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Analysis of DNA Content in Invasive Pancreatic Cancer: Special Reference to the Relationship Between the 4c Exceeding Rate of DNA Content and Pathological Findings

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The effectiveness of the analysis of DNA content in invasive pancreatic cancer to obtain a prognosis is controversial, as the DNA ploidy alone does not yield an accurate prognosis. On the other hand, image cytometry offers advantages over flow cytometry in that only tumor cells are used for DNA analysis and image flow cytometry is a reliable method to obtain accurate prognoses of patients with various cancers. In the present study, we retrospectively examined the DNA content of histological paraffin-embedded tumor material obtained from 35 patients (17 male, 18 female) who had been pancreatectomized because of invasive pancreatic cancer. For DNA analysis, all specimens were studied using the Quantitative Ploidy Analysis Program in the CAS 200 Image Analysis System (Cell Analysis System, Inc., Lombard, IL). In the present study, the DNA ploidy pattern, the DNA index (DI), and the 4c exceeding rate (ER) were calculated by analyzing the DNA histogram in each case. The main purpose of our study was to investigate the relationship between those three parameters and pathological findings in invasive pancreatic cancer.

The present study demonstrated that there was no significant relationship between the DNA ploidy pattern or the DI and pathological findings. However, there was a significant ($P<0.05$) relationship between the 4c ER and pathological findings such as tumor size, neural invasion, and tumor differentiation.

In conclusion, the 4c ER of the DNA histogram in the ICM correlated with an aggressive pathological state represented by tumor size, neural invasion, and tumor differentiation, and the analysis of the 4c ER provides important prognostic information regarding invasive ductal carcinoma of the pancreas.

Key words: invasive ductal carcinoma of the pancreas, DNA content, 4c exceeding rate, malignant potential

INTRODUCTION

Despite recent improvements in surgical and multidisciplinary treatment, the prognosis for patients with an invasive pancreatic cancer remains poor. After resection of the tumor, the mean survival time is 16 months. Furthermore, in a review of 144 reported series including approximately 37,000 patients with pancreatic ductal carcinoma, Gudjonsson found a median survival period of 4 months, with only 155 five-year survivors (1). For the treatment of patients with pancreatic cancer, an accurate prognosis can indicate pancreatic resection for cases with a good prognostic outcome and prevent patients with bad prognoses from having unnecessary surgery. Prognostically relevant factors based on the histological assessment of the resected pancreas are known but are controversial.

In pancreatic cancer, the DNA content has rarely been investigated, and the results are controversial (4,5). For DNA analysis, image cytometry (ICM)

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offers an advantage over flow cytometry (FCM) in that only tumor cells are used for DNA analysis. Weger *et a*l. (6) showed that, in pancreatic cancer, a DNA histogram measured by the flow cytometric method was not useful due to high background activity. Baisch *et al.* (4), therefore, raised the question of whether flow cytometry measurements of paraffin-embedded materials are precise enough to reveal the exact incidence of anueploidy in any tumor type (4). Moreover, some investigators have advocated that small anueploid populations could not be distinguished by the flow cytometric method (4-8).

These facts indicate that image analysis of the DNA content of tumor cells is an exact and reliable method to obtain an accurate prognosis of patients with pancreatic ductal carcinoma. However, few studies using ICM assessments of the cancer cell nuclei of primary ductal adenocarcinoma of the pancreas have been published. The aim of the present study was to assess the relationship between the nuclear DNA content of the tumor cells and pathological findings. The ultimate goal was to investigate whether the 4c ER, defined as the percentage of cells with an anueploid nuclear DNA content over 4c, had a strong relationship with pathological findings and whether the 4c ER might be a prognostic factor in pancreatic carcinoma.

PATIENTS AND METHODS

The investigation was performed using histological paraffin-embedded tumor material obtained from 35 patients (17 male / 18 female; mean age of the patients: 64.6±8.9 years) after resection of the invasive ductal adenocarcinoma of the pancreas at the First Department of Surgery, Shimane Medical University, from 1984 to 1994. Twenty-three tumors were located in the head of the pancreas. Of the twenty-three tumors, seven were found in the body, and three, in the tail of the pancreas. In two patients, the carcinoma had infiltrated the entire pancreas. For DNA analysis, the tumor area within the paraffin-embedded material was identified by a pathologist using hematoxylin and eosin-stained reference sections. 4μm of one section from the center was cut and dewaxed, dehydrated. Then, the sections were stained with Feulgen using a quantitative DNA staining kit (Cell Analysis System, Inc., Elmhurst, IL), a commercially available stain and reagent system. All slides were studied using the Quantitative Ploidy Analysis Program in the CAS 200 Image Analysis System (Cell Analysis System, Inc., Lombard, IL) At least 200 structurally identified neoplastic cell nuclei encountered in a systematic screening of each cancer were analyzed in each specimen, and DNA histograms were generated for each cell population. As a control, the DNA content of normal cells at the G0/G1 phase was defined as 7.18pg using a DNA calibration slide supplied by the CAS 200 Image Analysis System. The 4c was defined as a tumor which contains four nuclei. The DNA ploidy pattern and the DI of the neoplastic cells were calculated, and the 4c ER, defined as the percentage of cells with an aneuploid nuclear DNA content over 4c, was also calculated. Furthermore, the relationship between the 4c ER and prognostic pathological factors, such as clinical stage, tumor size, lymph node metastasis, perivascular invasion, tumor differentiation, and plexus invasion, was studied. The DI and 4c ER were expressed as the mean±standard deviation. Statistical analysis was conducted using the Student's t-test of the χ^2 square test. $P<0.05$ was considered statistically significant.

RESULTS

1) Relationship between the DNA ploidy pattern and pathological findings

In the present study, a diploid pattern of the DNA content was found in fifteen cases of pancreatic cancer, while an aneuploid pattern of the DNA content was found in twenty cases (Fig 1).

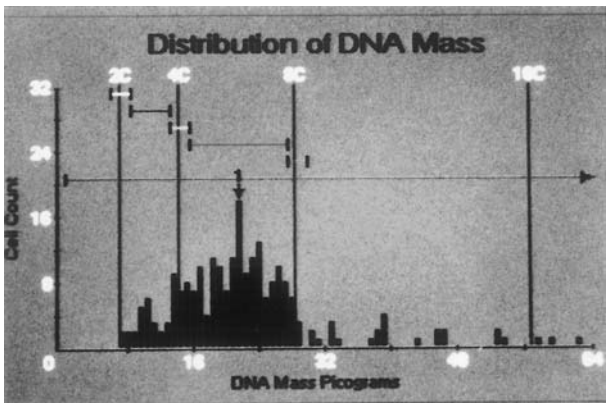

Fig. 1. DNA histogram of the aneuploidy pattern of the invasive ductal carcinoma of the pancreas in the present study.

Table 1 shows the relationships between the ploidy pattern of the DNA content and pathological findings in pancreatic cancer. Cases with tumor sizes over 6 cm and those with poorly differentiated tumors had a high incidence of the anueploid pattern of the DNA content. No significant association was observed between the DNA content and other histological measures.

Tabel 1: This table demonstrated relation between ploidy pattern and pathologic finding. The cases with tumor size over 6 cm and cases with poorly differentiated carcinoma had high rate of aneuploidy pattern, although there was no significant difference.

Pathologic Findings		diploid case (%)	aneuploid (%)
Tumor size			
	0-2cm(n=4)	3/4 (75.0)	1/4 (25.0)
	2-4cm(n=14)	8/14 (57.1)	6/14 (42.9)
	4-6cm(n=11)	4/11 (36.3)	7/11 (63.6)
	6cm< (n=6)	2/6 (33.3)	4/6 (66.7)
Retroperitoneal invasion			
	positive(n=25)	10/25 (40.0)	15/25(60.0)
	negative(n=10)	5/10 (50.0)	5/10 (50.0)
Anterior capsular invasion			
	positive (n=14)	8/14(57.1)	6/14(42.9)
	negative (n=31)	7/21(33.3)	14/20(66.7)
Perivascular invasion			
	positive (n=24)	10/24(41.7)	14/24(58.3)
	negative(n=11)	5/11(45.5)	6/10(60.0)
Neural invasion			
	positive (n=25)	11/25(44.0)	14/25(56.0)
	negative(n=10)	4/10 (40.0)	6/10(54.5)
Tumor differentiation			
	well (n=10)	6/10 (60.0)	4/10(40.0)
	modrately(n=19)	8/19 (42.1)	11/19(57.9)
	porrly(n=6)	2/6 (33.3)	4/6(66.7)

2) Relationship between the DI and pathological findings

Table 2 shows the relationship between the DI and pathological findings in pancreatic cancers. Cases with tumor sizes over 6 cm, retroperitoneal invasion, neural invasion, or poorly differentiated tumors had a high value of the DI. No significant association was observed between the DNA content and other histological measures.

3) Relationship between the 4c ER and pathological findings

Table 2 shows the relationship between the 4c ER and pathological findings in pancreatic cancer. The 4c ER of the cases with neural invasion (18.90±3.98%) was significantly higher (P<0.05) than that of those without neural invasion (7.89±3.78%). Moreover, the 4c ER of the cases with a poorly differentiated tumor (20.78±3.02%) was significantly higher (P<0.05) than that of those with a well- (8.68±2.34%) or moderately (13.98±1.98%) differentiated tumor.

Table 2:Relations between DNA Index, 4c ER and pathologic finding were demonstrated in this table. The DNA Index of the cases with neural invasion or poorly differentiated carcinoma was higher. And 4c ER of the cases with neural invasion or poorly differentiated carcinoma was significantly higher than that of the cases without those pathologic findings.

Pathologic Findings		DNA Index	4c ER (%)	
Tumor size				
	0-2cm(n=4)	1.23±0.67	6.6±1.62	
	2-4cm(n=14)	1.21±0.99	5.7±3.78	
	4-6cm(n=11)	1.89±0.56	8.9±2.99	
	6cm< (n=6)	1.97±1.08	10.6±2.78	
Retroperitoneal invasion				
	positive(n=25)	1.93±0.79	12.7±6.78	
	negative(n=10)	1.56±0.88	11.9±4.99	
Anterior capsular invasion				
	positive (n=14)	1.56±0.48	10.6±5.34	
	negative (n=31)	1.48±0.88	8.9±3.67	
Perivascular invasion				
	positive (n=24)	1.88±1.05	14.7±5.87	
	negative(n=11)	2.05±0.79	11.0±3.09	
Neural invasion				
	positive (n=25)	2.13±0.68	18.9±3.98	※1
	negative(n=10)	1.77±1.11	7.9±3.78	※1
Tumor differentiation				
	well (n=10)	1.35±0.88	8.7±2.34	
	modrately(n=19)	1.67±1.35	14.0±1.98	※2
	poorly(n=6)	2.56±0.79	20.8±3.02	※2

※1 p<0.05 by Student' t-test
※2 p<0.0001 by ANOVA

DISCUSSION

There is a relationship between the cytometrically assessed nuclear DNA distribution pattern of tumor cells and the length of a patient's survival. When the DNA ploidy of the tumor cells is of the so-called aneuploid type, the malignant disease is usually of an aggressive nature with rapid progress and a fatal outcome. Few studies using cytometrical DNA assessment of the cancer cell nuclei of primary ductal adenocarcinoma of the pancreas have been conducted. There have been reports regarding the prognostic significance of the tumor DNA ploidy in patients with pancreatic cancer (4,5). Eskelinen *et al.* reported that the DNA ploidy could be used to grade the aggressiveness of pancreatic cancer (9). On the other hand, Baisch *et al.* reported that the S+G2M fraction was significantly higher in pancreatic ductal carcinoma and was an independent prognostic factor, whereas the DNA ploidy was not of prognostic value (4).

Meanwhile, Yoshimura *et al*. reported that, in 86 patients with pancreatic ductal adenocarcinoma, the DNA aneuploid was observed in 42.9% of well-differentiated, 52.3% of moderately differentiated, and 71.4% of poorly differentiated tumors, and the prognosis for patients with retroperitoneal invasion and DNA aneuploidy was significantly worse than that for patients with DNA ploidy or those without retroperitoneal invasion (10). These results are controversial, indicating that the DNA ploidy alone does not yield an accurate prognosis. However, few studies using ICM assessments of the cancer cell nuclei of primary ductal adenocarcinoma of the pancreas have been published. The assessment of the DNA distribution by ICM with paraffin-embedded specimens is a valid method that has been used by some investigators (6, 11). From these facts, we assessed the DNA distribution by ICM in the present study.

In the present study, advanced carcinoma with either neural invasion or poor differentiation had a significantly higher 4c ER than that without those pathological findings. It is well known that the high incidence of a DNA aneuploid distribution reflects the well-known aggressive malignancy and high growth potential of this tumor entity. Weger *et al.* (6) reported that a pancreatic carcinoma with neoplastic cells with a triploid DNA content seems to be particularly aggressive and that triploid DNA could be of practical clinical significance for the determination of prognosis and the selection of therapy. The result of the present study was compatible with the result obtained by Weger *et al.* (6).

The present study demonstrated that the 4c ER in a DNA histogram had a strong correlation with the aggressive state of pathological findings such as tumor size, neural invasion, and tumor differentiation. In invasive pancreatic cancer, the most important pathological findings for early recurrence after pancreatectomy are neural invasion, tumor differentiation, and lymph node metastasis. This evidence suggested that the 4c ER of the DNA histogram in the ICM could be key to yield an accurate prognosis of patients with invasive pancreatic carcinoma.

It is very important to predict the clinical outcome of patients with pancreatic cancer, even if the cancerous lesion is completely resected, because the incidence of local recurrence after pancreatectomy is high and the prognosis is often extremely poor. The findings of the present study have implications for the prognostic information of invasive pancreatic cancer, and the evaluation of cellular kinetics by the 4c ER in the ICM adds important prognostic information.

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