Acta Urol. Jpn. 34: 949~954, 1989

THE STUDY ON CELL SURFACE ANTIGENS IN EPITHELIAL TUMOR OF THE UPPER URINARY TRACT: ABH-ISOANTIGEN AND THOMSEN-FRIEDENREICH ANTIGEN

Hidetoshi FUKATSU, Hitoshi NONOMURA, Yoshimasa MIYAGAWA, Masafumi WAKI, Yukio HATANO, Shinsuke HIRAIWA, Tadashi MURAMATSU, Eiji NISHIKAWA, Yoshiaki YAMADA, Takayoshi SATOH, Nobuaki HONDA and Akio SEGAWA

From the Department of Urology, Aichi Medical University

ABH-isoantigen (ABH-Ag) and Thomsen-Friedenreich antigen (T-Ag) were investigated by the Avidin-Biotin-Peroxidase Complex (ABC) method on 47 patients with epithelial tumor of the upper urinary tract (all patients underwent nephroureterectomy including the cuff of the bladder; 30 patients were diagnosed as transitional cell carcinoma of renal pelvis and 17 ureteral organs). The correlations between ABC expression for ABH-Ag and T-Ag with histological grade, stage and prognosis (5 year survival rate) were studied.

A correlation was observed between grade ($p<0.05$) and deletion of the antigenicity of ABH-Ag, but no correlation was evident with stage and prognosis. A high correlation was evident, however, between grade ($p<0.01$), stage ($p<0.01$) and prognosis ($p<0.01$) and deletion of the antigenicity of T-Ag. The analysis of ABC expression for ABH-Ag and T-Ag may therefore be valuable for predicting the malignant potential in transitional cell carcinoma of the upper urinary tract. T-Ag determination in particular may provide a useful prognostic probe should it find clinical application.

Key words: Renal pelvis and ureteral tumor, ABH-isoantigen, Thomsen-Friedenreich-antigeh

INTRODUCTION

Many studies have showed that blood group antigens are present not only on the surface of red cells but also in normal tissues[1], and they decrease or delete with cellular malignant change[2,3]. Blood group antigens have also been found in the urothelium, and there is an increasing body of literature concerning the prognostic value of such antigens in bladder tumors[4-7]. We previously investigated ABH-isoantigen (ABH-Ag) and Thomsen-Friedenreich antigen (T-Ag) expression by the Avidin-Biotin-Peroxidase Complex (ABC) method in bladder tumors and reported a high correlation between deletion of antigenicity and malignant potential[8]. In the present study, we investigated ABH-Ag and T-Ag expression in upper urothelial tumors and evaluated changes in antigenicity in relation to histological grade, stage, and patient survival.

SUBJECTS AND METHODS

Subjects were all admitted to the urology departments of hospitals affiliated with Aichi Medical University during the 13-year period from January, 1975 to December, 1987.

Specimens were surgically removed and subjected to histopathological examinations, and 47 cases of transitional cell carcinoma of the renal pelvis or ureter were diagnosed (30 cases involving the renal pelvis and 17 cases involving the ureter). Subjects included 34 males and 13 females ranging in age from 41 to 82 years (median age: 62.3), twenty subjects were blood type A, 11 were type B, four were type AB and 12 were type O. Grades were determined according to conventions

Table 1. Avidin-Biotin-Peroxidase Complex method

ABH-Isoantigen
1) deparaffinization
2) treat with 0.3%H_2O_2-methanol (30min)
3) wash tissue section with PBS
4) treat with normal serum goat (30min)
5) treat with DAKO Mouse Monoclonal Anti-Blood Group A.B.H (2 hour room temparature and one night at 4℃)
6) wash tissue section with PBS
7) treat with anti mouse serum goat (30min)
8) wash tissue section with PBS
9) traet with Avidin-Biotin Peroxidase Complex (30min)
10) wash tissue section PBS
11) DAB (diamino benzidine)
12) Mayer hematoxylin
13) mount
T-antigen
1) deparaffinization
2) treat with 0.3%H_2O_2-methanole (30min)
3) wash tissue section with PBS
4) treat with 10%NSS + PNA (0.005mg/ml) (2 hour .room temparature and one night at 4℃)
5) wash tissue section with PBS
6) treat with Avidin-Biotin-Peroxidase Complex (30min)
7) wash tissue section with PBS
8) DAB (diamino benzidin)
9) Mayer hematoxylin
10) mount
Cryptic T-antigen
1) deparaffinization
2) treat with 0.3%H_2O_2-methanole (30min)
3) wash tissue section with PBS
4) treat with neuraminidase type V (0.04unit/ml)
5) treat with 50%NSS + PNA (0.005mg/ml) The other same as T-antigen

for bladder cancer established by the Japanese Urological Association[9], and stages were determined by Batata classification[10], i.e., grades I and II were low grade and grade III was high grade, stages A and B were low stage and stages C and D were high stage.

Surgically isolated tissue specimens which had been embedded in paraffin were deparaffinized and stained for ABH-Ag and T-Ag by the ABC method. Procedural sequences are described in Table 1. ABH-Ag stained positively in over 20% of the cases with blood types A, B and AB, while less than 10% tested positive for blood type O. Positive responses were designated normal antigenicity and negative responses were designated abnormal antigenicity. The presence of T-Ag is masked in normal tissue by sialic acid residue and is exposed (T-Ag positiveness) only after neuraminidase treatment. Tissue which stained T-Ag positive after neuraminidase treatment (cryptic T-Ag positive) was classified as normal antigenicity, and other T-Ag positive and T-Ag negative tissue was classified as abnormal antigenicity. Determination of antigenicity was positive after staining in over 10% of the cases. Significant differences were determined by the χ^2 test.

RESULTS

1. Antigenicity and Grade

a) ABH-Ag

Among the 47 subjects, the tissues of 25 (53.2%) showed abnormal ABH-Ag expression: one out of eight grade I specimens (12.5%), eight of 17 grade II specimens (47.1%) and 16 of 22 grade III specimens (72.7%). That is, among the 25 specimens classified as low grade, 16 (64%) were normal and nine (36%) were abnormal. Among the 22 specimens classified as high grade, six (27.3%) were normal and 16 (72.7%) were abnormal. A higher frequency of abnormality was observed in the high classification compared to the low grade, and a significant correlation was noted between deletion of antigenicity and grade ($p<0.05$) (Table 2).

Table 2. Relationship between ABH-Ag and histological grade

	ABH-Ag		
Grade	Normal	Abnormal	Total
I	7	1	8
II	9	8	17
III	6	16	22
Total	22	25	47

Normal : ABH-Ag(+) Low grade(GI, II)
Abnormal : ABH-Ag(−) High grade(GIII) $P<0.05$

Table 3. Relationship between T-Ag and histological grade

	T-Ag		
Grade	Normal	Abnormal	Total
I	8	0	8
II	14	3	17
III	6	16	22
Total	28	19	47

Normal : Cryptic T-Ag(+) Low grade(GI, II)
Abnormal : T-Ag(+) or CrypticT-Ag(−) High grade(GIII) $P<0.01$

b) T-Ag

Tissues of 19 subjects (40.4%) showed abnormal T-Ag expression: none of 8 with grade I (0%), three of 17 grade II specimens (17.6%) and 16 of 22 grade III specimens (72.7%). Thus, among the 25 specimens classified as low grade, 22 (88%) were normal and three (12%) were abnormal. Among the 22 specimens classified as high grade, on the other hand, six (27.3%) were normal and 16 (72.7%) were abnormal. The frequency of abnormality was higher in the high grade classification, and a significant correlation was observed between deletion of antigenicity and grade ($p<0.01$) (Table 3).

2. Antigenicity and Stage

a) ABH-Ag

Among the 47 subjects, the tissues of 25 (53.2%) showed abnormal ABH-Ag expression: six out of 17 stage A specimens (35.3%), five of seven stage B (71.4%), six of 11 stage C (54.5%), and eight of 12 stage D specimens (66.7%). Of 24 specimens classified low stage, 13 (54.2%) were normal and 11 (45.8%) were abnormal. Among the 23 specimens classified as high stage, nine (39.1%) were normal and 14 (60.9%) were abnormal. Although a higher frequency of abnormality was observed in the high stage specimens, a significant correlation was not observed between antigenicity deletion and stage ($p>0.05$) (Table 4).

Table 4. Relationship between ABH-Ag and histological stage

	ABH-Ag		
Stage	Normal	Abnormar	Total
A	11	6	17
B	2	5	7
C	5	6	11
D	4	8	12
Total	22	25	47

Normal : ABH-Ag(+) Low stage(A, B)
Abnormal : ABH-Ag(−) High stage(C, D) $P>0.05$

b) T-Ag

Nineteen of the 47 specimens (40.4%) were abnormal: one of 17 stage A specimens (5.9%), two of seven stage B (28.6%), six of 11 stage C (54.5%), and 10 of 12 stage D specimens (83.3%). Twenty-one (87.5%) of the 24 low stage tissues were normal and three (12.5%) were abnormal, while seven (30.4%) of 23 high stage specimens were normal and 16 (69.6%) were abnormal. A higher frequency of abnormality was evident in the high stage tissues and a significant correlation was noted between antigenicity deletion and stage ($p<0.01$) (Table 5).

Table 5. Relationship between T-Ag and histological stage

Stage	T-Ag Normal	Abnormal	Total
A	16	1	17
B	5	2	7
C	5	6	11
D	2	10	12
Total	28	19	47

Normal : Cryptic T-Ag(+) Low stage(A, B)
Abnormal : T-Ag(+)or CrypticT-Ag(−) High stage(C, D) P<0.01

3. Antigenicity and Prognosis

Prognosis follow-up was possible in 43 cases. Survival rates were determined by the Kaplan-Meier method, and suirvival rates curves were tested for signficant differences by the generalized Wilcoxon method. The five-year survival rate for all 43 subjects was 62.8%. Although differences were observed in antigenicity differentiated 5-year survival rates for ABH-Ag, there were no significant correlations since 14 (73.7%) of 19 patients with ABH-Ag normal response, and 11 (45.8%) of 24 with ABH-Ag abnormal response survived for five years (Fig. 1). Significant differences were observed, however, for T-Ag wherein 22 (92%) of 24 patients testing normal and none (0%) of the 19 patients testing abnormal survived five years ($p<0.01$) (Fig. 2).

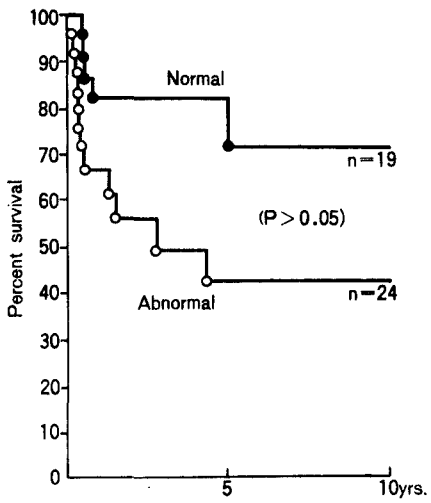

Fig. 1. Relationship between ABH-Ag and survival rate

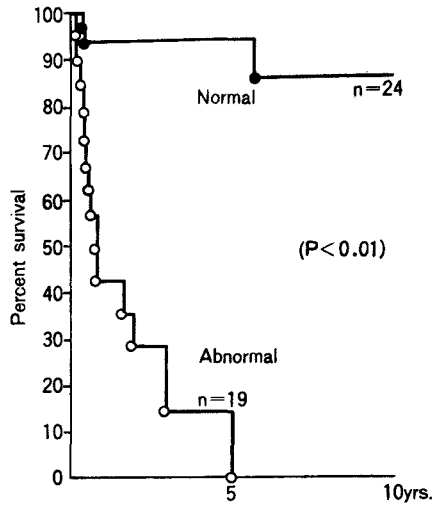

Fig. 2. Relationship between T-Ag and survival rate

DISCUSSION

Examination of ABH-Ag and T-Ag expression in the saccharide side chain of the cellular membrane[11], which occur with cell mutation or pre-mutation abnormal differentiation of the membrane, has been reported useful as a probe in determining a tumor's biological malignant potential. Numerous studies in the field of urology concern tumors of the bladder, and the usefulness of ABH-Ag and T-Ag expression as tumor markers has been confirmed[12,13], but there has been relatively few investigations into their applicability to epithelial tumors of the upper urinary tract.

Kagawa et al.[14] studied ABH-Ag and T-Ag expression in 38 cases of transitional cell carcinoma of the renal pelvis and ureter using the specific red cell adherence method and reported a correlation between ABH-Ag expression and grade, stage and survival rate, but observed no significant T-Ag correlation. Igawa et al.[15,16] investigated 39 cases of transitional cell carcinoma of the renal pelvis and ureter using the ABC method and did not observe any significant correlation between ABH-Ag and grade or stage, but did report a significant correlation between T-Ag and grade and stage ($p<0.01$). Results of multivariate studies show that ABH-Ag in Cox's

proportional hazards model and both ABH-Ag and T-Ag in linear discriminant analysis are important prognostic indicators. Sasaki et al.[17], in a study of 13 cases of tumor of the renal pelvis and ureter, reported the possibility of better prognostication by testing for the presence of ABH-Ag and T-Ag antigenicity. Among subjects in the present study, a significant correlation was observed between ABH-Ag and grade, but no correlation between ABH-Ag and stage or survival rate. On the other hand, significant correlations were found between T-Ag and stage, grade and survival rate. Based on these findings, examination of ABH-Ag and T-Ag antigenicity to determine biological malignant potential is considered a useful tool in making prognoses in cases of epithelial tumor of the upper urinary tract when used in conjunction with histopathological examination to determine histological malignant potential, and it is further thought that T-Ag is particularly useful as a prognosis probe.

REFERENCES

1) Holborrow EJ, Browin PC, Glynn LE, Hawes MD, Gresham GA, O'Brien TH and Coombs RR: The distribution of the blood group. An antigen in human tissue. Br J Exp Pathol **41**: 430-437, 1960
2) Kovarik S, Davidsohn I and Stejskal R: ABO antigen in cancer detection with the mixed cell agglutination. Arch Pathol **86**: 12-21, 1968
3) Springer GF, Desai PR and Banatwala I: Blood group MN antigens and precursors in normal and malignant human breast glandular tissue. J Natl Cancer Inst **54**: 335-339, 1975
4) Limas C and Lange PH: Altered reactivity for A,B,H antigens in transitional cell carcinomas of the urinary bladder. Cancer **46**: 1366-1373, 1980
5) Sasaki K: The study of ABH isoantigen of superficial urothelial tumor and carcinoma in situ of the bladder by the Avidin-Biotin-Peroxidase Complex (ABC) method. Jpn J Urol **74**: 1326-1338, 1983
6) Coon JS, Weistein RS and Summers JL: Blood group precusor T antigen expression in human urinary bladder carcinoma. Am J Clin Pathol **77**: 692-699, 1982
7) Oda Y: The study on Thomsen-Friedenreich antigen in bladder tumors. Acta Urol Jpn **31**: 407-418, 1985
8) Fukatsu H, Yamada H, Nonomura H, Miyagawa Y, Waki M, Hatano Y, Hiraiwa S, Muramatsu T, Nishikawa E, Yamade Y, Satoh T, Honda N and Segawa A: The study on Thomsen-Friedenreich antigen in bladder tumor. Acta Urol Jpn **34**: 260-267, 1988
9) Japanese Urological Association. The Japanese Pathological Society. General rule for clinical and pathological studies on bladder cancer. 1st ed pp. 62-86, Kanahara publication, Tokyo, 1980
10) Batata AM, Whitmore WF, Hilaris BS, Tokita N and Grabstald H: Primary carcinoma of the ureter. A prognostic study. Cancer **35**: 1626-1632, 1975
11) Springer GF, Desai PR, Yang H and Murthy MS: Carcinoma-associated blood group MN precursor antigens which all humans prossess antibodies. Clin Immunol Immunopathol **7**: 426-441, 1977
12) Summers JL, Coon JS, Ward RM, Falor WH, Miller AW and Weinstein RS: Prognosis in carcinoma of the urinary bladder based upon tissue blood group ABH and Thomsen-Friedenreich antigen status and karyotype of the intial tumor. Cancer Res **43**: 934-939, 1983
13) Kobayashi H: The study of cell surface antigen in bladder tumors. ABH-isoantigen and Thomsen-Friedenreich antigen. Acta Urol Jpn **33**: 535-544, 1987
14) Kagawa S, Takigawa H, Ghazizadeh M and Kurokawa K: Immunohistological detection of T antigen and ABH blood group antigens in upper urinary tract tumors. Brt J Urol **57**: 386-389, 1985
15) Igawa M, Kadomoto Y, Yamazaki A, Nakano H and Nihira H: Studies in ABH blood group antigen and Thomsen-Friedenreich antigen in renal pelvis and ureteral carcinomas. A comparison of pathological findings of tumors with ABH blood group antigens and Thomsen-Friedenreich antigen. Nishinihon J Urol **50**: 1143-1149, 1987
16) Igawa M, Kadomoto Y, Yamasaki A, Nakano H and Nihira H: Studies on ABH blood group antigen and Thomsen-Friedenreich antigen in renal pelvic and ureteral carcinomas. Estimation of the prognostic value of ABH blood group antigen and Thomsen-Friedenreich antigen by multivariate analyses. Nishinihon J Urol **50**: 1151-1155, 1988

17) Sasaki K, Maru A, Sakashita S and Koyanagy T: Carcinoma in situ of the urinary bladder. Clinical experience and preliminary study of Thomsen-Friedenreich antigen. Jpn J Urol 75: 269-277, 1984

(Accepted for publication Jan 11, 1989)

和文抄録

上部尿路上皮腫瘍における膜抗原の研究
—ABH-isoantigen と Thomsen-Friedenreich antigen—

愛知医科大学泌尿器科学教室（主任：瀬川昭夫教授）
深津　英捷，野々村仁志，宮川　嘉真，和気　正史
羽田野幸夫，平岩　親輔，村松　　直，西川　英二
山田　芳彰，佐藤　孝充，本多　靖明，瀬川　昭夫

上部尿路上皮腫瘍47例（すべて腎・尿管全摘出術兼膀胱部分切除術施行．30例は腎盂の移行上皮癌，17例に尿管の移行上皮癌）を対象に Avidin-Biotin-Peroxidase Complex（ABC）法を用いて ABH-isoantigen（ABH-Ag）と Thomsen-Friedenreich antigen（T-Ag）を検索し，その抗原性の変化と病理組織学的異型度，深達度および予後（5年生存率）との関係について検討した。

ABH-Ag では抗原性の消退と異型度との間に有意の相関（$p<0.05$）が認められたが，深達度および予後との間には有意の相関はみられなかった．一方，T-Ag では抗原性の消退と異型度，深達度ならびに予後との間に有意の相関（$p<0.01$）が認められた．以上より，上部尿路移行上皮癌における ABH-Ag および T-Ag の検索は，その癌の malignant potential を知る上で有益であり，予後予知因子としては T-Ag はより有用性が高いと考えられた．

（泌尿紀要 35：949-954，1989）