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Letters to the Editors

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Dear Sirs,

We read with interest the recent article in *Virchows Archiv A* entitled "Co-existence of two aneuploid stemlines in benign adenomas" by Joensuu et al. (1989). However, we would like to add some remarks on the technical aspects and the exceptional role of endocrine tumours in quantitative DNA investigations.

Studies by Vindelov (1989) have demonstrated that flow cytometry on cells prepared from formalin-fixed, paraffin-embedded tissue by enzyme digestion produces results which are difficult to interpret, since the usual evaluation of diploid standard values using trout or chicken erythrocytes is impossible using this technique. An internal standard using a mixture of nuclei from a paraffin block of normal tissue and a different block of tumour tissue as recommended by Hiddemann et al. (1984) and Schutte et al. (1985) is lacking – a fact that raises some questions with regard to the standardization of the results.

Fluorescence/light scatter dot plots for artefact exclusion are not given in the paper; doublets and triplets might be a serious problem in the preparation of cell suspensions from paraffin-embedded tissue. Furthermore, there are no data on the coefficients of variation of the different peaks, which are considered essential in the "Convention on nomenclature for DNA cytometry" (Hiddemann et al. 1984).

In Fig. 3, besides the diploid and the a1 peak, the a2 peak apparently represents a tetraploid stemline and the histogram shows a small peak in the octaploid region; it is questionable whether this kind of polyploidization justifies the interpretation of the DNA distribution as multiploid in a sense indicative of DNA aneuploidy usually associated with highly malignant, clinically unfavourable neoplasms.

When Hämmerli et al. (1968) showed that thyroid adenomas may have triploid stemlines (we found approximately 18% tetraploid adenomas, Mikuz et al. 1977), the inevitable conclusion "DNA aneuploidy equals malignancy" was no longer acceptable for endocrine tumours. Moreover, aneuploid stemlines can be found in the Arias-Stella reaction of the endometrial glands (Wagner and Richart 1986) as well as in seminal vesicles of older men (Mohr et al. 1974) – both tissues under endocrine control.

For these reasons we believe that the title of the paper is misleading, as it only refers to "adenomas" and not to "adenomas of endocrine tissues". However, the comparison between these (endocrine) adenomas and adenomas of the colon is even more misleading. For adenomas of the colon with severe atypia it is generally acceptable to use synonyms such as adenoma with focal carcinoma, intramucosal carcinoma or colonic carcinoma in situ (Fenoglio-Preiser et al. 1989). The fact that these adenomas were removed before they became invasive carcinomas does not permit the conclusion that these tumours do not fulfil the cytological and cytophotometric criteria of malignancy. These cases can also be considered examples of subjective visual underestimation of malignancy corrected by objective quantitation of DNA.

Although we do not question the authors' diagnosis of a pancreatic cystadenoma, the case history as well as the microphotographs of case 1 show some resemblance to the so-called "solid cystic tumour of the pancreas", a tumour of unclear biological dignity showing some late recurrences and doubtful histogenesis (Klöppel et al. 1981; Learmonth et al. 1985).

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Reply

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Dear Sirs,

(1) It has been known since the introduction of the technique that internal standards, such as trout and chicken erythrocytes, cannot be used successfully in flow cytometric DNA analyses performed from paraffin-embedded tissue, because they do not produce consistent ratios to the diploid G1 peak (Hedley et al. 1983). Therefore, in DNA aneuploid cases with a small DNA index it is not possible to identify the diploid peak with certainty. In practice, this is a small problem, because hypodiploid DNA aneuploid stemlines are rare (less than 2% of most solid tumours) and in the great majority of cases the peak with the lesser DNA content is correctly taken as the diploid peak (Hedley et al. 1983). The DNA indices of the aneuploid peaks found in the 3 DNA multiploid adenomas were rather large, (1.18, 1.35, 1.44, 1.66, 1.96, and 2.84) so falsely interpreted hypodiploid peaks are unlikely. To exclude the small possibility of flow-cytometry-induced artefact peaks the double-analysis method with external standards was used (Joensuu and Klemi 1988). Several groups, including our own, have shown that identical histograms can be produced from the same tissue before and after embedding it in paraffin, and flow cytometric DNA ploidy and/or S-phase determinations performed from paraffin-embedded tissue have good correlation with prognosis in several types of human cancer (Joensuu et al. 1986; Alanen et al. 1989; Hedley 1989).

(2) Forward and 90° scattergrams were recorded, but they provided little additional information in the present cases. When excessive nuclear doublets or triplets are present the histograms look very unlike the ones we obtained. In the present paper DNA histograms from each tumour are given as a separate figure, and therefore it is possible for all interested readers to calculate the approximate coefficient of variation value of any of the peaks in a few seconds (G1 peak width at half-maximum is divided by its distance from 0-channel, and the quotient is multiplied by 2.35).

(3) We agree that one of the aneuploid peaks with a DNA index 1.96 can be explained by DNA polyploidization, but the rest of the aneuploid peaks cannot. According to our study on DNA histogram classification (Joensuu and Kallioniemi 1989), many flow cytometrists classify a histogram with one hyperdiploid and one tetraploid peak as multiploid.

(4) Until recently DNA aneuploidy has been regarded as a conclusive (Barlogie et al. 1983) and a specific (Büchner et al. 1985) marker of malignancy. According to our results thyroid, parathyroid, pituitary, and adrenal adenomas are diploid, hyperdiploid with DNA index <1.5, or sometimes tetraploid, but rarely triploid, hypotetraploid or hypertetraploid (Joensuu and Klemi 1988).

(5) The heading is not misleading, since endocrine adenomas are adenomas, too. However, pancreatic cystadenoma cannot readily be classified as an "adenoma of endocrine tissues". It was stained for synaptophysin, neuron-specific enolase, and several peptide hormones, and proved negative. It is well established that the often premalignant colorectal adenomas may have a different biological course from endocrine adenomas. In our paper we do not compare endocrine and colorectal adenomas, although they are mentioned in one reference.

(6) One of the tumours was diagnosed as papillary cystadenoma and, accordingly, had a favourable prognosis. The "solid cystic tumour of the pancreas" has a favourable prognosis after complete resection and recurrence has been reported only in a few cases (Mathieu et al. 1989). Metastases are exceedingly rare; they have probably been described in only two patients (Klöppel and Maillet 1989).

In conclusion, multiploid DNA histograms were produced from three tumours that did not fulfil histological criteria for malignancy and were associated with a favourable prognosis. Although the malignant potential of these tumours may be discussed, from the practical and clinical point of view we recommend that at present the diagnosis of malignancy in endocrine adenomas should not rely merely on the presence of DNA aneuploidy, or even multiploidy. These findings should be regarded as additional information and judged together with clinical findings and histology.

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