New data on the immunohistochemical and morphological characteristics of ductal pancreatic adenocarcinoma

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Introduction

Pancreatic cancer was described in the 1760s by Giovanni Battista Morgagni in his classic book, De Sedibus et Causis Morborum per Anatomen Indigatis. And if until recently pancreatic cancer was a relatively rare disease, in recent years in most developed countries of the world it has come to the forefront of the overall cancer morbidity and mortality among malignant diseases of the digestive system. According to the literature, the frequency of occurrence of pancreatic cancer ranks 13th in the world and is one of the most unfavorable malignant neoplasms of the digestive system. In the United States and Japan, prostate cancer is among the five leading cancer morbidity and mortality [5, 12]. A radical method of treatment is surgical removal of the tumor, but only 15-20% of patients at the time of diagnosis are evaluated as resectable. The prognosis in patients with PC remains poor, the overall 5-year survival rate does not exceed 5% [5, 6, 14, 16].

Significant prognostic factors include age, size of the primary tumor, the state of the lines of resection, the presence of lymphogenous metastases, and the degree of malignancy (Grade) neoplasms [1, 5, 7, 8, 9, 10, 11, 12, 15, 16].

A tumor whose cells have high invasive and migratory properties, in a short time is capable of forming lymphogenous and distant metastases. It is known that to evaluate the invasive properties of malignant tumors and, possibly, to determine the increased metastatic potential, it is necessary to study the expression of various markers, including signal proteins Rac1 and RhoA, integrins of the family of β1 and β3 (Integrin β1, Integrin β3). (MMP2 and MMP9), epidermal growth factor and its receptor (EGF and EGFR), etc. In the tumor tissue, the expression of the transcription factors Snail and Twist, which may indicate the likelihood of epithelial- mesenchymal transition, which is the trigger mechanism for the development and successful implementation of the metastatic cascade program [1, 3, 4, 13]. From this point of view, the study of the morphology and immunohistochemistry of a tumor associated with increased invasive properties in pancreatic cancer seems very relevant.

Aim of research — to study the morphological and immunohistochemical features of ductal pancreatic adenocarcinoma.

Materials and methods

This study is retrospective. The morphological study of 84 patients with pancreatic cancer T1-4N0-2M0-1 at the age of 37 to 83 years, on average 61.5±10.0 years, who were treated in OGAUZ "G.K. Zherlova" and FGBU SibFNC of FMBA of Russia Medical Center №2 from 2007-2016.

Among the patients there were 43 (51%) men and 41 (49%) women, the average age of which was 58.6±9.1 years.

The prevalence of the tumor was determined according to the TNM classification (AJCC, 2010). IV (n=44.52.5%) and II (n=18.21%) stages of PCa were more often detected, III (n=7, 8.5%) and I (n=3.4%) were less frequently diagnosed.

A morphological study of the surgical or biopsy material obtained during radical or palliative interventions was carried out. In all cases, the histotype of the tumor was ductal adenocarcinoma of the pancreas (WHO recommendations, Geneva, 2010). The material was fixed in a 10-12% formalin solution. Posting of the material and preparation of histological preparations were carried out according to a standard procedure. The drugs were stained with hematoxylin and eosin. The study was carried out using a light microscope "Axio Lab.A1" (Carl Zeiss, Germany) and a histoscaner MIRAX MIDI (Carl Zeiss, Germany).

When studying the morphology of the primary tumor tissue, iron-like, trabecular, solid structures and

discrete groups of tumor cells were isolated in the infiltrative component.

The structures with a lumen, represented by one row relative to monomorphic cells with normochromic rounded nuclei, were considered as glandular. Trabecular structures were called either one series of small relatively monomorphic cells or structures consisting of 2-3 rows of cells of medium size with a moderate cytoplasm with rounded normochromic or hyperchromic nuclei.

Solid structures in the form of different in size and shape fields, consisting either of small cells with a moderate cytoplasm and monomorphic nuclei, or from large cells with abundant cytoplasm and polymorphic nuclei, were considered solid.

Clusters of 1 to 4 tumor cells, different in structure, were classified as discrete groups of cells. In most cases, a combination of different types of structures was identified. In each case, the number of different types of structures was counted, which varied from 1 to 4, respectively. In the structures of the infiltrative component of the tumor, the presence of such a feature as croweding, which morphologically manifested in an increase in the number of tumor cells per unit volume, was determined. In addition, in the stroma around different types of structures of the parenchymal component, the presence of retraction was determined. Retraction was considered an "arterial" gap between the cells of the parenchymal component and the surrounding matrix. In the stroma of the tumor, the severity of fibrosis and inflammatory infiltration was assessed according to the 3-point system (1 point — weak, 2 points — moderately, 3 points — pronounced).

Immunohistochemical study was performed according to a standard procedure. Antibodies from Dako to Ki67 (clone MIB-1, RTU, mouse) were used. Ki67 expression was evaluated as a percentage of positively stained cells in each variant of the parenchymatous component of the primary tumor of an invasive nonspecific carcinoma (in 10 fields of view per 1,000 cells with an increase of * 400). Antibodies from Abcam Anti-EGFR antibody [EP38Y] ab52894 (rabbit monoclonal 1: 100) were also used; Anti-P-catenin antibody [E247] ab32572 (rabbit monoclonal 1: 200); Anti-Integrin beta 3 antibody [EPR2417Y] ab75872 (rabbit monoclonal 1: 250); Anti-Integrin beta 1 antibody [4B7R] ab3167 (mouse mono-clonal 1:20); Anti-MMP2 antibody [6E3F8] ab86607 (mouse monoclonal 1: 200); Anti-Snail antibody (rabbit polyclonal 1: 1600); Anti-Twist antibody [Twist2C1a] — ChIP Grade ab50887 (mouse monoclonal 1:50). The expression of these markers in each of the present types of structures of the parenchymal component of the primary tumor was assessed by the following parameters: the presence or absence of expression; intensity of expression; percentage of tumor cells with positive marker expression (in 10 fields of view per 1000 cells at x400). The processing of the obtained data was carried out with the help of the program "Statistica 10.0".

Results and research

In 61% of cases with morphological study met adenocarcinoma of moderate differentiation degree in comparison with the cases with a high (15%; p=0.0000) and low degree of tumor differentiation (24%; p=0.0000). The parenchymal component of the neoplasms was represented by glandular, trabecular, solid structures and small groups of tumor cells.

In 88% of tumors were determined in zhelezistopodobnye structure, at least — trabecular (28%; p=0.0000), solid structure (19%; p=0.0000) and discrete groups of tumor cells (28%; p=0.0000). More often there was a combination of several types of structures (from 1 to 4). There were monomorphous tumors (61%), represented exclusively by ferruginous structures.

When studying cellular polymorphism severity revealed that tumors are more frequent polymorphism with moderate cells (51%), the tumors with a weak and a high degree of cellular polymorphism occur significantly less frequently (38%; p=0.04 and 11%; p=0.0000 respectively).

Neoplasms characterized by the presence of the expressed fibrous stroma (55%) had moderate rarer tumors (33%; p=0.002) and a slight degree of fibrosis (12%; p=0.0000). A study of the severity of inflammatory infiltration of the stroma of the tumor showed that tumors with poorly expressed inflammatory infiltration (10%) were less common than in cases. when moderate (45%, p=0.0000) or severe infiltration is observed (34%, p=0.0001). The phenomenon of crowding during histological examination was revealed in 19 (22%) patients. The retraction around the parenchymal structures of the tumor was determined in 12 (14%) cases.

The study examined the expression of markers associated with increased invasive properties of tumor tissue (Table 1).

Concerning other structures of tumor tissue, expression of MMP2 was much more common in

trabecular structures and in discrete cell groups. Positive expression of integrin β1 in discrete groups of tumor cells was observed less often than in other structures. The evaluation of integrin β3, EGFR, transcription factors Twist and Snail showed that positive expression of these markers was more often determined in trabecular structures (Table 1). Conversely, positive expression of β-catenin was less frequent in trabecular structures and in groups of tumor cells than in other structures of the infiltrative component.

Table 1

Distribution of patients with ductal adenocarcinoma of the pancreas depending on the presence of expression of the studied markers in different structures of the infiltrative component of the tumor

Type of structures of the infiltrative tumor component	Expression of markers Number of patients MMP2 Snail wist Integrinβ1 Integrinβ3 β-катенин EGFR						
Glandular-like	32/74 (43%)	34/74 (45%)	19/73 (26%)	55/74 (74%)	20/73 (27%)	51/74 (69%)	24/73 (32%)
Trabecular	17/20(85%) P_1=0,0006 P_3=0,001	19/23(82%) P_1=0,001 P_3=0,005	11/18(61%) P_1=0,002 P_3=0,004	13/22 (59%)	10/21(47%) P_1=0,04 P_3=0,03	10/24(42%) P_1=0,009 P_3=0,005	15/21(71%) P_1=0,0009 P_3=0,002
Solid	5/16 (31%)	7/17 (41%)	2/15 (13%)	14/16 (87%)	2/13 (15%)	15/18 (83%)	4/17 (23%)
Discrete cell groups	17/22(77%) P_1=0,003 P_3=0,003	20/24(83%) P_1=0,0008 P_3=0,004	15/26(58%) P_1=0,002 P_3=0,003	11/26(42%) P_1=0,003 P_3=0,003	12/26(46%) P_1=0,03 P_3=0,03	8/25(32%) P_1=0,0008 P_3=0,001	17/24(71%) P_1=0,0006 P_3=0,002

The expression of integrin β1, determined by 1-3 points, was lower in trabecular structures (n=13, 2,3±0,7) and in discrete groups of tumor cells (n=11, 2,4±0,6) with the correlation of this (n=55, 2.8±0.4) (p=0.0005 and p=0.003, respectively) and solid structures (n=14, 2.9±0.3) (p=0.003 and p=0.006, respectively). The expression of integrin β3 expression was high, in all structures it corresponded to three points and did not differ statistically significantly. The expression expression of MMP2, β-catenin, EGFR, and transcription factors Snail and Twist did not differ in different structures of the parenchymatous component of the tumor.

Expression of the studied markers in different structures of the infiltrative component of ductal adenocarcinoma of the pancreas is presented in Table. 2. The level of expression of MMP2 in all structures was high, ranged from 92.2 to 99.0%, did not differ in different structures of the parenchymatous component of the tumor (Table 2).

Table 2

Expression of markers in different structures of the infiltrative component of ductal pancreatic adenocarcinoma

Type of structures of the infiltrative tumor component	Expression of markers Number of patients MMP2 Snail Twist Integrinβ1 Integrinβ3 β -катенин EGFR						
Glandular-like	90,4+/-19% (n=32)	94,3+/-12,4% (n=34)	58,3+/-25,2% (n=19)	95,2+/-10,8% (n= 55)	96,3+/-6,4% (n=20)	88,2+/-18,6% (n=51)	64,3+/-16,4% (n=24)
Trabecular	96,7+/-6,7% (n=17)	96,9+/-4,6% (n=19)	61,1+/-29,1% (n= 11)	91,3+/-15,6% (n= 13) P_3=0,04	99,4+/-0,9% (n=10)	82,5+/-21,3% (n=10)	69,6+/-16,5% (n=15)
Solid	99,0+/-1,0% (n=5)	96,5+/-9,1% (n=7)	69,0+/-33,9% (n=2)	98,9+/.-2,9% (n=14)	93,5+/-6,3% (n=2)	89,9+/-16,7% (n=15)	75,2+/-18,7% (n=4)
Discrete cell groups	92,2+/-23,0% (n=17)	95,3+/-5,9% (n=20)	51,1+/-30,5% (n=15)	95,0+/-10,9% (n= 11)	94,9+/-6,1% (n= 12)	69,6+/-26,6% (n=8) p_1=0,008 p_3=0,01	58,5+/-22,4% (n= 17)

In trabecular structures, a smaller expression of integrin β1 was determined when compared with the localization of expression of this marker in solid structures. In turn, a lower expression of β-catenin was determined in discrete groups of tumor cells with respect to glandular and solid tumor structures. The level of expression of integrin β3, EGFR, Snail and Twist markers in different structures of the parenchymatous component of the tumor did not significantly differ (Table 2).

When studying the proliferative activity of the tumor, it was found out that the level of expression of Ki67 is higher in trabecular structures (n=22, 17.1±6.6%) compared with that in ferruginous (n=75, 11.2±7.8%; p=0.0009) and solid structures (n=19, 10.8±8.7%, p=0.006) of the parenchymal component.

A higher level of proliferative activity was observed in discrete cell groups (n=24, 19.4±7.8) compared with ferruginous (p=0.000) and solid structures (p=0.0007).

Conclusion

On the basis of this, it can be concluded that the ductal adenocarcinoma of the pancreas is characterized by a morphological heterogeneity, but more often the infiltrative component of the tumor is represented exclusively by the ferruginous structures and has a monomorphic structure, more often in neoplasms a moderate degree of differentiation is noted, and in the stroma there is a pronounced fibrosis with the phenomena of moderate or severe inflammatory infiltration. The study also determined the heterogeneity of the expression of markers associated with invasive growth. It has been established that, with respect to other tumor tissue structures, MMP2 expression is more often detected in trabecular structures and in discrete cell groups. The expression of integrin β1 in discrete groups of tumor cells was less positive compared to other structures of the tumor parenchyma. The evaluation of such markers as EGFR, integrins of the β3 family, transcription factors Twist and Snail revealed that along with discrete cells, the positive expression of these molecules is also more often determined in trabecular structures than in ferruginous and solid structures. Concerning the expression of β-catenin, the picture was the opposite, less often it was determined specifically in trabecular structures and in groups of tumor cells.

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In recent years, especially in developed countries, there has been an increase in the incidence of pancreatic cancer. Only 20% of tumors at the time of diagnosis are evaluated as resectable, but in these cases, the prognosis of the disease is unfavorable. The overall 5-year survival rate does not exceed 5%.

Pancreatic cancer was described in the 1760s by Giovanni Battista Morgagni in his classic book "De Sedibus et Causis Morborum per Anatomen Indigatis". Over the next 200 years, pathologists significantly improved our understanding of the macro- and microscopic features of this disease. At the same time, morphological research remained the basis of diagnostics for centuries. The introduction of immunohistochemical studies into clinical practice in the late 1970s and early 1980s radically changed our approach to diagnosing this disease. Evaluation of morphological features, as well as features of expression of markers that determine the invasive potential of such neoplasms, can serve in the future as a fundamental basis in solving questions concerning possible factors of prognosis upon malignant tumors of such a localization.

Aim of research — to study the morphological and immunohistochemical features of ductal pancreatic adenocarcinoma.

Materials and methods. The study included 84 patients with pancreatic cancer T1-4N0-2M0-1 stage, aged from 37 to 83, who underwent surgical treatment. Morphological study of the operating material was carried out. The condition for inclusion in the study was a histotype of the tumor, namely ductal pancreatic adenocarcinoma. Posting of the material, preparation of histological preparations, coloring, immunohistochemical examination were carried out according to a standard procedure.

Results and conclusion. The study made it possible to characterize the tumor morphology, as well as the features of expression of markers associated with more evident invasive characteristics of the tumor. The results of this work may be of interest in terms of their further comparison with the parameters of various forms of progression upon pancreatic cancer.