Fine mapping of the 1q21 breakpoint of the papillary renal cell carcinoma-associated (X;1) translocation

Received: 10 November 1995 / Revised: 3 February 1996

Abstract A combination of Southern blot analysis on a panel of tumor-derived somatic cell hybrids and fluorescence in situ hybridization (FISH) techniques was used to map a series of DNA markers relative to the 1q21 breakpoint of the renal cell carcinoma (RCC)-associated (X;1)-(p11;q21) translocation. This breakpoint maps between several members of the S100 family which are clustered in the 1q21 region and a conserved region between man and mouse containing the markers SPTA1-CRP-APCS-FcER1A-ATP1A2-APOA2. The location of the breakpoint coincides with the transition of a region of synteny of human chromosome 1 with mouse chromosomes 3 and 1.

Introduction

It is by now generally accepted that specific chromosomal aberrations play a crucial role in tumor development. Studies on tumor-specific translocations have led to the identification of several genes involved, among which are those encoding transcription factors (Rabbitts 1994). As a consequence of these translocations, genes may become deregulated or fusion genes may be formed which interfere with the normal programs of cell growth and differentiation, often by means of altered transcriptional control (Rabbitts 1994).

Renal cell carcinomas (RCCs) form a very heterogeneous group of tumors, both on a histological and a cytogenetic level (van den Berg et al. 1993; Stoerkel and van den Berg 1995). Chromophilic RCCs represent a subgroup of RCCs which mainly exhibit a papillary growth pattern, and therefore, are commonly referred to as papillary RCCs. The cytoplasm of this tumor type shows marked granularity with predominant eosinophilia. However, sometimes papillary RCCs show a clear cell-like appearance due to the disposition of glycogen and fat in their cytoplasm (Thoenes et al. 1986). Whereas chromosome 3 aberrations are frequently encountered in clear cell non-papillary cases, these anomalies appear to be uncommon in chromophilic papillary RCCs. Instead, a combination of gain of chromosomes 7 and 17, and loss of the Y chromosome has been found. In addition, t(X;1)(p11;q21) or variants thereof, have repeatedly been described in a subgroup of these tumors (de Jong et al. 1986; Tomlinson et al. 1991; Meloni et al. 1993; Dijkhuizen et al. 1995; Hernandez-Marti et al. 1995; Tonk et al. 1995; Zhao et al. 1995), sometimes as the sole cytogenetic anomaly present. Although all t(X;1)-positive patients described so far were male, a female patient with papillary RCC carrying this translocation has recently been identified as well (J. Couturier, personal communication). Despite the limited amount of data on the histological details of these tumors, there is growing evidence that this translocation is specific for chromophilic papillary RCCs with distinct clear cell features (i.e., the capacity to accumulate glycogen and fat) (Thoenes et al. 1986; Meloni et al. 1993; Tonk et al. 1995). In order to facilitate positional cloning of the t(X;1) breakpoint and the gene(s) involved, we have mapped a panel of markers of the proximal 1q region relative to this breakpoint. By doing so, the genomic interval of interest could be reduced considerably.

Materials and methods

Tumor cell lines and somatic cell hybrids

Two primary renal cell cultures (C189-12117 and C189-17872) were used in this study. C189-12117 contains the t(X;1) as the only abnormality, whereas C189-17872 also shows numerical aberrations next to the t(X;1) (Meloni et al. 1993). Tumor-derived somatic cell hybrids were obtained as described before (Geurts van Kessel et al. 1983; Sinke et al. 1993). Briefly, after fusion of A3 or Wg3h hamster cell lines and the t(X;1)-positive C189-12117 renal carcinoma cells, a panel of somatic cell hybrids was isolated in
which the reciprocal translocation chromosomes segregate. WgRe5 was chosen as the der(X) hybrid, A3Re3A as the der(1) hybrid (Sinké et al. 1993). As controls, two hybrid lines containing the normal X chromosome (Wieczorl et al. 1984) or normal chromosome 1 (GM13.139, Corell Repository) as the only human constituent, and parental hamster (A3) and mouse (A9) cell lines were included.

DNA probes and Southern blot analysis

The following chromosome 1 probes were used: the pB3 plasmid containing the genomic fragment of APOA2 was generously provided by Dr. H.R. Middleton-Price (Middleton-Price et al. 1988); the human probe for ATP1A2 by Dr. J.B. Lingrel (Shull and Lingrel 1987); plasmid pHtSp6 containing the SPTA1 cDNA by Dr. P.J. Curtis (Huebner et al. 1985); SPTA1-YACs were isolated from the CEPH mega yeast artificial chromosome (YAC) (Chumakov et al. 1992) and ICI YAC libraries (Anad et al. 1990) (ICI 6DA4; CEPH 638f3, 7489, 751b10, 770h10, 788b1) ranging from 240 kb to 1720 kb in size. The FcER1A probe was generously provided by Dr. P. Leder (Shimizu et al. 1988; Tepler et al. 1989), the human S100A4; S100A8(CAGA), cDNA by Dr. M.J. Vassan (Dornin et al. 1987; Wilkinson et al. 1988; Vassan et al. 1989); the SPRR1, 2, and 3 probes (pIC15B, pp930-2, and pSPR3) and YACs 1.2 and 5.2 by Dr. C. Backendorf (Gibbs et al. 1993); the pLM6 plasmid corresponding to the 5' end of NRTK1 by Dr. M.A. Pierotti (Martin-Zanca et al. 1989; Greco et al. 1993); cathepsin S cDNA and genomic fragment by Dr. H.A. Shapman (Shi et al. 1994); and probes corresponding to exon 1 and 9 of pbx1 by Dr. M. Cleary (Nourse et al. 1990). Probes were labeled by random-prime labeling and hybridized at 65°C in 0.5 M sodium phosphate buffer, 1 mM EDTA, and 7% SDS. Washes were performed in 40 mM sodium phosphate, 0.1% SDS.

Genomic DNA was isolated using standard protocols by proteinase K-SDS treatment followed by phenol-chloroform extractions and ethanol precipitation. After digestion and size selection on an agarose gel, the DNA was blotted onto Hybond N-Plus (Amersham) or Genescreen Plus (Dupont) membranes.

Fluorescence in situ hybridization

YAC probes were labeled with digoxigenin-11-dUTP (Boehringer Manheim) and centromere probes (chromosome 1-specific pUC 1.77) with Cy3-dCTP (BDS/KIMTEC) using a nick translation kit (Life Technologies). After coprecipitation with a 50-fold excess of Cot 1 DNA and heating for 10 min at 80°C, repetitive sequences present in the YACs were preannealed for 30 min at 37°C in 7 μl of 50% deionized formamide, 10% dextran sulfate, 2× SSC, 1% (v/v) Tween 20, pH 7.0. Chromosome slides were treated as described before (Dijkhuizen et al. 1995). After hybridization (50% formamide, 2× SSC) which was performed at 37°C for at least 48h, and washed (50% formamide, 2× SSC; 2× SSC), immunochemical detection was carried out using fluorescein isothiocyanate-conjugated sheep anti-digoxigenin (1:20; Boehringer Mannheim). The slides were mounted in anti-fade medium (1.4% w/v di-azobicyclo-(2,2,2)-octane(DABCO), Merck) containing 0.5 μg/ml 4,6-diamino-2-phenylindole (DAPI, Sigma) for counterstaining of the chromosomes. Slides were analyzed under a Zeiss Axiohot epifluorescence microscope equipped with appropriate filters. Digital images were captured as described before (Dijkhuizen et al. 1995) using the processing software program, BDS-image (Oncor).

Table 1 Localization of markers relative to the renal cell carcinoma-associated t(X;1) in band 1q21. DNA probes and yeast artificial chromosomes (YACs) were scored for absence (−) or presence (+) on der(X) and der(1) using Southern blot analysis (S) or fluorescence in situ hybridization (FISH, F).

<table>
<thead>
<tr>
<th>Marker/probe</th>
<th>Method</th>
<th>der(1)</th>
<th>der(X)</th>
<th>X-only</th>
<th>1-only</th>
</tr>
</thead>
<tbody>
<tr>
<td>YAC1.2†</td>
<td>F</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>YAC5.2†</td>
<td>F</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>SPRR1</td>
<td>S</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>SPRR2</td>
<td>S</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>SPRR3</td>
<td>S</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>S100A8/CAGA</td>
<td>S</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>S100A6/CACY</td>
<td>S</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>S100A4</td>
<td>S</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>SPTA1</td>
<td>S/F</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>FeER1A</td>
<td>S</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>ATP1A2</td>
<td>S</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>APOA2</td>
<td>S</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Cathepsin</td>
<td>S</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>NRTK1</td>
<td>S</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>pbx1</td>
<td>S</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

†SPTA1-containing YACs that were isolated from the ICI YAC and CEPH mega YAC libraries ranging in size from 240 to 1720 kb all mapped to der(X) in FISH experiments on CL89-12117 cells.

Fig. 1 Southern blot analysis of somatic hybrid cell lines using SPTA1 (a) and S100A4 (b) as molecular probes. A3 and A9 are the hamster and mouse controls, X and 1 the hybrids containing these chromosomes as the only human component, and RCC1 and RCC2 the C189-12117 and C189-17872 renal cancer cells containing the t(X;1). The DNAs were digested with EcoR1 (a) and HindIII (b). As a molecular marker, lambda DNA digested with HindIII was used.

Results and discussion

For fine mapping of the 1q21 breakpoint of the RCC-associated (X;1) translocation we mapped a series of DNA markers and YACs (Table 1) which were known to be located in or near the 1q21 region, relative to this break-
Fig. 2. A typical fluorescence in situ hybridization experiment of somatic cell hybrids (Sene et al. 1993) and controls. Probes were scored for the presence and absence of hybridization signal, which contained YACs 1 and 2 were scored as probes which both map probes to der(X) or der(1). DNA from the parental cell line were used as controls for the specificity of the signal. Those YACs 1 and 2 were scored as probes which both map to der(Y). The YAC signal in B, as indicated by arrow, is detected close to the centromere.

The Southern blot analysis was performed on our panel of somatic cell hybrids (Sene et al. 1993) and controls. Two other YACs within the 1q21 region were also analyzed by FISH (Table 1). The results from using YAC 2 are shown in Figure 2A. Again, this is in agreement with the Southern blot hybridizations using probes which both map to der(Y). Based on these results, and the map provided by Voelz et al. (1994), and the physical mapping of the closely linked markers SPT1A to APO1, the RBC-associated break point can be located between the SPT1A locus and the members of the S100 family.
Fig. 3 Diagram of chromosome 1 and localization of the renal cell carcinoma associated t(X;1) within band 1q21 (arrows). The asterisks indicate that there are more genes (S100 genes) or more gene copies within a family (SPRR1, 2, and 3 in a number of copies). The overall order was taken from Volz et al. (1993), Harden et al. (1994), and the report of the First International Workshop on Human Chromosome 1 Mapping (Dracopoli et al. 1994).

After testing the family that was tested, as indicated in Fig. 3. Since the order of genes in the SPTA1-APOA2 cluster is known (Dracopoli et al. 1994), we assume that also CRP and APCS map distal to the breakpoint. Recently, a clustering of at least nine S100 genes was described (Schaefer et al. 1995). Since the order and exact localization of all S100 genes is not completely known at present, we marked these S100 genes as S100* in Fig. 1. Moreover, it cannot be excluded that there are more (unidentified) members of the S100 family which map to this region. However, four of these genes (S100E/S1003, CAPL/S100A4, S100D/S100A5, and CACY/S100A6) were physically mapped in a direct head-to-tail order, based on overlapping genomic clones (Engelkamp et al. 1993). Based on this order, the marker we tested that is closest to and maps proximal to the breakpoint, is S100A4. Since the different S100 genes are highly homologous, this might increase the risk of recombination, and possibly, instability, which makes it a candidate region for breaks to occur. As expected, all other markers that are known to be located proximal to the S100 genes mapped to der(1) and thus proximal to the breakpoint. No differences in localization of the (X;1) breakpoint were observed between the two renal tumors that were examined, indicating that the exact location of the breakpoints must be confined to a relatively small area. Until now, no shifted bands (Southern blot analysis) or split signals (FISH) were detected with any of the probes used. The largest SPTA1 YACs were all still positive for FcER1A. Based on their size, and estimations that were made for the distance between SPTA1 and FcER1A (Kingsmore et al. 1989), these YACs must be extending for the largest part distal to the SPTA1 marker, and thus, away from the breakpoint.

NRTK1, which maps to 1q21-22 (Weier et al. 1995), has been reported to form the trk oncogene by linkage of the tyrosine kinase domain to foreign activating sequences (Martin-Zanca et al. 1989; Greco et al. 1993). Based on its function, it is a possible candidate in this region. However, since it is not located at the 1q21 breakpoint, this gene is not likely to be involved in the oncogenesis of these tumors. Another candidate which maps within 1q21 is cathepsin S. Abnormal levels of cathepsins (lysosomal proteinases) have been implicated in several types of cancer (Qian et al. 1989; Leto et al. 1992). However, this gene can also be excluded, based on its localization outside the restricted t(X;1) breakpoint region.

Interestingly, the 1q21 breakpoint coincides with the transition of a region of synteny of two different mouse chromosomes (1 and 3) to human chromosome 1 (Kingsmore et al. 1989; Dorin et al. 1990; Dracopoli et al. 1994).
be prone to recombination events and translocations. Further positional cloning will reveal whether the t(X;1) breakpoints in different tumors are clustered and map close to or within the same gene.

Acknowledgements The authors thank C. Backendorf for generously providing the SPRR probes and YACs. B. Janssen and the members of the Tumourcytogenetics Group are acknowledged for expert technical assistance. This work was supported by the Dutch Cancer Society, grant 94-733.

References


