Virchows Archiv A Pathol Anat (1991) 419:255–256

Virchows Archiv A
Pathological Anatomy and Histopathology

Letters to the editors

Dear Sirs,

We read with interest the article entitled "DNA ploidy and cell-cycle analysis in pancreatic and ampullary carcinoma: Flow cytometric study of formalin-fixed paraffin-embedded tissue" by Baisch et al. (1990). In this study 47 ductal adenocarcinomas of the pancreas were analysed by flow cytometry. DNA ploidy had no prognostic value for survival, but correlated weakly with the tumour stage and grade. Only 15% of the pancreatic adenocarcinomas were DNA aneuploid, which is considerably less than has been found in most human carcinomas, such as those of the breast, the bladder or the ovary, where typically about two-thirds of cancers are DNA aneuploid. We suggest that DNA aneuploidy is frequent also in pancreatic carcinoma, and that the low frequency of DNA aneuploidy observed by Baisch et al. may result from the selective nature of their series, which consisted only of patients who underwent surgery with probably curative intent.

In three other published series on DNA ploidy determination in pancreatic carcinoma (Alanen et al. 1990; Eskelinen et al. 1990; Porschen et al. 1990) the frequency of aneuploidy has varied between 52 and 61% (Table 1). However, if only patients who had been treated with radical surgery are examined, the frequency of DNA aneuploidy drops to 0–20%. In our series (Alanen et al. 1990) surgery was considered as radical only in 3 of the 38 cases with a non-diploid tumour, but in 12 of the 24 cases with DNA diploid carcinoma ($P=0.0002$), and the radically resected cancers also had a lower fraction of cells in the S-phase ($P=0.009$), indicating that resectable tumours form biologically a highly selected subgroup. There were only 3 radically operated carcinomas in the series by Eskelinen et al. (1990), but they were all diploid, whereas 24 carcinomas (55%) of the 44 non-radically treated cases were DNA aneuploid. Therefore, the results obtained by Baisch et al. are compatible with other existing data, as is their finding that patients without lymph node metastases have diploid tumours.

We found DNA ploidy to correlate well with prognosis ($P=0.0001$). A similar result was obtained by Eskelinen et al.; DNA aneuploid carcinomas had clearly inferior outcome ($P=0.004$). Furthermore, DNA index was an independent prognostic factor in a multivariate analysis, a result that has recently been confirmed by Porschen et al. (1990).

In our view, the present data (though scanty) from DNA flow cytometry performed from paraffin-embedded tissue fit well together and have given consistent results, provided that radically operated carcinomas are accepted to form a highly selected subgroup with frequent diploidy, low proliferation rate and probably low biological malignancy potential. This may be of clinical importance, because improved survival of patients treated with radical surgery as compared with those treated with palliative surgery only found in several non-randomized studies may partially be due to the slow progression rate of carcinomas that can be treated radically, and not the extent of surgery.

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Table 1. DNA ploidy in pancreatic carcinoma and in radically operated tumours

	Alanen et al.	Baisch et al.	Eskelinen et al.	Porschen et al.
No. of carcinomas operated	62	–	46	56
No. of aneuploid tumours (%)	38 (61%)	–	24 (52%)	29 (52%)
No. of radically operated carcinomas	15	47	3	–
No. of aneuploid tumours (%)	3 (20%)	7 (15%)	0 (0%)	–

Reply

Dear Sirs,

In their letter, Drs. Alanen Joensuu and Klemi point out that the 15% aneuploidy rate found in our series of ductal adenocarcinomas of the pancreas (Baisch et al. 1990) is much lower than that observed in three other recent investigations, one by their own (Alanen et al. 1990) and two by other groups (Eskelinen et al. 1991; Porschen et al. 1990) (one in abstract form), which ranged from 52% to 62%. We may add a fourth study by Weger et al. (1990) on the same subject, which also revealed a high non-diploid (tetraploid and aneuploid) DNA distribution pattern in more than 90% of the pancreatic carcinomas examined. There is thus no doubt that our data clearly differ from those of the other authors.

To explain this discrepancy, two possibilities may be considered: first, differences in the biology of the tumours studied, and second, methodological problems.

Alanen and collaborators suggest that diploidy characterizes the group of resectable pancreatic carcinomas, while aneuploidy is associated with non-resectability. The fact that our study with its high rate of diploidy included only resected tumours and that diploidy was also prevailing in the few radically operated carcinomas in the series of Alanen and Eskelinen seems to support this assumption. However, Weger and collaborators (1990), who included 48 patients with a Whipple resection in their series of 77 cases and recorded a non-diploid (tetraploid and aneuploid) pattern in 76 of 77 cases evaluated by image cytometry and 47 of 50 cases assessed by flow cytometry, probably encountered a much lower rate of diploidy in their patients treated by radical surgery than the other investigators. This implies that the high frequency of diploidy observed in our study may not only reflect a special tumour biology, i.e. tumours with limited extension and/or low proliferative capacity, but could also be due to problems related to the preparations of samples for DNA determination. The most important methodological problem encountered in flow cytometric studies of formalin-fixed, paraffin-embedded material is the relatively high amount of nuclear debris that may be obtained with the deparaffinization-disintegration technique. The resulting background noise on the histograms can then obscure low signals from aneuploid cells. In our study we used 30-μm-thick sections, as originally described by Hedley et al. (1983), while the other authors isolated the nuclei from 50-μm sections. Preservation of nuclei, however, seems to be better in samples from 50-μm sections than those from 30-μm sections (Eskelinen et al. 1990). For these reasons it might be possible that some pancreatic carcinomas with a very small fraction of aneuploid cells were missed in our study. As long as this possibility cannot be excluded, it remains speculative to explain the high rate of diploidy in our series solely by a special biology of these tumours.

In conclusion, we feel that only future studies will answer the question whether it is the biology of resectable pancreatic carcinomas or a problem related to the technique of flow cytometric examination that accounts for the differences in aneuploidy rates of pancreatic carcinomas. Such a study should be based on standardized techniques and well-defined biological and morphological parameters, factors that are urgently needed to make studies on the biological significance of DNA content in pancreatic carcinomas, but also in other comparable tumours.

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