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Chromosome imbalances in papillary renal cell carcinoma and first cytogenetic data of familial cases analyzed by comparative genomic hybridization

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Abstract. We used comparative genomic hybridization to analyze 17 tumor samples from 11 patients with papillary renal cell carcinoma (RCC), including three patients with hereditary papillary RCC. Whereas the most frequent aberrations confirmed data obtained by banding analyses, copy number increases on 5q, which previously were considered characteristic of nonpapillary RCC, were identified in two cases. In two complex cases belonging to the same family, a characteristic pattern of chromosomal aberrations was found: five of the six imbalances present in the less complex case were included in the karyotype of the other case, suggesting a genetically determined mechanism resulting in genomic instability of specific chromosomes or chromosomal subregions and/or selection of specific mutations.

Renal cell carcinomas (RCCs) with a papillary growth pattern account for approximately 5–10% of carcinomas of the kidney. Papillary RCC was first described by Mancilla-Jimenez and coworkers in 1976, who found a favorable prognosis of this tumor in comparison to that of nonpapillary RCCs (Mancilla-Jimenez et al., 1976). More recently, Kovacs et al. (1989) and Kovacs (1993) identified clear-cut genetic differences between papillary and nonpapillary RCCs. In clear-cell RCC, the most common type of nonpapillary RCC, loss of the short arm of chromosome 3 occurs in more than 90% of cases, whereas no such loss has so far been found in papillary RCC (Kovacs, 1993). Clear-cell RCCs, but not papillary RCCs, are characterized by mutations of the VHL gene (Gnarra et al., 1994). In RCCs with a papillary growth pattern, high incidences of trisomies and tetrasomies of chromosome 7 and of trisomies 16 and 17, as well as losses of the Y chromosome, have been found (Kovacs, 1993). Whereas considerable information is available on the genetics of nonpapillary clear-cell RCC, the genetics of papillary RCC has been studied by banding analysis in less than 50 cases.

This situation prompted us to perform an analysis of chromosomal gains and losses in papillary RCC using the technique of comparative genomic hybridization (CGH; Kallioniemi et al., 1992). For CGH, tumor DNA and a normal genomic reference DNA are cohybridized to normal metaphase chromosomes and are detected using different fluorochromes (Du Manoir et al., 1993; Joos et al., 1993; Kallioniemi et al., 1992). The ratios of fluorescent intensities generated by tumor-associated and reference DNAs denote regions with a normal genomic content as well as overrepresented and underrepresented sequences within the tumor DNA. Seventeen samples derived from 11 different patients were examined. For the first time, genetic changes in familial cases of papillary RCC were analyzed.

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Materials and methods

Tumors

Seventeen samples of tumor tissues or cell lines derived from 11 patients (eight males and three females ranging from 21 to 84 yr in age, with a median of 42 yr) were analyzed. The samples included nine primary tumors and one liver metastasis (STO3), five cell lines of primary tumor material, and two cell lines derived from lymph node metastases (UOK109 and UOK124, in both cases paraaortic lymph nodes). Two patients (STO2 and STO4) suffered from stage II, two patients (STO1 and STO5) from stage III, and the seven others from stage IV disease, according to the Robson classification. Three patients had hereditary papillary RCC. The clinical data, as well as the pedigrees of the respective families, have been published previously (Zbar et al., 1995; STO5 is patient III-5 in family 152; STO3 is patient III-2 in family 153; and STO4 is patient III-3 in family 153). Histological slides of the tumors were reviewed at the National Institutes of Health by one of us (M.J.M.). Only tumors with an estimated tumor cell content of more than 50% were included in this study. DNA was isolated by proteinase K digestion and phenol/chloroform extraction, as described before (Bergerheim et al., 1989).

Comparative genomic hybridization

Hybridization was performed as described earlier (Lichter et al., 1995). Briefly, tumor DNA and normal human DNA were labeled differentially and hybridized to slides of metaphase cells from the blood of a healthy donor. Following hybridization for 2–3 d and posthybridization washes, test and control DNA were detected via FITC and rhodamine, respectively. Chromosomes were counterstained with 4,6-diamidino-2-phenylindole (DAPI, 200 ng/ml), resulting in a Q-like-band pattern which was used for chromosome identification.

Digital image analysis

Image acquisition, processing, and evaluation were described in detail before (Du Manoir et al., 1995). Certain chromosomal regions are known to be critical in CGH analysis and, therefore, were not considered for quantitative image analysis. These regions were the distal part of 1p and the whole chromosome 19 (Du Manoir et al., 1995), as well as regions with a high content of repetitive sequences (heterochromatin blocks of centromeric regions and the long arm of the Y chromosome), which exhibit very low signal intensities due to suppression with Cot-1 DNA. This may result in gross variations of the ratio profiles from only small absolute variations of the hybridization intensities.

Results and discussion

Primary tumors

The CGH data are summarized in Fig. 1. In 9 of the 10 resected papillary renal cell tumor samples, chromosome gains or losses were identified by CGH analysis. The most frequent chromosome imbalances were gains of chromosomes 17 (six cases had an overrepresentation of the entire chromosome, while two additional cases had an overrepresentation of 17q [see also Fig. 2b]), 7 (five cases [see Fig. 2a]), 16 (four cases), and 1q (three cases). Two cases each exhibited gains of 3p, 3q, 8q, 20p, 20q, and 22q and of the whole chromosome 2, as well as losses of the whole chromosome 18 and chromosome bands 3q24→qter, 4q34→qter, 11q23→qter, and 12pter→p12.

These results are in agreement with data obtained by banding analysis (Kovacs, 1989; Kovacs, 1993). The combination of polysomies 7 and 17 does not occur in other types of RCC and has been shown to be characteristic of papillary RCCs, including both adenomas and carcinomas (Kovacs et al., 1991). Furthermore, it has been suggested (Kovacs et al., 1991) that chromosome changes in addition to these polysomies are indicative of malignant growth, irrespective of tumor size. In accordance with this hypothesis, in each of our 11 cases with carcinoma, aberrations other than gains of chromosomes 7 and 17 were present. Another aberration frequently found in banding studies is the loss of the Y chromosome in male patients (Kovacs et al., 1991, 1994). Due to its high content of repetitive DNA sequences, this chromosome is excluded from evaluation in CGH analyses (for an explanation, see the Materials and methods section of this paper), and, therefore, CGH data cannot be related to the cytogenetic findings regarding the Y chromosome.

Polysomies 16, 20, and partial polysomies of 3q, each of which was seen in at least two cases by CGH, were described amongst the most common additional imbalances (Kovacs, 1993). Other frequent aberrations associated with malignant growth in this tumor type are trisomies 8 and 12. These aberrations were diagnosed in one case each in this series. However, in additional cases, a partial trisomy 8 (UOK132) and a duplication of part of the long arm of chromosome 12 (UOK124) were found (see Fig. 1).

Cell lines

In five cases, DNA obtained from established tumor cell lines was examined. In all five samples overrepresentations of chromosome 7 and, in three of the five cases, overrepresentations of chromosome 17 were identified. In four of these cases, material from the corresponding primary tumors was available. Although the number of cases is low, differences between CGH findings in primary tumor material and derived cell lines are evident. These results are listed in Table I.

Overrepresentation of 5q in primary tumors and cell lines

In contrast to the banding data reported to date, in our series an unequivocal overrepresentation of at least part of 5q was seen in two patients (bands 5q33→qter in a cell line derived from UOK109met and the entire length of 5q in primary tumor material of UOK124). In another two cases (a cell line derived from UOK120 and primary tumor material of UOK132), the ratio profiles for parts of 5q showed a clear shift toward overrepresentation without exceeding the threshold value, suggesting the presence of a subclone with a gain of 5q material. Such a gain has been considered characteristic of nonpapillary RCC (Presti, 1991; Meloni et al., 1992). In these tumors, the excess 5q material is often associated with an unbalanced t(3;5), resulting in monosomy of 3p and trisomy of 5q segments. In one series, this translocation was found in 20 (27%) of 75 patients (Kovacs, 1993). Because translocations cannot be diagnosed by CGH, it remains unclear whether the mechanism of overrepresentation of 5q in papillary RCC is associated with a t(3;5). However, none of the four patients with shifted ratio profiles for 5q exhibited a loss of chromosome sequences on 3p. Loss of 3p material was seen only in one case (UOK146), which did not exhibit any imbalances with respect to chromosome 5. The ratio profiles for chromosomes 5 in the four relevant cases are shown in Fig. 2c.

Cytogenetic findings in familial cases of papillary RCC

In addition to clear-cell RCC in von Hippel-Lindau disease and transitional cell carcinoma associated with Lynch syn-

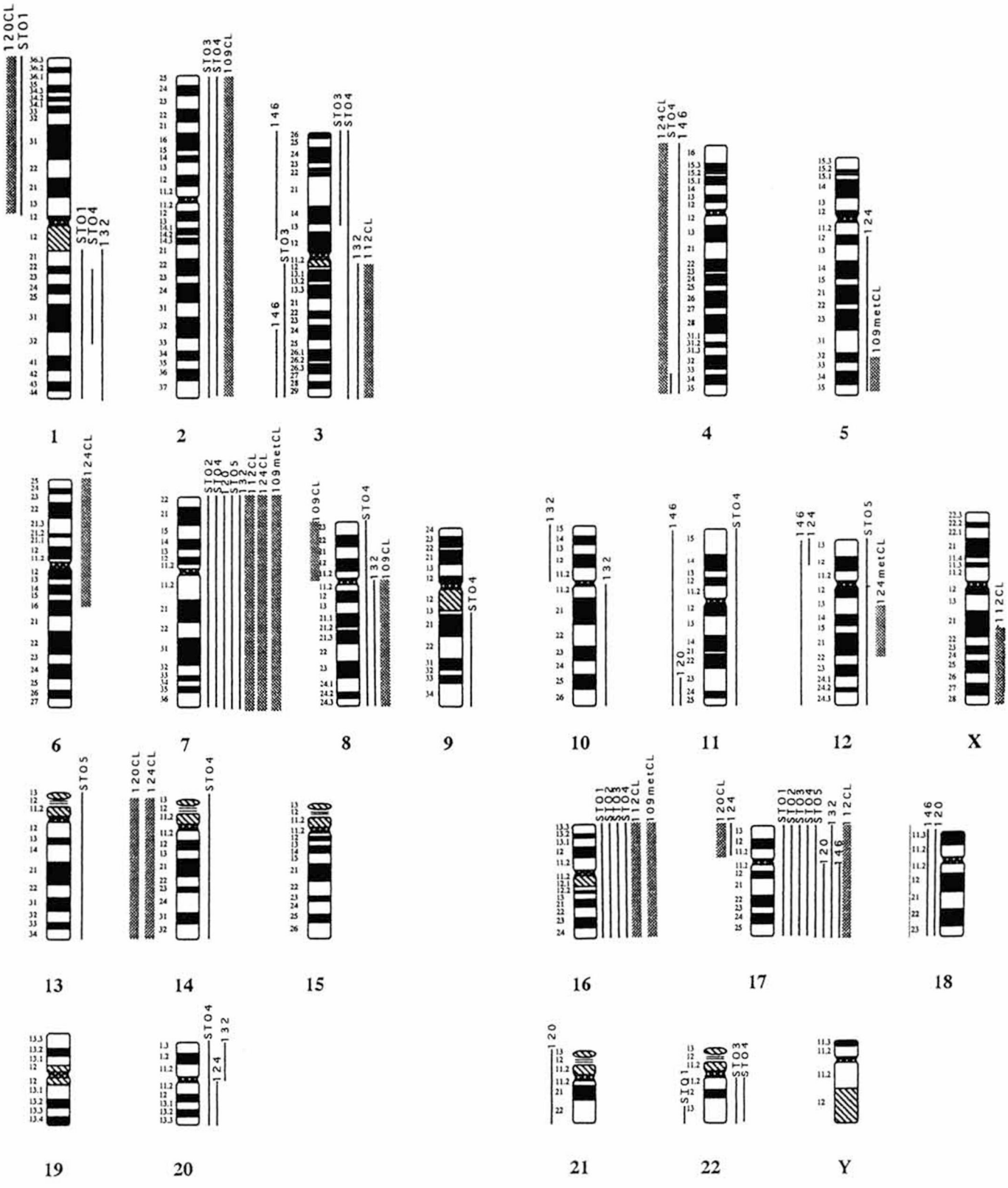

Fig. 1. Summary of all chromosomal gains and losses identified in 11 cases of papillary renal cell carcinoma. Vertical lines on the right side of a chromosome indicate a gain of genetic material, while those on the left side of a chromosome indicate loss of genetic material. The thick gray lines represent aberrations that were identified only in the cell line, but not in cryopreserved tumor material of the respective case (case numbers are provided for each line).

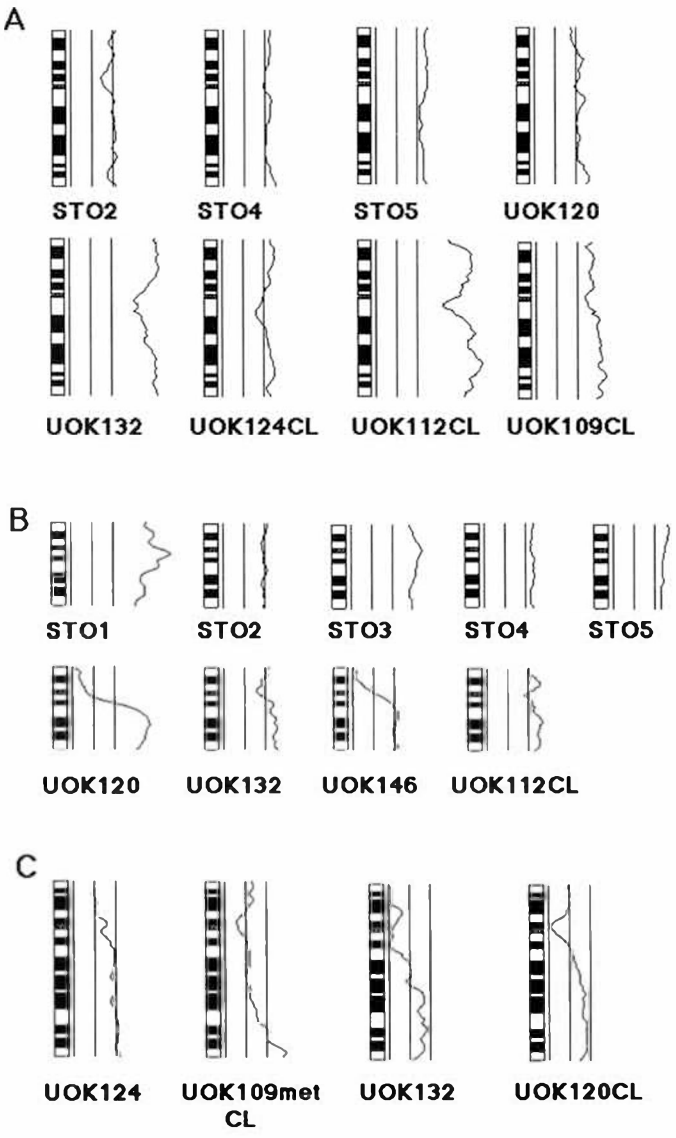

Table I. Results of comparative genomic hybridization (CGH) in established cell lines of papillary renal cell carcinomas and corresponding tumors

Cell line	CGH karyotype[a]
UOK 109CL	rev ish enh(2,7,8q), dim(8p)
UOK109metCL	rev ish enh(2,5q32q35,7,8q,16), dim(8p)
UOK112TU	No imbalances detected[b]
UOK112CL	rev ish enh(Xq21q28,3q,7,16,17)
UOK120TU	rev ish enh(7,17q), dim(11q23q25,18,21)
UOK120CL	rev ish enh(7), dim(1p,11q23q25,14,17p,18,21)
UOK124TU	rev ish enh(5q12q35,20q), dim(12p,17p)
UOK124CL	rev ish enh(5q32q35,6p,6q12q16,7), dim(4,14)
UOK124metCL	rev ish enh(6p,12q13q22)
UOK132TU	rev ish enh(1q,3q,7,8 q,10q,17,20p), dim(10p)
UOK132CL	rev ish enh(1q,3q,7,8q,20p), dim(10p)

[a] CGH karyotypes are listed according to ISCN (1995) nomenclature (Mitelman, 1995).

[b] In this case, CGH of the primary tumor tissue revealed a normal chromsomal content, whereas analysis of the tumor cell line showed chromosomal imbalances typical of papillary renal cell carcinomas. This may be due to a high percentage of normal cells within the particular tissue portion that was used for DNA isolation in this patient.

Fig. 2. Average ratio profiles of the relevant chromosomes 7 (**A**), 17 (**B**), and 5 (**C**). The ratios of FITC to rhodamine fluorescence are plotted along the respective chromosomes. The mode of the intensity ratio (central line) and the thresholds for overrepresentation (right line) and underrepresentation (left line) are shown. For each chromosome, the case number is provided. (**A**) Ratio profiles of the eight cases with a gain of chromosome 7. In all cases, the whole chromosome is overrepresented. (**B**) Ratio profiles of the nine cases with an overrepresentation of chromosome 17. In UOK 120 and UOK 146, only 17q is overrepresented, and the profile of the short arm is shifted toward underrepresentation. (**C**) Ratio profiles of cases with changes on 5q. In UOK124 and the cell line of a lymph-node metastasis of UOK 109, the profiles exceed the diagnostic thresholds for overrepresentation. In two additional cases (UOK132 and the cell line of UOK120) a clear shift of the ratio profile is seen. However, the ratio values are below the thresholds for overrepresentation. This finding is suggestive of the presence of subclones with gains of 5q material in tumor samples UOK132 and UOK120CL.

drome type II, papillary RCC is a third type of kidney cancer with clear evidence for familial cases (Zbar et al., 1995). Altogether, 10 families with familial papillary RCC have been described (Zbar et al., 1995). In the present study, cytogenetic data of cases from two different families are provided for the first time. All three cases had complex imbalances affecting 4 (STO5), 6 (STO3), and 13 (STO4) chromosomal regions, respectively. The only chromosomal imbalance present in all three cases was a gain of chromosome 17. Interestingly, in the two cases belonging to the same family (STO3 and STO4), a characteristic pattern of chromosomal changes was demonstrated: The copy number karyotype of case STO4 with chromosomal imbalances affecting 13 different chromosome regions [CGH result: rev ish enh(1q22q32,2,3,4q33q35,7,8,9q, 11,14,16,17,20,22q)] included five of six aberrations identified in the less complex case [STO3; CGH result: rev ish enh(2, 3p14p26,16,17,22q), dim(3q)]. Apart from gains of chromosomes 16 and 17, the other three imbalances shared by these cases in the same family were not present in any of the other eight tumors. To our knowledge, the presence of such closely related genetic alterations in tumors of siblings has not been demonstrated before. This finding suggests a genetically determined mechanism resulting in genomic alterations that affect specific chromosomes or chromosomal subregions, or, alternatively, selection of cells with mutations involving specific chromosome subregions.

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