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# Different DNA ploidy patterns for the differentiation of common subtypes of renal tumors

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**Abstract.** *Objectives*: The common subtypes of renal tumors are conventional or clear cell carcinoma, papillary carcinoma, chromophobe carcinoma and oncocytoma. Each subtype has its distinct histogenesis and clinical evolution. DNA ploidy is viewed as a marker of gross genomic aberrations. The aim of this study is to evaluate the DNA ploidy in the common subtypes of renal tumors to increase our understanding of renal tumor biology and to broaden clinical application of DNA ploidy. *Methods*: 38 renal tumor samples (13 clear cell RCCs, 12 papillary RCCs, 7 chromophobe RCCs, and 6 oncocytomas) were studied. Five biopsies of different parts of each fresh tumor were subjected to a flow cytometric analysis of DNA ploidy. *Results*: All tumors except one papillary RCC generated interpretable DNA histograms. Flow cytometric analysis of oncocytomas showed the diploid pattern (29/30 frequencies) while the chromophobe RCC never showed the diploid pattern (0/55 frequencies) ($p < 0.01$). 3/7 chromopbobe RCCs possessed the hypodiploid stemline. The hypodiploid stemline appeared neither in conventional RCCs (0/63 frequencies) nor in papillary RCCs (0/50 frequencies). The diploid pattern was dominant in conventional and papillary RCCs. 10/13 (76.9%) of clear cell RCCs and 9/11 (81.8%) of papillary RCCs possessed a homogeneous DNA ploidy pattern while only 1/7 (14.3%) has a homogeneous DNA ploidy pattern. 6/7 chromophobe RCCs had multiple aneuploid stemlines. *Conclusions*: Flow cytometric analysis reveals that conventional and papillary RCCs are more homogeneous than chromophobe RCC. Each subtype of renal tumors possesses a specific DNA ploidy pattern. The analysis of DNA ploidy is useful for the differentiation of common subtypes of renal tumors in morphologically difficult cases.

Keywords: DNA ploidy, renal tumor, differentiation

## 1. Introduction

Renal cell carcinoma (RCC) is the most frequent tumor of adult kidney. RCC has no longer been considered as a unique disease. New classification of histogenetic subtypes has been introduced in epithelial renal tumors according to specific chromosome alterations: conventional (clear cell) carcinoma, papillary carcinoma, chromophobe carcinoma, benign oncocytoma, etc. [5,25,33]. Recent data emphasize the differential diagnosis because the clinical course of each subtype is different [6]. Currently, the standard diagnosis of RCC remains in post-operative morphological study under microscope. However, the morphological and cytological features of different subtypes may be similar as granular/eosinophilic cells may occur in conventional carcinoma, papillary, chromophobe and oncocytoma [19,33].

Cancer is regarded as a genetic disease. Its development and progression are characterized by the various genetic changes. Recent studies have demonstrated gene expression profiling in RCC. Each subtype of RCC possesses its distinct pattern of gene expression [30,31]. Another common change occured in RCC is the shift in DNA ploidy, which is considered as a mark of gross genomic aberrations [26]. DNA ploidy study yields information about biological abnormal-

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ities of tumor evolution as well as provides a more objective assessment than conventional histopathology alone [4,22,27]. The pattern of DNA ploidy was reported to correlate with the histogenetic subtypes in some kind of tumors [13].

There has been considerable interests in the application of adjunctive techniques, such as immunohistochemistry, to the differential diagnosis of renal tumors [32]. New techniques such as FISH or CGH have been explored to differentially diagnose RCC by detecting chromosome aberrations [15,29]. In this study, we have used the flow cytometry for detecting the DNA ploidy in the common subtypes of renal tumors. The aim of this study was not only to increase our understanding of the biological evolution of renal tumors but also to broaden the clinical application of DNA ploidy study.

## 2. Materials and methods

### *2.1. Pathological diagnosis*

The tumor was pathologically diagnosed as conventional or clear cell carcinoma, papillary carcinoma, chromophobe carcinoma and oncocytoma according to the UICC/AJCC guidelines [28]. 38 renal tumor samples (13 clear cell RCCs, 12 papillary RCCs, 7 chromophobe RCCs, and 6 oncocytomas) were included.

### *2.2. Tumor tissue samples*

5 pieces of typical tumor from different areas were obtained immediately after the kidney was taken out. The renal tumor samples were put into RPMI 1640 solution for DNA ploidy analysis. These tumor biopsies were performed by a pathologist in order to assure that the tumor piece was consisted mainly of tumor cells. The tumor was consecutively enrolled. This research protocol was approved by a local research committee. The tumor was graded according to the Fuhrman criteria and staged according to 1997 TMN system [9,28].

### *2.3. DNA ploidy analysis*

Flow cytometric analysis of DNA ploidy was performed by a flow cytometric specialist. We used the method of technical control as we previously described with some improvements [7,18]. Briefly, the tumor tissue was mechanically dissociated to obtain a final suspension of an approximate concentration of $10^6$ nuclei/ml. The cell suspension was stained with propidium iodide. A suspension of human lymphocytes of the same gender were used as external and internal diploid reference. Flow cytometric analysis was performed with a Diva cell sorter from BD Bioscience (Mountain View, CA), equipped with an enterprise laser from Coherent (Palo Alto, CA), emitting 120 mW at 488 nm. Computer analysis of DNA histogram was done by using ModFit 3.1, a multiple option cell cycle fitting that determines the DNA index (DI) and cell cycle fractions in cell populations. The DI of a sample was calculated as the ratio of the modal channel number of diploid reference peak and the modal channel number of a peak of the sample. For the accuracy of DNA measurement, the coefficient of variation was calculated for each DNA histogram. The coefficient of variation was always less than 5%. Diploid reference, human lymphocytes, was characterized by a DI equal to $1.00 \pm 0.1$. Each aneuploid peak present in a sample was characterized by a DI value. Samples that had at least one G0/G1 peak with a DI inferior or superior to $1.00 \pm 0.1$ were defined as DNA aneuploidy.

Five samples were taken from each tumor to perform DNA flow cytometry. A tumor's ploidy status was based on the analysis of all samples [1,20,24]. A tumor was considered as diploid if all analyzed samples had a normal DNA content and aneuploid if at least one sample was aneuploid. A tumor was considered homogeneous only if all analyzed samples had the same ploidy DI. A tumor was regarded as heterogeneous when both diploid and aneuploid samples or at least two different aneuploid peaks were found in the same tumor regardless of whether they were within the same sample or in different samples. Multiploid tumors were defined by the presence of more than one aneuploid peak in at least one sample.

### *2.4. Statistics*

The frequency of ploidy pattern between the chromophobe RCCs and oncocytomas was compared with the $\chi^2$ test.

## 3. Results

There were 32 renal carcinomas. Their grade and stage were as follows: 6 grade I, 18 grade II, 7 grade III and 1 grade IV; 8 pT1a, 6 pT1b, 6 T2, 11 pT3a and 1 pT3b. There were four patients with metastasis (M+ or N+).

Table 1
DNA index distribution in subtypes of renal tumors

| | Hypodiploid DI $< 1.0$ | Diploid DI $= 1.0$ | Aneuploid DI $> 1.0$ |
|---|---|---|---|
| Conventional | | | |
| $N = 13$ | 0/13 | 7/13 | 6/13 |
| (63 DIs) | 0/63 | 32/63 | 31/63 |
| Papillary | | | |
| $N = 11$ | 0/11 | 6/11 | 5/11 |
| (50 DIs) | 0/50 | 33/50 | 27/50 |
| Chromophobe | | | |
| $N = 7$ | 3/7 | 0/7 | 6/7 |
| (55 DIs) | 14/55 | 0/55 | 41/55 |
| Oncocytoma | | | |
| $N = 6$ | 0/6 | 5/6 | 1/6 |
| (30 DIs) | 0/30 | 29/30 | 1/30 |

All tumors except one papillary RCC generated interpretable DNA histograms. The pattern of DNA ploidy was summerized in Table 1. The representative DNA histograms are shown in Fig. 1. Flow cytometric analysis was successful in all biopsies from the chromophobe RCCs (35 biopsies) and oncocytomas (30 biopsies). Three chromophobe RCCs showed multiple ploidy stemlines, resulting 55 numbers of DIs. 3/7 chromopbobe RCCs possessed the hypodiploid stemline. Flow cytometric analysis of oncocytoma showed the diploid pattern (29/30 frequencies) while the chromophobe RCC never showed the diploid pattern (0/55 frequencies) ($p < 0.01$). 63 of 65 biopsies were interpretable for the conventional RCCs and 50 of 60 biopsies were interpretable for the papillary RCCs. The diploid pattern was dominant in the conventional and papillary RCCs.

10/13 (76.9%) of clear cell RCCs and 9/11 (81.8%) of papillary RCCs possessed a homogeneous DNA ploidy pattern while only 1/7 (14.3%) has a homogeneous DNA ploidy pattern. Multiple aneuploid stemline was observed neither in the clear cell RCC nor in the papillary RCC. Among the seven chromophobe RCC, three had two aneuploid stemlines and the other three had three aneuploid stemlines.

## 4. Discussion

The common subtypes of renal tumors are conventional or clear cell RCC, papillary RCC, chromophobe RCC and oncocytoma. The clear cell RCC is the most common and the most aggressive. The treatment methods will be tailored according to each subtype. A radical nephrectomy should be performed to the clear cell carcinoma while a conservative surgery or watchful waiting should be applied to the benign oncocytoma. It is evident that the differentiation of each subtype is clinically meaningful.

The cytogenetic features of each subtype are well documented. For example, the loss of 3p appears in clear cell RCC while trisomies of 7, 12, 16, 17 and 20 and the loss of the Y chromosome occur in papillary RCC. Recently, the analysis of gene expression profiling was performed in order to find new molecular markers that can potentially be used for more accurate diagnosis and prognosis prediction [30,31]. DNA ploidy, viewed as a marker of gross genomic aberrations, is largely studied in RCC for its prognostic value. However, this marker for the differentiation of common subtypes of renal tumors has not been fully documented.

Our results demonstrated the similar DNA ploidy pattern between the clear cell RCC and papillary RCC. In these two types of tumors, the homogeneous DNA ploidy pattern is dominant. We believe that a single stemline is a crucial mechanism for the tumor progression in these two types of tumors. In fact, most RCCs show only simple chromosomal changes. This was reflected by relative simple karyotypes with little cytogenetic intratumor heterogeneity [11]. On the contrary, about half of chromophobe RCC possessed hypodipolid stemline and 6/7 chromophobe RCCs had multiple aneuploid stemlines. This may suggest that multiple stemlines are an important event during the progression of chromophobe RCC. Thus, Flow cytometric analysis reveals that conventional and papillary RCCs are more homogeneous than chromophobe RCC. Based on SKY and CGH data, Alimov et al. also found that the clear cell RCCs are genetically more homogeneous than the other types of kidney cancer [3]. In our previous study, we demonstrated that clear cell RCC acquired DNA aneuploidization mainly in large tumors [18]. Considering that normal cells are diploid and that diploid stemlines may evolve into aneuploid stemlines, the appearance of aneuploid stemlines may be a late event in clear cell and papillary RCCs. The DNA aneuploidy has been suggested to predict poor survival in RCC [1], suggesting that some tumors may acquire a more aggressive phenotype through the subsequent evolution of gross genomic aberrations. Since the diploidy was never detected in chromophobe RCC, the gross genomic aberrations appeared early during the tumor development and progression of chromophobe RCC. A recent study in thymic tumors also

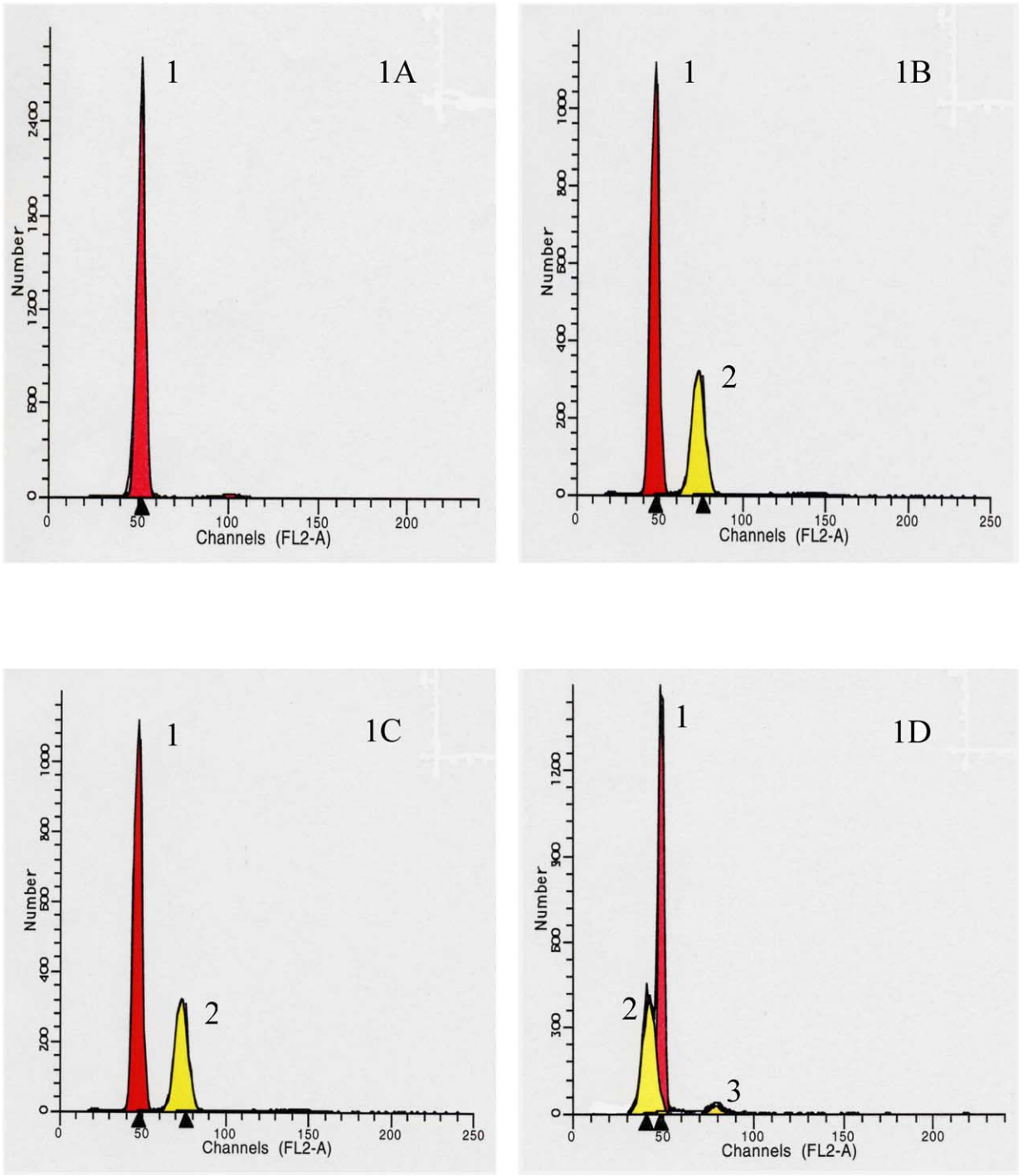


Fig. 1. Examples of DNA content in renal tumors. The vertical axis indicates the number of nuclei analyzed; the horizontal axis indicates the DNA fluorescence. Peak 1 is the diploid reference of mixture of human lmyphocytes. 1A is an oncocytoma: DNA index = 1, CV = 3.3%. 1B is a clear cell RCC: DNA index of the peak 2 = 1.55, CV = 3.2%. 1C is a papillary RCC: DNA index of the peak 2 = 1.56, CV = 5.0%. 1D is a chromophobe RCC: DNA index of peak 2 = 0.84, CV of peak 2 = 4.8%; DNA index of peak 3 = 1.73, CV of peak 3 = 5%.

demonstrated the ploidy analysis correlated with the histogenetic subtypes [13]. The DNA ploidy pattern also supports the concept of histological subtypes of renal tumors. The comparison of DNA ploidy of different subtype of renal tumors revealed the different evolution of gross genomic aberration. The gross genomic aberrations as detected by flow cytometry are rare in oncocytoma.

The majority of renal tumors can be correctly diagnosed by routine pathology. However, the similarity may cause the difficulty in some cases. The major difficulty for the differentiation of renal tumors may lie in the granular/eosinophilic variant of chromophobe RCC and oncocytoma [19,33]. So far, there have been no powerful marker for this differentiation. Oncocytoma is considered as a benign tumor. Our results show that the majority of oncocytomas are diploid tumors, which supports the benign feature of this tumor. This result agreed with the previous reports [14,23]. We have never found a diploid sample in the chromophobe RCC, which is similar with a recent study [2]. Based on these results, we believe that the measurement of ploidy status provides a powerful means of distinguishing the benign oncocytoma from the chromophobe RCC.

Recently, there is a tendency to develop techniques for robust and objective classification of renal tumors [10,12,16,17,21]. Such techniques include FISH, CGH, etc. The techniques of flow cytometry for DNA ploidy has been well developed for the utilization in surgical pathology of solid tumors [4,8]. The technique is comparable with the immunohistochemistry in term of cost, labor intensity and standardization. In addition, the flow cytometry technique can provide an objective discrimination. Therefore, flow cytometry can be used as an ancillary examination for difficult morphological cases of renal tumors.

In conclusion, we made an analysis of DNA ploidy for the common subtypes of renal tumors. Flow cytometric analysis reveals that conventional and papillary RCCs are more homogeneous than chromophobe RCC. Each specific subtype of renal tumors possesses a specific DNA ploidy pattern. The DNA ploidy pattern is useful for the differentiation of common subtypes of renal tumors in morphologically difficult cases.

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