Patterns of Aneuploidy in Stage IV Clear Cell Renal Cell Carcinoma Revealed by Comparative Genomic Hybridization and Spectral Karyotyping

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We report the use of spectral karyotyping (SKY) and comparative genomic hybridization (CGH) to describe the numerous genomic imbalances characteristic of stage IV clear cell renal cell carcinoma (CCRCC). SKY and CGH were performed on 10 cell lines established from nephrectomy specimens, and CGH on uncultured material from five of the primary renal tumors. The mutational status of *VHL* (3p25) and *MET* (7q31), genes implicated in renal carcinogenesis, were determined for each case. Each case showed marked aneuploidy, with an average number of copy alterations of 14.6 (±2.7) in the primary tumors and 19.3 (±4.6) in the cell lines. Both whole-chromosome and chromosome-segment imbalances were noted by CGH: consistent losses or gains included +5q23→ter (100%), −3p14→ter (80%), and +7 (70%). All *VHL* mutations and 83% of the genomic imbalances found in the primary tumors were also found in the cell lines derived from them. SKY showed many complex structural rearrangements that were undetected by conventional banding analysis in these solid tumors. All cases with *VHL* inactivation had 3p loss and 5q gain related primarily to unbalanced translocations between 3p and 5q. In contrast, gains of chromosome 7 resulted primarily from whole-chromosome gains and were not associated with mutations of *MET*. SKY and CGH demonstrated that genomic imbalances in advanced RCC were the result of either segregation errors [i.e., whole chromosomal gains and losses (7.8/case)] or chromosomal rearrangements (10.7/case), of which the majority were unbalanced translocations.

INTRODUCTION

Loss of chromosome arm 3p DNA including loss of the von Hippel–Lindau (*VHL*) gene that maps to 3p25 is a common and early finding in the majority of sporadic and hereditary clear cell renal cell carcinomas (CCRCCs) (Latif et al., 1993; Gnarra et al., 1994). Classical cytogenetic studies have shown that, whereas small tumors also have gains of 5q and 7 and loss of Y, later-stage (i.e., advanced) tumors undergo complex chromosomal rearrangements associated with loss of 8p, 9p, 14, and 17 (Kovacs and Frisch, 1989; Kovacs and Kung, 1991; Reiter et al., 1993; Zhao et al., 1995; Beroud et al., 1996; Yang et al., 2000; Gunawan et al., 2001; Presti et al., 2002). A comprehensive study of advanced CCRCCs is important for understanding the chromosomal mechanisms associated with aggressive disease for which there is no effective treatment. Recently, molecular cytogenetic techniques have improved the characterization of chromosomal aberrations in solid tumors. Spectral karyotyping (SKY) allows the simultaneous visualization of all human chromosomes in different colors and has particular advantages for detecting subtle rearrangements in highly aneuploid cancers (Schröck et al., 1996; Knutsen and Ried, 2000). Comparative genomic hybridization (CGH) maps DNA copy number changes to normal chromosomes (Kallioniemi et al., 1992; Ried et al., 1997). Together, SKY and CGH can be used to elucidate the structural changes that occur in tumors and the genomic consequence of such alterations. To characterize more accurately the complex aberrations of high stage CCRCC, we have applied SKY, CGH, and mutation analysis for *VHL* and the proto-oncogene *MET*, and 3p allelotyping to 10 CCRCC cell lines. We were also interested in how the cytogenetic

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data of tumors in long-term culture compared with uncultured material and have therefore applied CGH to primary tumors from which the cell lines were derived, where possible.

MATERIALS AND METHODS

Specimens

Ten cell lines were established from CCRCC tumors resected by radical nephrectomy from patients with stage IV disease as part of an IRB-approved immunotherapy protocol (Anglard et al., 1992). Each tumor was of high nuclear grade and of the clear-cell ("conventional") histological type. Archived primary tumors and cell line stocks were maintained at −80°C, and the latter were grown in RPMI 1640 medium supplemented with 10% FCS (Clontech, Rockville, MD) at 37°C in a humidified 5% CO_2 incubator. DNA was prepared from flash-frozen sections of primary tumor and from the cell lines at passages 15–25. The samples were digested with proteinase K (Boehringer-Mannheim, Mannheim, Germany) at 50°C for 2 hr (cell lines) or overnight (primary tumors), then precipitated under high-salt conditions (PureGene DNA Purification Kit; Gentra Systems, Minneapolis, MN).

Molecular Analyses

Mutations in *VHL* and *MET* were assessed by denaturing high-performance liquid chromatography (DHPLC), as described (Nickerson et al., 2000), and confirmed by direct sequencing of all three *VHL* exons and exons 16–19 of the *MET* proto-oncogene by use of ABI Prism 310 or 3700 genetic analyzers (Applied Biosystems, Foster City, CA). *VHL* promoter methylation assays for lines UOK121 and UOK143 were reported previously, and were performed in similar fashion for UOK171 and UOK181 (the cases without detectable *VHL* mutation) (Herman et al., 1994). In addition, UOK171 and UOK181 were assessed for deletions of *VHL* by Southern blotting and fluorescence in situ hybridization (FISH) analysis (Stolle et al., 1998). 3p loss of heterozygosity (LOH) for six of the cases (UOK111, 115, 117, 121, 122, 130) was previously demonstrated by restriction fragment length polymorphism (RFLP) analysis (Anglard et al., 1992), and was assessed at *D3S1110* and *D3S1038* (loci flanking *VHL* at 3p25–26) in the remainder of cases by fluorescence-based PCR with an ABI Prism Sequencing machine and Genescan and Genotyper software, according to published methods (Cawkwell et al., 1993).

Cytogenetic Analyses

Metaphase chromosomes for CGH, SKY, and FISH were obtained by routine methods (Barch et al., 1997). SKY probes were hybridized to metaphase chromosomes as described (Schröck et al., 1996; Macville et al., 1999). Images were acquired with SkyView software (Applied Spectral Imaging, Migdal Haemek, Israel) by use of a spectral cube with a custom-designed SKY-3 optical filter (Chroma Technology, Brattleboro, VT) and a charge-coupled device camera (Hamamatsu, Bridgewater, NJ) connected to a DMRXA microscope (Leica, Wetzlar, Germany). For CGH, 1 μg of biotin-labeled tumor DNA and digoxigenin-labeled normal donor DNA (sex-matched) were cohybridized to sex-matched normal human lymphocyte metaphase chromosomes and detected, and images were acquired with Q-CGH software (Leica Imaging Systems, Cambridge, UK). A signal ratio of tumor to reference of <0.5 and >1.5 for a particular chromosomal region was interpreted as a loss or gain, respectively. High-level gains (HLGs) were defined as areas of tumor-to-signal ratios of >2.0 (Ried et al., 1997). The average (± SD) number of copy alterations (ANCAs) was the mean number of chromosomal or chromosomal segment imbalances per case as detected by CGH (Ried et al., 1999). FISH analysis was performed on selected metaphase spreads from the tumor cell lines, as described, with an FITC-labeled centromere 7 enumeration probe (CEP 7; Vysis, Downers Grove, IL) and a cosmid 182b3 probe specific for *MET* exon 16 (a generous gift from Dr. Steve Scherer, Hospital for Sick Children, Toronto, Canada).

RESULTS

VHL and *MET* Studies

Of the 10 CCRCC cell lines studied, eight (80%) had evidence of *VHL* inactivation by mutation or promoter methylation (Table 1). These cases all had 3p LOH near the *VHL* locus and are referred to as *VHL*(−/−), to signify inactivation and loss of the two copies of the gene, respectively (Table 1) (Anglard et al., 1992). Five uncultured tumors (UOK121, 130, 143, 154, and 181) were available for analysis and demonstrated the same *VHL* gene status (data not shown). The other two lines (UOK171 and UOK181) had no detectable methylation, deletion, or mutation of *VHL*, and are correspondingly referred to as *VHL*(+/+) (Table 1). By FISH, we found a mean of one to three extra copies of chromosome 7 in all 10 of the CCRCC lines, which suggested a possible role for *MET*

TABLE 1. Summary of Selected Cytogenetic Findings in 10 Cases of Metastatic Conventional RCC

Case (UOK no.)	Status of *VHL* (3p25)[a]	3p LOH	Status of *MET* (7q31)	Ploidy	Aneuploidy of chromosomes 3 and 7		
					SKY		CGH[b]
	VHL (−/−)						
111	MS T→A @ nt 562	yes	Normal	⟨3n⟩	der(3)t(3;8)(p13;q11.2)	−3p, +3q	++7
115	Del @ nt 522.523	yes	Normal	⟨4n⟩	der(3)t(3;5)(p11;p12)	−3p	Normal
117	MS T→C @ nt 686	yes	Normal	⟨3n⟩	der(3)t(3;5)(p12;q21)	−3p, +3q	+7
121	Methylated[c]	yes	Normal	⟨3n⟩	der(3)t(3;5)(p13;q13)	−3p	+7
122	MS C→T @ nt 550	yes	Normal	⟨4n⟩	der(3)t(3;5)(p12;q13)	−3p	+7
130	MS T→C @ nt 671	yes	Normal	⟨3n⟩	del(3)(p12)	−3p	−7p++q
143	Methylated[c]	yes	Normal	⟨3n⟩	der(3)t(3;5)(p12;q11.2)	−3p	++7
154	Del @ nt 820	yes	Normal	⟨4n⟩	der(3)t(3;5)(p12;q13)	−3p, +3q	++7
	VHL (+/+)						
171	Normal	no	Normal	⟨3n⟩	der(3)t(3;11)(p21;p15), der(3)t(3;17)(q29;q11.2)	+3q	Normal
181	Normal	no	Normal	⟨3n⟩	Normal	Normal	−7q

[a]MS, missense mutation; nt, nucleotide; Del, deletion.
[b]Losses, gains, and HLGs of chromosomes detected by CGH are indicated by −, + and ++, respectively.
[c]Methylation data from Herman et al. (1994).

(7q31) (data not shown). However, direct sequencing and DHPLC of *MET* exons 16–19 in each did not reveal any activating *MET* mutations (Table 1).

SKY Analysis

SKY analysis revealed the 10 cell lines to be either near-triploid or tetraploid and to have multiple unbalanced translocations. No examples of reciprocal, balanced translocations were noted. Complete clonal karyotypes are listed in Table 2, with the corresponding color SKYGRAMS available online (http://www.ncbi.nlm.gov/sky/skyweb.cgi) (also see Fig. 1). In the eight *VHL*(−/−) lines, translocations or deletions of 3p (8/8 cases, breakpoints 3p11 to 3p13, with telomeric loss) and extra copies of chromosome 7 (7/8 cases) were seen. In the *VHL*(+/+) cell lines, no gains of chromosome 7 or 7q were observed. Although no whole-arm deletions were seen, a del(3)(p25) was seen in UOK181, and, in UOK171, two unbalanced translocations involving chromosome 3 [der(3)t(3;17) and der(3)t(3;11)x2] were present. Both *VHL*(+/+) lines had the following recurrent structural aberrations: i(8)(q10), translocations involving chromosome 14, and losses of chromosomes 14 and 22.

SKY refined previously published G-banded karyotypes of four of these CCRCC lines (UOK117, UOK121, UOK122, and UOK130) (Zhao et al., 1995) (Table 2). Numerous unbalanced translocations that were undetected by conventional banding were revealed by SKY (Table 2 and Fig. 2). SKY was most helpful in unequivocally resolving the donor moiety of derivative chromosomes; for example, the donor arm for the 3p derivative chromosome in UOK122 was previously thought to be 7q, but, by SKY, the derivative proved to be a der(3)t(3;5)(p12;q13). In addition, SKY identified telomeric breakpoints that were not detected with conventional banding (see 10qter, 14, 15, 17qter, and 22 breakpoints, Fig. 2, and online SKYGRAMS).

CGH Studies

We performed CGH to assess the global genomic imbalances in the DNA copy number as a result of the chromosomal rearrangements identified by SKY, especially those rearrangements of low frequency (<50% of metaphase cells) or of unclear origins (Fig. 3 and at http://www.ncbi.nlm.gov/sky/skyweb.cgi). The *VHL*(−/−) lines demonstrated loss of chromosome segments 3p11–13 to 3pter (8/8 cases) and gains of chromosome 7 (7/8 cases). Four of the cases showed HLG of 7 or of 7q. In contrast, the two *VHL*(+/+) lines

TABLE 2. Karyotypes Determined by SKY*

A. *VHL*(−/−) lines

i. UOK111: 72–75⟨±3n⟩,XXX,+X, i(1)(p10)×2,der(1)t(1;17)(p13;q11.2),+der(1)t(1;17)(p13;q11.2),+der(1)t(1;5)(p22;q22)×2,der(3)t(3;8)(p13;q11.2),+der(3)t(3.8)(p13;q11.2),−4,+6,+7,−8,+10,−14,−17,i(18)(p10),+20,dic(9;22)(?p12;q13)del(9)(p?22)del(22)(p12)×2[3],dic(22;22)(p11.2;p11.2)del(22)(q13.3),+dic(22;22)(p11.2;p11.2)del(22)(q13.3)[2] [cp5]

ii. UOK115: 41–86⟨±4n⟩,XX,−Yx2, i(1)(q10),del(3)(p21),der(3)t(3;5)(p11;p12)×2,−4,i(5)(p10),der(6)t(5;6)(q22;q21)×2,del(7)(q11.2q22),del(9)(p12),del(12)(q22),−13,der(13)t(X;13)(?;p11.2),−14×2,der(18)t(5;18)(?;p11.2) [cp8]

iii. **UOK117:** 49–71⟨±3n⟩,XXY,**der(3)t(3;5)(p12;q21),+der(3)t(3.5)(p12;q21),i(5)(p10),+del(7)(q32),+i(8)(q10),i(9)(q10),+1(9)(q10),+10−15,+i(16)(q10),+17,−20,der(22)t(2;22)(q11.2;p11.2)** [cp8]

iv. **UOK121:** 50–80⟨±3n⟩,XXY,**i(2)(p10),+i(2)(p10),der(3)t(3;5)(p13;q13),der(4)t(4;4)(q35;p12),dup(4)(p16p12),del(5)(q11,2),+der(6)t(6;11)(q21;q14),**+7,**−8,−9,+del(11)(q12),**+13,**−14 der(14)t(9;14)(p13;p11.2)×2,**−15,**−15,+17,**+18,**+del(18)(q12.3q21.2),del(19)(p13.1),+del(20)(p11.2),+21,+22** [cp10]

v. **UOK122:** 70–78⟨±4n⟩,**XX,**−Y,**−Y, der(1)t(1;22)(p12;q11.2),der(3)t(3;5)(p12;q13)×2,−4,del(4)(q12q31.1),−6,der(6)t(6:9)(p11.2;q22.1),**+7,**−8,**i(8)(q10),**−9×2,i(11)(p10),del(11)(q14),del(12)(q24),+del(12)(q24)×2,**−13,**−13,−14,−14,i(15)(q10),**+16, **+del(16)(q23q23.2),−17,i(17)(q10),−18,**−18,−19,i(19)(q10),−21,**−22,−22** [cp5]

vi. **UOK130:** 52–82,**XXY**⟨±3n⟩,**dic(1)(p12p22),+dic(1)(p12p22),der(2)t(2;9)(q31;?)del(2)(p23)×2,−3,**del(3)(p12),−4,−6,i(7)(q10),−8,der(9)t(5;9)(p13;q32)×3,−10,**+12,**−13,**−15,dic(15;15)(qter;qter),del(17)(p13),der(18)t(13;18)(q14;q11.2)×2,**−19,**−19** [cp3]

vii. UOK143: 43–76⟨±3n⟩,XX,−Y, +der(1;14)(q10;q10)[2],der(3)t(3;5)(p12;q11.2),−6,+7,+7,i(8)(q10),−9,del(11)(q12),+der(11)t(9:11)(q13;q13),+12,+del(12)(q22),−13,der(14)t(11;14)(q11.2;p11.2),der(14)t(5;14)(?;p11.2),+15,+17,+del(19)(p13.2),+20 [cp9]

viii. UOK154: 82–95⟨±4n⟩,XXXX,+X, +der(1)t(1;8)(p36;q11.2),del(2)(q11.2),der(3)t(3;5)(p12;q13),+der(3)t(3;5)(p12;q13),−4,del(5)(q15),−6,−6,+7,+7,+der(7)del(p21)del(q21),+10,der(11)t(1;11)(q12;q21)×2,−13,−14×2,der(14)t(9;14)(?;q32),−15,del(17)(p13)×2,+del(17)(p13),−18×2,18,+19,+20,+20,+20,der(21)t(21;22)(p11.2;p12)×2 [cp6]

B. *VHL*(+/+) Lines

i. UOK171: 45–129⟨±3n⟩,X,−X, −X,del(2)(p23)×3,der(3)t(3;17)(q29;q11.2),der(3)t(3;11)(p21;p15)×2,der(4)t(2;4)(p?23;q35)×2,del(5)(q12q22),+del(5)(q12q22),der(6)t(3;6)(p21;p12),i(8)(q10),dic(8;9)(p12p12),der(9)t(9;21)(p12;q11.2),+10,der(11)t(3;11)(q21;q14),+der(12)t(9;12;9;12;9;12;9)(9?::12p12→12q12::9?::12?::9?::12?::9?),−13×2,−14,der(14)t(14;20)(p11.2;p11.2)×2,der(16)t(12;16)(?;q12.1)+der(16)t(12;16)(?;q12.1),+i(16)(q10),−17,+19,−20,−21,−22 [cp13]

ii. UOK181: 60–67⟨±3n⟩,X,i(X)(p10), −Y,del(1)(p32),+dic(1;1)(:p12→q32::p11→q32:),+dic(1;21)(21qter→21p13::1p32→q11:),der(2)t(2;15)(q33;q24),del(3)(p25),−4,−5,−6,der(6)t(6;12)(q25;q15),del(7)(q11.23q22),der(7)t(6;7)(?;q11.2),del(8)(p22),+i(8)(q10),i(9)(p10),der(10)t(10;22;22)(10qter→10p13::22qter→22q12::22q12→qter),der(10)t(10;12)(p12;pter?),+der(10)t(10;12)(p12;?p13.3),del(11)(q13),−14,der(14)t(14;18)(p11.2;p11.2),−15,−16,der(16)t(10;16)(?qter;p13.3),+del(17)(q25),−18,−22,der(22)t(5;22)(q13;p11.2) [cp9]

*Cell lines in boldface are those with previously published G-banded karyotypes (see Zhao et al., 1995). The aberrations refined and/or revealed by SKY and not seen by G-banding are shown in boldface.

had no detectable 3p loss or chromosome 7 gain. UOK171 [*VHL*(+/+)], which, by SKY, had a clonal non-reciprocal translocation involving a breakpoint at 3p21, had no net gain or loss of DNA from the 3p arm by CGH. All 10 cell lines evidenced gains at the minimally overlapping region of 5q23 to 5qter. Copy number abnormalities that occurred in ≥50% of the lines overall included loss of 4, 6q, 8p, 9q, 11q, 14, and Y, and gain of 5p, 12, 17, 19, 20, and X.

SKY and CGH analysis identified the copy number changes as resulting primarily from either numerical (42%) or structural aberrations (58%), the majority of which were non-reciprocal translocations (Table 3). Compared to the eight *VHL*(−/−)

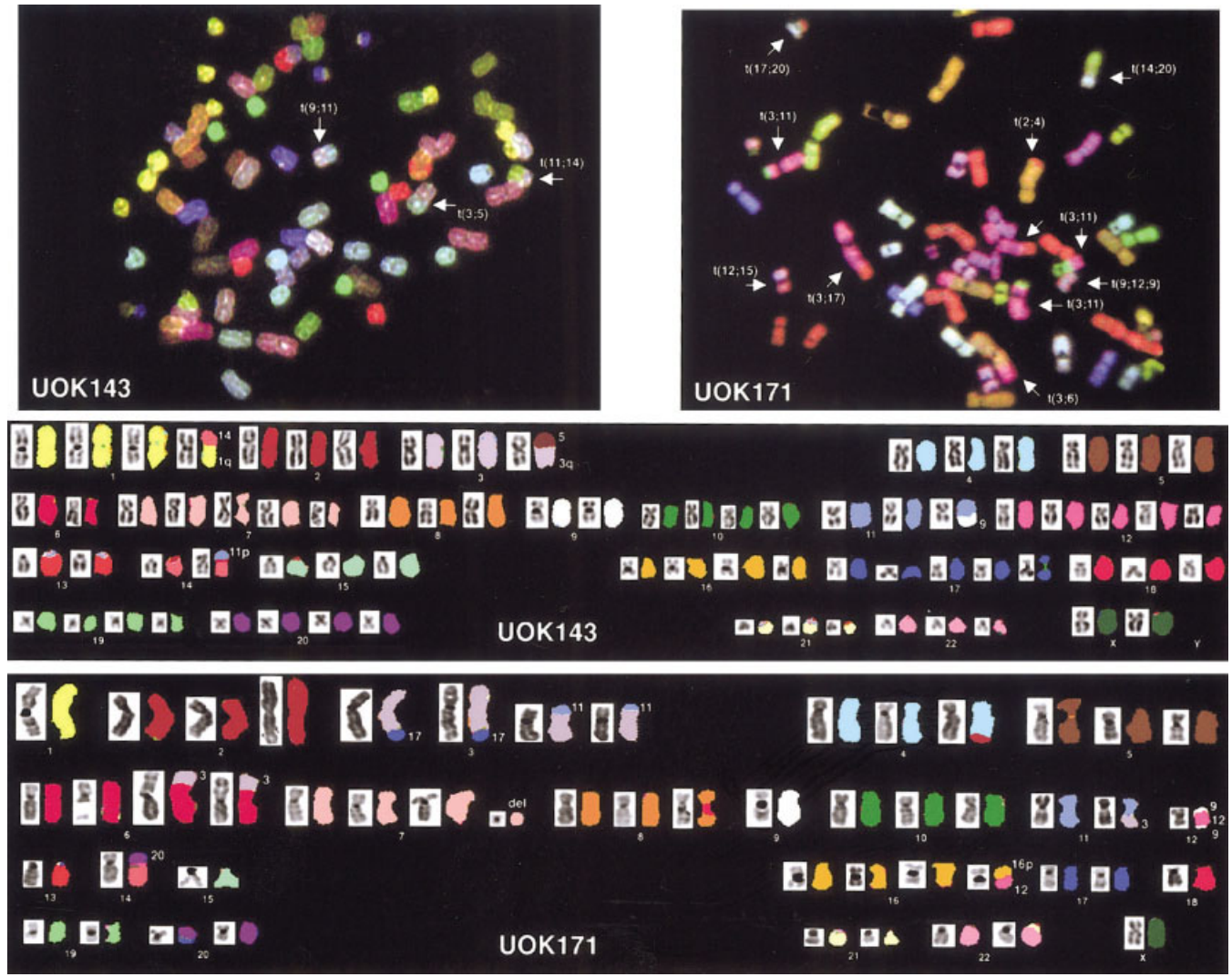

Figure 1. SKY representation of two cases of advanced CCRCC. One (UOK143) has 3p LOH and methylated *VHL* [*VHL*(−/−)], whereas the other (UOK171) has no detectable *VHL* mutation or LOH [*VHL*(+/+)]. 3p translocations are noted in both UOK143 and 171, but are associated with net 3p loss only in UOK143 and the other seven *VHL*(−/−) cell lines. [See online karyograms (, under "pavlovich").] In contrast, the unbalanced translocations in UOK171 between chromosomes 3 and 6, 11, and 17 did not result in net 3p loss by CGH, nor did they in the other *VHL*(+/+) cell line UOK181.

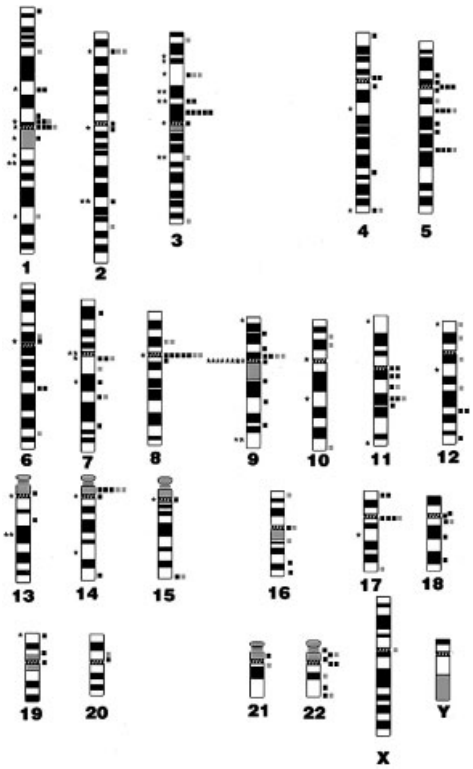

Figure 2. Breakpoints as determined by G-banding (*) or SKY analysis for the RCC cell lines [black boxes, *VHL*(−/−) cases; gray boxes, *VHL*(+/+) cases]. As indicated, SKY detected numerous breakpoints that were not identified by conventional G-banding.

CCRCC lines, the two *VHL*(+/+) cases had a greater number of structural alterations/case, primarily the result of unbalanced translocations, although the sample size precluded any statistical validation.

To determine the possible consequences of culture conditions as contributors toward the aneuploidy often observed in established cell lines, we performed CGH on DNA from five available primary renal tumors (cases UOK121, 130, 143, 154, and 181). The majority of the chromosomal gains and losses detected in the primary tumors were maintained in the derived cell lines. Consistent alterations between the primary tumors and the cell lines included gains of chromosomes and chromosome arms 5, 7, 9p, 12, 17, 20, 21, and X, and losses of 2q, 3p, 9q, 14, 15, 18q, and Y. Overall, 72 copy number alterations were noted in the five primary CCRCC tumors, only 12 of which were not present in the corresponding cell lines (83% concordance). Gains of 4q (two tumors) and 13 (two tumors) were among the few CGH changes unique to the primary tumors. Only two HLGs were noted in the primary tumors: the X chromosome in UOK121 and 9p in UOK181, both of which were maintained in the corresponding cell lines. The ANCA of the five primary tumors and of the five cell lines derived from them was 14.6 ± 2.7 and 21.0 ± 2.4, respectively. The ANCA of all 10 RCC lines was 19.3 ± 3.3, which reflects a high amount of chromosome-level genetic imbalance (Table 3).

DISCUSSION

Advanced CCRCC has no effective treatment. We therefore conducted a genome-wide screening for genomic imbalances and chromosomal translocations of 10 CCRCC cell lines and primary tumors, potentially to guide the search for critical disease loci. With this aim, we used molecular cytogenetic techniques to complement 3p LOH and molecular analysis of the *VHL* and *MET* genes in 10 cases of advanced (stage IV) CCRCC. Our plan was to define the patterns of aneuploidy present in these cell lines, compare the lines with the tumors from which they were derived, and assess for any obvious differences between *VHL*(−/−) and *VHL*(+/+) cases. SKY proved invaluable for detecting and categorizing subtle chromosomal rearrangements, such as complex translocations and insertions not identifiable by banding-based analysis (Fig. 3 and online CGH and SKYGRAMS). CGH complemented and reinforced the SKY data, particularly where the intercellular chromosomal copy number varied widely in large metaphase spreads.

Cell lines have been suspected of harboring aberrations not relevant to tumorigenesis. Our findings revealed, however, that cell lines at passages 15–25 have the same *VHL* inactivation status, and maintain 3p loss, 5q gain, and 7 gain (when present) and the majority of copy number aberrations in culture compared to the uncultured primary tumors. Only 17% of the genomic imbalances found in the primary tumors were not detectable in their respective cell lines. An additional 40% of chromosomal imbalances were found in the cell lines and may represent a selection of aggressive subclones from the primary tumor that have retained most of their genetic aberrations but acquired others in vitro. It is evident that the majority of changes present in the original tumor are maintained in the cell line, validating the use of such lines for research purposes. However, the additional chromosomal imbalances encountered in the cell lines should be considered whenever scientific conclusions are made based solely on data from cells in long-term culture.

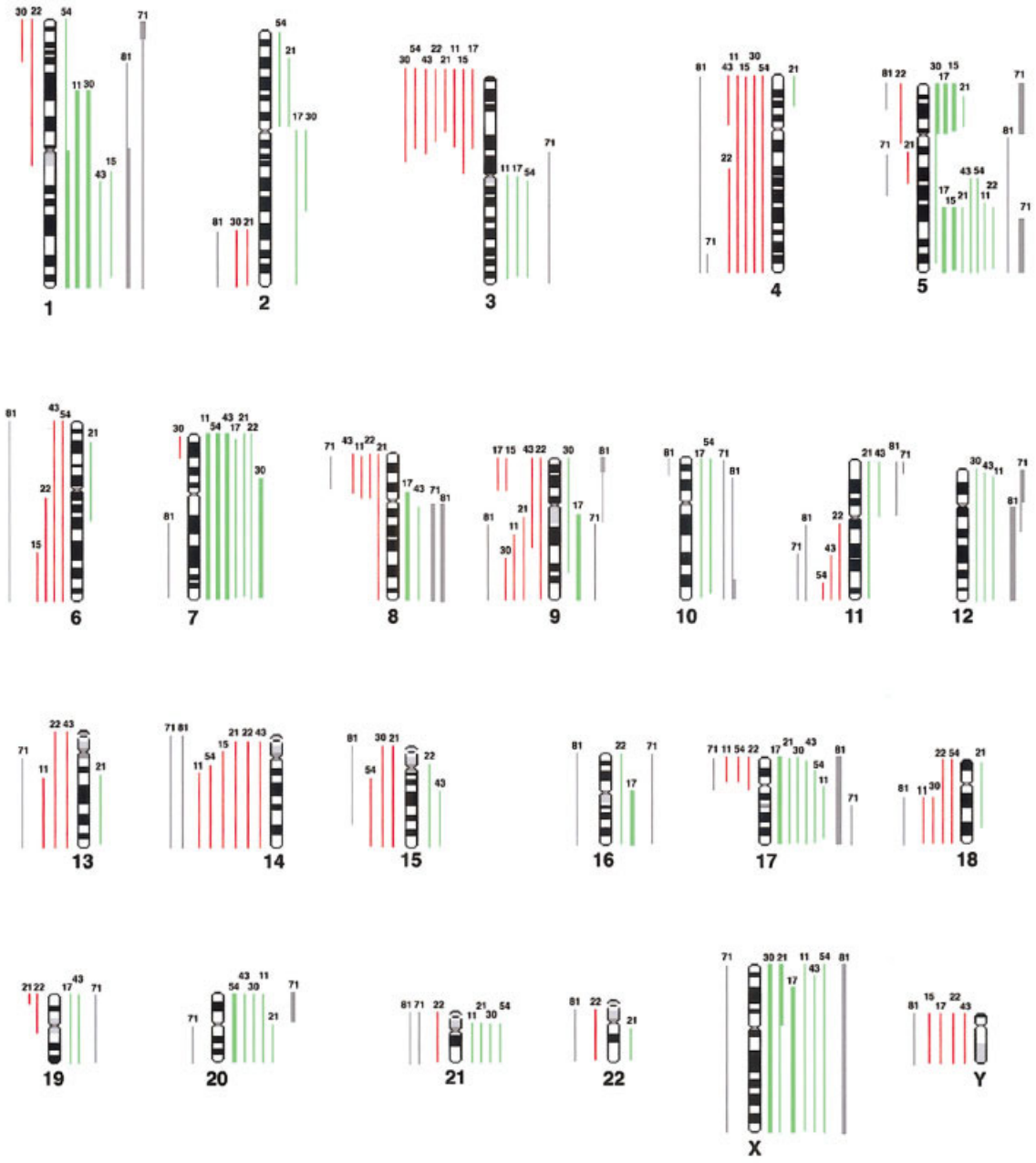

Figure 3. Composite CGH result of 10 RCC cell lines from cases of stage IV CCRCC. The cell lines are abbreviated as follows: UOK111 as "11," UOK115 as "15," and so forth. The vertical lines to the right and left of each chromosomal ideogram represent chromosomal gains and losses, respectively. The thicker bars indicate high-level gains. The *VHL*(+/+) cases, UOK171 and UOK181 ("71" and "81," respectively), have gray gain and loss bars placed lateral to the bars of the eight *VHL*(−/−) cases.

By use of SKY analysis, we found differences from the previously published karyotypes obtained by classical G-banding (Zhao et al., 1995). Our results for stage IV tumors show that *VHL* inactivation, loss of 3p, and gain of 5q are commonly maintained through tumor progression to stage IV. However, the near diploidy of the early cancer cell is replaced by a high degree of polyploidy and, on average, >10 numerical and/or structural aberrations per cell. The *VHL* inactivation status appears to suggest an influence on the pattern of aneuploidy. CCRCCs with inactive *VHL* always had 3p loss as a result of 3p terminal deletions, either isolated or arising from non-reciprocal transloca-

TABLE 3. Aberrations in Ten Advanced-Stage Cell RCC Lines

Case (UOK no.)	Types of aberrations (SKY)[a]		Genomic aneuploidy (CGH)	
	Numerical	Structural	No. of Alterations[b]	No. of HLG[c]
111	8	7	18	2
115	4	10	9	2
117	4	7	15	7
121	9	11	22	1
122	13	12	21	0
130	7	8	19	4
143	9	10	20	1
154	15	9	20	3
171	7	14	24	6
181	2	19	25	7
Sum	78	107	193	33
Mean	7.8	10.7	19.3	3.3

[a]Numerical = aberrations due to gains or losses of whole chromosomes unrelated to structural abnormalities, and resulting in copy number alterations on CGH; Structural = aberrations including unbalanced translocations, deletions, isochromosomes, and dicentrics resulting in copy number alterations on CGH.
[b]Determined by ANCA (average number of copy alterations by CGH).
[c]HLG = number of high level gains by CGH.

tions (typically with chromosome arm 5q), consistent with previous reports (Kovacs and Frisch, 1989; Kovacs and Kung, 1991). On the other hand, both cases of *VHL*(+/+) wild-type cancers had retention of 3p material surrounding the *VHL* locus by CGH and LOH, and exhibited recurrent gains of chromosome arm 8q (resulting from isochromosome 8q), unbalanced translocations involving chromosome 14, and losses of chromosomes 14, 15, 18, and 22.

Specific chromosomal alterations that were previously associated with a poor clinical outcome were found in our lines, consistent with their being derived from patients with metastatic disease: losses at 8p, 9p, and 14, and gains of 10 and 17 (Beroud et al., 1996; Reutzel et al., 2001; Presti et al., 2002). Some changes previously associated with a good-prognosis CCRCC (gains of 5q31-ter) were present in all 10 cases, irrespective of *VHL* status, whereas others (chromosome 7 gains) were found in all eight *VHL*(−/−) cases, but not in either *VHL*(+/+) case (Moch et al., 1996; Gunawan et al., 2001). Because our tumors were all metastatic (stage IV) at presentation, neither 5qter nor chromosome 7 gains appeared protective from metastatic disease in these cases. The identification of genes mutated or up-regulated at 5qter, 7, 10, and 17 may lead to a better understanding of their specific roles in the genesis and/or progression of renal neoplasia (Bugert et al., 2000; Amare Kadam et al., 2001). One candidate gene may be the proto-oncogene *MET*, which has demonstrated importance in hereditary and sporadic papillary RCC and which maps to 7q31 (Schmidt et al., 1997; Zhuang et al., 1998). Although we found no activating mutations of *MET*, we did not exclude the possibility that an increased *MET* copy number alone may play a role in the pathogenesis of CCRCC.

In the generation of the highly aneuploid genome of stage IV CCRCC, our data show that the major mechanisms leading to copy number change are segregation errors (resulting in whole-chromosome losses or gains) and non-homologous chromatid exchange (resulting in non-reciprocal translocations and DNA losses). Amplifications arising from genome-wide reduplication of marker chromosomes were not seen and do not appear to be the mechanism for DNA gain in CCRCC. No balanced translocations were noted. Through LOH and CGH analyses, a recent report implicated sister chromatid exchange as the cause of interstitial 3p LOH in RCC (Alimov et al., 2000). Our genome-level SKY and CGH analyses support other mechanisms; however, our data do not specifically exclude the possibility that interstitial chromosomal deletions may be a precursor to more widespread genomic instability. More thorough LOH analyses along the whole 3p arm, in conjunction with CGH and SKY from early-stage and late-stage tumors, would be required to address this question.

In summary, our results demonstrate that molecular cytogenetics can be used to quantitate and finely characterize the aneuploidy of the high-stage solid tumor. The tumors analyzed here represent the terminal aspects of CCRCC development, and lie in stark contrast to their near-diploid pre-meta-

static progenitors (Presti et al., 1998; Phillips et al., 2001). That *VHL* inactive (−/−) and *VHL* wild-type (+/+) RCCs achieve the same histology (clear cell) and clinical disease (chemotherapy-refractory, metastatic) demonstrates that divergent tumors may share common pathways of genetic signaling to achieve a similar phenotypic result (Clifford et al., 1998). Combining these cytogenetic data with cDNA global expression profiling will help determine which genes are the targets of DNA copy number change in advanced tumors as the basis for diagnostic and therapeutic manipulation.

REFERENCES

Alimov A, Kost-Alimova M, Liu J, Li C, Bergerheim U, Imreh S, Klein G, Zabarovsky ER. 2000. Combined LOH/CGH analysis proves the existence of interstitial 3p deletions in renal cell carcinoma. Oncogene 19:1392–1399.

Amare Kadam PS, Varghese C, Bharde SH, Narasimhamoorthy NK, Desai S, Advani SH, Havaldar R, Kulkarni JN. 2001. Proliferating cell nuclear antigen and epidermal growth factor receptor (EGFr) status in renal cell carcinoma patients with polysomy of chromosome 7. Cancer Genet Cytogenet 125:139–146.

Anglard P, Trahan E, Liu S, Latif F, Merino MJ, Lerman MI, Zbar B, Linehan WM. 1992. Molecular and cellular characterization of human renal cell carcinoma cell lines. Cancer Res 52:348–356.

Barch M, Knutsen T, Spurbeck J. 1997. The AGT cytogenetic laboratory manual. Philadelphia: Lippincott/Raven.

Beroud C, Fournet JC, Jeanpierre C, Droz D, Bouvier R, Froger D, Chretien Y, Marechal JM, Weissenbach J, Junien C. 1996. Correlations of allelic imbalance of chromosome 14 with adverse prognostic parameters in 148 renal cell carcinomas. Genes Chromosomes Cancer 17:215–224.

Bugert P, Pesti T, Kovacs G. 2000. The tcf17 gene at chromosome 5q is not involved in the development of conventional renal cell carcinoma. Int J Cancer 86:806–810.

Cawkwell L, Bell SM, Lewis FA, Dixon MF, Taylor GR, Quirke P. 1993. Detection of allele loss in colorectal tumours using microsatellites and fluorescent DNA technology. Br J Cancer 67:1262–1267.

Clifford SC, Prowse AH, Affara NA, Buys CH, Maher ER. 1998. Inactivation of the von Hippel–Lindau (VHL) tumour suppressor gene and allelic losses at chromosome arm 3p in primary renal cell carcinoma: evidence for a VHL-independent pathway in clear cell renal tumourigenesis. Genes Chromosomes Cancer 22:200–209.

Gnarra JR, Tory K, Weng Y, Schmidt L, Wei MH, Li H, Latif F, Liu S, Chen F, Duh F-M, Lubensky IA, Duan R, Florence C, Pozzatti R, Walther MM, Bander NH, Grossman HB, Brauch H, Pomer S, Brooks JD, Issacs WB, Lerman MI, Zbar B, Linehan WM. 1994. Mutations of the VHL tumour suppressor gene in renal carcinoma. Nat Genet 7:85–90.

Gunawan B, Huber W, Holtrup M, von Heydebreck A, Efferth T, Poustka A, Ringert RH, Jakse G, Fuzesi L. 2001. Prognostic impacts of cytogenetic findings in clear cell renal cell carcinoma: gain of 5q31-qter predicts a distinct clinical phenotype with favorable prognosis. Cancer Res 61:7731–7738.

Herman JG, Latif F, Weng Y, Lerman MI, Zbar B, Liu S, Samid D, Duan DS, Gnarra JR, Linehan WM, Baylin S. 1994. Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. Proc Natl Acad Sci USA 91:9700–9704.

Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F, Pinkel D. 1992. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 258: 818–821.

Knutsen T, Ried T. 2000. SKY: a comprehensive diagnostic and research tool. A review of the first 300 published cases. J Assoc Genet Tech 26:3–15.

Kovacs G, Frisch S. 1989. Clonal chromosome abnormalities in tumor cells from patients with sporadic renal cell carcinomas. Cancer Res 49:651–659.

Kovacs G, Kung HF. 1991. Nonhomologous chromatid exchange in hereditary and sporadic renal cell carcinomas. Proc Natl Acad Sci USA 88:194–198.

Latif F, Tory K, Gnarra J, Yao M, Duh FM, Orcutt ML, Stackhouse T, Kuzmin I, Modi W, Geil L, Schmidt L, Zhou F, Li H, Wei MH, Chen F, Glenn G, Choyke P, Walther MM, Weng Y, Duan DSR, Dean M, Glavac D, Richards FM, Crossey PA, Fergusin-Smith MA, Le Paslier D, Chumakov I, Cohen D, Chinault AC, Maher ER, Linehan WM, Zbar B, Lerman MI. 1993. Identification of the von Hippel–Lindau disease tumor suppressor gene. Science 260:1317–1320.

Macville M, Schröck E, Padilla-Nash H, Keck C, Ghadimi BM, Zimonjic D, Popescu N, Ried T. 1999. Comprehensive and definitive molecular cytogenetic characterization of HeLa cells by spectral karyotyping. Cancer Res 59:141–150.

Moch H, Presti JC Jr, Sauter G, Buchholz N, Jordan P, Mihatsch MJ, Waldman FM. 1996. Genetic aberrations detected by comparative genomic hybridization are associated with clinical outcome in renal cell carcinoma. Cancer Res 56:27–30.

Nickerson ML, Weirich G, Zbar B, Schmidt LS. 2000. Signature-based analysis of MET proto-oncogene mutations using DHPLC. Hum Mutat 16:68–76.

Phillips JL, Ghadimi BM, Wangsa D, Padilla-Nash H, Worrell R, Hewitt S, Walther MM, Linehan WM, Klausner RD, Ried T. 2001. Molecular cytogenetic characterization of early and late renal cell carcinomas in von Hippel–Lindau (VHL) disease with spectral karyotyping (SKY). Genes Chromosomes Cancer 31:1–9.

Presti JC Jr, Moch H, Gelb AB, Huynh D, Waldman FM. 1998. Initiating genetic events in small renal neoplasms detected by comparative genomic hybridization. J Urol 160:1557–1561.

Presti JC Jr, Wilhelm M, Reuter V, Russo P, Motzer R, Waldman F. 2002. Allelic loss on chromosomes 8 and 9 correlates with clinical outcome in locally advanced clear cell carcinoma of the kidney. J Urol 167:1464–1468.

Reiter RE, Anglard P, Liu S, Gnarra JR, Linehan WM. 1993. Chromosome 17p deletions and p53 mutations in renal cell carcinoma. Cancer Res 53:3092–3097.

Reutzel D, Mende M, Naumann S, Störkel S, Brenner W, Zabel B, Decker J. 2001. Genomic imbalances in 61 renal cancers from the proximal tubulus detected by comparative genomic hybridization. Cytogenet Cell Genet 93:221–227.

Ried T, Liyanage M, du Manoir S, Heselmeyer K, Auer G, Macville M, Schröck E. 1997. Tumor cytogenetics revisited: comparative genomic hybridization and spectral karyotyping. J Mol Med 75: 801–814.

Ried T, Heselmeyer-Haddad K, Blegen H, Schröck E, Auer G. 1999. Genomic changes defining the genesis, progression, and malignancy potential in solid human tumors: a phenotype/genotype correlation. Genes Chromosomes Cancer 25:195–204.

Schmidt L, Duh F-M, Chen F, Kishida T, Glenn G, Choyke P, Scherer SW, Zhuang Z, Lubensky IA, Dean M, Allikmets R, Chidambaram A, Bergerheim UR, Feltis TJ, Casadevall C, Zamarron A, Bernues M, Richard S, Lips CJM, Walther MM, Tsui L, Geil L, Orcutt ML, Stackhouse T, Lipan J, Slife L, Brauch H, Decker J, Niehans G, Hughson MD, Moch H, Störkel S, Lerman MI, Linehan WM, Zbar B. 1997. Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. Nat Genet 16:68–73.

Schröck E, du Manoir S, Veldman T, Schoell B, Wienberg J, Ferguson-Smith MA, Ning Y, Ledbetter, DH, Bar-Am I, Soenksen D, Garini Y, Ried T. 1996. Multicolor spectral karyotyping of human chromosomes. Science 273:494–497.

Stolle C, Glenn G, Zbar B, Humphrey JS, Choyke P, Walther M, Pack S, Hurley K, Andrey C, Klausner R, Linehan WM. 1998. Improved detection of germline mutations in the von Hippel-Lindau disease tumor suppressor gene. Hum Mutat 12:417–423.

Yang ZQ, Yoshida MA, Fukuda Y, Kurihara N, Nakamura Y, Inazawa J. 2000. Molecular cytogenetic analysis of 17 renal cancer cell lines: increased copy number at 5q31–33 in cell lines from nonpapillary carcinomas. Jpn J Cancer Res 91:156–163.

Zhao WP, Gnarra JR, Liu S, Knutsen T, Linehan WM, Whang-Peng J. 1995. Renal cell carcinoma. Cytogenetic analysis of tumors and cell lines. Cancer Genet Cytogenet 82:128–139.

Zhuang Z, Park WS, Pack S, Schmidt L, Vortmeyer AO, Pak E, Pham T, Weil RJ, Candidus S, Lubensky IA, Linehan WM, Zbar B, Weirich G. 1998. Trisomy 7-harbouring non-random duplication of the mutant MET allele in hereditary papillary renal carcinomas. Nat Genet 20:66–69.