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Prognostic Value of Cytogenetic Analysis in Clear Cell Renal Carcinoma: A Study on 131 Patients with Long-Term Follow-Up

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Abstract. *Background and Aims: cytogenetics analysis has a role in diagnosis of conventional renal cell carcinoma, but the role about prognosis is still matter of debate. This study reviews the authors' experience in CCRC cytogenetic analysis. Patients and Methods: Data from 131 patients with clear cell renal carcinoma who underwent cytogenetic analysis of the tumour karyotype at the host institute between 1997 and 2002 were prospectively collected. In all cases the cytogenetic analysis was carried out by a single experienced geneticist and the morphological features of the neoplasia were evaluated by a single experienced uropathologist. Results: Patients were followed for an average period of 67.3 months, median of 73 months, range 12-136 months, with a planned follow-up. The statistical association among chromosome alterations, clinico-pathological features and disease-free survival were investigated. At univariate analysis, symptoms at diagnosis, tumour diameter, Fuhrman's grading, TNM stage and sarcomatoid differentiation were all significantly correlated with survival, whereas among chromosomal abnormalities, deletion of chromosomes 19, 20 and 22 showed a significant impact on survival. At multivariate analysis of these factors, TNM stage and deletion of chromosome 19 maintained an independent and statistically significant association with disease-free survival. Conclusion: Although these results may be considered as preliminary, it is possible to conclude that the alterations of the tumour karyotype may contribute to determining CCRC prognosis.*

The current classification of parenchymal renal tumours was defined by the Heidelberg and Rochester's consensus conferences (1) and discriminates five histotypes: conventional, papillary, chromophobic, collecting ducts and unclassifiable. Among them, the conventional or clear cell renal carcinoma (CCRC) is the most frequent, accounting for 60-70% of all renal carcinomas. This classification is important because the definition of the tumour histotype is based on the integration between the microscopic morphological picture and the alteration pattern of the tumour karyotype at cytogenetic analysis. Hence, tumour karyotype analysis in renal neoplasia has a proven diagnostic role, while there is a lack of evidence regarding its role in the definition of the prognosis. This study reviews the authors' experience in CCRC cytogenetic analysis.

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Key Words: Conventional renal carcinoma, karyotype analysis, chromosome alterations.

Materials and Methods

In the host institute (University of Brescia, Italy) from 1997 to 2002 and for the purposes of a research project, cytogenetic analysis was performed in all patients who underwent surgery for renal tumour, for a total number of 283 cases. All cases were staged pre-operatively with abdominal computerised tomography (CT) or magnetic resonance imaging (MRI) and chest X-ray or CT; a brain CT and a bone scintigraphy were performed only in the cases with clinical evidence of advanced disease, locally or distant, or based on specific symptoms. Generally, a healthy contralateral kidney implied radical nephrectomy in case of neoplasias larger than 4 cm, centrally-localised, or with pre-operatory suspicion of advanced disease. Otherwise, nephron-sparing surgery was indicated for organ-confined neoplasias smaller than 4 cm. All histology samples were evaluated by a single experienced uropathologist (R.T.) and all karyotypes were obtained and evaluated by a single expert cytogeneticist (P.B.). Karyotypes were prepared from tumour specimens, minced in collagenase overnight. After five days in culture, the cells were harvested in conformity using a standard procedure described elsewhere (2). Chromosome preparations were G-banded and their karyotypes were expressed according to the International System for Human Cytogenetic Nomenclature (3). Twenty G-banded metaphases were analysed for each tumour. All patients were followed in a reserved follow-up outpatient unit, with blood and urine tests, abdominal ultrasound or CT and chest X-ray or CT, every six months in the first two years and then yearly for a prolonged period of time; in the case of nephron-sparing surgery,

Table I. *Patient characteristics*.

Characteristic	No. patients (%)
Asymptomatic diagnosis	80 pts (61.0%)
Side of the neoplasia	Right 65 pts (49.6%)
	Left 66 pts (51.4%)
Type of surgery	Nephrectomy 110 pts (84.0%)
	Nephron-sparing surgery 21 pts (16.0%)
Mean tumour diameter	5.65 cm (1-19 cm)
Invasion of perirenal tissues	30 pts (22.9%)
Invasion of adjacent organs	2 pts (1.5%)
Venous invasion	34 pts (26.0%)
Lymph node metastases	3 pts (2.3%)
Distant metastases	19 pts (14.5%)
TNM stage 1	68 pts (51.9%)
TNM stage 2	9 pts (6.9%)
TNM stage 3	35 pts (26.7%)
TNM stage 4	19 pts (14.5%)
Fuhrman's grading 1	5 pts (3.8%)
Fuhrman's grading 2	45 pts (34.4%)
Fuhrman's grading 3	53 pts (40.5%)
Fuhrman's grading 4	28 pts (21.4%)
Sarcomatoid differentiation	11 pts (8.4%)

Table II. *Frequencies of chromosomal alterations*.

Chromosomal alteration	No. patients (%)	Chromosome alteration	No. patients (%)
–1	11/131 (8,4)	–11	10/131 (7,6)
–1p	9/131 (6,9)	+12	16/131 (12,2)
+2	10/131 (7,6)	–13	14/131 (10,7)
–3	56/131 (42,7)	–14	29/131 (22,1)
–3p	41/131 (31,3)	+14q	7/131 (5,3)
–3q	12/131 (9,2)	–15	16/131 (12,2)
–4	13/131 (9.9)	–16	7/131 (5,3)
+4	8/131 (7,1)	+16	10/131 (7,6)
+5	14/131 (10,7)	–17	11/131 (8,4)
+5q	10/131 (7,6)	–18	18/131 (13,7)
–6	15/131 (11,5)	+19	9/131 (6,9)
+6q	8/131 (6,1)	–19	7/131 (5,3)
+7	23/131 (17,6)	+20	20/131 (15,3)
–7	8/131 (6,1)	–20	7/131 (5,3)
–8	18/131 (13,7)	–21	11/131 (8,4)
–9	19/131 (14,5)	–22	15/131 (11,2)
–10	12/131 (9,16)		

an additional abdominal CT was performed four months after the operation, aimed at ruling out any residual disease.

For this study, the clinical (age at diagnosis, gender, symptoms at diagnosis, side of the neoplasia), surgical (nephrectomy or conservative surgery), pathologic (tumour diameter, Fuhrman's grading (4), TNM 2002 stage, sarcomatoid differentiation) and follow-up (total follow-up time, disease-free survival, state of the patient at last available check) data were collected for non-familiar CCRC patients for whom the cytogenetic analysis of the tumour karyotype was available, thus ruling out the cases where the karyotype could not be evaluated due to a lack in the cell culture growth and those with normal karyotype (46 XX or XY). For each detected karyotype alteration, the distribution of the analysed pathologic factors was compared for the cases with or without the alteration. Survival analysis evaluated the impact of the pathological elements and the chromosome alterations on disease-free survival.

Statistical analysis. For the survival analysis, only deaths with CCRC listed as the underlying cause were considered as events. Disease-free survival was defined as the interval from the date of surgery to the first relapse, first metastasis, death, or the last follow-up visit. The log-rank test was used to compare survival distributions between subgroups. The prognostic impact of chromosome alterations, adjusted for the other prognostic factors, was assessed on multivariate analysis using the Cox proportional hazard regression model. For all tests two-tailed *p*-values were used, considered as statistically significant when lower than 0.05. The software SPSS for Windows (SPSS Inc Chicago- IL, USA) was used for all statistical calculations.

Results

The data of 131 (out of 283) patients were analysed (74 males, 57 females; mean age 62.9 years, range 27-85 years) with pathologic tumour karyotype, whose clinical, surgical and pathologic data are reported in Table I. Of the remaining 152 patients excluded from the analysis, there were 55 cases with normal karyotype (46 XX or XY), 36 with unavailable karyotype due to the lack of growth of cellular culture, 48 with non clear-cell histology and 13 with CCRC but with insufficient follow-up time. The 131 patients included into the study were followed after surgery for a mean period of 67.3 months (median 73 months, range 12-136 months, standard deviation 36 months). Nineteen patients (14.5%) had metastases at diagnosis, while disease progression was observed in 15 (13.3% of 112 M0) at a mean interval of 34.3 months from surgery (range 6-95 months). Twenty-two patients (17.9%) died because of the disease within 13.3 months after surgery.

The cytogenetic analysis highlighted a predominantly diploid tumour karyotype (74% of cases), with a mean number of 55.5 chromosomes (range 37-166) and a mean number of 5.7 chromosome alterations per patient (range 1-24). An involvement of chromosome 3 was observed in 75.6% of cases (99/131), as short arm deletion (-3p) in 41.1% of them. Among the remaining chromosomes, the most involved were (in decreasing order): Y (40.6% of males), 7 (29.8% of cases), 14 (25.2%), 6 (22.1%) and 20 (20.6%). Table II shows the chromosome alterations detected.

By comparing disease-free survival among the cases with a given chromosome alteration and those lacking it, a statically significant correlation was detected with deletion of chromosomes 19, 20 and 22, which highlighted a negative impact of these chromosomes on survival (Figure 1A, 1B

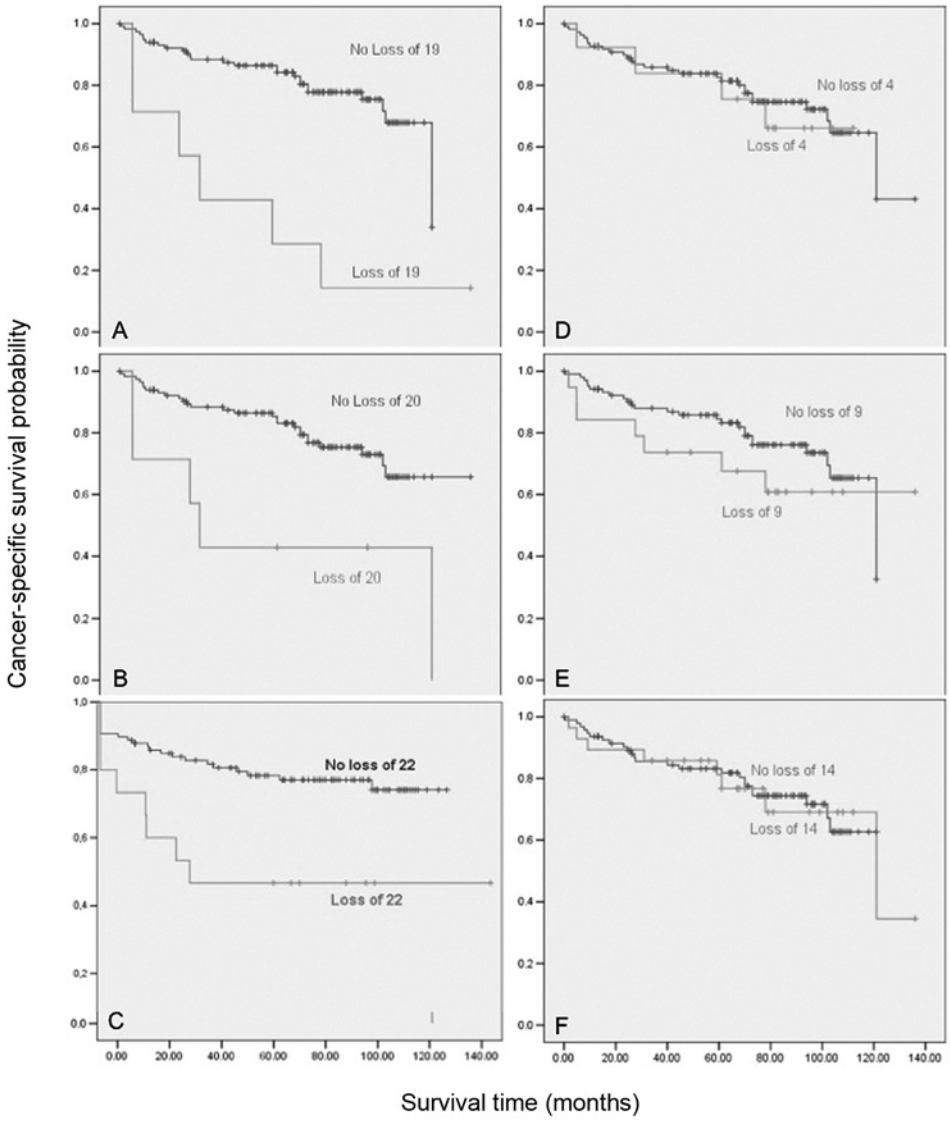

Figure 1. *Survival curves of patients with and without loss of genetic material on chromosomes 19 (A), 20 (B), 22 (C), 4 (D), 9 (E), 14 (F).*

and 1C). In contrast, all other analysed chromosome alterations had no significant impact on survival. Table III shows the results of survival analysis which estimates the impact of pathologic features and chromosome alterations on disease-free survival. At multivariate analysis, a high TNM stage (3 or 4) and the loss of genetic material of chromosome 19 were the only factors that confirmed an independent prognostic impact.

Discussion

The classification of the parenchymal renal neoplasia in Heidelberg and Rochester's consensus conferences (1) was a

Table III. *Uni- and multi-variate analyses of disease-free survival.*

	Univariate *p*-value	Multivariate *p*-value†
Incidental *vs*. symptomatic diagnosis	0.012	0.125
Diameter <5 cm *vs*. >5 cm	0.001	0.630
G1/G2 *vs*. G3/G4	<0.001	0.080
TNM stage 1-2 *vs*. 3-4	0.001	**0.004**
Sarcomatoid differentiation absent *vs*. present	<0.001	0.077
–19 absent *vs*. present	<0.001	**0.015**
–20 absent *vs*. present	0.006	0.530
–22 absent *vs*. present	0.007	0.883

†Multivariate *p*-values in plot denote statistical significance.

remarkable breakthrough, since it led to a definition of the tumour histotype which combined the morphological features and the tumour karyotype alteration profile. The classic cytogenetic analysis has therefore a well-established diagnostic role (5), which is relevant in clinical practice in combination with the microscopic evaluation, especially for cases where it cannot not be conclusive: a correct determination of the tumour histotype contributes to a correct assessment of the prognosis (6) and, in the case of metastasis, it influences the choice of the systemic therapy, moreover when it is a targeted therapy. The chromosome alterations which characterise the tumour histotype (7) are termed primary, since they would determine the first steps in cancer growth, while secondary alterations appear at a later stage and, thus, would regulate the neoplasia progression, which is still a rather unpredictable event for renal carcinoma, in spite of the many validated prognostic factors currently available (8). Hence, at least theoretically, knowing the profile of the chromosome alterations, given its extreme specificity in every single patient, would contribute to better prognosis of the disease. Some authors have highlighted a negative prognostic impact of chromosome alterations –8p, –9p and –14q, correlated to a more advanced staging, a higher grading and a lower global survival (9-15), while others have suggested the favourable prognostic role of chromosome alteration +5q (16). Nevertheless in the above mentioned studies there were some limitations regarding the retrospective design, the small number of cases or the short follow-up time. In addition, it should be noted that the tumour genome was more often analysed with comparative genomic hybridisation and fluorescence *in situ* hybridisation, which are faster and simpler since they do not require a culture of the tumour cells and may be applied on already included material; such methods do not allow, though, an overview of the entire karyotype, as classic cytogenetic analysis does, and may not detect some alterations since they only analyse a few portions of the tumour chromosomal pool selected in a pre-analytical phase.

Klatte *et al*. recently published the first study to prove the prognostic impact of some chromosomal alterations in CCRC patients by means of the classic cytogenetic analysis (17). The study revealed an unfavourable prognostic value for chromosome alterations –Y, –4p, –9p and –14q together with a favourable role for chromosome alteration –3p.

The present study prospectively evaluated a monocentric series of consecutive CCRC patients by means of the classic cytogenetic analysis, with a smaller number of cases than in the study by Klatte *et al*. (131 *vs*. 246), but followed up for a longer mean time period (67 *vs*. 25 months). Additionally, CCRC has its own typical chromosome alteration pattern, with high prevalence of chromosome 3 and the frequent involvement of chromosomes Y, 7, 14, 6 and 20, although chromosome +5q was also detected at a lower frequency (7.6%). At univariate evaluation, an impact on survival with statistical significance was detected for all the clinical-pathological factors considered, whereas among chromosome alterations, only the loss of chromosomes 19, 20 and 22 had a negative and significant impact on disease-free survival, in spite of their low incidence. At multivariate analysis, only TNM stage 3 or 4 and the loss of chromosome 19 confirmed an independent impact on survival. As opposed to Klatte *et al*. (17), neither any unfavourable impact on survival was detected for alterations in chromosomes 4 (alteration present *vs*. absent, log-rank test p=0.739, Figure 1D), 9 (p=0.312, Figure 1E) and 14 (p=0.878, Figure 1F) nor a favourable impact on survival was detected for alterations for chromosome 3 (p=0.146). This discordance is important, considering the strict overlapping in the design of the two studies. A possible explanation for such divergence may be given by interpreting the variability of the cytogenetic profiles and the different impact of the alterations as an evidence of the biological heterogeneity of CCRC. In any case, in the light of such results it is possible to speculate that in chromosomes 19, 20 and 22 there are some secondary alterations which lead to the mutation of the genes that foster CCRC progression. Actually in chromosome 22 (22q13.1), the *PDGF* beta gene may be found; its expression in CCRC is regulated by *HIF* alpha, which on turn is regulated by *VHL*. The PDGF beta receptor, together with the VEGF receptor, is one of the key-role tyrosine kinases in tumour neoangiogenesis processes (18), which are used by two common metastatic renal carcinoma targeted-therapies in the clinical setting, namely sunitinib and sorafenib (19). On chromosome 20 (20p13) it is possible to find the *FKBP12* gene which codes a protein to inhibit the *mTOR* activity and which, again, regulates the activity of *HIF* with an alternative pathway to that of *VHL* (20). *FKBP12* is the elective ligand of temsirolimus (21), another targeted therapy coded in the clinical setting for metastatic renal carcinoma. Finally, on chromosome 19 (19p13.3) there is the gene *ANGPTL4*, which, in the condition of intracellular hypoxia, codes a protein which favours endothelium cell apoptosis. This event may reduce the ability of some carcinomas to progress, CCRC being one of them, as has already been proven (22). Although exclusively in a speculative way, the above evidence puts a genetic basis to explain the outcomes of the present study, specifically how the involvement of chromosomes 19, 20 and 22 may affect CCRC progression. Anyway, it should be noted that the very low prevalence of cases with these alterations would render routine cytogenetic analysis for prognosis or guidance to targeted therapy out of a research context impractical.

In conclusion, by analysing a monocentric set of cases of CCRC patients followed for a long period, a possible prognostic value of cytogenetic analysis was observed, since alterations in chromosomes 19, 20 and 22 were associated with a significantly lower disease-free survival.

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