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ABH Isoantigens, Histology and DNA Ploidy in 36 Consecutive Patients with Transitional Cell Bladder Cancer. Status of Tumor and Biopsies Taken from Visually Normal Urothelium

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Summary. Tumors and biopsies from visibly normal urothelium in 36 consecutive patients with transitional cell bladder carcinoma were analysed for histological pathology, DNA ploidy and ABH isoantigens. Tumor isoantigen deletion correlated strongly with malignant histology (p = 0.016) and aneuploidy (p = 0.005). In 4/12 patients with ABH isoantigens present on the tumor, and in 6/8 with isoantigens absent, isoantigen changes were found in normal looking urothelium, usually with normal histology and ploidy. It was concluded that the ABH isoantigen change was an early event in bladder carcinoma.

Key words: ABH isoantigens, Transitional cell bladder carcinoma, Field disease, Cytoflow DNA, Histopathology.

Introduction

The blood group (ABH) isoantigens are normally present on urothelium. Continued presence of antigen indicates that a low grade, low stage transtitional cell bladder carcinoma will not become invasive. Deletion might herald an invasive carcinoma [2, 4–6, 11]. The predictive value of ABH deletion is not sufficient in a clinical setting [17] but determination of the antigens are of great interest as a research tool.

The A, B and H isoantigens are not expressed if a glycosyl-transferase fails to attach the terminal carbrohydrate molecule responsible for the antigenic expression of the glycoprotein or glycolipid precursor. This failure presupposes changes in expression of the glycosyl-transferase gene [9, 14]. A change in the expression of a single gene is likely to precede the cellular alterations registered by histopathology or even by ploidy determination. Thus ABH isoantigen deletion could be an observable indicator of an early stage in malignant urothelial transformaion.

Mapping studies of the urthelium in transitional cell bladder carcinoma have shown histopathological abnormalities, ranging from hyperplasia to carcinoma in situ, in areas of mucosa with a grossly normal appearance [7, 10]. Malignant changes in ploidy [15], T-antigen [3] and ABH isoantigens are also found. We studied consecutive material from bladder carcinoma to observe the extent of ABH isoantigen changes in newly diagnosed bladder tumors.

Material and Methods

Thirtynine consecutive patients with bladder carcinoma diagnosed during 1982 and 1983 at the Department of Urology, South Hospital, Stockholm, Sweden were invesitgated. Three patients were excluded: two had squamous cell carcinoma and one had an adenocarcinoma of the bladder. Thirtysix patients were diagnosed as having transitional cell carcinoma. Their mean age wai 69 ± 7 years (range 54–91). Eight of them were females.

At diagnosis the bladder was cold cup biopsied from left and right walls, trigone, and from the visible tumor. In addition, resection material from tumor and tumor base was assessed. All biopsies were analysed for ABH isoantigens, cytoflow ploidy, histological grade (WHO) and classification (UICC). Patient status after 2.5–3.5 years of follow up was reviewed.

ABH Isoantigen Analysis

The formaldehyde fixed paraffin embedded biopsy was recut in 4 μm sections and attached to chromium treated glass slides. The specimens were deparaffinized three times in toluene for ten minutes and rehydrated in decreasing concentrations of alcohol. Staining was done with indirect immunofluorescence for the A and B isoantigens and with direct lectin fluroescence for the H isoantigen [1]. The specimen was washed in PBS and incubated for 45 minutes at room temperature with polyclonal anti A or anti B blood typing sera (Ortho Diagnostic Systems, Don Mills, Ont. Canada) diluted 1:10 with PBS. After a PBS rinse the second antibody, fluorescein isothiocyanate (FITC) labelled sheep antihuman Ig (SBL, Stockholm, Sweden) diluted 1:10 with PBS, was added and incubated for 30 minutes at room temperature. After rinsing and mounting in 2% propylgallate glycerol [8], the specimen was inspected in a Zeiss fluorescence microscope. The staining of the H isoantigen was performed by a direct lectin method using Ulex Europaeus FITC (Vector, Burlingame, CA, USA) diluted 1:20 in PBS. The

Table 1. ABH isoantigen status of tumor at diagnosis related to pathological stage (UICC), grade (WHO) and cytoflow ploidy

	ABH isoantigen status			
	pos	Neg	Not assessable	Sum
Stage (UICC)				
Ta	13	7	3	23
T1	0	0	0	0
T2	0	2	0	2
T3	1	5	2	8
T4	0	1	0	1
Tis	1	1	0	2
Sum	15	16	5	36
Grade (WHO)				
G1	5	2	0	7
G2	8	6	3	17
G3	2	8	2	12
Sum	15	16	5	36
Ploidy				
Diploid	12	5	2	19
Aneuploid	3	9	2	14
Not assessable	0	2	1	3
Sum	15	16	5	36

Table 2. ABH isoantigen status of tumor at diagnosis related to 2.5–3.5 years follow-up patient status

Follow-up patient status	Primary tumor ABH isoantigen status			
	Pos	Neg	Not assessable	
Alive, TUR treated	14	8	3	
Alive, Cystectomized		4	2	
Alive, Irradiated	1	3		
Dead (Tumor)		1		
ψ	15	16	5	36

incubation time was 30 min at 37 °C. One control with "wrong" blood group serum was used per section. Vascular endothelium staining was used as positive internal control.

The specimen was regarded as isoantigen positive if 75% or more of the tumor or, when only benign tissue was present, the urothelial area expressed specific staining.

Cytoflow Ploidy Analysis

For cytoflow ploidy assessment [15] the biopsy was mechanically disrupted to form a cell suspension that was fixed in 96% ethanol for 12 hours. RNA was removed with ribonuclease and the cytoplasm digested for 10 min at 37 °C with 0.5% pepsin. Staining of the cell nuclei was done with ethidium bromide for 30 minutes, followed by analysis in a cytofluorometer. The fluorescence intenstiy was sorted in a 256 multichannel analyser and presented as a DNA histogram. Human Ficoll-prepared lymphocytes were used as external standard. For this study the biopsies swere sorted as diploid or aneuploid.

Results

213 biopsies from 36 patients were analysed. For 92 biopsies (43%) it was not possible to determine the ABH isoantigen status. Unspecific fluorescence was the main obstacle in the antigen determinations. It usually affected the peripheral parts of the biopsy and thus in the urothelium; in five of the cancers the isoantigen status thus not known. The data was analysed for the remaining 31 patients.

Tumor category (UICC) and ABH isoantigens were well correlated (Table 1; $p = 0.006$ Pearson correlation coefficients, two tailed significance). Thirteen of 20 assessable tumors with stage pTa expressed normal isoantigens, while higher grade carcinoma expressed isoantigens in one of eight patients with exophytic tumors and in one ot two with pTis (Carcinoma in Situ).

The grade (WHO) of the tumor correlated well with ABH isoantigen status ($p = 0.016$; Table 1). Low grade tumors expressed normal isoantigen (5/7), while G3 tumors were deleted (2/10 expressed isoantigens).

Cytoflow ploidy also correlated to ABH isantigen status, as shown in Table 1 ($p = 0.005$). Diploid tumors were isoantigen positive in 12 of 17 assessable instances, while aneuploid tumors were positive in 3 of 12 cases.

In 15 patients with ABH isoantigen positive tumor biopsies, 11 were diploid, in stage pTa and with histological grade 2 or lower. Three patients had an indication of more aggressive disease in one or two markers, while one woman with Carcinoma in Situ and normal ABH isoantigens had a high grade, aneuploid bladder carcinoma. Among 16 patients with isoantigen deleted tumors, 13 showed aggressive tumor in other markers as well, 8 of them in more than one. Three patients with ABH isoantigen negative tumors were normal in the other three parameters. None of these three has as yet developed an invasive carcinoma.

Table 2 presents patient status at follow-up 2.5–3.5 years after the primary diagnoses. Of 22 ABH isoantigen assessable patients that had been treated by transuretheral resections (TUR), fourteen had expressed normal isoantigens at diagnosis. None of them had developed invasive disease. Nine ABH isoantigen assessable patients with aggressive disease (1 pTa, 2 pT2, 6 pT3; 2 G2, 7 G3) had been radically treated by cystectomy (4) and by full dose irradiation (5). One irradiated patient had died of his cancer, the only death in this series. Only one of these nine patients had tumors expressing normal isoantigens ($p = 0.02$, Fishers exact test, two tailed).

The biopsies taken from normal looking urothelium were technically difficult to assess for ABH isoantigen. Often the small cold cup biopsy showed heavy unsepcific fluorescence. The analysis therefore had to be confined to 20 patients (Table 3). The ABH isoantigen status of the

Table 3. ABH isoantigen, histopathology and cytoflow ploidy status of biopsies of visually normal urothelium related to ABH isoantigen status of the tumor

	Tumor	
	ABH present $n = 15$	ABH deleted $n = 16$
Biopsy ABH		
present	8	2
deleted	4	6
Biopsy histology		
benign	12	12
malignant	2	4
Biopsy ploidy		
diploid	13	13
aneuploid	1	3

normal-looking urothelium tended to match that of the tumor biopsy (14/20 cases), although this relationship was not significant ($p = 0.17$ Fishers exact test two tailed). Four out of the six non-concordant biopsies were deleted, while the tumor contained ABH isoantigen. Of these four patients, one also had an aneuploid biospy and one had concomitant Cis; the other two had G2 tumor but histologically normal mapping. In 10 of 20 cases, normal-looking urothelium failed to express isoantigens. The biopsies from normal-looking bladder urothelium mostly had benign histology (24/30 cases) and a diploid DNA pattern (26/30 cases). ABH isoantigen delection occurred more extensively than did histological and ploidy changes.

Discussion

The importance of specimen treatment for the ABH isoantigen assessment has been stressed by Limas and Lange [12]. They showed a general decrease in isoantigen expression in formaldehyde fixed tissue as compared to freshly processed tissue. This could give false negative isoantigen expression.

Fourty-three percent of our specimens were not assessable. The biopsies from normal-looking urothelium had strong unspecific fluorescence, making ABH isoantigen determinations impossible. This was possible a result of mechanical maltreatment of the specimens by the cold-cup biopsy forceps.

For the visible tumor there was, as expected [1], a strong correlation between ABH isoantigen status and tumor category, tumor grade and ploidy. Tumor category and grade are the common clinical parameters for predicting bladder cancer behavious. Ploidy determination has proved useful in predicting tumor behaviour [15]. The ABH isoantigen was deleted in tumors shown to be aggressive also by other means.

In this material 8/9 patients that later recieved radical cancer treatment had tumors deleted of ABH isoantigens at diagnosis. As only one patient died of this cancer during the observation period, and he was radically treated anyhow, ABH isoantigen determination at the time of diagnosis would not have provided further assistance in treatment planning for these patients.

The biopsies from normal-looking urothelium showed the same ABH isoantigen expression as the tumor in 14 out of 20 of our patients. Stein [13] in 1981 found that in 89% of 103 random biopsies ABH assessment agreed with that of the tumor. He concluded that the ABH isoantigen changes were the earliest measureable changes in the malignant potential of the urothelium; in 25 tumors with normal ABH isoantigen all biopsies were normal. We, on the other hand, found that four of twelve patients with ABH isoantigen normal tumors lacked isoantigen in at least one mapping biopsy. In two of them biopsies also showed other malignant changes, indicating more widespread malignant urothelial involvement. Weinstein [16], in a study of nine bladders from patients with Cis, found deletion of approximately half of the sections of histologically normal epithelium and regarded this as representing low grade Carcinoma in Situ. Furhter study of the same nine specimens also showed abnormalities of the T-antigens expression [3].

Widespread urothelial changes can be observed in transitional cell bladder carcinomas as an indication of involvement of urothelium far from the visible tumor. The ABH isoantigen deletion is one of these observable changes. These changes occur more extensively than do histological and ploidy changes. For deletion to occur, the only required change is in the expression of a glycosyl-transferase enzyme, while histological and observable ploidy changes are of greater magnitude. It might therefore be that ABH isoantigen deletion is one of the first signs of malignant urothelial change. This indicates that bladder mapping with ABH isoantigen estimation in some cases provides additional information compared to isoantigen evaluation of the tumor only. It is not yet known whether this may have a prognostic value.

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