Characterization of Bladder Papilloma by Two-Parameter DNA-RNA Flow Cytometry

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ABSTRACT

Two-parameter flow cytometry (FCM) studies of 0.9% NaCl solution bladder irrigation specimens were performed on 48 patients with histologically orderly or atypical papilloma of the urinary bladder in order to assess the value of RNA as a possible second parameter, along with DNA, in the detection of bladder tumors. DNA, RNA, and nuclear diameter measurements were obtained for each of 5000 cells/sample, and analyses were based on the distributions of those values. With the use of DNA content alone, 22 cases (46%) were classified positive by FCM. With RNA content as an additional parameter, 40 cases (83%) were positive. Two cases were suspicious, and 6 cases were normal by both parameters.

Of 28 patients with papillomas showing histological atypia, 16 had positive DNA histograms, including 3 patients with aneuploid stemlines, but 24 of the 28 had positive RNA histograms. Of 20 patients with orderly papillomas, 6 patients had positive DNA histograms, including 3 patients with aneuploid DNA stem cell lines, but 16 of the 20 patients had positive RNA histograms.

Thus, the probability of positive DNA histograms is higher in atypical papillomas (57%) than in orderly papillomas (30%), whereas elevated (positive) RNA is more characteristic of all papillomas without distinction between those that are histologically atypical (86% positive) or orderly (80% positive). For patients at risk of developing papillary bladder tumors, two-parameter DNA-RNA FCM appears to offer greater diagnostic sensitivity than does FCM based on DNA content alone.

INTRODUCTION

Papillomas of the urinary bladder are papillary tumors composed of cytologically benign epithelium. Following endoscopic excision or fulguration, the 5-year life expectancy of patients with papilloma is normal. While these tumors rarely, if ever, invade or metastasize (except iatrogenically), their presence does signify increased risk of other (recurrent) papillomas and/or carcinoma (5, 7, 12—16). From 30 to 68% of patients with initially solitary tumor and 52 to 88% with 2 or more tumors will develop recurrences (5, 7, 12, 13, 15). The rate of subsequent frank carcinoma is in the range of 5 to 15% (7, 12, 14), and the probability of carcinoma is increased in patients with atypical as compared to orderly papillomas (12). Thus, lifetime surveillance is recommended for these patients. Both conventional cytology and flow cytometry of DNA content are useful in detecting carcinoma but are of limited value in recognizing the cytologically benign papillomas. The exfoliated cells of papillomas have diploid DNA values (6, 9), as do normal bladder epithelial cells, although they differ from the latter in at least some cases in proliferative activity, cell configuration, and amount of cytoplasm. Since the latter features are likely to affect RNA content, the present study was undertaken to determine whether papillomas could be identified by FCM parameters on the basis of combined DNA and RNA distributions.

MATERIALS AND METHODS

During the 17-month period, March 1980 through July 1981, bladder irrigation specimens were obtained from 48 patients with bladder papilloma just prior to therapeutic endoscopic treatment. Twenty-one patients were being treated for the first time at Memorial Hospital; 27 patients had a history of recurrent papilloma for as long as 7 years. Specimens were obtained by barbotage with 0.9% NaCl solution through the resectoscope with an Ellik evacuator. Each specimen was promptly refrigerated and processed within 24 hr. The cellular sediment was concentrated by centrifugation at 1500 rpm for 10 min, discarding the supernatant and resuspending the cell pellet in Hank’s balanced salt solution to a concentration of 10⁶ cells/ml. The cell suspension was then vortexed and sieved through a 54-μm nylon mesh filter to remove tissue fragments and cell groups.

Staining was carried out by the 2-step acridine orange technique described by Darzynkiewicz et al. (3). Briefly, this involves treating 0.2 ml of the cell suspension with 0.4 ml acid detergent [0.1% (v/v) Triton X-100-0.08 N HCl, 0.15 M NaCl] followed in 30 sec by 1.2 ml acridine orange-staining solution (6.0 μg chromatographically pure acridine orange-10⁻³ M EDTA-0.15 M NaCl-0.1 M citrate-phosphate buffer at pH 6). The acridine orange binds to double-helical DNA by intercalation and fluoresces green in blue light and to single-stranded RNA in a crystalline lattice that fluoresces red (8). Any double-stranded RNA is denatured to its single-stranded form by the EDTA in the presence of acridine orange. The fluorescence emission at the 2 different wavelength bands is separated optically and quantified for each of the 5000 cells/sample in an Ortho FC-200 flow cytometer. Measurements are stored in a Data General Nova 1220 minicomputer and subsequently recalled for analysis.

Instrument calibration was carried out with acridine orange-stained human peripheral blood lymphocytes prior to examination of the bladder specimens. The magnitudes of green (DNA) fluorescence, red (RNA) fluorescence, and green fluorescence pulse width (nuclear diameter) for each cell in a given sample were displayed in 2-dimensional scattergrams of DNA versus RNA or DNA versus pulse width. In these computer-generated displays, dead or dying cells, squamous cells, granulocytes, and bladder epithelial cells could be identified as de-
A positive FCM diagnosis of bladder cancer was made if the bladder epithelial cells showed (a) a distinct aneuploid stem cell line or (b) a tetraploid stem cell line with greater than 15% hyperdiploid cells (DNA content greater than the normal complement 2c). A sample was considered suspicious but not definitely cancer if there was a tetraploid population with 10 to 15% hyperdiploid cells or more than 10% hyperdiploid cells with no aneuploid stem cell line. A specimen with less than 10% hyperdiploid cells and no aneuploid stem cell line was considered normal.

RNA was evaluated as an additional parameter in this study. A normal bladder irrigation specimen has only a single symmetrical RNA peak representing the normal diploid bladder epithelial cells (Chart 1). A RNA histogram was obtained on the selected bladder epithelial cell population in each case and inspected for the presence of an abnormal RNA distribution (asymmetry or presence of an extra population of cells with higher amounts of RNA). The normal RNA distribution in each case was obtained by constructing a Gaussian distribution on the basis of the left side of the curve (cells with RNA equal to or less than the modal value). If more than 10% of the cells had greater RNA values than did the upper limit of normal, the specimen was considered abnormal (Chart 2). In some cases, a distinct second population with high modal value was seen (Chart 3).

The bladder papillomas were subclassified as “orderly” or “atypical” according to their histological pattern. Thus, the tumors composed of uniform epithelial cells in an orderly arrangement were classified as “orderly” papillomas (Fig. 1). The tumors with a disordered arrangement of generally less uniform, although still cytologically benign, epithelium were classified as “atypical” (Fig. 2). Variations in cell orientation, size, and configuration accounted for the disarranged appearance of the atypical papillomas; nuclear variability was minimal, although somewhat more evident in the atypical than in the orderly papillomas. DNA modal values, RNA modal values, and percentage of hyperdiploid cells were analyzed and compared in the orderly versus atypical papillomas.

RESULTS

The 48 cases of papilloma included 20 cases classified as orderly and 28 cases as atypical. By present FCM criteria, on the basis of DNA distribution (9), 22 cases (46%) were positive. Six of the 22 cases had aneuploid stem cell lines with modal DNA values ranging between 3.0c and 3.8c. Ten cases (21%) were suspicious, and 16 cases (33%) were negative. RNA distribution showed distinctive abnormal populations in 40 cases (83%), inconclusive changes in 2 cases (4%), and no abnormal population in 6 cases (13%) (Table 1).

The FCM findings are shown separately for patients with orderly and atypical papillomas in Table 1. Sixteen of the 28 patients with atypical papillomas (57%), but only 6 of the 20 patients with orderly papillomas (30%) had positive FCM on the basis of DNA. On the other hand, RNA was equally likely to be positive in atypical (86%) and orderly (80%) papillomas. Representative DNA and RNA histograms are shown in Charts 1 to 3.

All of the patients with positive or suspicious FCM based on DNA were positive by the RNA criterion. Of the 16 patients that were negative by DNA measurements, 8 patients became positive when RNA content was added as a parameter, 2 patients became suspicious, and 6 patients remained unrecognized (negative).

DISCUSSION

FCM provides a rapid, objective, and quantitative method of analysis of exfoliated bladder epithelium in irrigation speci-
FCM findings for 48 cases of papilloma of the urinary bladder

<table>
<thead>
<tr>
<th>Classification by DNA</th>
<th>Positive</th>
<th>Suspicious</th>
<th>Negative</th>
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<tr>
<td>Orderly papilloma</td>
<td>6</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Atypical papilloma</td>
<td>16</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>22 (46)</td>
<td>10 (21)</td>
<td>16 (33)</td>
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<table>
<thead>
<tr>
<th>Classification by RNA</th>
<th>Positive</th>
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<th>Negative</th>
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<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Atypical papilloma</td>
<td>24</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>40 (83)</td>
<td>2 (4)</td>
<td>6 (13)</td>
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* Numbers in parentheses, percentage.

merit particularly close and careful follow-up. In fact, 2 of these 6 cases have developed frank noninvasive carcinoma during the 7-month period of this study. The 16 remaining patients with positive FCM had an increased proportion of hyperdiploid and/or tetraploid cells, which is consistent with an increased proliferative rate. If one excludes the 6 cases with aneuploidy, it is of interest that 13 of 26 atypical papillomas (46%) but only 3 of 20 orderly papillomas (15%) had an increase in hyperdiploid or tetraploid cells. The implication, of course, is that there is a greater proliferative rate of bladder epithelium (within the tumor or elsewhere) with atypical than with orderly papillomas.

Both orderly and atypical papillomas were characterized by a high proportion of cells containing increased RNA. With the use of RNA content as a diagnostic criterion, 40 of the 48 cases (83%) were positive by FCM, with no significant difference between atypical and orderly tumors. Thus, RNA does appear to be a useful feature in identifying papillomas that might not be recognized by DNA measurements alone. However, the specificity of this feature was not investigated. Proliferative or regenerative processes could also be expected to contain increased RNA.

A larger range of clinical specimens must be studied before the potential limitations can be defined, but in a patient known to be at risk of developing bladder tumors, the presence of a cell population with high-RNA content by FCM should be viewed with suspicion even in the absence of DNA abnormalities. Two-parameter DNA/RNA measurements by FCM are...
feasible, and they appear to increase the diagnostic value of FCM on the basis of DNA values alone.

REFERENCES


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