Urol Res (1995) 23:381–386

ORIGINAL PAPER

M. Kushima · R. Kushima · T. Hattori · T. Tomoyoshi

Heterogeneity and progression of renal cell carcinomas as revealed by DNA cytofluorometry and the significance of the presence of polyploid cells

Received: 6 March 1995 / Accepted: 18 May 1995

Abstract With special attention to the presence of polyploid cells, we examined heterogeneity and progression of renal cell carcinomas. We separated 116 blocks from 51 tumors into several specimens according to the histologic findings, and analyzed their DNA ploidy patterns. Thirty-one tumors (61%) were aneuploid, 29 of which showed intratumoral DNA ploidy heterogeneity. Diploid cell lines were observed in 47 tumors (92%). Polyploid cells in the diploid component were more frequently found in tumors with mixed diploid and aneuploid patterns than in the purely diploid tumors. Of the diploid cases, higher stage cases tended to have a higher incidence of polyploid cells than the lower stage cases. The incidence of aneuploid cases and DNA heterogeneity became greater as the tumors progressed. Aneuploid cases had a poorer outcome than did the diploid cases. If diploid cases with polyploid cells were classified as aneuploid cases, the difference in the survival rate between the diploid and aneuploid cases became more significant. Diploid renal cell carcinomas with polyploid cells may be an intermediate stage between diploidy and aneuploidy. Analyzing renal cell carcinomas for the presence of polyploid cells is useful for differentiating diploidy, which is actually aneuploid, from pure diploidy.

Key words Renal cell carcinoma · DNA ploidy · Polyploid cells · Heterogeneity · Progression · Survival rate

M. Kushima (✉) · T. Tomoyoshi
Department of Urology, Shiga University of Medical Science, Seta Ohtsu, Shiga 520-21, Japan
Fax: (+81) 775 48 2400

R. Kushima · T. Hattori
Department of Pathology, Shiga University of Medical Science, Seta Ohtsu, Shiga, Japan

Renal cell carcinoma is known to be an unpredictable malignancy, and metastasis can develop from early stage tumors. It is generally agreed that both histopathologic grading and clinical stage are good prognostic indicators [5, 10]. The relationship between outcome and nuclear DNA content has been examined using paraffin blocks. Some investigators reported that aneuploid carcinomas have a worse prognosis than diploid carcinomas [1, 3, 5, 6, 10, 14], but diploid carcinomas with a poor outcome have been observed frequently [4, 12], so this issue remains controversial. Intratumoral heterogeneity in DNA ploidy has been reported recently [2, 3, 12, 13, 15], and aneuploid cell populations have sometimes been found in predominantly diploid tumors when they were examined in detail. It has been reported that abnormal DNA content and heterogeneous populations begin to appear as tumors, reaching 2.0–5.0 cm in diameter, and that the occurrence of nondiploid stem lines and heterogeneous DNA content may parallel both tumor growth and more aggressive behavior [2]. Although in most of these studies the nuclear DNA content was measured using flow cytometry [3–6, 10, 13, 15], some studies utilized static cytofluorometry [1, 2, 12–14], which is useful for recognition of the diploid cell line of a tumor, especially for the detection of polyploid cells whose DNA content exceeds that of the cell cycle of the stem cell [11].

In the present study, we chose tumor tissues from as many sites as possible from each tumor and analyzed each with static cytofluorometry in order to address the issue of the heterogeneity in DNA ploidy in renal cell carcinomas. We determined the nuclear DNA content in detail as well as whether or not polyploid cells were present. It was also examined whether the nuclear DNA content was correlated with the histopathologic grading, tumor stage, size of tumor or outcome in patients with renal cell carcinomas. We also examined how the nuclear DNA content changes with tumor progression.

Materials and methods

Tumor samples

We examined 51 tumors obtained from 46 patients, including 1 with multiple tumors in both kidneys and another with multiple tumors in only 1 kidney, who were treated at the Hospital of Shiga University of Medical Science and at our affiliated hospitals from 1979 to 1991. A total of 116 blocks from 51 tumors were studied (1–7 blocks per tumor, mean 2.3 blocks).

Specimen preparation

Each tumor was fixed in 10% formalin, embedded in paraffin, and 5-μm and 100-μm serial sections were made alternately. The 5-μm sections were stained with hematoxylin and eosin (H&E) for histologic examination, and the 100-μm sections were used for cell isolation. The histopathologic grading (grade 1 ~ 3), histologic structure and histologic cell types of the specimens were reviewed and classified according to the "General rule for clinical and pathological studies on renal cell carcinoma" [9]. These three grades were defined as follows: grade 1: nuclei of tumor cells resemble those of normal tubular cells, and occasionally show pyknosis. Grade 2: nuclei of tumor cells are larger than those of grade 1, and are occasionally irregular or slightly pleomorphic. Nucleoli are often prominent. There are no bizarre nuclei. Grade 3: nuclei are more irregular and more pleomorphic than those of grade 2. Bizarre giant nuclei are often observed. The worst grade of each tumor component was taken as the histopathologic grading of the tumor.

Cell isolation [7]

The 100-μm sections were dewaxed and stained lightly with hematoxy for easy identification of the desired areas. They were resected by micromanipulation under a stereoscopic microscope, guided by observation of the adjacent 5-μm H&E sections under a low-powered microscope to dissect the material correctly. The tumor tissues were chosen according to the histologic findings, for a total of 169 sites (mean 3.3 sites for each tumor). After bleaching with 10 mM EDTA-2 Na, the dissected tissues were digested with 100 μg/ml proteinase K for 30 min at 37 °C, and homogenized to facilitate mechanical cell isolation. The supernatants of free-cell suspensions were transferred to other tubes and centrifuged for 5 min at 350 *g*. The cell pellets were rinsed twice with phosphate buffer, and the cell suspensions were smeared on non-fluorescent glass slides with an automatic smear maker (Auto Smear CF-120, Sakura Seiki, Tokyo, Japan).

Static cytofluorometry of the nuclear DNA content [7]

The smears were stained for 1 h at room temperature with 50 ng/ml DAPI (4′,6-diamidino-2-phenylindole dihydrochloride) in a solution containing 10 mM TRIS (pH 7.4), 10 mM EDTA-2 Na, 100 mM NaCl and 10 mM 2-mercaptoethylamine hydrochloride. Static cytofluorometry for nuclear DNA content was performed with an epi-illumination cytofluorometer (Nikon P1). We measured the fluorescence intensities of the nuclear DNA-DAPI complexes with a ×40 objective lens, and the nuclear fluorescence intensities of about 30 lymphocytes, plasma cells, neutrophils, endothelial cells and other stromal cells were measured to determine the internal standard of the 2C (diploid, G_0/G_1) DNA content in the specimens. The nuclear fluorescence intensities of 400 randomly chosen cancer cells were measured, and a histogram of the DNA distribution pattern was drawn for each specimen.

Examination of DNA ploidy patterns by static cytofluorometry [8, 11]

We defined DNA ploidy patterns as follows. (1) When only one peak of G_0/G_1 cells was evident in the histogram of the nuclear DNA content, this population was considered to have a single stem cell line. (2) If an additional peak, distinct from and greater than one-fifth of the largest peak, was observed, and a corresponding $S + G_2$ fraction was also seen, we assumed that the specimen had another stem cell line in addition to the largest peak. These stem lines were classified as diploid and aneuploid, and we classified the DNA ploidy pattern of each histogram accordingly. If the peak of the stem line was near 2C (2 ± 0.2C), we classified the stem line as diploid and the histogram that had a single peak of the diploid cell line was classified as having a diploid pattern. If the peak of a stem line was different from 2 ± 0.2C, we classified the stem line as aneuploid and the histogram that had more than one peak of the aneuploid cell line was defined as having an aneuploid pattern. Cells were defined as polyploid when the DNA content of the cells exceeded that of the G_2M-phase cells of the largest stem cell line (Fig. 1). If several stem cell lines within a tumor were present, we defined it as a heterogeneous tumor in terms of its nuclear DNA ploidy. Tumors that had more than one aneuploid cell line were classified as aneuploid tumors.

Statistical analysis

We used the TNM classification and stage classification of the Union Internationale Contre le Cancer (UICC) [16] for the clinical staging. The chi-square test and Fisher's exact probability test were used for statistical comparisons, while the survival ratios were calculated using the Kaplan-Meier method and the generalized Wilcoxon's method was used for statistical analysis. A P value of less than 0.05 was considered statistically significant.

Results

Using static cytofluorometry, we determined the ploidy patterns in 149 of 169 specimens (88%). The CV value of the G_0/G_1 peaks of the control cells was $8.6 + 3.5\%$. Eighty-one of the 149 specimens (54%) showed an aneuploid pattern. Comparing the ploidy patterns with the histopathologic grading, aneuploid patterns were seen more frequently in grade 2 specimens than in grade 1 specimens. The ploidy pattern of the grade 3 specimens could not be determined because of their small sample number . However, comparing the presence of polyploid cells with histopathologic grading in diploid specimens, all grade 3 specimens had some polyploid cells (six out of six specimens) (Table 1).

Twenty of 51 tumors (39%) were diploid tumors with no other stem cell lines present. Thirty-one tumors (61%) exhibited an aneuploid pattern with more than one aneuploid stem cell line present (Table 2). Twenty-nine tumors (57%) had intratumoral DNA ploidy heterogeneity, of which 27 tumors had a diploid cell line. A diploid cell line was observed in 27 of the 31 aneuploid tumors (87%), and thus a total of 47 of 51 tumors (92%) had a diploid cell line.

Concerning the relationship between the histopathologic grading and the DNA ploidy patterns, the

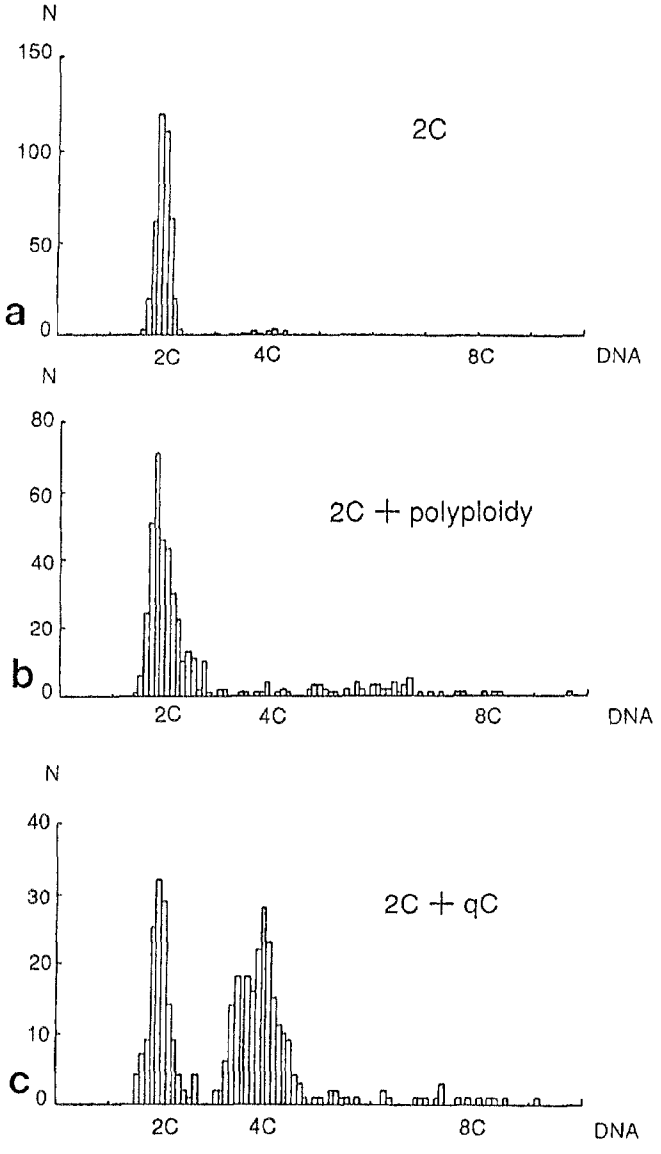


Fig. 1a–c DNA histograms by static cytofluorometry: **a** diploid pattern, **b** predominantly diploid with polyploid cells present, **c** aneuploid pattern

incidence of an aneuploid pattern was significantly higher in the higher grade tumors. This was especially true for the grade 3 tumors; seven of eight tumors (88%) had an aneuploid pattern. The incidence of intratumoral DNA ploidy heterogeneity became greater as the histopathologic grading increased. In 20 diploid tumors, we divided the tumors according to the incidence of polyploid cells, which we compared with the histopathologic grading. No grade 1 tumor had any polyploid cells, which were more frequently observed in the higher grade tumors, and one grade 3 tumor also had polyploid cells (Table 3).

Classifying the tumors by the maximum diameter in 25-mm intervals, the incidence of aneuploidy or heterogeneity in DNA ploidy was more frequent as the tumor

Table 1 DNA ploidy pattern and presence of polyploid cells within predominantly diploid specimens according to grading in 149 specimens

Grade	Diploid		Aneuploid (%)	Total
	Polyploid cells			
	(−) (%)	(+) (%)		
G1	33(55)	4(7) *	23(38) ***	60
G2	16(20)	9(11) ****	54(68) ***	79
G3	0	6(60) **	4(40)	10
Total	49(33)	19(13)	81(54)	149

*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.0005$, ****: $P < 0.001$

Table 2 DNA ploidy patterns obtained from the 51 tumors

Histogram	Number of tumors (%)
Homogenous tumor	
diploidy	20(39)
aneuploidy	2
Total	22(43)
Heterogenous tumor	
2 aneuploidy	2
diploidy + 1 aneuploidy	16
diploidy + 2 aneuploidy	8
diploidy + 3 aneuploidy	2
diploidy + 5 aneuploidy	1
Total	29(57)

Table 3 DNA ploidy pattern, presence of ploypoid cells in the diploid tumors, and tumor heterogeneity of the DNA content according to grading in the 51 tumors

Grade	Diploid		Aneuploid (%)	Total	Heterogeneity (%)
	Polyploid cells				
	(−) (%)	(+) (%)			
G1	8(73)	0	3(27) *	11	3(27)
G2	9(28)	2(6)	21(66) *	32	19(59) *
G3	0	1(12)	7(88)	8	7(88)
Total	17(33)	3(6)	31(61)	51	29(57)

*: $P < 0.05$

diameter increased. All of the tumors larger than 76 mm were aneuploid tumors, and all the tumors larger than 101 mm showed heterogeneity (Table 4). In the purely diploid tumors, only 3 of 20 tumors (15%) had polyploid cells, whereas 9 of 16 tumors (56%) with both diploid and aneuploid components harbored polyploid cells in the diploid component (Table 5).

We compared the TNM classification and staging with the DNA ploidy patterns of the 46 cases (Table 6).

Table 4 Size, DNA ploidy pattern and tumor heterogeneity of the DNA content in 48 tumors

Size (mm)	Diploid (%)	Aneuploid (%)	Total	Heterogeneity (%)
1 ~ 25	7(70)	3(30)	10	3(30)
26 ~ 50	9(50)	9(50)	18	8(44)
51 ~ 75	2(29)	5(71)	7	5(71)
76 ~ 100	0	7(100)	7	6(86)
101 ~	0	6(100)	6	6(100)
Total	18(37)	30(63)	48	28(58)

*: $P < 0.05$, **: $P < 0.01$

Table 5 DNA ploidy pattern of the tumors and the presence of polyploid cells within the diploid component of the tumors

DNA ploidy pattern	Polyploid cells		Total
	(−) (%)	(+) (%)	
Diploidy	17(85)	3(15)	20
Diploidy + Aneuploidy	7(44)	9(56)	16 ($P < 0.01$)

The incidence of aneuploidy was higher in the higher pT or M cases and in those with more advanced stage disease. The incidence of heterogeneity in DNA ploidy was also higher in the higher pT or M cases and in those with more advanced stage disease. Concerning the presence of polyploid cells in the diploid cases in relation to the staging, diploid cases from patients with more advanced stage disease tended to have polyploid cells more frequently than those from patients with earlier stage disease (Table 7).

Of the 42 cases for which the survival data were available, the survival rate was significantly lower in those with the higher grade disease (between grade 1 and 2: $P < 0.05$; between grade 1 and 3: $P < 0.01$). It also was lower in those with a higher pT. A significant difference was noted between those with pT2 and pT3 disease ($P < 0.005$), and the survival rate was significantly lower in those with higher stage disease

Table 7 Stage and presence of polyploid cells in the diploid cases

Stage	Polyploid cells		Total
	(−) (%)	(+) (%)	
Stage 1	4(80)	1(20)	5
Stage 2	8(100)	0	8
Stage 3	2(67)	1(33)	3
Stage 4	1(50)	1(50)	2

($P < 0.00005$ between stage 2 and stage 4; $P < 0.005$ between stage 3 and stage 4). The survival rate of the patients with tumors larger than 51 mm was significantly lower than that of those with smaller tumors ($P < 0.01$).

The survival rate was significantly lower in the aneuploid cases than in the diploid cases ($P < 0.05$). When the diploid cases with polyploid cells were included with the aneuploid cases, a more significant difference was found ($P < 0.005$) (Fig. 2). Comparing the survival rate with the DNA ploidy patterns for the grade 2 cases that were the largest in number, significantly higher survival rates were seen in the purely diploid cases than in the aneuploid cases or in the diploid cases with polyploid cells ($P < 0.05$), whereas there was no significant difference in the survival rate between the aneuploid cases and the diploid cases with or without polyploid cells (Fig. 3). Comparing the survival rate with the DNA ploidy patterns for the stage 2 cases that were largest in number, the survival rate was significantly higher in the diploid cases than in the aneuploid cases ($P < 0.001$).

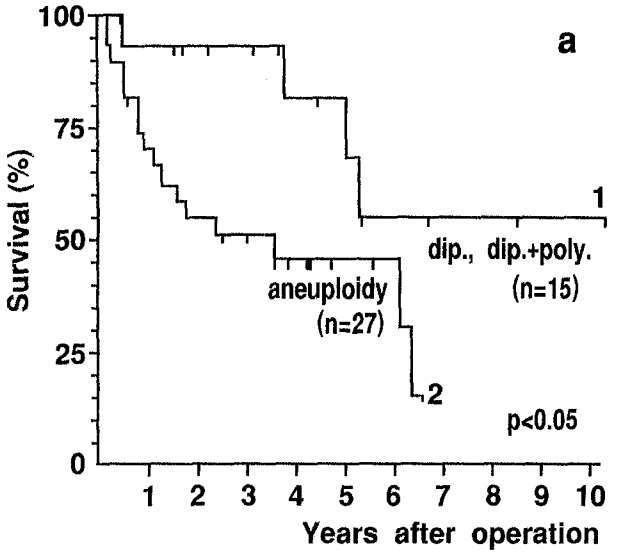
Discussion

Recently, many studies on the nuclear DNA content of various human tumors, including renal cell carcinomas, embedded in paraffin blocks have been reported using flow cytometry (FCM) [3–6, 10, 13, 15] and static

Table 6 PT, M, stage and DNA ploidy pattern, and tumor heterogeneity in the DNA content in 46 cases

	Diploid (%)	Aneuploid (%)	Total	Heterogeneity (%)
pT1	5(83)	1(17)	6	1(17)
pT2	9(43)	12(57)	21	10(48)
pT3	4(22)	14(78)	18	14(78)
pT4	0	1(100)	1	1(100)
M0	17(49)	18(51)	35	16(46)
M1	1(9)	10(91)	11	10(91)
Stage 1	5(83)	1(17)	6	1(17)
Stage 2	8(44)	10(56)	18	8(44)
Stage 3	3(33)	6(67)	9	6(67)
Satge 4	2(15)	11(85)	13	11(85)
Total	18(39)	28(61)	46	26(57)

*: $P < 0.05$, **: $P < 0.01$



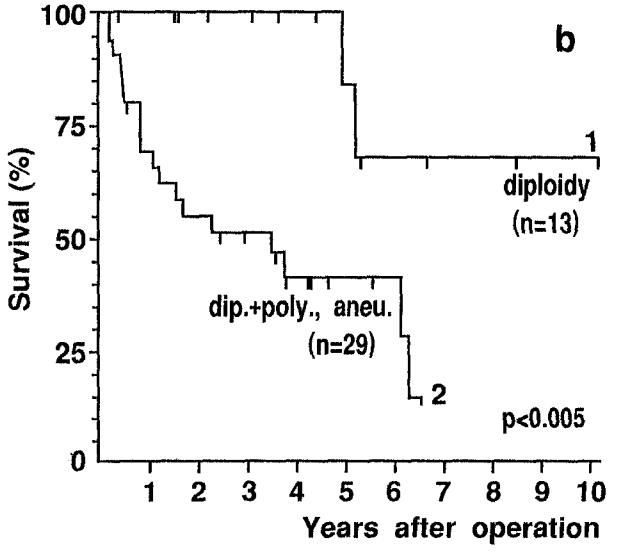


Fig. 2a, b Survival curves according to DNA ploidy: **a** *1* diploidy and diploidy + polyploidy ($n = 15$), *2* aneuploidy ($n = 27$) ($P < 0.05$); **b** *1* diploidy ($n = 13$), *2* diploidy + polyploidy and aneuploidy ($n = 29$) ($P < 0.005$)

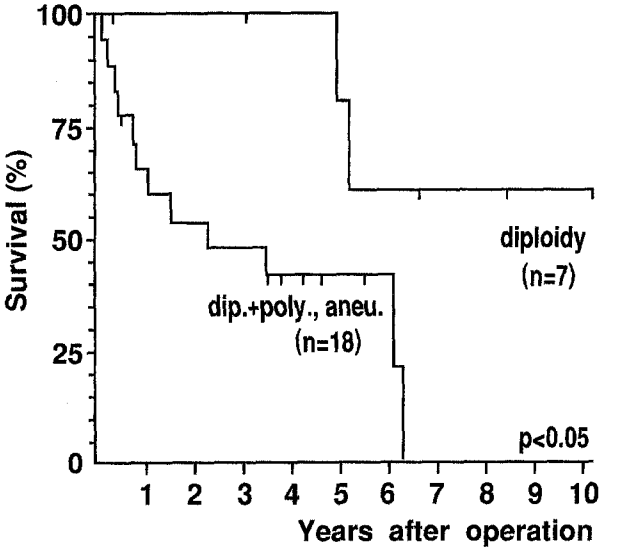


Fig. 3 Survival curves of the grade 2 cases according to DNA ploidy

cytofluorometry [1, 2, 12–14]. Some investigators reported that aneuploid renal cell carcinomas show high histologic grading [5, 6] and higher stage disease and have a poorer prognosis [1, 5, 10, 14], whereas others reported that there was no correlation between the nuclear DNA content and the clinical prognosis [4, 12]. In these studies, various methods were used for the measurement of the nuclear DNA content, the sampling of the specimens or the assessment of the DNA ploidy patterns. Many investigations have used FCM. In a previous study, we measured the DNA content by FCM and static cytofluorometry in the same material, and reported that the flow cytometric study appeared to have limited ability to detect the diploid cell line of a tumor and to detect the presence of polyploid cells that constitute much smaller populations than the stem cell line [11]. The DNA ploidy patterns have usually been classified into diploidy and aneuploidy (nondiploidy), or occasionally tetraploidy. Yoshida [17] reported that most nephroblastomas show diploidy, and that the outcome was poor in diploid cases. He suggested that subtle derangements of cell-cycle regulation could cause very rapid growth of these cells, resulting in a fatal outcome for the patient, indicating that the diploid cells of tumors must be different from normal diploid cells in their biologic behavior. For this kind of study, not only the heterogeneity in DNA ploidy but also the presence of polyploid cells must be considered. In this study, in order to examine the diploid patterns in detail, we carefully screened for the presence of polyploid cells using static cytofluorometry.

The present study found aneuploid cell lines in 61% of tumors. This is similar to values of 41–65% reported by other investigators. Since Lanigan et al. [12], who reported the incidence of aneuploid tumors to be 41%, examined multiple blocks from each tumor in only 14 of 90 tumors, it is possible that he missed some aneuploid cell lines. Heterogeneity in the DNA ploidy patterns was observed in 57% of tumors in this study. Ljungberg et al. [15] measured eight specimens from each tumor and observed the heterogeneity in the nuclear DNA content in 45% of tumors by FCM for fresh specimens. Currin et al. [3] also reported that 29% of tumors showed heterogeneity when they analyzed two or more specimens from paraffin-embedded tissues.

Diploid cell lines were observed in 93% of tumors showing heterogeneity in DNA ploidy, and adding

purely diploid tumors, 92% of all tumors demonstrated diploid cell lines. Ljungberg et al. [15] reported diploid cell lines in 85% of tumors showing heterogeneity, and of all tumors 80% had diploid cell lines. Renal cell carcinoma is characterized as a tumor that contains a diploid cell line very frequently. The presence of polyploid cells in the diploid component was more frequent in tumors with mixed diploid and aneuploid patterns than in the purely diploid tumors. In the predominantly diploid cases, higher stage cases tended to have polyploid cells more frequently than the lower stage cases. The incidence of aneuploid cases and heterogeneity in DNA ploidy increased with the progression of tumors. This suggests that, if the cancer can be regarded as monoclonal in origin, most renal cell carcinomas begin as diploid tumors, then polyploid cells develop within the diploid population, and eventually aneuploid cell lines arise from the polyploid cells as the tumors progress. Banner et al. [2] reported that abnormal DNA content and heterogeneous populations began to appear in tumors from 2.0 to 5.0 cm in diameter. In this study, aneuploid cell lines were observed in three tumors smaller than 2.5 cm. A small aneuploid tumor of 1.0 cm was seen in the patient with multiple bilateral kidney tumors, so it is possible that the small aneuploid tumor was a metastasis, and it might have been aneuploid originally.

As the histopathologic grading progressed, not only the incidence of aneuploid cell lines became higher, but also the incidence of polyploid cells in the diploidy became higher. The nuclear DNA content was correlated with the histopathologic grading and it may be correlated with the biologic behavior of the tumor cells.

The nuclear DNA content is a useful index for the prognosis of patients with renal cell carcinomas as is the histopathologic grading, TNM classification and clinical stage. Aneuploid cases have a poorer prognosis than do the diploid cases. Grouping the diploid cases with polyploid cells into the aneuploid cases, a more significant difference in the survival rates could be detected between the diploid and aneuploid cases. In this classification, the nuclear DNA content yields more detailed information for grade 2 renal cell carcinomas, which are the most common forms. From this point of view, diploid cases with polyploid cells should actually be regarded as aneuploid cases. Detailed analysis of the nuclear DNA content may add more useful information for follow-up studies or for the consideration of adjuvant therapies.

In conclusion, using the advantage of static cytofluorometry, we examined the DNA ploidy patterns of renal cell carcinomas and carefully inspected for the presence of polyploid cells. Most renal cell carcinomas appear to begin as diploid tumors. As tumors progress, some polyploid cells may appear within the diploid component and then some aneuploid stem lines may arise from the polyploid cells. The outcome of patients with aneuploid tumors or diploid tumors with polyploid cells was poor, suggesting that the diploid predominant tumors with polyploid cells should be treated differently from the purely diploid tumors without polyploid cells.

Acknowledgements The authors wish to thank Dr. T. Konishi of the Department of Urology and Dr. S. Hamada of the Department of Pathology, Shiga University of Medical Science, for their encouragement during the course of this study.

References

1. Al-Abadi H, Nagel R (1988) Prognostic relevance of ploidy and proliferative activity of renal cell carcinoma. Eur Urol 15:271
2. Banner BF, Brancazio L, Bahnson RR, Ernstoff MS, Taylor SR (1990) DNA analysis of multiple synchronous renal cell carcinomas. Cancer 66:2180
3. Currin SM, Lee SE, Walther PJ (1990) Flow cytometric assessment of deoxyribonucleic acid content in renal adenocarcinoma: does ploidy status enhance prognostic stratification over stage alone? J Urol 143:458
4. Ekfors TO, Lipasti J, Nurmi MJ, Eerola E (1987) Flow cytometric analysis of the DNA profile of renal cell carcinoma. Pathol Res Pract 182:58
5. Grignon DJ, Ayala AG, El-Naggar A, Wishnow KI, Ro JY, Swanson DA, McLemore D, Giacco GG, Guinee VF (1989) Renal cell carcinoma. A clinicopathologic and DNA flow cytometric analysis of 103 cases. Cancer 64:2133
6. Grignon DJ, El-Naggar A, Green LK, Ayala AG, Ro JY, Swanson DA, Troncoso P, McLemore D, Giacco GG, Guinee VF (1989) DNA flow cytometry as a predictor of outcome of stage 1 renal cell carcinoma. Cancer 63:1161
7. Hamada S, Itoh R, Fujita S (1988) DNA distribution pattern of the so-called severe dysplasia and small carcinomas of the colon and rectum and its possible significance in the tumor progression. Cancer 61:1555
8. Hamada S, Namura K, Fujita S, Kushima R, Hattori T (1990) DNA ploidy and proliferative activity of human pulmonary epithelium. Virchows Arch [B] Cell Pathol 58:405
9. Japanese Urological Association, The Japanese Society of Pathology, Japan Radiological Society (1992) General rule for clinical and pathological studies on renal cell carcinoma, 2nd edn. Tokyo, Kanehara, p 82
10. Kloppel G, Knofel WT, Baish H, Otto U (1986) Prognosis of renal cell carcinoma related to nuclear grade, DNA content and Robson stage. Eur Urol 12:426
11. Kushima M, Konishi T, Okada Y, Tomoyoshi T, Kushima R, Hattori T (1994) Tumor heterogeneity in DNA ploidy of renal cell carcinomas as revealed by static cytofluorometry and flow cytometry. Jpn J Urol 85:473
12. Lanigan DJ, McLean PA, Murphy DM, Donovan MG, Leader M (1992) Image analysis in the determination of ploidy and prognosis in renal cell carcinoma. Eur Urol 22:228
13. Ljungberg B, Stenling R, Roos G (1985) DNA content in renal cell carcinoma with reference to tumor heterogeneity. Cancer 56:503
14. Ljungberg B, Forsslund G, Stenling R, Zetterberg A (1986) Prognostic significance of the DNA content in renal cell carcinoma. J Urol 135:422
15. Ljungberg B, Stenling R, Ross G (1987) Flow cytometric DNA analysis of renal-cell carcinoma. A study of fine needle aspiration biopsies in comparison with multiple surgical samples. Anal Quant Cytol Histol 9:505
16. UICC International Union Against Cancer (1979) TNM classification of malignant tumours. 4th edn. Springer, Berlin Heidelberg New York, p 136
17. Yoshida S (1992) DNA value and prognosis of renal tumors differences between childhood and adult tumors. Acta Pathol Jpn 42:185