Thymidine phosphorylase expression associated with clinical characteristics in pancreatic cancer

Thesis by

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Thymidine phosphorylase expression associated with clinical characteristics in pancreatic cancer

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Sung Kwan Shin

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Abstract

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Thymidine Phosphorylase (TP) is a enzyme that maintains of homeostasis of nucleic acids. The enzymatic activity of TP is indispensable for its angiogenic activity. The tumor growth is dependent on angiogenesis. Recent studies have demonstrated that angiogenesis in human solid tumors is a risk factor for metastasis and recurrence. Intratumoral microvessel density, a marker of angiogenesis grade, has been shown to be correlated with

expression of TP. The incidence of pancreatic cancer in Korea has been increased three-fold in last 20 years, as the life style gradually became Westernized. At the time of diagnosis, local invasion or metastasis is commonly found, so a surgery of curative aim is not feasible in most cases and the prognosis of pancreatic cancer is very poor. There has been a few reports studied the relationships between the extent of neovascularization and clinicopathologic characteristics, world-widely, whereas there has been no report in Korea. Therefore the expression of TP was investigated in 43 patients with invasive ductal adenocarcinoma of the pancreas and the correlation between TP expression and clinicopathological findings and clinical outcome was examined.

1. Of the 43 patients, 28 were male and 15 were female and the male to female ratio is 1.87:1. The average age at the time of surgery was 57.7± 9.82years.

2. Eight patients (18.6%) had stage I disease, 13 (30.2%) had stage II disease, 14 (32.6%) had stage III disease, and 8 (18.6%) had stage IV disease.

   Twenty-eight of 43 pancreatic cancers (65%) were positive for TP. In T stage, the degree of local invasion was significantly correlated to TP-positivity (p=0.03). There was a significant difference in TP expression between earlier stages of tumors (stage I + stage II) and the tumors with advanced stages (stage III + stage IV)(p=0.01).

3. Of 27 patients who underwent curative resection, 23 patients was diagnosed metastasis postoperatively. Liver metastasis occurred in 14 patients. Peritoneal and lung metastasis occurred in 11 patients and a patient respectively. Local recurrence including

lymph node metastasis was found in 8 patients. There was significant difference in TP expression between the patients with postoperative hepatic recurrences and those without them (p= 0.05).

4. There was a statistically significant difference between TP-positive tumors and TP-negative tumors in all patients (p=0.019).
5. The average microvessel count in TP-positive tumors was significantly higher than that in TP-negative tumors (p< 0.001).

In conclusion, this study demonstrated that TP expression in pancreatic cancer significantly correlated with the presence of postoperative hepatic metastasis, T stage, TNM stage, and poorer prognosis in patients with pancreatic cancers. Although additional studies are need to clarify the mechanism of TP

mediated angiogenesis, invasiveness, and metastasis, these results could suggest that inhibitors of this enzyme suppress the growth of TP expression tumors and might be valuable in the treatment of patients with pancreatic cancers

Key Words: pancreatic cancer, thymidine phosphorylase, microvessel count

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I. Introduction

Thymidine Phosphorylase (TP) is a enzyme that maintains of homeostasis of nucleic acids. TP catalyzes the reversible phophorolysis of thymidine, deoxyuridine and their respective bases and 2-deoxyribose phosphate[1]. TP also catalyzes the transfer of deoxyribose from one deoxynucleoside to another base to form a second deoxynucleoside[2]. In 1987, platelet derived endothelial cell growth factor (PD-ECGF) was purified to homogeneity from human platelets[3,4]. PD-ECGF stimulates chemotaxis of endothelial cells and [^{3}H]thymidine incorporation

by endothelial cells *in vitro* and has angiogenic activity *in vivo* [5]. This enzyme has been demonstrated to be identical to TP[6,7]. Although the enzymatic activity of TP is indispensable for its angiogenic activity[8], the mechanism is still unclear. TP activity in plasma has shown to be elevated in patients with cancers and tumor-bearing animals[9,10] and its expression in some solid tumors is higher than that of in the adjacent normal tissue[11,12].

The tumor growth is dependent on angiogenesis[13-15]. When tumors reach the size of a few millimeters, these new vessels may facilitate the invasion of tumor cells into vasculature and subsequent metastasis[16], thus angiogenesis correlates with the probability of metastases. Recent studies have demonstrated that angiogenesis in human solid tumors is a risk factor for metastasis and recurrence[17-19]. Intratumoral microvessel density, a marker of angiogenesis grade, has been shown to be correlated with expression of TP and is regarded as a potent prognostic indicator in various type of malignant tumors[20-24].

Even though pancreatic cancer shows a high prevalence second to colon cancers in Western countries[25] and ranks as the fifth leading cause of cancer death in the United States[26], it had

been a relatively rare disease ranks as tenth malignancy in Korea[27]. However, the incidence of pancreatic cancer in Korea has been increased three-fold in last 20 years, as the life style gradually became westernized[28,29]. Since the pancreatic cancer is localized in the retroperitoneal space, clinical manifestations are vague and non-specific, which often leads to delayed diagnosis[30]. At the time of diagnosis, local invasion or metastasis is commonly found, so a surgery of curative aim is not feasible in most cases. This contributes to 5-year survival rate of 0.4 ~ 3% and extremely poor prognosis of pancreatic cancer. Even if curative resection has been accomplished, the 2-year survival rate does not exceed 10%, because of high incidence of recurrence[31]. Therefore a more effective management as adjuvant treatment is essential to be identified. Parameters such as age, size of tumor, location, invasion to adjacent organs, lymph node metastasis, histologic type, degree of differentiation, treatment modality, and DNA ploidy are known to be helpful to assess the prognosis of pancreatic cancer[32,33]. As mentioned above, the prognosis of pancreatic cancer is very poor, but a few reports are available regarding the relationships between the

extent of neovascularization and clinicopathologic characteristics world-widely[34,35] and there has been no report in Korea.

The aims of this study were to examine the expression of TP, to study the relationship between TP expression and clinico pathological findings, and to investigate the prognostic significance of TP in pancreatic cancer.

Ⅱ. Materials and Methods

1. Subjects

Forty-seven patients with pancreatic cancers who had undergone surgical resection of the pancreas in Severance Hospital of Yonsei University College of Medicine between January 1990 and March 2000 were evaluated. None had received prior chemotherapy or radiation therapy.

2. Analysis of clinical and pathologic characteristics

Based on out patients and hospitalization records, clinical findings such as age, sex, location of tumor, invasion of adjacent organs were reviewed. The recurrence or survival was investigated during follow up period. All patients who had survived at least 60 days after operation were studied, to exclude postoperative mortality-related bias. At the time of surgery, pancreatic cancers were staged according to TNM system[36]. The M stage was determined from the intraoperative

findings, chest and bone radiography, ultrasonography, computed tomography, magnetic resonance imaging and laboratory tests reflecting bone and liver metastasis.

Histopathological diagnosis was confirmed at the department of pathology of Severance Hospital of Yonsei University College of Medicine. Cell type and degree of differentiation of pancreatic cancers were examined.

3. Immunohistochemical staining

Samples were fixed by 10% formaldehyde in phosphate-buffered saline (PBS), embedded in paraffin, and cut into consecutive 4μm thick sections. The sections were deparaffinized with xylene and dehydrated with 100%, 90%, 80%, 70%, 50%, 30% ethanol and then distilled water succeedingly. Endogenous peroxidase was blocked by immersing the slides in 3% hydrogen peroxide in distilled water for 15 min. at room temperature. After washing three times with PBS for 5 min each, the sections were blocked by soaking in 10% normal donkey serum in PBS for 1 hour at room temperature. After washing

three times with PBS for 5 min each, the blocked sections were incubated in 0.8 μg/ml monoclonal antibody directed against TP (1:250 dilution; Neomarker Corp., Fremont, CA, USA) and in anti-factor VIII polyclonal antibody (von Willebrand factor, 1:300 dilution; Dako Corp., Santabarbara, CA, USA) for 2 hours, respectively at room temperature. After 2 hours, washing three times with PBS for 5 min each, the slides were incubated for 30 min with biotinylated antimouse IgG (Cat No: KO 0690, Dako Corp., Carpinteria, CA, USA) at room temperature, washed three times in PBS for 15 min each, and incubated with streptoavidin peroxidase conjugated (Cat No: KO 0690, Dako Corp., Carpinteria, CA, USA) for 30 min at room temperature. After three 15-min washes with PBS, the sections were incubated with AEC (3-amino-9-ethylcarbazole) chromogen (Dako Corp., Carpinteria, CA, USA) and washed in distilled water. Then, the slides were counter stained with hematoxylin before mounting.

4. Evaluation of immunostaining and microvessel counting

For microscopic examination, we examined at least 200 cells

to determine whether they were stained with monoclonal antibody against TP. Tumor samples were considered to be TP positive when more than 5% of the cancer cells were stained, since less than 5% of cells were stained in most normal tissues, except liver[37].

Microvessel counts were assessed by light microscopy after staining for factor VIII. After screening the areas with intense neovascularization at low power (x 100 field; x10 objective and x 10 ocular), microvessel in the areas with highest number of discrete microvessels stained with anti-factor VIII polyclonal antibody were counted in a x 400 field (x 40 objective and x 10 ocular), because the microvessel count was more precise than in a x 100 field. The evaluation of TP expression and microvessel counts was assessed by two investigators without knowledge of the clinicopathologic findings.

5. Statistical analysis

Demographic and clinicopathological characteristics were compared between TP-positive and TP-negative tumors using the $\chi 2$-test or Student's t-test. Differences between Kaplan-

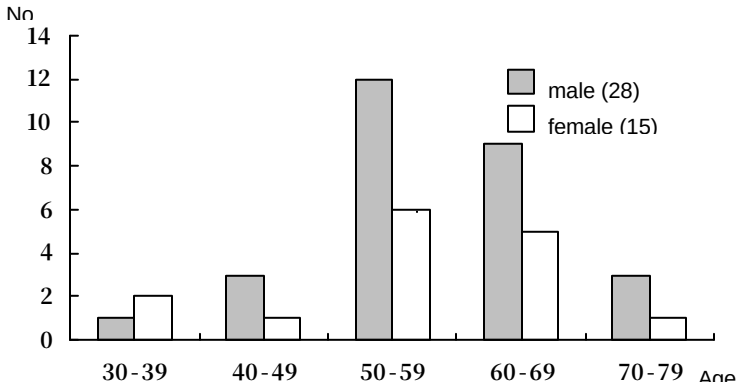
Meier survival curves based on the expression of TP were analyzed by log-rank test. The relationship between TP expression and microvessel count was determined by Student's t-test. A value of $p < 0.05$ was taken to be statistically significant. All of statistical analysis was performed using Window-SPSS release 10.0 for personal computer.

III. Results

1. Clinicopathlogic findings

From January 1990 to march 2000, total 43 cases with invasive ductal adenocarcinoma of the pancreas were evaluated. Of the 43 patients, 28 were male and 15 were female and the male to female ratio is 1.87:1. The average age at the time of surgery was 57.7± 9.82 years. The cancer was prevalent in the 6th and 7th decades of life (74.5%)(Fig.1).

Fig.1 Distribution of sex and age



The locations of pancreatic cancer were head (26 cases, 60.5%), body (4 cases, 9.3%), and tail of pancreas (13

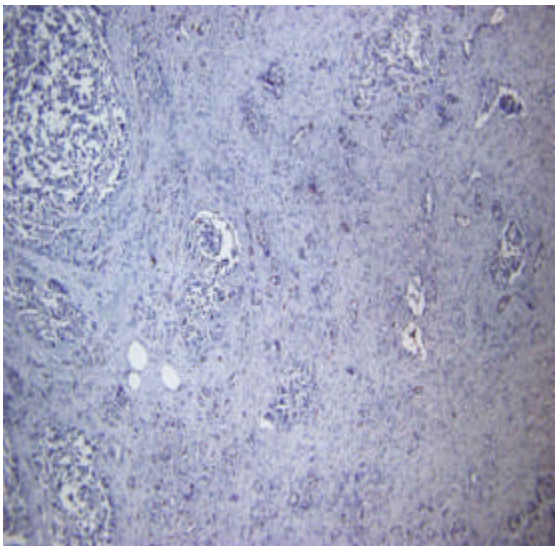
cases,30.2%). Eight patients (18.6%) had stage I disease, 13 (30.2%) had stage II disease, 14 (32.6%) had stage III disease, and 8 (18.6%) had stage IV disease. Twenty-seven patients (63%) underwent curative resection and the remaining 16 patients (37%) underwent palliative surgery of pancreatic carcinima. Five patients (11.6%) had distant metastasis at the time of surgery. Among 43 cases, 24 cases (56%) were accompanied by chronic pancreatitis histologically. The duration of follow-up period was from 5 months to 98 months (median 16 months) and median survival was 16.6 months. The survival rates of 1 and 2 years according to TNM stage were 75/46% in stage I, 75/25% in stage II, 41/9% in stage III and 0/0% in stage IV respectively. In 27 patients who underwent curative resection, twenty-three patients was diagnosed metastasis postoperatively. The duration of postoperative recurrences was 1.5 months to 23.1 months (mean 8.7±5.35 months). Liver metastasis occurred in 14 patients. Peritoneal and lung metastasis occurred in eleven and one patient respectively. Local recurrence including lymph node metastasis was found in 8 patients.

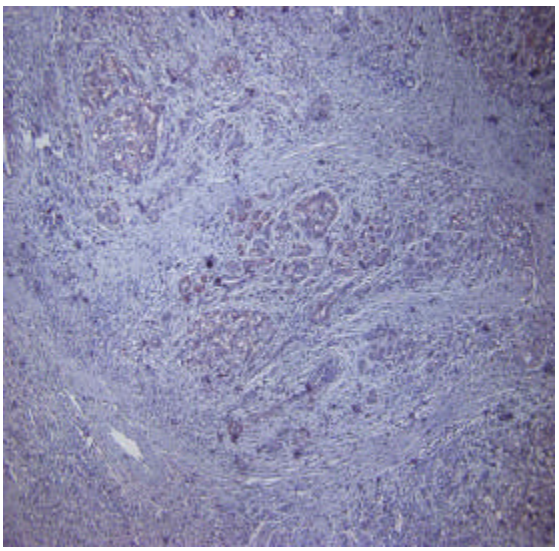
2. Expression of thymidine phosphorylase

Most non-neoplastic pancreatic duct cells were not stained with the anti-TP antibody. In contrast, the tumor cell of ductal carcinoma showed strongly cytoplasmic expression(Fig.2). Twenty-eight of 43 pancreatic cancers (65%) were positive for TP. No significant correlation was found between TP expression and age, sex, N stage, M stage, the presence of chronic pancreatitis or the presence of portal vein invasion. In T stage, the degree of local invasion was significantly correlated to TP-positivity ($p=0.03$, table 1). There was a significant difference in TP expression between earlier stages of tumors (stage I + stage II) and the tumors with advanced stages (stage III + stage IV)($p=0.01$, table 1).

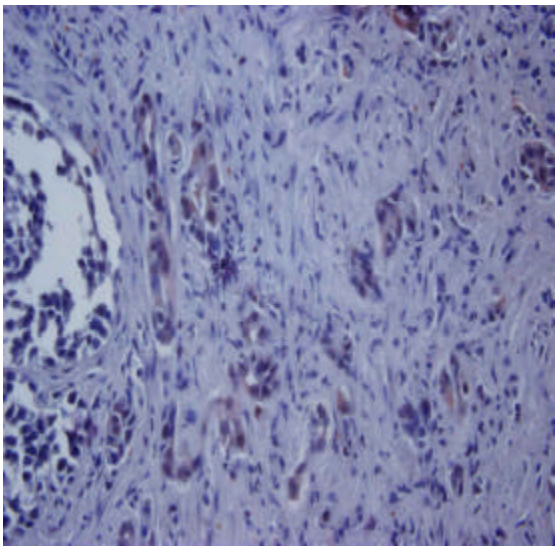
Fig. 2 Immunohistochemical staining for thymidine phosphorylase in pancreatic cancers

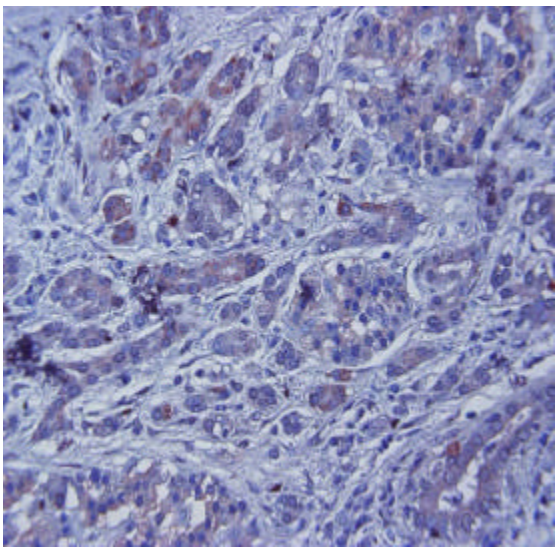
(a)





(b)





(a) and (b) show that mainly cytoplasmic expression of thymidine phosphorylase (a, x100 ; b, x400).

Table 1. Comparison of thymidine phosphorylase expression and clinicopathological findings

	Thymidine phosphorylase		P*
	Positive (n=28)	Negative (n=15)	
Sex			0.56
Male	18	10	
Female	10	5	
Age	57.5±10.3	57.8±9.2	0.92
T stage			0.04
T1	2	3	
T2	2	5	
T3	20	7	
T4	4	0	
Invasiveness			0.03
T1+T2	4	8	
T3+T4	24	7	
N stage			0.12
N0	14	11	
N1	14	4	
M stage			0.06
M0	22	15	
M1	6	0	
TNM stage			0.04
Ⅰ	2	6	
Ⅱ	8	5	

Ⅲ	10	4	
Ⅳ	8	0	
Earlier vs advanced			0.01
Ⅰ+Ⅱ	10	11	
Ⅲ+Ⅳ	18	4	
Portal vein invasion			0.42
No	2	0	
Yes	26	15	

*: χ2-test

3. Relationship between recurrence and thymidine phosphory lase expression

To investigate the relationship between the types of recurrence and TP expression in pancreatic cancer, the initial sites of recurrence were divided into postoperative hepatic metastasis, local recurrence including nodal recurrence, peritoneal recurrences, and lung metastasis. There were significant differences in TP expression between postoperative hepatic recurrences and those without them (p= 0.05), but not between patients with local, peritoneal, pulmonary recurrences those without them (p=0.42, 0.31, and 0.35, respectively)(Table 2).

Table 2. Comparison of thymidine phosphorylase expression and type of tumor recurrence

	Thymidine phosphorylase		P*
	Positive (n=28)	Negative (n=15)	
Hepatic metastasis			0.05
No	16	13	
Yes	12	2	
Local recurrence			0.42
No	22	13	
Yes	6	2	
Peritoneal recurrence			0.31
No	22	10	
Yes	6	5	
Lung metastasis			0.35
No	28	14	
Yes	0	1	

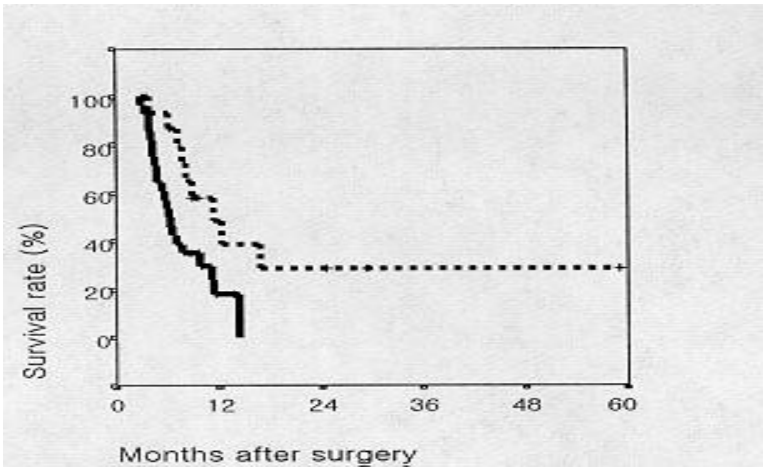
*: Fisher's exact test

4. Prognostic relevance of thymidine phosphorylase expression

At the time of this analysis, the median follow-up time was 16 months, ranging from 5-98 months. All of patients died of pancreatic cancer and the median survival time was 10.4 months, ranging from 5-28 months. The 43 patients were staged

according to the operative findings as mentioned above. The difference in survival between patients with TP-positive tumors and those with TP-negative tumors was calculated using the Kaplan Meier method. There was a statistically significant difference between TP-positive tumors and TP-negative tumors in all patients (p=0.019, Fig. 3,).

Fig. 3 Kaplan Meier survival curve for all patients



A comparison of survival curves between patients with thymidine phophorylase-positive tumors (thick lines) and thymidine phosphoylase-negative tumors (dashed lines) is shown (p=0.019).

5. Correlation between thymidine phosphorylase expression and microvessel count

The average microvessel count in TP-positive tumors was significantly higher than that in TP-negative tumors ($p < 0.001$)(table 3 and fig. 4).

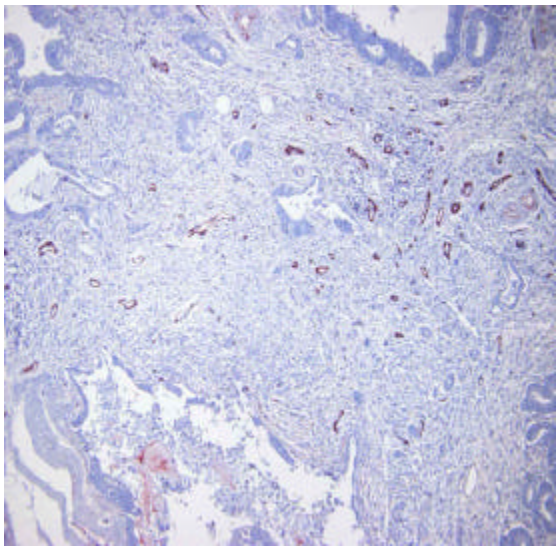
Table 3 Correlation between thymidine phosphorylase expression microvessel count in pancreatic cancers

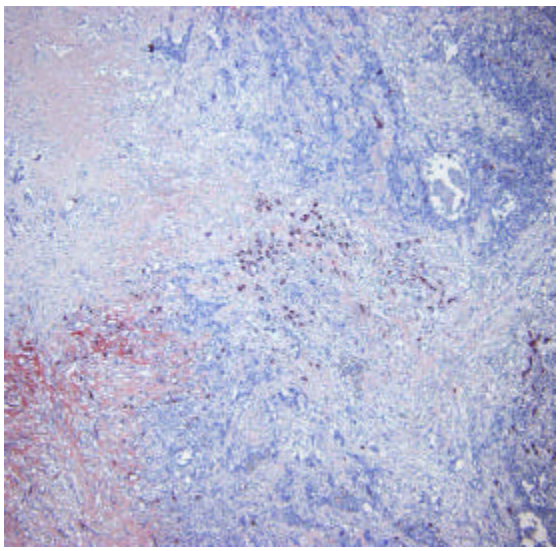
Thymidinie phosphrylase	Microvessel count (mean ± SD)	P*
Positive	27.68±8.92	
		< 0.001
Negative	13.07±4.38	

*: Student's t-test

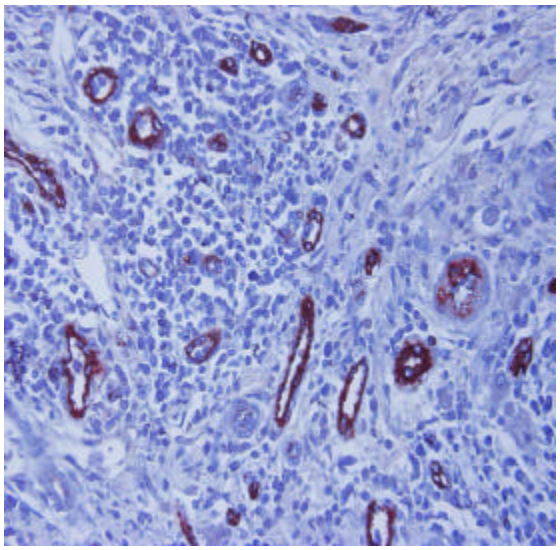
Fig. 4 Immunohistochemical staining for factor VIII in pancreatic ductal carcinoma

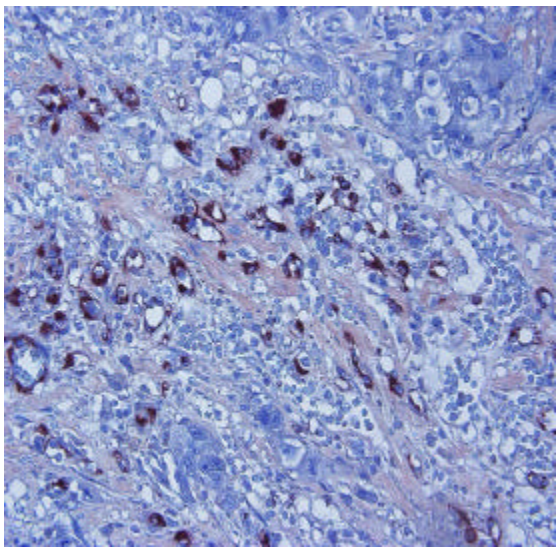
(a)





(b)





(a) was photographed at x 100 and (b) was at x 400

IV. Discussion

Neoangiogenesis is a requirement for tumor growth and metastasis, and is induced by angiogenic growth factors produced by tumor cells and/or infiltrating cells[14]. Considerable attention has been paid to the mechanism of tumor angiogenesis in recent years. Most workers has been directed toward the identification of the various peptide growth factors that stimulate proliferation and motility of endothelial cells to induce new blood vessel formation. Interestingly, several investigators have shown that the existence of hypoxic conditions within tumors increases the gene expression of a variety of proteins, including the angiogenic factor, platelet-derived endothelial cell growth factor (PD-ECGF) in tumor cells.[38] PD-ECGF is a 55 kDa polypeptide existing in vivo as a 110kDa homodimer and an endothelial cell mitogen initially purified to homogeneity from human platelets[3,4]. PD-ECGF has been found to have complete sequence identity between 120 amino acids of human thymidine phosphorylase(TP), an enzyme involved in pyrimidine nucleoside metabolism[1,2], and sequence of PD-ECGF, and also been demonstrated that PD-

ECGF has TP activity[6,7]. These observations and similar reports from other laboratories suggest that human TP is identical to PD-ECGF[42,43]. This study was focused in the expression of TP among a variety of angiogenic factors, since pancreatic cancer is composed of abundant fibrotic components and has been considered to be more hypoxic than other malignancies.

TP has been demonstrated to have angiogenic activity, and its enzymatic activity is needed for angiogenesis[8,44]. In ovarian tumors, areas of increased blood flow, a measure of angiogenesis, are associated with elevated TP expression[45]. Expression of thrombomodulin, a marker protein for endothelial cells, significantly correlates with TP activity of colorectal cancers, suggesting that this enzyme may be important in angiogenesis in human colorectal carcinomas[11,40]. Similar observations have been reported for breasts, gastric, and esophageal cancers[24,46,47]. The present study demonstrated a significant correlation TP expression and T stage, TNM stage system, and postoperative hepatic metastases in pancreatic cancer.

Angiogenesis is necessary for rapid tumor growth and allows

the vascularized tumor to extend vertically into the deep tissues beneath the basement membrane. During the vascular phase, tumor cells may be shed into the circulation[48]. The clinical significance of these findings has been documented in studies of breast[23], gastric[18], and colorectal cancer[17], where microvessel density has been shown to be significantly correlated with the occurrence of metastases and recurrence of disease. Intratumoral microvessel density (MVD), a marker of angiogenesis grade, has been demonstrated to be a potent prognostic indicator in various types of malignant tumors[20]. In the present study, TP-positive tumors had a significantly higher microvessel count than did TP-negative tumors. The angiogenic property of TP may be catalyst for invasion to adjacent organ and hepatic metastases. Recently, O'Brein and his collegues reported that the expression of TP was 33-fold higher in invasive bladder cancers than it was in superficial bladder cancers, and that expression of vascular endothelial cell growth factor was higher in superficial cancers than it was in invasive cancers[12]. With this observation as the model, the present study suggest that increased TP in pancreatic carcinomas may enhance

the tumor's invasiveness and ability to metastasize via its angiogenic property.

The prognostic significance of tumor PD-ECGF expression is not completely clear. There were inconsistent results in studies of colorectal carcinoma. In two studies, active expression of PD-ECGF in tumor cells was correlated with high microvessel count, advanced tumor stage, and poor survival in colorectal cancer [40,49] whereas another study failed to find a correlation between PD-ECGF expression and tumor vascularity[50]. In the latter study, a high expression of PD-ECGF in tumor stromal macrophages was predictive of good prognosis, a finding that was attributed to the role of PD-ECGF expressed by stromal cells in enhancing immune reaction against the tumor cells. Correlation of TP expression with the patient's prognosis was found in this study. There was statistically significant difference in overall survival. The patients with TP-positive tumors showed poorer prognosis than those who with TP-negative tumors. These finding was compatible with the reports of other investigators[34,35], but no significant difference in survival between patients with TP-positive and negative tumors was demonstrated in stage I and

Ⅱ or stage Ⅲ and Ⅳ (data was not shown).

Carcinoma cells transfected with wild type TP complementary DNA (cDNA) grew larger in nude mice than did those transfected with mutant cDNA without TP activity[43]. This finding and similar report from another laboratory suggest that TP activity is required for rapid tumor growth[40]. Carcinoma cells around the necrotic area or in the fibrous tissues of the pancreas showed a high level of TP expression. Among numerous clinical variables examined, the significant relationship between T category and TP expression was documented in this study. These results suggest that TP has another function, in addition to angiogenesis, related to tumor progression. These findings suggest that TP expression in pancreatic cancer enhances the abilities of tumor invasion and/or metastasis through its angiogenic properties.

Over the past few years, rapid progress has been observed in the translation of angiogenesis research into clinical applications. There is now fairly convincing evidence in support of a prognostic value of tumor angiogenic activity in cancer patients, hence the assessment of angiogenesis may become a part of routine prognostic evaluation in cancer patients in the near

future. In addition, evaluation of tumor angiogenic activity may have other far-reaching implications in cancer management. Antiangiogenic therapy is now being evaluated in clinical trials, and the potential relevance of circulating angiogenic factors in patients undergoing antiangiogenic treatment is a particularly interesting area for future research. It is my contention that inhibitors of TP suppress the growth of TP expression tumors and might be valuable in the treatment of patients with pancreatic cancers.

V. Conclusions

Forty three patients with pancreatic cancers who had undergone surgical resection of pancreas in Severance Hospital of Yonsei University, college of medicine between January 1990 and March 2000 were evaluated in order to study the relationship between TP expression and clinicopathological findings, and to investigate the prognostic significance of TP in pancreatic cancer. Through the investigation of the expression of TP in pancreatic cancer and examination of the correlation between TP expression and clinicopathological findings and clinical outcome, the results as below were obtained.

1. Of the 43 patients with ductal adenocarcinoma of the pancreas, 28 were male and 15 were female and the male to female ratio is 1.87:1. The average age at the time of surgery was 57.7± 9.82years.
2. Eight patients (18.6%) had stage I disease, 13 (30.2%) had stage II disease, 14 (32.6%) had stage III disease, and 8 (18.6%) had stage IV disease.
3. Twenty-eight of 43 pancreatic cancers (65%) were

positive for TP. In T stage, the degree of local invasion was significantly correlated to TP-positivity (p=0.03). There was a significant difference in TP expression between earlier stages of tumors (stage I + stage II) and the tumors with advanced stages (stage III + stage IV)(p=0.01).

4. Of 27 patients who underwent curative resection, 23 patients was diagnosed metastasis postoperatively. Liver metastasis occurred in 14 patients. Peritoneal and lung metastasis occurred in 11 patients and a patient respectively. Local recurrence including lymph node metastasis was found in 8 patients. There were significant differences in TP expression between the patients with postoperative hepatic recurrences and those without them (p= 0.05).
5. There was a statistically significant difference between TP-positive tumors and TP-negative tumors in all patients (p=0.019).
6. The average microvessel count in TP-positive tumors was significantly higher than that in TP-negative tumors

($p < 0.001$).

In conclusion, this study demonstrated that TP expression in pancreatic cancer significantly correlated with the presence of postoperative hepatic metastasis, T category, TNM category, and poorer prognosis in patients with pancreatic cancers. Although additional studies are need to clarify the mechanism of TP mediated angiogenesis, invasiveness, and metastasis, these results could suggest that inhibitors of this enzyme suppress the growth of TP expression tumors and might be valuable in the treatment of patients with pancreatic cancers.

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국문요약

췌장암에서 Thymidine Phosphorylase의 발현 양상과 임상적 특징과의 상관관계 분석

Thymidine phosphorylase(TP)는 핵산의 항상성 유지에 필요한 효소이자 종양의 성장, 주변조직으로의 침윤 및 전이를 결정하는 요소인 맥관 형성에 관여하는 혈관형성인자의 하나로 알려져 있다. 종양의 맥관 형성은 여러 고형암에서 원격전이와 재발의 위험요인으로 알려져 있어 맥관 형성의 지표로서 TP의 발현 여부는 향후의 예후를 시사할 수 있을 것으로 생각된다. 췌장암은 조기진단이 어려워 진단당시에 이미 주변장기로 전이된 경우가 많아 근치적 절제가 불가능한 경우가 대부분이며, 근치적 절제를 시행하더라도 5년 생존율이 5-10%에 불과한 예후가 매우 불량한 악성 종양이다. 그러나, 췌장암의 불량한 예후와 악성 경향에도 불구하고 종양의 맥관 형성과

환자의 임상적 특징과의 상관관계에 대한 보고는 전 세계적으로 매우 드물며, 우리나라에서는 전무하다.

이에 저자는 1990년 1월부터 2000년 3월까지 연세대학교 의과대학 세브란스 병원에서 췌장암으로 진단 받고 췌장 절제술이 가능하였던 43예를 대상으로, 추적관찰 기간 동안의 재발여부 및 연구 시점을 기준으로 했을 때의 생존율 등을 포함하는 임상적 특징을 검토하고, 절제조직의 병리학적 병기, 세포 유형, 분화도 등의 병리적 특징을 조사하였다. 조직을 이용한 TP에 대한 면역조직화학 염색을 실시하고 미세혈관밀도를 알아보기 위해 von Willebrand factor (factor VIII)에 대한 면역조직화학 염색을 실시하여, TP의 발현과 환자들의 임상적·병리적 특징과의 관련 여부를 분석하고, 미세혈관밀도와 TP 양성 여부와의 상관관계를 조사하여 다음과 같은 결과를 얻었다.

1. 대상 환자는 총 43명으로 남자 28명, 여자 15명이었으며

췌장암 진단 당시의 평균 나이는 57.7±9.82세였다. 남녀 모두 50대에서 가장 높은 빈도를 보였다.

6. 췌장암의 위치는 두부가 26예(60.5%), 체부가 4예(9.3%), 미부가 13예(30.2%)였으며, 근치적 절제가 가능하였던 환자들은 27예(63%), 고식적 절제를 시행한 경우는 16예(37%)였고, 췌장암 수술당시 원격전이가 된 경우는 5예(11.6%)였다. 만성 췌장염이 동반된 경우가 24예(56%)였고, 췌장암의 조직형은 모두 선암이었다. 췌장암의 병리학적 병기는 1기가 8예(18.6%), 2기가 13예(30.2%), 3기가 14예(32.6%), 4기가 8예(18.6%) 였다.
7. 추적 기간은 5개월에서 98개월까지였고(중앙값 16개월), 중앙생존기간은 16.8개월이었고, 1년과 2년 생존율은 1기가 75%, 46%, 2기가 75%, 25%, 3기가 41%, 9%, 4기는 0%였다.
8. TP 양성군은 28예(65%), TP 음성 군은 15예(35%)였다. TP 양성 군과 음성 군의 임상적, 병리적 특징을 비교하였을 때,

성별비, 연령, 만성 췌장염의 동반여부, TNM 병기중 N과 M 병기, 간문맥의 침윤 여부는 양군 사이에 차이를 보이지 않았으나, T병기(p=0.03), TNM병기(p=0.01)에 있어서는 주변조직으로의 침습 정도가 높을수록 진행된 병기 일수록 TP양성의 빈도가 유의하게 높았다.

9. 미세혈관밀도는 광학 현미경의 고 배율 시야(x400)에서 평균 22.5±10.35개 였으며, TP 양성 군은 27.68±8.92개, TP 음성 군은 13.07±4.38개로 양성 군에서 의미 있게 높은 결과를 보였다.($p< 0.001$)

10. 췌장암의 재발은 근치적 절제가 가능하였던 27예 중 23예에서 재발하였으며, 재발부위는 간 전이가 14예, 폐 전이가 1예, 복막 전이가 11예였으며, 원발 부위의 임파절 전이를 포함한 국소재발이 8예였다. TP 양성 여부와 재발부위의 관계를 분석하였을 때 TP 양성 군에서 의미 있게 간 전이의 빈도가 높았다(p=0.05).

11. TP 양성 군과 음성 군에 있어서 5년 생존율을 비교하였을 때 전체 생존율은 TP 음성 군에서 의미 있게 높게 나타났다 ($p=0.0019$).

이상의 결과를 종합하여 보면 췌장암에 있어서 TP가 양성으로 표현되는 것은 수술 후 간 전이와 관련이 있고 불량한 예후 인자로서 역할을 한다는 것을 알 수 있었다. 비록 아직까지 TP로 매개되는 신생혈관형성, 주변조직으로의 침습의 정확한 기전은 밝혀지지 않았지만, TP에 대한 억제제가 TP를 발현하는 종양의 성장을 억제할 수 있을 것으로 생각되며, 이는 현재 적절한 항암제가 없는 췌장암의 치료에 도움이 될 것으로 판단된다.

핵심되는 말 : 췌장암, thymidine phosphorylase, 미세혈관밀도