Relationship between nuclear morphometry, DNA content and resectability of pancreatic cancer

Yin-Cheng He, Wei Peng, Jian-Guo Qiao, Jun Cao, Ji-Wei Chen

AIM: To investigate the association of nuclear morphometry and DNA content with resectability of pancreatic cancer.

METHODS: A total of 36 patients with pancreatic adenocarcinoma were divided into resectable group and unresectable group. The nuclear morphometry and DNA contents of tumor cells were analyzed by IBAS autoimage analyzer from paraffin-embedded materials. Localization size, histological type and grade, and clinical stage of the tumor were evaluated. Factors influencing resectability of pancreatic cancer were investigated using stepwise regression analysis.

RESULTS: Statistical significance was found in nuclear DNA content (integrated optical density, IOD) of tumor cells (1.64±0.41 vs 2.96±0.55), DNA ploidy, ages (46.5±5.3 years vs 58.6±8.7 years) and tumor volumes (298.1±101.5 cm³ vs 634.7±512.5 cm³) in both groups (P<0.05), and no difference was found in the nuclear morphometry (P>0.05). The rates of diploid/tetraploid and aneuploid were 66.7 % and 33.3 % in resectable group respectively, and 38.9 % and 62.1 % in unresectable group, respectively. The nuclear morphometry and DNA content (IOD) of tumor cells were measured by IBAS image analyzer (Kontron Company, Germany). The light was a halogen lamp (12V, 0.55), DNA ploidy, ages (46.5±5.3 years vs 58.6±8.7 years) and tumor volumes (298.1±101.5 cm³ vs 634.7±512.5 cm³) in both groups (P<0.05), and no difference was found in the nuclear morphometry (P>0.05). The rates of diploid/tetraploid and aneuploid were 66.7 % and 33.3 % in resectable group respectively, and 38.9 % and 62.1 % in unresectable group, respectively. There is a high correlation between resectability of pancreatic cancers and their DNA contents, DNA ploidy status and clinical stage.

CONCLUSION: There is a high correlation between resectability of pancreatic cancers and their DNA contents, DNA ploidy status and clinical stage.

INTRODUCTION
Pancreatic cancer is a highly malignant tumor, and has the most dismal prognosis among abdominal malignancies. The overall five-year survival for all the patients is only 0.4 %. Patients who undergo radical resection have five-year survival rates between 10-24 % [1,3,4]. The traditional approach to patients has been surgical procedure, but approximately 10 % to 20 % of cancers of the pancreatic head, body and tail can be resected for potential cure[7,9]. The reason is the topographical peculiarities of the pancreas and the biological aggressiveness are involved. Recent results indicated that there was a relation between DNA ploidy, DNA content of tumor cells nuclei and the biological behaviour of tumors[10-15]. The present study was to investigate the effect of the clinical characteristics, nuclear morphometry and DNA contents of pancreatic cancer on its resectability.

MATERIALS AND METHODS
Patients and group
A total of 36 patients with pancreatic carcinoma were enrolled in this study. There were 20 men and 16 women with a mean age of 52.7±8.4 years (range 32-72 years). They were followed up from 1999 to December 2002. No patient had received preoperative chemotherapy and radiotherapy. The patients were divided into: resectable group and unresectable group (18 patients per group). In resectable group, 15 patients underwent radical resection with Whipple’s procedure, 2 splenopancreatectomy and 1 total pancreatectomy. In unresectable group, palliative bypass of the biliary tree and/or the duodenum was performed in 14 patients and biopsy in 4 patients. Criteria for unresectability included definite liver metastases, tumor spread to the whole pancreas proved by needle biopsy on operation, obstruction or invasion of the portal or mesenteric veins, and/or tumor encasement of the celiac or superior mesenteric arteries. Localization size, histological type, histological grade, and clinical stage of the tumor were evaluated for each patient.

Preparation of sections
From paraffin blocks, areas with high contents of neoplastic parenchymal cells were selected. Sections about 50 to 100 μm thick were cut from these parts and deparaffinized. The two 5 μm slides were prepared, one was stained with hematoxylin and eosin for confirmation of the presence of pancreatic cancer cells in the 50 μm sections, the other was stained with Feulgen method for DNA measurement.

Feulgen reaction
The cell nuclei were stained by the classic Feulgen reaction. The sections were deparaffinized and washed with distilled water. Cells were hydrolyzed (1 N HCl at 25 °C for 2-3 minutes, 1N HCl at 60 °C for 10-12 minutes, 1 N HCl at 25 °C for 2-3 minutes) and washed with distilled water again, then stained with Schiff’s reagents for 80 minutes at room temperature, treated with freshly prepared sulfuric acid rinse 3 times (3-6 minutes), and washed in running tap water for 10 minutes. After dehydration, sections were coverslipped for DNA analysis.

Image analysis
Morphological characteristics and nuclear DNA content of tumor cells were measured by IBAS image analyzer (Kontron Company, Germany). The light was a halogen lamp (12V, 100W). Measurements were made using an interferential filter centered at 546 nm. Lymphocytes admixed with the tumor cells on the sections were used as internal control cells in the

Abstract

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CONCLUSION: There is a high correlation between resectability of pancreatic cancers and their DNA contents, DNA ploidy status and clinical stage.

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procedure. At least 100 structurally identified neoplastic cell nuclei, selected at random on three different areas, were analyzed in each specimen. The structural identification was based on conventional cytodiagnostic criteria such as nuclear shape and chromatin texture. Morphological characteristics [nuclear areas, perimeter (µm), length of minor axis (µm), length of major axis (µm), form factor (FF)] and nuclear DNA content of tumor cells (integrated optical density, IOD) were analyzed. FF was calculated using the following equation: $FF = 4\pi A/P^2$. Where $A$ is nuclear areas ($\mu m^2$), and $P$ is nuclear perimeter ($\mu m$).

**DNA ploidy**

The same investigator made all measurements with no previous knowledge of coded clinical data. The DNA histograms were divided into three groups[16]. Type A with one stemline within the diploid region (1.5-2.5 C) and a G2 fraction in the tetraploid region, type B with one stemline in the tetraploid region (3.5-4.5 C) and a G2 fraction in the octoploid region, independent of the presence of a diploid stemline, and type C with a stemline outside the diploid/tetraploid region or a mosaic pattern.

**Statistical analysis**

All the data were input into a microcomputer, and statistical evaluation was carried out using the SPSS 10.0 statistical package. Differences between the mean values were analyzed for significance using the Student’s $t$ test. Fisher’s exact probability test (two tailed) was used to assess the constituent ratio between two groups because of N of valid cases <40. To establish a relation between independent variables ($X$, resectability), stepwise regression analysis was used. The difference was considered significant when $P$ value was less than 0.05.

**RESULTS**

**Histological examination**

The 36 cases consisted of 33 ductal adenocarcinoma, 1 acinus cell carcinoma, 1 mucinous carcinoma, and 1 adenosquamous carcinoma.

**Nuclear morphometry and DNA contents**

Nuclear morphometry and DNA contents are shown in Table 1. Statistical significance was found in IOD, ages and tumor volumes between resectable group and unsectable group ($P<0.05$). No difference was found in the nuclear morphometry of tumor cells when resectable group with unsectable group were compared ($P>0.05$, Table 1).

**Nuclear DNA content of tumor cells (IOD) versus clinical pathologic parameters**

Relationships between IOD and clinical pathologic characteristics are presented in Table 1. Except for clinical stage (correlation coefficient $r=0.683, P<0.05$), no statistically significant relationship was found between IOD and age ($r=0.201, P>0.05$), tumor cell sources ($r=0.209$), histological grade ($r=0.167$), site ($r=0.235$), size ($r=0.312$), nuclear areas ($r=0.184$), perimeter ($r=0.085$), minor axis ($r=0.202$), major axis ($r=0.206$) and form factor ($r=0.149$).

**Correlation analysis of resectability**

Relationships between resectability and clinical pathologic characteristics were analyzed using Pearson correlation analysis. Correlation coefficients "r" are listed in Table 1. Age, clinical stage, site, size, IOD and ploidy status were covariates independently associated with resectability of pancreatic cancer.

| Table 1 Relationship between DNA contents, clinical characteristics and resectability |
|-------------------------------------------------|-----------------|-----------------|------------------|
| Variable Factor | Resectable group | Unsectable group | Correlation coefficient (r) |
|-----------------|-----------------|-----------------|------------------|
| $X_1$ Age (years) | 46.5±5.3 | 58.6±0.7* | 0.536* |
| $X_2$ Tumor cells (n) | | | 0.387 |
| Ductal | 17(94%) | 16(89%) |
| Other | 1(6%) | 2(11%) |
| $X_3$ Clinical stage (n)* | | | 0.605* |
| Stage I | 9(50%) | 0(0%)* |
| Stage II | 8(44%) | 3(17%) |
| Stage III | 1(6%) | 11(61%)* |
| Stage IV | 0(0%) | 4(22%) |
| $X_4$ Histological grade (n) | | | 0.394 |
| Kloppel I | 5(28%) | 3(17%) |
| Kloppel II | 6(33%) | 9(50%) |
| Kloppel III | 7(39%) | 6(33%) |
| $X_5$ Site (n) | | | -0.545* |
| Head of pancreas | 16(89%) | 10(56%)* |
| Other | 2(11%) | 8(44%)* |
| $X_6$ Size (cm²) | 298.1±101.5 | 634.7±312.5* | 0.579* |
| $X_7$ Nuclear areas (µm²) | 33.7±8.42 | 34.7±6.93 | 0.478 |
| $X_8$ Perimeter (µm) | 24.9±4.02 | 25.6±3.02 | 0.367 |
| $X_9$ Minor axis (µm) | 5.56±0.68 | 5.67±0.57 | 0.482 |
| $X_{10}$ Major axis (µm) | 8.3±1.10 | 8.5±0.93 | 0.434 |
| $X_{11}$ Form factor | 0.75±0.05 | 0.70±0.06 | 0.376 |
| $X_{12}$ IOD | 1.64±0.41 | 2.96±0.55* | 0.787* |
| $X_{13}$ DNA ploidy (n) | | | 0.759* |
| Diploid/ tetraploid | 12(67%) | 7(39%)* |
| Aneuploid | 6(33%) | 11(61%)* |

* $P<0.05$, vs resectable group, $P>0.05$, $T$ test, $P<0.05$, resectable group vs unsectable group

**Stepwise regression analysis of resectability**

Resectability predictors were evaluated in a stepwise regression model. In this model, stepwise regression analysis demonstrated that IOD ($X_1$), ploidy status ($X_3$) and clinical stage ($X_8$) were statistically significant resectable indicators after backward elimination ($F=2.80$). The regression equation for resectability was $Y=-9.2053+3.5428X_1+2.5392X_3+2.3001X_8$ ($RR=0.8780, P<0.01$). IOD remained to be the most important predictor, ploidy status was the second important one, followed by clinical stage according to variable $F$ values.

**DISCUSSION**

The prognosis of pancreatic cancer though remarkable diagnosis and therapeutic advances, have led many surgeons to question whether patients with pancreatic cancer should be submitted to radical surgery[17-20]. Nevertheless, the only chance for longterm survival and cure is undoubtedly related to the feasibility of radical surgery. Therefore, many surgeons have investigated the resectability of pancreatic cancer. Although the biologic behavior and location of the tumor are the most common predictors reliable factors that may predict resectability before laparotomy are waiting to be found[21-26].

DNA aneuploidy is one of the markers of malignant tumour cells. Aneuploid DNA pattern may be related to the development of distant organ metastases, invasion and prognosis[27-29]. Weger et al[16] investigated the relationship between DNA ploidy status and resectability of pancreatic cancer, in which 77 cases were studied by automatic DNA image cytometry, and the authors found that the radical resectable rates of diploid, tetraploid and aneuploid tumors were 87.5%, 48.6% and 25.9% respectively. Joensuu et al found that only 3 of 15 resected pancreatic cancers had...
aneploid DNA content, whereas 35 of 47 nonresected pancreatic cancers had aneuploid, and the patients with diploid tumors lived longer than patients with aneuploid cancers. These findings suggested that the biological behavior of tumor could influence life span and resectability[40], which was in agreement with our study. Nuclear morphology (nuclear area, perimeter, length of minor axis, length of major axis and form factor) was not significant between resectable group and unresectable group (P>0.05), but the tumor cellular DNA contents and ploidies in unresectable group were different from those in resectable group (P<0.05). It is suggested that there may be some difference between both groups at molecular levels, despite no difference was found in nuclear morphology. Table 1 shows that the rates of diploid/tetraploid and aneuploid were 66.7% and 33.3% in resectable group, and 38.9% and despite no difference was found in nuclear morphometry. The tumor cellular DNA contents and DNA ploidy levels, accompanied by high DNA contents and DNA ploidy levels, were 66.7% and 33.3% in resectable group, and 38.9% and 35 of 47 nonresected pancreatic cancers had aneuploid, and the patients with diploid

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DNA measurements as a guide to select patients for surgical

4 Zheng M, Liu LX, Zhu AL, Qi SY, Jiang HC, Xiao ZY. K-ras gene the clinical application of preoperative imaging techniques and plan for radical or palliative procedure. We are convinced that of tumors is principally determined by DNA contents and resectability of tumors and their quantitative DNA contents, accompanied by high DNA contents and DNA ploidy levels. According to our findings, there was a high correlation between resectability of tumors and their quantitative DNA contents, DNA ploidy status. That is to say, radical or palliative resection of tumors is principally determined by DNA contents and ploidy status of tumor nuclei. Tumor tissue could be easily obtained in most cases by ultrasound guided percutaneous fine needle aspiration biopsy before operation[31]. Therefore, DNA image analysis for aspirated cellular materials may provide important preoperative information, and may help surgeons plan for radical or palliative procedure. We are convinced that the clinical application of preoperative imaging techniques and DNA measurements as a guide to select patients for surgical resection will become mature in the near future.

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