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Original Article

Tumor size and clinical stage are independent risk predictors for the high occurrence and poor prognosis of postoperative liver metastasis in patients with radically resectable pancreatic cancer

Weiwei Sheng, Ming Dong, Jianping Zhou, Yuji Li, Fanmin Kong, Yulin Tian

Department of General Surgery, Gastrointestinal Surgery, The First Hospital of China Medical University, Shenyang 110001, China

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Abstract: There is no detailed data for illustrating the predictors that influence the occurrence and prognosis of postoperative liver metastasis (PLM) in patients with radically resectable pancreatic cancer (PC). 189 consecutive non-metastatic PC patients and 20 nude mice were entered into this study. We retrospectively determined the clinical factors that influenced the occurrence and prognosis of PLM, and constructed liver metastasis models in vivo for further biology research. PC recurred in 145 (76.7%) of the 189 patients, and 68 cases occurred in liver metastasis (46.8%). Its occurrence was positively associated with tumor size, T stage, lymph metastasis (classified as UICC stage IIB) and UICC stage (IA+IB vs. IIA+IIB). Multivariate analysis identified tumor size ≥ 3.5 cm (P = 0.005), lymph metastasis (P = 0.032) and UICC stage (P = 0.021) as independent risk factors for PLM. Meanwhile, PLM was an independent unfavorable prognostic indicator for the survival of 189 PC patients. Tumor size (P = 0.024) and lymph metastasis (P = 0.026) were independent risk prognostic indicators in 68 patients with PLM. In vivo, high incidence of liver metastasis was found in nude mice implanted with PC cells in both spleen and tail vein injected manners (100% and 80%). Meanwhile, tumor size and number of liver metastases were closely associated with the poor survival of nude mice. Tumor size and clinical stage are independent risk indicators for the high occurrence and poor prognosis of PLM in PC patients. Two liver metastasis models further reveal the aggressive biology of tumor size- and number- dependent liver metastasis in PC.

Keywords: Postoperative liver metastasis, occurrence, prognosis, predictor, pancreatic cancer

Introduction

Pancreatic adenocarcinoma (PC) is one of the most malignant digestive tumors with a total 5-year survival rate less than 5% [1]. Radical surgery may provide long-term benefits. However, even following curative resection, the reported 5-year survival rate remains low (7-24%) [2]. The poor prognosis is mainly due to its strong local recurrence (50-80%) and a risk of developing distant metastases (25-50%) [3].

Postoperative liver metastasis (PLM), as the most common distant metastasis in PC patients, is a main cause of treatment failure following surgical resection and a major factor associated with the poor prognosis in PC [4]. However, as yet, there was no detailed data for illustrating the definite predictors that influence the occurrence and prognosis of PLM in patients with radically resectable PC. Therefore, in order to prevent initiation and development of PLM in early stage, we retrospectively determined the clinical factors that influenced the occurrence and prognosis of PLM in PC patients. Meanwhile, two liver metastasis models were established to further evaluate the aggressive biology of liver metastasis derived from PC in vivo.

Materials and methods

Patients

This study was approved by the institutional review board of China Medical University and a

Table 1. Clinical characteristics of the 189 PC patients

Parameters		No. of patients
Cases		189
Age (years)	≤ 60	105
	> 60	84
Gender	Male	120
	Female	69
Tumor location	Head	127
	Body-tail	62
Tumor size (cm)	< 3.5	101
	≥ 3.5	88
Differentiation	Well	47
	Moderate to poor	142
T stage	T1+T2	70
	T3	119
Lymph nodes metastasis	N0 (negative)	130
	N1 (positive)	59
UICC stage	IA+IB	52
	IIA+IIB	137
Perineural invasion	Absent	133
	Present	56
Vascular permeation	Absent	127
	Present	62
Pre-therapeutic CA19-9 level	< 37 U/ml	52
	≥ 37 U/ml	137
Obstructive jaundice[a]	Absent	42
	Present	85

a, Obstructive jaundice was only presented in PC that located within head of the pancreas. Serum indirect bilirubin was higher than 34.2 umol/L (2 mg/dl).

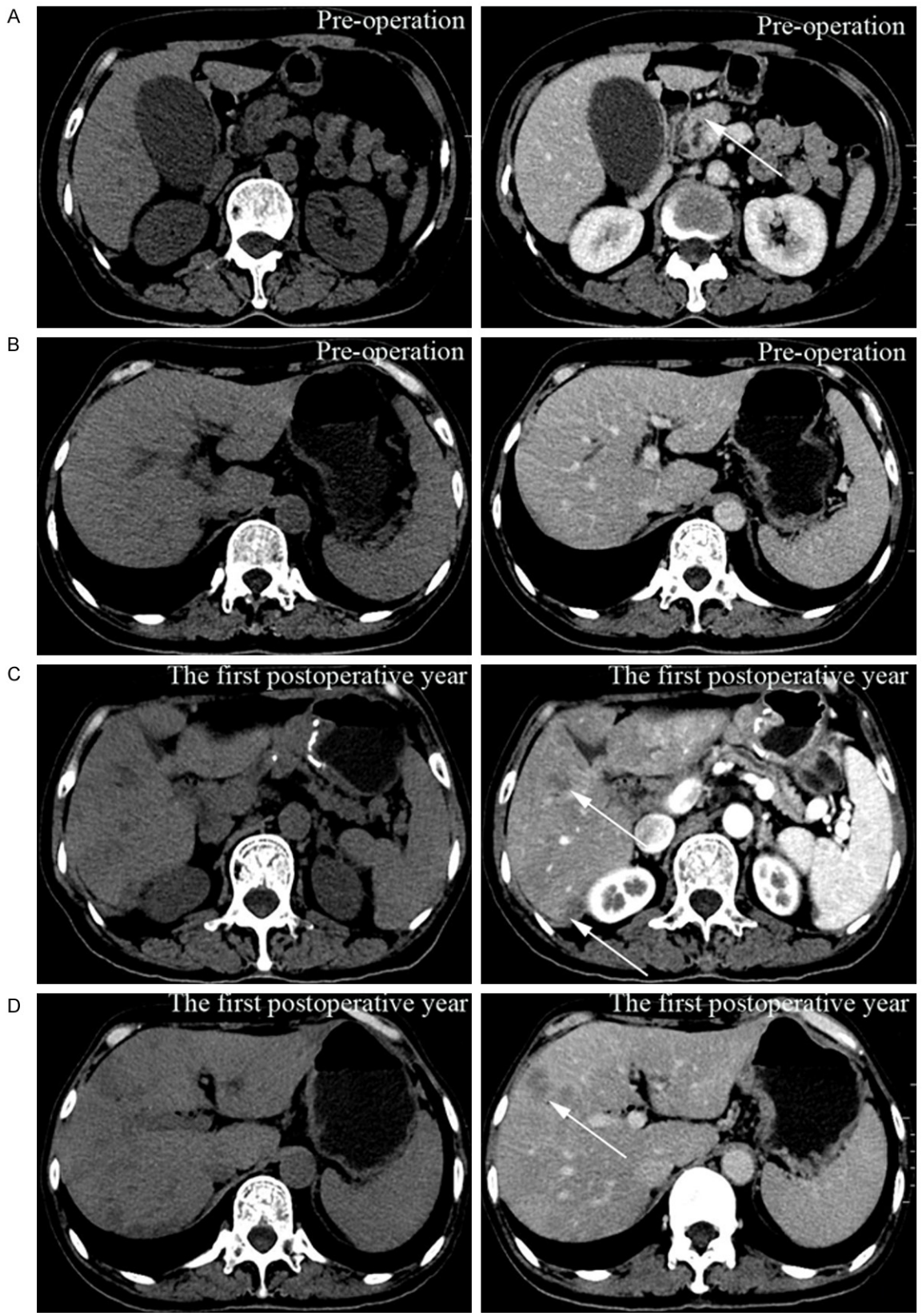
consent form was signed by each participating patient. All tumors were histologically proven to be invasive ductal adenocarcinomas of the pancreas. Preoperative tumor markers, abdominal ultrasound scan (US), contrast computed tomography (CT)/positron emission tomography (PET), contrast nuclear magnetic resonance (MRI) and surgical exploration were used to ensure whether all PC patients meet our resection criteria as Sugiura et al. previously reported [5], including: a) no distant metastasis, b) no evidence of tumor extension to the superior mesenteric artery or hepatic artery in PC, c) according to 7th edition of UICC 2010 TNM classification, all patients were identified as UICC stage ≤ IIB and T stage ≤ T3. Based on above criteria, between 2004 and 2014, 189 consecutive PC patients underwent radical pancreatectomy (127 cases of pancreatoduodenectomies, and 62 cases of distal pancreatectomies) were entered into this study. In order to achieve R0 resection, some cases underwent surrounding organs resection, such as spleen, adrenal gland, colon, left gastric artery, portal and superior mesenteric vein. In order to avoid bias in clinical significance analyze, patients who accept neoadjuvant treatment were excluded into this study. Namely, patients we recruited did not accept neoadjuvant treatment and postoperative chemoradiation based on their own decision in view of the side and indefinite therapeutic effect regard to above treatments.

Follow-up

After surgery, patients regularly underwent laboratory examinations, including tumor markers, liver function, US, abdominal CT/PET or contrast MRI. If the disease can't be diagnosed by noninvasive examination, needle biopsy could be performed for pathological diagnosis. We characterized the newly developed hepatic lesion as PLM, if the liver metastasis showed no definite evidence of other metastasis or recurrence elsewhere [6]. Patient follow-up examinations were performed every 3 months for the first 2 postoperative years, every 6 months for > 2 years, and yearly thereafter. In order to exclude the liver micrometastases that were not detected in pre-operation, PLM that was diagnosed within the postoperative follow-up time < 3 months were excluded from this study as Park et al. suggested [6]. Locoregional recurrence was defined as tumor recurrence within the tumor bed and regional lymph nodes. Distant metastasis was defined as metastasis to another part of the body, such as the liver, lung, bone and distant lymph. A dedicated table for patients' characteristics was summarized in **Table 1**. Abdominal CT for making a definite diagnosis of PLM was shown in **Figure 1**.

Materials

Human SW1990 PC cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). MiaPaCa-2 cells were obtained from the American Type Culture Collection (ATCC, USA). These cell lines were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Pre-operation
Pre-operation
B
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C
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The first postoperative year
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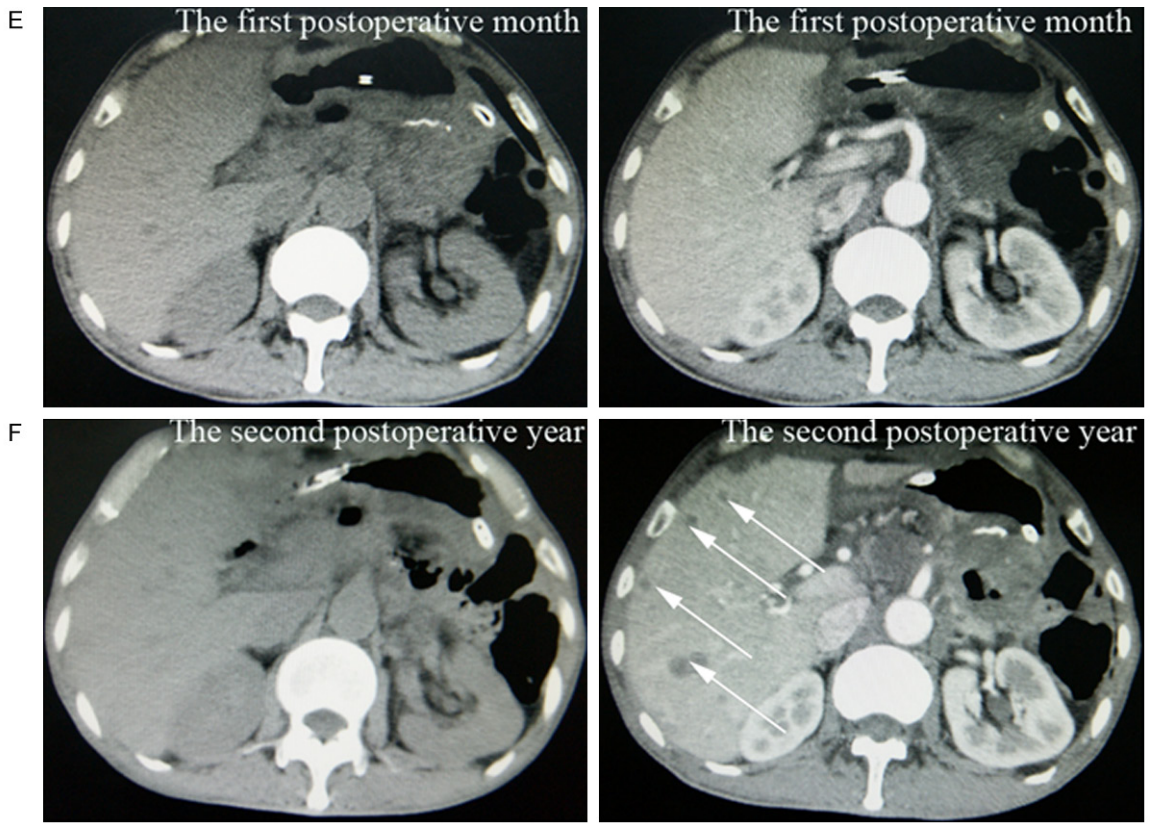

Figure 1. Abdominal CT of the PC patients in preoperative and postoperative phases, respectively. (A) In pre-operation, Plain and contrast enhanced CT of PC in the head of pancreas as arrows suggested. No liver metastasis was found. (B) Plain and contrast enhanced CT of the same PC patient in another CT slice, no liver metastasis was found. (C) Plain and contrast enhanced CT of the same PC patient underwent pancreaticoduodenectomy in the first postoperative year. Several liver metastases were found as arrows suggested. (D) Plain and contrast enhanced CT of the same postoperative patient in another CT slice, several liver metastases were found as arrows suggested. (E) Plain and contrast enhanced CT of another PC patient underwent distal pancreatectomy and splenectomy in the first postoperative month, no liver metastasis was found. (F) Plain and contrast enhanced CT of the same postoperative PC patient in the second postoperative year. Several liver metastases were found as arrows suggested.

maintained in recommended growth media with 10% fetal calf serum (Hyclone, Logan, UT). 20 cases of BALB/c female mice, 5 weeks of age, were obtained from Vital River Laboratory Animal Technology (Beijing, China).

Liver metastasis model in spleen injected manner

Animals were maintained according to institutional regulations in facilities approved by the Animal Care Committee of China Medical University, in accordance with Chinese government guidelines for animal experiments.

Under the guide of previous study [7], Miapaca-2 cells were harvested from subconfluent cultures via treatment with trypsin for 2 min, and resuspend the cells in FBS-free 1640 to produce single-cell suspensions for late spleen injection. A small horizontal laparotomy incision of about 2 cm was made in left abdominal flank under pentobarbital anesthesia. The spleen was identified and exposed. A total of 1×10^6/ml Miapaca-2 cells suspended in 100 μl of FBS-free 1640 were injected into the splenic capsule, being careful to avoid possible bleeding and leakage of tumor cells from the injection site. No unexpected mortality was observed in any of the mice. The mice were sacrificed upon 20% weight loss, and the survival days of nude mice were calculated from the injecting day to killing day. The liver metastases were investigated immediately, and then fixed for hematoxylin and eosin (HE) staining. Because liver metastases in spleen injected manner exhibited in focal lesion growth pattern (**Figure 3B**),

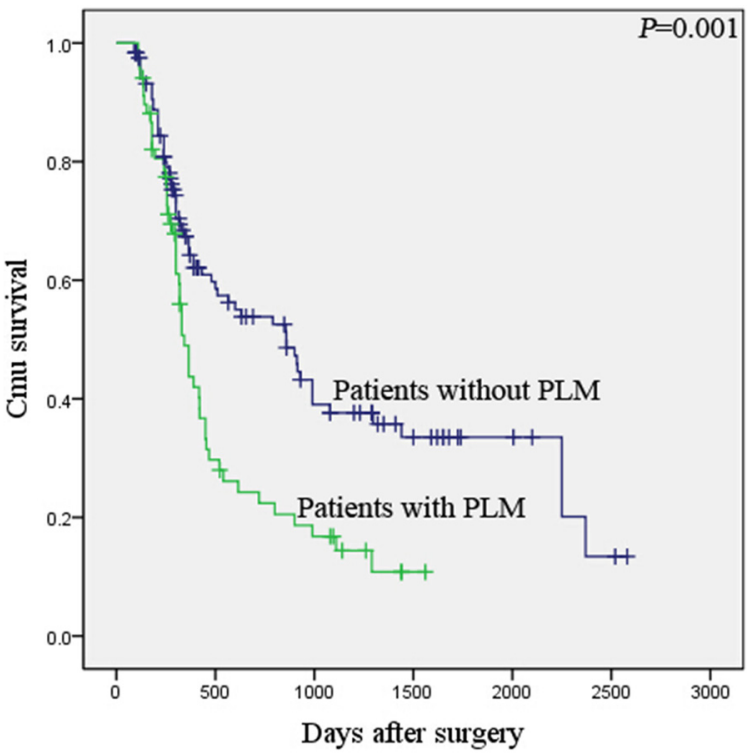

Figure 2. Kaplan-Meier analysis of overall survival of PC patients with PLM.

thus, tumor volumes were used in statistical analysis and were calculated by the following formula: length × width × height × 0.52 in millimeters by vernier calipers.

Liver metastasis model in tail vein injected manner

A total of 5×10^6/ml SW1990 cells suspended in 100 μl of FBS-free 1640 were injected into the tail vein of the other 10 cases of nude mice. A cotton swab was held over the injection site for 1 min to prevent leakage from tail vein. The mice were sacrificed upon 20% weight loss, and the survival days of nude mice were calculated from the injecting day to killing day. Liver metastases were investigated immediately, and then fixed for HE staining. Because liver metastases in tail vein injected manner exhibited in multiple points growth pattern (**Figure 4B**), thus, the number of liver metastases was calculated in subsequent statistical analysis.

Statistical analysis

Statistical analysis was performed using SPSS software 13.0 (SPSS, Chicago, IL, USA). The relationship between PLM and clinicopathological parameters was analyzed using a Chi-Squared test. A logistic regression analysis was performed to determine the impact of the pathologic findings that were significant with regard to PLM in the univariate analysis. The Kaplan-Meier method was used to estimate survival in PC patients and nude mice, and the differences were analyzed by the log-rank test. The variables that were found to be significant by the univariate analysis were subjected to a multivariate Cox proportional hazards regression analysis in a stepwise manner. A value of $P < 0.05$ was considered to be statistically significant.

Results

The status of postoperative recurrence

PC recurred in 145 (145/189; 76.7%) of the total 189 patients. Locoregional recurrence developed in 78 patients (53.8%); distant metastasis developed in 88 patients (60.7%), including 68 in liver metastasis (46.8%), 7 had lung metastasis (4.8%), 10 had the distant lymph nodes metastasis (6.9%), 3 had bone metastasis (2.1%), of whom 13 had both locoregional recurrence and liver metastasis, 8 had both locoregional recurrence and distant lymph node metastasis. Without doubt, liver metastasis is the most common distant metastasis in postoperative PC patients.

Prognostic factors of patients who underwent curative pancreatectomy for PC

Patients with PLM had a significantly worse overall survival ($P = 0.001$) (**Figure 2**; **Table 2**). The median survival of patients who developed PLM was 11.4 months, whereas the median survival of patients free from PLM was 28.6 months. 55 cases of deaths were found in 68 PC patients with PLM (80.9%). In addition, univariate analysis showed clinicopathological factors, such as, lymph nodes metastasis ($P = 0.003$), UICC stage ($P < 0.001$) and vascu-

Table 2. Univariate and multivariate analysis for prognostic factors of patients who underwent pancreatectomy for PC

Parameters	Median survival (days)	Univariate analysis *P* (log rank)	Multivariate analysis hazard ratio (95% CI)	*P*
Age (< 60/≥ 60 years)	485/450	0.757	—	
Gender (male/female)	450/615	0.507	—	
Tumor location (Head/Body-tail)	480/343	0.683	—	
Tumor size (< 3.5/≥ 3.5 cm)	790/330	0.073	—	
Well/Moderate to poor Differentiation	629/432	0.097	—	
T stage (T1+T2/T3+T4)	510/450	0.072	—	
Lymph nodes metastasis (N0/N1)	629/330	0.003	1.109 (0.927-2.121)	0.109
UICC stage (IA+IB/IIA+IIB)	1440/390	< 0.001	1.390 (0.788-2.451)	0.256
Perineural invasion (absent/present)	510/418	0.330	—	
Vascular permeation (absent/present)	615/365	< 0.001	1.699 (1.115-2.589)	0.014
CA19-9 level (< 37 U/ml/≥ 37 U/ml)	858/420	0.181	—	
Obstructive jaundice (absent/present)	600/480	0.337	—	
Liver metastasis after surgery (Negative/Positive)	858/343	0.001	1.592 (1.079-2.350)	0.019

lar permeation ($P < 0.001$) were also associated with patients' prognosis. In multivariate model, PLM ($P = 0.019$) and vascular permeation ($P = 0.014$) were independent unfavorable prognostic indicators in PC (**Table 2**).

Association of PLM with clinicopathological characteristics

An analysis of the relationship between PLM and clinicopathological characteristics was shown in **Table 3**. PLM occurrence was positively associated with tumor size ($P = 0.002$), T stage ($P = 0.004$), lymph nodes metastasis ($P = 0.004$) and UICC stage ($P = 0.003$), but had no relationship with age, gender, tumor location, differentiation, perineural invasion, pre-therapeutic CA19-9 level and obstructive jaundice ($P > 0.05$). A multivariate analysis (Logistic regression analysis) identified tumor size ≥ 3.5 cm ($P = 0.005$), UICC stage (IIA+IIB) ($P = 0.021$) and lymph nodes metastasis ($P = 0.032$) as independent risk factors for PLM (**Table 3**).

Univariate and multivariate analysis for prognostic factors in 68 cases of PC patients with PLM

The clinical predictors for the poor prognosis of patients with PLM were rarely reported in previous studies. In univariate analysis, tumor size ($P = 0.004$), lymph nodes metastasis ($P = 0.007$), UICC stage ($P = 0.004$) and vascular invasion ($P = 0.043$) were positively associated with the poor prognosis in 68 PC patients with PLM. Multivariate analysis identified tumor size ($P = 0.024$) and lymph nodes metastasis ($P = 0.026$) (also classified as UICC stage IIB) as independent risk prognostic factors for PLM patients (**Table 4**).

Two liver metastasis models in vivo

In order to further investigate biology of liver metastasis in PC, we constructed two liver metastasis models in vivo. Human Miapaca-2 cells were injected into the spleen of 10 cases of nude mice. Primary spleen tumor and liver metastasis which showed in focal growth pattern were proved by histopathological examination with HE staining and were successfully formed in all nude mice (100%) (**Figure 3**). Liver was the first planting site for PC cells in this model, which is an easy approach for liver metastasis model constructing.

Human SW1990 cells were injected into the tail vein of the other 10 cases of nude mice. Liver metastases which showed in multiple points growth pattern were found in 8 nude mice (8/10; 80%). However, no lung metastasis was found in all mice in this model (**Figure 4**). Theoretically, the lung was the first planting site

Table 3. Association of postoperative liver metastasis with clinicopathological characteristics in univariate and multivariate analysis

Parameters	No. of patients	Univariate analysis			Multivariate analysis	
		Postoperative liver metastasis		*P*	Odds ratio (95% CI)	*P*
		Absent	Present			
Cases	189	121	68			
Age(years)						
≤ 60	105	71	34	0.249	—	
> 60	84	50	34			
Gender						
Male	120	78	42	0.712	—	
Female	69	43	26			
Tumor location						
Head	127	83	44	0.585	—	
Body-tail	62	38	24			
Tumor size (cm)						
< 3.5	101	75	26	0.002	2.508 (1.328-4.737)	0.005
≥ 3.5	88	46	42			
Differentiation						
Well	47	35	12	0.085	—	
Moderate to poor	142	86	56			
T stage						
T1+T2	70	54	16	0.004	2.477 (0.792-7.747)	0.119
T3	119	67	52			
Lymph nodes metastasis						
N0 (negative)	130	92	38	0.004	2.266 (1.074-4.784)	0.032
N1 (positive)	59	29	30			
UICC stage						
IA+IB	52	42	10	0.003	2.184 (1.127-4.233)	0.021
IIA+IIB	137	79	58			
Perineural invasion						
Absent	133	87	46	0.539	—	
Present	56	34	22			
Vascular permeation						
Absent	127	87	40	0.066	—	
Present	62	34	28			
Pre-therapeutic CA19-9 level						
< 37 U/ml	52	36	16	0.358	—	
≥ 37 U/ml	137	85	52			
Obstructive jaundice[a]						
Absent	42	30	12	0.312	—	
Present	85	53	32			

a, Obstructive jaundice was only presented in PC that located within head of the pancreas. Serum indirect bilirubin was higher than 34.2 umol/L (2 mg/dl).

for tumor cells in tain vein circulation. It indicated that PC had a specific metastatic ability into liver.

In Miapaca-2 injected spleen model, nude mice with the tumor size of liver metastasis ≥ 0.5 mm^3 had a significant poor overall survival (*P* =

Table 4. Univariate and multivariate analysis for prognostic factors in 68 cases of PC patients with PLM

Parameters	Median survival (days)	Univariate analysis *P* (log rank)	Multivariate analysis hazard ratio (95% CI)	*P*
Age (< 60/≥ 60 years)	365/343	0.593	—	
Gender (male/female)	330/365	0.137	—	
Tumor location (Head/Body-tail)	420/317	0.176	—	
Tumor size (< 3.5/≥ 3.5 cm)	450/320	0.004	2.159 (1.109-4.202)	0.024
Well/Moderate to poor Differentiation	468/343	0.086	—	
T stage (T1+T2/T3+T4)	420/320	0.071	—	
Lymph nodes metastasis (N0/N1)	420/257	0.007	1.896 (1.078-3.336)	0.026
UICC stage (IA+IB/IIA+IIB)	432/320	0.004	2.846 (0.840-9.646)	0.093
Perineural invasion (absent/present)	365/330	0.443	—	
Vascular permeation (absent/present)	365/320	0.043	1.485 (0.840-2.625)	0.173
CA19-9 level (< 37 U/ml/≥ 37 U/ml)	421/330	0.087	—	
Obstructive jaundice (absent/present)	450/418	0.144	—	

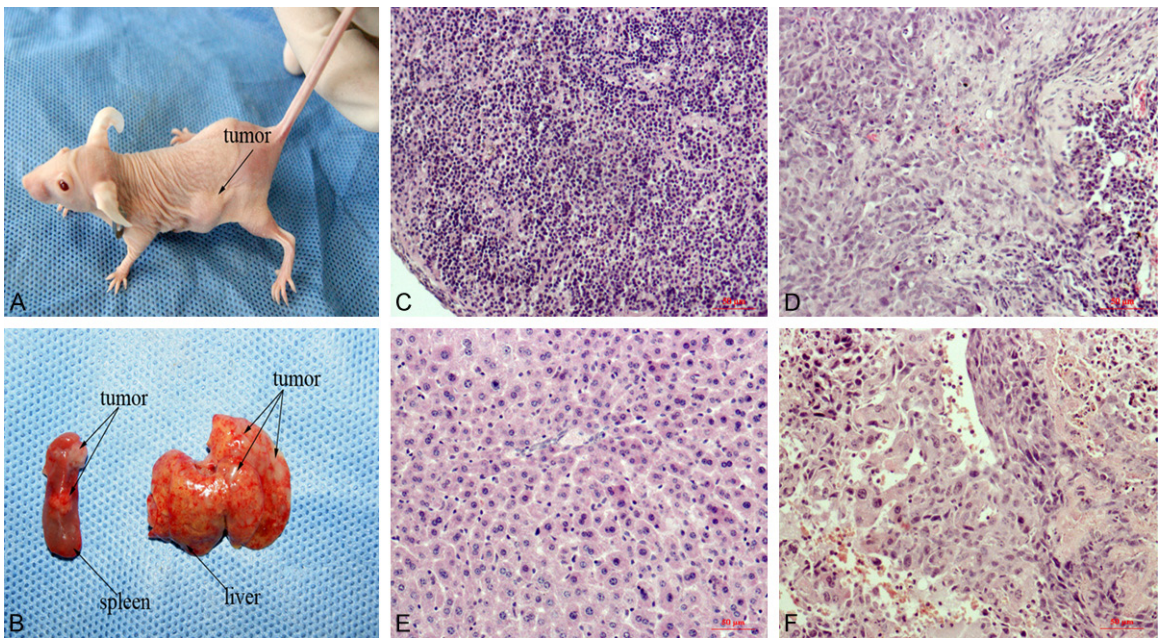

Figure 3. Liver metastasis model in spleen injected manner. (A) Primary splenic tumor implanted with Miapaca-2 PC cells was formed in nude mice as arrows suggested. (B) Both primary splenic tumor and liver metastases were formed in focal lesion growth pattern as arrows suggested. (C, D) HE staining for normal spleen (C) and primary tumor (D), respectively. (E, F) HE staining for normal liver (E) and metastatic tumor (F), respectively.

0.037) (**Figure 5A**), while nude mice with the number of liver metastases ≥ 5 had a significant poor overall survival, compared with the nude mice with the number of liver metastases < 5 or without liver metastases in SW1990 injected tail vein model ($P = 0.002$) (**Figure 5B**). It indicated that tumor size and number of liver metastases were closely associated with the poor overall survival of nude mice.

Discussion

In order to avoid bias in the difference of disease-progression and treatments toward PC patients, all patients were recruited with the

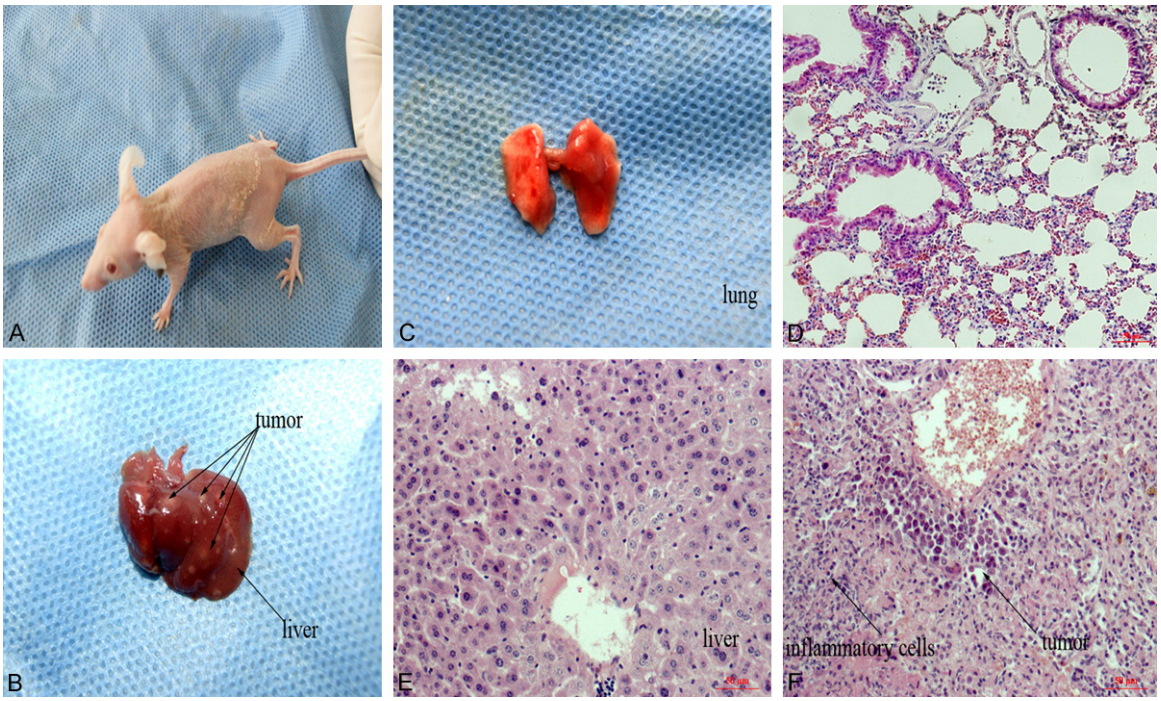

Figure 4. Liver metastasis model in tail vein injected manner. (A) 5-6 weeks later, the nude mice were exhausted after tail vein injection of SW1990 PC cells. (B) Liver metastases were formed in multiple points growth pattern as arrows suggested. (C, D) No lung metastasis was found in autopsy lung (C) and HE staining (D), respectively. (E, F) HE staining for normal liver (E) and metastatic tumor (F), respectively.

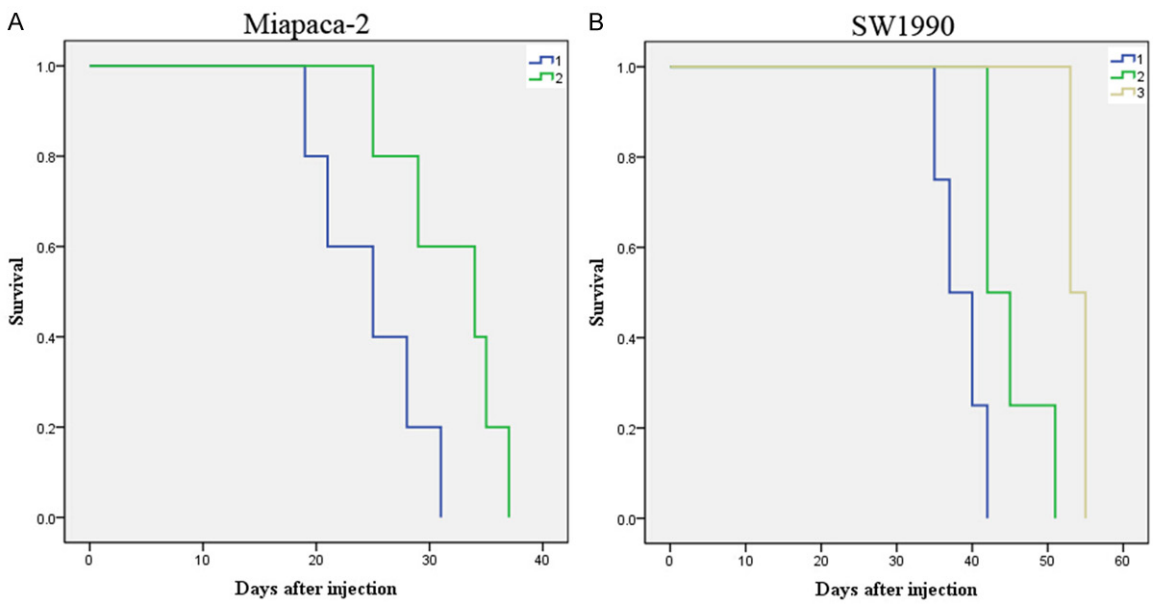

Figure 5. The effect of tumor size and the number of liver metastases in the overall survival of nude mice in spleen and tail vein injected models. A. In Miapaca-2 injecting spleen model, nude mice with the tumor size of liver metastasis ≥ 0.5 mm^3 had a significant poor overall survival, compared with nude mice with tumor size < 0.5 mm^3. 1. 5 cases of nude mice with tumor size ≥ 0.5 mm^3; 2. 5 cases of nude mice with tumor size < 0.5 mm^3. B. In SW1990 injecting tail vein model, nude mice with the number of liver metastases ≥ 5 had a significant poor overall survival, compared with the nude mice with the number of liver metastases < 5 or without liver metastases. 1. 4 cases of nude mice with the number of liver metastases ≥ 5; 2. 4 cases of nude mice with the number of liver metastases < 5; 3. 2 cases of nude mice without liver metastasis.

UICC stage ≤ IIB and were achieved histological R0 resection. Meanwhile, they didn't accept preoperative neoadjuvant treatment and postoperative chemoradiation based on their own decisions. Therefore, the clinical data was objective in statistic analysis.

The incidence of PLM is different in various reports. In 145 recurred postoperative patients of our study, 68 cases occurred in PLM (46.8%). In other studies, 13 cases of PLM were found in 55 recurred PC patients (23.6%) as Tani et al. reported [8]. Hishinuma et al. showed that recurrence was confirmed for 22 of 24 PC patients. 18 (75%) of the 24 patients had local recurrence, 12 (50%) had hepatic metastasis, and 11 (46%) had both. In addition, PLM appeared in about half of the 64 patients (45.3%) as Matsumoto et al. reported [4]. In any case, PLM is the most common distant metastasis in PC patients.

The effect of PLM in the prognosis of postoperative PC patients remains controversial. In present study, PLM was an independent unfavorable prognostic indicator of PC patients. The mortality rate of patients with PLM was 80.9%. Moreover, the size and number of liver metastases were closely associated with the poor survival of nude mice. Inoue et al. also reported that 13 (65.0%) of the 20 deaths were caused by liver metastases who underwent curative resection of PC [9]. The 1-, 3-, and 5-year survival rates of the patients who developed postoperative liver metastases were 31, 3.4, and 0%, respectively; their median survival was 10 months [4]. But Tani et al. showed that the liver metastasis as an initial recurrent site had no impact on the median survival time in 55 postoperative recurred PC patients [8]. Nonetheless, PLM was the main poor prognostic indicator for postoperative PC patients.

As yet, there was no detailed data for illustrating the definite predictors that influence the occurrence of PLM except for dynamic monitor of CA19-9 level [10-14]. In our present study, tumor size ≥ 3.5 cm, lymph nodes metastasis and UICC stage (IIA+IIB) were identified as independent risk factors for the occurrence of PLM. According to UICC 2010 TNM classification, PC with lymph nodes metastasis was also classified as UICC stage IIB, which further verified the crucial role of clinical stage (I+IIA vs. IIB or IA+IB vs. IIA+IIB) in the initiation and progression of PLM. In addition, we found that only preoperative CA19-9 level can't be identified as a predictor for PLM. Indeed, the predictors for PLM remain controversial in previous studies. For example, Fujioka et al. showed that G3 histological grading and venous system invasion were independent risk factors for PLM in 174 PC patients [15]. In another study, female and low tumor grade were found as independent risk factors for recurrence with liver metastasis in 55 resected PC patients [8]. But Gaku et al. showed that only tumor size (≥ 3 cm) was significantly correlated with PLM in 64 PC patients [4]. Our study showed a new range of tumor size (≥ 3.5 cm) in predicting PLM, which increased radical resection rate of PC despite under the threat of PLM. In addition, in 41 patients underwent extended radical pancreatectomy combined with intraoperative radiotherapy, preoperative biliary drainage and jaundice were factors influencing PLM [16]. But our study showed obstructive jaundice in PC that located in the head of pancreas was not associated with the occurrence and prognosis of PLM patients. Indeed, obstructive jaundice is not a marker for the advanced progression of PC. Conversely, patients with obstructive jaundice are usually diagnosed and accepted for surgery earlier than patients with PC in the body and tail without jaundice. Taking together, the majority of all studies on predictors for PLM were questionable in terms of sample size and statistical methods, being largely based on small retrospective analyses as Stocken et al. suggest [17]. In general, tumor size, clinical stage and the dynamic monitor of CA19-9 level (not only preoperative level) are definite predictors for the occurrence of PLM.

In addition, our study showed that tumor size and clinical stage were independent risk prognostic factors for PLM patients, which was rarely reported in other studies. Meanwhile, tumor size and number of liver metastases were also closely associated with the poor overall survival of nude mice. Thus, enlarged tumor size and advanced clinical stage not only increased the occurrence of PLM, but also co-contributed to the poor prognosis of PC patients with PLM. Both of them might coordinately participate in the aggressive development and progression of PLM.

Finally, previous studies showed portal vein and primary pancreas injected manners for constructing liver metastasis models [18]. Our current study showed two new liver metastasis models, both of which were easy for operation. Tail veil and spleen injected models were reliable for avoiding large vascular embolization and the leakage of injected tumor cells. High incidence and short survival of tumor size- and number- dependent liver metastasis in these two models further verified the specific metastatic ability and aggressive biology of PC cells implanted into the liver. Meanwhile, two models were useful for clarifying the molecular mechanism of this specific metastatic ability as Chung et al. suggested [19]. For example, as Eguchi et al. report [19], MAL2 gene was highly expressed in liver metastasis cultured PC cells. Meanwhile, its high expression was associated with a high rate of distant metastasis, which is a promising predictive marker for distant metastasis in postoperative PC patients.

In conclusion, PLM, as the most common distant metastasis in PC, is an independent unfavorable prognostic indicator for PC patients. Tumor size and clinical stage are independent risk predictors for the high occurrence and poor prognosis of PLM. Patients with tumor size ≥ 3.5 cm and advanced clinical stage (IIB or IIA+IIB stages) should accept postoperative chemoradiation to prevent PLM as Habermehl and Tajima et al. suggest [2, 20]. Two liver metastasis models further reveal the aggressive biology of tumor size- and number- dependent liver metastasis in PC, which supply a platform for investigating the molecular mechanism of the initiation PLM in PC.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Ming Dong, Department of General Surgery, Gastrointestinal Surgery, The First Hospital of China Medical University, Shenyang 110001, China. Tel: +86-24-83282886; Fax: +86-24-83282886; E-mail: cmumingdong@sohu.com

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