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Rearrangements of Chromosome 3 in Nonfamilial Renal Cell Carcinomas from Japanese Patients

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Cytogenetic studies were successfully carried out in 5 tumor tissues from Japanese patients with nonfamilial renal cell carcinoma, histologically diagnosed as clear cell subtype. Mitotic cells were obtained by a combined method of enzymatic disaggregation and short-term culture (6–12 days). The modal chromosome numbers were found to be diploid or near-diploid in all the cases examined. Every case showed characteristic structural and numerical abnormalities. Rearrangements in the short arm of chromosome 3 were observed as clonal abnormalities in all the cases, including a translocation t(3;6) resulting in a partial loss of 3p (3 cases), a terminal deletion of 3p (one case) and 2 different translocations involving 3p and 8p (one case). The other clonal abnormalities were a whole or partial trisomy of chromosome 7 and a loss of Y chromosome. The overall results in the present study were consistent with those of our previous data in American patients, and suggest that the rearrangements of chromosome 3 leading to a partial loss of its short arm may play primary and significant role(s) in the development of renal cell carcinoma.

Key words: Chromosome rearrangement — Partial 3p deletion — Renal cell carcinoma

Nonrandom chromosome changes have been identified in a variety of human cancers and leukemias.[1, 2] The results of cytogenetic analyses in hematological diseases have suggested that such specific chromosome rearrangements may be one of the significant steps associated with the pathogenesis of human malignancies.[3] Although cytogenetic studies in solid tumors have been far less numerous than those in leukemias and lymphomas, chromosomal data in solid tumors have been gradually accumulated in recent years, mainly due to the improvement in culture techniques.[4]

As to renal cell carcinoma (RCC), a few reports on chromosome analysis have been presented. A constitutional translocation between 3p and 8q was detected in patients with hereditary renal cancer,[5] and a translocation involving 3p and 11q was identified in tumor cells from another hereditary renal carcinoma.[6] We have previously reported that rearrangements in the short arm of chromosome 3 were frequently observed in tumor cells from nonfamilial cases.[7, 8] The specificity of the 3p rearrangements in RCCs has also been demonstrated in more recent studies.[9–11]

In this study, we have analyzed the karyotypic changes in RCCs from Japanese patients in order to confirm the presence of the 3p rearrangements and to see if any other nonrandom chromosome abnormalities might exist in these tumors.

Materials and Methods

All tumor samples were obtained at operation from patients with renal cell carcinoma. All of these were histologically diagnosed as clear cell subtype of sporadic RCC. None of the patients had been treated with radiation and/or chemical agents prior to surgery. Metastasis to other organs was not detected in these patients. The samples were washed in Hanks' buffer solution containing antibiotics, minced into small pieces with scissors, and digested with 0.8% collagenase type II (Sigma, Inc.) for 1–2 hr at 37°. The suspension was then mixed with Hanks' solution, and passed through a stainless steel mesh to obtain a single cell suspension. The cells collected by centrifugation were washed 2–3 times with Hanks' solution and in-

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cubated in RPMI 1640 medium supplemented with 10% fetal calf serum in an atmosphere of 5% CO_2.

Harvesting of cells was carried out when the tumor cells became attached to the 25 cm^2 culture flasks and grew out sufficiently with higher mitotic indices. After colcemid treatment (0.01–0.02 μg/ml) for 5 hr, the cells were detached by treatment with 0.025% trypsin including EDTA, and treated with 0.075*M* KCl for 30 min at 37°. The cells were then fixed 3 times in freshly prepared 3:1 methanol/acetic acid. Air-dried preparations were made, and chromosome analysis was performed by quinacrine[12] and trypsin-Giemsa[13] banding techniques according to the ISCN (1985).[14] Chromosome abnormalities were judged clonal either when an identical structural abnormality was observed in at least 2 cells or when 3 or more cells gained or lacked the same chromosome.

Results

A total of 10 tumor samples were obtained from Japanese patients (5 males and 5 females), aged 45–76 years. Among these, mitotic cells suitable for banding analysis were found in 5 cases (4 males and 1 female) (success rate 50.0%) after 6–12 days of culture. The results of cytogenetic analyses are summarized in Table I.

Case 1: Modal chromosome number was 46 (range 41–46). Out of 25 metaphases analyzed by banding, 19 cells had an identical structural change in the short arm of chromosome 3. Based on careful analyses of banding patterns, the rearranged chromosome 3 was identified as a der(3)t(3;6)(p11;p11) (Fig. 1). The karyotype was thus partial monosomy for 3p(p11-pter) and trisomy for 6p. Trisomy of chromosome 7 and a loss of Y chromosome were found in 17 cells and 18 cells analyzed, respectively.

Case 2: Modal chromosome number was 45 (range 43–45), and only 6 metaphases were karyotypically analyzable. All of these showed a structural abnormality in the short arm of chromosome 3, which was similar to that observed in Case 1. Trisomy 7, monosomy 6q and a loss of Y chromosome were also observed in all the cells karyotyped (Fig. 2).

Case 3: Out of 16 metaphases analyzed in detail, 14 cells consistently showed 2 different translocations, t(3;8)(p13;p23) and t(3;?;8)(p13;?;p23), which resulted in a partial loss of the p13-pter region (Figs. 3 and 4). All the cells except for one cell with a normal karyotype showed trisomy 7 as the only clonal

Table I. Results of Karyotypic Analyses in 5 Renal Cell Carcinomas

Case No.	Age/Sex	Culture period (days)	No. cells analyzed	Karyotype and no. of cells
1	64M	10	25	46,X,−Y,−3,+7,+der(3)t(3;6)(p11;p11)=11 8 cells with additional non-clonal chromosome abnormalities 46,XY=5 41,X,−Y,−3,−14,−18,−21,t(13;14)(p11;p11)=1
2	76M	12	6	45,X,−Y,−3,−6,+7,+der(3)t(3;6)(p11;p11)=3 3 cells with additional non-clonal chromosome abnormalities
3	47M	7	16	47,XY,−3,+7,−8,+der(8)t(3;8),[a]+der(8)t(3;?;8)[b]=10 4 cells with additional non-clonal chromosome abnormalities 44,XY,−3,+7,−8,−16,−22,+der(8)t(3;8)=1 46,XY=1
4	61M	7	30	45,X,−Y,−3,−6,+7,+der(3)t(3;6)(p13;p12)=14 44,X,−Y,−3,−6,+7,−14,+der(3)t(3;6)(p13;p12)=6 6 cells with additional non-clonal chromosome abnormalities 3 near-tetraploid cells with 2 sets of the above rearranged chromosome 37,X,−Y,−2,−3,−6,−10,−12,−13,−14,−18,del(9)(q13)=1
5	49F	6	32	46,XX,−2,+der(2)t(2;7)(q35;q11),del(3)(p13p25)=29 2 cells with additional non-clonal chromosome abnormalities 1 near-tetraploid cell with 2 sets each of the above rearranged chromosomes

a) Band composition of the der(8) was defined as: 8qter→cen→8p23::3p13→3qter.
b) Band composition of the der(8) was: 8qter→cen→8p23::?→?::3p13→3qter.

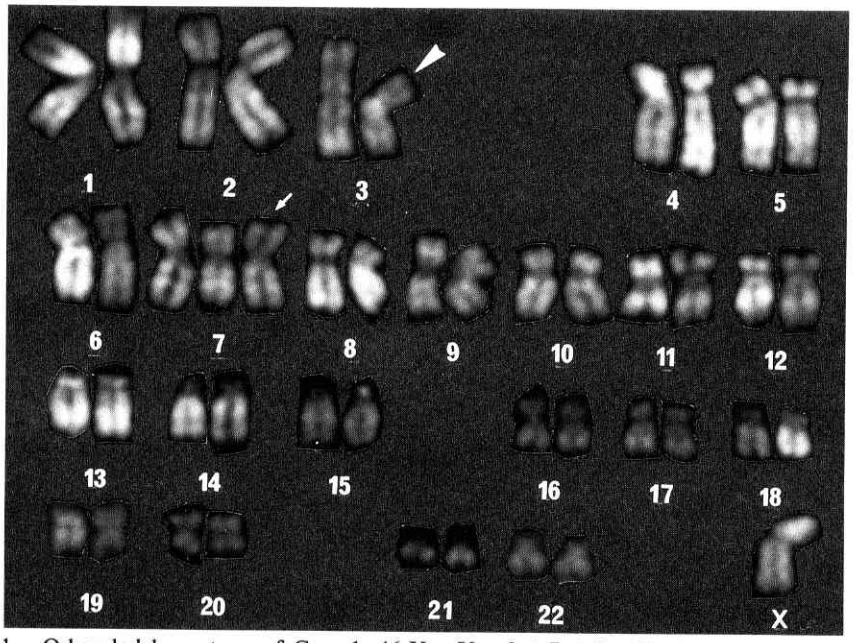

Fig. 1. Q-banded karyotype of Case 1: 46,X,−Y,−3,+7,+der(3)t(3;6)(p11;p11). Note the rearranged chromosome 3 (arrowhead) and an extra copy of chromosome 7 (arrow).

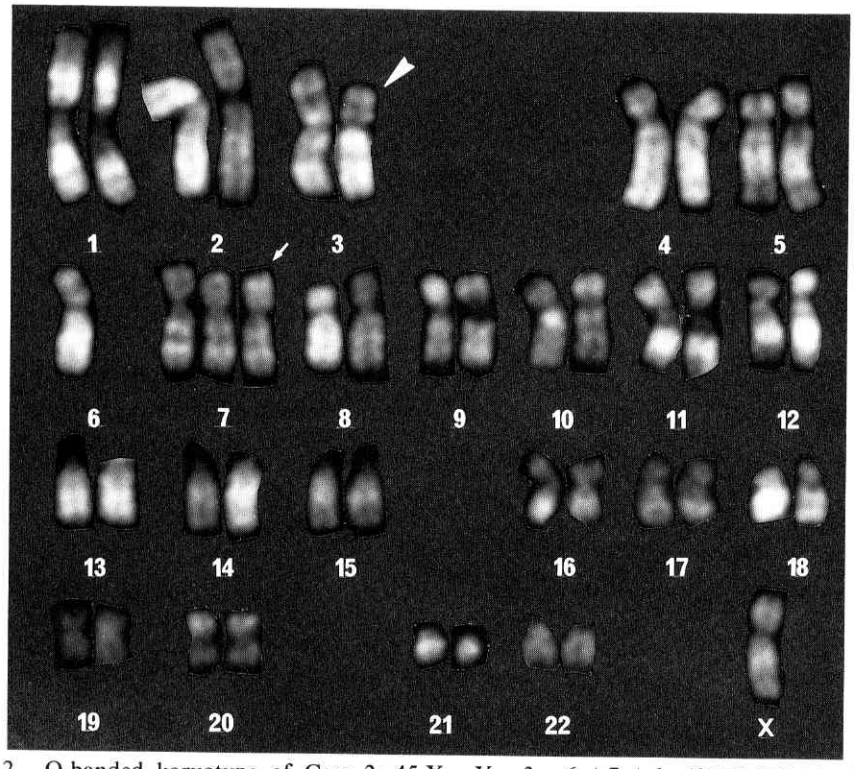

Fig. 2. Q-banded karyotype of Case 2: 45,X,−Y,−3,−6,+7,+der(3)t(3;6)(p11;p11). Note the rearranged chromosome 3 (arrowhead) and an extra copy of chromosome 7 (arrow).

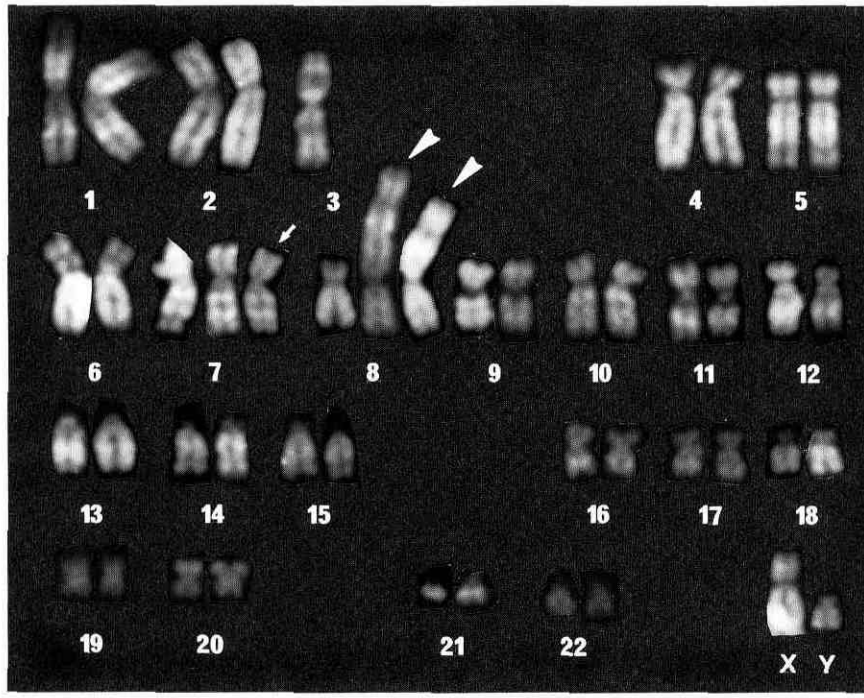

Fig. 3. Q-banded karyotype of Case 3: 47,XY, −3, +7, −8, +der(8)t(3;8)(p13;p23), +der (8)t(3;?;8)(p13;?;p23). Note two translocations involving chromosomes 3p and 8p (arrowhead) and an extra copy of chromosome 7 (arrow).

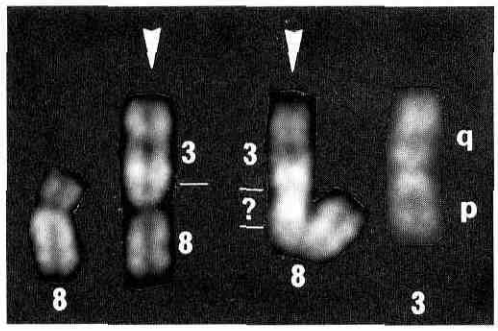

Fig. 4. Q-banded partial karyotype of Case 3 showing two different translocations involving chromosomes 3 and 8.

change in number. The modal chromosome number in this case was 47 (range 44–47).

Case 4: Modal chromosome number was 45 (range 37–90), and a total of 30 metaphases including 3 near-tetraploid cells were karyotyped in detail. Twenty-nine cells showed an abnormal 3p which appeared to be the result of translocation between 3p and 6p with breakpoints of p13 and p12, respectively (Fig. 5). Trisomy 7, monosomy 6q and a loss of Y chromosome were found with high frequencies. Monosomy 14 was also found in 5 cells.

Case 5: A total of 32 cells were analyzed, all of which showed two types of rearrangements simultaneously. One was an interstitial deletion of 3p with breakpoints of p13 and p25. The other was a der(2) resulting from a translocation between chromosomes 2 and 7, and the karyotype indicated partial trisomy of 7q (Fig. 6).

In summary, a total of 109 metaphases from 5 primary tumors were karyotypically analyzed in detail. Only 6 metaphases showed a normal karyotype (Cases 1 and 3) and appeared to be derived from normal kidney or fibroblastic cells grown in short-term culture. The remaining 103 cells (94.5%) showed abnormal karyotypes with clonal and non-clonal changes in structure and number. A total of 7

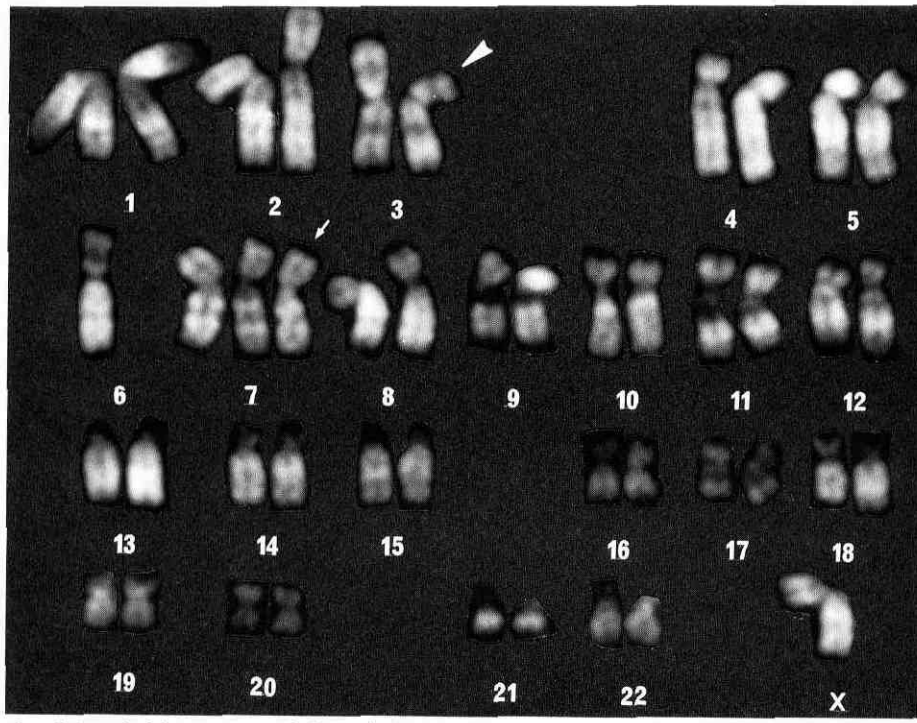

Fig. 5. Q-banded karyotype of Case 4: 45,X, −Y, −3, −6, +7, −18, +der(3)t(3;6)(p13;p12), +der (18)t(18;?)(q23;?). Note the rearranged chromosome 3 (arrowhead) and an extra copy of chromosome 7 (arrow).

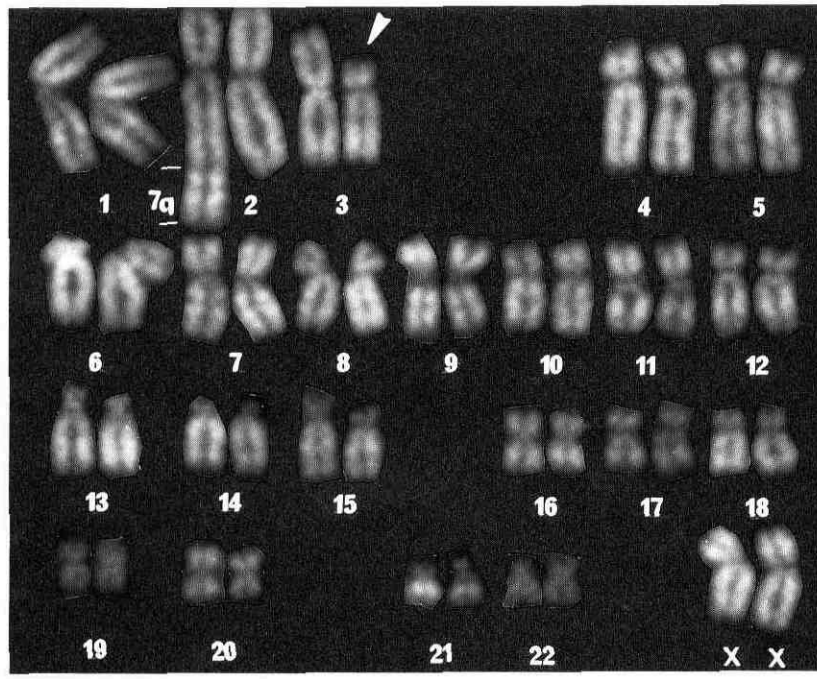

Fig. 6. Q-banded karyotype of Case 5: 46,XX, −2, +der(2)t(2;7)(q35;q11),del(3)(p13p25). The arrowhead indicates the rearranged chromosome 3.

clonal rearrangements were identified in those 5 tumors (Table I), and abnormalities of chromosome 3p were involved in 6 clones (85.7%). Trisomy 7 was observed in 4 cases, and a partial trisomy of 7q in one case as clonal changes. A loss of Y chromosome was also found in 3 of 4 male patients.

Constitutional karyotypes of these patients were all normal in cultured peripheral lymphocytes.

Discussion

A combined technique of enzymatic disaggregation (collagenase type II) and short-term culture has been shown to be highly effective for obtaining analyzable metaphases from solid tumors.[4,7] Wake *et al.*[4] reported that mitotic cells were obtained in 85% of various kinds of solid tumors with this enzyme. In the present study using the same method, mitotic cells adequate for banding analysis were successfully obtained in 5 of 10 tumor samples from Japanese patients with nonfamilial RCC, although the mitotic index varied from one to another. The success rate was almost the same as that (55%) in our previous study dealing with RCCs from American patients.[7]

The modal chromosome numbers of the 5 tumors were either diploid or near-diploid. Each sample showed characteristic clonal chromosome abnormalities. Among these, the partial loss of chromosome 3p, either by a deletion or translocation, was found in all the cases studied here, with the commonest deleted region being 3p13-p25. In our previous study,[7] 8 of 12 RCCs showed the loss of chromosome 3p as clonal abnormalities, which resulted from translocation, deletion, or isochromosome. Furthermore, the 3p rearrangements were common to almost all the cells observed in each specimen, apart from karyotypically normal cells which appeared to be nonmalignant cells. This suggests that the tumors were derived from a single cell with this type of chromosome abnormality.

The specificity of the chromosome 3p rearrangements in RCCs has also been established in more recent studies.[9-11] Combining the results of our previous[7] and present series with those reported by others,[9-11,15] the partial loss of 3p is seen in 49 (75.4%) of 65 RCC tumor specimens. Furthermore, some of the RCCs showed the 3p rearrangement as a sole karyotypic change.[10,15,16] From these data, it appears that the chromosome 3p rearrangements leading to a partial deletion of variable segments may play an early and possibly crucial role in the process of RCC development or progression.

A similar rearrangement of chromosome 3p has frequently been observed in other kinds of solid tumors,[2] and del(3p) was found to be non-random in small cell lung carcinoma.[17] However, the high frequency of this chromosome change in RCCs is quite characteristic compared with those in other tumors.

It should also be noted that a constitutional chromosome translocation t(3;8) was found in a family with heritable RCC,[5,18] and that an acquired translocation t(3;11) was identified in tumor cells from a patient with familial RCC whose lymphocytes showed a normal chromosome constitution. All the available data in familial and nonfamilial RCCs suggests that the specific rearrangement of chromosome 3p may be an initiating cytogenetic event in tumor induction. Thus, one could speculate that the 3p rearragement is primarily associated, by analogy to retinoblastoma[19] and Wilms' tumor,[20] with the development of homo- or hemizygosity for a recessive mutation at the locus of chromosome 3p. Recent molecular analysis using RFLP markers has demonstrated frequent allele loss at loci on the 3p chromosome region.[21] Further accumulation of data is required on both chromosomal and molecular events in the corresponding region of chromosome 3p.

Among the translocations involving chromosome 3p in this study, 3 cases showed the translocation t(3;6). The 6p rearrangement has not been reported in other studies[9-11] except for one case with i(6p),[7] and the significance of the recurrent t(3;6) observed here remains unknown.

In addition to the 3p abnormalities, a whole or partial trisomy of chromosome 7 was seen in 4 of the present cases. It is interesting to note that the erythropoietin (EPO) gene was recently localized to chromosome 7 at the region of q11-q22,[22] and that the production of this hormone was frequently demonstrated in cultures of human renal carcinoma cells.[23,24] EPO is a hemopoietic growth factor

to regulate the production of red blood cells, and is produced in man primarily in adult kidney and in fetal liver. Furthermore, the receptor gene of epidermal growth factor (EGFR) has also been mapped on the region of p11-13 of this chromosome.[25] It has been suggested that over-expression of EGFR may be a characteristic feature in various human tumors.[26] Although, in the present study, we did not examine the production of either EPO or EGFR in the tumor cells, a possible causal relationship between the chromosome 7 abnormalities and altered expression of EPO and EGFR may be significant for an understanding of the mechanism of RCC development or progression.

Acknowledgments

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