The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/21824

Please be advised that this information was generated on 2019-04-07 and may be subject to change.
Distinct Xp11.2 Breakpoints in Two Renal Cell Carcinomas Exhibiting X;Autosome Translocations


Department of Medical Genetics, University of Groningen (T.D., E.v.d.B., R.P.F., A.B., B.d.J.), and Department of Human Genetics, University Hospital Nijmegen (M.W., M.W., A.G.v.K.), the Netherlands, and the Institute of Pathology, University of Mainz, Germany (S.S.)

Several human renal cell carcinomas with X;autosome translocations have been reported in recent years. The t(X;1)(p11.2;q21) appears to be a specific primary anomaly, suggesting that tumors with this translocation form a distinct subgroup of RCC. Here we report two new cases, one with a t(X;10)(p11.2;q23), the other with a t(X;1)(p11.2;p34). The common breakpoint in Xp11.2 suggests that they belong to the above-mentioned subset of RCC. Using FISH in conjunction with X-specific YAC clones, we demonstrate that the two new cases exhibited distinct breakpoints within Xp11.2.

INTRODUCTION

Renal cell carcinomas (RCCs) arise from tubular epithelium. The incidence of RCC peaks in the sixth decade of life (Medeiros and Weiss, 1990). Approximately 70% of the patients are males. This disparity between the sexes is even more striking when the tumor occurs at an early age.

Cytogenetic and molecular genetic investigation of RCC has revealed consistent karyotypic abnormalities corresponding to the different histologic RCC subtypes (Kovacs et al., 1991; Kovacs, 1993; Van Den Berg et al., 1993). A deletion of the short arm of chromosome 3 occurs in the clear cell type, which is the most common type of RCC. Also, a (partial) trisomy of chromosome 5, especially the 5q22-pter segment, is frequently found in the clear cell tumors. A different set of abnormalities is found in the chromophlic (or granular), tubulopapillary tumors. Instead of 3p and 5q aberrations, this subtype is characterized by combinations of gain of chromosomes 7 and 17 and loss of the Y chromosome, together with additional copies of chromosomes 12, 16, and/or 20. Renal oncocytoma shows loss of the Y chromosome in combination with loss of chromosome 1. Recently, a subset of oncocytomas was described with abnormalities involving 11q13 (Van Den Berg et al., 1994). In the chromophobic tumor type, loss of the Y chromosome and loss of chromosomes 1, 6, 14, 15, and 22 may be found. In addition, telomeric associations are observed in this subtype.

Meloni et al. (1993) described four cases of papillary RCC in which a t(X;1)(p11.2;q21) was observed, in one case as the sole abnormality. This unique translocation had first been described by de Jong and coworkers in 1986 in a case of RCC in a 2-year-old boy. In addition, three other cases of RCC have been published (Yoshida et al., 1985; Tomlinson et al., 1991; Ohjimi et al., 1993), two containing X;autosome translocations (Yoshida et al., 1985; Tomlinson et al., 1991) and one with a del(X) (Ohjimi et al., 1993), again with breaks in Xp11.2. This suggests that tumors carrying Xp11.2 anomalies represent a new subgroup of RCC.

We present two new cases of RCC carrying translocations involving Xp11.2. Using FISH in conjunction with Xp11.2-specific YAC clones, we determined the relative locations of the Xp11.2 breakpoints in these tumors. The results obtained were compared with data from the literature, and attempts were made to correlate the different breakpoints with tumor histology.

MATERIALS AND METHODS

Patient Material and Cytogenetic Analysis

The first patient was a 52-year-old male who presented with a 12 cm diameter RCC in the left kidney. The tumor had a papillary growth pattern (Fig. 1b), but also showed distinct clear cell features (Fig. 1a). The second patient was a 77-year-old male with a 3×2 cm RCC in the left kidney. This tumor was of the chromophobic papillary type, grade II. Both tumors were classified according to Thoenes et al. (1986).

Received December 12, 1994; accepted April 19, 1995.

Address reprint requests to T. Dijkhuizen, Department of Medical Genetics, University of Groningen, Antonius Deusinglaan 4, 9713 AW Groningen, The Netherlands.

© 1995 Wiley-Liss, Inc.
Figure 1. Case 1 showing the clear cell features of this tumor with areas of (a) a compact (solid) growth pattern and (b) a papillary growth pattern, HE, 480 x.

Fresh representative samples of both tumors were submitted for cytogenetic investigation. Part of the tissue was cultured for 5–7 days in RPMI 1640 supplemented with FCS (16%), glutamine, and antibiotics. The cultures were harvested and chromosome preparations were made according to standard cytogenetic techniques. The chromosomes were G-banded using trypsin, and karyotypes were described according to the ISCN 1991.

Fluorescence In Situ Hybridization

All fluorescence in situ hybridization (FISH) procedures on metaphase cells were essentially as described previously (De Leeuw et al., 1994b). The probes used were: the centromere X-specific alphoid sequence probe pBamX5, the OATL1 YAC #2, and the OATL2 YAC #7. All probes were labeled with either biotin-11-dUTP (Sigma, St. Louis, MO) or digoxigenin-11dUTP (Boehringer Mannheim, Germany) using a nick-translation kit (GIBCO Life Technologies, Gaithersburg, MD). The labeled DNA was precipitated in the presence of sonicated herring sperm DNA (50-fold concentration). In the case of the YACs, a 50-fold amount of sonicated total human DNA was coprecipitated for preannealing purposes. This mixture was dissolved in 6 µl of a hybridization FDST solution (50% v/v deionized formamide, 10% w/v dextran sulphate, 2 × SSC, 1% Tween-20, pH 7.0). Prior to hybridization, the probe was denatured at 80°C for 10 min, chilled on ice, and incubated at 37°C for 4 hr (200 ng YAC DNA per reaction), allowing preannealing. In the case of centromeric probes, no preannealing was performed and the probe concentration was 10 ng per hybridization. Metaphase
The slides were pretreated with RNase A (100 μg/ml in 2 × SSC at 37°C for 1 hr). Subsequently, the slides were denatured in 70% formamide, 2 × SSC, pH 7.0, at 70°C for 2 min and incubated with the probes under an 18×18-mm coverslip in a moist chamber for 2 days.

Immunocytochemical detection of the hybridizing probes was achieved using FITC-conjugated avidin (Vector Laboratories, Burlingame, CA; 1:500 diluted) followed by a two time amplification using rabbit anti-FITC (Vector Laboratories; 1:250 diluted), and mouse anti-rabbit conjugated FITC in the case of biotinylated probes (green signals). For digoxigenin probes, rhodamine-conjugated sheep anti-digoxigenin (Boehringer Mannheim; dilution 1:20) was used followed by Texas Red-conjugated donkey anti-sheep (red signals) (Jackson Immunoresearch, West Grove, PA; dilution 1:100). The slides were mounted in antifade medium (9 parts DABCO, Merck, Darmstadt, Germany, in glycerol and 1 part 0.2 M TRIS/HCl, pH 8.0) containing 4,6-diamino-2-phenylindole (DAPI, 0.5 μg/ml, Sigma) for counterstaining of the chromosomes.

Slides were viewed under a Zeiss Axiophot epifluorescence microscope equipped with appropriate filters for the visualization of FITC, rhodamine/Texas Red, and DAPI fluorescence as well as the simultaneous visualization of FITC and Texas Red fluorescence (Omega double band pass filter, Brattleboro, VT). Separate digital images (for Texas Red, FITC, and DAPI, respectively) were recorded using a Photometrics high-performance CH250/A cooled CCD-camera interfaced onto a Macintosh Iic computer. The images were superimposed and displayed in red-green-blue pseudocolors on the computer screen using the image analysis and processing software program BDS-image (Biological Detection Systems, Rockville, MD). Photographs were made from the computer screen on Kodak EPP 100 Plus colorslide film using a Polaroid Quickprint.

RESULTS

Banding Analysis

A total number of 10 cells were analyzed from case 1. Eight cells showed a balanced translocation involving chromosomes X and 1, as well as other changes, giving the karyotype 51,Y,t(X;1)(p11.2;p34),+5,der(6)t(1;6)(q11;q11), +7, +8, +11, +20 (Fig. 2a).

Eleven cells were analyzed from case 2. The karyotype was 52,−Y;+t(X;10)(p11.2;q23), +3, +5, +7, +7, +12, +16, +17 (Fig. 2b). The constitutional karyotype of both patients was 46,XY.

FISH Analysis

Two OAT-specific YACs, one corresponding to the OATL1 (YAC #2; insert size 500 kb) cluster and the other corresponding to the OATL2 (YAC #7; insert size 600 kb) cluster on the human X chromosome, were used. Metaphase spreads of both tumors were analyzed with these YACs in conjunction with FISH. The results of the FISH analyses are given in Figure 3. In case 1, all sequences contained within YAC #2 were translocated to chromosome 1 in the X;1-translocation (Fig. 3c), whereas all sequences contained within YAC #7 were retained on the X chromosome (Fig. 3d). The breakpoint of case 1 is, therefore, distal to the OATL2 locus and proximal to the OATL1 locus (Fig. 4). For case 2, all sequences contained within YAC #2 and YAC #7 were retained on the X chromosome (Fig. 3a,b), indicating that the X;10-translocation in this case was distal to the OATL1 locus (Fig. 4).

DISCUSSION

The cytogenetic and histologic data of all cases of RCC with X;autosome translocations are summarized in Table 1. Histologically, most of the cases were described according to the WHO classification. They include four papillary tumors (Meloni et al., 1993), one clear cell tumor (Tomlinson et al., 1991), and one metastasis of a papillary tumor (Ohjimi et al., 1993). Three of the cases were classified according to the criteria proposed by Thoenes and Störlke (De Jong et al., 1986; the present two cases). Two of them revealed a papillary growth pattern with distinct clear cell features, whereas the third tumor (case 2) was a chromophilic tubulopapillary RCC. The remaining case was published by Yoshida et al. (1985) but lacks a description of the pathologic findings.

Cytogenetic analysis revealed a t(X;1)(p11.2;q21) in five of ten cases (De Jong et al., 1986; Meloni et al., 1993), in two of them as the sole anomaly. This led to the conclusion that these tumors might represent a new cytogenetic subtype of RCC, especially since this abnormality has never been described in any other malignancy. The other cases exhibited a t(X;1)(p11.2;p34) (Yoshida et al., 1985; our case 1), a t(X;17)(p11.2;q25) (Tomlinson et al., 1991), a t(X;10)(p11.2;q23) (our case 2), and a del(X)(p11) (Ohjimi et al., 1993).

The common denominator in cases of RCC with...
Figure 2. a: One of the metaphases of case 1 revealing the 51,Y,t(X;1)(p11.2;p34), + 5,der(6)t(1;6) (q11;q11), + 7, + 8, + 11, + 20 chromosomal pattern. b: One of the metaphases of case 2 revealing the 52,—Y,t(X;10)(p11.2;q23), + 3, + 5, + 7, + 12, + 16, + 17 chromosomal pattern.

X; autosome translocations is the breakpoint in Xp11.2, suggesting an important role for this region in tumor development. Cytogenetically, a similar breakpoint in Xp11.2 has been observed in synovial sarcoma, with the specific translocation t(X;18)(p11.2;q11.2). Recently, De Leeuw and Suijkerbuijk (De Leeuw et al., 1993, 1994a; Suijkerbuijk et al., 1993) mapped two alternative Xp11.2 breakpoints in these neoplasms to the OATL1 and the OATL2 regions, respectively, using FISH and Xp11.2-specific YAC clones. The genes at the OATL1/OATL2 regions as well as those at 18q11.2
have recently been cloned (Clark et al., 1994; De Leeuw et al., 1995).

In order to determine the relative breakpoint positions at Xp11.2 in the RCCs, four cases (Suikerbuijk et al., 1993, and the present two cases) were examined with the OATL1 and OATL2-specific YACs and other probes (Sinke et al., 1993; Suikerbuijk et al., 1993). The examination of the first two cases revealed a breakpoint location distal to the OATL2 and proximal to the OATL1 region (Suikerbuijk et al., 1993). Surprisingly, the relative breakpoint of at least one of the present two cases appears to be different. In case 2, it is located distal to both the OATL1 and OATL2. In case 1 the relative breakpoint is distal to the OATL2 and proximal to the OATL1 region. The YAC #7 signal, which was previously found to be split by the translocation in RCCs with a t(X;1)(p11.2;q21) (Suijk-

---

**Figure 3.** FISH on case 2 (a,b)- and case 1 (c,d)-derived metaphase cells using a paint for chromosome 10 (a; green), OATL1 (YAC2 (b; green) or OATL2 YAC7 (d; green), in conjunction with the chromosome X centromere probe (b; red), the chromosome 1 centromere probe (c; red), or a combination of chromosome 1 and X centromere probes (d; red). a: The normal 10, the der(10) and the der (X) are marked by a large arrowhead, a small arrowhead, and an arrow, respectively. b: The der (X) is marked by an arrow. c,d: The chromosome 1 centromeres are marked by arrowheads, whereas the YAC signals are indicated by arrows. In b and d, the X centromeres (red) are not marked. Chromosomes are counterstained in blue (DAPI).
Figure 4. Diagram representing the short arm of the X chromosome and the relative locations of the different breakpoints in Xp11.2 (arrows); SS1 and SS2, two alternative synovial sarcoma-associated breakpoints; X;1*, t(X;1)(p11.2;q21) breakpoint observed in papillary renal cell carcinoma; X;1 and X;10, two breakpoints corresponding to the new cases reported here.

TABLE I. Histologic and Cytogenetic Data of RCC With X;Autosomal Translocations

<table>
<thead>
<tr>
<th>Reference</th>
<th>Case</th>
<th>Age (yr)</th>
<th>Histology</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>De Jong et al., 1986</td>
<td>2</td>
<td>68</td>
<td>Clear cell; papillary + compact</td>
<td>46,Y,t(X;1)(p11.2;q21)</td>
</tr>
<tr>
<td>Tomlinson et al., 1991</td>
<td>1</td>
<td>55</td>
<td>Clear cell; alveolar</td>
<td>46,Y,t(X;17)(p11.2;q25)</td>
</tr>
<tr>
<td>Ohjimi et al., 1993</td>
<td>24</td>
<td>24</td>
<td>Metastatic RCC; papillary</td>
<td>45,Y,del(X)(p11.1)(q23?),add(13)(p11),+add(13)(p11),+add(16) (p11),−17,−18</td>
</tr>
<tr>
<td>Yoshida et al., 1985</td>
<td></td>
<td></td>
<td></td>
<td>46,Y,t(X;1)(p11.2;p34.1)</td>
</tr>
<tr>
<td>Meloni et al., 1993</td>
<td>1</td>
<td>68</td>
<td>RCC; granular + papillary (G4)</td>
<td>49,Y,t(X;1)(p11.2;q21),+7,+15,+17</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>55</td>
<td>RCC; papillary (G3)</td>
<td>41,Y,t(X;1)(p11.2;q21),i(1)(q10),der(3)t(3;12)(q12;q12),−4,−5,inv(7) (q11.2p22),−9,−10,−11,−13, add(16),+17,+18,+20/40,idem,Y</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td>RCC; papillary (G3)</td>
<td>45,Y,t(X;1)(p11.2;q21),−22</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>24</td>
<td>RCC; papillary</td>
<td>46,Y,t(X;1)(p11.2;q21)/46,idem,inv (13)(q12q22)</td>
</tr>
<tr>
<td>This series</td>
<td>1</td>
<td>52</td>
<td>Clear cell; papillary + compact (G2)</td>
<td>51,Y,t(X;1)(p11.2;p34),+5,der(6)c(1;6)(q11;q11),+7,+8,+11,+20</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>77</td>
<td>Chromophilic; papillary (G2)</td>
<td>52,−Y,t(X;10)(p11.2;q23),+3,5,7,+7,+12,+16,+17</td>
</tr>
</tbody>
</table>

Since Xp11.2 contains various cross-hybridizing low copy repeated motifs (Sinke et al., 1993), the breakpoint in case 1 may be similar to the one found by Suijkerbuijk et al. (1993). This suggests that there is a subtype of RCC with this specific Xp11.2 breakpoint, possibly with a distinct histology, involving both clear cell features and a papillary growth pattern.

Case 2 was a chromophilic tubulopapillary RCC without any clear cell features, and had, besides
the Xp11.2 translocation, a chromosomal pattern frequently associated with this subtype (Van Den Berg et al., 1993), i.e., -Y, +7, +7, +12, +16, and +17. Moreover, the FISH results indicate that the breakpoint in this case was located distal to the OATL1 region, whereas the breakpoints in the other three cases examined with FISH are located between the OATL1 and OATL2 regions. In this case the Xp11.2 translocation might be coincidental.

Alternatively, distinct sequences may be involved in the development of specific subsets of renal tumors, analogous to the findings in synovial sarcoma. In these sarcomas, two related regions in Xp11.2 are involved in the translocation with chromosome 18. This alternative involvement seems to correlate with two histologic subtypes of this neoplasm. Biphasic synovial sarcomas display a break predominantly in the OATL1 region, whereas the monophasic subtype appears to be associated with the OATL2 region (De Leeuw et al., 1994b, 1995). It is tempting to speculate that in RCC the breakpoint differences may also reflect subtle differences in tumor histology. The other cases were classified according to the criteria of the WHO (Mostofi et al., 1981), and the cell type is not mentioned. Reexamination of these cases, using the classification of Thoenes and Störkel (Thoenes et al., 1986), might reveal histologic patterns similar to that of our case 1.

An interesting finding is that four of ten patients with an RCC carrying an X;autosomal translocation were of relatively young age (1, 2, 24, and 24 years old). Overall, the incidence of RCC increases with age. In all of these young patients, except one, the X;autosomal translocation was the sole aberration, indicating its crucial role in tumorigenesis. The fourth case, published by Ohjimi et al. (1993), had a del(X) and was a metastasis. The additional chromosomal abnormalities found by the authors are probably a reflection of the metastatic potential of this tumor. The case described by Yoshida et al. (1985) also had t(X;1) as the sole aberration, but the age of the patient was not provided.

Thus far, all RCCs with Xp11.2 abnormalities have developed in male patients. Whether or not an X-linked tumor suppressor gene is involved, as proposed by Tomlinson et al. (1991) and Meloni et al. (1993), is not clear. The fact that all of the cases developed in males should perhaps not be overrated since 70% of all cases of RCC occur in males and, including the present cases, only ten cases have yet been published. RCC in male patients frequently shows loss of the Y chromosome. This is especially true for the chromophilic (papillary) type, in which Y chromosome loss is found in 84% of cases (Kovacs et al., 1994). In the cases of RCC with Xp11.2 abnormalities, Y chromosome loss is found in only 20% of the cases. This percentage closely resembles the findings in clear cell RCC, in which the Y chromosome is lost in 22% of male patients. There might be a relation between this observation and the finding of at least three tumors with distinct clear cell features.

In conclusion, RCCs with a breakpoint in Xp11.2 may represent a new subtype of RCC with a distinct histology and an aberrant age distribution. A more detailed description of the pathologic findings of these neoplasms, in addition to the exact determination of the breakpoints and genes involved in Xp11.2, might shed light on this subject.

REFERENCES


DISTINCT Xp11.2 BREAKPOINTS IN TWO RCCs