American Journal of Pathology, Vol. 132, No. 1, July 1988

DNA Aneuploidy in Adenomas of Endocrine Organs

HEIKKI JOENSUU, MD, PhD, and PEKKA J. KLEMI, MD, PhD

From the Departments of Radiotherapy and Pathology, University Central Hospital of Turku, Turku, Finland

The nuclear DNA content of 44 pituitary, 49 thyroid, 54 parathyroid, and 17 adrenal adenomas was analyzed from paraffin-embedded tissue with flow cytometry. Interpretable histograms of good quality (CV < 7%, mean CV, 4%) were obtained in 96% of the cases. Unequivocal evidence of DNA aneuploidy was found in 29% of pituitary, 25% of thyroid, 35% of parathyroid, and in 53% of adrenal adenomas. Excluding the multiploid (N = 2) and tetraploid adenomas (N = 5), the DNA indices of aneuploid adenomas were generally small (mean, 1.34). Patients with a diploid thyroid or parathyroid adenoma had a lower mean age at diagnosis than patients with a nondiploid adenoma. None of the adenomas gave rise to metastases after conservative surgery. It is concluded that DNA aneuploidy is common in endocrine adenomas and that the presence of DNA aneuploidy is not incompatible with a benign histologic diagnosis. The usefulness of DNA aneuploidy as a conclusive sign of malignancy in clinical practice is questioned. (Am J Pathol 1988, 132: 145–151)

WITH FLOW CYTOMETRY the nuclear DNA content of thousands of cells can be measured in less than 1 minute. Because DNA aneuploidy (abnormal nuclear DNA content) has been suggested to be a conclusive,[1] most common malignancy-specific[2] marker of malignancy, and DNA aneuploidy is present in the majority of human cancers,[1] flow cytometry appeared to be a method for rapid and objective distinction between benign and malignant tumors. Furthermore, in several types of human cancer the presence of DNA aneuploidy is associated with poor prognosis.[3,4]

After finding aneuploidy in histologically benign tumors, some authors have reexamined their histologic sections and changed the diagnosis from benign to malignant.[5] DNA aneuploidy also has been used to confirm the presence of malignancy in histologically equivocal cases.[6] However, DNA aneuploidy has been reported to be present both in premalignant and histologically benign diseases.[7–16]

Large and systematic studies on the occurrence of DNA aneuploidy in histologically benign diseases are few. This study reports the result of DNA content analysis of a series of 164 endocrine adenomas and 41 control samples. The analysis was done from paraffin-embedded material, because for archival material a long follow-up is often available and the nature of the tissue analyzed can be verified easily. Good quality histograms, well suited for the detection of aneuploidy, can be obtained from formalin-fixed and paraffin-embedded tissue.[17] The result of the present study indicate that DNA aneuploidy may be present in both histologically and clinically benign endocrine adenomas.

Material and Methods

Patients

The formalin-fixed and paraffin-embedded tissue blocks of 44 pituitary, 49 thyroid, 54 parathyroid, and 17 adrenal adenomas, treated from 1977 to 1985 were selected at random from the archives of the Department of Pathology, Turku University Central Hospital. In addition, paraffin blocks thought to contain normal thyroid tissue (N = 33, obtained from 21 patients), normal adrenal gland (N = 3), or benign lymph node (N = 5) were analyzed as controls. The original histologic diagnoses of the 205 cases were reviewed by 1 author (P. K.), but in none of the cases

Supported by the Cancer Society of Finland.
Accepted for publication February 28, 1988.
Address reprint requests to Heikki Joensuu, MD, PhD, Department of Radiotherapy University Central Hospital of Turku, SF-20520 Turku, Finland.

was the diagnosis of adenoma changed to carcinoma. The medical records of the patients were reviewed. The mean age of the 164 patients with adenoma was 51 years (range, 2–78 years) and 30% were male.

The histologic typing of the tumors was done according to the WHO.[18] Thyroid adenomas were subclassified according to Meissner and Warren.[19] Twelve of the pituitary adenomas were eosinophilic, 6 basophilic, and 26 chromophobic. The parathyroid adenomas were single except in 1 case. One patient with a parathyroid adenoma was known to have a multiple endocrine neoplasia (MEN) syndrome.

The adenomas were treated usually with conservative surgery. In thyroid and parathyroid adenomas the extent of surgery was described by the surgeon as "extirpation," "enucleation," or "subtotal lobectomy." Thyroidectomy was done in 2 cases with a concomitant papillary carcinoma. Pituitary adenomas were removed either in craniotomy or transsphenoidally, adrenal adenomas by adrenalectomy.

Flow Cytometry

The preparation of a single cell suspension from paraffin-embedded tissue was done as described earlier.[10,20] Two 50-μ sections were cut. One or more 5-μ sections were cut between and immediately adjacent to the 50-μ sections, and were stained for light microscopy to confirm the nature of the tissue used for DNA analysis. One 50-μ section was deparaffinized with xylene, rehydrated in a series of decreasing concentrations of ethanol, washed, resuspended in a 0.5% pepsin solution for 60 minutes at 37 C, and stained with propidium iodide,[21] whereas the other 50-μ section was stored for possible reanalysis. The resulting suspension was filtered through a silk mesh prior to flow cytometry.

Flow cytometry was done with a FACStar flow cytometer (Becton-Dickinson Immunocytometry Systems, Mountain View, CA). A 488 nm argon ion laser line run at 600 mW was used for fluorescence excitation. A 585 ± 42 nm band-pass filter was used in front of the red photomultiplier to block the laser light. For each histogram 20,000 particles were analyzed.

Careful control systems were used. The samples were run in batches of about 20. Before and after running each batch standard fluorescent beads were run, and a coefficient of variation (CV) of about 2% was obtained consistently. After running all the samples of the batch, the same samples were rerun in the reversed order. With few exceptions practically identical histograms were produced in both runs, thus reducing the possibility of artifactual peaks. Further, a few samples taken from histologically normal tissue were analyzed with adenomas. In a few cases with uncertain histogram classification, a reanalysis was done from the stored 50-μ section, and again identical histograms were usually obtained. Thus, all samples were run at least twice, and a few blocks were analyzed 4 times.

Classification of Histograms

The conventional classification of histograms to diploid, aneuploid, tetraploid, and multiploid was considered insufficient for the full description of the data, and therefore the "near diploid" and "diploid with an increased G2/M peak" groups were introduced. The rules for classification of the histograms are given below:

Diploid (D)

One symmetrical G0/G1 peak is seen (Figure 1A).

Near diploid (ND)

An asymmetrical G0/G1 peak is repeatedly obtained. Two G0/G1 peaks cannot be identified (Figure 1B).

Diploid with an increased G2/M peak (I)

One symmetrical G0/G1 peak, the percentage of G2/M cells at 4N (tetraploid region, DI 1.95–2.05) exceeds 11% (mean + 3 SD of 37 nonneoplastic samples, Figure 1C). A peak at 6N (indicating the presence of triplets and hence, also the presence of doublets) is not present. The G2/M% of these cases ranged from 11.1–21.1% (mean, 14.6%) and the S% from 2–8.5% (mean, 4.7%).

Tetraploid (T)

Both a peak at 4N (tetraploid region) and another at 8N, indicating proliferative activity of cells with tetraploid DNA content, are present (Figure 1D). No peak at 6N was allowed. The peak at 4N must include >20% of the total number of cells. Similar cases with <20% cells at 4N were classified either as diploid or to increased G2/M group (N = 3, all adrenal adenomas, G2/M 8.9–13.6%).

Aneuploid (A)

Two G0/G1 peaks can be seen (Figure 1E), or an asymmetrical G0/G1 peak with 2 corresponding and clearly identifiable G2/M peaks are seen (N = 3).

Multiploid (M)

More than 1 aneuploid peak is seen (Figure 1F).

Unequivocal evidence of aneuploidy was considered to be present if the histogram was classified either as tetraploid, aneuploid or multiploid (T + A + M). Suggestive evidence of aneuploidy was considered to be present in near diploid cases and in cases with an

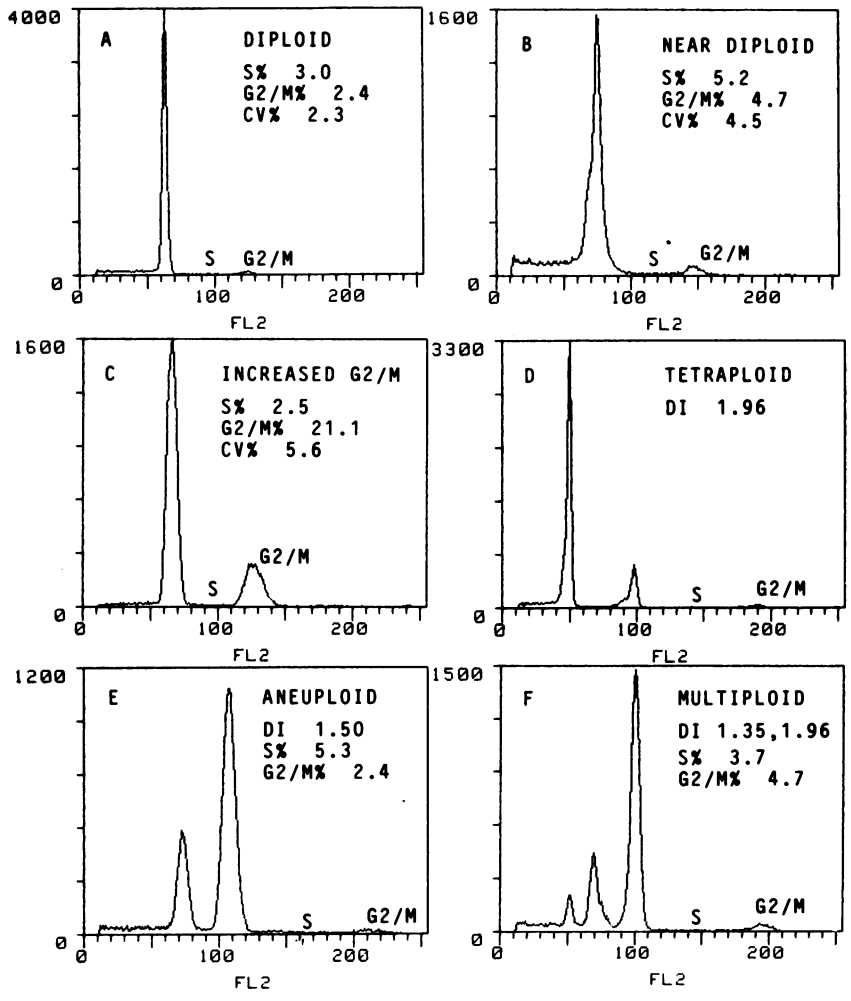

Figure 1—Examples of different types of histograms produced from paraffin-embedded tissue. For the definitions, see Materials and Methods. Histogram B was obtained from an adrenal adenoma, the rest from parathyroid adenomas. The tetraploid case shown (panel D) has 20.2% of the total number of cells at 4N (modal channel number 98), which was the lowest percentage accepted. CV, coefficient of variation; DI, DNA index. The number of particles analyzed is given on the vertical axis and DNA fluorescence (FL2, DNA content) on the horizontal axis.

increased G2/M peak (ND + I). No evidence of aneuploidy was present if the histogram was diploid (D).

When calculating the DNA index (DI), the modal channel number of an aneuploid peak was divided by the modal channel number of the diploid peak. The peak with the least DNA content was taken as the diploid peak. The percentage of S- and G2/M-phase cells was calculated using the rectilinear method.[22] The height of the S-phase was measured near the G2/M peak to avoid counting cell debris. The cell cycle analysis was not done if overlapping cell populations (most aneuploid cases) or excessive cell debris (N = 10) was present.

The chi-square test, Fisher's exact test and Student's *t*-test were used in statistical calculations.

Results

The summary of the results of DNA content analysis is shown in Table 1. Unequivocal evidence of DNA aneuploidy was found in 29% of pituitary, 25% of thyroid, 35% of parathyroid, and 53% of adrenal adenomas, and no evidence of aneuploidy in 71%, 56%, 50%, and 18% of the cases, respectively. Two cases were clearly multiploid (DIs 1.44 and 2.70, and 1.35 and 1.96, Figure 1).

The DIs of aneuploid tumors (N = 44) were generally small (mean, 1.34; range, 1.08–1.92). The mean DIs of aneuploid pituitary, thyroid, parathyroid, and adrenocortical adenomas were 1.38, 1.27, 1.42, and

Table 1—DNA Ploidy in Human Endocrine Adenomas

Ploidy*	Pituitary adenomas N (%)	Thyroid N (%)	Parathyroid N (%)	Adrenal N (%)
Diploid	27 (71%)	27 (56%)	27 (50%)	3 (18%)
Near diploid	—	8 (17%)	4 (7%)	1 (6%)
Increased G2%	—	1 (2%)	4 (7%)	4 (24%)
Tetraploid	—	—	5 (9%)	—
Aneuploid	11 (29%)	12 (25%)	13 (24%)	8 (47%)
Multiploid	—	—	1 (2%)	1 (6%)
Total	38 (100%)†	48 (100%)†	54 (100%)	17 (100%)

* For classification of histograms see Materials and Methods and Figure 1.

† The total of 7 histograms were not classified.

1.27, respectively. In addition to the tetraploid and multiploid adenomas only 1 pituitary, 2 thyroid, 4 parathyroid, and 1 adrenal adenoma had a DI > 1.50.

Pituitary Adenomas

Aneuploid tumors tended to be more common in males (8/19) than in females (3/19, $P = 0.07$). The mean age of the patients with an aneuploid adenoma (49 years) did not differ from that of the patients with a diploid adenoma (54 years). Four of the 12 eosinophilic, 2 of the 6 basophilic, and 5 of the 20 chromophobic adenomas were aneuploid (NS). One of the 5 adenomas considered to be intrasellar was aneuploid. Complete blood hormone analyses were usually not done before surgery, but 10 adenomas were known to secrete prolactin, and 6 of these were aneuploid. Further, in 2 of the remaining 4 diploid cases plasma prolactin level was only marginally elevated. Two of the 5 patients with an elevated plasma growth hormone level were aneuploid.

Thyroid Adenomas

The patients with a diploid thyroid adenoma (N = 27) had a lower mean age at diagnosis (42 years) than the patients with a nondiploid adenoma (N = 21, mean, 48 years, $P = 0.04$). The difference in the mean age was not significant if the patients with an unequivocally aneuploid adenoma were compared with the rest of the patients. The occurrence of DNA aneuploidy was not associated with sex. All 3 Hürthle cell and the only clear cell adenoma were aneuploid, but no association of aneuploidy could be found with the simple, fetal or embryonal types. The largest diameter of adenoma was stated in the surgical report in 25 cases (16 D, 1 I, 4 ND, and 4 A). Seven of the diploid, 2 near diploid and 2 aneuploid adenomas were 3.0 cm or larger in diameter. The mean largest diameter of the diploid adenomas was 3.0 cm, and that of the 4 aneuploid adenomas 3.4 cm.

Parathyroid adenomas

The mean age at diagnosis of the patients with a diploid parathyroid adenoma (N = 27) was lower than that of the patients with a nondiploid adenoma (N = 27, 52 *vs.* 59 years, $P = 0.01$). Again, the difference in the mean age was not significant if the patients with an unequivocally aneuploid adenoma were compared with the rest of the patients. DNA aneuploidy was not associated with sex. The size of the adenoma was indicated in the surgical report in 52 cases (28 D, 3 ND, 3 I, 5 T, 12 A, and 1 M). The mean largest diameter of the 28 diploid adenomas was 1.8 cm, and that of the 12 aneuploid adenomas 1.7 cm. The mean largest diameter of the adenomas with unequivocal evidence of aneuploidy was 1.9 cm.

Fasting serum calcium content was not elevated in 2 cases (1 D, 1 T). Fasting serum parathormone (fS-PTH) content was measured in 28 patients with a diploid and in 19 patients with a nondiploid parathyroid adenoma (2 ND, 3 I, 4 T, 9 A, 1 M), and was found to be elevated in all but 2 patients with a diploid and in 1 patient with an aneuploid adenoma. In 8 patients fS-PTH was found to be >5 μ/1, and 5 of these were aneuploid.

The only adenoma known to be associated with a MEN syndrome had an increased number of G2/M-phase cells (11.9%).

Adrenal Adenomas (Table 2)

The mean largest diameter of the 9 aneuploid or multiploid adenomas was 8.2 cm as compared with only 2.9 cm in the rest of the cases. Five of the aneuploid or multiploid adenomas were larger than 5 cm in diameter, as compared with only 1 in the 8 remaining cases (NS). The mean ages at diagnosis of these 2 groups did not differ significantly (45 *vs.* 50 years). A large DI tended to be associated with a large adenoma size.

Ploidy of the Control Samples

The 39 control samples with an interpretable histogram were classified as diploid, except in 4 cases. In 1 case with aneuploidy a typical thyroid adenoma was unexpectedly found in the control section. Another patient, who was discovered, after review of her medical records, to suffer from Basedow's disease had >11% G2/M phase cells in repeated analyses from 2 different tissue blocks (11.5 and 11.8%). Finally, 1 histogram produced from a microscopically normal thyroid gland, and obtained from a patient with a concomitant papillary carcinoma with DI 1.68, was aneuploid (DI 1.12) in repeated analyses (N = 4).

Quality of Histograms and Cell Cycle Analysis

Unclassifiable histograms were obtained in 5 cases (3 pituitary adenomas and 2 control samples). All histograms with CV >7% were excluded (3 pituitary and 1 thyroid adenoma, CV 7.5–8.7%). Thus, only 9 (4%) of the total of 205 samples analyzed were excluded. The mean CV was 4%.

The mean percentage of S phase cells (3.6%; SD, 1.6%; range, 1.1–8.8%; N = 87) and G2/M phase cells (4.4%; SD, 2.0%; range, 1.3–9.5%) was low in diploid and near diploid adenomas. The percentages of S and

Table 2—DNA Ploidy in Adrenal Adenomas

Case	Sex/Age	Function	Size*	Ploidy	DI	Follow-up
M.H.	M/2	Virilizing	6 cm	Multiploid	2.70, 1.44	Alive, 8 year
M.M.	F/64	Pheochromocytoma	8 cm	Aneuploid	1.67	Dead, 1 year†
R.A.	M/52	Nonsecreting	30 cm	Aneuploid	1.35	Alive, 5 year
T.R.	F/43	Virilizing	3 cm	Aneuploid	1.33	Alive, 6 year
K.M.	F/32	Virilizing	11 cm	Aneuploid	1.18	Alive, 5 year
H.A.	F/59	Nonsecreting	8 cm	Aneuploid	1.18	Alive, 2 year
S.K.	M/72	Nonsecreting	3 cm	Aneuploid	1.18	Alive, 2 year
L.H.	M/43	Conn's syndrome	2 cm	Aneuploid	1.12	Alive, 6 year
V.M.	F/35	Conn's syndrome	3 cm	Aneuploid	1.11	—
K.K.	F/41	Cushing's syndrome	2 cm	G2 19.4%	1.00	Alive, 11 year
M.P.	F/37	Conn's syndrome	2 cm	G2 13.6%	1.00	Alive, 3 year
K.M.	F/56	Cushing's syndrome	3 cm	G2 11.2%	1.00	Alive, 3 year
F.Y.	M/59	Nonsecreting	2 cm	G2 11.1%	1.00	Alive, 2 year
N.P.	F/28	Cushing's syndrome	4 cm	Near diploid	1.00	Alive, 2 year
L.L.	F/64	Cushing's syndrome	6 cm	Diploid	1.00	Alive, 3 year
L.E.	F/66	Nonsecreting	3 cm	Diploid	1.00	Alive, 3 year
A.A.	M/46	Nonsecreting	1 cm	Diploid	1.00	Dead, 4 year‡

* The largest diameter given.
† Died from cerebral infarction.
‡ Died from histologically diagnosed renal cancer.

G2/M phase cells could be calculated only for 10 aneuploid stemlines due to overlapping cell populations, and they were not found to be different from those of diploid and near diploid tumors (mean S phase, 4.4%; SD, 1.6%; range, 2.9–7.7%; mean G2/M phase, 4.1%; SD, 2.0%; range, 0.9–6.4%).

Follow-up

According to the hospital records, the patients with an unequivocally aneuploid adenoma had been followed up for the total of 195 years of life (mean, 3.8 years; range, 0–11 years). None of the adenomas was known to have given rise to metastases. A questionnaire was posted to the local authorities for possible death certificates in cases with unequivocal aneuploidy and with incomplete follow-up data. Including this additional information, 3 of the 51 patients with unequivocally aneuploid adenoma had died during the total of 281 years of life (mean, 5.5 years) following surgery, but none from cancer.

Evidence of Clonal Heterogeneity

Three tissue blocks obtained from the same multiploid adenoma (case M. H., Table 2) were analyzed. In 2 analyses, only 1 aneuploid stemline with DI 2.70 was seen, whereas the histogram produced from the third block revealed a large aneuploid stemline with DI 1.44 in addition to the 2.70 stemline, indicating clonal heterogeneity within the same adenoma. In the analyses of 2 tissue blocks from a multiploid parathyroid adenoma (Figure 1F) the same stemlines with DIs 1.35 and 1.96 were obtained.

Discussion

Primary pituitary tumors seldom have a malignant clinical course or give rise to metastases, and therefore the presence of aneuploidy in many pituitary adenomas is surprising. Anniko et al[7] have studied pituitary adenomas by flow cytometry, and found aneuploidy in 49% (23/47). They also found that pituitary adenomas with secretion of prolactine, alone or concomitantly with growth hormone, often had an aneuploid DNA pattern, which is in concordance with the present results.

Unequivocal aneuploidy was found in 25% of follicular thyroid adenomas, which is in agreement with the literature review (Table 3).[5,8–11,23,24] Mattfeldt et al[11] studied 24–80 sections in each of their 6 aneuploid cases, but saw no evidence of angioinvasion or capsular penetration, which was compatible with the benign histology. Similarly, in another study[10] the aneuploid thyroid adenomas were subserially sectioned, but in none of the cases was invasive growth seen,

Table 3—DNA Aneuploidy in Follicular Adenomas of the Thyroid

		Aneuploid	
Author	N	N	%
Johannessen et al, 1982	4	1	25
Greenebaum et al, 1985	19	7*	37
Flint et al, 1985	1	–	–
Kraemer et al, 1985	5	1	20
Christov, 1986	26	–	–
Joensuu et al, 1986	67	18	27
Mattfeldt et al, 1987	13	6	46
TOTAL	134	33	24

* Adenomas were reclassified as noninvasive low grade follicular carcinomas after finding DNA aneuploidy and after review of the histology.

whereas Greenebaum et al[5] reviewed the histologic diagnosis of 3 aneuploid adenomas, which led to the modified diagnosis of noninvasive low grade follicular cancer in all three.

The mean age of the patients with a diploid thyroid adenoma was lower than that of the rest of the patients. The probability of DNA aneuploidy in differentiated thyroid carcinoma increases with patient age at diagnosis, and the increase is more rapid after the age of 40.[4] Hence, in both benign and malignant thyroid neoplasms DNA aneuploidy appears to be associated with aging.

The flow cytometric studies on parathyroid and adrenal adenomas are also few. Bowlby et al[12] found aneuploidy in 5% (3/56) of parathyroid adenomas, and 21% (12/56) had >15% cells in the tetraploid region, whereas all their 16 adrenal cortical adenomas were diploid.[25] Several authors[26-29] have suggested that DNA aneuploidy correlates with malignant behaviour in adrenal neoplasms, and the presence of aneuploidy has been used to confirm malignancy in a case of adrenal neoplasm without capsular invasion or clinical recurrence.[6] DNA aneuploidy and large DIs were associated with a large adenoma size (Table 2), but no evidence of malignant behaviour was obtained.

One control sample containing microscopically normal thyroid tissue was aneuploid with DI 1.12 in repeated analyses. This patient had a concomitant aneuploid papillary carcinoma. Barlogie et al[14] found DNA aneuploidy in 7 patients with a previously established diagnosis of cancer among the group of 209 patients with normal histology or reactive changes. Reid et al[15] reported aneuploidy in 1 patient with Barrett's esophagus in a sample containing specialized metaplastic epithelium, as did Hammarberg et al[30] in histologically normal mucosa from patients with ulcerative colitis. In some cases DNA aneuploidy may be present even if no abnormality is seen in light microscopy.

The quality of the histograms was good, strict criteria were used for DNA aneuploidy, and precautions were taken to exclude the possibility of artifactual peaks. According to our experience, similar histograms with the same DI are obtained both from fine-needle aspiration biopsy material drawn before surgery and from the corresponding paraffin-embedded tissue.[10,17] Small differences in the DNA content of different types of normal cells[31] do not explain the present findings because only large (>8%) changes were considered to be an unequivocal sign of aneuploidy. Erroneous histologic diagnoses of low-grade carcinoma as adenoma do not explain the result because the adenomas were selected at random, the diagnoses were reviewed by 1 of the authors, and none of the tumors had a clinical course compatible with malignancy.

The number of stemlines with an abnormal DNA content may be larger than now reported because some of the ND peaks may be caused by aneuploid stemlines with DI near the resolution limit of the method, and some of the increased G2/M peaks may be caused by tetraploid stemlines with a low proliferation rate. Chromosomal abnormalities can be demonstrated by chromosome analysis in a considerable proportion of diploid tumors in DNA flow cytometry.[32] Because clonal heterogeneity may occur in some adenomas, more aneuploidy might have been found by analyzing several sections of each block. Stenzinger et al[13] found clonal heterogeneity in 2 congenital melanocytic nevi with flow cytometry.

Based on the present results, aneuploid endocrine adenomas should be relatively common tumors in the general population. For example, in the population of Framingham the prevalence of thyroid nodules was 4.2%, and they were detected at a rate of about 0.1% per year in the general population.[33] Because thyroid carcinomas are rare tumors, most aneuploid thyroid tumors are expected to be histologically benign. In a series of 187 consecutive patients with thyroid tumors, 13 of the operated 63 tumors were aneuploid, and 7 of the 13 aneuploid tumors were histologically benign, although malignant thyroid tumors were overrepresented in the material.[34]

The majority of the aneuploid adenomas had a DI value in the hypotriploid range (mean DI, 1.34), whereas many human carcinomas have a mean DI of aneuploid tumors in the hypertriploid range.[35] The number of S phase cells was constantly low both in diploid and nondiploid tumors. These findings are in agreement with results obtained from fresh material,[34] the percentage of proliferative cells is often larger in malignant thyroid neoplasms than in benign ones, and the DIs larger in aneuploid follicular thyroid carcinomas than in adenomas.

It is concluded that DNA aneuploidy occurs commonly in several types of human endocrine adenomas. The prognosis of aneuploid adenomas appears to be as good as that of diploid ones after conservative surgery, but little is known about their tendency to progress into histologically and clinically malignant neoplasms if left in place. In thyroid neoplasms, the probability of a neoplasm, both histologically benign and malignant, to be aneuploid increases with advancing age. The presence of DNA aneuploidy is compatible with benign histology, and, from the clinical point of view, its use as a definitive criterion for malignancy is questioned.

References

1. Barlogie B, Raber M, Schuman J, Johnson TS, Drewinko B, Swartzendruber DE, Göhde W, Andreeff M,

Freireich EJ: Flow cytometry in clinical cancer research. Cancer Res 1983, 43:3982
2. Büchner Th, Hiddemann W, Wörmann B, Kleinemeier B, Schumann J, Göhde W, Ritter J, Müller K-M, von Bassewitz DB, Roessner A, Grundmann E: Differential pattern of DNA-aneuploidy in human malignancies. Path Res Pract 1985, 179:310–317
3. Friedlander ML, Hedley DW, Taylor IW: Clinical and biological significance of aneuploidy in human tumors. J Clin Pathol 1984, 37:961–974
4. Joensuu H, Klemi P, Eerola E, Tuominen J: Influence of cellular DNA content on survival in differentiated thyroid cancer. Cancer 1986, 58:2462–2467
5. Greenebaum E, Koss LG, Elequin F, Silver CE: The diagnostic value of flow cytometric DNA measurements in follicular tumors of the thyroid gland. Cancer 1985, 56:2011–2018
6. Klein FA, Miller NL, Hackler RH: Flow cytometry in feminizing adrenocortical carcinoma. J Urol 1985, 134: 933–935
7. Anniko M, Tribukait B, Wersäll J: DNA ploidy and cell phase in human pituitary tumors. Cancer 1984, 53: 1708–1713
8. Johannessen JV, Sobrinho-Simoens M, Lindmo T, Tangen KO: The diagnostic value of flow cytometric DNA measurements in selected disorders of the human thyroid. Am J Clin Pathol 1982, 77:20–25
9. Kraemer BB, Srigley JR, Batsakis JG, Silva EG, Goepfert H: DNA flow cytometry of thyroid neoplasms. Arch Otolaryngol 1985, 111:34–38
10. Joensuu H, Klemi P, Eerola E: DNA aneuploidy in follicular adenomas of the thyroid gland. Am J Pathol 1986, 125:373–376
11. Mattfeldt T, Schürmann G, Feichter G: Stereology and flow-cytometry of well differentiated follicular neoplasms of the thyroid gland. Virchows Arch A 1987, 410:433–441
12. Bowlby LS, DeBault LE, Abraham SR: Flow cytometric DNA analysis of parathyroid glands. Am J Pathol 1987, 128:338–344
13. Stenzinger W, Suter L, Schumann J: DNA aneuploidy in congenital melanocytic nevi: Suggestive evidence for premalignant changes. J Invest Dermatol 1984, 82: 569–572
14. Barlogie B, Drewinko B, Schumann J, Göhde W, Dosik G, Latreille J, Johnston DA, Freireich EJ: Cellular DNA content as a marker of neoplasia in man. Am J Med 1980, 69:195–203
15. Reid BJ, Haggitt RC, Rubin CE, Rabinovitch PS: Barrett's esophagus: Correlation between flow cytometry and histology in detection of patients at risk for adenocarcinoma. Gastroenterology 1987, 93:1–11
16. Ingh van den HF, Griffioen G, Cornelisse CJ: Flow cytometric detection of aneuploidy in colorectal adenomas. Cancer Res 1985, 45:3392–3397
17. Klemi PJ, Joensuu H: Comparison of DNA ploidy in routine fine needle aspiration biopsy and paraffin embedded samples. Anal Quantit Cytol Histol, in press
18. Willams ED, Siebenmann RE, Sobin LH: Histological typing of endocrine tumours. International Histological Classification of Tumours No. 23, World Health Organization, Geneva, 1980
19. Meissner WA, Warren S: Tumours of the thyroid gland, Atlas of Tumour Pathology, Second series, Fascicle 4. Edited by HI Firminger. Washington DC, Armed Forces Institute of Pathology, 1968
20. Hedley DW, Friedlander ML, Taylor IW, Rugg CA, Musgrove EA: Method for analysis of cellular DNA content of paraffin-embedded pathological material using flow cytometry. J Histochem Cytochem 1983, 31: 1333–1335
21. Vindeløv LL, Christensen IJ, Nissen NI: A detergent-trypsin method for the preparation of nuclei for flow cytometric DNA analysis. Cytometry 1983, 3:323–327
22. Baisch H, Göhde W, Linden WA: Analysis of PCP-data to determine the fraction of cells in the various phases of the cell cycle. Rad Environm Biophys 1975, 12:31–39
23. Flint A, Lovett EJ, Stoolman LM, McMillan K, Schnitzer B, McClatchey KD, Hudson JL: Flow cytometric analysis of DNA in diagnostic cytology. Am J Clin Pathol 1985, 84:278–282
24. Christov K: Flow cytometric DNA measurements on human thyroid tumors. Virchows Arch (Cell Pathol) 1986, 51:255–263
25. Bowlby LS, DeBault LE, Abraham SR: Flow cytometric analysis of adrenal cortical tumor DNA. Cancer 1986, 58:1499–1505
26. Amberson JB, Vaughan ED, Gray GF, Naus GJ: Flow cytometric analysis of nuclear DNA from adrenocortical neoplasms. Cancer 1987, 59:2091–2095
27. Klein FA, Kay S, Ratliff JE, White FKH, Newsome HH: Flow cytometric determinations of ploidy and proliferative patterns of adrenal neoplasms: An adjunct to histological classification. J Urol 1985, 134:862–866
28. Taylor SR, Roederer M, Murphy RF: Flow cytometric DNA analysis of adrenocortical tumors in children. Cancer 1987, 59:2059–2063
29. Hosaka Y, Rainwater LM, Grant CS, Farrow GM, Heerden van JA, Lieber MM: Pheochromocytoma: Nuclear deoxyribonucleic acid pattern studied by flow cytometry. Surgery 1986, 100:1003–1009
30. Hammarberg C, Slezak P, Tribukait B: Early detection of malignancy in ulcerative colitis. Cancer 1984, 53: 291–295
31. Iversen OE, Laerum OD: Trout and salmon erythrocytes and human leukocytes as internal standards for ploidy control in flow cytometry. Cytometry 1987, 8: 190–196
32. Smeets AWGB, Pauwels RPE, Beck JML, Geraedts JPM, Debruyne FMJ, Laarakkers L, Feitz WFJ, Vooijs GP, Ramaekers FCS: Tissue-specific markers in flow cytometry of urological cancers. III. Comparing chromosomal and flow cytometric DNA analysis of bladder tumors. Int J Cancer 1987, 39:304–310
33. Vander JB, Gaston EA, Dawber TR: The significance of nontoxic thyroid nodules: Final report of a 15 year study on the incidence of thyroid malignancy. Ann Intern Med 1968, 69:537–540
34. Joensuu H, Klemi PJ, Eerola E: Diagnostic value of DNA flow cytometry combined with fine needle aspiration biopsy in thyroid tumors. Anal Quantit Cytol Histol 1987, 9:328–334
35. Tribukait B: Clinical DNA flow cytometry. Med Oncol Tumor Pharmacother 1984, 1:211–218