Allelic Losses at Chromosome 17p in Human Renal Cell Carcinoma Are Inversely Related to Allelic Losses at Chromosome 3p

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ABSTRACT

Recent studies have demonstrated that allelic losses at chromosome 17p are associated with the genesis of a wide variety of human cancers. In order to assess whether the rearrangement of chromosome 17p was responsible for the genesis of renal cell carcinoma (RCC), we used restriction fragment length polymorphism analysis of chromosome 17p. We studied 48 RCCs, including 6 metastatic RCCs, from 43 patients with 5 polymorphic probes to loci within or near the p53 gene. Allelic losses at chromosome 17p were detected in only 6 of the 36 informative cases (17%), and no definitive correlation was demonstrated between allelic losses at 17p and the tumor stages. The 6 RCCs with allelic losses at 17p were histopathologically classified as a clear cell type in one, a mixed cell type in one, and granular cell types in the other four cases. Allelic losses at 17p in the clear cell type of RCC were infrequent (6%, 1 of 18), and were not detected even in the metastatic tumor from a highly advanced RCC case. This finding suggests that allelic losses at 17p could be random genetic rearrangements in the case of the clear cell type of RCC. On the other hand, allelic losses at 17p in the granular cell type of RCC were demonstrated with a significantly higher frequency (44%, 4 of 9). We previously reported that allelic losses at 3p were specific to the clear cell type of RCC (Ogawa et al., Cancer Res., 51:949–953, 1991). Examination of the association of allelic losses at 17p with those at 3p revealed that none of the informative RCCs with allelic losses at 17p showed allelic losses at 3p. Conversely, 17 of 25 informative RCCs with retention of 17p alleles lost alleles at 3p. Thus, an inverse relationship was demonstrated with statistical significance (P < 0.01). These data suggest that the types of rearrangement on chromosome 17p and/or chromosome 3p can differentiate between the histopathological subtypes of RCC.

INTRODUCTION

It is currently accepted that an accumulation of genetic aberrations causing an activation of oncogenes and/or inactivation of tumor suppressor genes is necessary for human carcinogenesis (1). Attempts to identify tumor suppressor genes by RFLP analysis have revealed several chromosomal losses in a number of different malignancies: allelic losses at chromosomes 5q, 17p, and 18q in colorectal cancer (2); at 9q, chromosome 3p rearrangement was different between the histopathological subtypes of RCC. However, it remains unknown whether allelic losses on the short arm of chromosome 17 are implicated in the tumorigenesis and histogenesis of RCC. Therefore, we performed a RFLP analysis of chromosome 17p on 48 RCC specimens, including 6 metastatic RCCs, from 43 patients. Furthermore, we studied the relationship between allelic losses at chromosome 17p or 3p and the histopathological features of RCC.

MATERIALS AND METHODS

Samples. Forty-one primary RCCs and 6 metastatic RCCs from 43 patients treated at our hospital or other community hospitals were analyzed. Thirty-nine of the 41 primary RCC specimens were obtained from radical nephrectomies, and the other 2 were from autopsies. One of the 6 metastatic RCCs was obtained from hepatic resection of an advanced RCC case, and the remaining 5 specimens were from 3 autopsy cases. Tumor specimens were frozen immediately and stored at −70°C until the extraction of DNA. In addition, normal kidney tissues from the patients were also stored at −70°C to serve as normal controls.

Histological Examination. Histological evaluation was performed by examining at least 2 sections from each tumor specimen. Tumors were classified according to the modified WHO classification (22) as described previously (19).

Polymorphic Probes. The following 5 probes, which are mapped to genomic loci symbols, locations, and the restriction endonucleases used to analyze the loss of heterozygosity on chromosome 17p: CH237.3 (23); pYNZ22 (24); pBP53 (25); pHF12–2 (26); and pMCCT53.1 (27). The corresponding locus symbols, locations, and the restriction endonucleases used to demonstrate the DNA polymorphism are listed in Table 1 in accordance with Human Gene Mapping 10.5 (28).

DNA Isolation and Allele Deletion Analysis. High molecular weight DNA from the tissue samples was prepared according to methods described previously (19). Restriction endonuclease digestion of these samples, agarose gel electrophoresis, Southern hybridization, labeling of the probes by the random primer method, autoradiography, and the determination of allelic losses were also performed as described previously (19).

RESULTS

Normal DNA from 36 of the 43 patients was heterozygous at one or more loci on chromosome 17p. The frequency of loss

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of heterozygosity at each locus is shown in Table 1. The overall frequency of the allelic loss at chromosome 17p was 17%, representing 6 of the 36 informative cases (Tables 1 and 2). Fig. 1 shows a representative Southern hybridization analysis blot demonstrating the loss or retention of heterozygosity in tumors at D17S5 locus.

Primary RCCs. Of the 36 informative cases, primary tumors were available in 34 cases (Table 2). Although the number of advanced stage tumors was small, no positive correlation was found between the loss of heterozygosity at 17p and the tumor stage (data not shown). In particular, none of the 8 primary tumors from the highly advanced cases (Robson's stage IV (30)) showed any allelic losses at 17p (data not shown).

Metastatic RCCs. Six metastatic RCC samples from 4 advanced cases were analyzed. Of these 4, 3 cases were informative (patients 38, 39, and 40 (Table 2)). Both primary and metastatic tumors were obtained in one case (patient 39), and tumor samples from three different metastatic sites were available in one case (patient 40). No histopathological differences were seen among the tumor samples in a single individual. Of the three informative cases, loss of heterozygosity at 17p was demonstrated in only one RCC case (patient 38), which was a granular cell type, papillary, grade 2 adrenal gland metastatic tumor. All tumor samples examined from patients 39 and 40 showed retention of heterozygosity at 17p.

Relationship between Allelic Losses at 17p or 3p and Histopathological Features. Table 3 shows the comparative histopathological features of the tumors and the loss of heterozygosity at 17p in the 36 informative cases. Four of the 9 granular cell type RCCs (44%) showed allelic loss at chromosome 17p, whereas only 1 of the 18 clear cell type RCCs (6%) did. The incidences were significantly different (Fisher's exact test, \(P < 0.05\)). With respect to the histological configuration of the RCCs, 2 of the 6 papillary tumors (33%) showed allelic loss at 17p. However, there was no obvious correlation between allelic losses at 17p and the histological configuration or cytological grading. We have previously demonstrated that allelic losses at chromosome 3p were specific to the clear cell type of RCC (19). Thus, we attempted to uncover the relationship between allelic losses at chromosome 17p and 3p. The loss of heterozygosity at chromosome 3p was tested with the 7 polymorphic probes p627, pBH302, pH3H2, pHF12-32, B67, pMS1-37, and pEFD145; the results of RFLP studies of chromosome 3p

**Table 1. Polymorphic probes used, endonucleases used, and the loss of heterozygosity on chromosome 17p in renal cell carcinomas**

<table>
<thead>
<tr>
<th>Locus symbol</th>
<th>Probe</th>
<th>Restriction enzyme</th>
<th>Map location</th>
<th>Allelic loss/informative cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D17S28</td>
<td>pYNH37.3</td>
<td>TaqI</td>
<td>17p13.3</td>
<td>0/4 (0)</td>
</tr>
<tr>
<td>D17S5</td>
<td>pYNZ22</td>
<td>Psl</td>
<td>17p13.3</td>
<td>3/23 (13)</td>
</tr>
<tr>
<td>p53</td>
<td>BHP53</td>
<td>BamHI</td>
<td>17p13.1</td>
<td>1/19 (5)</td>
</tr>
<tr>
<td>D17S1</td>
<td>pHF12-2</td>
<td>MspI</td>
<td>17p13</td>
<td>2/12 (17)</td>
</tr>
<tr>
<td>D17S31</td>
<td>pMCT35.1</td>
<td>MspI</td>
<td>17p13.1-11.2</td>
<td>1/17 (6)</td>
</tr>
<tr>
<td>Total (chromosome 17p)</td>
<td></td>
<td></td>
<td></td>
<td>6/36 (17)</td>
</tr>
</tbody>
</table>

*According to Human Gene Mapping 10.5 (28).
allelic losses at 17p, together with those at other chromosomes, might contribute to the progression of RCC (21, 35). Nevertheless, we could not demonstrate any definitive correlation between allelic losses at 17p and the tumor stage or cytological grade. However, the incidence of allelic loss at 17p in the granular cell type RCC was significantly higher (44%, 4 of 9) than in the clear cell type RCC (6%, 1 of 18). We did not find any allelic loss in a metastatic, clear cell type RCC, even in a highly advanced stage (patient 39). There have been three studies (21, 33, 34) in which both the allelic states on chromosome 17p and the histopathological features of a given RCC specimen were clearly described. Although one study (34) showed allelic losses at 17p in clear cell type tumors, the other two studies (21, 33) support our observations because all of the 8 RCCs with allelic losses at 17p were histopathologically classified as granular cell, mixed cell, or sarcomatous cell types. In particular, Presti et al. (21) showed that all of the 12 pure, clear cell RCCs retained their heterozygosity at the D17S5 locus. We also demonstrated that the papillary type of RCCs showed a relatively frequent loss at 17p (33%, 2 of 6). This observation agrees with the results by Presti et al. (21), who reported that all of the 3 papillary RCCs examined cytogenetically showed trisomy 17, and an allelic loss at 17p was detected in one of these tumors by RFLP analysis. From these observations, it is possible that allelic losses at chromosome 17p may have an important role in the tumorigenesis of papillary or non-clear cell type RCCs, but not in the tumorigenesis of the clear cell type of RCC. Together with our previous study (19) demonstrating that allelic losses at chromosome 3p were specific to the clear cell type RCC, the present data suggest that the types of rearrangements of chromosome

### Table 3 Histopathological features and the loss of heterozygosity on chromosome 17p

| No. of cases with loss of heterozygosity/no. of informative cases (%) |
|------------------|-----------------|
| All cases        | 6/36 (17)       |
| Cell type        |                 |
| Clear cell       | 1/18 (6)        |
| Granular cell    | 4/9 (44)        |
| Mixed            | 1/7 (14)        |
| Other            | 0/2 (0)         |
| Configuration    |                 |
| Papillary        | 2/6 (33)        |
| Nonpapillary     | 4/30 (13)       |
| Cytological grade|                 |
| 1                | 3/23 (13)       |
| 2                | 3/11 (27)       |
| 3                | 0/2 (0)         |

* Including two patients in whom only metastatic tumors were available.
* Clear versus granular (P < 0.05).
* Dominant architectural configuration.
3p and/or 17p can differentiate between the histopathological subtypes of RCCs.

One of the more interesting findings in our study was the inverse relationship between allelic losses at chromosomes 17p and 3p. Although it is now accepted that the tumor suppressor gene(s) responsible for the genesis of RCC resides on chromosome 3p, the gene(s) have not yet been isolated. Hosoe et al. (41) localized the gene in the von Hippel-Lindau disease, an autosomal dominant trait characterized by a predisposition to develop RCC, to locus 3p26. This indicates that a putative suppressor gene may reside at 3p26. Yamakawa et al. (42) demonstrated 2 commonly deleted regions at 3p13-14.3 and 3p21.3 in sporadic RCC. More recently, a receptor protein-tyrosine phosphatase γ has been proposed as a candidate for the tumor suppressor gene at 3p21 (43). These observations suggest that several tumor suppressor genes may exist on the short arm of chromosome 3 and that allelic losses at 3p result in the inactivation of multiple tumor suppressor genes. On the other hand, recent RFLP studies (44, 45) postulate the existence of another tumor suppressor gene, in addition to the p53 gene, on the short arm of chromosome 17. Thus, allelic losses at 17p observed in our study may also indicate the inactivation of another tumor suppressor gene as well as the p53 gene. In view of the inverse relationship between allelic losses at 17p and 3p, we can speculate that certain tumor suppressor gene(s) on chromosome 3p is comparable to the p53 or another tumor suppressor gene on chromosome 17p with regard to their growth suppressing function. Recently, several genes have been isolated as candidates for tumor suppressor genes, and they are considered to have important roles in signal transduction (46). From this viewpoint, it is possible that tumor suppressor genes can influence each other in various growth suppressive pathways via some sort of feedback. However, the precise mechanisms of tumor suppression remain uncertain, and the relationship between the tumor suppressor genes also remains to be elucidated.

ACKNOWLEDGMENTS

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REFERENCES

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