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Adrenomedullary hyperplasia and phaeochromocytoma. DNA cytophotometric findings in 47 cases*

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Summary. Fifty adrenalectomy specimens containing normal (n = 3), hyperplastic (n = 4) or neoplastic (n = 43) medullary tissue were subjected to quantitative cytophotometric measurements of DNA content. Differing evaluation schemes were applied for interpretation of DNA distribution patterns. Of the 43 phaeochromocytomas (PCC), 16 were inherited as part of the syndrome of multiple endocrine neoplasia type 2a (MEN 2a). Five of 27 sporadic PCCs followed a malignant course. Three benign and three malignant PCCs lacked endocrine activity.

In normal medulla and in adrenomedullary hyperplasia, diploid or euploid DNA distributions were found. In contrast, 87% (33/38) of the benign and all 5 malignant PCCs exhibited non-diploid or aneuploid DNA histograms. No differences in DNA content existed between functioning and non-functioning PCCs or between sporadic and hereditary tumours.

In this study, in contrast to earlier communications, DNA cytophotometry did not discriminate between benign and malignant adrenomedullary tumours. In addition, as opposed to the findings in a variety of other endocrine tumours, DNA measurements did not appear to be a useful tool to assess the prognosis of an individual malignant PCC.

Key words: Adrenal gland – Phaeochromocytoma – DNA cytophotometry – Prognosis

Introduction

It is generally agreed that the biological behavior of phaeochromocytomas can not be assessed by conventional microscopy. Histology fails to show substantial differences between clinically benign and malignant adrenomedullary tumours, and criteria indicative of invasion and malignancy are an unreliable guide to the future outcome of the case (Remine et al. 1974; Scott and Halter 1984; Russel and Rubinstein 1989). The only absolute criterion for diagnosing a malignant phaeochromocytoma is the presence of secondary tumour deposits in sites where chromaffin tissue is not found normally (Remine et al. 1974; Amberson et al. 1987).

Cytophotometric DNA measurements have been shown to be a useful auxiliary method for correct typing (benign versus malignant) and – in the case of malignant tumours – for determining the degree of aggressiveness (low versus high grade) in a variety of tumours of different sites (Auer et al. 1980; Forrslund et al. 1984; Ljuneberg et al. 1986; Hamper et al. 1989). DNA data on phaeochromocytomas is scanty (Lewis 1971; Klein et al. 1985; Hosaka et al. 1986; Amberson et al. 1987). Since the diagnostic and prognostic value of this method is judged discrepantly by these groups, we performed DNA cytophotometry on a selection of 50 adrenalectomy specimens, belonging to different types of medullary disease.

Material and methods

Formalin-fixed paraffin-embedded material of 50 adrenalectomy specimens was gathered from the surgical pathology files of different institutes and departments of pathology. For documentation of preoperative symptoms, clinical case histories were reviewed.

The series comprised 3 normal controls, 4 cases of adrenomedullary hyperplasia, and 43 PCCs. Of the 27 sporadic tumours, 22 cases lacked morphological or clinical features of malignancy. Three of these showed admixtures of PCC elements with ganglioneuroma. In three other patients, extensive preoperative laboratory investigations had failed to demonstrate endocrine abnormalities. Five sporadic PCCs followed a malignant clinical course; three of these lacked endocrine activity. Sixteen tumours were neoplasms inherited in the setting of MEN 2a. Each time, preoperative laboratory investigations were diagnostic of PCC. In four patients bilateral disease was found.

In 25 out of 43 PCC patients, follow-up information exceeding a postoperative interval of 3 months was achieved, amounting to

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Table 1. Summary of clinical, morphological, and DNA cytophotometric findings in 4 cases of adrenomedullary hyperplasia and 43 phaeochromocytomas

No.	Diagnosis	Sex (f/m)	Age (yrs)	Side (r/l)	Size (cm)	Weight (g)	Clinical findings				DNA measurements			Follow-up	
							bp	uA	uN	va	Histogram	P 90	MGi	(months)	
1	Hyperplasia	m	43	l		6	+	+	+	+	II	40.0	0.523	9	NED
2	Hyperplasia	f	66	r		6	+	+	+	+	II	50.0	0.464	7	NED
3	Hyperplasia	f	39	l		5	+	+	+	+	II	40.0	0.410	4	NED
4	Hyperplasia	m	53	l		5	+	+	+	+	I	37.1	0.101	1	NED
5	PCC spor	m	46	r	3	18	+	+	+	+	IV	72.8	1.343	4	NED
6	PCC spor	f	29	l	6	49	n	+	+	n	II	72.9	0.578	72	NED
7	PCC spor	m	46	r	7	95	n	+	+	0	IV	91.4	2.072	48	NED
8	PCC spor	m	69	l	8	200	+	0	0	0	IV	98.6	2.261	48	NED
9	PCC spor	m	50	l	6	67	+	+	+	+	IV	75.7	1.671	36	NED
10	PCC spor	f	44	l	6	80	+	+	+	0	III	87.1	0.983	6	NED
11	PCC spor	f	52	r		72	+	+	+	+	II	31.4	0.373	op death	
12	PCC spor	m	23	r	5	35	+	+	+	n	IV	97.1	1.580	180	NED
13	PCC spor	m	59	l	5	50	+	+	+	+	III	94.3	0.626		
14	PCC spor	f	48	l	5	40	+	0	0	0	III	85.7	1.196	6	NED
15	PCC spor	f	61	r	3	19	+	+	+	+	III	75.7	1.036		
16	PCC spor	m	22	r	5	32	+	+	+	0	IV	95.7	2.244		
17	PCC spor	f	73	r	3	14	+	+	+	+	III	81.4	0.768		
18	PCC spor	m	16	l	5	48	+	n	+	+	IV	100.0	3.453	60	NED
19	PCC spor	f	17	l	6	70	+	n	n	n	III	44.3	0.830	4	NED
20	PCC spor	m	19	r	4	72	+	+	+	0	III	65.7	0.598		
21	PCC spor*	m	67	r	6	50	+	n	n	n	IV	85.7	1.179	11	NED
22	PCC spor*	f	74	l	3	20	+	n	n	n	III	91.4	0.747	4	NED
23	PCC spor*	f	42	r	5	51	n	n	n	n	III	88.6	1.081	9	NED
24	PCC spor	f	70	r	2	13	n	0	0	0	IV	97.1	1.517	48	NED
25	PCC spor	f	64	r	8	57	+	+	+	+	IV	100.0	2.377	26	NED
26	PCC spor	f	78	r	6	63	+	+	+	+	III	60.0	0.428		
27	PCC spor	m	52	l	10	220	+	+	+	+	IV	88.6	1.005	13	death
28	PCC spor	m	55	r	11	270	+	+	+	+	III	81.4	0.765	24	rec
29	PCC spor*	f	36	l	12	352	+	n	n	n	IV	100.0	2.708	3	death
30	PCC spor*	f	64	l	15	1850	n	n	n	n	IV	100.0	1.516	2	death
31	PCC spor*	m	32	l	22	2100	n	n	n	n	IV	100.0	1.987	1	met
32	PCC her	f	53	r	7	95	+	+	+	+	II	27.1	0.188		
33	PCC her	f	49	r	6	57	+	+	+	+	III	82.8	0.669		
34	PCC her	m	62	r	8	66	+	+	+	+	IV	90.0	1.199		
35	PCC her	f	40	l	6	32	+	+	+	+	II	47.1	0.470	79	NED
36	PCC her	f	52	l	8	85	+	+	+	0	IV	71.4	1.128	37	NED
37	PCC her	f	60	r	4	15	+	+	+	+	III	60.0	1.614		
38	PCC her	m	34	r	2	13	n	+	+	0	III	84.3	0.803		
39	PCC her	m	39	l	8	140	n	+	+	+	IV	100.0	1.317	67	NED
40	PCC her	m	12	r	3	11					IV	100.0	1.313		
41	PCC her			l	6	50					IV	98.6	1.736		
42	PCC her	m	29	l	3	9	+	+	+	+	IV	91.4	1.637	17	NED
43	PCC her			r	9	77					III	85.7	0.793		
44	PCC her	f	52	l	1	5	+	+	+	+	III	74.3	0.722		
45	PCC her			r	2	5					II	44.3	0.369		
46	PCC her	f	23	r	4	32	n	+	+	0	III	75.4	1.132		
47	PCC her			l	3	25					IV	88.6	1.682		

Side: tumour localisation (right/left adrenal). Clinical findings: bp-blood pressure; uA-urinary adrenaline; uN-urinary noradrenaline; va-vanillyl-mandelic acid (n = normal, + = elevated, 0 not evaluated). DNA measurements: Histogram-type (Auer); P 90 value (Forrslund); MGi MG-index (Böcking). Follow-up: NED-no evidence of disease; rec-recurrent disease; met-metastases; op-intraoperative. Hyperplasia: Adrenomedullary hyperplasia; PCC: Phaeochromocytoma; spor: sporadic; her: hereditary. spor*: non-functioning tumour

a mean observation period of 36 (range 1–180) months. One of the 22 patients with benign sporadic PCC had died intraoperatively. The others showed continuous symptom free survival. Three of the 5 patients with malignant sporadic PCC had died 2 to 13 months after surgery, the remainder still suffer from distant metastases. None of the 12 patients with hereditary PCC had an apparently malignant tumour; 5 of these cases suffered from metastatic medullary thyroid carcinoma continuously. Two of the 8 subjects in the hereditary category presenting originally with unilateral disease later developed a contralateral PCC.

The values for tumour diameters and weights were taken from the original pathological descriptions. For reclassification, routinely H&E stained slides were used. For diagnosis of adrenomedullary hyperplasia, morphometric analyses of total medullary volumes were performed (Dralle et al. 1990). In non-functioning adrenal tumours, the diagnosis of PCC was dependent on the immunocytochemical demonstration of synaptophysin-positivity (Jahn et al. 1985) and negativity for the adrenocortical marker D11 (Schröder et al. 1989).

For cytophotometric determination of DNA content 6 µm-thick paraffin sections were used. After dewaxing, RNA was destroyed by hydrolysis in 1N hydrochlorid acid at 60° C for 15 min.

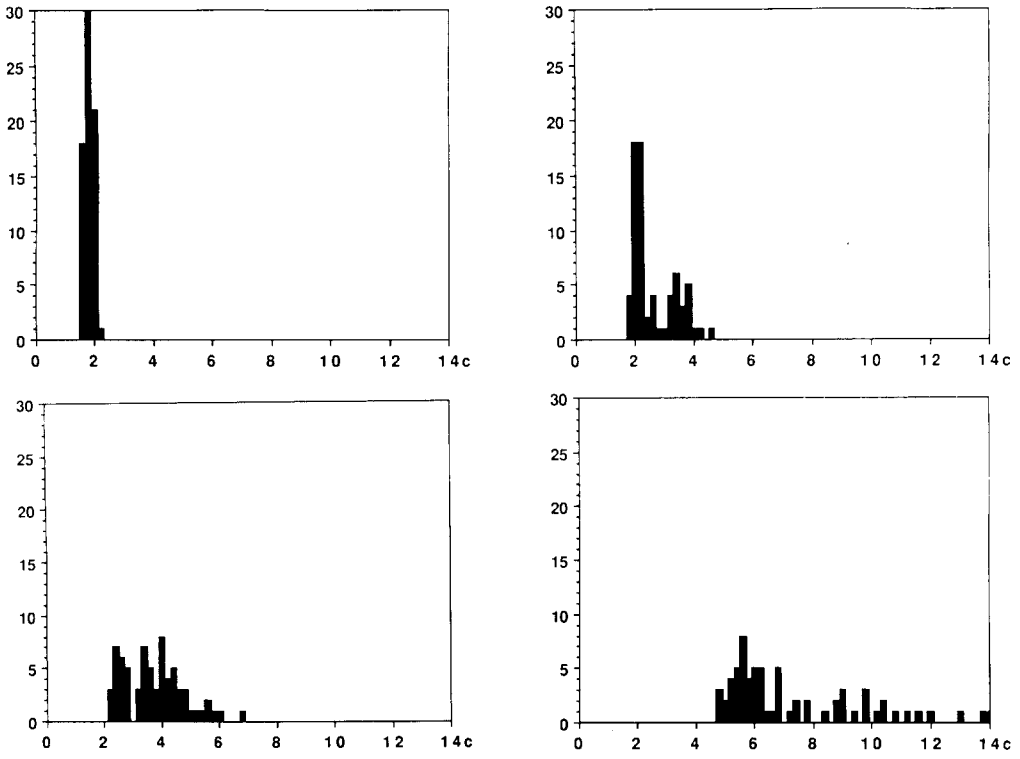

Fig. 1. Examples of typical histograms: **A** (*upper, left*) DNA histogram type I (diploid; normal adrenal medulla); **B** (*upper, right*) type II (euploid; adrenomedullary hyperplasia case No 1); **C** (*lower, left*) type III (non-diploid; non-functioning benign phaeochromocytoma case No 21); **D** (*lower, right*) type IV (aneuploid; non-functioning malignant phaeochromocytoma case No 29)

Afterwards, Schiff's reagent was applied for 1 h and the sections were subsequently treated with 1% sodium metabisulphite. Coverslips were mounted with "Eukitt" (refraction index 1.494). Single cell DNA-measurement was performed in the scanning mode on a LEITZ-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 for each case. Data were processed on-line by a EUROCOS-computer using specially adopted commercial software (LEITZ, Hamburg). Determination of diploid values was performed using normal adrenomedullary cells from the 3 control specimens noted above; the mean ± 2 standard deviations of their cellular DNA content was defined as a 2c region. Evaluation of the data was done using three different schemes: (i) classifying the histograms according to Auer et al. (1980) into four different types (I–IV); (ii) determining the P90 value according to Forsslund et al. (1984); (iii) calculating the MG-index according to Böcking et al. (1984).

Results

Morphological and clinical findings are summarized in Table 1. Cytophotometrically, the adrenal medulla of the three control specimens was characterized by single distinct modal DNA values in the diploid region of normal cells (type I) (Fig. 1 A). The same finding was obtained for one of 4 cases of adrenomedullary hyperplasia (No 1–4), the remaining three cases exhibiting euploid type II histograms, 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 (Fig. 1 B).

Two of the 22 benign sporadic PCCs (9%) (No 5–26) also showed type II histograms. For 10 lesions (45,5%), type III populations were demonstrated, defined as having two peaks but differing from the type II populations in that the histograms showed a sizeable number of cells with DNA amounts similar to those of control cells in DNA synthesis. The positions of the two peaks, as a rule, deviated somewhat from the 2c and 4c values of normal populations (Fig. 1 C). In 10 cases (45,5%), type IV specimens were seen with a very pronounced and irregular aneuploidy, DNA amounts per cell ranging from levels near 2c up to values beyond 6c or even 14c (Fig. 1 D). Of the 5 clinically malignant PCCs (No 27–31), one showed type III and four exhibited type IV histograms. Among the 16 hereditary PCCs (No 32–47), type II, III and IV histograms were found in 3, 6 and 7 (19%, 37%, 44%) cases, respectively.

No systematic discrepancies between sporadic, hereditary, functioning or non-functioning, benign or malignant PCCs were found by using DNA histogram typing or either of the other DNA data evaluation schemes.

Discussion

Four studies on the diagnostic and prognostic value of DNA determinations in PCC have been reported. In a cytophotometric investigation, Lewis (1971) found benign behaviour in each of 12 lesions with a diploid DNA content, whereas distant metastases were demonstrated for each of 3 aneuploid PCCs. The author suggested that such measurements allowed discrimination between metastasizing and benign PCCs and a similar conclusion was drawn by Hosaka et al. (1986) based on a flow cytometric study of 62 PCCs using the technique of Hedley et al. (1983). Each of the 18 patients with normal DNA histograms followed a benign clinical course, whereas 8 of 26 patients classified as DNA tetraploid/polyploid and 7 of 18 patients exhibiting DNA aneuploid peak

had evidence of malignancy, the differences between these groups being statistically significant. According to this series, DNA ploidy measurements thus seemed to provide useful prognostic information, although a clear-cut discrimination between benign and malignant neoplasms was not produced by this method. Klein et al. (1985) considered flow cytometric DNA determination to be an accurate objective method of identifying adrenal malignancy. However, only one (clinically benign euploid) PCC case was included in their study of 4 normal and 7 neoplastic adrenal tissues.

In contrast with these studies, Amberson et al. (1987) in a recent flow cytometric investigation, found aneuploidy to be a phenomenon frequently also encountered in benign adrenal PCC. Of 19 such tumours, only 6 were diploid, whereas 9 clearly exhibited aneuploid DNA content.

The findings presented herein parallel the observations of the latter study. We found non-diploid DNA values in 38 of 43 PCCs (88%), 21 of which clearly exhibited pronounced aneuploidy. Clinically malignant behaviour could only be established in 5 of these tumours. The remaining 33 non-diploid lesions lacked morphological evidence of malignancy. In 20 of these, follow-up disclosed an uneventful postoperative course (the observation period being longer than 12 months in 13 of 20 patients).

On the basis of these results, DNA measurements thus supply neither diagnostic nor prognostic information for patients with PCC. In addition, DNA data are not associated with a particular genetic background of disease (sporadic versus hereditary tumours) or with the presence of clinical symptoms (functioning versus non-functioning neoplasms).

Our study thus provides another example of the fact that DNA determinations are of limited value in differential diangosis especially among endocrine and neuroendocrine neoplasias. Aneuploidy has been shown not to be diagnostic of malignancy in thyroid, parathyroid and adrenal cortical adenomas (Joensuu et al. 1986, 1989) and carcinoids of different sites (Olinici and Cäluser 1988). It should be mentioned, however, that significant prognostic information is gained by this method for follicular thyroid carcinomas (Cohn et al. 1984; Joensuu et al. 1986) and medullary carcinomas of the thyroid (Bäckdahl et al. 1985; Schröder et al. 1988). Since medullary thyroid carcinomas and PCCs are of the same neuroendocrine lineage and frequently occur together as manifestations of MEN 2 syndrome, the irrelevance of DNA measurements in adrenomedullary tumours is even more remarkable. There must be a continued search for other procedures enabling a clear discrimination between risk groups in PCC patients.

References

Amberson JB, Vaughan ED, Gray GF, Naus GJ (1987) Flow cytometric determination of nuclear DNA content in benign adrenal pheochromocytomas. Urology 30:102–104

Auer G, Caspersson TO, Wallgren AS (1980) DNA content and survival in mammary carcinoma. Anal Quant Cytol Histol 2:161–165

Bäckdahl M, Tallroth E, Auer G, Forrslund G, Granberg PO, Lundell G, Löwhagen T (1985) Prognostic value of nuclear DNA content in medullary thyroid carcinoma. World J Surg 9:980–987

Böcking A, Adler CP, Common HH, Hilgarth M, Granzen B, Auffermann W (1984) Algorithm for a DNA-cytophotometric diagnosis and grading of malignancy. Anal Quant Cytol Histol 6:1–8

Cohn K, Bäckdahl M, Forsslund G, Auer G, Lundell G, Löwhagen T, Tallroth E, Willems JS, Zetterberg A, Granberg PO (1984) Prognostic value of nuclear DNA content in papillary thyroid carcinoma. World J Surg 8:474–480

Dralle H, Schröder S, Gratz KF, Gorte R, Padberg B, Hesch RD (1990) Sporadic unilateral adrenomedullary hyperplasia with hypertension cured by adrenalectomy. World J Surg (in press)

Forsslund G, Cedermark B, Öhmann U, Erhardt K, Zetterberg A, Auer G (984) The significance of DNA distribution patterns in rectal carcinoma. Dis Colon Rectum 27:579–584

Hamper K, Schimmelpennig H, Caselitz J, Arps H, Berger J, Askenstein U, Auer G, Seifert G (1989) Mucoepidermoid tumors of the salivary glands. Cancer 63:708–717

Hedley DW, Friedlander ML, Taylor IW, Rugg CA, Musgrove EA (1983) Method of analysis of cellular DNA content of paraffin-embedded pathological material using flow cytometry. J Histochem Cytochem 31:1333–1335

Hosaka Y, Rainwater LM, Grant CS, Farrow GM, van Heerden JA, Lieber MM (1986) Pheochromocytoma: nuclear deoxyribonucleic acid patterns studied by flow cytometry. Surgery 6:1003–1010

Jahn R, Schiebler W, Quimet C, Greengard P (1985) A 38.000-dalton membrane protein (p38) present in synaptic vesicles. Proc Natl Acad Sci USA 82:4137–4141

Joensuu H, Klemi P, Eerola E, Tuominen J (1986) Influence of cellular DNA content on survival in differenciated thyroid cancer. Cancer 58:2462–2467

Joensuu H, Klemi PJ, Alanen KA (1989) Co-existence of two aneuploid stemlines in benign adenomas. Virchows Arch [A] 415:175–180

Klein FA, Kay S, Ratliff JE, White FK, Newsome HH (1985) Flow cytometric determinations of ploidy and proliferation patterns of adrenal neoplasms: an adjunct to histological classification. J Urol 134:862–866

Lewis PD (1971) A cytophotometric study of benign and malignant phaeochromocytomas. Virchows Arch [B] 9:371–376

Ljunegberg B, Stenling R, Roos G (1986) DNA content and prognosis in renal cell carcinoma. Cancer 57:2346–2350

Olinici CD, Cäluser I (1988) Cytometric study of carcinoid tumors and their metastases. Arch Geschwulstforsch 58:403–409

Remine WH, Chong GC, van Heerden JA, Sheps SG, Harrison EG (1974) Current management of pheochromocytoma. Ann Surg 179:740–748

Russell DS, Rubinstein LJ (1989) Pathology of tumours of the nervous system, 5th edn. Edward Arnold, London Melbourne Auckland, pp 945–974

Schröder S, Böcker W, Baisch H, Bürk CG, Arps H, Meiners I, Kastendieck H, Heitz PU, Klöppel G (1988) Prognostic factors in medullary thyroid carcinomas. Survival in relation to age, sex, stage, histology, immunohistochemistry, and DNA content. Cancer 61:806–816

Schröder S, Niendorf A, Dietel M, Beisiegel U (1989) Immunocytochemical differential diagnosis of adrenocortical neoplasms through new monoclonal antibody D11 (A1193). Proc Am Assoc Cancer Res 30:300

Scott HW, Halter SA (1984) Oncologic aspects of pheochromocytoma: the importance of follow-up. Surgery 96:1061–1066