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Ploidy Level Determinations in High-Grade and Low-Grade Malignant Variants of Prostatic Carcinoma[1]

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ABSTRACT

Ploidy level determinations of prostatic carcinoma in 213 patients selected on the basis of survival time were analyzed. Two extreme groups were chosen: extreme group 1, 131 patients who died from prostatic cancer within 3 years of diagnosis; and extreme group 2, 82 patients who survived for more than 15 years after diagnosis. All patients were diagnosed by fine needle aspiration biopsy. The DNA measurements were performed on Feulgen stained, destained May-Grünwald-Giemsa smears. The DNA distribution patterns were studied in three benign prostatic lesions as a base for analyses of prostatic carcinoma. By choosing two extreme groups it was possible to evaluate in detail the optimal limits for defining the diploid 2c region and the tetraploid 4c region. Various limits were tested in order to determine those which most clearly separate extreme group 1 from extreme group 2. We found that the optimal upper diploid limit was 2.5c and the optimal tetraploid limits were 3.5c–4.5c.By using these limits to determine the percentage of aberrant tumor cells, *i.e.*, non-2c and non-4c, and combining this with the modal value (in c units) of the tumor cell population the tumors could unambiguously be divided into D-, T-, and A-types. D/T-type tumors were found only in extreme group 2 and had <30% aberrant tumor cells, while A-type tumors (high-grade aneuploid) had >50% non-2c and non-4c tumor cells. All A-type tumors were found in extreme group 1.

In order to investigate whether the classification of tumors into D-, T-, and A-type was valid in general and could also be applied to patients with survival time between the two extreme groups (3–15 years), a material of 79 patients with a wide range of survival times was tested. The tumors were classified according to the above-mentioned criteria into A-, D-, and T-type tumors. All patients who died within 5 years of diagnosis had A-type tumors. All patients who lived >5 years from diagnosis had D- or T-type tumors.

INTRODUCTION

Nuclear DNA content, as determined by quantitative cytophotometry, is an important prognostic feature in tumors of the prostate (1–4), as well as in several other tumor types (5–20). Tumors with abnormally increased, clearly aneuploid DNAvalues generally show a more malignant clinical course than tumors with predominantly diploid (or near diploid) DNA values. In most of the above-cited studies, performed with slide cytophotometric methods, frequency of cells with elevated DNA values above the normal diploid level (usually more than 2.5c units) was used successfully as a gross parameter of aneuploidy. However, in prostatic carcinoma (1, 4) as well as in breast cancer (1, 4) some of the tumors with abnormally high DNA contents, possibly representing tetraploid (or near tetraploid) tumors, show a more favorable clinical course than most of the tumors with abnormally increased, clearly aneuploid DNA values.

The degree of prognostic information, provided by ploidy level, as determined from nuclear DNA content, in relation to other prognostic parameters is still somewhat unclear. One reason for this is that different methods for DNA determinations and different definitions of ploidy derived from DNA data have been used (4, 7, 12, 14, 17, 19, 21–23).

The present study was undertaken to work out the optimal DNA criteria, obtainable from cytophotometric measurements on cytological slides, which in the most powerful way to discriminate low-grade malignant variants from high-grade malignant variants of prostatic carcinoma.

MATERIALS AND METHODS

Clinical Material. During the period 1960–1969, 3874 patients with a primary diagnosis of prostatic carcinoma were registered in the Swedish Cancer Register from Stockholm County. From the material 213 patients, diagnosed by fine needle aspiration biopsy, were selected on the basis of survival time. The only treatment these patients received was estrogen. Two extreme groups were chosen. Extreme group 1 consists of 131 patients with progressive tumor disease and death from cancer in less than 3 years after diagnosis as documented in clinical records and autopsy protocols. Extreme group 2 consists of 82 patients who survived for more than 15 years after diagnosis. Fifty-four patients in group 2 had died from cancer after 15 years and 28 were still alive at the time of this study (1988).

To study the DNA histograms in nonmalignant diseases of the prostate 10 cases of prostatitis, 9 cases of prostate hyperplasia, and 7 smears consisting of cell material from vesica seminalis were analyzed.

To investigate whether ploidy determination was also valid for all prostatic tumors and not only for tumors belonging to the extreme groups, another patient material consisting of 79 patients was studied. All patients were diagnosed as having prostatic carcinoma by fine needle aspiration biopsy at the Karolinska hospital and registered in the Swedish Cancer Register in 1966. These patients were treated only with estrogen. Only patients who died from their prostatic carcinoma or were still alive at the time of the study in 1988 were used. Seventy-four patients died of prostatic carcinoma, and 5 were still alive. The follow-up time was 22 years or until death.

Cell Material. The original, May-Grünwald-Giemsa stained smears, on which the primary diagnosis of prostatic carcinoma or the 3 benign diseases of the prostate was based, were used for cytophotometric DNA analysis. After destaining in absolute methanol (99.8%) for 1–3 weeks (3, 12), the cells were refixed in 10% buffered neutral formalin and stained according to the Feulgen procedure involving acid hydrolysis in 5 N HCl at 22°C for 1 h (11, 24).

DNA Measurements. The cytophotometric measurements of Feulgen stained cell nuclei were performed in a modified photographic method described by Adams (25). A Leitz photomicroscope with a plan apochromatic 40×/1.0 oil objective (refraction index, 1.518), in a monochromatic light (wavelength 546 nm) was used. The film, Kodak technical Pan 2415, exposure time 29 DIN = 640 ASA, was developed in Kodak D19 for 4 min at 22°C and fixed in Kodak 3000 for 8 min.

In each slide granulocytes were used as internal control cells and given the value 2c denoting the normal diploid content of DNA. All measured values were expressed in c units in relation to the corresponding granulocyte control cells. Ten to 20 granulocytes and 50 to 100 tumor cells or benign epithelium cells were measured on each slide: 2c region, the diploid region with an upper limit of 2.5c; 4c region, the tetraploid region, 3.5c–4.5c; modal value, defined as the most frequent c value using a class width of 0.5c; abnormality, cells outside and between the 2c and 4c region, *i.e.*, the non-2c and non-4c cells.

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RESULTS

Three benign prostatic lesions were analyzed with respect to nuclear DNA content in order to provide background information for ploidy level determinations in prostatic carcinoma. Fig. 1 shows that all cases of prostatitis had a high proportion of cells in the 2c region and a smaller number of cells in the 4c region varying from 1 to 13% of the total cell number. The same DNA distribution is shown in the cases of prostatic hyperplasia (Fig. 2), exhibiting up to 14% of the cells in the 4c region. Essentially the same DNA pattern is seen in vesica seminalis (Fig. 3), although a somewhat higher proportion of cells in the 4c region was found.

From Figs. 1–3, arbitrary limits defining the diploid and the tetraploid regions were estimated empirically. Based on the control cell populations (granulocytes) and in agreement with previous observations (12) 2.5c was used as an upper limit of the 2c region. Also in benign prostatic lesions 2.5c seems to be an accurate limit of the 2c region. Only small fractions of the cells belonging to the 2c peak exceeded the 2.5c limit. The 4c region is well defined by the limits 3.5c and 4.5c in benign prostatic lesions (see further below). This is particularly evident from Fig. 3 (vesica seminalis), where a substantial proportion of cells with 4c DNA content can be seen.

DNA distribution patterns obtained from cytophotometric DNA measurements of prostatic cancers are exemplified in Fig 4. Some prostatic carcinomas show a DNA distribution pattern similar to that of benign prostatic lesions, with the majority of cells in the 2c and 4c regions (Fig. 4, *left*). These tumors are referred to as D- or T-tumors, respectively. On the other hand, prostatic carcinoma were also found showing a DNA distribu-

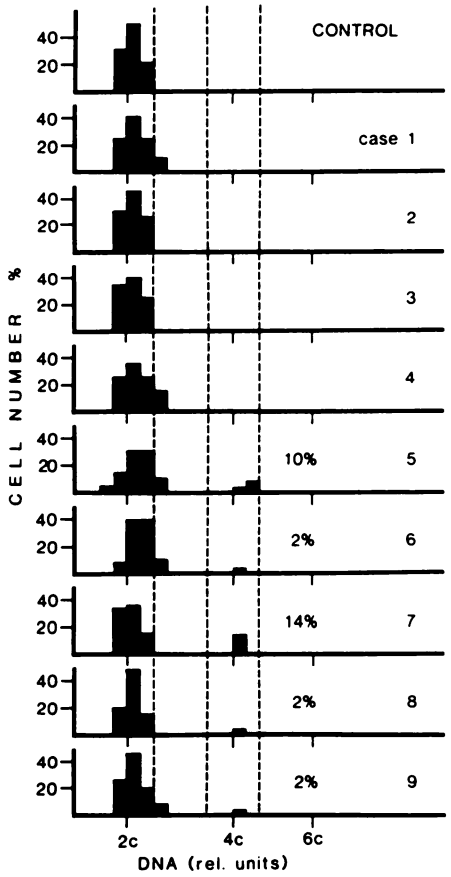

Fig. 2. DNA distribution patterns in 9 cases of prostate hyperplasia. The numbers stated in the figure denote the percentage of cells in the 4c region.

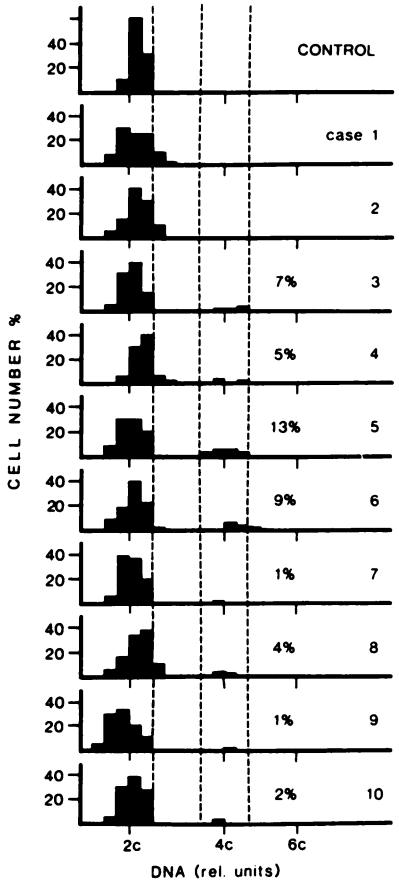

Fig. 1. DNA distribution patterns in 10 cases of prostatitis. The numbers stated in the figure denote the percentage of cells in the 4c region.

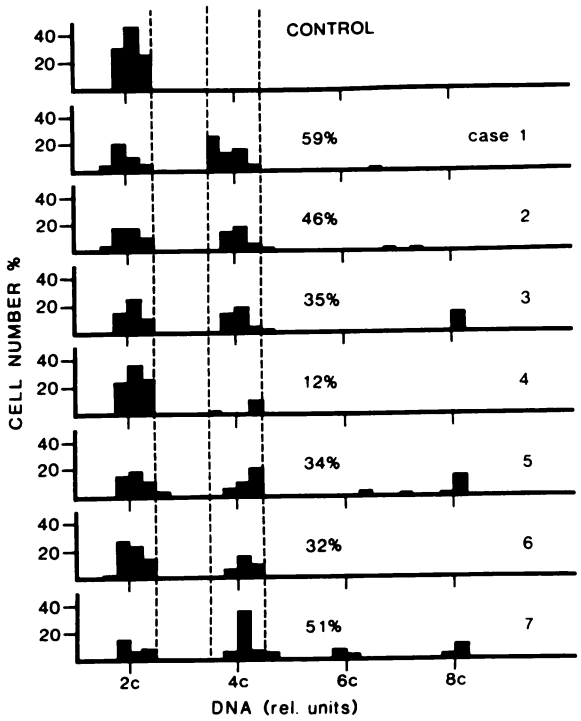

Fig. 3. DNA distribution patterns in 7 cases with cell material from vesica seminalis. The numbers stated in the figure denote the percentage of cells in the 4c region.

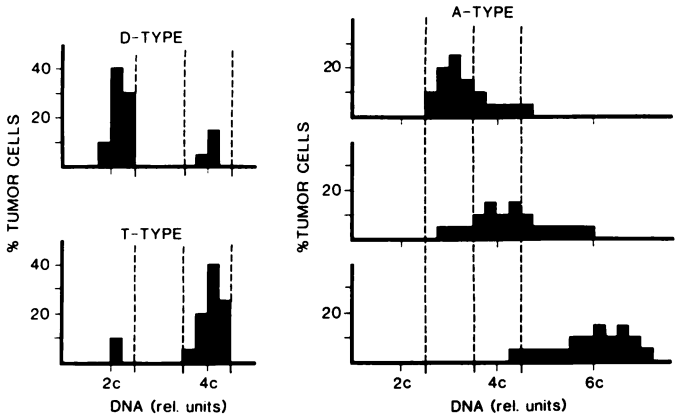

Fig. 4. DNA distribution patterns in prostatic carcinoma. D-type denotes a predominantly diploid (or near diploid) DNA distribution pattern. T-type denotes a tetraploid (or near tetraploid) DNA distribution pattern. A-type denotes a highly abnormal, aneuploid DNA distribution.

tion pattern which considerably deviates from that of benign prostatic lesions and that of D- or T-tumors (Fig. 4, *right*). These tumors are referred to as A-tumors. The accuracy with which D-, T-, and A-tumors can be separated from each other is dependent on which limits are used for defining the 2c and 4c regions. This was studied in detail by comparing high-grade malignant tumor variants (extreme group 1, death from cancer within 3 years) with low-grade malignant variants (extreme group 2, alive more than 15 years after diagnosis). Different upper limits for the 2c window (2.25c, 2.5c, and 2.75c) were tested as well as the range of the 4c window (3.6c–4.4c, 3.5c–4.5c, and 3.2c–4.8c). In order to determine the limits that give the best discrimination between extreme groups 1 and 2 the percentage of tumor cells outside these windows (non-2c plus non-4c) was used as a denominator of ploidy abnormality . Fig. 5 shows that the best separation of the two extreme groups is obtained using 2.5c as an upper limit for the 2c region and a width of the 4c region of 3.5c–4.5c (Fig. 5, *B:2*). As can be seen in Fig. 5 the limits 2.5c and 3.5c–4.5c manage to separate the highly malignant tumors, extreme group 1, from the low-malignant tumors, extreme group 2, to such an extent that a gap of 20% was obtained (Fig. 5, *B:2*). The lowest percentage of non-2c and non-4c cells in extreme group 1 was 50%, and the highest percentage in extreme group 2 was 30%. Using the other limits tested, separations with a smaller gap, nonseparation, or even an overlapping of the two extreme groups were obtained. In extreme group 1 all of the patients had tumors with more than 50% abnormal (non-2c and non-4c) tumor cells, and all of the patients in extreme group 2 had tumors with less than 30% abnormal tumor cells.

The tumors were further characterized by combining the percentage of cells outside the 2c and 4c regions with the modal DNA value of the tumor population (Fig 6). Three different categories were found. Extreme group 1 (highly malignant) showed modal values between 2.5c and 7.25c. In extreme group 2 (low-malignant), tumors with modal values around 2c (1.75c–2.5c) or around 4c (3.75c–4.25c) were found. By combining these two parameters the tumors can be accurately separated into D-, T-, and A-tumors.

To investigate whether the classification of tumors into D-, T-, and A-type was valid in general and could be applied also to patients with survival time between the two extreme groups (3–15 years), 79 patients with prostatic carcinoma were analyzed. In Fig. 7 survival time is plotted against percentage abnormal tumor cells (non-2c and non-4c cells).

In this material it is evident that patients with A-tumors die within 5 years of diagnosis, while patients with D- or T-tumors survive from at least 5 years after diagnosis. Five patients are still alive (Fig. 5, O) at the time of this study (1988). This material also demonstrates that there is a separation between A-tumors on one hand and D- and T-tumors on the other and that this method of tumor classification is thus as accurate for patients surviving for 3–15 years as for the extreme groups.

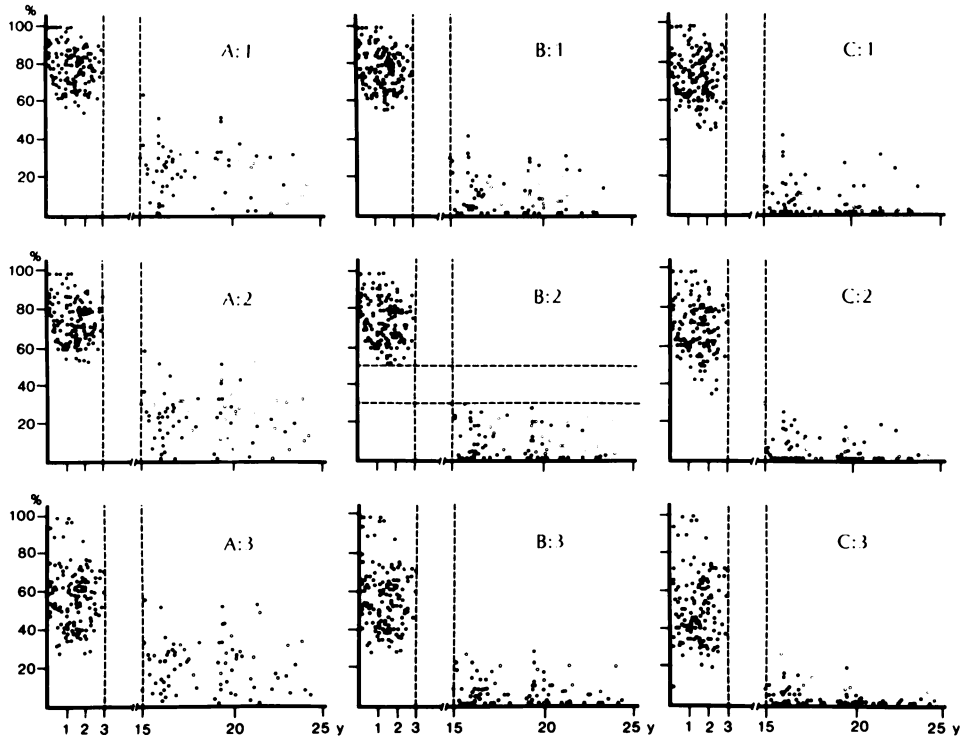

Fig. 5. Relationship between survival time (in years) and percentage of tumor cells which are non-2c and non-4c in 213 cases of prostatic carcinoma. Extreme group 1 (dead <3 years), 131 cases; extreme group 2 (alive 15> years), 82 cases. 2c region: *A* = 2.25c; *B* = 2.5c; *C* = 2.75c. 4c region: *1* = 3.6c–4.4c; *2* = 3.5c–4.5c; *3* = 3.2c–4.8c.

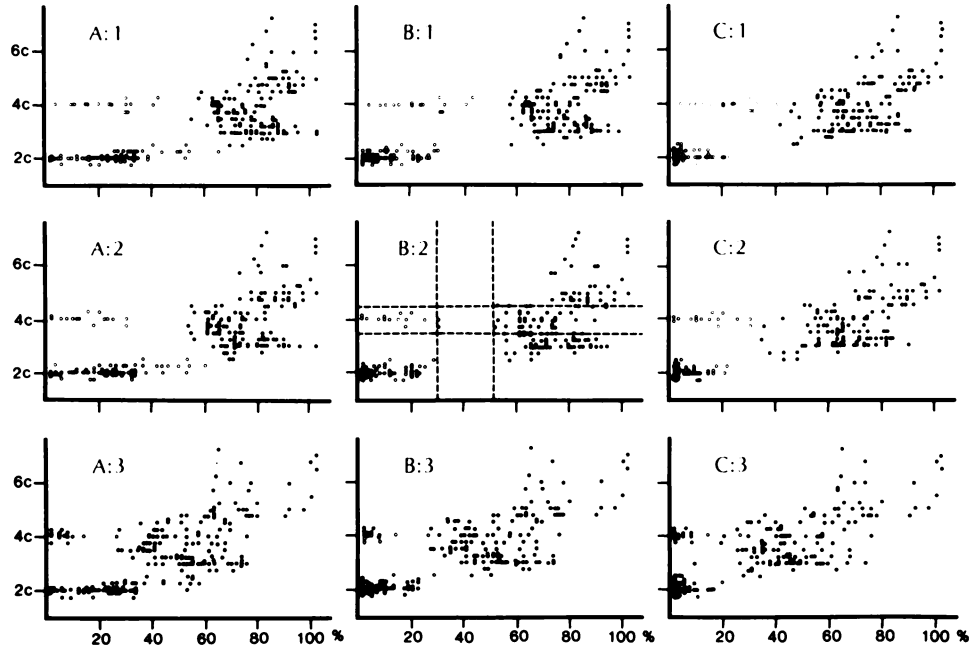

Fig. 6. Relationship between percentage of tumor cells which are non-2c and non-4c and the modal value in c units of the tumor cell population. ●, extreme group 1 (dead <3 years), 131 patients; ○, extreme group 2 (alive >15 years), 82 patients. 2c region: *A* = 2.25c; *B* = 2.5c; *C* = 2.75c. 4c region: *1* = 3.6c–4.4c; *2* = 3.5c–4.5c; *3* = 3.2c–4.8c.

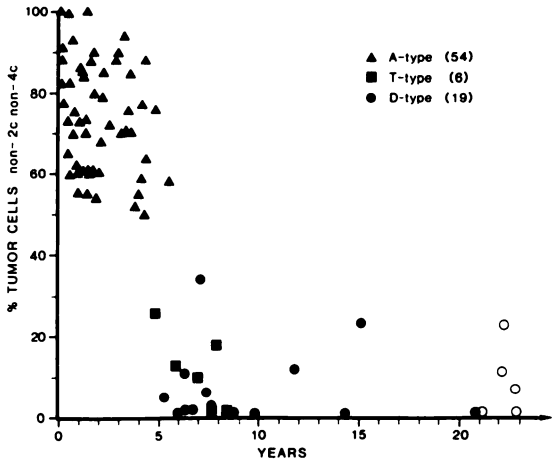

Fig. 7. Ploidy classification of 79 patients with prostatic carcinoma. Survival time plotted in relation to percentage of tumor cells which are non-2c and non-4c. ○, patients with D-type tumors still alive at the time of this study (1988).

DISCUSSION

The data of the present paper clearly show that high-grade malignant tumors can be objectively and accurately discriminated from low-grade malignant tumors on the basis of ploidy level as determined from quantitative cytophotometric DNA analysis of Feulgen stained tumor cell nuclei. However, the precision by which the high-grade malignant tumors can be distinguished from the low-grade variants is strongly dependent on how the cytophotometric Feulgen DNA analysis is performed and how ploidy level is calculated and defined from the cytophotometric data. By studying normal control cells and cells from benign prostatic lesions an approximate estimation of the range of Feulgen DNA values in the 2c, diploid region and in the 4c, tetraploid region could be made.

It was found that the low-grade malignant variants had DNA distributions similar to those of normal cells or cells from benign prostatic lesions; *i.e.*, most of the tumor cell nuclei had DNA values in the diploid, 2c region, or in the tetraploid, 4c region. The high-grade malignant tumors, on the other hand, had most of the tumor cell nuclei outside these regions. Using frequency of tumor cell nuclei with DNA values outside the normal diploid or tetraploid region as a measure of aberration from normality, it was possible to define tumors objectively as D- or T-tumors and A-tumors. However, the diploid and tetraploid regions had to be defined quantitatively.

Previously developed methods have made it possible to use old archival cytological slides for accurate quantitation of nuclear DNA content (3, 4, 12, 26). This enables the use of a retrospective patient material with a known clinical course to be used for the precise determination of the limits defining the 2c and 4c regions. Two extreme groups reflecting high-grade and low-grade malignancy were selected based on the clinical course. Extreme group 1 consisted of patients who died of their cancer within 3 years, and extreme group 2 consisted of patients who survived more than 15 years after diagnosis. DNA measurements from these high-grade malignant tumors (extreme group 1) and low-grade malignant tumors (extreme group 2) were analyzed in detail.

The accuracy by which high-grade and low-grade malignant tumors could be separated on the basis of DNA distribution pattern was greatly influenced by the choice of the limits defining the 2c and 4c regions. Small variations in the limits could make it impossible to unambiguously separate extreme group 1 from extreme group 2 on the basis of the DNA values. This is extremely important in classification of malignancy grade based on DNA measurements. By combining modal values of frequency of "abnormal" cells (non-2c and non-4c cells) all tumors could be unequivocally categorized into highly aneuploid (A-type), near-diploid (D-type), or near-tetraploid (T-type). This method of categorizing tumors into A-, D-, and T-type seems to be valid for all prostatic tumors and not only for the extreme groups used in the methodological analyses.

Characteristic features of the highly aneuploid A-type tumors were the rapid progression of the tumor diseases leading to death within 5 years. In contrast, D- and T-tumors progressed much more slowly, leading to death at the earliest 5 years after diagnosis. Many patients who still had the tumor disease were still alive 15–20 years after diagnosis. This supports previous findings about the relation between the degree of aneuploidy and degree of malignancy in prognostic carcinoma (3, 4, 20, 27, 28).

The reason why A-type tumors are more malignant than D/T-type tumors remains to be clarified. The clearly aneuploid and variable cytophotometric DNA pattern in the A-type tumors may reflect a high degree of genomic instability in such tumor cell populations, an instability which in itself may lead to a rapid generation of new phenotypes that may be a prerequisite for a rapid progression of the tumor disease. A genetic instability of this kind could be the result of an increased instability at the karyotypic level. The tumor cells could for example have acquired the ability to gain and lose chromosomes more rapidly. This would favor the development of the specific karyotypes (or the specific gene dosage imbalances) required for reaching the various stages in the progression of the tumor disease. Alternative hypotheses including increased mutability, high frequency of recombination events, gene amplification, or other DNA rearrangements must be considered.

Although the genetic background for D/T-type and A-type tumors is not understood at present, cytophotometric determination of nuclear DNA content undoubtedly provides us with a powerful diagnostic method for discriminating between low-grade and high-grade malignant tumors.

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