Evaluation of DNA Nuclear Pattern as a Prognostic Determinant in Resected Pancreatic Ductal Adenocarcinoma

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From a cohort of 72 patients who underwent radical resection for ductal adenocarcinoma of the pancreas between 1951 and 1980, 62 paraffin-embedded specimens were analyzed by flow cytometry. Patients were divided into two groups according to the length of survival: long-term survivors (19 patients who survived 3 or more years after operation) and short-term survivors (43 patients who died within 12 months after resection). In 30 specimens (48%), the nuclear DNA pattern was diploid, whereas 32 were nondiploid (two tetraploid and 30 aneuploid). There were no significant differences in the number of diploid/nondiploid patterns, the fraction of cells in DNA synthetic (S) phase, or the DNA index between the two groups. These data suggest that there is no difference in the DNA content analysis of patients with pancreatic ductal adenocarcinoma when comparing long-term with short-term survivors following resection.

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CARCINOMA OF THE pancreas is currently the sixth most common malignant tumor[1] and is the fourth leading cause of death from cancer in men.[2] Ductal adenocarcinoma with its dismal long-term prognosis is the most common type of pancreatic malignancy. Median survival after resection varies from 10 to 20 months, with a 5-year survival of approximately 2% regardless of therapy.[3,4] Although several histopathologic classifications are presently used in ordinary ductal adenocarcinomas,[5,6] there are no reliable prognostic factors that permit the surgeon to identify and separate that population of patients in whom a radical resection might be of benefit from the group in whom the risk/benefit balance favors a palliative (nonresective) procedure.

In 1986, we published the results of 79 patients who underwent resection for ductal adenocarcinoma of the pancreas, in whom we sought prognostic factors that may influence survival.[7] Although we found that certain histopathologic characteristics such as Broders' grades 3 and 4 in the primary tumor, a round-cell infiltrate at the tumor margin, and epithelial atypia in the uninvolved pancreatic ducts were significantly associated with a poor prognosis, we did not believe that all of these features could be identified in small biopsy specimens before resection, nor did we think that the statistical differences were strong enough as prognostic markers to change the therapeutic approach. We have continued to elucidate and define more significant prognostic markers.

Deoxyribonucleic acid (DNA) content analysis (DNA ploidy) has been demonstrated to provide significant prognostic information for several malignant tumors.[8-12] Very few investigations, however, have studied ductal pancreatic carcinoma, and they have shown disparate results. Schwab et al.,[13] for example, reported that 98% of 45 patients with resected ductal adenocarcinoma had nondiploid nuclear DNA distribution. They found that triploid tumors had a significantly more malignant course when compared with nontriploid DNA patterns. Another study by Joensuu et al.[14] showed that 38% of 62 patients with histologically diagnosed pancreatic carcinoma had tumors with a diploid distribution, and that they had significantly better prognosis when compared with aneuploid tumors, although these patients were not stratified by type of treatment.

The aim of the present study was to investigate the relationship between DNA ploidy patterns and survival in a population of patients undergoing resection for ductal adenocarcinoma of the pancreas at the Mayo Clinic from 1951 to 1980. Specifically, we hypothesized that if ploidy pattern correlated with survival, recognition of this pattern should be more apparent in our long-term survivors. Therefore, we examined ploidy pattern in 72 patients who underwent complete resection of this tumor.

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Methods

From the total of patients undergoing resection for ductal adenocarcinoma of the pancreas at the Mayo Clinic between 1951 and 1980, 79 patients were selected for study to obtain two dichotomous groups of long-term and short-term survivors with no overlapping. These included 23 patients who survived 3 years or more after operation (long-term survivors) and 56 patients who died within 6 months after resection (short-term survivors). Of this group, included in our study of 1986, 72 formalin-fixed and paraffin-embedded archival samples were available for analysis by flow cytometry. Because of poor-quality histograms, we were unable to interpret 10 of the 72 samples evaluated (14%). These patients were excluded from analysis. Hematoxylin-eosin–stained slides were reexamined and the histopathologic diagnosis reconfirmed (LHW). Nuclear DNA content of tumor cells was measured by flow cytometric analysis of nuclei isolated and stained with propidium iodide by standard methods.[15] Nuclear DNA content was measured on a FACS analyzer (Becton Dickinson, Sunnyvale, CA). Histograms were generated by analysis of more than 10,000 nuclei per sample. "Cell cycle" analysis of the histograms was performed using a rectangular computer model program.[16] The DNA histogram patterns were analyzed without knowledge of the clinical course of survival status of the patients.

DNA ploidy patterns were divided into three groups: (1) Histograms with a single symmetric G1 peak were classified as DNA diploid (normal). (2) Histograms with greater than or equal to 13% nuclei in the 4C peak were classified as DNA tetraploid. The value of 13% for the normal upper limit of 4C cells was determined from the 3 standard deviation upper limit observed with normal human pancreatic tissue. (3) If a G1 peak separate from the 2C or 4C peaks was present, the histogram was classified as DNA aneuploid (Fig. 1).

Statistics

Patients were divided in a similar fashion to the previous study into two groups according to survival. Patients who survived 3 years or more after operation were considered long-term survivors (group I), whereas those who died within 12 months after resection were considered as short-term survivors (group II). All hospital deaths (within 30 days of operation) and patients who survived for at least 1 but less than 3 years after operation were excluded from our prior study and have not been included in the present analysis.

Comparison of the two groups was performed with the chi square test for discrete variables and with two-sample t test for continuous variables, or rank-sum test when variables were nongaussian or variances were unequal.

Results

The study group thus consisted of 62 specimens, of which 19 corresponded to patients with long-term and 43 to patients with short-term survival.

DNA Ploidy and Outcome

In group I, 12 patients (63%) had DNA diploid tumors, and 7 (37%) had nondiploid DNA pattern, compared with 18 (42%) and 25 (58%), respectively, in group II (not significant [NS], chi square test). The comparative ploidy distribution is shown in Fig. 2.

The percentage of cells in the S-phase fraction in the 30 patients with DNA diploid tumors was calculated. The mean S of the 12 cancers in group I was 9.6% (standard deviation [SD], 2.7) whereas that of the 18 cancers in group II was 9.5% (SD, 4.1). Figure 3 demonstrates that there were no significant differences in the distribution of percentage of S-phase cells when comparing both groups. A similar analysis comparing the percentage of cells in

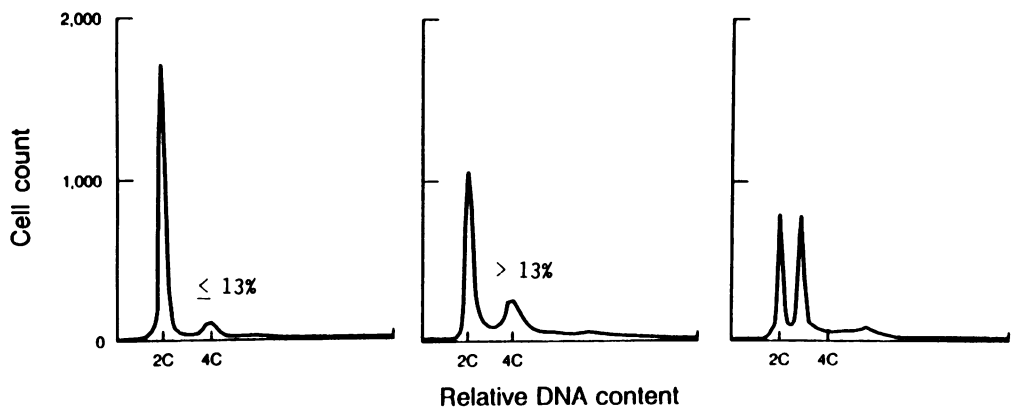

FIG. 1. Nuclear DNA histogram patterns. (Left) DNA diploid, (center) DNA tetraploid, and (right) DNA aneuploid.

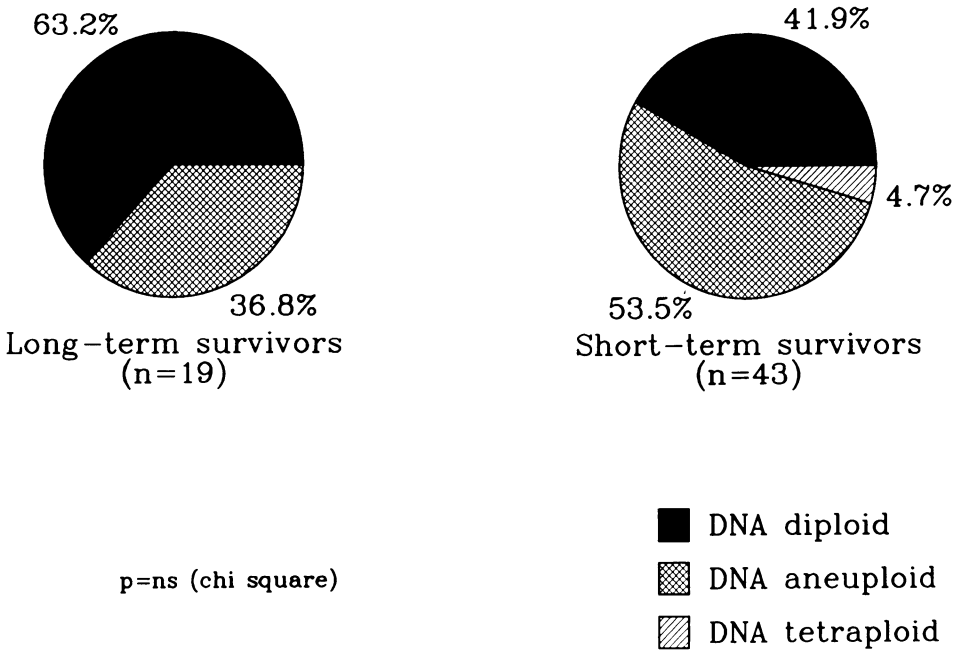

FIG. 2. Distribution of nuclear DNA ploidy patterns according to length of survival.

S+G2M-phase showed nonsignificant differences between either group.

The DNA index of the 19 patients with long-term survival varied from 1 to 1.86 (mean ± SD, 1.1 ± 0.2) and that of the 43 patients with short-term survival varied from 1 to 2 (mean ± SD, 1.2 ± 0.2) (NS, rank-sum test) (Fig. 4).

There were six 5-year survivors. The ploidy pattern was diploid in four and aneuploid in two. The distribution of DNA nuclear patterns of the patients who survived 3, 4, and 5 or more years is presented in Table 1.

Ploidy and Tumor Grade

Four tumors were classified as Broders' grade 1, 29 grade 2, 26 grade 3, and 3 as grade 4. The distribution of nuclear DNA ploidy patterns for the various histologic grades of the tumors is shown in Figure 5. There was a significant correlation between histologic grade and ploidy pattern. The four grade 1 tumors showed DNA diploid pattern, the three grade 4 were nondiploid, and grades 2 and 3 showed an increasing number of nondiploid distribution as the tumors became less differentiated ($p < 0.01$, Wilcoxon rank-sum test).

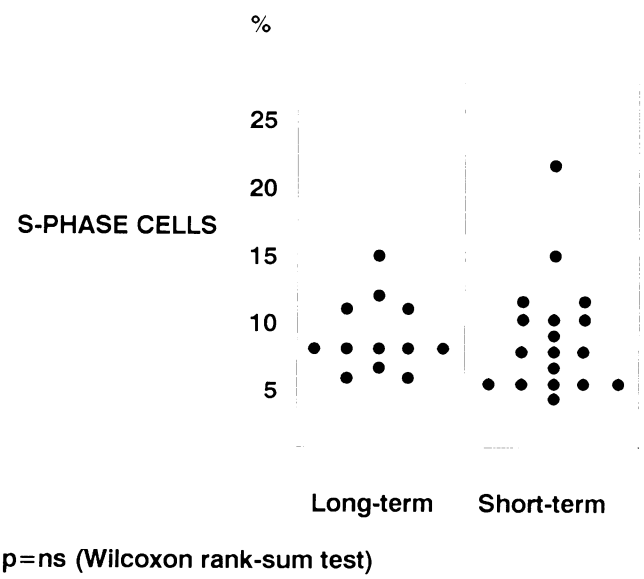

FIG. 3. Distribution of 30 patients with diploid tumors according to percentage of cells in S-phase fraction derived from cytometric cell cycle analysis.

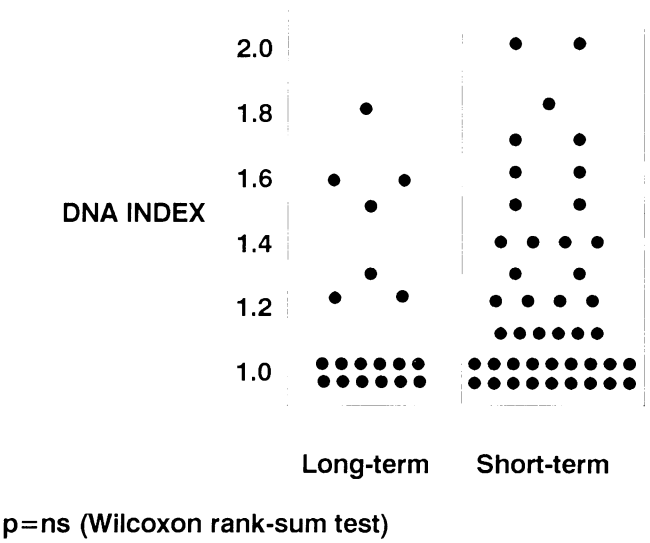

FIG. 4. Distribution of tumors according to DNA index.

TABLE 1. *Distribution of the DNA Ploidy Pattern in the 19 Long-Term Survivors According to Length of Survival*

	Diploid	Aneuploid
Survival (yr)	n (%)	n (%)
3–3.9 (n = 8)	4 (50)	4 (50)
4–4.9 (n = 5)	4 (75)	1 (25)
≥5 (n = 6)	4 (66)	2 (34)

Discussion

After many years of clinical experience on the management of patients with pancreatic ductal adenocarcinoma, controversy remains regarding the superiority of curative over palliative surgery and the predictable identification of patients in whom the risks of radical surgery would be justified.[14] Although the biologic behavior is most commonly predictably poor, reasons for the continuing controversy are attributed to the rare exceptions to this biologic behavior of the tumor and the lack of reliable factors that may predict survival before resection is undertaken.

Histomorphologic grading of tumors has demonstrated conflicting prognostic information.[4–6] Many diverse clinical features and intraoperative findings have failed to reliably predict survival.[7,17] Recently, however, lymph node status, vessel invasion, and the number of units of blood transfused were found to be significant determinants of long-term survival.[18]

The first study evaluating the prognostic value of nuclear DNA content in pancreatic cancer was published by Weger et al.[19] in 1987. The authors studied, by semiautomatic DNA image cytometry, 77 cases who were also clinically followed over the long term. Of 15 patients with near tetraploid tumors, eight were still alive 70 months after diagnosis, whereas all 16 patients with triploid tumors had died within 18 months. There were no diploid tumors. These results suggested that DNA analysis of ductal adenocarcinoma of the pancreas could be helpful as a prognostic predictor and as an adjunct for treatment planning. This initial study was followed by a similar analysis from the same institution[13] in which the authors included a better-defined population of 45 patients who underwent radical pancreatoduodenectomy for ductal adenocarcinoma of the pancreas. The differences in the population did not change the earlier results, and this subsequent study confirmed a significantly more malignant course for triploid tumors when compared with their nontriploid counterparts.

In contrast, Joensuu et al.[14] in 1989 reported a series of 63 patients with pancreatic carcinoma in whom the ploidy study demonstrated 24 patients with diploid and 38 with nondiploid cancers. Although the survival rate 2 years after diagnosis was zero, the prognosis for the patient with diploid tumors was significantly better than that of the patients with nondiploid tumors.

In the present study, we found 30 diploid tumors in 62 patients with ductal adenocarcinoma of the pancreas. We found no significant differences in the number of diploid/nondiploid tumors or in the DNA index between the group of patients who survived at least 3 years after pancreatoduodenal resection *versus* those patients who died within 6 months after operation. Moreover, the S-phase and S + G2M fractions of the 30 diploid tumors, which have been suggested to improve the predictive power of DNA measurements in certain endocrine tumors,[20] were similar between both groups.

Our results do not fully support previously reported findings.[13,14,19] The reason for the wide variation in the occurrence of diploid/tetraploid patterns among the different series is unknown. Variations are unlikely related to the characteristics of the population, the cytometric methods employed,[21] or technical problems caused by degradation of the nuclear contents as a result of storage.[22] More interesting, however, are the different findings in the value of DNA ploidy as a prognostic factor. The lack of an association between ploidy and survival in our series may be explained by the characteristics of the study design. We are aware that small differences in the behavior of diploid/nondiploid tumors may have been missed in our study. Our study design was intentionally stratified to show a difference, however. The principal aim of this analysis was to assess if the power of DNA ploidy studies in this particular tumor was strong enough to produce different DNA expression in populations with diametrically opposite clinical courses (<1-year *versus* ≥3-year survival). We do think that the relationship between DNA modal

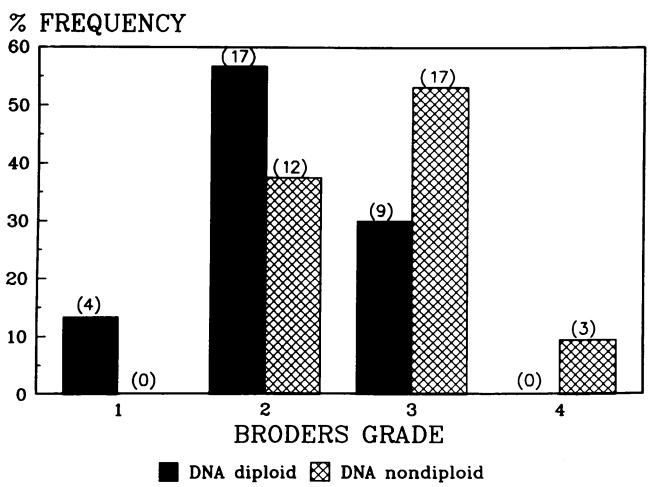

FIG. 5. Distribution of nuclear DNA ploidy patterns according to Broders' classification.

value and survival time still require confirmation by longitudinal studies in a well-defined patient population.

The absence of DNA diploid distribution in pancreatic cancer, in addition to the finding of 100% DNA diploid content in presence of chronic pancreatitis, had been proposed to be of some help in solving the differentiating diagnosis between these two entities,[21] because the presence of DNA aneuploidy would strongly indicate the existence of pancreatic carcinoma. Our results of almost 50% of DNA diploid ductal adenocarcinomas strongly refute this suggestion.

Pancreatic ductal adenocarcinoma remains a disease today with a frustratingly poor prognosis. Efforts should continue to elucidate the cause, to evaluate more efficient adjuvant therapy, and to search for predictable prognostic markers. These efforts are particularly important because operative therapy can today be performed quite safely with good results, unfortunately in the short term only.

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