type, adult or fetal, was found to express OmS.

Category 2. Antigens in this category are not expressed by normal urothelial cells but are expressed by other normal cell types.

Category 3. These antigens are expressed by normal urothelial cells as well as by other cell types. Normal urothelium of ureter and bladder show uniform staining with antibodies defining T16 and T87. J143 is limited to the basal cell layers of normal urothelium. In contrast to the absence of OmS antigen expression in certain individuals, categories 2 and 3 antigens are found in all individuals.

Antigenic Phenotype of Bladder Tumors. A panel of 46 bladder tumors were typed for expression of categories 1, 2, and 3 antigens (Table 2). Antibodies to OmS and category 2 antigens reacted with a proportion of bladder tumors, whereas antibodies to category 3 antigens reacted with all bladder tumors except one. According to their expression of categories 1 and 2 antigens, bladder tumors could be divided into distinct subsets.

Comparison of Antigenic Phenotype and Histopathology of Bladder Tumors. The 46 bladder tumors listed in Table 2 were classified according to histopathological features (Table 3; Fig. 2). Twenty-seven tumors were superficial bladder tumors, of which 19 were papillary and 8 were flat. The other tumors were muscle invasive or metastatic. Table 3 and Fig. 2 show that tumor subsets defined by monoclonal antibodies correlated with...
expression in normal urothelium. To investigate this further,
tumor dissemination at other sites. T138+ invasive bladder tumors, 7 patients had evidence of
but only few of the superficial tumors. Tl 38 shows a particularly
or metastatic tumors. In contrast, T43 and T138 antigens are
of Om5 expression in normal urothelium was approximately
specimens of normal urothelium and bladder tumors were
striking correlation with metastatic potential; all 4 metastatic specimens studied were T138* and of the 16 patients with
the histopathology of the tumor. Om5 antigen is expressed by
24 of 27 superficial bladder tumors but by only 3 of 19 invasive
or metastatic tumors. In contrast, T43 and T138 antigens are
found on a high proportion of invasive and metastatic tumors
but only few of the superficial tumors. T138 shows a particularly
striking correlation with metastatic potential; all 4 metastatic
specimens studied were T138* and of the 16 patients with
T138+ invasive bladder tumors, 7 patients had evidence of
tumor dissemination at other sites.

Om5 Expression in Normal Urothelium and in Bladder Tumors. The incidence of Om5+ superficial bladder tumors appears to be higher than would be predicted from data on Om5+
expression in normal urothelium. To investigate this further,
specimens of normal urothelium and bladder tumors were
obtained from a larger series of patients (Table 4). The incidence
of Om5 expression in normal urothelium was approximately
the same in individuals with bladder tumors (60%) as it was in
individuals with other diseases (55%). In contrast, 88% of 61
superficial bladder tumors showed Om5 expression, suggesting
that either superficial bladder tumors occur preferentially in
individuals with Om5+ urothelium or that individuals with
Om5+ urothelium developed Om5+ superficial bladder tumors.
To address this most directly, normal urothelium and tumor
specimens were obtained from the same individual and typed
for Om5 expression. Table 5 shows results of this comparative
study in a series of 11 patients with either superficial or invasive
bladder tumors. All specimens were also typed for T16, providing
a control to ensure origin from urothelium. In five of seven
patients with superficial tumors, the tumor and normal urothe-
lum showed an identical Om5 phenotype; however, in two
patients (patients 1 and 2) the normal urothelium was Om5~
whereas the superficial bladder tumor was Om5* (Fig. 3). Other
patients showed loss of Om5 in invasive bladder tumors (Table
5). Patient 7 is particularly instructive, as this patient had both
a superficial and invasive tumor. Normal urothelium and super-
ficial tumor were Om5+, whereas the invasive bladder tumor
was Om5~.

### DISCUSSION

The seven antigenic systems analyzed in this report provide
valuable probes in the study of normal and transformed urothe-
lial cells. Category 3 antigens (T16, T87, J143) represent lin-
eage or differentiation markers that are expressed by both fetal
and adult urothelium and that persist during tumor progression
and development. Category 2 antigens (T43, T138, T23), on
the other hand, are not expressed by fetal or adult urothelium
nor by most superficial bladder tumors, but do appear in a
transformation-related pattern on a subset of bladder cancers.
The category 1 antigen, Om5, has unique features that qualify
it as a differentiation antigen restricted to urothelium and as a
transformation-specific antigen. The typing of normal urothe-
lum indicates two Om5 phenotypes with approximately equal
frequency of Om5+ and Om5~ individuals. The fact that super-
ficial tumors show a higher frequency of the Om5+ phenotype
suggests that either Om5+ individuals have a higher suscepti-
bility for this tumor type or that Om5 antigen is induced in the
preneoplastic or neoplastic urothelial cells of Om5~ individuals.
Three lines of evidence suggest that Om5 may, in fact, be an
example of antigen induction associated with transformation:
(a) normal urothelium from bladder cancer patients do not

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**Table 1** Characteristics and reactivity of a panel of mouse monoclonal antibodies generated against human bladder tumor cells

<table>
<thead>
<tr>
<th>Category</th>
<th>Antibody designation</th>
<th>Molecular weight of antigen</th>
<th>Chromosome assignment of locus coding for antigen</th>
<th>Reactivity with normal tissues*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Om5 (γ1)</td>
<td>Not known</td>
<td>Not known</td>
<td>Urothelium Kidney Prostate Others</td>
</tr>
<tr>
<td>2</td>
<td>T43 (γ1)</td>
<td>gp 85*</td>
<td>11 (25)*</td>
<td>Positive or negative Negative Negative Positive Negative</td>
</tr>
<tr>
<td></td>
<td>T138 (μ)</td>
<td>gp 25</td>
<td>Not known</td>
<td>Negative Proximal tubule Negative Negative Negative</td>
</tr>
<tr>
<td></td>
<td>T23 (μ)</td>
<td>Not known</td>
<td>Not known</td>
<td>Negative Negative Negative Negative</td>
</tr>
<tr>
<td>3</td>
<td>T16 (γ2B)</td>
<td>gp 48,42</td>
<td>Not known</td>
<td>Positive Distal and collecting tubules Positive Breed ducts, skin adnexae, stratified epithelium α, β</td>
</tr>
<tr>
<td></td>
<td>T87 (γ1)</td>
<td>gp 60</td>
<td>1 (26)</td>
<td>Positive Distal and collecting tubules Positive Breed ducts and acini, colon, thyroid, and endometrium, fetal thymocytes</td>
</tr>
<tr>
<td></td>
<td>J143 (γ1)</td>
<td>gp 140,30</td>
<td>17 (27)</td>
<td>Positive Glomerulus Negative Stratified epithelial, α', β' some basal membranes, smooth muscle</td>
</tr>
</tbody>
</table>

* Reactivity by indirect immunofluorescence or avidin-biotin complex immunoperoxidase tests.

**Table 2** Reactivity of monoclonal antibodies with fetal and adult urothelium and bladder tumors

<table>
<thead>
<tr>
<th>Monoclonal antibodies</th>
<th>Reactivity</th>
<th>Category 1, Om5</th>
<th>Category 2, T43 T138 T23 T16 T87 J143</th>
<th>Category 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal urothelium (9)*</td>
<td>Positive</td>
<td>1</td>
<td>8 9 9 9</td>
<td>9 9 9</td>
</tr>
<tr>
<td>Adult urothelium (22)</td>
<td>Positive</td>
<td>12</td>
<td>22 22 22</td>
<td>22 22 22</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>21</td>
<td>21 21 21 21 21</td>
<td>40 1</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, number of individuals tested.

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PATIENT 1

Om5

T43

T16

PATIENT 2

Fig. 1. Expression of Om5 (category 1), T43 (category 2), and T16 (category 3) antigens by normal urothelium from two patients. Indirect immunofluorescence test. x 400.

Table 3 Comparison of antigenic phenotype and histopathology of bladder tumors from 46 patients

The reactivity of bladder tumors with monoclonal antibodies to category 1 (Om5) or category 2 (T43, T138, T23) antigens was determined by indirect immunofluorescence and avidin-biotin complex immunoperoxidase tests.

<table>
<thead>
<tr>
<th>Bladder tumor</th>
<th>Om5</th>
<th>T43</th>
<th>T138</th>
<th>T23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papillary superficial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1, 2, 3, 4, 5, 7, 8, 10, 16, 19, 28, 30, 35, 44, 48, 11, 13, 34, 42</td>
<td>Om</td>
<td>Om</td>
<td>Om</td>
<td>Om</td>
</tr>
<tr>
<td>In situ</td>
<td>Om</td>
<td>Om</td>
<td>Om</td>
<td>Om</td>
</tr>
<tr>
<td>31, 32, 33, 29, 40, 46, 43, 17</td>
<td>Om</td>
<td>Om</td>
<td>Om</td>
<td>Om</td>
</tr>
<tr>
<td>Muscle invasive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>47, 37, 39, 9, 21, 6, 12, 14, 15, 20, 22, 23, 36, 38, 49</td>
<td>Om</td>
<td>Om</td>
<td>Om</td>
<td>Om</td>
</tr>
<tr>
<td>Metastatic</td>
<td>Om</td>
<td>Om</td>
<td>Om</td>
<td>Om</td>
</tr>
<tr>
<td>24, 27, 41, 52</td>
<td>Om</td>
<td>Om</td>
<td>Om</td>
<td>Om</td>
</tr>
</tbody>
</table>

* Specimen number.
+ Positive reactivity.
+ Negative reactivity.
+ t, Proven distant metastasis in this patient.
Fig. 2. Expression of OmS (category 1) and T43 (category 2) antigens by papillary superficial (A), in situ (B), muscle invasive (C), and metastatic bladder tumors (D). Indirect immunofluorescence tests. A, B, C, × 200; D, × 400.

Table 4 Reactivity of OmS antibody with normal urothelium and bladder tumors as determined by indirect immunofluorescence and avidin-biotin complex immunoperoxidase

<table>
<thead>
<tr>
<th>Human tissues</th>
<th>No. of specimens tested</th>
<th>No. of specimens with OmS expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal urothelum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Individuals with bladder tumors</td>
<td>59</td>
<td>35 (60)*</td>
</tr>
<tr>
<td>Individuals with other diseases</td>
<td>22</td>
<td>12 (55)</td>
</tr>
<tr>
<td>Bladder tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papillary superficial</td>
<td>37</td>
<td>32 (87)</td>
</tr>
<tr>
<td>In situ</td>
<td>24</td>
<td>21 (88)</td>
</tr>
<tr>
<td>Muscle invasive</td>
<td>20</td>
<td>4 (20)</td>
</tr>
<tr>
<td>Metastatic</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.

show higher frequency of Om5 antigen expression than normal urothelium from patients with other neoplastic or nonneoplastic diseases; (b) Om5* cells disappear from bladder washings of patients with Om5* tumors after successful intravesical treatment with Bacillus Calmette-Guérin (17); and (c) in the 11 patients where bladder tumors and normal urothelium from the same individual were tested, 2 patients were identified with Om5* normal urothelium and Om5* tumors. These characteristics of Om5 antigen, particularly the Om5*→Om5* conversion associated with transformation, shows interesting parallels with TL3 antigens of the mouse (18). In normal mice, TL antigens are also differentiation antigens with expression restricted to thymocytes. Not all mouse strains express TL, permitting the definition of TL+ and TL− strains. However, TL+ leukemias occur in TL− strains, indicating that TL structural information is universal in the mouse and that leukemogenesis has disrupted a normal regulatory mechanism controlling TL expression. Further studies on the anomalous appearance of Om5* tumors in Om5* individuals and information about the structure and regulation of Om5 antigen will be

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3 The abbreviation used is: TL, thymus leukemia.
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Fig. 3. Expression of Om5 (category 1) and T16 (category 3) antigens by normal urothelium and superficial papillary bladder tumor from the same individual (patient 2 in Table 5). Indirect immunofluorescence test. × 400.

Table 5 Comparative Om5 phenotype of normal urothelium and bladder tumors

<table>
<thead>
<tr>
<th>Patient</th>
<th>Normal urothelium</th>
<th>Papillary or flat superficial tumors</th>
<th>Invasive tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Om5</td>
<td>T16</td>
<td>Om5</td>
</tr>
<tr>
<td>1</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>2</td>
<td>○</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>3</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>4</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>5</td>
<td>•</td>
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<tr>
<td>6</td>
<td>•</td>
<td>•</td>
<td>•</td>
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<tr>
<td>7</td>
<td>•</td>
<td>•</td>
<td>•</td>
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<tr>
<td>8</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>9</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>10</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>11</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
</tbody>
</table>

* C, negative reactivity; ○, positive reactivity.

necessary before the significance of Om5 antigen induction in bladder tumors can be evaluated.

Although phenotyping with monoclonal antibodies has been useful in defining lineage and differentiation markers of human tumors (19, 20), little is known about the relationship of antigen phenotype to biological characteristics of tumors, such as invasiveness and metastasis. In the case of bladder tumors, there have been many studies of A, B, and H blood group antigenic expression, with the finding that expression of these antigens is reduced in more aggressive bladder tumors (21–23). In the present study, we have also observed a correlation between antigenic phenotype and known clinical features of the disease. The less aggressive superficial tumors tend to have the Om5+ T43− T138− phenotype, whereas invasive and/or metastatic tumors are Om5− T43+ T138+. Appearance of T43 and T138 in tumors derived from normal urothelium not expressing these antigens could be a reflection of the greater proliferative rate of tumor cells, particularly those with more aggressive characteristics. The fact that these antigens are expressed at higher levels in cultured cell types (7) is consistent with this hypothesis. Another possibility is that antigen induction reflects malignancy-related alterations of gene regulation leading to activation of normally silent genes.

Each of the antigens analyzed in this study has a characteristic pattern of expression in normal tissues, ranging from the restricted expression of Om5 to the more general expression of T23. It is difficult to make general statements about the significance of these patterns without knowing the function of these surface molecules. However, the finding that T138, an antigen expressed on invasive and metastatic tumors is also a marker restricted to endothelial cells (7) raises the possibility that T138 may be involved in the ability of cancer cells to invade blood or lymphatic vessels, a prerequisite for cancer dissemination. Cote et al. (24) have recently described another endothelial marker, BT11, that is expressed by invasive tumors. Although these associations between endothelial markers and tumor invasiveness may be fortuitous, the appearance of endothelial antigens during tumor progression deserves further study.
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