The prognostic significance of clinicopathological features and apoptosis inhibitor proteins in pancreas ductal adenocarcinoma

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Abstract

Introduction : **Pancreas ductal adenocarcinoma (PDAC) is among tumors with unfavorable prognosis. The aim of this study was to determine potential prognostic factors in PDAC.**

Materials and methods : **We retrospectively evaluated a total of 117 cases according to clinicopathological parameters and survivin and livin expression. We investigated the relationship between these parameters and their prognostic significance.**

Results : **In univariate analysis, lymph node metastasis, surgical margin positivity, tumor size over 4 cm and survivin expression were associated with poorer survival but livin expression was not correlated with survival-time. In multivariate analysis, only lymph node metastasis was independent factor predicting a poorer outcome.**

Conclusions : **In present study, the lymph node metastasis was the strongest predictor of survival. Our finding suggest that survivin could be a target for the treatment of advanced stage resectable PDAC. Our results need to be supported by studies on larger series with advanced techniques.** (Acta gastroenterol. belg., **2014**, 77, **229-234**).

Key words : pancreas ductal adenocarcinoma, apoptosis inhibitor proteins, prognosis, Survivin, Livin.

Introduction

Pancreas ductal adenocarcinoma (PDAC) is the most frequent neoplasm of the pancreas (approximately 85-90% of all pancreas neoplasms) and has a poor prognosis (1). Tumor size, histological type, differentiation, perineural and lymphovascular invasion, regional lymph node metastasis and surgical margin positivity are known as the poor prognostic indicators (2,3,4,5). Perineural and lymphovascular invasion commonly appear even in the smallest primary tumors and suggest a propensity for early distant spread (6). The molecular analysis of PDAC has provided a lot of knowledge about cancer genes and classical cancer signaling cascades. The finding about pancreatic cancers that have mutations in apoptotic pathways emphasize the role of apoptosis (7,8).

Apoptosis, also called 'Cellular Suicide', is a programmed cell death mechanism against cellular damage. The caspase protease family are accepted as key proteins for apoptosis. Proteins within the apoptosis mechanism that inhibit both the caspase protein family and the other proapoptotic proteins with an antagonistic effect are called intracellular apoptosis inhibitor proteins (IAPs) (9). All IAP proteins contain one to three baculovirus IAP repeat (BIR) domains that are required for antiapoptotic activity, and most of them also possess a carboxyl-terminal RING domain (9,10). Eight IAP family members have been defined in humans so far : NIAP, cIAP1, cIAP2, XIAP, IAP-like protein 2, Apollon, Livin/ML-IAP and Survivin (9).

Survivin, the smallest member of IAPs, is not expressed in most human tissues except in some rapidly dividing cells (cervical basal cells, CD34+ bone marrow cells, thymocytes, etc.) (11). It is expressed in the majority of tumors such as pancreas cancer, ovarian cancers, breast cancers, papillary urothelial cancers. Besides, its expression is thought to be related to poor prognosis and resistance to treatment (12,13,14,15).

Livin is one of the last defined IAPs. Two different subtypes with proapoptotic and antiapoptotic effects have been defined (16). A high expression of livin is associated with an unfavorable prognosis in osteosarcoma and neuroblastoma, recurrence in superficial bladder cancers and resistance to chemotherapy in pancreatic cancer cells while its expression is not correlated with the prognosis in some tumors such as hepatocellular carcinoma, nasopharynx carcinoma, and non-small cell lung carcinoma (12,17,18,19,20,21,22). There seem to be no article with large series about the prognostic value of immunohistochemically (IHC) livin expression in PDAC.

In order to understand the impact of clinical, pathological and immunohistochemical features on prognosis, we retrospectively the evaluated surgical specimens from patients who had PDAC.

Materials and methods

Clinicopathological data

From 2000 to 2008, 146 patients underwent pancreatic resection for pancreatic mass in our institution. 117 patients were diagnosed with PDAC. Hematoxylin-Eosin-stained sections of the 117 patients were reevaluated by two pathologists and pathological findings were recorded

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in a standard format : histopathological diagnosis with grade of differentiation ; tumor site and size ; tumor node metastasis ; extension to the duodenum, peripancreatic soft tissue ; neural or lymphovascular invasion ; concurrent chronic pancreatitis and PanIN ; and resection margin status (pancreatic, common bile duct, and retropancreatic resection margin). The localization was classified as head, body or tail ; the tumor size as ≤ 4 cm and > 4 cm ; the differentiation degree as well, moderate and poor ; the PanIN degree as (we regarded the highest PanIN degree observed in the patient as the dominant pattern.) PanIN-I, PanIN-II and PanIN-III ; and other parameters as present or absent. The presence of macroscopic and/or microscopic tumor at the surgical margin was considered as surgical margin positivity. The clinical features of the cases and the macroscopical features of the tumors were obtained from the hospital archive system. The prognostic information was obtained from the archive records of Local Cancer Monitoring and Follow-up Center. Survival time was calculated from the date of resection to the date of death or to the date of the latest follow-up. The follow-up data of the patients were updated in January 2011.

Immunohistochemistry

4-micron-thick sections were obtained for IHC investigation by choosing one of the blocks that had gone through the routine process and best reflected the tumor features. Survivin (Clone D-8, Santa Cruz Biotechnology, Inc, 1:50 delusion, catalog number : sc-17779) and livin (Clone T-21, Santa Cruz Biotechnology, 1:25 delusion, catalog number : sc- 133741) primary antibodies were used for IHC. The survivin antibody was incubated at 37°C for 32 minutes and the livin antibody at 25°C for 100 minutes. The positive control was breast carcinoma for survivin and lymph node metastasis tissue of malignant melanoma for livin.

Immunohistochemically, cytoplasmic staining was considered positive for survivin and livin. At least 10 high power fields were evaluated for each tumor and the staining rate of the tumor cells was determined. A mean percentage of cytoplasmic positive tumor cells was determined and a level of immunoreaction was graded into four categories as follows : 0, < 5% ; 1+, 5-25% ; 2+, 26-50% ; 3+, 51-75% ; 4+, > 75% positive cells. In tumours showing heterogeneity of staining, the immunostaining was judged according to the prominent pattern.

Statistical analysis

The statistical analyses were performed with the SPSS software version 19.0. The correlation between survivin, livin expressions and clinicopathological characteristics was analyzed by using the chi-square test. Fisher's exact test was used for the comparisons of the categorical variables. A combination of scoring was performed, defining a score of immunoreaction from 0 as negative and 1 and 4 as positive. Grouping according to the median percentage as a cut- off point did not lead to any different results. Survival was univariately analyzed by the Kaplan-Meier method with a log-rank test for the comparison of subgroups. Multivariate survival analysis was performed by the Cox proportional hazard model (forward selection strategy using a likelihood ratio statistic) including the report of relative risks and their 95% confidential interval. A p value ≤ 0.05 was considered statistically significant in all statistical analyses.

This study followed the Declaration of Helsinki on medical protocol and ethics and the regional Ethical Review Board approved the study.

Results

Patients characteristics

There were 75 men (64.1%) and 42 women (35.9%). The median age of these 117 patients was 63 years (range, 38-84 years). The age distribution of the patients showed that 76 patients (64.9%) were between 51-70 and there were only 4 patients under the age of 40. The mortality risk increased with increasing age (Odds ratio : 1.015 (95% confidence interval : 0.995-1.036)) but we found no statistical significance between age and survival (p = 0.150). A single tumor focus was found in all the cases. The smallest tumor size was 1 cm and the largest 10 cm. They were macroscopically a hard, solid, grey-white masses with irregular borders and no cystic features. Tumor was seen in the retropancreatic resection margin in 17 (39.5%) cases, in pancreas margin in 15 (34.9%) cases, in common bile duct margin in 5 (11.6%) cases, in both pancreas and common bile duct margin in 3 (7%) cases, and in both pancreas margin and retropancreatic surgical margin in 3 (7%) cases.

Correlation between clinicopathological data

Statistical analyses were showed a positive correlation between pancreatic head localization and lymphovascular invasion (p = 0.040) and duodenum invasion (p = 0.001) ; between peripancreatic fat tissue invasion and perineural invasion (p = 0.008) and regional lymph node metastasis (p = 0.009) ; and between surgical margin positivity and tumor size over 4 cm (p = 0.020), regional lymph node metastasis (p = 0.020) and chronic pancreatitis (p = 0.040).

Correlation between clinicopathological data and survival time

The tumor was localized in the head of the pancreas in 110 patients, in the body in 4 patients and in the tail in 3 patients. The median survival of the patients in whom the tumor was localized in head of pancreas was 20.04 months, and it was 9.25 months when the tumor was localized in the body and it was 7 months when the tumor was localized in the tail. Peripancreatic soft tissue invasion was not detected in only two patients. In seven

Table 1. — **Results of univariate survival analysis according to various prognostic factors**

Parameters	Criteria	Number of cases	Mean survival time (month)	1-year-survival (%)	2-year-survival (%)	p Value
Sex	Men	75	15.93	44	19	0.098
	Women	42	25.43	49	26	
Size	≤ 4	85	23.18	49	29	0.001
	> 4	32	10.22	38	3	
Differentiation	Well	0	–	–	–	0.894
	Moderate	99	19.05	46	22	
	Poor	18	19.37	42	16	
Lymphovascular invasion	Present	67	20.65	50	26	0.462
	Absent	50	17.03	40	16	
Invasion of duodenum	Present	76	19.33	46	22	0.929
	Absent	41	18.72	45	21	
Chronic pancreatitis	Present	59	15.71	44	14	0.085
	Absent	58	23.48	47	30	
PanIN	Present	93	18.37	44	22	0.603
	Absent	24	22.57	51	20	
PanIN-Grade	PanIN I	29	19.07	40	20	0.691
	PanIN II	50	16.18	36	19	
	PanIN III	14	19.81	63	27	
Surgical margin	Positive	43	10.91	35	8	< 0.001
	Negative	74	25.28	53	33	
Lymph node metastasis	Present	90	13.92	37	11	< 0.001
	Absent	27	39.37	76	61	
Survivin	Positive	52	13.10	38	16	0.012
	Negative	65	24.16	52	26	
Livin	Positive	58	19.00	43	23	0.736
	Negative	59	19.75	49	20	

patients, perineural invasion was not seen and median survival time for these patients was 27 months (range ; 11-34 months). In almost all the patients, the tumor was localized in the head and it invaded the perineural and peripancreatic soft tissue invasion so survival analyses were not performed for localization, perineural invasion and peripancreatic soft tissue invasion and Patients characteristics and Univariate analysis according to clinicopathological data are shown in Table 1.

Correlation between immunohistochemical factors and clinicopathological data

There was no immunohistochemical staining with survivin in normal pancreas ducts but staining was seen in the base of some acinus cells, in some endocrine island cells, and in some cases in the stromal cells and chronic inflammation cells. A positive expression of survivin (score 1-4) was found in 52 (44.4%) of PDAC (Fig. 1). In 46 (88.5%) patients with survivin expression, there was also regional lymph node metastasis and this finding was significant ($p = 0.008$). In 22 (75.9%) of 29 patients with PanIN-I, survivin expression was not seen. However, survivin expression was observed in 50% of patients with PanIN-II and 64.3% of patients with PanIN-III. As the grade of PanIN lesions went up, survivin expression went up, too ($p = 0.05$).

Immunohistochemically, there was no livin expression in 59 (50.4%) patients while expression (score 1-4) was present in 58 (49.6%) (Fig. 2). Lymphovascular invasion was observed in 67.2% of patients with livin expression and this finding was significant, statistically ($p = 0.03$). However, there was no relationship between livin expression and the other clinicopathological data ($p > 0.05$).

Survival

The mean survival time was 19.47 ± 2.24 months and the median survival time 12 months (range ; 1-89 months). The survival rates of the cases were 79.8% in the 6th month (standard error : 0.041), 46.1% in the 12th month (standard error : 0.052), 26.3% in the 18th month (standard error : 0.046), and 22% in the 24th month (standard error : 0.043). Univariate analysis revealed the poor prognostic factors as lymph node metastasis ($p < 0,01$),

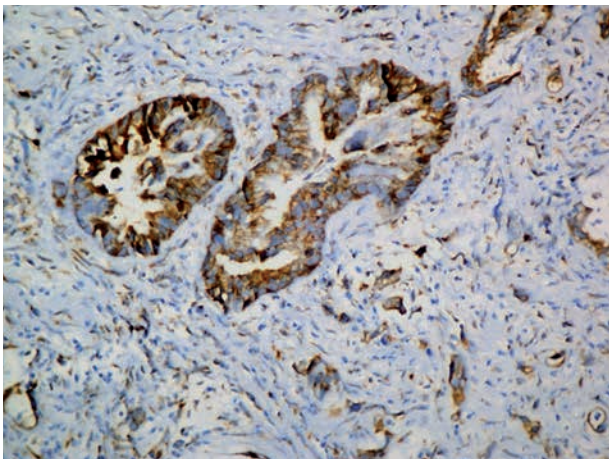

Fig. 1. — Positive staining for survivin in tumor cells (× 40)

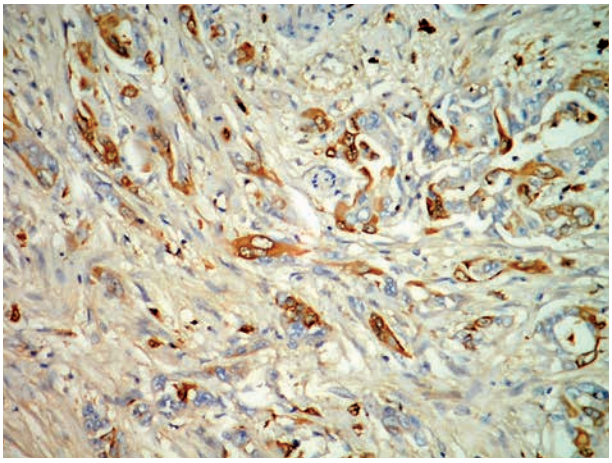

Fig. 2. — Positive staining for livin in tumor cells (× 40)

surgical margin positivity ($p < 0,001$), a tumor size over 4 cm ($p = 0,001$) and survivin expression ($p = 0,01$).

According to statistical analysis, livin expression was not correlated with survival-time ($p = 0.73$). Kaplan-Meier survival curves of the patients based on survivin expression were shown in Figure 3. In multivariate analysis, only lymph node metastasis ($p = 0.007$; RR 2.3) was independent factor predicting a poorer outcome. Results of multivariate survival (Cox regression) analysis were shown in Table 2.

Discussion

Approximately 85-90% of pancreas cancers are ductal adenocarcinomas and they have a very unfavorable prognosis (1,23). The most important factor for prognosis is resectability (24). Besides, many studies on the prognostic value of histological factors have reported the importance of histological type of the tumor, localization, size, differentiation, local spread, regional lymph node metastasis and surgical margin positivity (2,3,4,25,26,27). Perineural invasion commonly appear in even the smallest primary tumors and suggest a propensity for early

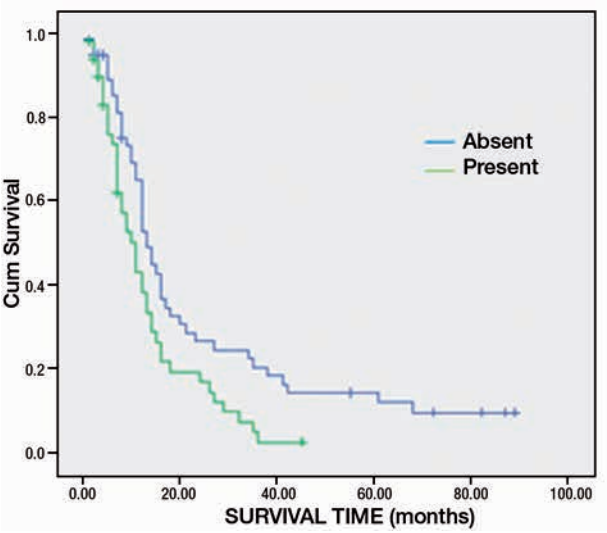

Fig. 3

distant spread but it was reported not to have a prognostic value in multivariate analysis (2,6,25). In this study, perineural invasion was significantly correlated with peripancreatic soft tissue invasion. This finding supports the notion that perineural invasion can be used as a tool for spread to neighboring tissues (6,28). However, we did not evaluate the prognostic value of perineural invasion because, in almost all the cases, the tumor had perineural invasion.

Some authors reported that poor differentiation was correlated with poor prognosis (29). However, we did not find a prognostic value of differentiation. This result seems to have been affected by the fact, in most patients, the tumor had moderate differentiation and no patient had a well- differentiated tumor. Studies investigating the prognostic value of differentiation and localization in large series are required.

According to a meta-analysis evaluating prognostic factors of PDAC and including 25930 patients from the 1980-2008 period, large tumor size was poor prognostic factor. This analysis also claimed that it is more possible to observe negative surgical margins in patients with small tumors, probably due to easier resection. The critical tumor size ranged between 2 cm and 5 cm according to the authors (3). Besides, a study on a large series reported that the retroperitoneal surgical margin positivity was significantly associated with tumor size (30). Our study results have shown that surgical margin positivity was associated with the tumor size over 4 cm. ($p = 0.020$) As is known, the status of regional lymph nodes can provide a guide for managing PDAC. There are many studies associating regional lymph node metastasis with short-term survival (3,25-28). The univariate analysis results of our study showed that tumor size over 4 cm and surgical margin positivity were poor prognostic factors. Besides, according to multivariate analysis, regional lymph node metastasis was the most important factor on prognosis. Results of our study showed that the early

Table 2. — **Results of multivariate survival (Cox regression) analysis**

Parameter	p Value	Relative risk	95% confidential interval
Lymph node metastasis	0.007	2.349	1.2-4.3
Surgical Margin positivity	0.103	1.503	0.9-2.4
Size (< 4 cm)	0.059	1.609	0.9-2.6
Survivin positivity	0.264	1.301	0.8-2.0

diagnosis and complete resection may be quite important for prognosis.

The tumors localized in the pancreas head are known to provide a basis for distal chronic pancreatitis secondary to progressive obstruction. There are also many articles showing that chronic pancreatitis increases the risk of PDAC (31,32). In the light of these findings, it is not surprising to find signs of chronic pancreatitis accompanying the tumor in PDAC. In the present study, chronic pancreatitis was significantly correlated with the surgical margin positivity. This information could be interpreted as chronic pancreatitis making it harder for the surgeon to palpate the tumor during the surgery and also making safe surgical border resection more difficult. It may be possible, in larger and more comprehensive studies, to elucidate the effect of a chronic pancreatitis on the surgical margin.

The accumulation of genetic and somatic mutations (mutation in the K-ras and the CDKN2A, Tp53, SMAD4/DPC4 and BRCA2 tumor suppressor genes) is a well-known step of PDAC carcinogenesis but the contribution of apoptosis to carcinogenesis is not clearly defined (7,8,33). The lack of apoptotic cells in PanIN-I and PanIN-II lesions indicate that antiapoptotic mechanisms become effective in the early stages of PDAC carcinogenesis (34). The observation of proteins such as cIAP2 and survivin from the apoptosis inhibitor family in various degrees in PanIN lesions and the high expression of survivin and livin in PDAC are accepted as the basis of this hypothesis (12,35). Many studies have shown the expression of apoptosis inhibitor proteins in both PanIN and PDAC (12,34,35). Bhanot et al (35) reported that survivin expression showed a positive correlation with the PanIN grade. There are only a few studies investigating the relationship between immunohistochemical survivin expression and the histological features in PDAC. According to these studies, survivin expression was not correlated with tumor size, grade, perineural and lymphovascular invasion and lymph node metastasis (36,37). However, in our study, survivin positivity was significantly associated with high grade PanIN lesions and advanced stage tumor which had regional lymph node metastasis. These two findings suggest that PanIN lesions use antiapoptotic mechanisms when progressing to PDAC and antiapoptotic mechanisms are effective in PDAC progression.

The increased expression of survivin is seen in many tumors and it is reported to be associated with short-term survival and recurrence and resistance to chemotherapy-radiotherapy (12-14,21,22,38,39). According to our results of multivariate analysis, survivin positivity was not independent prognostic factor in PDAC. However, survivin positivity was seen more in the patients who had advanced stage with regional lymph node metastasis. Although the number of cases was low, this finding suggest that survivin could be a target for the treatment of advanced stage resectable PDAC, but what about the inresectable tumors ? The studies on large series which contain the patients with inresectable tumor may illuminate the way to the answer to this question.

Livin is one of the IAP family members that was defined last and studies on the association between its high expression and prognosis have provided contradictory results. It has not been found to show an effect on prognosis in tumors such as hepatocellular carcinoma, nasopharyngeal carcinoma, and non-small cell lung carcinoma while there was a relationship with short-term survival and poor differentiation in tumors such as osteosarcoma, neuroblastoma and a relationship with early recurrence in bladder carcinoma (17-22). Articles evaluating its effect mechanism have stated that livin may have both an antiapoptotic and apoptotic effect on tumorogenesis (16,40). The number of studies on the association between livin expression and survival in PDAC is inadequate. In our study, livin positivity was not correlated with prognosis. More studies are needed to elucidate the effect mechanism and prognostic significance of livin in PDAC.

There are many studies on the treatment of PDAC that has high fatality within a short period. Scientists seeking targeted treatment or investigating the causes of chemotherapy resistance try to use the molecules playing a role in pathogenesis as target and apoptosis inhibitor proteins are among these important targets (12,41,42,43). Finding histological and molecular prognostic markers that may be effective in choosing treatment options and elucidating new treatments are becoming more and more important.

Conclusion

The present study aimed to investigate histological factors that may be important in determining treatment and in estimating the prognosis, and the molecules that may be targeted for treatment in PDAC. This study showed that only regional lymph node metastasis are the indipendent prognostic factor in PDAC. Our results need

to be supported by studies on larger series with advanced techniques.

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