Urol Res (1985) 13:43–45

Urological Research

Fluorescence Methods for Measuring the A, B, and H Isoantigens on Cytological Material from Bladder Carcinoma

E. Borgström[1,2], B. Wahren[2] and H. Gustafson[1]

[1] Department of Urology, Karolinska Hospital, S-10401 Stockholm, Sweden
[2] Department of Virology, National Bacteriological Laboratory, S-10521 Stockholm, Sweden

Accepted: July 11, 1984

Summary. Fluorescence methods for determining ABH isoantigens on transitional cells were developed for the study of cytologic material. Indirect immunofluorescence was used for A- and B-antigens, fluorescein-labeled Ulex Europaeus lectin for the H-antigen. Isoantigens of 151 patients with bladder carcinoma were studied. Patients with a malignant cytology showed deletion in 31/65 cases compared to 12/62 cases when the cytology was benign. Cytoflow ploidy determinations were abnormal in 41/120 cases measured; cells from 21 of these 41 had isoantigen deletion compared to 25/79 cases with a diploid pattern. The ABH isoantigen deletion shows some relation to malignant cytology, abnormal ploidy and a visible tumor, but is probably an independent parameter.

Key words: Blood group isoantigens, Cancer markers, Human bladder carcinoma, Bladder washings.

Introduction

The relationship between the invasive potential of low stage urothelial carcinoma and deletion of the ABH isoantigens has aroused considerable interest [6–8, 14, 15, 17, 18]. The antigen measurements are usually done by the Specific Red Cell Adherence Test (SRCA) of Davidsohn on formaldehydefixed tissue sections [5, 13]. The only possibility of quantifying the antigens lies in red cell counts and even then, exact localization is ruled out by the large size of the indicator red cells. The peroxidase-anti-peroxidase (PAP) method gives a better morphological picture [1, 2, 20] but is liable to be disturbed by interference from endogenous peroxidases.

We therefore developed two fluorescence methods for the ABH isoantigens on cytologic material from bladder cancer. Bladder washings are done routinely in the diagnostic and check-procedures ups for bladder carcinoma. The ABH isoantigen status of our patients can thus be followed. Individual cells can be identified as transitional or other cell types. This appeared to be a suitable material for measurements of deletion of antigens from the cell surface.

Material and Methods

Of the 151 patients in the present series, 109 were male and 42 female. Twenty-two had newly diagnosed tumors and 129 were part of our follow-up program: 84 cases with tumor in the bladder and 45 without visible tumor at this check-up. The histological gradings at presentation were 38 tumors of grade 1 (G1), 89 of grade G2 and 24 of grade G3, according to the WHO classification [16].

During cystoscopy in patients with bladder carcinoma we did bladder washings routinely with physiological saline. This cell suspension is used for smears [9], for cytofluorometric ploidy measurements [22], and for ABH isoantigen determinations.

Ploidy Measurements

The degree of ploidy was determined by a cytofluorometric method [22]. This method denudes the transitional cell nucleus with pepsin. The RNA was digested with ribonuclease and subsequently the DNA was stained with ethidium bromide before analysis in a cytofluorometer.

Isoantigen Determination

Cells from the bladder washings were centrifuged, resuspended in phosphate buffered saline (PBS), pH 7.4, with 1% bovine serum albumin (Miles, Slough, England), and allowed to dry overnight on glass slides.

For A- and B-isoantigen determination, human anti-A and anti-B antibodies (Dako, Copenhagen, Denmark) were spread on the specimen and incubated at 20 °C for 45 min. After rinsing with PBS, rabbit anti-human IgM labeled with fluorescein-isothiocyanate (FITC; Dako), diluted 1:20 with PBS, was added, the slide incubated for 30 min, rinsed and mounted.

For H-isoantigen determination, Ulex Europaeus agglutinin 1 FITC (Vector Laboratories, Burlingame, Cal., USA), was diluted 1:20 in PBS and spread on the specimen. After incubation for 30 min at 37 °C, the slide was rinsed and mounted.

Table 1. Cystoscopic assessment of the tumor at the time of the study

Isoantigen	Number of cases with	
	Papillary tumor < 2 cm	Tumor > 2 cm or solid
Present	52	15
Deleted	14	25

Table 2. Cytology at the time of the study

Isoantigen	Number of cases with	
	Benign Cytology	Malignant Cytology
Present	50	34
Deleted	12	31

Table 3. DNA measurements at the time of the study

Isoantigen	Number of cases with	
	Diploidy	Aneuploidy
Present	54	20
Deleted	25	21

Red blood cell suspensions of the A, B and 0 blood groups were used as positive controls in addition to red rells ordinarily present in each cell preparation. Anti-A serum on B-blood type cells and vice versa served as negative controls. All specimens were stained for all three antigens and inspected in a Zeiss fluorescence microscope without knowledge of the patient's blood group. The slides were considered negative (deleted) for the ABH isoantigen if 25% or more ot the transitional cells lacked specific staining. The χ^2 test was used for statistical analyses.

Results

Technical Points

Bovine serum albumin as a diluent of the cell suspension gives low background staining, good adherence of cells to the slide and preserves the cells well. Citrus pectin, ordinarily used in cytological work, did not retain the cells through the staining process. Fixation is important. Air drying, in our hands, gave higher fluorescence intensity compared to fixation with either methanol, methanol-acetone, methanol-acetic acid, formaldehyde or glutaraldehyde.

As a rule there is no doubt whether a cell population is stained. In a negative cell population usually all or almost all transitional cells lacked staining. Cases where the subpopulation without specific staining exceeded 25% were considered negative. No patient was assigned to the wrong blood group on the basis of the tests.

Blood Group Isoantigen Determinations in Patients with Bladder Carcinoma

There was no statistical differences between presence or absence of the ABH isoantigens in cells from patients of different blood groups ($p = 0.9$).

The cystoscopy report recorded presence of tumor in 106 cases (Table 1). The appearance of these tumors ranged from papillary, less than 2 cm in diameter in 66 cases, to larger than 2 cm in diameter or solid in the others. Among the 66 cases of smaller tumors ABH deletion was found in 14, while in the group with larger and/or solid tumors deletion was found in 25 out of 40 cases. The difference is highly significant ($p < 0.001$).

ABH isoantigen determination correlated well with cytology ($p < 0.001$, Table 2). Where the cytologist reported benign-looking cells in the bladder washing specimen, we found deletion in only 12 out of 62 cases. With malignant cytologic pattern isoantigens were present or deleted with about equal frequency.

Aneuploid cell populations, either tetraploid or non-tetraploid, were recorded in 41 out of 120 patients (Table 3). In 21 of the 41 aneuploid tumors deletion was found, compared to 25 of the 79 diploid tumors ($p < 0.05$).

Discussion

Fluorescence methods for measuring the ABH isoantigens were developed because of several shortcomings in the specific red cell adherence method, especially its low efficacy for determining the H-antigen. This problem seems to be related to the large size of the indicator red cells, making attachment difficult to the small Ulex Europaeus lectin molecule already bound to the urothelial cell [3, 20]. Our method for the H-antigen uses a fluorinated lectin and seems to perform just as well as the indirect immunofluorescence method for the A- and B-antigens. Fluorescence methods for determining the blood group isoantigens [1, 4, 11] have been published for formaldehyde fixed tissue material and shown to give results comparable to SRCA regarding deletion in bladder cancer.

Measurement of the isoantigens on cytologic material has several advantages. Bladder washings are done at every cystoscopy follow-up on patients with bladder carcinoma. This enables us to monitor the isoantigen status of the patient and we do not have to rely solely on the state at the initial diagnosis. Deparaffination of formaldehyde fixed histological slides is unnecessary since the cytology slide is

prepared directly for the ABH measurement. The cells are spaced individually on the slide, making it possible to assess or measure single cell fluorescence. Bladder washings have been used previously [12] to detect the presence of the ABH isoantigens but with the SRCA method. The results of the ABH status reported on cytologic material are similar to determinations on tissue specimens [19].

Often we found that deletion of the blood group antigens were an all – or – non phenomenon, i.e. involved the whole of the cell population investigated. This is in accordance with the report of Weinstein et al. [23], who observed blood group antigen deletion even on histologically normal biopsies from cystectomy specimens containing carcinoma in other parts. Stein et al. [21] reported on random biopsies from 103 bladders with ABH positive or negative tumors. In 92 of those bladders the antigen pattern in non-tumor areas agreed with that of the tumor.

The DNA pattern of the tumor cells, studied with cytofluorometric technique, is a parameter that correlates well with the progressive potential of superficial tumors [11].

In this material diploid cell populations generally retained their ABH isoantigens while deletion was more frequent among aneuploid populations.

Isoantigens were generally present in small papillary tumors, cells with benign cytology, and diploid cells. In larger tumors, cells with malignant cytology or with aneuploidy, however, isoantigens were either present or absent with a less predictable frequency. These findings may allow a further subdivision on the basis of isoantigen content as to the prognosis. Such studies are in progress.

Our fluorescence methods for ABH isoantigen measurements are easy to perform and assess. Quantification might be possible with a suitable photometer microscope. When applied to a patient material, there is good correlation with cytology and the urologist's clinical assessment of the bladder at cystoscopy.

Acknowledgements. This study was supported by the Cancer Society of Stockholm and the Japanese-Swedish Urological Research Foundation.

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Dr. E. Borgström
Department of Urology
Karolinska Hospital
S-10401 Stockholm
Sweden