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# Abnormalities at Chromosome Region 3p12–14 Characterize Clear Cell Renal Carcinoma

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**ABSTRACT:** In an effort to determine whether or not any characteristic chromosomal abnormalities exist in renal cancer, cytogenetic findings were correlated with tumor histology in nine cases of renal adenocarcinoma. Metaphase preparations adequate for analysis were obtained from cultures harvested between day 3 and day 21. Model chromosome number was diploid in three cases, hypodiploid in three, and hyperdiploid in the remaining three. One clear cell adenocarcinoma failed to reveal any chromosomal abnormality. Two tumors, a tubular/papillary carcinoma and an acinar/papillary carcinoma, showed the clonal abnormalities  $del(1)(p21), +2, +7, +8, +12, +13, +16, +17, -21$  and  $t(2;10)(q14-21;q26), +7q, +11q, -18$ , respectively. Interestingly, five of six clear cell tumors studied had clonal abnormalities affecting the short arm of chromosome #3 in the 3p12–21 region, and in the remaining case, of 15 karyotyped metaphases suitable for interpretation, one showed a deletion in 3p. These data indicate that clear cell carcinoma of the kidney may be associated with a nonrandom chromosomal abnormality involving the 3p12–14 region.

## INTRODUCTION

Almost all human cancers, when studied appropriately, will show changes in chromosome structure or number [1, 2]. These findings take on added significance when it is noted that the chromosome breakpoints identified in human tumors frequently correlate with the location of cellular oncogenes [3]. There are few cytogenetic studies of primary renal carcinomas. Cohen et al. described a family with hereditary renal carcinoma and a constitutional balanced translocation between chromosomes #3 and #8:  $t(3;8)(p21;q24)$  [4]. Subsequently, high resolution G-banding analysis documented that the chromosome #3 breakpoint occurred at 3p14.2, rather than 3p21 [5]. Another familial renal carcinoma was reported with a  $t(3;11)(p13-14;p15)$  limited only to tumor cells [6]. Wang et al. described abnormalities of chromosomes #3 and #6 in fresh renal tumors and cell lines [7]. Recently, Yoshida et al. described rearrangements involving chromosome #3 in eight of 12 nonfamilial renal

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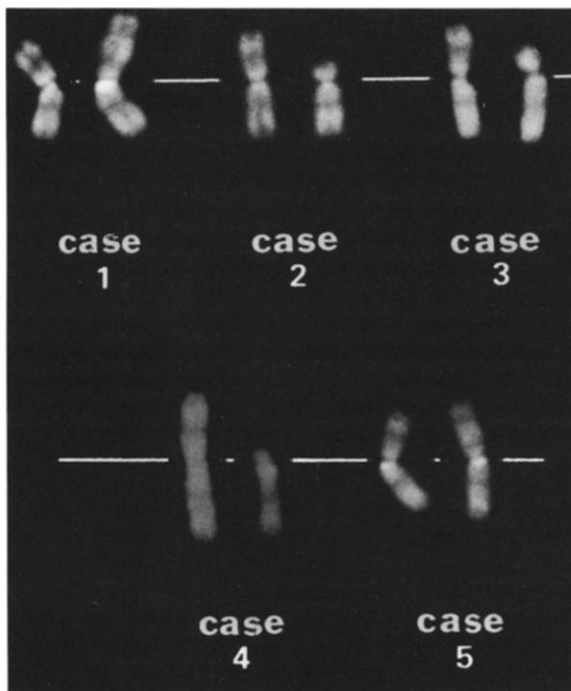
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carcinomas [8]. Deletion of 3p was noted in four cases, translocations involving chromosome #3 in four cases, and an *i*(3p) in one case. Additional clonal changes were noted in these and in two other tumors. In two additional sporadic renal carcinomas studied, clonal abnormalities were noted, but none affected the 3p13-14 region [9, 10]. In an effort to confirm these reports and see if any additional chromosomal abnormalities might exist in these tumors, cytogenetic findings were correlated with tumor histology in nine cases of primary, nonfamilial renal adenocarcinoma.

## MATERIALS AND METHODS

Primary tumor samples were obtained at the time of surgery from patients with either local or metastatic renal carcinoma. Tumor samples were transferred immediately to the laboratory, where they were minced and then disaggregated either mechanically or enzymatically. In the latter cases, tumor samples were exposed to either warm trypsin (0.025%) or collagenase (0.1%), hyaluronidase (0.01%) and DNase (0.002%) [11]. The cells were washed three times with Hank's balanced salt solution or RPMI 1640 medium. Aliquots of tumor cells were transferred to 25-cm<sup>2</sup> plastic culture flasks and incubated in RPMI 1640 medium supplemented with 15% fetal calf serum, 1% glutamine, and antibiotics (penicillin 100 µg/ml and streptomycin 100 µg/ml) in an atmosphere of 5% CO<sub>2</sub>. Medium was changed after 24–48 hours and dead or nonattached cells were removed. Proliferating cultures were exposed to colcemid (0.01 to 0.02 µg/ml) for 3–12 hours depending on the mitotic

**Figure 1** Partial karyotype of five clear cell adenocarcinomas with clonal abnormalities at the 3p13-14 region. Cases 2 and 3 show *del*(3)(p13), whereas, case 4 shows a deletion occurring more proximally, *del*(3)(p12). Cases 1 and 5 show *dir ins*(3)(p13p21).



activity of the culture. The cultered cells were detached using 0.025% trypsin with EDTA. Cells were then treated with 0.075 M KCl, fixed with methanol/acetic acid (3:1), and applied to standard, glass microscope slides. Chromosomes were analyzed using either Q- or G-banding, as previously described [12]. Metaphase chromosomes were analyzed and modal number was recorded [13]. The clinical stage along with the particular histologic type of tumor was recorded in each case.

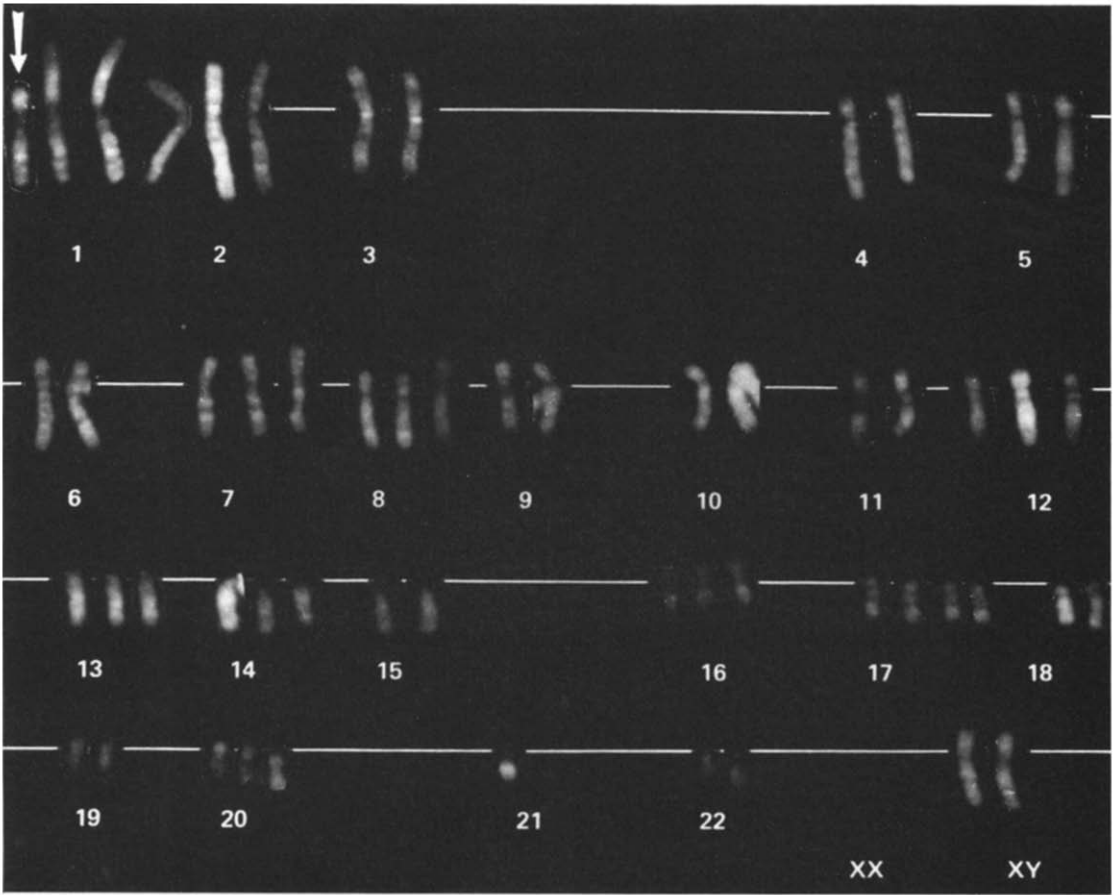
## RESULTS

Metaphase preparations adequate for interpretation were obtained from nine renal adenocarcinomas after 3–21 days of culture (Table 1). The tumors were removed from four women and five men, aged 38–73 years. All tumor stages were represented, but histology varied. Modal chromosome number was 46 in three cases, hypodiploid in three, and hyperdiploid in the remaining three cases. Several interesting cytogenetic abnormalities were noted. The most common clonal abnormality involved the short arm of chromosome #3. Five clear cell carcinomas showed clonal abnormalities in this region (Fig. 1). These abnormalities seemed to be localized to the 3p12-21 region and included del(3)(p13) in two cases, dir ins(3)(p13p21) in two cases, and del(3)(p12) in one case. The origin of the inserted segments could not be determined with certainty. An additional clear cell carcinoma had 15 metaphases adequate for interpretation and in one del(3)(p13) was noted. One renal adenocarcinoma (case 7) showed no chromosomal abnormalities. Case 8 had a tubular/papillary carcinoma that contained del(1)(p21→pter) plus either addition or loss of

**Table 1** Clinical and cytogetic features of renal cell carcinoma

Case number	Sex/Age (yr)	Histology	Stage	Chromosome number		Karyotype
				Mode	Range	
1	M/64	Clear cell	IV	45	42–78	45,XY,dir dup(1)(q12→q44),dir ins(3)(p13p21),-15
2	F/52	Clear cell	I	44	36–83	44,X,-Xdel(3)(p13),-9
3	F/41	Clear cell	IV	63	38–79	63,XX+X,del(3)(p13),+1,+2,+4,+9,+10,+12,+13,+14,+15,+16,+19,+21,+22,+7q+,11q+,i(8q)
4	F/73	Clear cell	IV	46	43–89	46,XX,del(3)(p12)
5	F/54	Clear cell	III	49	32–72	49,XX,dir ins(3)(p13p21),+2,+7,+16
6	M/61	Clear cell	IV	46	32–83	46,XY,del(3)(p13) <sup>a</sup>
7	M/38	Clear cell/papillary	I	46	33–47	46,XY
8	M/68	Tubular/papillary	II	52	29–56	52,XY,del(1)(p21),+2,+7,+8,+12,+13,+16,+17,-21
9	M/58	Acinar/papillary	II	45	41–87	47,XY,t(2;10)(q14-21;q26),7q+,11q+,-18

<sup>a</sup>This abnormality was seen in only one of 15 metaphases karyotyped, therefore, we cannot establish its clonal nature.

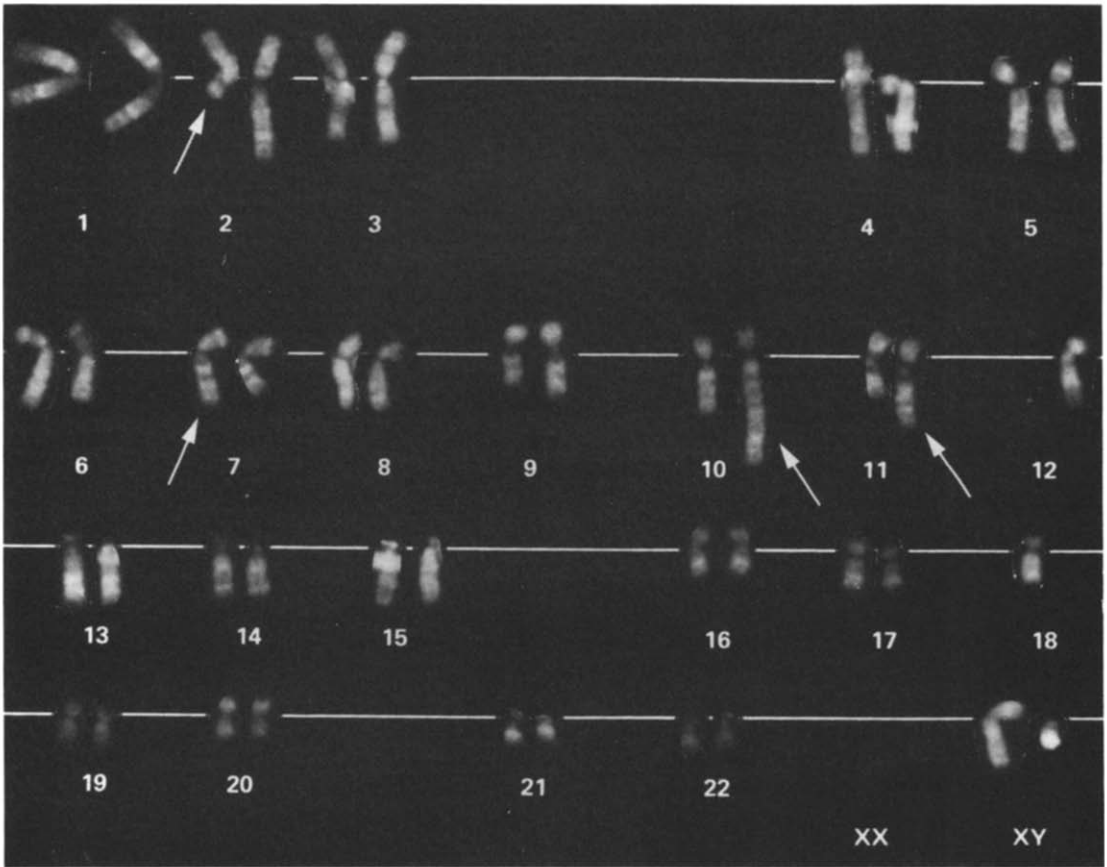


**Figure 2** A tubular/papillary adenocarcinoma (case 8). The karyotype shown is not completely representative of the clonal karyotype, which possesses a sex chromosome constitution of XY and fails to show consistent gain of chromosome #21.

several chromosomes (Fig. 2). Case 9 had a papillary tumor that showed  $t(2;10)(q14-21;q26)$  and  $7q+$ ,  $11q+$  abnormalities (Fig. 3). Additional clonal abnormalities were noted in several of the tumors.

## DISCUSSION

The consistency of chromosome #3 rearrangements in cancers of the kidney suggested in previous reports is confirmed in the present study, where six of nine tumors displayed abnormalities at region 3p. These were demonstrated to be clonal in five cases, whereas, in the sixth case only a single cell with a 3p abnormality was encountered. Four of the five tumors with clonal abnormalities at chromosome region 3p12-14 possessed few additional cytogenetic changes. This strongly suggests that rearrangement at this site may play an early and perhaps critical role in tumor induction or progression. We also found that #3 abnormalities were confined to tumors with a clear cell histology. It is not clear from several previous reports if abnormalities of chromosome #3 occurred in any other histologic subtype. In the



**Figure 3** An acinar/papillary adenocarcinoma (case 9) showing  $t(2;10)(q14-21;q26)$ ,  $7q+$ ,  $11q+$ .

present study, the rarer papillary adenocarcinomas did not display abnormalities in this region. One clear cell adenocarcinoma failed to show any chromosome abnormalities. The metaphases examined in this case may have been derived from surrounding normal kidney cells that proliferated in culture. It may also be that a submicroscopic abnormality was present, but undetectable by the standard banding techniques used.

Several human tumors including small cell lung carcinomas, salivary gland tumors, rhabdomyosarcomas, and ovarian carcinomas manifest chromosomal abnormalities at the 3p13-14 region. This suggests that this chromosomal region may contain a gene or genes whose deletion or deregulation is important in the induction or growth of these tumors [14-17]. The nature of such a gene remains to be determined. The *c-ras* oncogene has been mapped more distally along chromosome region 3p and expression of this gene has not been studied in renal cancers [18]. Drabkin et al. recently described translocation of *c-myc* to the 3p14.2 breakpoint in a case of hereditary renal cell carcinoma associated with a  $t(3;8)(p14.2;q24.13)$  chromosomal translocation [19]. Only one tumor in the present report (case 3) showed detectable abnormalities of chromosome #8 and this was  $i(8q)$ . A possible *c-ras* related gene has been localized to the 3p12-14 region in human germ line chromosomes [20]. No mutations of *c-Ki-ras* oncogene were reported in a study of

ten human renal carcinomas using restriction fragment length polymorphisms for this gene [21]. Trent et al. studied several rhabdomyosarcomas with abnormalities at region 3p13-14 and were unable to find a H-ras related sequence in this region. In addition, they also failed to detect structural alterations or amplification of the c-Ha-ras or N-ras genes [16].

A certain degree of concordance exists between fragile sites, oncogene locations, and chromosomal breakpoints found in a variety of solid and hemopoietic neoplasms [22]. The 3p13-14 region has been reported to be a common fragile site [23]. Interestingly, Pathak and Goodacre have described a patient with a renal carcinoma that showed a t(3;8). The patient did not have a constitutional translocation, but his peripheral lymphocytes showed an increased expression of the fragile site at the 3p13-14 region after treatment with FUdR and caffeine, suggesting that somatic expression of a constitutional fragile site may lead to a clonal karyotypic abnormality in the tumor [24].

Balanced chromosomal translocations involving oncogene locations are common in hemopoietic neoplasms [25]. These rearrangements are thought to lead to oncogene activation and neoplastic transformation. Alternatively, solid tumors including the renal adenocarcinomas in the present study rarely show balanced translocations and such tumors are more frequently characterized by either nonreciprocal chromosome translocations or deletions [26]. Certain solid tumors may be a product of gene loss with activation of a tumor-promoting recessive allele. Retinoblastomas and Wilms' tumors, both, are thought to arise in such a fashion [27-29]. Whether or not such a gene exists at the 3p12-14 site and is responsible for the induction of renal carcinoma and other solid tumors with similar chromosomal abnormalities remains to be determined.

Our data confirm that renal adenocarcinoma is associated with a nonrandom chromosomal abnormality at the 3p12-14 region. In the present study, this abnormality was confined to tumors with a clear cell histology. Because several solid tumors possess similar abnormalities, continued study of this region may provide important insights into the genetic mechanisms of cancer induction or maintenance.

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