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Nuclear DNA content of borderline tumors of the ovary: correlation with histology and significance for prognosis*,**

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Summary. Scanning-DNA cytophotometry was applied to Feulgen stained sections of 22 borderline tumors of the ovary (BOT). The DNA content was related to conventional histology. In 11 cases clinical follow up for more than 5 years was available. The DNA measurements disclosed two subgroups in the group of BOT. One showed a nuclear DNA content not exceeding tetraploidy (4c) indicating proliferative activity without malignant change and a second one exhibited DNA values higher than 4c indicating malignant transformation. Correlation of histological evaluation with the DNA content revealed a good agreement in 15 cases. However, a discrepancy was found in 7 cases: either the histological evaluation aroused suspicion for malignant potential but the histogram showed DNA values not higher than 4c ($n=4$), or histology showed well differentiated lesions with an atypical histogram ($n=3$). Clinical monitoring revealed no recurrence or tumor spread in all but one case of the group of lesions with DNA values up to 4c, whereas in the group with atypical DNA histograms (DNA values >4c) relapse appeared in 6 out of 7 cases. The results suggest that DNA analysis has prognostic significance for BOT.

Key words: Ovarian carcinoma – Borderline – Malignancy – DNA-cytophotometry

Introduction

Determination of the DNA content of tumor cells has often been demonstrated to be valuable in assessing the prognosis of malignant tumors, e.g.

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in carcinomas of breast (Fossa et al. 1983; Auer et al. 1984), urinary bladder (Trubkait et al. 1982; Baisch et al. 1986), thyroid (Sprenger et al. 1977; Bjelkenkranz et al. 1982), prostate (Seppelt and Sprenger 1984), ovary (Atkin et al. 1970; Friedlander et al. 1983; Erhardt et al. 1984), in acute leukemia (Secker-Walker et al. 1982; Ritter et al. 1984). Since it is possible to applicate DNA cytophotometry to archival paraffin embedded tissue (Auer et al. 1980; Friedlander et al. 1984), retrospective analyses can be performed correlating cellular DNA content and follow up data of patients.

Among the epithelial tumors of the ovary, 10 up to 20% are diagnosed as lesions with a dignitity intermediate between benign and malignant (Hart and Norris 1973; Annual Report-FIGO 1979; Kjoerstadt and Abeller 1983; Dietel et al. 1982). These tumors are termed "borderline tumors of the ovary" (BOT) or synonymously "carcinomas of low malignant potential" (Serov and Scully 1973). Since the morphological criteria to diagnose a BOT are not well defined this diagnosis implicates subjective judgement and thus can be a source of confusion which often results in ambiguous ways of treatment.

In the present study 22 cases diagnosed histologically as borderline tumors were reclassified on the basis of their nuclear DNA content. The histograms were related to the histological appearance. In 11 cases the clinical follow up for more than 5 years was available and the significance of DNA measurements for prognostic statements could be revealed retrospectively. The results underline the usefulness of DNA determinations to improve prediction on the individual clinical course of BOT.

Material and methods

Tumor material and follow up. Paraffin embedded tissue from 40 tumors diagnosed histologically as BOT in routine work of the Dept. of Gynecopathology and the Institute of Pathology (University of Hamburg) was taken and reexamined by Prof. Dr. H.-E. Stegner (head of the Dept. of Gynecopathology) and one of the authors (M.D.). From these, 22 blocks were selected for DNA-measurements. H.E. and Feulgen stained sections were prepared in parallel to ensure a precise localisation of the area to be examined. For control 10 unequivocal benign and 20 clearly malignant tumors were examined additionally. In 11 cases the clinical course of the tumor disease could be monitored continuously for more than 5 years.

Feulgen staining procedure. The tumor material was sectioned at 8 μm previously (Dietel et al. 1985) shown to reveal an optimal number of intact nuclei and lowest number of nuclei overlappings. For quantitative analysis the Feulgen staining was performed. RNA was destroyed by hydrolysis for 15 min in 1 N hydrochloric acid at 60° C. Sections were then washed twice in PBS and subsequently stained with Schiff's reagent for 1 h at 25° C. Differentiation of nuclei was obtained with 1% sodium metabisulphite, followed by successive rinses in distilled water, graded alcohols, and xylene. The cover slips were mounted with Eukitt (refractive index $n = 1.494$).

Scanning-DNA cytophotometry. The nuclear DNA content was determined with a Leitz MPV-compact microscope cytophotometer (Leitz, Wetzlar, FRG). Per case 50–100 individual nuclei were scanned in 0.5 μm steps. These were performed by means of a motor driven microscope table directed by a specially adopted microcomputer (Lang-Elektronik, Rechternbach, FRG). Absorbance of the single measuring point was determined with a spot of 2.54 μm^2 in projection to the area of measurement. The determination of extinction was done at a wavelength of

587 ± 9.5 nm. All data recorded from the photometer were transformed and stored by means of an Eurocos-Computer (SOCOS) directly interfaced to the MPV-compact. The software was purchased from Leitz and adopted by one of the authors (H.A.)

The Feulgen-DNA content of the individual nucleus was calculated by summing up the product of individual extinction (e_i) and square of step width for all measuring spots within the nucleus. For standardization, the diploid nuclear DNA content (2c) of normal ovarian surface epithelia and of ovarian fibroblasts was measured. The mean value ± SD was defined as 2c range, twice this value correspondingly as 4c. The histograms were drawn in relation to the 2c value.

Results

Control tumors with unquestionable dignity

DNA histograms of normal surface cells, fibroblasts, and benign epithelial tumors (mucinous and serous cystadenomas) showed almost exclusively diploid DNA values (Fig. 1). Very few nuclei exhibited a slightly higher DNA content indicating benign cell proliferation. No value exceeded 4c. All but 1 ovarian carcinomas exhibited atypical histograms with severe aneuploidy of the tumor cells (Fig. 2).

Borderline tumors (carcinomas of low malignant potential)

Among the 22 specimens diagnosed histologically as BOT, 15 cases were found to consist of cells with maximal tetraploid (4c) cells (Fig. 3A). The DNA pattern varied from clearly benign (most cells diploid) to those with many cells between 2c and 4c and a peak around 4c indicating proliferative potency. Seven cases were found with DNA values higher than 4c (Fig. 4A) with several cells suspicious of malignant dedifferentiation.

In 15 cases correlation of the DNA histograms with the respective histological appearance showed a good agreement, i.e. morphological signs of

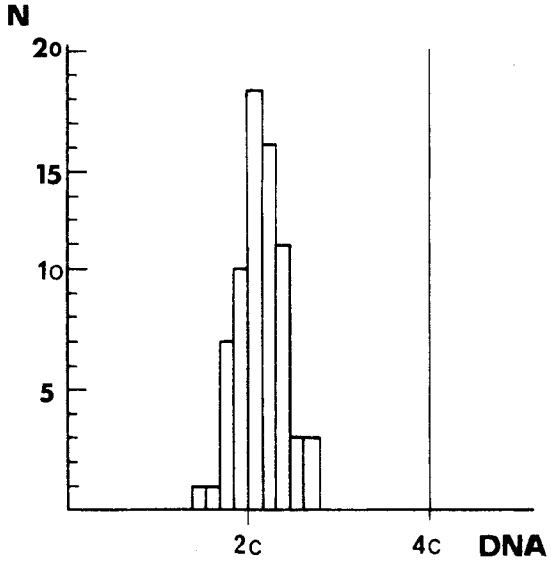


Fig. 1. Example of the DNA histogram of a benign, non-proliferating ovarian cystadenoma showing a single peak of diploid nuclei at 2c ± SD

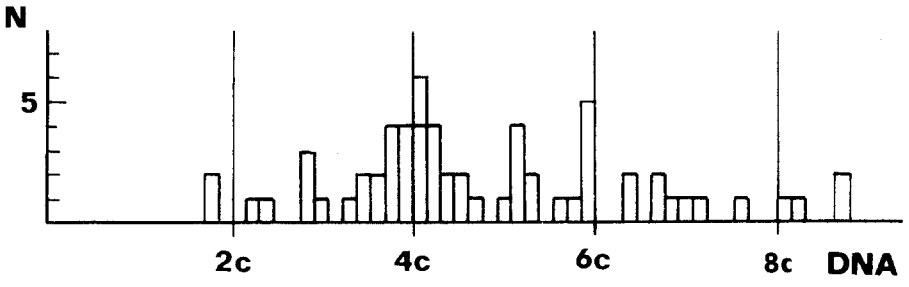
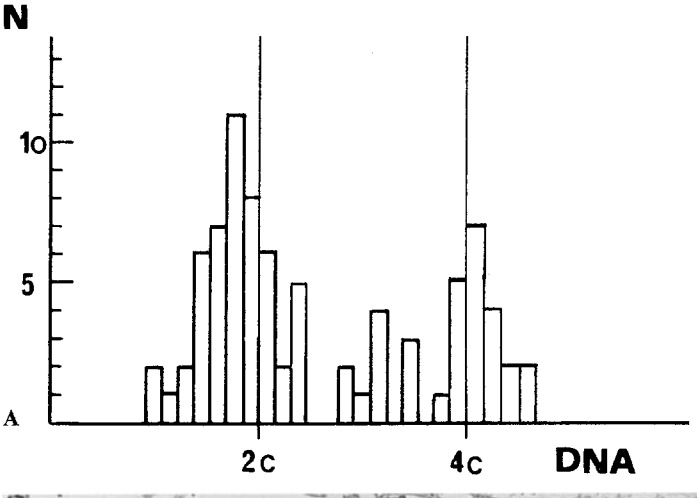


Fig. 2. DNA histogram of an ovarian carcinoma revealing many nuclei with atypical DNA values higher than 4c+SD characteristic for malignant dedifferentiation



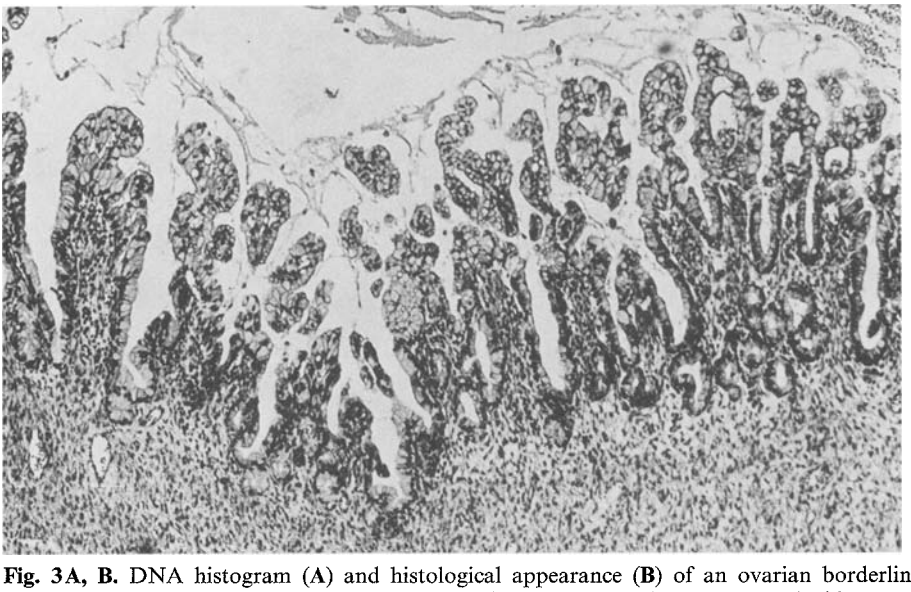
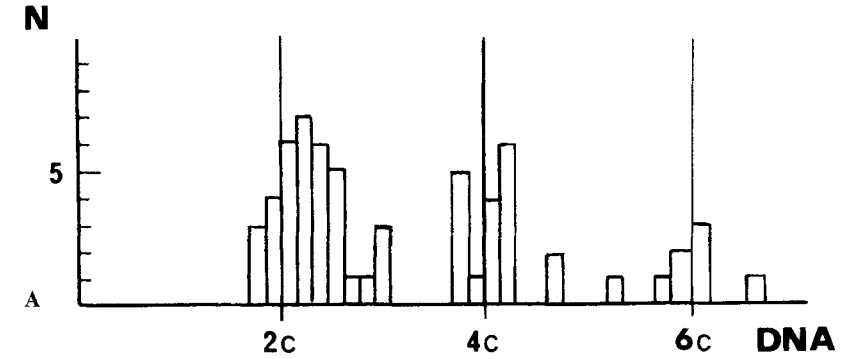


Fig. 3A, B. DNA histogram (A) and histological appearance (B) of an ovarian borderline tumor. On the basis of histology malignant potential was suspected. In contrast, the histogram is typical for proliferative potential of benign character with nuclear DNA values not exceeding 4c+SD. The patient showed no signs of recurrence within 5 years. (B: × 100)



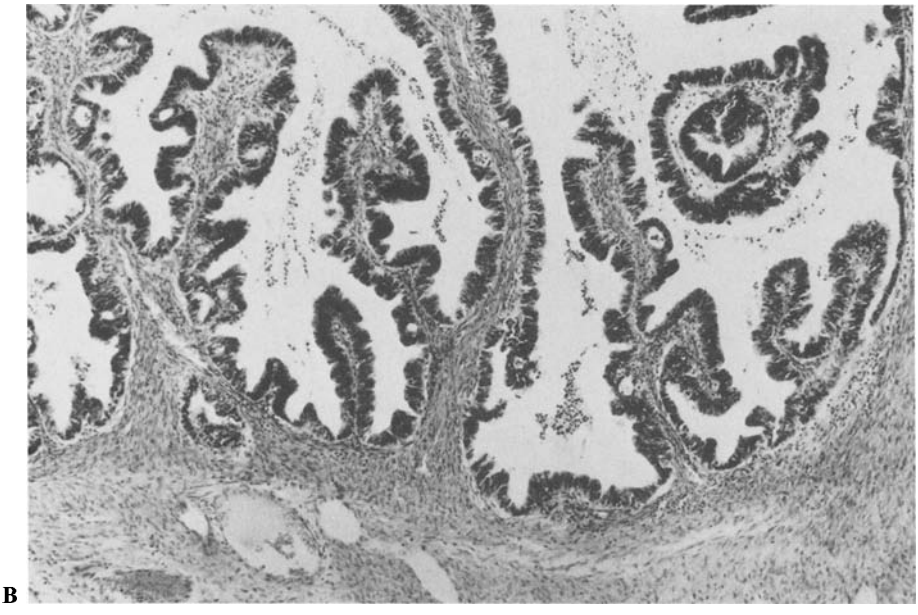


Fig. 4A, B. DNA histogram (**A**) and histological appearance (**B**) of an ovarian borderline tumor. Histology of this case revealed a relatively benign appearance whereas many atypical DNA values higher than 4c+SD are disclosed by cytophotometry. This indicates malignant transformation. A relapse developed within 7 month. (**B**: × 100)

high proliferative activity with distinct cellular abnormality were related to histograms with nuclear DNA values higher than 4c and relatively benign histology showed unsuspicious histograms without nuclei exceeding 4c. However, in 7 cases this agreement could not be stated, i.e. 4 lesions histologically suspicious of a malignant transformation (Fig. 3B) showed histograms with DNA values not higher than 4c, while 3 relatively well differentiated lesions were related to histograms with atypical DNA values (Fig. 4B).

Clinical follow-up of the borderline tumors

Clinical follow up for more than 5 years could be obtained from 11 patients. Four of these were in the group with DNA values not exceeding a maximum

of 4c. Up to now, only in one case a relapse was found, while none of the other patients developed local recurrence, peritoneal spread, or distant metastases. The tumors of the remaining 7 patients showed atypical cells in DNA cytophotometry. In 6 of these a peritoneal spread was diagnosed within a period of 4 month up to 5 years after primary tumor removal. One case showed no reappearance of the disease within 4 years of follow-up.

Discussion

At the beginning of this investigation it was intensively discussed whether to use flow cytophotometry of cell suspensions or scanning cytophotometry of tissue sections. Since the areas of interest in ovarian borderline tumors are often circumscript (Hart and Norris 1973; Scully 1979; Dietel et al. 1983, 1985) an enzymatic desintegration of the paraffin material would contain a great number of non-tumorous cells. This could be cause of unprecise results in flow cytophotometry. Since in scanning-cytophotometry the areas of interest can be selected exactly, this procedure was chosen for the present study.

The group of borderline tumors of the ovary integrates lesions which possess a dignity intermediate between benign and malignant (Hart and Norris 1973; Serov and Scully 1973; Dietel et al. 1982; Kjoerstadt and Abeller 1983; Ovarian Tumor Panel 1983). Thus, the diagnosis "BOT" does not always allow a precise prediction on course of the individual case. To add information to the dignity of these lesions the present study was undertaken investigating the nuclear DNA content of BOT. The measurements revealed two different groups, one with DNA values in the range up to tetraploidy indicating proliferating lesions but without hints for a malignant transformation. The second group contained a portion of cells with hypertetraploid nuclei indicative of malignancy. It has to be emphasized that in several cases this discrimination could not have been established by means of conventional histology alone. This is in agreement with findings reported similarly by other authors (Sachs et al. 1974; Shiromizu 1980; Erhardt et al. 1985).

In several studies non-aneuploid ovarian carcinomas have been described (Atkin 1970; Friedlander et al. 1984). In the present series and others reported previously (Dietel 1983; Dietel et al. 1985) no ovarian carcinoma was found containing a normal DNA pattern. However, from the number of cases examined up to now we can not exclude principally the existance of such tumors. In any case, the incidence of up to 30% (Friedlander et al. 1984) appears to be rather high. The discrepancy may be due to methodological differences between flow cytophotometry and scanning cytophotometry.

In 11 cases the clinical follow-up for more than 5 years was known. The results obtained so far indicate that borderline tumors with an atypical DNA pattern have a higher risk to develope recurrence than those without the described signs of malignant change. In this study the follow-up period is not long enough to allow statements on different survival rates of both groups. Nevertheless, comprehending our results and those of others (Sachs

et al. 1974; Erhardt et al. 1984, 1985) it is suggested that in addition to conventional histology borderline tumors should be examined by DNA-cytophotometry as an objective method potent to improve the statements on prognosis. This is of special importance since borderline tumors often do appear in young fertile women (Dietel 1982; Kjoerstad and Abeller 1983). The improved estimation of prognosis might allow an individually adopted therapy with organ preservation in selected cases.

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