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Cytogenetic and Morphologic Typing of 58 Papillary Renal Cell Carcinomas: Evidence for a Cytogenetic Evolution of Type 2 from Type 1 Tumors

Bastian Gunawan, Anja von Heydebreck, Thekla Fritsch, Wolfgang Huber, Rolf-Hermann Ringert, Gerhard Jakse, and László Füzesi[1]

Institute of Pathology [B. G., T. F., L. F.] and Department of Urology [R-H. R.], University of Göttingen, 37075 Göttingen; Department of Computational Molecular Biology, Max Planck Institute for Molecular Genetics, 14195 Berlin [A. v. H.]; Division of Molecular Genome Analysis, German Cancer Research Center, 69120 Heidelberg [W. H.]; and Department of Urology, University of Aachen, 52057 Aachen [G. J.], Germany

ABSTRACT

We evaluated clinical characteristics, patient outcome (mean follow-up, 47 months), and cytogenetic abnormalities in the largest as yet reported cytogenetic series of 47 primary and 11 secondary papillary renal cell carcinomas for differences between the recently proposed type 1 and type 2 subtypes. Secondary tumors were more often of type 2 morphology ($P = 0.02$), whereas primary type 2 tumors were associated with higher clinical stage ($P = 0.001$) and worse patient outcome ($P = 0.02$). Although both subtypes had at least one of the primary chromosomal gains at 17q, 7, and 16q, type 2 tumors had moderately lower frequencies of primary gains at 17p (61 *versus* 94%; $P = 0.007$) and 17q (72 *versus* 97%; $P = 0.02$). On the other hand, type 2 tumors overall had more chromosomal alterations than type 1 tumors ($P = 0.01$), particularly gains of 1q (28 *versus* 3%; $P = 0.02$) and losses of 8p (33 *versus* 0%; $P = 0.001$), 11 (28 *versus* 3%; $P = 0.02$), and 18 (44 *versus* 9%; $P = 0.01$). Hierarchical clustering suggested cytogenetic patterns common but not restricted to type 2 morphology, one characterized by multiple additional gains, and another predominantly showing additional losses. These findings provide genetic evidence that type 1 and type 2 tumors arise from common cytogenetic pathways and that type 2 tumors evolve from type 1 tumors. Independently of type, losses of 9p were statistically correlated with advanced disease ($P = 0.0008$) and may serve as a potential adverse prognostic marker in papillary renal cell carcinomas.

INTRODUCTION

The morphological classification of renal epithelial tumors, based on variations of histology and concepts of histogenesis proposed by Thoenes *et al.* (1), distinguishes clear cell RCC[2] (70–80%), papillary (formerly chromophilic) RCC (10–15%), chromophobic RCC (<5%), and the rare Bellini duct carcinoma (<1%; Refs. 1, 2). Evolving cytogenetic and molecular genetic correlates of the major morphological categories have provided a firm genetic basis and validation of the current classification (2–6). Accordingly, papillary RCC is recognized as a RCC variant with distinct genetic features, characterized primarily by trisomies or tetrasomies 7 and 17 and loss of chromosome Y, as well as additional gains of chromosomes 3q, 12, 16, and 20 (3, 5, 7–9). Recently, Delahunt and Eble (10) proposed a subcategorization of papillary RCCs into type 1 tumors with single-layered small cells and pale cytoplasm and type 2 tumors with pseudostratified large cells and eosinophilic cytoplasm. Preliminary comparisons of clinicopathological features and patient survival between these two subtypes have suggested a prognostic use to this categorization, with type 2 morphology being associated with poorer patient outcome (11). Earlier CGH and molecular genetic studies reported the two subtypes to be correlated with specific genetic abnormalities. Whereas Jiang *et al.* (12) found DNA copy number gains of 7p and 17p to be more frequent in the 9 type 1 tumors of their 25 papillary RCCs, Sanders *et al.* (13) reported differences in allelic imbalance frequency on 17q and 9p between their 17 type 1 and 8 type 2 tumors. These findings were considered as evidence that type 1 and type 2 tumors arise from distinct genetic pathways. However, a common set of genetic alterations that uniquely distinguished between type 1 and type 2 tumors in both these series could not be established.

To test the current premise that type 1 and type 2 tumors represent distinct genetic subgroups of papillary RCCs, we herein present the largest as yet reported cytogenetic study of papillary RCCs, including 47 primary and 11 relapse tumors. We sought to identify (*a*) single cytogenetic aberrations and (*b*) global cytogenetic patterns that distinguished between 35 type 1 and 23 type 2 papillary RCCs. Moreover, cytogenetic alterations were evaluated in relation to classical indicators of prognosis and patient survival to investigate whether cytogenetic changes may add valuable prognostic information.

MATERIALS AND METHODS

Patients and Tumor Samples. The series comprised 58 tumor samples (47 primary tumors and 11 secondary tumors, including 10 metastases and 1 local recurrence) from 50 adult patients (41 men and 9 women; mean age at diagnosis, 62 years; range, 36–82 years) for which cytogenetic analysis could successfully be performed between 1989 and 2002. From 1 patient, 4 synchronous primary tumors were available; from 2 patients, both primary and corresponding secondary tumors could be obtained; and from another 2 patients, two to three metastases were included. All tumor samples met the diagnostic criteria for papillary RCC exhibiting at least 75% papillary or tubulopapillary architecture. Grading was performed by the Fuhrmann grading system (14). The tumors were reviewed and subclassified as type 1 and type 2 (Fig. 1) according to criteria described previously (10). Primary tumors were staged according to the TNM[2] system (15). Survival data could be obtained for 38 of the 44 patients with primary tumors (mean follow-up, 47 months; median, 41 months) by reviewing the clinic records, direct communication with the attending physicians, and from the local cancer registry. Recurrence-free survival was defined as the time between surgical treatment of primary tumors and clinically detectable relapse.

Cytogenetic Analysis. For classical cytogenetic analysis, viable tumor samples were excised immediately after surgery by experienced pathologists and submitted to short-term culture and chromosome analysis. Chromosomes were banded using routine G- and 4′,6-diamidino-2-phenylindole-banding techniques. For cases investigated by 4′,6-diamidino-2-phenylindole banding, image acquisition and analysis of metaphase spreads were performed on a Quips Genetics Workstation using the Quips Karyotyping Software (Applied Imaging, Newcastle, United Kingdom). Clonality criteria and karyotype descriptions follow the recommendations of International System for Human Cytogenetic Nomenclature (16). For mathematical analyses, cytogenetic abnormalities were expressed as copy number changes of chromosome arms by calculating the ratio of copy numbers for p- and q-arms of each chromosome to the underlying ploidy level (haploidy, $n = 1$; diploidy, $n = 2$; triploidy, $n = 3$; tetraploidy, $n = 4$). Ratios >1 were regarded as gains and ratios <1 as losses, whereas ratios of 1 indicated maintenance of chromosomal balance.

Statistical Analysis. The associations between clinicopathological variables (tumor size, TNM stage, clinical stage, Fuhrmann grade, and type) and

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[1] To whom requests for reprints should be addressed, at Institute of Pathology, Georg August University Hospital Göttingen, Robert-Koch-Str. 40, D-37075 Göttingen, Germany. Phone: 49-551-398687; Fax: 49-551-398627; E-mail: fuezesi@med.uni-goettingen.de.

[2] The abbreviations used are: RCC, renal cell carcinoma; CGH, comparative genomic hybridization; TNM, tumor-node-metastasis.

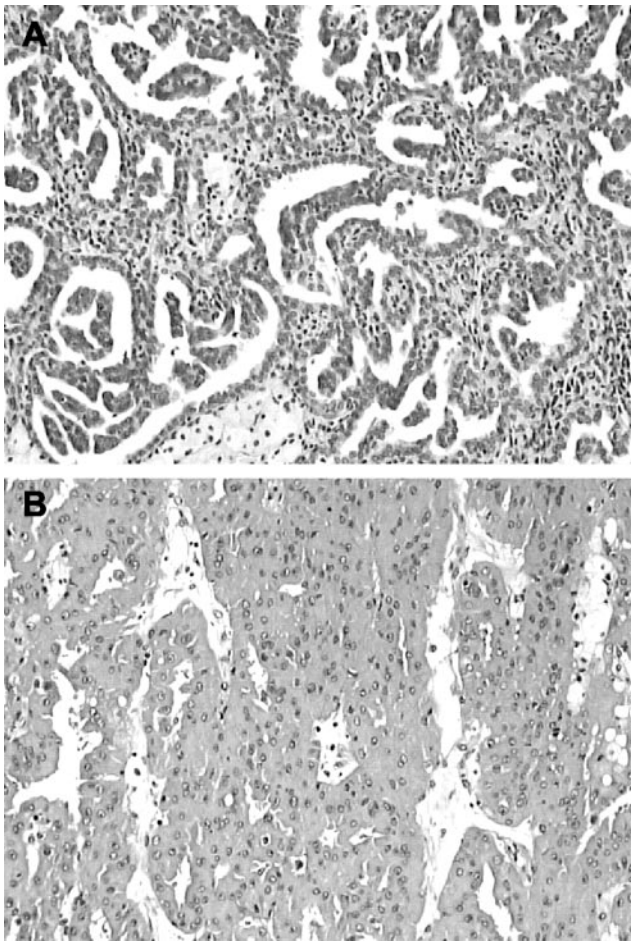

Fig. 1. Type 1 papillary RCC composed of slender papillae covered by a single layer of tumor cells with scanty cytoplasm. Foamy macrophages are seen in some papillary cores (*A*). Type 2 papillary RCC composed of papillae covered by a pseudostratified layer of large tumor cells with voluminous eosinophilic cytoplasm (*B*).

cytogenetic abnormalities were evaluated using the two-sample Wilcoxon test and Fisher's exact test for contingency tables. The conditional independence of cytogenetic and clinical variables, given the tumor type, was tested with the Cochran-Mantel-Haenszel test (17). Survival rates based on cytogenetic abnormalities and clinicopathological parameters were plotted by the Kaplan-Meier method. Statistical differences in survival times between different groups of patients were determined with the log-rank test. All statistical analyses were conducted using the software system R (18).

Hierarchical Clustering. Hierarchical clustering of the tumors was based on similarity of chromosome arm copy number patterns. The chromosome arms considered in the distance computation were restricted to autosomal arms, which were either gained or lost in at least 10% of all tumors. Aberrations of the sex chromosomes were not included. In the case of varying copy numbers for different bands on a chromosome arm, the copy number ratio with the largest deviation from 1 was selected. We considered the matrix $X = (x_{ij})$, where x_{ij} is the copy number of chromosome arm j in tumor i. The distance between any two rows i, i′, or columns j, j′, of X was defined as $\Sigma_j 1x_{ij} - x_{i'j}1$ or $\Sigma_i 1x_{ij} - x_{ij'}1$, respectively. On the basis of this distance measure, we applied complete linkage hierarchical clustering to both the rows and the columns of X.

RESULTS

Clinicopathological Characteristics. The clinicopathological characteristics of the 58 papillary RCCs from 50 patients are presented in Table 1. Primary tumors with higher Fuhrmann grades 3 and 4 were more often of higher T-stage ($P = 0.004$), positive M-stage ($P = 0.0008$), and higher clinical stage ($P = 0.002$). Among the 38 patients with available follow-up, higher Fuhrmann grades 3 and 4 were associated with shorter recurrence-free and overall survival ($P = 0.006$ and $P = 0.00006$). Overall survival was also significantly shorter for patients with higher T-stages (pT_3 and pT_4; $P = 0.0008$), N-positive tumors ($P = 0.004$), and M1-tumors ($P = 1 \times 10^{-7}$). Accordingly, patients in lower clinical stages (stage I and II) had a more favorable outcome (26 patients, 0 deaths), as opposed to patients in higher clinical stages (stage III and IV; 12 patients, 6 deaths). The differences in overall survival were highly significant ($P = 0.00004$).

Next, we sought to identify clinicopathological correlates for the type 1 and type 2 subtypes of papillary RCCs defined by Delahunt and Eble (10). Table 2 documents the distribution of Fuhrmann grade, tumor stages, as well as primary and secondary papillary RCCs by tumor type. Generally, patients with primary papillary RCCs had more often tumors of type 1 morphology, whereas patients with secondary RCCs had predominantly tumors of type 2 morphology ($P = 0.02$). Moreover, type 2 tumors were of significantly higher Fuhrmann grades than type 1 tumors ($P = 0.0002$). Within patients with primary papillary RCCs, type 2 tumors were more frequent high T-stage ($P = 0.003$), positive N-stage ($P = 0.009$), positive M-stage ($P = 0.0005$), and high clinical stage ($P = 0.001$) tumors. Accordingly, there was a significant association of type 2 morphology with poorer recurrence-free ($P = 0.006$) and overall patient survival ($P = 0.02$).

Cytogenetical Characteristics. All 58 papillary RCCs had aberrant karyotypes with a mean of 8.3 changes/tumor (median, 6; range, 2–24; Table 1). The number of changes was higher in the 11 secondary tumors (mean, 13.5; median, 14; range, 6–24) than in the 47 primary tumors (mean, 7.1; median, 6; range, 2–21). In general, chromosomal gains dominated over chromosomal losses, leading to hyperdiploid to hypotriploid modal chromosome numbers in most cases. The most common change was gain at chromosome 17q occurring in 51 (87.9%) cases, of which 47 were trisomies or tetrasomies and the remaining 4 were unbalanced translocations leading to partial gain of 17q. In most cases, gain of 17q was combined with polysomy 7 and/or gain at 16q occurring in 48 (82.8%) cases each. All tumors regardless of underlying clinicopathological characteristics had at least one of the chromosomal gains at 17q, 7, or 16q. Notably, there were some differences in the frequencies of these chromosomal abnormalities between localized and advanced tumors. Gain of chromosome 7 was significantly associated with lower T-stage ($P = 0.009$) and was more frequent in tumors of lower clinical stage, although this difference did not reach statistical significance ($P = 0.09$). Similar trends were observed for gain of 17p, which as a tendency occurred more often in tumors of lower T-stage ($P = 0.06$), negative N-stage ($P = 0.07$), and lower clinical stage ($P = 0.09$).

Additional presumably secondary changes included gains at chromosomes 20 (50%), 3q (43.1%), 12 (41.4%), 2 (20.7%), 8q (19%), 5q (17.2%), 13q (17.2%), and 1q (12.1%), as well as loss of Y chromosome, which occurred in 41 (87.2%) of 47 tumors from male patients and autosomal losses involving chromosomes 18 (22.4%), 11q (15.5%), 22q (15.5%), 9p (13.8%), 14q (13.8%), 8p (12.1%), 19p (12.1%), 2q (10.3%), 4p (10.3%), and 15q (10.3%). Several of these less frequent chromosomal abnormalities were correlated with advanced disease. Statistically significant associations were observed between (*a*) higher T-stage and losses of 8p ($P = 0.007$), 9p ($P = 0.004$), and 11q ($P = 0.007$); (*b*) positive N-Stage and gain of 3q ($P = 0.03$) and loss of 9p ($P = 0.04$); (*c*) positive M-stage and loss of 8p ($P = 0.002$); as well as (*d*) higher clinical stage and losses of 8p ($P = 0.002$), 9p ($P = 0.002$), and 11q ($P = 0.03$). These resulted in significantly decreased recurrence-free intervals for chromosomal losses at 8p ($P = 0.004$), 9p ($P = 0.00003$), and 18 ($P = 0.01$).

Table 1 *Clinicopathological and cytogenetic findings in 58 papillary RCCs from 50 patients*

No.	Age (yr)/Sex	Stage (TNM)	Grade	Type	Follow-up in months	Karyotype
1	38/M[a]	I ($T_1N_0M_0$)	1	1	NED, 14	44–49,XY,+7,+17[cp25]/45,XY,−16[1]
2[b]	51/M	I ($T_1N_0M_0$)	1	1	NED, 141	47–49,X,−Y,+7,r(15),+16,+17[cp22]
3	60/F	I ($T_1N_0M_0$)	1	1	NED, 64	49,XX,+13,der(14)t(14;15)(q22;q21),+16,+17[cp28]
4	68/M	I ($T_1N_0M_0$)	1	1	NED, 113	46–51,X,−Y,inv(1)(p36q21),+5,+7,+16,+17[cp14]
5	62/M	I ($T_1N_0M_0$)	1	1	NED, 104	51–53,X,−Y,+2,+3,+7,+7,+12,t(16;17)(p13;q12),+der(16)t(16;17)(p13;q12),+17[cp15]
6	63/M	I ($T_1N_0M_x$)	1	1	NA	50,X,−Y,+3,+7,+16,+17,+20[17]
7	69/M	I ($T_1N_0M_0$)	1	1	NED, 100	50,X,−Y,+6,+7,+16,+17,+20[cp3]
8	67/M	I ($T_1N_0M_0$)	1	1	NED, 25	48,X,−Y,+7,+16,+17[2]/48–50,idem,+3[cp38]/96–98,idemx2,+3,+3[cp2]
9	62/M	I ($T_1N_0M_0$)	1	1	NED, 94	47–50,X,−Y,+7,+12,+13,+17[cp58]/49,idem,der(22)t(3;22)(q12;p1)[4]/48–50,idem,add(22)(p1)[cp3]
10	39/F	I ($T_1N_0M_0$)	1	1	NED, 59	46–49,X,−X,+7,+7,+17,+20[cp48]
11	80/M	I ($T_1N_0M_0$)	1	1	NED, 62	47–50,X,−Y,+7,+16,+17,+20[cp34]/49–50,idem,add(2)(q37)[cp3]
12	72/F	I ($T_1N_0M_0$)	1	1	DOO, 22	47–51,XX,+3,+7,+16,+17,+20[cp19]
13	81/M	I ($T_1N_0M_0$)	1	1	NED, 46	50–58,XY,+2,+3,+5,+7,+7,+8,+12,+12,+16,+17,+20,+mar1[cp16]
14	59/M	I ($T_1N_0M_0$)	1	1	NED, 20	47–50,X,−Y,+7,+16,+17,+20[cp20]
15	51/M	I ($T_1N_xM_0$)	1	1	NED, 5	50–51,X,−Y,+7,+12,+13,+16,+17,+20[cp41]/51,idem,der(8)t(4;8)(q21;q24)[2]
16	54/M	I ($T_1N_0M_0$)	2	1	DOO, DP, 74	50–54,X,−Y,+3,+5,+7,+7,+12,+16,+17,−18,+20[cp40]
17	63/M	I ($T_1N_0M_0$)	2	1	DOO, 0	47–49,X,−Y,+2,+7,+12,+13,−14,+17[cp12]/46–50,idem,del(2)(q21)[4],add(14)(q32)[14], add(16)(p13)[9],der(16)t(5;16)(q13;p13)[6],add(16)(q24)[2][cp25]/48,idem,der(14)t(3;14)(q11;q32)[2]/48, idem,der(16)t(3;16)(q11;p13)[2]
18	71/M	I ($T_1N_xM_0$)	2	1	NED, 4	40–45,X,−Y,der(6)t(6;17)(q24;q12∼21),+7,+12,−18[cp12]/45–46,XY[cp2]
19[b]	47/M	I ($T_1N_0M_0$)	4	1	NED, 145	49,X,−Y,+7,+7,t(11;12)(q22;q13),+16,+17[cp3]
20[b]	74/M	II ($T_2N_0M_0$)	1	1	NA	47–51,X,−Y,+7,+8,der(9)t(3;9)(q11;q34),+16,+17,+20[cp87]/47–48,idem,−21[cp5]/48–49,idem, −22[cp3]/51,idem,+7[2]/50,idem,der(19)t(3;19)(q11;p13)[2]
21	49/M	II ($T_2N_xM_x$)	1	1	NA	50,X,−Y,der(2)t(2;3)(q37;q12),−5,+7,+7,−9,+12,der(15)t(9;15)(q12;p12),+16,+17,+18, der(19)t(5;19)(q13;q13),+20[23]
22	47/M	II ($T_2N_0M_0$)	1	1	NED, 96	46–47,X,−Y,+7,+16[cp25]
23	65/F	II ($T_2N_0M_0$)	1	1	DP, 43	47–51,XX,+8,+16,+17,+20[cp17]/88–98,idemx2[cp4]/46–51,idem,−4[cp3]
24[b]	61/M	II ($T_2N_0M_0$)	2	1	NED, 39	47–48,X,−Y,+7,+16,+17[cp19]/46–48,idem,der(13)t(3;13)(q11;q34)[cp81]/46–47,idem,der(13)t(3; 13)(q11;q34),−22[cp4]
25	72/M	II ($T_2N_0M_0$)	2	1	NED, 37	48–49,X,−Y,+7,+16,+17,+20[cp22]
26[b]	54/M	II ($T_2N_0M_0$)	2	1	NED, 132	48,X,−Y,+7,+7,+17[cp5]/93–96,idemx2,−5,−9[cp4]/97,idemx2,−10,+20,+mar1[1]
26b[b]	54		1	1		48–50,X,−Y,+7,+7,+12,−14,+16,+17,+20[cp63]/46–49,idem,der(13)t(3;13)(q11;p13)[cp16]/50, idem,der(19)t(3;19)(q11;p13)[cp12]/49,idem,add(13)(p13)[cp3]
26c[b]	54		1	1		47–50,X,−Y,+7,+7,del(11)(q21),+17,der(21)t(3;21)(q11;p12)[cp60]
26d[b]	54		1	1		44–48,X,−Y,+7,+7,+17[cp98]/92,idemx2,−2,−7,−8,−22[1]
27	65/F	III ($T_{3b}N_0M_0$)	1	1	NED, 63	47–49,XX,+12,+16,+17[cp40]
28	64/M	III ($T_{3a}N_xM_x$	2	1	NA	39–42,XY,der(1)t(1;14)(p13;q11),−9,−11,add(13)(p11),−14,+add(16)(q12),+17,−18,add(18)(p11), −20,−21,−22[cp12]
29	54/M	III ($T_1N_1M_0$)	2	1	AD, 48	51,XY,+X,+3,+7,+8,+16,+17[cp5]/95–102,idemx2[15]
30	68/F	IV ($T_1N_0M_1$)	1	1	DOTD, 5	32–35⟨1n⟩,X,add(1)(q12),+add(1)(p13)x2,+2,+5,+7,del(9)(p21),+del(11)(q13),+12,+16,+17, +r,+mar1[cp8]/64–65,idemx2,−5,−del(11)(q13),−r[cp4]/66–68,idemx2,−5,+6, −del(11)(q13),−r[cp4]
31	70/M	IV ($T_{3a}N_2M_0$)	1	1	AD, 3	47–50,X,−Y,+7,+12,+17,+der(20)t(3;20)(q12;q13.3)[cp26]/45–49,idem,del(9)(p13)[cp34]/49, idem,dup(3)(q21q2??)[3]
32b[b]	55/M	Metastasis	1	1		44–50,X,−Y,+3,der(5)t(5;14)(p15;q11),+7,+7,add(8)(q21),−14,−15,+16,+17,+20[cp14]
33[b]	82/F	I ($T_1N_0M_0$)	2	2	NED, 73	46–49,X,add(X)(q22),add(1)(q42),+4,−6,+8,+16,+der(1;17)t(1;17)(p11;q25)add(1)(q42), +der(1;17)t(1;17)(p11;q25)add(1)(q42),−18,add(19)(p13)[cp95]/45–47,idem,−22[cp4]/49–50,idem, +add(X)(q22)[4],+13[2][cp5]
34	75/F	I ($T_1N_xM_x$)	2	2	NED, 7	51–56,X,inv(X)(p11q26),+2,+3,+7,+der(7)inv(7)(p11p22)inv(7)(p11q11),+12,+13,+16,+17, +20,+22[cp22]
35	76/M	I ($T_1N_xM_x$)	2	2	NA	47–49,X,−Y,+3,+7,+16,+17[cp24]/47–48,idem,−18[cp3]/47–48,idem,−22[cp3]/45–46,XY[cp2]
36	62/M	II ($T_2N_0M_0$)	2	2	NED, 48	38–49,X,−Y,+7,+7,+16[cp30]/97,idemx2,+20?[1]/42–47,idem,−7[12],−11[6][cp16]
37	59/M	III ($T_{3a}N_0M_0$)	3	2	NED, 54	43–48,X,−Y,1qh+,+2,del(11)(q15q21),−14,+16,+17,+20[cp8]/90–96,idemx2[cp3]
38	61/M	III ($T_{3b}N_xM_0$)	2	2	NED, 12	42–44,X,−Y,add(8)(p23),−15,add(15)(q24∼26),der(16)t(16;17)(q22;q21),+der(16)t(16;17)(q22;q21), +der(16)t(16;17)(q22;q21),−17,add(19)(p13),−22[cp26]
39	70/M	IV ($T_1N_2M_1$)	2	2	AD, 9	45–50,X,−Y,+3,+7,+7,add(8)(p23),+12,−18,+20[cp41]/90–99,idemx2[cp7]/48–49, idem,inv(14)(p13q13)[cp3]/43–47,idem,dup(14)(q11q32)[cp4]
40	74/M	IV ($T_{3b}N_1M_1$)	3	2	DOTD, 0	53–59,XX,+add(1)(p12),−2,dup(3)(p21p24),+7,+der(12)t(1;12)(q23;q13),+i(12)(q10), +16,+17,+19,+20,+20,+mar1,+mar2[cp9]/51–56,idem,+19[cp5]/60,idem,+5,+7,+19[cp2]/56–62, idem,+2,+7,+19[cp3]/114,idemx2,+2,+2,+7,+7,−i(12)x2,+19,+19[cp1]/58–61,idem,+2, +i(3)(q10),+12,+12,−i(12)(q10),+13,+19[cp3]
41	70/M	IV ($T_4N_0M_1$)	3	2	DOTD, 3	43–45,X,−Y,+16,−18[cp28]/43–45,idem,−9[cp3]/44–45,idem,−8[cp2]
42	71/F	IV ($T_1N_2M_1$)	4	2	DOTD, 4	50–51,XX,+3,+7,+16,+17,+20[cp27]
43	36/M	IV ($T_{3a}N_0M_1$)	4	2	DOTD, 6	52–55,X,−Y,add(2)(p25),del(3)(p11p12),add(4)(p15),+add(4)(p15),−5,+7,+7,add(11)(p13), der(11)t(5;11)(q13;p14),+12,der(15)t(X;15)(q11;p11),der(16;16)t(16;16)(q24;q24)del(16)(p13),+17, +18,+19,+20,+mar1,+mar2,3–6dmin[cp31]/50–53,idem,−8[cp4]/43–46,XY[cp22]
44	46/M	III ($T_{3a}N_1M_x$)	2	2	AD, 3	52,XY,+3,+7,+10,+16,+17,+20[2]/56–59,idem,+X,−Y,+der(1)t(1;3)(p11;q11),+2,−3,+7, +8,−10,+12,+16,[cp3]/96,idemx2,−Y,−Y,+2,−3,−3,−10,−10,−21[cp2]/92,idemx2,−Y,−Y, −3,−3,−9,der(11)t(1;11)(q11;q24)x2,−10,−10,+12,−13,−14,−15,−17,add(17)(p12)x2,−19, −21[1]/75–86,idemx2,−2,−3,−3,−5,−9,−9,−10,−10,−11,der(11)t(1;11)(q11;q24),−13,−14,−14, −15,−16,−17,−17,add(17)(p12)x2,−18,−19,−21[cp7]/78–80,idemx2,−1,−2,−3,−3,−4,−5,−9, −9,−10,−10,−11,der(11)t(1;11)(q11;q24),−13,−14,−14,−15,−16,−17,−17,add(17)(p12)x2,−18, −19,−21[cp4]/73–82,idemx2,del(1)(q11),−2,−3,−3,−5,−9,−9,−10,−10,−10,−11, der(11)t(1;11)(q11;q24),−12,−13,−13,−14,−14,−15,−16,−16,−17,−17,add(17)(p12)x2, −21[cp6]/46,XY[2]
44 b	46	Metastasis	2	2		90–95,XX,−Y,−Y,+7,+7,−9,der(11)t(1;11)(q11;q24)x2,−13,−14,−15,+16,+16,+add(17)(p12), +add(17)(p12)[cp6]/91–93,idem,+20,+20[cp7]/89–93,idem,−18,+20,+20[cp9]/94–95, idem,+i(1)(q10),+20,+20[cp2]
45	76/M	IV ($T_4N_1M_1$)	4	2	AD	70–73,XX,−Y,−Y,−1,−2,−4,+7,+7,−8,add(8)(p11)x2,−9,−9,−10,−10,−11,−11,−12,−12,−13, −13,add(13)(p11),−14,−14,−15,−15,+16,+16,+16,+16,−17,−18,add(19)(p13)x2,−20,−20, −21,−21,−21,−22,−22,+mar1,+mar2,+mar3,+mar4[cp15]/63–67,idem,−16,−17, −add(19)(p13)[cp4]

Table 1 *Continued*

No.	Age (yr)/Sex	Stage (TNM)	Grade	Type	Follow-up in months	Karyotype
45b	76	Metastasis	2	2		64–74,XX,−Y,−Y,−1,−2,−4,+7,+7,−8,add(8)(p11)x2,−9,−9,−10,−10,−11,−11,−12,−12,−13,−13,add(13)(p11),−14,−14,−15,−15,+16,+16,+16,+16,−17,−18,add(19)(p13)x2,−20,−20,−21,−21,−21,−22,−22,+mar1,+mar2,+mar3,+mar4[cp25]
46b	36/M	Metastasis	3	2		61–63⟨2n⟩,XY,+2,+4,+5,+7,+8,+8,+10,+12,+12,+13,+13,+16,+17,+17,+20,+20,+mar1,+mar2[cp8]/121–127,idemx2,−mar2[4],−mar2[3][cp5]/61–63,idem,−12[cp11]/57–64,idem,−10[cp7]/63,idem,−8[cp4]
46c	37	Metastasis	1	2		60–62,X,−Y,+2,+5,+5,+7,+8,+8,+10,+12,+12,+13,+13,+16,+17,+17,+20,+20,+mar[cp20]
46d	38	Metastasis	2	2		62–64⟨2n⟩,XY,+X,+2,+3,+4,+5,+7,+8,+10,+12,+13,+13,+16,+17,+17,+19,+20,+20,+21[cp3]
47b	68/M	Metastasis	2	2		42–48,X,−Y,i(8)(q10),+16,−18,+20[cp10]
48b	65/M	Local Recurrence	3	2		46–51,X,−Y,+7,+7,+del(12)(q12q15),+16,+17,+mar1[cp15]/50–51,idem,+2[cp2]/50–51,idem,+20[cp2]/46,XY[cp2]/91,XXYY,−5,−17,+mar[1]
48c	65	Metastasis	3	2		48–50,X,−Y,+7,+7,+del(12)(q12q15),+16,+17[cp8]/42–46,XY[cp31]
49b	74/M	Metastasis	3	2		67–71,XX,+i(1)(q10),+del(7)(p36),+2,+3,+i(5)(q10),+7,+7,+7,+8,+8,+12,+12,+16,+16,+17,+20,+20,+21,+21,+22,+mar1,+mar2,+mar3,+mar4,+mar5[cp20]
50b	63/M	Metastasis	3	2		44–48,XY,i(1)(q10),−2,del(3)(p12p21),−4,del(9qh−)(q33),−11,+12,+16,+17,−18,add(18)(p11),add(21)(p13),+r,+mar1[cp4]/45–49,idem,−17[cp3]/43–46,idem,−17,−22[cp22]

[a] F, female; M, male; NED, no evidence of disease; NA, not available; DOTD, died of tumor disease; DOO, died of other; DP, disease progression; AD, advanced disease.
[b] Published earlier.

Independently of type, only loss of 9p retained a statistically significant association with advanced disease, particularly with higher T-stage ($P = 0.002$) and higher clinical stage ($P = 0.0008$).

Subsequently, we sought to identify particular chromosomal changes that most strongly defined the division of tumors by type. Although there was no universal chromosomal marker that made the distinction between all 32 patients with type 1 tumors and 18 patients with type 2 tumors, there was a significantly higher number of chromosomal changes in type 2 tumors than in type 1 tumors ($P = 0.01$). As regards differences in the frequencies of aberrations, statistical analysis revealed gains of 17p (94 *versus* 61%; $P = 0.007$) and 17q (97 *versus* 72%; $P = 0.02$) to be more common in type 1 tumors than type 2 tumors, whereas additional changes were more prevalent in the type 2 group than in the type 1 group. Among these, losses of 8p (0 *versus* 33%; $P = 0.001$) were exclusively seen in 6 patients with type 2 tumors. Other abnormalities, which were statistically more common but not restricted to type 2 morphology, included losses of chromosomes 11 (3 *versus* 28%; $P = 0.02$) and 18 (9 *versus* 44%; $P = 0.01$) and gains of chromosome 1q (3 *versus* 28%; $P = 0.02$).

Hierarchical Clustering. Having identified single chromosomal abnormalities that appear to make the distinction by type on a genetic basis, we subsequently sought to establish particular patterns of cytogenetic alterations in the type 1 and type 2 groups. We used hierarchical clustering to group tumors on the basis of similarities in their copy number changes of chromosome arms calculated in relation to the underlying ploidy level. The same clustering was performed to group chromosomal arms on the basis of similarity in the pattern with which their copy numbers varied over all samples. The data were transformed into a matrix format, with each row representing the copy numbers for a single chromosome arm over all tumor samples, and each column representing the copy numbers for all chromosome arms in a single tumor. Clustering based on the global cytogenetic profile, approximated by a selected set of chromosomal alterations occurring in at least 10% of the 58 tumors, revealed little variation in gains and losses among most of the tumors (Fig. 2). In fact, there was only one small branch with highly correlated patterns of cytogenetic alterations defined by high level gains of 7, 16, 17, 12, 20, as well as 1q, 5, 8, and 13. Other less correlated patterns of cytogenetic changes comprised chromosomal losses, including losses of 8p and 18, and lack of 17p gain. These patterns partly correlated with type 2 morphology. Overall, however, hierarchical clustering revealed no distinct pattern of cytogenetic changes that unequivocally identified type 1 or type 2 tumors. Surprisingly, even clusterings using a selected set of chromosomal changes that most strongly defined the division of tumors by type in univariate analyses disclosed no evident patterns that could readily be assigned to type 1 or type 2 morphology (data not shown).

Table 2 *Clinicopathological characteristics by tumor type*

	Type 1 $n = 35$		Type 2 $n = 23$		
	n	%	*n*	%	*P*
Fuhrmann grade					
Grade 1/2	34	97%	12	52%	0.0002
Grade 3/4	1	3%	11	48%	
Patients with primary tumors	$n = 31$		$n = 13$		
T-stage					
$pT_{1/2}$	28	90%	6	46%	0.003
$pT_{3/4}$	3	10%	7	54%	
N-stage					
pN_0	25	81%	5	38%	0.009
$pN_{1/2}$	2	6%	5	38%	
M-stage					
pM_0	27	87%	4	31%	0.0005
pM_1	1	3%	6	46%	
Clinical stage					
Stage I/II	26	84%	4	31%	0.001
Stage III/IV	5	16%	9	69%	
Patients with secondary tumors	$n = 1$		$n = 5$		0.02

DISCUSSION

This study comprises the largest as yet reported genetic series of papillary RCCs diagnosed according to the subclassification recently proposed by Delahunt and Eble (10). In general, we could confirm the prognostic use to this subdivision (11) in that type 2 morphology was associated with higher T-stage, clinical stage, and Fuhrmann grades, positive N-stage and M-stage, and poorer patient survival. The histogenetic background to this subdivision remains largely unclarified, but current views favor the possibility that type 1 and type 2 papillary RCCs represent two distinct tumor entities (11–13). Although earlier molecular genetic studies have suggested distinct genetic pathways in type 1 and type 2 papillary RCCs (12, 13), tumor type-specific cytogenetic profiles have thus far not been identified.

In this study, we could confirm earlier CGH and allelotyping studies (12, 13) in that type 1 tumors are more frequently characterized by gains at 17p and 17q (94 and 97%) than type 2 tumors (61 and 72%). An additional and novel aspect, however, is that gain of 17p and polysomy 7 also appear to more frequent in low stage tumors than in advanced tumors, underlining their role as primary events in papillary RCC tumorigenesis that may be lost or obscured during later tumor progression.

Overall, there was only little variation in cytogenetic patterns

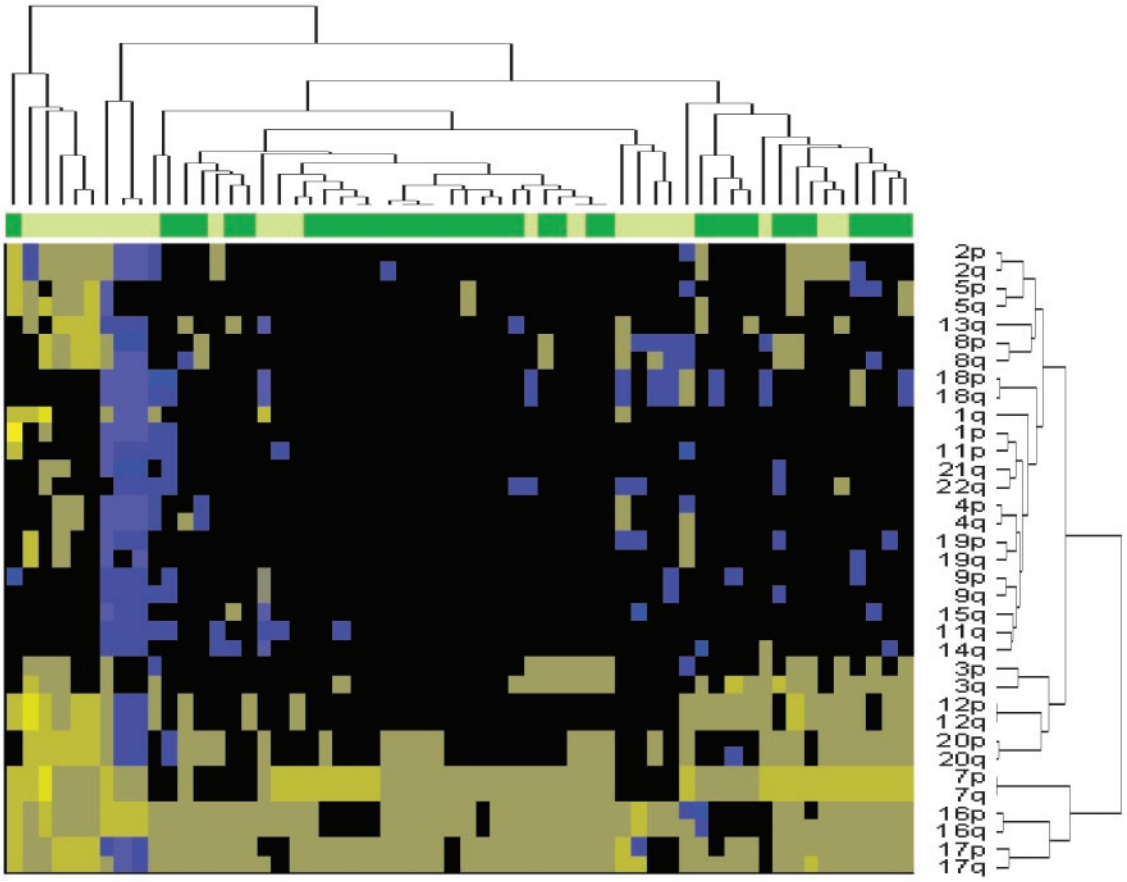

Fig. 2. Hierarchical clustering of 58 tumors on the basis of chromosome copy number levels. The 35 autosomal arms, which were either lost or gained in at least 10% of the tumor samples, are depicted in the right dendogram. The individual tumor samples represented in the top dendogram are color coded according to type 1 (*dark green*) or type 2 (*light green*) morphology. Shown are the color-coded ratios of chromosomal copy numbers to the underlying ploidy level, with *yellow* representing gains, *blue* representing losses, and the color intensity representing the magnitude of the deviation. *Black* indicates chromosomal balance. Each row represents the ratios for a separate chromosome arm over all tumor samples, and each column represents the ratios for all chromosome arms in a separate tumor.

between type 1 and type 2 papillary RCCs as regards the most frequent chromosomal changes +17, +7, +16, +20, +3q, and +12. Hierarchical clustering suggested two cytogenetic patterns that appeared to be common but not restricted to type 2 morphology. One was characterized by combined high-level gains (ratios $\geq$ 2) of various chromosomes, including those commonly gained as primary and secondary aberrations, and another by weakly correlated patterns of less common secondary chromosomal losses (ratios $<$ 1), including losses at 17p. More significantly, our series revealed increased numbers of chromosomal abnormalities in type 2 tumors. Aberrations that were statistically more common in tumors with type 2 than type 1 morphology tumors included losses of 8p, 11, and 18 and gains of 1q. Similar to what has been well established in other malignancies, accumulation of secondary chromosome abnormalities generally reflects genetic tumor progression. This is also consistent with earlier views that in addition to +7 and +17, further chromosomal polysomies accumulate during progression of small papillary tumors toward distinctly malignant papillary carcinomas (7, 8, 19). On the other hand, primary gains of chromosomes 7 and 17 may be lost again in the course of tumor progression as a result of increasing genetic instability. Thus, the present findings provide strong evidence to the concept that type 1 and type 2 papillary RCCs arise from common cytogenetic pathways and that type 1 tumors with fewer changes give rise to type 2 tumors with more aberrant karyotypes. The interpretational discrepancy between molecular genetic and cytogenetic data highlights the need for a multidisciplinary and comprehensive approach to assess the genetic evolution of papillary RCC.

As regards the prognostic value of cytogenetic changes, several secondary chromosomal abnormalities were found to be correlated with advanced disease. In particular, losses of 8p, 9p, and 11q were significantly associated with higher T-stage and higher clinical stage, loss of 8p with positive M-stage, and loss of 9p and gain of 3q with positive N-stage. Significant differences in patient outcome were observed for losses of 8p, 9p, and 18. Losses at chromosomes 8p and 18 have previously been implicated with advanced disease and higher grade in clear cell RCCs (3, 20, 21) and may represent new candidates for prognostic markers in papillary RCCs. Interestingly, the only chromosomal alteration that retained a statistical significant association with advanced disease when eliminating the influence of type was loss of 9p. This observation argues for a role of a tumor suppressor gene at 9p in the progression of papillary RCCs independently of type. Schraml *et al.* (22) have earlier shown allelic loss at the D9S171 locus on 9p13 in papillary RCCs to be associated with short patient survival independently of tumor grade and stage. This chromosomal locus is also subject to allelic losses in clear cell RCCs as determined by CGH, cytogenetic, or microsatellite analysis and has previously been linked to metastasis and tumor progression in clear cell RCCs (20, 23, 24). Future studies will eventually have to establish the prognostic use of 9p abnormalities in papillary RCCs.

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