Tumor Behavior in Transitional Cell Carcinoma of the Bladder in Relation to Chromosomal Markers and Histopathology

V. Ramesh Babu,1 Michael D. Lutz, Brian J. Miles, Riad N. Farah, Lester Weiss, and Daniel L. Van Dyke

Medical Genetics and Birth Defects Center [V. R. B., D. L. V. D., L. W.], Department of Urology [M. D. L., B. J. M., R. N. F.], Henry Ford Hospital, Detroit, Michigan 48202

ABSTRACT

Tumor cells from direct harvests and short term cultures were karyotyped from 15 patients with transitional cell carcinoma of the bladder. There were two tumors with an apparently normal diploid karyotype, eight with counts up to 50 and with marker chromosomes, and five with counts of 60 or more and with markers. The median interval between recurrences was 3 months for the near-diploid, and 3 months for the near-polyploid tumors. One patient whose tumor was normal diploid had a recurrence at 5 months and the second patient whose tumor had normal diploid tumor had no recurrence over 15 months. Four tumors (27% of the series) had a rearrangement involving band 3p14: three had +der(5)(3;5)(p14;p14) and one had +der(6)(1;3)(p21;p14;p23). Duplication 3p14 → 3pter was observed in four tumors, and deletion 11p15 → 11pter in five. Three other abnormalities were observed in three cases each: deletion 5p14→5pter, duplication 1q23→1q32 and deletion 6q21→6qter. Trisomy 7 was observed as a sole clonal abnormality in one carcinoma in situ. Thirteen of 15 patients had recurrence of their tumor. Tumor progression (either in stage or grade) was evident in seven recurrent tumors. Among the seven with tumor progression, three had 11p deletion, two had 11p deletion plus 3p duplication, one had 3p duplication, and one had trisomy 7. Four of the five that had 11p deletion underwent cystectomy and three have died. Three of eight near-diploid tumors progressed and four of five near-tetraploid tumors progressed. It will be important to characterize any cytogenetic changes that are of prognostic value, since the categorization of bladder tumors by other methods has been problematic.

INTRODUCTION

Transitional cell carcinomas of the bladder are a heterogeneous group. Histopathological characteristics have not reliably predicted tumor recurrence or progression. Other potential predictors of recurrent or invasive disease have included ABO blood group surface antigens, Thomsen-Freidenreich antigen, DNA content, chromosome modal number, and marker chromosomes (1–7). Of these, cytogenetic studies have been the more useful predictors of tumor behavior. Falor et al. (5) found that of 65 patients followed for up to 11 years, tumors recurred in all 45 patients whose tumors had a marker chromosome, but in only two of 20 whose tumors did not have a marker chromosome. However, the identity of the marker chromosomes was unclear since banding methods were not employed. With banding, nonrandom abnormalities of chromosomes 1, 5, 6, 7, 9, 11 (including 11p deletion), and 13 were described (8–12). Molecular genetic confirmation was provided for deletion of segments of 11p (13). Gibas et al. (9, 10) described i(5p), monosomy 9, and trisomy 7 as the primary changes in some subgroups of bladder cancer patients. We report here a study of G-banded karyotypes of direct and cultured tumor cells from 15 patients with transitional cell carcinoma of the bladder. Duplication 3p14 → 3pter and deletion 11p15 → 11pter were observed in four and five tumors, respectively. All five patients with 11p deletion had tumor progression (two of these also had 3p duplication), four of these underwent cystectomy, and three have died.

MATERIALS AND METHODS

A brief clinical and pathological description of each patient is given in Table 1. Tissue specimens for karyotyping were obtained prior to recurrence, chemotherapy or radiation in all but four instances (cases 7, 8, 9, and 12). The specimens from cases 9 and 12 were obtained at cystectomy. Cases 7 and 8 had previous recurrences. The tumor material was transported within 2 h to the cytogenetic laboratory in sterile medium F10 supplemented with 15% fetal bovine serum. Each sample was minced with scalpels and the tissue fragments were dissociated with 0.8% Collagenase in Hanks' buffer and F10 medium for 2–3 h at 37°C. Whenever possible, both direct harvest and short term culture methods were used. For direct preparations, a final concentration of 0.01 μg/ml colcemid was added for 2–3 h to the cell suspension. For short term cultures, 0.5–1 ml of dissociated material was mixed with 4 ml fresh medium F10 supplemented with 15% fetal bovine serum and antibiotics, and distributed among two to five flasks. The total volume in each flask was brought to 3 ml with additional medium. When sufficient growth was observed, standard cytogenetic harvesting techniques were used. Karyotypes from 14-day or more (long term) cultures were excluded from the study, since these were found to have fibroblastic appearance in culture, and apparently normal karyotypes.

Microscope slides were G-banded using Trypsin and Giemsa. The number of cells analyzed varied from three to 40. To define a clonal abnormality, we followed the recommendations of the international workshops on chromosomes in leukaemia (14). Any structural abnormality present in two cells was considered clonal. Chromosome loss in three cells or more, or chromosome gain in two cells or more, was also considered clonal.

RESULTS

Chromosomes were obtained on all 15 specimens in the series. Each tumor was categorized into one of three chromosomal groups: normal, near diploid, and near polyploid. Tumors in the normal group had apparently normal diploid karyotypes; tumors in the near-diploid group had 43–50 chromosomes including markers; and tumors in the near-polyploid group had 69–130 chromosomes including markers.

The clonal abnormalities are listed in Table 2 and a detailed description of each tumor karyotype is provided in the Appendix. Chromosomes 1, 3, 5, 6, and 11 were involved in clonal structural rearrangements in five or more tumors. Chromosome 7, 8, and 18 abnormalities were observed in three tumors each. Chromosome 9 and Y abnormalities were observed in two tumors each. Chromosomes 2, 4, 10, 12, 16, and X were abnormal in one tumor each.

In three tumors (cases 11, 13, and 15), an unbalanced translocation involving the short arms of chromosomes 3 and 5 was observed: −5,+der(5)(3;5)(p14;p14). In these tumors, there were in addition at least three or four normal copies of chromosome 3, but only one or two normal copies of chromo-
some 5. Thus, there was a net increase of region 3p14 — 3pter and a net decrease of region 5p14 — 5pter (Fig. 1). In one tumor, a similar translocation involving 6p was observed: −6,+der(6)(3;6)(p14;p23) (case 12, Fig. 2). Deletion of 11p15 — 11pter was observed in two tumors (cases 5 and 13), and one entire 11p was lost in three tumors (cases 7, 12, and 14) (Fig. 3). Duplication 1q21-23 — 1qter (Fig. 3) and deletion 6q11-21 — 6qter (Fig. 1) were observed in three tumors each. Loss of the Y chromosome was observed in two tumors. Trisomy 7 was observed as a sole clonal abnormality in the only carcinoma in situ studied in the present series (Fig. 4).

The median duration between the last two recurrences or since the last clinical presentation was 3 months for near-diploid tumors, and 3 months for near-polyploid tumors. One patient whose tumor was normal diploid had a recurrence at 5 months. Two patients have been free of disease (no recurrence) for the last 15 months: case 2 in the normal group, and case 4 in the near diploid group with a balanced translocation t(6;12) (Fig. 2). The mean duration between the last two recurrences was 5.5 months for tumors with 3p duplication (cases 11 and 15), 4 months for tumors with 11p deletion (cases 5, 7, and 14), and under 2 months for tumors with both 3p duplication and 11p deletion (cases 12 and 13).

Three of 8 near-diploid tumors (cases 5, 7, and 9) have progressed either in grade or stage. Two of these had 11p deletion (cases 5 and 7) and one had trisomy 7 (case 9). Two patients in the near-diploid group underwent cystectomy (cases 5 and 9), and one patient died 2 months after the most recent recurrence (case 7). Four of five near-polyploid tumors have progressed. One of these had 3p duplication (case 15), one had 11p deletion (case 14), and two had both (cases 12 and 13). All three patients with near-polyploidy and 11p abnormalities underwent cystectomy and the two who had 3p and 11p abnormalities have died within 2 months of their cystectomy.

DISCUSSION

We have observed a characteristic rearrangement of 3p in four of 15 tumors: t(3;5)(p14;p14), t(3;5)(p14;p23) in three tumors and t(3;5)(p14;p23) in one. In each case the translocation was unbalanced with a net increase of region 3p14 — 3pter and decrease of region 5p14 — 5pter or 6p23 — 6pter. Constitutional deletion of 3p14-3p21 was observed in one bladder cancer patient (15), but the tumor was not karyotyped. Atkin and Baker (8) described a marker chromosome containing "most of 3p" in one of 10 bladder cancers. Because of the 6p involvement in the translocation in one case, it appears that 3p duplication may be more important than 5p or 6p deletion at some stage of tumorigenesis in one group of bladder cancers. Other
rearrangements of 3p have been observed in other epithelial tumors, including deletion of 3p14-3p21 in small cell carcinoma of the lung (16), translocations in familial renal cell carcinoma (17-18), and mixed parotid gland tumor (19). The present observation of 3p duplication in four of 15 (27%) patients further implicates 3p genes in the pathogenesis of epithelial tumors. Deletion or inactivation of 3p genes has been postulated for the pathogenesis of the other epithelial tumors (20). In the neuroblastoma, an ectodermal tumor, duplication or amplification of the oncogene N-myc has been correlated with tumor stage and behavior (21). Duplication of the oncogene ras-1, which resides at 3p25, may likewise be important in the pathogenesis of some bladder tumors.

Duplication 3p was associated with a high risk for tumor progression in this series. All four patients who had 3p duplication developed one or multiple recurrences, and the median duration between the last two recurrences was 2.5 months. Three of the four duplication 3p tumors progressed and two patients underwent cystectomy and later died (one was lost to follow-up). In comparison, progression was seen in only four of 11 tumors without 3p duplication.

Consistent with some of the previous cytogenetic studies (8-10), deletion of 11p was observed in five of 15 (33%) tumors. The breakpoints were 11p15 in two, and 11p11 in three. Vanni et al. (11) described involvement of band 11p15 in the formation of a marker chromosome in two bladder tumors. The H-ras-1 oncogene was active in some bladder cancer cell lines (22-23).

By molecular analysis using insulin and ras oncogene probes, both localized to 11p15, Fearon et al. (13), observed loss of 11p genes in tumor cells from five of 12 bladder cancer patients. Thus, genes important in the pathogenesis of bladder cancer appear to reside at or distal to 11p15. Combining our results with those of previous studies, translocations or deletions involving 11p15 were observed in 24 of 48 (50%) bladder tumors. Since loss of 11p genes is common in bladder as well as Wilms (24) tumors, deletion or rearrangement of these genes probably play an important role in initiation or progression of these tumors.

Deletion 11p was associated with a poor prognosis in this series. All five patients with 11p deletion had progression, whereas tumor progression was evident in only 2 of 10 patients without 11p deletion. Four underwent cystectomy, and three have died. The median duration between the last two recurrences was 3 months. Therefore, 11p deletion and 3p duplication may be associated with high risk for progression.

Deletion 6q and duplication 1q were both observed in three tumors. Deletion 6q has been reported in malignant melanomas (25), ovarian cancer (26), and acute lymphocytic leukemia (27). Duplication 1q has been described in several cancers including breast (28), cervix (28), ovary (28-29), and testis cancers (30). Further, duplication 1q has been reported as a secondary change as the tumor progresses, rather than as a primary event, in ovarian and hematological malignancies (29). The significance of these two changes in bladder cancer is likely to be clarified as the sample size increases.
TRANSITIONAL CELL CARCINOMA OF THE BLADDER

In 1952, Melicow suggested that carcinoma in situ was a distinct entity that represented bladder cancer's earliest stage (31). However, more recent data indicate that two forms of carcinoma in situ exist: one with and one without infiltrative capabilities (32). Our observation of trisomy 7 (case 9) in one carcinoma in situ is distinct from our other bladder tumors, in terms of the chromosome abnormalities. The duration between the last two recurrences was 2 months and the patient underwent cystectomy, so this tumor probably represented the infiltrative form. Gibas et al. (10) described trisomy 7 as a sole abnormality in one grade 3 invasive (T3) bladder carcinoma. It is plausible that trisomy 7 is associated with aggressive tumor behavior in a discrete subgroup of bladder carcinoma.

Gibas et al. (9, 10) described i(5p), monosomy 9, and trisomy 7 as the primary changes in some subgroups of bladder cancer patients. These were also observed in our series, but only trisomy 7 was observed as a sole abnormality. It is uncertain whether 3p duplication, deletion 5p or deletion 11p represents primary or secondary events, because none was observed as a sole abnormality. It is possible that duplication 3p and deletion 11p are secondary changes associated with tumor progression. Further studies will perhaps clarify the nature of the later changes.

Two tumors in the normal group had an apparently normal karyotype. The recurrent tumor in one patient apparently regressed to grade 1 from grade 2. Even though the cultures in the normal group were harvested within 3 and 5 days, it is possible that only normal fibroblasts instead of tumor cells grew in these cultures. It is also possible that a chromosome change was present but beyond the resolution of a midmetaphase preparation. Interpretation of normal karyotypes from short

Fig. 2. A near-tetraploid karyotype from the recurring, invasive grade 3 tumor of case 12. Arrows, structural abnormalities: t(1;3;6)(q21;p14;p23), i(8q), and i(11q). m1-m5, are unidentified markers. In all other cells, the der(1) and der(3) were presumably lost and there were, in addition to one copy of der(6), at least three or four normal copies of number 3 plus two or three copies of number 6.

Fig. 3. Partial karyotypes illustrating unbalanced rearrangements of chromosomes 1 and 11 from case 7 (A); case 13 (B, C); case 12 (D); case 14 (E); and case 6 (F). Some structurally normal chromosomes are placed upside down. In A, the derived 11 from a t(1;11)(q23;p11) is compared with a number 1 at left and number 11 at right. In B and C, derived 11s from a t(1;11)(q21;p15) are compared with a number 1 at left and number 11 at right. In D, the isochromosomai(11q is compared with a number 11. In E, the derived 11 from a t(8;11)(q11;p11) is compared with a number 8 at left and number 11 at right. In F, the duplication 1q21→1q32 chromosome is compared with a normal number 1.

Fig. 4. The karyotype from a grade 3, invasive carcinoma in situ tumor, case 9: 47,XY,+7. Arrow, trisomy 7.
term cultures of tumor material continues to be a problem. It will be of interest to karyotype recurrences of these tumors, should they occur.

From previous cytogenetic studies, it appeared that in general near-diploid tumors had a lesser tendency to invade and a higher level of differentiation compared to tumors in the triploid range (33). In order to address the potential value of ploidy as a prognostic indicator, we divided our tumors into three groups: normal (two cases discussed above), near-diploid, and near-polyploid. Four of eight near-diploid tumors were invasive, whereas four of five near-polyploid tumors were invasive. Three of eight near-diploid tumors progressed, whereas four of five near-polyploid tumors progressed. However the median duration between recurrences was similar for near-diploid and near-polyploid tumors (3 months for both), and neither tumor progression nor tumor invasiveness was restricted to near-polyploid tumors. Thus although ploidy alone appears to be correlated with invasion and progression, it is not a completely reliable prognostic indicator thereof.

It appears that specific chromosome changes may confer differing potentials for progression in bladder cancer. Of the seven tumors that progressed, three had 11p deletion, one had 3p duplication, two had both these changes, and one had trisomy 7. The only other patient (case 11) with a near-polyploid tumor and 3p duplication was lost to follow-up. Of the four near-diploid tumors that had recurrence and abnormalities other than 7, 3p, or 11p none has progressed. This suggests that a higher likelihood of progression may be reliably predicted by certain characteristic chromosome changes including 3p duplication, 11p deletion, and trisomy 7. Considering the heterogeneity of transitional cell carcinomas of the bladder, it is not surprising to find several different characteristic chromosome changes for this neoplasia. The number of patients with each chromosome change is too small to permit clinical application at present. If larger studies reveal any subgroup with a characteristic change, cytogenetic studies of bladder cancer will undoubtedly prove useful for patient management, just as cytogenetic studies of leukemia are becoming increasingly useful.

**APPENDIX**

Detailed descriptions of clonal tumor karyotypes.

**Case 1.** Seven cells from 5-day cultures had an apparently normal karyotype: 46,XY. The patient had a recurrence within 5 months of the initial study. The tumor regressed to a lower malignant grade 1 from grade 2.

**Case 2.** Ten cells from 3-day cultures had an apparently normal karyotype: 46,XX. Seven cells had apparently random chromosome losses. The tumor has not recurred in the last 15 months.

**Case 3.** Four cells from 2-day cultures had a karyotype of 46,X,Y,del(4)(p11),del(10)(q22). The patient had a recurrence within 3 months of the first study and the tumor retained the grade and stage.

**Case 4.** Five cells from 2-day cultures had a karyotype of 45,X,Y,-18,t(6;12)(p25;q21). One of the five cells had two other chromosomes missing as a probable artifact of slide preparation. The tumor has not recurred in the last 18 months.

**Case 5.** Thirteen of 19 cells from overnight cultures had a karyotype of 46,XX,t(1;18)(q3;23),t(2;6)(q11; q13),del(9)(q2),t(11;11)(p15;q23). Two cells had the above modal karyotype, but normal 11s. Two cells had the modal karyotype, normal 11s, and del(6)(q21). Two cells had modal karyotype, normal 11s, and del(7)(q22). The patient had five recurrences in 10 months. At the last recurrence, the tumor progressed from noninvasive to invasive and the patient underwent cystectomy. Karyotypes were also obtained from the first recurrent tumor and the tumor obtained at cystectomy. All of the marker chromosomes identified in the initial study were recognized, although the morphology of the chromosomes was suboptimal in the recurrent tumors. The tumor apparently did not undergo karyotype evolution. In the initial study, three cells had a karyotype of 45,XX,-Y. These cells may have been of stromal origin, since loss of the Y chromosome was also observed in four of 100 cells of lymphocyte cultures of this 70-year-old man.

**Case 6.** Nine cells from 2-day cultures had a karyotype of 46,XY,dup(1)(q21q32). One cell was 45,XY,-9 with normal 1s. The patient had recurrence within 3 months of the first surgery with no change in tumor grade or stage.

**Case 7.** All 20 cells analyzed from direct preparation had a t(1;11) with a net duplication of 1q23→1qter and deletion of 11p11→11pter, three markers, and apparently random loss and gain of chromosomes. One cell had a karyotype of 46,XX,-8,-11,-14,-18,-19,20,+2,+22,+der(11)(11:11)(q23p11),+3 mar. The markers may have been derived from chromosomes 8 and 14, and did not appear to contain 11p material. The patient had five recurrences in his last 3 years and died 2 months after the most recent recurrence.

**Case 8.** Four cells from 3-day cultures had the following karyotypes: 45,X,-Y,46,XY,-del(16)(q22); 43,X,Y,-3,-22,del(16)(q22); and 42,XX,-Y,-10,-15,-16. With the exception of del(16)(q22) and -Y, the other chromosome losses were probably random. Y Chromosome loss was also observed in one of 100 cells of the patient's peripheral lymphocytes. The tumor has recurred twice in the last 5 years with no change in grade or stage.

**Case 9.** From tumor material obtained at cystectomy, four of five cells from 14-day cultures had a karyotype of 47,XXY,+7. One cell was 48,XY,+5,+18,-22,del(4)(p12),+del(7)(q22),t(14;14)(p32;q35).

**Case 10.** Of 11 cells from 11-day cultures, six had 43–47 chromosomes with abnormal but different karyotypes. The only clonal abnormality recognized was +18. There were two to four unidentified markers in each cell. Another five cells had counts of 43–47 but were not completely analyzable.

**Case 11.** Thirty-four of 40 cells from 3-day cultures had 80–92 chromosomes and six cells had 42–48 chromosomes. There were three or four unidentified markers in each near diploid cell. One X chromosome was lost in each near diploid cell. One near-diploid cell had a karyotype of 44,X,-5,-11,-12,-15,-16,-17,+8,t(1;9)(p11;p11),+der(5)(3;5)(p14;q14),t(6;8)(p11;p11),+3 mar. Complete analysis of the polyplid cells was not possible, but two copies of each of the t(1;9), der(5)(3;5), and t(6;8) were present. The patient had a recurrence 3 months after the initial surgery with no change in grade or stage.

**Case 12.** Fifteen cells from 2-day cultures had 60–90 chromosomes. Four of the markers were identified as i(8q), i(11q), der(6)(1;3;6)(q21;p14;p23), and t(7?)(q32?). One cell had a karyotype of 69,XYY,+2,+4,6,7,9,-10,-11,-12,-13,-11,13,-14,15,16,17,17,+19,+19,+20,21,1+der(3),+der(6)(1;3;6)(q21;p14;p23),+i(6q),+i(8q),+i(11q),+i(11q),+5 mar. The patient died 4 months after cystectomy due to renal failure.

**Case 13.** Fourteen cells from 5-day cultures had counts of 62–134 chromosomes. Each cell had a der(11)(11:11)(q21;p15) with a net duplication 1q and deletion 11p. In three cells a der(5)(3;5)(p14;p14) was observed, with a net duplication 3p and deletion 5p. One cell had a karyotype of 62,XY,+1,+1,2,2,3,3,7,8,10,-11,12,+13,-13,15,18,-21,+22,t(12;2p23),t(7;8)(q27;?),+der(11)(11;11)(q21;p15),+i(5p),+i(5p),+3 mar. Four cells had one chromosome with one homogeneously stained region (HSR) below the centromere about the size of 18, and one cell had four double minutes (DM) without HSR. The patient had a recurrence within 2 months after the surgery and the nodal karyotype changed from near-tetraploid to near-triploid with the original markers plus a new del(6)(q21). The HSR and DM were not observed at this recurrence. The patient underwent cystectomy and died 2 months later.

**Case 14.** Three cells from 5-day cultures had 90–120 chromosomes. Of several markers four were identified as del(1)(X)(q21), t(3;7)(q27;?), 7(5p), and t(8;11)(p11;q11). The patient had a first recurrence in 4.5 months with progression from grade 2 to 3, and underwent cystectomy 2 months after the second recurrence.

**Case 15.** Eight cells from a direct preparation had 70–93 chromo-
somes. Of four markers, two were identified as del(6)(q21) and der(5)(3;5)(p14;p14), with a net duplication 3p14 → 3pter and deletion 5p14 → 5pter. One cell had the following changes from tetraploidy:

-1, -2, -2, +3, -5, -8, -9, -10, -11, -12, -13, -14, +15, -16, -18, -19, +20, +21, +X, der(5)(3;5)(p14;p14), +der(5)(3;5)(p14;p14), del(6)(q21), +del(6)(q21), del(9)(q22), +mar1, +mar2, +3 mar3.

The patient had three recurrences in 8 months. The last recurrence showed progression from grade 2 to 3. Between the second and third recurrence, the karyotypic mode changed from near-tetraploid to near-triploid with the same markers.

ACKNOWLEDGMENTS

We thank Drs. Joseph Cerney, Ray Littleton, and Brian Shumaker for clinical material and Timothy Drumheller, Regina Puskorius, Tonya Moore, and Erika Clark for technical help.

REFERENCES

Tumor Behavior in Transitional Cell Carcinoma of the Bladder in Relation to Chromosomal Markers and Histopathology

V. Ramesh Babu, Michael D. Lutz, Brian J. Miles, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/47/24_Part_1/6800

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/47/24_Part_1/6800.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.