DNA and S-phase representation in human growth hormone producing pituitary adenomas

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Abstract. The DNA contents of 33 pituitary adenomas from patients with acromegaly were analysed with flowcytofluorometry. Degrees of ploidy and the proliferation rate, expressed as percentage of cells in S-phase, were determined. The aim was to compare these morphological and functional tumour properties with clinical and laboratory parameters to establish a possible relation and to further elucidate the characteristics of these tumours. In 15 tumours (45%) diploid DNA pattern were found, while 18 (55%) showed varying degrees of aneuploidy. The frequency of cells in S-phase showed wide variations and were equally distributed in diploid and aneuploid tumours. Duration of symptoms, age at diagnosis, preoperative growth hormone (GH)- and prolactin (Prl)-levels, tumour size and grade of invasive tumour growth as determined by radiological estimations, did not correlate to ploidy or grade of proliferation.

The lack of correlation between DNA pattern and proliferation rate in relation to clinical, laboratory and radiological parameters in tumours causing acromegaly contrasts to the documented relation between the degree of ploidy, cells in proliferation and grade of malignancy reported in tumours of other sites. The 55% aneuploid GH producing tumours indicate a certain malignant transformation. The high frequency of cells in S-phase in several GH secreting tumours completes the malignant morphological and functional cell properties. The benign character of tumours causing acromegaly is therefore in contrast to these findings.

The lack of clinical significance of the DNA pattern and the frequency of cells in proliferation in GH producing tumours and the benign character despite malignant cell properties in most of these tumours are difficult to explain. The possibility of unknown factors regulating growth rate, invasiveness and the lack of metastazation in tumours causing acromegaly cannot be excluded.

The DNA pattern of tumours from various sites has been related to their malignancy (for review, see Sandberg 1980; Barlogie et al. 1983; Tribukait 1984a) and in addition the amount of cells in proliferation may predict the growth of a tumour and its clinical course (Gustafsson et al. 1982; Hansson et al. 1982; Tribukait 1984b).

Experience from analysis of the DNA content from malignant tumours show that they can deviate slightly from the diploid modes of chromosomes or they may also show gross chromosomal aberrations (Sandberg 1980). The malignant qualities of clinically evaluated tumours include growth rate, invasiveness and metastases. Grade of malignancy has been compared to the existence of diploid DNA pattern or gross chromosomal aberrations in some tumour forms. Generally, in tumours from various sites, a better survival was found for diploid tumours with the exception of carcinomas of the cervix (Atkin & Kay 1979; Tribukait 1984b; Atkin 1984; Fu et al. 1982).

The possible biological significance of the proliferative properties of tumours can be calculated from the clinical follow-up of the patients. The proportion of S-phase cells in bladder tumours, irrespective of treatment modality, is related to death in tumour disease. Patients with tumours containing <10% of cells in S-phase survived in 95% over a 4-year-period, whereas survival in cases with tumours in which S-phase exceeded 20% was only 35%. Furthermore, in cervix carcinomas, where diploidy or aneuploidy was not related to survival, increasing frequencies of S-phase cells was
correlated to shorter survival with a high significance (Tribukait 1984b). Thus, the degree of ploidy and frequency of cells in proliferative phase may reflect basic tumour characteristics.

Pituitary adenomas of varying endocrine activity including growth hormone (GH) producing tumours have been studied with regard to DNA pattern and proliferation rate. Diploid as well as aneuploid DNA contents and low to high proportions of cells in proliferative phase have been found in GH-, prolactin (Prl)- and non-secreting pituitary adenomas (Anniko et al. 1981a,b, 1983).

The aim of the present work was to study the nuclear DNA contents and the amount of cells in proliferation in GH producing pituitary tumours in relation to the duration of the disease, signs and symptoms, secretory activity as reflected by hormone levels as well as tumour size and the degree of invasive tumour growth based on radiological findings in the sella region.

Material and Methods

During the period 1980–1984, 33 consecutive patients, 6 men and 27 women with acromegaly due to pituitary adenomas, were evaluated before and after transsphenoidal microsurgery. The patients were evaluated with regard to duration of symptoms, pre- and post-operative plasma GH and serum Prl levels. The roentgenological examination included plain sellar films, encephalography and computed tomography. Tumour size was estimated based on tomographic films from pre-operative encephalographies. The largest diameter of the tumour was measured and the tumour was graded as class I, when the diameter was < 15 mm; class II ≥ 15–25 mm, class III ≥ 25–< 35 mm and class IV tumours with sella diameter ≥ 35 mm. The degree of invasive tumour growth was based on the presence of local bone erosions such as demineralisations and erosions, (−) denotes adenomas with no signs of invasive growth, (+) tumours with moderate signs of invasiveness and (+++) denotes tumours with large destructions of the sella and surrounding bone. Visual acuity and perimetry were performed in all patients. GH and Prl levels given in tables and figures are the means of 4 fasting morning samples. GH was determined by a double antibody radioimmunoassay (RIA) (Cerasi et al. 1966), and Prl by a commercial RIA-kit (Biodata, Switzerland).

After removal, one piece of the pituitary adenoma was taken for light microscopy, which in all cases showed pituitary adenomas. Another piece of the pituitary tumour was placed in isotonic sterile saline solution at room temperature for DNA-analysis with a rapid flow-cytofluorometer technique (Tribukait et al. 1975). The material was processed by squeezing it through fine mesh nylon gauze with TRIS-EDTA buffer, pH 7.5. After centrifugation, the cell material was fixed in 96% ice-cold ethanol. The fixed cells were washed in a TRIS-EDTA buffer with 1 mg/ml RNA-ase, a method which has been proved in various cell systems to remove all RNA. A suspension of single-cell nuclei was obtained by pepsin treatment, thus eliminating all non-specific fluorescence. After washing in the buffer, the nuclei were stained using 2.5 × 10⁻⁵ M ethidium bromide in TRIS-EDTA buffer with a molarity of 395 mOsm. This high molarity further reduces the risk of unspecific binding of ethidium bromide. All preparations were checked microscopically. The DNA contents of the cell nuclei were then analysed using a rapid flow-cytofluorimeter ICP II (Phywe, West Germany, now Ortho instruments, USA) with a flow rate of up to 1000 cells/sec. The excitation and emission wave-lengths were 455–490 nm and 590–630 nm, respectively. The output was sorted with a 256 multi-channel analyser and the number of cells in proliferation, S-phase, was calculated. The results were corrected for background noise. The degree of aneuploidy was calculated in relation to the DNA contents of normal human lymphocytes, given the value 2c, denoting the normal diploid DNA content. The average number of cells analysed for each histogram was 20 000.

\[ \chi^2 \]-analysis was used in the statistical evaluations.

Results

The patients were grouped according to tumour diploidy and aneuploidy (Table 1). The two groups were similar with regard to age, sex, duration of symptoms and pre-operative GH levels. The mean GH levels was 47 µg/l, median 35 µg/l and range 5–225 µg/l in patients with diploid tumours. The corresponding levels in patients with aneuploid tumours were 41, 26 and 7–181 µg/l. The frequency of patients with concomitant hyperprolactinaemia was similar in the two groups, 47% (7/15) in patients with diploid and 39% (7/18) in patients with aneuploid tumours. Tumour size, degree of invasive tumour growth, frequency of suprasellar extensions and visual field defects were also similar in the two groups (Table 1).

The frequency of cells in S-phase varied in diploid tumours between 3.5–17.8% and in the aneuploid group from 3.3 to 19.5%. Twenty-seven per cent (4/15) of the diploid tumours and 44% (8/18) of the aneuploid tumours showed high proliferation rate, i.e. ≥ 10% of the cells in S-phase. These 12 adenomas with a frequency of cells in
Clinical, hormonal, roentgenological data and the frequency of pituitary cells in S-phase in 33 patients with acromegaly. The patients were numbered according to their pre-operative GH levels. Fourteen patients with pre-operative hyperprolactinaemia, S-Prl > 25 µg/l, are indicated by numbers in italics. Patients No. 1–15 had tumours with diploid DNA pattern, No. 16–33 tumours with aneuploid DNA pattern.

### Diploid adenomas

<table>
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<th>S-Prl</th>
<th>Tumour class</th>
<th>Grade of invasive tumour growth</th>
<th>Suprasellar extension</th>
<th>Visual field defects</th>
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| Mean         | 41  | 8.9 | 47                         |                           |       |              |                               |                       |                     | 8.1       |
| range        | 25–62| 2–20| 5–225                      | 5–60                      |       |              |                               |                       |                     | 3.5–17.8  |

### Aneuploid adenomas

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| Mean         | 47  | 9.2 | 41                         |                           |       |              |                               |                       |                     | 9.2       |
| range        | 33–60| 1–30| 7–181                      | 5–560                     |       |              |                               |                       |                     | 3.3–19.5  |
Duration of clinical symptoms in 33 patients with acromegaly related to increasing tumour size, expressed as class I-IV.

Fig. 2.
Degree of invasive tumour growth in 33 patients with acromegaly correlated to increasing tumour size, expressed as class I-IV.
S-phase $\geq 10\%$ (patients No. 1, 6, 9, 15, 18, 19, 22–24, 26, 29, 33) did not differ from the other 21 adenomas in the material with regard to DNA contents, age at diagnosis, duration of symptoms, GH levels, hyperprolactaemia, tumour size or invasive tumour growth (Table 1).

The size of the pituitary tumour did not correlate to the subjective duration of clinical symptoms referable to acromegaly (Fig. 1). There was a correlation ($P < 0.01$) between tumour size and degree of invasive tumour growth, i.e., tumour invasiveness was more often seen in large tumours (Fig. 2). Patients with large tumours (class III-IV) had higher GH levels than patients with small tumours (class I-II) ($P < 0.05$, Fig. 3). In contrast, there was no correlation between GH levels and tumour invasiveness (Fig. 4).

There was no relation between GH levels and the frequency of cells in S-phase (Fig. 5) or between tumour size and proliferation rate (Fig. 6). Furthermore, there was no correlation between tumour invasiveness and S-phase representation (Fig. 7). Of the three adenomas with the highest frequency of cells in S-phase one was small (Class II) and two were large tumours (Class III) (Fig. 6). All three showed invasive tumour growth (Fig. 7).

4/18 aneuploid tumours showed two cell lines, i.e., two populations of DNA (patients No. 16, 20, 24, 27; Table 1). The frequency of cells in S-phase in these tumours was 5.2, 6.3, 10.9, and 7.9%, respectively. These 4 patients varied in age at diagnosis, duration of symptoms, tumour size and invasive tumour growth and the tumours showed isolated GH as well as GH and Prl hypersecretion. However, in the group of tumours with isolated GH secretion, 1/11 (9%) aneuploid tumours showed two aneuploid cell lines while 3/7 (43%) of tumours with GH and Prl secretion had two aneuploid cell lines (Fig. 8).
The amount of cells in proliferation, S-phase, related to plasma-GH in 33 patients with acromegaly.

Fig. 5.

The frequency of cells in proliferation, S-phase, in 33 patients with acromegaly, correlated to increasing tumour size, expressed as class I-IV.

Fig. 6.

The amount of cells in proliferation, S-phase, in relation to degree of invasive tumour growth in 33 patients with acromegaly.

Fig. 7.
of metastases
GH
benign
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adenomas.
with
DNA
clearly
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levels
near-diploid
malignant
Melmed
diagnosis,
turn
frequency
producing
contents
GH
shows
than
with
pituitary
al.
(n = 33)
lines.
which
were
abnormally
found
in
GH+
PRL
secreting
adenomas. Lines are drawn to connect four tumours containing two cell lines.

Discussion
GH producing pituitary adenomas grow slowly, metastases are exceedingly rare and, at the time of diagnosis, the beginning of the disease can usually be traced back several years (for review, see Melmed et al. 1983; Hulting et al. 1982). The benign character of pituitary GH producing adenomas is further underlined by our finding that the frequency of tumour invasiveness tended to increase with increasing size of the adenomas which in turn was associated with higher plasma GH levels than the smaller adenomas. However, this study shows that GH producing pituitary adenomas, despite their benign clinical course, display a notable frequency of aneuploid tumours, 55%, clearly indicating abnormally high number of chromosomes which are found preferently only in malignant tumours. A malignant transformation of near-diploid pituitary adenomas has also been demonstrated by direct cytogenic examination of chromosomes (Mark 1971). Separation of acidophil adenomas into densely granulated and sparsely granulated varieties has shown that the former have a high hormone content and regular nuclei and the latter a low hormone content and pleomorphic nuclei (Lewis & van Noorden 1972; Ezrin et al. 1982; Trouillas et al. 1980). Clinical parameters such as duration of symptoms and age at diagnosis, GH and PRL levels, as well as radiological findings regarding tumour size and invasive tumour growth, did not differ in patients with diploid tumours compared to those with aneuploid tumours. Whether tumours secreting both GH and PRL really exhibit in a higher frequency two aneuploid cell lines has to be proven in a larger material. Therefore, the analysis of the DNA contents of these tumours was of no prognostic value for the clinical aspects of the disease. This is in contrast to the relation between aneuploidy and malignancy seen in solid tumours from other sites with the exception of squamous cell carcinomas of the cervix as already mentioned (Atkin & Kay 1979; Tribukait 1984b; Friedlander et al. 1983, 1984; Schwabe et al. 1983; Coulson et al. 1984; Atkin 1984; Fu et al. 1982; Auer & Zetterberg 1984; Wolley et al. 1982).

12/33 adenomas showed high proliferation rate, i.e. the adenomas had ≥ 10% of the cells in S-phase. Four tumours showed diploid and eight tumours aneuploid DNA patterns and they represented 4/15 (27%) of the diploid tumours and 8/18 (44%) of the aneuploid adenomas. Since the proportion of diploid cells in aneuploid tumours was not more than about 2.5% (Anniko et al. 1983), it is justified to assume that also in diploid tumours normal cells are negligible and, thus, do not influence the estimation of the proportion of S-phase of diploid tumours (Tribukait 1984b). Thus, a high frequency of cells in S-phase was not confined to adenomas with aneuploid DNA contents. Interestingly, the 12 patients with highly proliferative adenomas were not characterized by any particular clinical, laboratory or radiological findings indicating biologically aggressive tumours. Therefore, in acromegaly no conclusions regarding the clinical course can be made from the amount of cells in proliferation. This is in contrast to previous reports on bladder carcinomas and melanomas (Gustafsson et al. 1982; Tribukait 1984a; Hansson et al. 1982), indicating that patients with a proportion of S-phase cells ≥ 10% had significantly shorter sur-
vival times. Also with carcinomas of the cervix (Tribukait 1984b) a positive correlation has been documented between cells in S-phase and death in tumour disease. One may therefore speculate whether the amount of cells in S-phase estimated from the cellular DNA-contents in pituitary adenomas really represents cells in proliferation or whether these cells are in fact resting in S-phase.

The morphological and the functional tumour cell properties in combination, i.e. the degree of ploidy and the amount of cells in S-phase in one tumour taken together, were not related to any of the clinical or laboratory parameters that were investigated in GH producing tumours. One explanation may be that genetically based basal tumour characteristics may prevent functional cell properties to be clinically expressed.

However, the lack of correlation of the DNA pattern and proliferation rate in GH producing tumours in comparison to clinical and laboratory parameters contrasts to the findings in tumours from other sites and remains to be elucidated. The benign clinical character of tumours causing acromegaly despite a large proportion of aneuploid tumours and several tumours with high proliferation rate also has to be explained. The possibility of unknown factors regulating growth rate, invasiveness and the lack of metastazation in these tumours cannot be excluded.

Acknowledgments
This work was supported by grants from the Swedish Medical Research Council (grant No. 6865), the Bergvall's Foundation, Swedish Society of Medical Sciences, Söderbergs Foundation and The Cancer Society of Stockholm.

References


Received on December 19th, 1984.