Nonrandom Chromosomal Changes in Transitional Cell Carcinoma of the Bladder

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ABSTRACT

Nine cases of transitional cell carcinoma (eight from the urinary bladder and one from the ureter; six noninvasive and three invasive) were subjected to detailed cytogenetic analysis with a G-band method. The synchronization of primary cultures with methotrexate for high-resolution banding was performed in five cases. In the remaining four cases, the chromosomes were obtained from short-term cultures after prolonged (16 hr) exposure to Colcemid.

Two cases were near-tetraploid, one was hypotripliod, and six were near-diploid (three hyperdiploid and three hypodiploid). All but one case showed various structural abnormalities in the karyotype. The chromosomal changes ranged from the presence of only two abnormal chromosomes (markers) to complex karyotypes with as many as 15 markers. In most tumors, the origin of the marker chromosomes could be readily deciphered. The nonrandom chromosomal aberrations included: (a) an isochromosome of the short arm of chromosome 5 (three cases); (b) monosomy of chromosome 9 found in four cases (this was the sole abnormality in one case); (c) involvement of chromosome 8 as an isochromosome of the long arm (two cases) or loss of the short arm due to deletion (one case) or translocation (one case); and (d) interstitial deletion of chromosome 13 (three cases).

Our results indicate that the formation of i(5p) and monosomy 9 may be the primary karyotypic changes in two subgroups of transitional cell carcinoma. Involvement of chromosomes 8 and 13, on the other hand, seems to be a result of secondary karyotypic evolution. Two invasive tumors showed the presence of secondary clones, with additional structural chromosome aberrations superimposed on those already existing in the main cell population. In both cases, the additional aberrations involved the short arm of chromosome 11, resulting in loss of genetic material from the short arm. The short arm of chromosome 11 is the putative site of an oncogene which has been isolated from human bladder carcinoma cell lines. Deletion of the 11p was also seen in one case of noninvasive transitional cell carcinoma localized in the ureter; the material from 11p was probably translocated to chromosome 13.

These findings suggest that the loss of genetic material from the short arm of chromosome 11 is a secondary event in the karyotypic evolution of transitional cell carcinoma, probably related to the invasive behavior of the tumor.

INTRODUCTION

TCC of the bladder has been analyzed cytogenetically by a number of authors (19). Most of these studies were performed with a direct technique and nonbanded preparations; furthermore, the poor morphology of the chromosomes due to technical factors prevented precise analysis of chromosomal rearrangements and, therefore, the establishment of specific abnormalities which, in this cancer, still remain undefined (5, 6, 18–20). Nevertheless, some clinically relevant information has been extracted from these early data on chromosome changes in bladder cancer. Invasiveness of the tumors has been shown to positively correlate with ploidy of the tumors, and the likelihood of recurrence of superficial tumors has been related to the presence of structurally abnormal chromosomes (markers) which can be detected sometimes even in unbanded preparations (7, 8, 18–20).

Recent advances in cytogenetic technology of tumors (12, 24, 26) have made possible an analysis of increasing numbers of solid tumors, with the result that the number of cancers with known specific (primary) chromosomal defect is growing rapidly (1, 2, 10, 11, 13, 23, 25, 27). Also, the discovery of a correlation between the loci of some human cellular oncogenes and the breakpoints of specific chromosomal rearrangements in certain leukemias and lymphomas has contributed to a widened interest in the cytogenetics of specific cancers (17, 27). The finding that some oncogenes may be displaced in cancer cells as a result of chromosomal rearrangements, particularly translocations (4, 5, 22), for the first time yielded some clues to the possible mechanism of carcinogenesis at the chromosomal level (17, 21, 27). Thus, the knowledge of chromosomal changes in cancer can contribute both to the understanding of the basic aspects of carcinogenesis and, at the same time, provide clinically relevant information, as has been amply demonstrated for leukemias and lymphomas (17, 19, 27).

The oncogene from the human EJ bladder carcinoma cell line has been recently localized to the short arm of chromosome 11 and the oncogene from the T24 cell line, which, according to some authors, is identical with EJ, has been also localized to chromosome 11 (6, 14). These findings raised the natural question as to whether the p-arm (short arm) of chromosome 11 is involved in chromosomal aberrations in bladder cancer. In this paper, we wish to report on 9 cases of TCC. Our findings indicate that the short arm of chromosome 11 is not a primary site of chromosomal aberrations in TCC of the bladder. In fact, the preliminary evidence indicates that a loss of genetic material from 11p may be a secondary karyotypic event in the clonal evolution of the tumor cell population, probably signifying or associated with invasive behavior of the tumor. We have also identified 4

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nonrandom chromosomal abnormalities in our material, 2 of them probably being the primary changes in different subgroups of TCC.

MATERIALS AND METHODS

The tumor samples were transported to the laboratory in RPMI Medium 1640 supplemented with 17% fetal calf serum and antibiotics (penicillin, 100 units/ml; streptomycin, 100 /µg/ml; and gentamicin, 50 /µg/ml). Most samples were received 24 hr after removal of the tumor in hospitals outside the Buffalo area. Depending on the consistency of the tumor, the samples were disaggregated either mechanically, by mincing them with curved scissors or, additionally, digested with a 0.8% collagenease solution at 37° for 2 hr. After disaggregation, all of the material was distributed to culture flasks with a small amount of medium (1 to 1.5 ml of medium/25-sq cm flask). No attempts were made to separate pieces of tissue from single-cell suspensions, since we observed that most outgrowths start from cell clusters and small pieces of tissue. After 24 hr of incubation, the nonattached material was gently removed, and fresh medium was added. The culture medium used was RPMI Medium 1640 supplemented with 17% fetal calf serum, L-glutamine, and antibiotics (used in Cases 1 to 4). Recently, we started to supplement this medium with bovine insulin (5 /µg/ml), hydrocortisone (5 /µg/ml), triiodothyronine (0.2 /µm), transferrin (5 /µg/ml), and glutathione (10 /µg/ml) (used in Cases 5 to 9; all chemicals were purchased from Sigma Chemical Co., St. Louis, MO). The cultures were observed daily under the microscope and, after the area covered by epithelial outgrowths was sufficiently large (at least a 1-sq cm combined area), they were harvested for cytogenetic analysis (usually after 2 to 5 days of culture). Cases 1 to 4 were harvested after 16 hr exposure to Colcemid (0.01 µg/ml). Cases 5 to 9 were processed according to the high-resolution technique developed by Yunis (26). The cultures were exposed for 17 hr to 10^-7 M MTX. After that time, the medium was removed, the cultures were washed with a prewarmed medium and, finally, fresh medium supplemented with 10^-3 M thymidine was added. The cultures were then incubated for an additional 7 hr; Colcemid was added for the last 3 hr at a final concentration of 0.02 µg/ml. All cultures were harvested following an in situ exposure to a hypotonic solution composed of KCl (3 g/liter), ethylenebis(oxyethylenedinitri)tetraacetic acid (EGTA, 0.2 g/liter), and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes, 4.8 g/liter). The pH of this solution was adjusted to 7.4, and the treatment was performed for 0.5 to 1 hr at 37°.

This rather unconventional type of hypotonic treatment has been developed for a dual purpose: (a) to detach the cells and separate them from each other in a tight multilayer epithelial outgrowth; and (b) to deliver a hypotonic shock for optimal chromosome spreading. This type of hypotonic treatment produces excellent spreads of chromosomes (Fig. 5) and is routinely used in our laboratory for cultures of epithelial tumors. After the hypotonic treatment, the cells were spun down and fixed in methanol:acetic acid (3:1). Slides were prepared in conventional way, and G-banding was performed by the method of Yunis (26).

RESULTS

Relevant clinical information on the patients is summarized in Table 1. Cytogenetic findings were as follows.

**Case 1.** Of 28 metaphases analyzed, 21 had a modal karyotype of 48,XX,-8,+i(8q),+i(8q). The remaining 7 metaphases showed random loss of different chromosomes, but always retained at least one marker. A representative karyotype of this case is shown in Fig. 1.

**Case 2.** Fifteen cells were analyzed in this case; the modal chromosome number was found to be 45. The karyotypic changes were very complicated with the presence of as many as 15 marker chromosomes. The origin of the marker chromosomes is described as: m1, 1p+; m2, 2q-; m3, del(6)(q15); m4, 7q+; m5, 10p+ (the additional material on 10p may have originated from the q-arm of chromosome 11); m6, del(13)(q14q22); m7, i(17q); m8, 19p+ (submetacentric; size of B group chromosomes); m9, ring chromosome of unknown origin; m10, very small chromosome of unknown origin; m11, acrocentric (D-group size) with a very strong band in the middle of the long arm; m12, small metacentric (F-group size), origin unknown; m13, 12q+ (the additional material may be derived from 1p); m14, metacentric, C-group size, origin unknown; and m15, submetacentric, C-group size, origin unknown.

Markers m1, m3, m4 to m11, and m13 were present in the majority of cells, whereas the remaining markers were seen only occasionally. Most cells showed monosomy of chromosomes 9 and 11; both chromosome 5 homologues were absent in all cells studied.

**Case 3.** Seven cells were karyotyped in this case, and the modal chromosome number was found to be 44. Four cells had a marker interpreted as 10q+, with 2 of the cells showing additionally a deletion, del(8)(p11). One of the remaining 3 cells had an inv(7)(p12;q36) as the only abnormality, and 2 cells contained structurally abnormal chromosomes of uncertain origin.

**Case 4.** The karyotype of this tumor was near-tetraploid. Eight cells analyzed in detail showed the following structural abnormalities. m2, 2q+; m3, del(3)(q21); m4, 4p+; m5, i(8q); m6, idic(17)(p12); m8 and m9, small metacentric and small submetacentric, E-group size; m10 and m11, originated from a translocation t(1;11)(q11;q23).

**Case 5.** Twenty-four metaphases were analyzed in this case. The modal karyotype was 47,XX,-7,-9,-14,-15,+t(7p12;13q13)+del(14)(q22),+i(15q),+19. A representative karyotype of this case is displayed in Fig. 2. Markers m2 and m3 were present in all the cells analyzed. The interstitial deletion of chromosome 7 (m1) could be seen with clarity only in metaphases with elongated chromosomes, but was probably present in all cells. A secondary clone was discovered in this case (Fig. 3). It contained markers m1 to m3 and, additionally, a translocation t(8q;11q) and t(1;11) in one cell each, which resulted in the formation of markers m4 to m6. A third cell was found which contained both of the above-mentioned translocations. The translocation t(1;11) seemed to be reciprocal, but part of the p-arm of chromosome 11 was probably lost. In the case of t(8q;11q), the short arms of chromosomes 8 and 11 were lost.

**Case 6.** Of 37 metaphases analyzed, 10 showed a modal karyotype of 47,X,-2,-6,-10,-13,+i(2p),+i(5p),+del(6)(q12q21),+del(10)(q24),+del(13)(q12q21),+del(X)(q26). The remaining cells were either hypo- or pseudodiploid with apparently...
random loss of different chromosomes. A representative karyotype of this tumor is shown in Fig. 4.

**Case 7.** Fifteen cells were analyzed in this case; all of them showed a monosomy of chromosome 9. The modal karyotype was 45,XX,--9.

**Case 8.** Thirty-two cells were analyzed in detail in this case. The chromosome number was near-tetraploid (Figs. 5 and 6). Chromosomes 3 and 13 were pentasomic in the majority of the cells, and chromosome 22 was frequently present in 6 copies. The Y-chromosome was consistently lost. Two fine structural defects were present in all 32 metaphases. One was a chromosome 12 with an abnormal short arm, and the second was a marker chromosome usually present in 2 to 3 copies/cell. Its origin was not established, but it may have been partly derived from chromosome 17 (Fig. 5). A detailed analysis of the karyotypes showed the presence of 3 populations of cells. The main population (19 cells) contained only the above-mentioned markers and usually 5 copies of chromosome 13. The secondary clone (9 cells) contained additionally marker m2, resulting from a translocation, t8;13(q13;p11) (Fig. 5). The third clone (4 cells) contained, in addition to markers m1 and m3, either a deletion of 11p or a translocation(11;16)(q13;q13) (Fig. 6). The deletion of 11p was presumably interstitial (p11 and p12) and, in the case of (t11;16), the whole short arm of chromosome 11 had been apparently lost. Most cells in this case contained a variable number of very small double minute chromosomes.

**Case 9.** Seventeen metaphases were analyzed in this case. The modal chromosome number was in the hypotripliod range (58 to 60). Eleven marker chromosomes were consistently present (Fig 7). The origin of most of these markers could be deciphered as m1, dup(1)(pter→cen→q11::p35→p11::q12→qter); m2, the lower part of this acrocentric marker consisted of the distal part of 1q; m3, 2q+; m4, (5q); m5, del(11)(p13) (most of the 11p has probably been translocated to chromosome 13, forming marker m7); m6, t(11;X)(q11;q22) (marker m10 was the second product of this translocation); m8, del(13)(q12q14); m9, t(10;19)(10qter→19q1 ter); and m11, large submetacentric (part of its short arm may have been derived from 3q, and the distal part of its long arm resembled chromosome 15).

Monosomy 9 was consistently present in all cells; chromosomes 3, 4, 7, and 12 were trisomic in most metaphases, and 4 to 5 small unidentified markers were also present in most cells.

**DISCUSSION**

In establishing the primary (specific) chromosome changes in cancers, certain materials and conditions are more optimal than others. Since, generally cancers (including TCC) tend to have complex and multiple chromosome changes accompanied by hyperdiploidy, it is very difficult to decide as to which of these changes is the primary one. However, near-diploid tumors serve as optimal material for establishing primary changes, particularly those tumors containing only a few, and ideally only one, karyotypic change. The use of established cell cultures also has its limits, since selection of a particular cell type may occur in vitro, which may not be representative of the major cell type in vivo, and the identity of the established cell lines is frequently uncertain as recently demonstrated for EJ and T24 bladder carcinoma cell lines (15). Furthermore, long-term in vitro culture conditions may favor cells with a particular karyotype, as has been observed in recent studies on a number of bladder cancer cell lines.4 (9). Thus, it was shown that most of the cells had a high chromosome number and apparently required a combination of karyotypic changes for survival in vitro; although these changes probably contained within them the primary one(s), their complexity makes the pinpointing of the primary changes very difficult. We recently described the karyotypic changes in established cell lines from bladder cancers and found a number of nonrandom abnormalities, i.e., 8p−,9p−,t(15;18) and involvement of chromosome 16, common to the lines. This combination of karyotypic changes was apparently necessary for survival in vitro, as has been pointed out by Hastings and Franks (9).

Alternatively, such combination of markers may provide cells with a proliferative advantage over cells with other chromosomal constitutions. If one assumes that established cell lines retain the primary karyotypic change, e.g., involvement of chromosomes 8 and 9 (also observed by us in the present study), their use may be of some value in establishing the chromosomes associated with the nonrandom karyotypic changes in bladder cancer. However, in establishing the primary chromosome change in a cancer, direct or nearly direct (short-term culture) cytogenetic examination of tumors with a near-diploid chromosome range with limited karyotypic changes offers the best chances of success.

Our initial series of bladder cancers included 15 tumors, only 10 of which showed growth in culture; of these 10 tumors, 7 yielded analyzable metaphases, with 3 showing normal karyotypes. The remaining 4 cases could be analyzed in detail (Cases 1 to 4 of the present report), but the number of metaphases available in 2 of them was very small. The contracted and fuzzy appearance of the chromosomes was observed in the majority of metaphases in all but one tumor (Case 1) in this series. These technical difficulties prompted us to apply a high-resolution chromosome technique, with the use of MTX synchronization, in the next series of 10 cases. Five of these tumors (Cases 5 to 9 of the present report) yielded large numbers of high-quality metaphases, and all of them turned out to be abnormal. Thus, the application of MTX synchronization has increased the success rate of the karyotypic analysis from about 25% to 50%; even more important is the quality of chromosome preparations, which has also been greatly improved with the number of metaphases suitable for detailed analysis being significantly increased. The high-resolution chromosome technique, originally developed for lymphocyte cultures, is now routinely applied in some laboratories for the cytogenetic study of bone marrow in leukemias and samples of lymphoma. The application of this method allowed Yunis et al. (28) to demonstrate that all cases of acute nonlymphocytic leukemia have chromosomal defects. However, the applicability of these techniques to solid tumors has not been conclusively demonstrated. Our experience suggests that MTX synchronization is the method of choice in the cytogenetic studies of TCC.

The chromosomal changes observed by us in TCC were of different types and ranged widely in complexity. The near-diploid cases with relatively simple karyotypic changes showed isochromosomes, deletions, or simple numerical abnormalities as predominant changes (Cases 1, 3, and 5 to 7). Translocations, on
the other hand, were very rarely seen in these cases. The polyplid and karyotypically complex cases (Cases 2, 4, 8, and 9) showed the presence of many translocations, although iso-chromosomes, deletions, and numerical abnormalities were also frequently seen. Thus, it appears that translocations may be secondary changes in TCC, in contrast to leukemias and lymphomas in which they are a cardinal, primary change. TCC of the bladder is not an exception in this respect, since most solid tumors with known specific defects are characterized by deletions and isochromosomes (small-cell lung cancer, Wilms' tumor, retinoblastoma, neuroblastoma, and testicular tumors), with only 2 tumors, i.e., mixed-parotid gland tumor and papillary cystadenocarcinoma of the ovary, being characterized by specific translocations (1, 2, 10, 11, 13, 23, 25, 27). It appears, therefore, that the mechanism of carcinogenesis which is emerging from the molecular and cytogenetic studies of Burkitt's lymphoma, i.e., the misplacing of an oncogene in the process of chromosomal translocations, is probably not universal and does not apply to most solid tumors, including TCC. Other mechanisms related to chromosomal changes in cancers, including TCC, may be operative (21, 27).

Four chromosomal abnormalities appear to be nonrandom in our material; none of them was a translocation. (a) An isochromosome of the short arm of chromosome 5 has been found in 3 cases (Cases 1, 6, and 9), all of which were noninvasive tumors. Case 1 was particularly informative, since an i(5p) was one of only 2 structural changes in this tumor, the second being i(8q). This finding suggests that the formation of i(5p) may be an initial chromosomal change in a subgroup of TCC, although the number of cases in our study is too small to justify speculation on the biological or clinical characteristics of these tumors. (b) The apparently nonrandom abnormality was monosomy of chromosome 9 observed in 3 near-diploid tumors and in one near-triploid case (Cases 2, 5, 7, and 9). In none of the 3 near-diploid cases was the i(5p) present. In Case 9, which was near-triploid and had a very complicated karyotype with several markers of unknown origin, parts of the missing chromosome 9 could have been involved in the formation of marker chromosomes and, thus, be present in the cancer cells. Therefore, it appears to us that simple monosomy of chromosome 9 may initiate malignant transformation in a subgroup of TCC. This suggestion is strongly supported by the finding in Case 7 in which monosomy of chromosome 9 was the only karyotypic abnormality. (c) Another nonrandom abnormality was involvement of chromosome 8. An i(8q) was found in 2 cases (Cases 1 and 4), and loss of the short arm of chromosome 8 was observed in 2 other cases (Cases 3 and 8), but in the secondary clones. Thus, it appears that involvement of chromosome 8 is a secondary, noninitiating event in the karyotypic evolution of TCC. (d) Finally, an interstitial deletion of proximal to the centromere region of chromosome 13 was observed in 3 cases (Cases 2, 6, and 9). All these cases had either an i(5p) or monosomy of chromosome 9, indicating that this may also be a secondary, noninitiating event in the karyotypic evolution of TCC.

The localization of an oncogene from the EJ bladder cancer cell line to the short arm of chromosome 11 prompted us to scrutinize this chromosome with particular emphasis. However, in only one case (Case 9) was the short arm of this chromosome involved in a translocation which appeared to be balanced. In the remaining cases, the main clones did not show any visible abnormalities of 11p, although a very small deletion could not be positively excluded. However, in 2 tumors (Cases 5 and 8), both of them invasive carcinomas, we found evidence of the presence of secondary clones. These secondary clones showed additional structural aberrations, mostly translocations, which were superimposed on the already existing abnormalities of the main cell population. In both cases, it appeared that these additional abnormalities led to the loss of genetic material from the short arm of chromosome 11. In Case 8, there was also another secondary clone in which chromosome 8 was involved in a translocation. Thus, it appears to us that the short arm of chromosome 11, the site of an EJ oncogene, may be involved in the later stages of progression of TCC of the bladder. It also seems that the loss of 11p may be an important event, signifying the onset of invasion by the tumor. Certainly, more studies are needed to verify this suggestion since, in a small series of tumors, the occurrence of a deletion of 11p only in invasive tumors may be a coincidence. However, our study indicates that rearrangements of the short arm of chromosome 11 are not a primary event in TCC. The c-ras oncogenes which are activated in various kinds of epithelial tumors (3, 16) may, therefore, not be involved in the initial steps of carcinogenesis. This suspicion is supported by the fact that chromosome 11, the site of the c-ras oncogene, which is activated in lung cancer, is not involved in chromosomal changes in small-cell carcinoma of the lung. Instead, a deletion of chromosome 3 has been demonstrated to be specific for that cancer (25). The data presented in this paper and the earlier findings in established bladder carcinoma cell lines (9) also indicate that the c-ras oncogenes may not play an initiating role in carcinogenesis in human epithelial cells.

All but 2 of our patients have received some form of chemotherapy during the last 5 years, but none was treated at the time of tumor removal. The chemotherapy may have contributed to the formation of complex secondary rearrangements in some cases, although there was no obvious relationship between the intensity of chemotherapeutic treatment in the past and the complexity of the karyotype. The study of untreated cases at diagnosis will be probably more informative and optimal, now that technical obstacles to the cytogenetic analysis of TCC have been largely removed.

REFERENCES


Fig. 1. Representative karyotype of Case 1, with 2 marker chromosomes, i(5p) and 2 copies of i(8q). This karyotype is based on the metaphase in lower right corner.
Fig. 2. Modal karyotype of Case 5. Marker m1 is chromosome 7 with an interstitial deletion of its short arm, m2 is a deleted chromosome 14, and m3 is an isochromosome of the long arm of chromosome 15. Note the presence of monosomy of chromosome 9.

Fig. 3. Karyotype of the secondary clone of Case 5. A, Marker m4 originated from the translocation t(1;11), and the marker m6 consists of the long arms of chromosomes 11 and 8. B, both products of the translocation t(1;11), i.e., markers m4 and m5, along with normal homologues 1 and 11 from the same cell. Note the presence of markers m2 and m3, which are present in the primary clone.
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Fig. 4. Representative karyotype of Case 6. Five marker chromosomes were consistently present in this case; m1 is an isochromosome of the short arm of chromosome 2, m2 is an isochromosome of the short arm of chromosome 5, m3 is a chromosome 6 with deletion of its long arm, m4 is chromosome 10 with a deletion of its long arm, m5 is chromosome 13 with an interstitial deletion, and m6 is an X with a deletion of its long arm.

Fig. 5. Karyotype of Case 8 showing the secondary clone with the presence of 3 marker chromosomes. Only markers m1 and m2 were present in the main clone. Marker m1 is chromosome 12 with an abnormal short arm, and m2 is a result of a translocation t(8;13). The origin of m3 is unknown. This karyotype is based on the metaphase in lower right corner.
Fig. 6. Karyotype of a secondary clone of Case 8, with a translocation t(11;16) resulting in the loss of the short arm of chromosome 11 and formation of marker m4 (A). Deletion of the short arm of chromosome 11 (m5) in 2 different cells. Normal homologues 11 from the same cell are displayed for comparison (B).

Fig. 7. Representative karyotype of Case 9. For description of the markers, see text.
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