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DNA cytophotometry in adrenocortical tumours: a clinicomorphological study of 66 cases*

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Summary. Surgical specimens of 66 adrenocortical tumours were investigated by conventional microscopy and DNA cytophotometry. Histologically, 50 neoplasms were classified as adenomas and 16 as carcinomas. In only 8 of the latter cases were distant metastases and/or a lethal outcome recorded. On single cell scanning cytophotometry either non-euploid or aneuploid DNA histograms were identified in 24 of 50 adenomas (48%) and in 14 of 16 carcinomas (88%). The two carcinomas exhibiting euploid DNA distributions fell into the group of 7 malignancies which are recurrence-free so far. From these findings it is concluded that DNA measurements have no diagnostic and only limited prognostic value in neoplasms of the adrenal cortex.

Key words: Adrenocortical tumours – DNA cytophotometry – Differential diagnosis – Prognosis

Introduction

In several neoplasms of endocrine and neuroendocrine lineage, there is the dilemma of assessing their biological potential correctly (benign versus malignant). This applies especially for adrenocortical tumours (ACTs), in which malignancy cannot be demonstrated by a single morphological variable. Different authors have addressed this problem by defining a histological index of malignancy, according to which a variety of microscopic features has to be evaluated, graded and added to make a score which results in the diagnosis of carcinoma when a specifically defined threshold is crossed (Hough et al. 1979; Weiss 1984; van Slooten et al. 1985). Some of these schemes are, however, hampered by the fact that the biological potential of individual ACTs remains indeterminate even after application of the respective classification procedures (Hough et al. 1979; Weiss 1984). In addition, aggressive behaviour has been described for ACTs which had only been typed as borderline lesions histologically (Gandour and Grizzle 1986). The failure of conventional light microscopy to predict the clinical course of any given ACT precisely has stimulated several groups to perform DNA measurements on this type of neoplasm. Considerable discrepancies came to light when the DNA data of ACTs reported in the literature were compared, and all of the respective studies so far utilized the method of flow cytometry. We performed DNA scanning cytophotometry on a selection of 66 clinically well-documented ACTs and correlated our results with the findings of conventional histology and the further evolution of disease.

* Dedicated to Prof. G. Seifert, former director of the Institute of Pathology, University of Hamburg, on the occasion of his 70th birthday

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Material and methods

Formalin-fixed, paraffin-embedded material obtained from surgical specimens of 66 primary ACTs was analysed using conventional histology and single cell scanning DNA cytophotometry. Microscopically, the criteria listed in Table 1 (e.g. nuclear features, mitotic activity, structural findings and indicators of microinvasive growth) were evaluated and added to histological indices of malignancy as defined by Hough et al. (1979), Weiss (1984) and van Slooten et al. (1985).

Information relating to the preoperative history was noted from the patient's medical reports. The values for tumour diameters and weights were taken from the original pathological descriptions. In each case, the development of disease following surgery was documented until spring 1990 (mean postoperative observation period 29 months, range 1–96 months).

Cytophotometric determinations of DNA content were performed as described previously (Padberg et al. 1990). Briefly, paraffin sections of the primary tumours were cut at a 6-μm thickness and stained according to the Feulgen technique. Coverslips were mounted with "Eukitt" (refraction index 1.494). Single cell DNA measurement was performed in the scanning mode on a Leitz-

Table 1. Definition of histological indices of malignancy in adrenocortical tumours[a]

Criterion	Parameter	Score		
		Weiss 1984	Hough et al. 1979	van Slooten et al. 1985
Nuclear grade	Moderate or strong	1	0.39	2.1
Nuclear hyperchromasia	Moderate or marked			2.6
Nucleoli	Abnormal structure			4.1
Mitotic activity	>5/50 HPF	1		
	>10/100 HPF		0.60	
	>2/10 HPF			9.0
Atypical mitoses	Present	1		
Clear cells	<25% of the tumour	1		
Architecture	Diffuse pattern	1	0.92	
Structure	Mainly abnormal			1.6
Venous structures	Invasion present	1	0.92[b]	
Sinusoidal structures	Invasion present	1		3.3[c]
Tumour capsule	Invasion present	1	0.37	
Necroses	Present	1	0.69	
Regressive changes	Present			5.7
Broad fibrous bands	Present		1.00	

[a] Weiss (1984): 1–3, benign; 4, borderline; 5–9, malignant; Hough et al. (1979): 0.17 ± 0.26, benign; 1 ± 0.58, indeterminate; 2.91 ± 0.9, malignant; van Slooten et al. (1985): <8, benign; ≥8, malignant

[b] Vascular invasion

[c] Capsular and/or vascular invasion

MPV-cytophotometer based on a Leitz-Orthoplan microscope. The measuring spot was 2.54 μm^2; the steps of the scanning process were 0.5 μm wide. Absorption of the probes was determined at a wave length of 560.0 ± 9.5 nm. The number of cells examined was 70–100 for each case. Data were processed on-line by a Eurocos computer using specially adopted commercial software (Leitz, Wetzlar, FRG). Determination of diploid values was performed using 210 normal adrenocortical cells from 3 control specimens; the mean ± 2 SD of their cellular DNA content was defined as the 2 c region. Evaluation of the data was done using two different schemes: (i) classifying the DNA histograms according to Auer et al. (1980) into four different types I–IV (for details, see Results); (ii) determining the P90 value according to Forsslund et al. (1984).

Results

On reclassification in line with the three above-mentioned criteria catalogues (Hough et al. 1979; Weiss 1984; van Slooten et al. 1985: Table 1), the results of which were identical each time, 50 tumours were typed as adenomas and 16 as carcinomas. The mean tumour weights were 67 (8–1080) g for adenomas and 674 (20–3000) g for carcinomas; 9 adenomas weighed more, 2 carcinomas less than 50 g. At the resection of the primary tumour, the mean patient age was 53 (range 20–79) years in the former and 45 (range 2–76) years in the latter group. For both types of neoplasm, a clear predominance of female patients was found (M:F adenomas 1:3.5; carcinomas 1:2.2). Preoperatively, in the adenoma patients the following hormonal symptoms had been documented: hyperaldosteronism (Conn's syndrome) in 20 of 50; hypercortisolism (Cushing's syndrome) in 8 of 50; virilization in 1 of 50; feminization in 1 of 50; 20 of 50 patients had not shown endocrine abnormalities (non-functioning ACTs). Clinicomorphological findings in the total of 16 malignant ACTs are given in Table 2. At the end of the observation period, 48 adenoma patients showed symptom-free survival; 2 patients had previously died from unrelated causes. Of the carcinoma patients, 6 had died from the tumour, 1 from other causes; 2 patients were alive with persistent tumour manifestations, while 7 continued to live symptom-free 4–61 months after surgery.

On cytophotometry, 5 of 50 adenomas (10%) but none of the 16 carcinomas were characterized by single distinct modal values in the diploid region (diploid type I histograms, Fig. 1a) as recorded for each of the three control specimens. Twenty-one of the 50 adenomas (42%) and 2 of 16 carcinomas (12.5%) exhibited DNA distributions defined as having either a distinct modal value in the tetraploid or near-tetraploid region or showing two well-defined peaks around the 2c and 4c regions (euploid type II histograms, Fig. 1b). In 19 of 50 adenomas (38%) and 6 of 16 carcinomas (37.5%), non-euploid type III populations (Fig. 1c) were found, having two peaks but differing from the type II populations in that the histograms exhibited a sizeable number of cells with DNA amounts similar to those of the control cells in DNA synthesis. The position of the two peaks, as a rule, deviated somewhat from the 2c and 4c values of normal populations. In 5 of 50 adenomas (10%) and 8 of 16 carcinomas (50%), aneuploid type IV specimens (Fig. 1d) were seen with a pronounced irregularity and DNA amounts per cell ranged from levels near 2c up to values beyond 8c or even 12c.

To summarize, diploidy was observed exclusively in a small number of benign adrenocortical neoplasms, while aneuploidy occurred five times more frequently among malignant when compared with benign ACTs. Systematic differences could, however, not be detected between these two groups, either upon DNA histogram typing or when determining the respective P90 values (data for the group of the malignant ACTs included in Table 2). Neither was any association demonstrable between the DNA content of tumours and the presence or absence of hormonal function. When comparing the cytophotometric findings to the follow-up data, no clear-cut correlation was found between the DNA value of tumours and the further course of disease. Yet both of the two carcinoma cases exhibiting euploid DNA distributions fell into the group of 7 malignancies which are recurrence-free so far.

Table 2. Clinicomorphological findings in 16 malignant adrenocortical tumours

No.	Age, sex	Histological indices of malignancy DNA							Follow-up (months)	
		Tumour weight	Clinical symptoms	Hough et al. (1979)	Weiss (1984)	van Slooten et al. (1985)	Auer	p90		
1	47 M	130 g	Cush.	3.60	6	16.8	III	41.4	7	DFC
2	69 F	58 g	Cush., Vir.	2.97	7	24.3	III	41.4	2	DFC
3	44 F	1500 g	Cush., Vir.	2.60	6	18.4	IV	43.0	90	AWT
4	02 M	34g	Cush., Pub. pr.	2.31	3	9.4	III	47.1	28	NED
5	62 M	190 g	Cush.	2.97	7	21.7	III	48.6	25	NED
6	51 F	113 g	Cush.	3.50	7	19.6	III	72.9	26	DFC
7	57 F	1300 g	Cush., Vir.	4.52	6	21.7	IV	75.7	1	DFC
8	02 F	20 g	Cush., Vir.	2.83	6	22.7	IV	77.1	7	NED
9	76 F	250 g	Cush.	3.97	6	15.3	IV	98.6	61	NED
10	31 F	1440 g	Vir.	3.92	5	11.1	IV	90.0	28	AWT
11	40 F	750 g	Non-funct.	3.60	5	9.4	II	22.9	55	NED
12	40 F	54 g	Non-funct.	2.60	6	22.5	II	24.3	35	NED
13	44 F	995 g	Non-funct.	3.97	6	24.3	III	50.0	96	DFC
14	61 F	3000g	Non-funct.	3.97	7	21.7	IV	77.1	57	DOC
15	45 M	280 g	Non-funct.	3.89	7	28.4	IV	85.7	1	DFC
16	24 M	1890 g	Non-funct.	4.52	7	28.4	IV	92.5	31	NED

Cush., Cushing's syndrome; Vir., virilization; Non-funct., non-functioning; Pub. pr., pubertas praecox; DFC, death from carcinoma; AWT, alive with tumour; NED, no evidence of disease; DOC, death from other causes

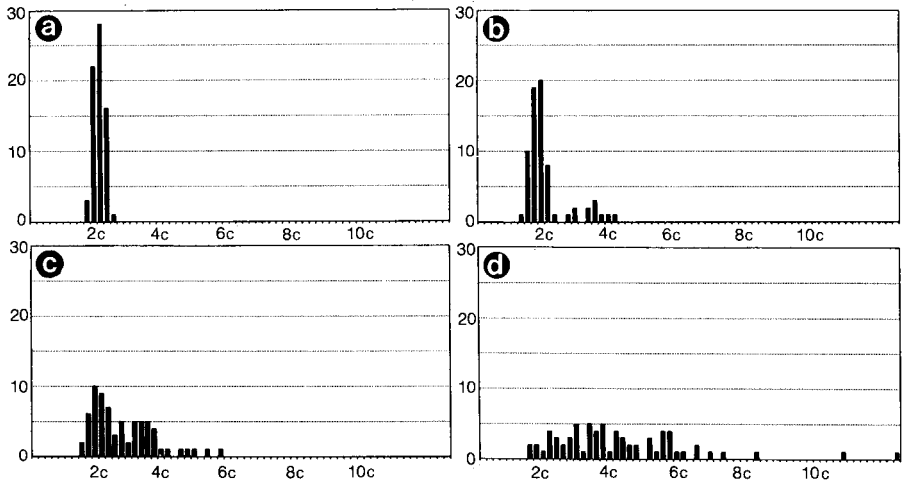

Fig. 1. Examples of typical histograms: **a** diploid type I (non-functioning adenoma); **b** euploid type II (adenoma associated with Conn's syndrome); **c** non-euploid type III (carcinoma associated with Cushing's syndrome; case 5 from Table 1); **d** aneuploid type IV (non-functioning carcinoma; case 15)

Discussion

Our series underlines the relevance of the three commonly used classification schemes for the histological discrimination of benign and malignant ACTs (Hough et al. 1979; Weiss 1984; van Slooten et al. 1985). All neoplasms typed in this way as adenomas followed an uneventful postoperative course, although 9 of these weighed more than 50 g and some considerably more – a point to remember in the differential diagnosis with adrenocortical carcinoma (Schteingart et al. 1968), as recently re-emphasized by Rosai (1989). Surprisingly, however, 7 of our 16 ACTs typed as carcinomas have remained recurrence-free so far. Hence, the malignancies in our material exhibited an altogether more favourable behaviour than such neoplasms in several other reports communicated in the literature.

As far as we have been able to ascertain, nine different groups have communicated DNA findings in ACTs, all of them utilizing the method of flow cytometry on either paraffin-embedded or on fresh material. The flow cytometric data reported in the literature and the cytophotometric findings presented herein are summarized in Table 3. With regard to the detection of aneuploidy exclusively among carcinomas three of the groups (Klein et al. 1985; Bowlby et al. 1986; Kojima et al. 1988) assumed DNA measurements to be superior to conventional histology in the discrimination between benign and malignant tumours of the adrenal cortex. This assumption was, however, refuted by the findings of Joensuu and Klemi (1988), who demonstrated euploidy and aneuploidy each to occur in approximately 50% of benign ACTs – a result in line with our observations and those of Cibas et al. (1990) – reflecting the fact that by no

Table 3. DNA findings in adrenocortical tumours

References	n[a]	Adenomas		Carcinomas	
		Euploid	Aneuploid	Euploid	Aneuploid
Klein et al. (1985)[b]	6	2/2	0/2	0/4	4/4
Bowlby et al. (1986)[c]	22	16/16	0/16	1/6	5/6
Amberson et al. (1987)[c]	48			1/5	4/5
Hosaka et al. (1987)[c]	52			22/52	30/52
Taylor et al. (1987)[b+c]	10			5/10	5/10
Joensuu and Klemi (1988)[c]	17	8/17	9/17		
Kojima et al. (1988)[c]	15	9/9	0/9	0/6	6/6
Rainwater et al. (1989)[c]	26	19/20	1/20	3/6	3/6
Cibas et al. (1990)[c]	43	24/30	6/30	4/13	9/13
Present study[d]	66	26/50	24/50	2/16	14/16

[a] Total number of adrenocortical tumours investigated
[b] Flow cytometry using fresh material
[c] Flow cytometry using paraffin-embedded material
[d] Cytophotometry

means all carcinomas exhibit aneuploid DNA distributions. Several more recent cytometric studies concur with this statement by describing euploid DNA values for up to 50% of adrenocortical carcinomas (Amberson et al. 1987; Hosaka et al. 1987; Taylor et al. 1987; Rainwater et al. 1989). In each of these four series, however, an association between the DNA content of tumours and the aggressiveness of disease was suggested since aneuploidy was reported to be a phenomenon only encountered among ACTs with virulent behaviour. It has yet to be stressed in this context that Cibas et al. (1990), in their comprehensive study of 43 ACTs, failed to demonstrate such differences in survival between patients with diploid versus aneuploid tumours. Our data appear to parallel the findings of the latter study, since not only our 2 euploid, but also 3 of our 8 aneuploid adrenocortical carcinomas followed an uneventful course.

The discrepancies between our results and that of others might partly be explained by differences in the cytometric methodology; yet with regard to the small number of cases included in some of the cited studies selection bias could also have contributed to this discordance. Since none of the two evaluation schemes chosen to interpret our DNA data provided significant results, we conclude that cytophotometry is of no diagnostic and of only limited prognostic value in adrenocortical tumours. Rather, from our results, conventional histology remains the most effective means of assessing the malignancy of individuals ACTs, while DNA measurements – in order to determine euploidy – might only be regarded as a supplementary tool to recognize carcinomas of the adrenal cortex with low malignant potential.

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