DNA Ploidy and Cell Phase in Human Pituitary Tumors

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The flow-cytofluorometric technique for nuclear DNA analysis was used in the study of 47 consecutive cases with pituitary tumors. The material consisted of 18 tumors secreting growth hormone (GH), 10 secreting GH and prolactin (PRL), 10 prolactinomas, 2 secreting thyroid-stimulating hormone (TSH), 2 secreting ACTH, and 5 were nonsecreting. An aneuploid DNA pattern occurred in 23 of 47 (49%) cases ranging from 1.4 to 3.6 N thus deflecting from the normal diploid 2N chromosome content of somatic nonreproductive cells. In four tumors two cell lines occurred, but one of them was always dominant, comprising 80% to 90% of all cells analyzed which totally amounted to 20,000 to 30,000 cells. The highest percentage of aneuploid tumors occurred in those with a concomitant secretion of GH and PRL (8 of 10, 80%). The percentage of diploid cells in tumors with an aneuploid nuclear DNA content was very low, 2.3 ± 2.9% (+0.65% SE). In 11 cases with aneuploidy, diploid cells were not detected. The mean age in patients with diploid and aneuploid tumors was rather similar in the different endocrinologic types of tumors.


PITUITARY TUMORS, independent of endocrinologic type, exhibit large variations in the degree of hormonal activity and in the local growth pattern. The morphology of pituitary tumors shows a benign structure except in extremely few cases with malignancies.1–3 So far, it has not been possible to correlate any parameter to the clinical course of pituitary tumor disease.

In malignant solid tumors, the number of chromosomes in tumor cells often differs from the normal number of 46. Very often not only the number but also the structure of chromosomes is changed.4–6 An indirect method of establishing different chromosomal numbers and of utilizing this criterion in the assessment of a large number of tumor cells is offered by the cytophotometric measurement of cell nuclei, i.e., DNA analysis with regard to ploidy level and the percentage of cells in different phases of the cell cycle.7,8

There are no general correlations between the nuclear DNA pattern and the morphologic growth pattern and/or criteria for malignancy. This varies considerably between different types of solid tumors. Thus, each type of tumor including subgroups has to be evaluated individually with regard to nuclear DNA ploidy and proliferation rate. Recently, Anniko and associates10,11 showed that an aneuploid DNA pattern occurred in many morphologically benign pituitary tumors. Studies on the DNA pattern in human pituitary tumors are few.

The aim of the current study was to analyze a large number of pituitary tumors with regard to DNA ploidy and percentage of cells in different phases of the cell cycle. Correlations of the DNA pattern to a large number of clinical parameters have been made.

Patients and Methods

Patients

This study is based on 47 consecutive patients with pituitary tumors operated on at the Department of Oto-Rhino-Laryngology, Karolinska Hospital, Stockholm, Sweden. The hormonal types of tumor and age of the patients at operation are illustrated in Table 1 and Figures 1A to 1C.

Methods

All patients with pituitary tumors underwent a complete endocrinologic, ophthalmologic, and roentgenologic investigation.12

The genetic material is represented by the number of chromosome sets (N) which is 2N (diploid) in most somatic cells. An indirect calculation of the amount of chromosome material in a tumor or normal cell nucleus can be achieved by analysing its DNA content with a cytophotometric method. These analyses can either be done on identified cells with a single-cell method or with

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TABLE 1. Age of Patients With Pituitary Tumors Subjected to DNA Analysis After Surgery

<table>
<thead>
<tr>
<th>Type of tumor</th>
<th>&lt;20</th>
<th>20-29</th>
<th>30-39</th>
<th>40-49</th>
<th>50-59</th>
<th>60-69</th>
<th>&gt;70</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td></td>
<td>18</td>
</tr>
<tr>
<td>GH + PRL</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>PRL</td>
<td>3</td>
<td>3</td>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>TSH</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>ACTH</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Nonsecreting</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>1</td>
<td>12</td>
<td>14</td>
<td>13</td>
<td>3</td>
<td>3</td>
<td>47</td>
</tr>
</tbody>
</table>

GH: growth hormone; PRL: prolactin; TSH: thyroid-stimulating hormone; ACTH: adrenocorticotropic hormone.

Results

The occurrence of diploid and aneuploid pituitary tumors with regard to age at operation and sex is illustrated in Figures 1A–1C and 2. Aneuploid tumors occurred in a total of 23 of 47 (49%) cases. Patients with pituitary tumors, except in two cases, were found in the 3 decades between 30 and 60 years of age. Patients between 30 and 39 years of age at operation had aneuploid tumors in 4/12 (33%) cases. In the two following decades aneuploidy occurred in 10/14 (71%) and 7/13 (54%) cases, respectively. The mean age in patients with diploid and aneuploid tumors was rather similar in the different endocrinologic types of tumors: growth hormone (GH) secreting, 49 versus 52 years; GH and PRL secreting, 43 versus 41 years; prolactinomas, 49 versus 49 years; and nonsecreting tumors, 53 versus 49 (1 case) years. The occurrence of aneuploid tumors in the two sexes was rather similar with regard to age, except in the period 30 to 39 years of age. Aneuploidy was found in 4/8 (50%) women but in 0/4 (0%) in men.

The ploidy level of the different endocrinologic types of tumors and in the total material is illustrated in Figures 2 and 3. Pituitary tumors with an aneuploid nuclear DNA content occurred mainly in acromegaly with a concomitant secretion of GH and PRL (8/10, 80%). In tumors secreting GH or PRL only, an aneuploid DNA level occurred in 6/18 (33%) and 7/10 (70%) cases, respectively. Only 1/5 (20%) of nonsecreting tumors showed an aneuploid DNA content. A hypodiploid ploidy occurred only in tumors with a concomitant secretion of GH and PRL. (3/13, 23% of the cell lines). The percentage of diploid cells was very low in our 22/24 cases with aneuploid nuclear DNA content, 2.3 ± 2.9%. In 11 cases diploid cells were not detected.

In four pituitary tumors two cell lines occurred with different DNA ploidy levels. In these four tumors one cell line always dominated comprising 80% to 90% of all cells analyzed, which totally amounted to 20,000 to 30,000 cells. In the tumor secreting both GH and PRL with 19.1% diploid cells and an aneuploid cell line at N = 2.4 the latter dominated.

There is no relationship between the age of the patient at operation and the ploidy level of the pituitary tumor.

A comparison of pituitary tumors with a high DNA ploidy (N = 3-4) and a near diploid level (N = 2.2) is shown in Tables 2 and 3. Both groups comprise six cases. The mean age in the high ploidy level group was 42 years and 51 years in the near-diploid group. Patients with acromegaly had low or normal GH serum levels in the N = 2.2 group (three cases) in contrast to a 6-to-10-fold increase in the high ploidy group (four cases). The latter group contained three cases with a concomitant secretion of GH and PRL (3/8 aneuploid tumors of this type). The percentage of cells in different phases of the cell cycle was similar between tumors of the two DNA ploidy levels.

The serum hormone levels of GH and PRL in tumors causing acromegaly or prolactinomas could not be correlated to the DNA ploidy.

Discussion

Pituitary neoplasms are generally considered as benign tumors. Only in extremely rare cases does metastasis occur. Clinically severe signs and symptoms can occur due to hormone secretion and/or to local growth pattern of the tumor. Despite the benign histologic features of pituitary tumors the DNA values in many tumors, as shown in this study, are characteristic of malignant neoplasms.

Objective methods for the determination of genetic material include chromosome analyses, DNA determination in individual cells, scanning methods, and flow-cytometric methods, the last of these has the advantage that it allows the analysis of a very large number of cells. The certainty of the assessment of the degree of ploidy is thus increased. Furthermore, this method makes an automatized flow technique on a large number of morphologically unidentified cell^13,14 In the current study flow-cytofluorometry has been performed. The method used for cell preparation, fixation and staining for DNA analysis has been described earlier.13,14
it possible to assess the degree of proliferation, which is a significant biological parameter in the case of tumor diseases. Quantitative DNA analysis of tumor cell nuclei makes it possible to detect gross chromosomal abnormalities. An important limitation with this method is also that an aneuploid DNA pattern may not always necessarily represent true aneuploidy if most or a substantial portion of the cells are in various stages of the S-phase.

However, this was not the case in our material. Minor changes in diploid cells such as marker chromosomes can only be determined by chromosome analysis. Such studies by Mark\textsuperscript{15,16} showed that an aberrant chromosome pattern occurred in several pituitary tumors, i.e., the tumors have to be classified as pseudodiploid, but without any relation to malignant transformation.

The DNA distribution pattern of pituitary tumors shows both diploid and aneuploid tumors, the latter with a high variability of DNA content without obvious subgroupings. Many of the aneuploid pituitary tumors have a DNA content in the near-diploid region. Thus, the characteristic DNA distribution pattern (profile) of pituitary tumors in general is in the 2N or near 2N region with only a few exceptions. Diploid pituitary tumors can contain up to 19.9% tetraploid cells. The normal percentage of tetraploid cells in diploid tumors is $6.3 \pm 2.3\%$; range, 2.4% to 11.4% tetraploid cells (Anniko and Tri-bukait, unpublished observations, 1981; 24 diploid tumors).

In our material, three cell lines with a hypodiploid DNA ploidy occurred. Such a feature is extremely rare,
independent of type of tumor. However, the biological significance of the different degrees of aneuploidy is as yet unknown. A higher ploidy level is often reflected by an increased metabolic activity of the tumor. However, so far it is not known which part of the genome is responsible for hormone secretion in pituitary tumors.

Flow-cytometric analysis by Kawamoto and associates showed 67% to 90% diploid cell in nine pituitary tumors and with remaining cells in a continuous range from diploid to tetraploid rather than forming an S-phase and tetraploid peaks. We have earlier analyzed both the DNA ploidy and the percentage of cells in each cell phase in a total of 29 pituitary tumors. Tumors were subgrouped into diploid and aneuploid. Minimal and moderate degrees of mitosis and nuclear polymorphism occurred more frequently in aneuploid tumors than in diploid tumors. The ploidy levels and percentage of cells in different phases of the cell cycle remained constant during 1 to 10 days in organ culture.

In the current study, several clinical parameters were analyzed with regard to DNA ploidy pattern, e.g., patient age, sex, endocrinologic type of tumor, and serum hormone level. Pituitary tumors with secretion of PRL, alone or concomitantly with GH, revealed an aneuploid nuclear DNA pattern more often than any other hormone type of tumor. The group of tumors with a concomitant secretion of PRL and GH comprised 10 cases with a total of 13 cell lines. In addition, the three cases with a hypodiploid DNA ploidy also occurred in this group. As compared with all other endocrinologic types of pituitary tumors, the variability of the DNA pattern in this group was considerable.

### Table 2. Clinical Data on Patients With Pituitary Tumors With a DNA Ploidy of 3-4

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age/sex</th>
<th>Tumor type</th>
<th>GH (µg/ml)*</th>
<th>PRL (µg/L)†</th>
<th>DNA ploidy</th>
<th>G1 (%)</th>
<th>G2 (%)</th>
<th>S (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43/F</td>
<td>GH</td>
<td>118 pmol/L</td>
<td>Normal</td>
<td>3.4</td>
<td>89.9</td>
<td>4.9</td>
<td>5.2</td>
</tr>
<tr>
<td>2</td>
<td>33/F</td>
<td>GH+PRL</td>
<td>110</td>
<td>172</td>
<td>3.6</td>
<td>89.7</td>
<td>5.2</td>
<td>5.1</td>
</tr>
<tr>
<td>3</td>
<td>47/F</td>
<td>GH+PRL</td>
<td>100</td>
<td>38</td>
<td>3.2‡</td>
<td>86.8</td>
<td>8.6</td>
<td>5.2</td>
</tr>
<tr>
<td>4</td>
<td>34/F</td>
<td>GH+PRL</td>
<td>2183 pmol/L</td>
<td>29</td>
<td>3.2</td>
<td>87.2</td>
<td>6.9</td>
<td>10.3</td>
</tr>
<tr>
<td>5</td>
<td>48/M</td>
<td>PRL</td>
<td>Normal</td>
<td>145</td>
<td>3.2</td>
<td>88.8</td>
<td>3.7</td>
<td>7.4</td>
</tr>
<tr>
<td>6</td>
<td>49/M</td>
<td>Nonsecreting</td>
<td>—$</td>
<td>—$</td>
<td>3.0</td>
<td>85.2</td>
<td>6.4</td>
<td>8.4</td>
</tr>
</tbody>
</table>

* Reference <9.  
† Reference <25.  
‡ This tumor comprised two stem lines with ploidy levels of 3.2 (91.6%) and 2.2 (8.4% of the cells).  
§ Panhypopituitarism was diagnosed.

GH: growth hormone; PRL: prolactin.

### Table 3. Clinical Data on Patients With Pituitary Tumors With a DNA Ploidy of 2.2

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age/sex</th>
<th>Tumor type</th>
<th>GH (µg/ml)*</th>
<th>PRL (µg/L)†</th>
<th>TSH (µU/ml)‡</th>
<th>G1 (%)</th>
<th>G2 (%)</th>
<th>S (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45/F</td>
<td>GH</td>
<td>629 pmol/L</td>
<td>Normal</td>
<td>Normal</td>
<td>85.2</td>
<td>6.7</td>
<td>8.1</td>
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<tr>
<td>2</td>
<td>55/M</td>
<td>GH</td>
<td>132</td>
<td>Normal</td>
<td>Normal</td>
<td>83.7</td>
<td>7.9</td>
<td>8.4</td>
</tr>
<tr>
<td>3</td>
<td>56/F</td>
<td>GH</td>
<td>4</td>
<td>Normal</td>
<td>Normal</td>
<td>74.5</td>
<td>6.9</td>
<td>18.6</td>
</tr>
<tr>
<td>4</td>
<td>49/F</td>
<td>PRL</td>
<td>Normal</td>
<td>73</td>
<td>Normal</td>
<td>86.1</td>
<td>5.4</td>
<td>8.5</td>
</tr>
<tr>
<td>5</td>
<td>58/M</td>
<td>PRL</td>
<td>Normal</td>
<td>28</td>
<td>Normal</td>
<td>79.8</td>
<td>17.1</td>
<td>3.1</td>
</tr>
<tr>
<td>6</td>
<td>43/F</td>
<td>TSH</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>81.8</td>
<td>10.9</td>
<td>7.3</td>
</tr>
</tbody>
</table>

* Reference <9.  
† Reference <25.  
‡ Reference <7.

GH: growth hormone; PRL: prolactin; TSH: thyroid-stimulating hormone.
From our total material of 47 cases, no absolute correlations were possible to establish in subgroups, i.e., different endocrinologic types of pituitary tumors as described for some other types of solid tumors, e.g., in the bladder carcinoma and osteosarcomas. The comparison of pituitary tumors with an aneuploidy level at \( N = 3 \) to 4 (six cases) respectively near-diploid, \( N = 2.2 \) (six cases), showed that patients in the former group in general had a higher serum hormone level independent of endocrinologic type of tumor. These patients also had an estimated shorter duration of pituitary tumor disease, approximately 4 to 8 years. Patients with the near-diploid nuclear DNA level had an estimated duration of disease between 10 and 20 years. The increased serum hormone level can possibly reflect an increased metabolic function. However, the degree of pituitary tumor disease outside the sella turcica is not reflected merely by the serum hormone level, but also in the peripheral response to hormonal stimulation.

Pituitary tumors with a high percentage of S-phase cells appear to have a more aggressive clinical course than tumors with a small fraction of S-phase cells. A high proportion of S-phase cells occurred only in aneuploid tumors (six cases). In several tumors there is a connection between nuclear DNA content and the malignancy of the individual tumor. However, Ganzer found no correlation between the DNA distribution patterns and the histologic grade in malignant tumors of the head and neck region.

The clinical significance of the DNA pattern in tumors is still to be evaluated and is likely to vary between different types of tumors. In several types of solid tumors, therapy and prognosis seem to have an intimate relationship to the ploidy pattern. Further studies are warranted to analyze the nuclear DNA characteristics in a large material and a long-term clinical follow-up of the cases before flow-cytomfluorometric data on pituitary tumors can be used in clinical diagnosis and not remain limited to basic research.

Conclusions

The total material of 47 cases comprised 24 cases with a diploid and 23 cases with an aneuploid DNA pattern. With the flow-cytomfluorometric technique it is not possible to investigate if the diploid group of pituitary tumors comprises diploid tumors only or also those with a pseudodiploid pattern.

Aneuploidy occurred mostly in tumors with a concomitant secretion of GH and PRL (8/10, 80% cases).

Tumors with an aneuploid DNA content contained 2.3 ± 2.9% diploid cells. In 11 cases with aneuploidy, diploid cells were not detected.

Tumors comprising two cell lines occurred in 4/47. One cell line always dominated comprising 80% to 90% of all tumor cells analyzed, which totally amounted to 20,000 to 30,000 cells.

In the total material no distinct correlation exists between degree of ploidy and serum hormone levels. However, the comparison of aneuploid pituitary tumors with a ploidy level of 3 to 4 with a near-diploid revealed that in the former group all six cases had a high serum hormone level. In contrast, the near-diploid group of tumors (six cases) all showed normal or near-normal serum hormone levels.

Patients with a pituitary tumor with an aneuploid DNA pattern had an estimated case history of 4 to 8 years in contrast to patients with diploid or near-diploid tumors with an estimated duration of disease between 10 and 20 years.

REFERENCES

17. Tribukait B, Hammarberg C, Rubio C. Ploidy and proliferation in colorectal adenocarcinomas related to Dukes’ classification and to...


20. Gustafsson H. DNA analysis by flow-cytofluorometry in carcinoma of the urinary bladder, thesis. Department of Urology Karolinska Hospital and Department of Medical Radiobiology, Karolinska Institute, Stockholm, Sweden, 1982.

21. Kreicbergs A. Malignancy grading of chondrosarcoma. A cytochemical, morphological and clinical study, thesis. Department of Orthopaedic Surgery and Department of Tumour Pathology, Karolinska Hospital and Department of Medical Radiobiology, Karolinska Institute, Stockholm, Sweden, 1982.


