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ORIGINAL ARTICLE

Yoshimitsu Minari · Yoshinori Nio
Noriyuki Hirahara · Ming Dong

Heterogeneic distribution of thymidine phosphorylase between primary tumors and metastatic lesions of human pancreatic ductal carcinoma: implications for the efficacy of chemotherapy with 5-FU or its derivatives

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Abstract *Purpose:* It has been suggested that the expression of thymidine phosphorylase (TdRPase) correlates with the malignant potential of various cancers, but its involvement in human invasive ductal carcinoma (IDC) of the pancreas has not been reported. In the present study, the distribution and clinical significance of TdRPase in IDCs and benign diseases of the pancreas were assessed, especially in relation to the efficacy of chemotherapy with 5-FU or its derivatives. *Method:* The expression of TdRPase in 148 specimens of pancreatic IDCs (66 primary lesions, 46 nodal lesions and 36 distant metastases from 126 patients) and in 24 specimens of benign diseases (4 cystadenomas, 3 hyperplasias, and 17 chronic pancreatitises) was examined by immunohistochemical staining with anti-TdRPase monoclonal antibody and evaluated in terms of three grades of immunoreactivity: negative 0, low 1, or high 2. *Results:* Positive TdRPase staining (low and high immunoreactivity) was detected in 71% (47/66) of the primary lesions, in 46% (21/46) of the involved nodes, in 53% (19/36) of various lesions of distant metastasis, and in 37% (9/24) of the benign diseases. The staining intensity was significantly higher in the IDC tissues than in the benign disease tissues, and significantly lower in the metastatic lesions than in the primary lesions. TdRPase reactivity did not correlate with the survival rate in both resectable and unresectable IDCs. In patients with both primary tumor and nodal involvement, however, high TdRPase activity in involved nodes was significantly associated with a poor prognosis. On the other hand, although adjuvant chemotherapy was found to improve the survival of patients, TdRPase activity in the tumor did not show any significant relationship with the efficacy of chemotherapy with 5-FU or its derivatives. *Conclusions:* The present study suggested that in pancreatic IDC the activity of TdRPase in primary lesions is different from that in metastatic lesions, and that DNA is synthesized mainly through the salvage pathway in primary lesions and through a de novo pathway in metastatic lesions. This may be one of the reasons for the heterogeneity in chemosensitivity of human pancreatic IDC.

Key words Thymidine phosphorylase · Pancreatic cancer · Chemotherapy · Tumor heterogeneity

Y. Minari · Y. Nio (✉) · N. Hirahara · M. Dong
First Department of Surgery,
Shimane Medical University, 89-1 Enya-cho,
Izumo, Shimane 693-8501, Japan
E-mail: fsurgery@shimane-med.ac.jp
Tel.: +81-853-202225
Fax: +81-853-202222

Introduction

Invasive ductal carcinoma (IDC) of the pancreas grows rapidly and is very resistant to a variety of cancer therapies including surgery, radiation, and chemotherapy. The prognosis of patients with the disease is very poor, and 85% of patients with unresectable pancreatic IDC die within a year due to liver metastasis or peritoneal dissemination [10]. Even when patients undergo curative surgery, 50% die within a year [8, 17]. Although the malignant potential of pancreatic IDC is very high, there are no precise indices for such potential, which is reflected in growth, invasion, and metastasis. Tumor growth correlates with DNA synthesis in tumor cells. Tumor growth and proliferation have been evaluated by measuring ^{3}H-thymidine (TdR) uptake [19, 31], the BrdU labeling index [24], cell cycle by flow cytometry, and various cell proliferation markers including the expression of proliferating cell nuclear antigen and argyrophilic nucleolar organizer regions [2, 3, 18].

TdR phosphorylase (TdRPase), which consists of 440 amino acids and has a molecular weight of 55 kDa [4, 26], is a key enzyme for the phosphorylation of pyrimidine in DNA synthesis. Two major pathways are known in the metabolism of pyrimidine in DNA synthesis: the de novo pathway and the salvage pathway. TdRPase promotes phosphorylation of pyrimidine and subsequent production of thymine and 2′-deoxy-D-ribose in the salvage pathway, and is considered to play an important role in

the proliferation, invasion, and metastasis of cancer cells [4, 26]. Higher activity of TdRPase is found in cancerous tissue than in nonmalignant tissue [9, 11, 12, 13, 14, 20, 30]. Furthermore, TdRPase exerts a significant influence on the effect of 5-fluorouracil (5-FU) or its derivatives because 5-FU inhibits de novo DNA synthesis [15, 29]. Some tumor cells can, however, survive after chemotherapy with 5-FU by synthesizing DNA through the salvage pathway. Thus, although the relationship between TdRPase and the efficacy of cancer chemotherapy, especially with 5-FU or its derivatives, is very important, the biological role of TdRPase in tumor cells has not been sufficiently clarified.

In the present study, we examined the expression of TdRPase in various lesions of human pancreatic IDC, its correlation with clinicopathological factors including clinical stage, patient survival, and its implication in the efficacy of adjuvant chemotherapy (ACT) with 5-FU or its derivatives after pancreatic IDC surgery.

Materials and methods

Patients

The present study included 102 patients with pancreatic IDC and 24 patients with benign diseases. The profiles of the patients are summarized in Table 1. Clinically the cancers were staged according to the UICC (TNM) classification (1997) [6]. All patients underwent surgery between October 1980 and December 1998 and were followed up in our department. Of the 102 patients, 66 with resectable pancreatic IDC underwent pancreatoduodenectomy, or distal or total pancreatectomy with lymphadenectomy, and 46 of these 66 demonstrated nodal involvement. The remaining 36 patients with unresectable IDC underwent bypass or exploratory surgery, and specimens of distant metastases were obtained during surgery. A total of 148 specimens of pancreatic IDC (66 primary lesions, 46 involved nodes and 36 distant metastases) and 24 specimens of benign diseases were examined.

Adjuvant chemotherapy

In Japan, under the universal coverage of the health insurance system, the use of anticancer agents is strictly restricted by the Japanese Ministry of Health and Welfare. Accordingly, ACT regimens mainly involve the use of approved agents including 5-FU, mitomycin-C (MMC), Adriamycin (ADR), cyclophosphamide (CPA), and UFT (a 4:1 mixture of uracil and Ftorafur). The adjuvant chemotherapy is summarized in Table 2. Of the 66 patients with resectable IDC, 36 received ACT and 30 did not receive any ACT (surgery alone, SA). Some of the SA group received salvage chemotherapy after their pancreatic cancer recurred. Of the 36 patients in ACT group, 18 received UFT alone, 1 received HCFU (carmofur) alone, 15 received combination regimens including UFT, and 2 were treated with other regimens. Of the 36 patients with unresectable cancer, 22 did not receive any ACT and 14 received ACT (two UFT alone, one 5-FU alone, ten combination regimens including UFT, one other combination regimen).

Anti-TdRPase monoclonal antibody (mAb)

The anti-TdRPase mAb was kindly supplied by Nippon Roche Company, Tokyo, Japan. The hybridomas producing mouse anti-TdRPase mAb were cloned from the spleen cells of mice immunized with human TdRPase, which was purified from a human colonic cancer xenograft HCT116 [22]. The antibody was diluted at 100 μg/ml for immunohistochemical staining.

Immunohistochemical staining

The specimens were fixed in 10% formalin and embedded in paraffin blocks, sliced into 3-μm sections and prepared on silanized slides (DAKO Japan Company, Tokyo, Japan). The slides were deparaffinized three times in xylene for 5 min each time and rehydrated three times in 90% ethanol for 5 min each time. Endogenous peroxidase was blocked with methanol containing 0.3% H_2O_2 for 5 min. The specimens were washed in phosphate-buffered saline (PBS; Gibco, Biocult, Glasgow, U.K.), heated in 10 m*M* citrate buffer (pH 6.0) three times in a microwave oven (800 W) for 5 min each time [1, 27]. After washing with PBS, nonspecific reaction was blocked with 10% normal rabbit serum (Nichirei) in a moist chamber for 10 min. The slides were incubated with anti-TdRPase mAb in a moist chamber at 8ºC for 12 h, washed three times with PBS for 5 min each time, and incubated with anti-mouse IgG + IgA + IgM antibody (Nichirei) diluted at 10 μg/ml for 20 min at room temperature. After washing three times with PBS for 5 min each time, the slides were incubated with streptoavidin-conjugated peroxidase (Nichirei) diluted at 100 μg/ml for 10 min, and with 3,3-diaminobenzidine-tetrahydrochloride solution (Nichirei) for 20 min. The slides were then counterstained with 1 ml veronal acetate-buffered methyl green solution (pH 4.0) for 1 h. After washing twice with 100% ethanol, the slides were dehydrated with 100% xylene, covered with coverglasses in

Table 1 Profile of the patients with pancreatic diseases

	pTNM stage	Invasive ductal carcinoma		Benign diseases ($n = 24$)	Overall ($n = 126$)
		Resectable ($n = 66$)	Unresectable ($n = 36$)		
Patient characteristics					
Male		31	25	17	73
Female		35	11	7	53
Age (years)		65 ± 10	68 ± 9	53 ± 13	63 ± 12
Invasive ductal carcinoma					
Overall		66	36	–	102
	I	10	–	–	10
	II	3	–	–	3
	III	32	–	–	32
	IV	21	36	–	57
Benign diseases					
Chronic pancreatitis		–	–	17	17
Cystadenoma		–	–	4	4
Hyperplasia		–	–	3	3

Table 2 Adjuvant chemotherapy (the remaining patients, i.e. 30 resectable and 22 unresectable, were treated with surgery only) (*UFT* Ftorafur + uracil, *5-FU* 5-fluorouracil, *CPA* cyclophosphamide, *MMC* mitomycin-C, *ADR* Adriamycin, *HCFU* carmofur, *CDDP* cisplatin; the last two of these drugs are not approved by the Japanese Ministry of Health and Welfare for the treatment of pancreatic cancer; the others are)

Adjuvant chemotherapy	Resectable ($n = 66$)	Unresectable ($n = 36$)	Overall ($n = 102$)
Total receiving	36	14	50
Single agent			
Total	19	3	22
UFT alone	18	2	20
HCFU alone	1	–	1
5-FU alone	–	1	1
Combination regimens			
Total	17	11	28
UFT + 5-FU	1	0	1
UFT + CPA	12	6	18
UFT + CPA + 5-FU + CDDP	1	–	1
UFT + MMC + 5-FU	1	–	1
UFT + CPA + CDDP	–	2	2
UFT + CPA + CDDP + MMC + ADR	–	1	1
5-FU + CDDP	1	–	1
5-FU + CDDP + VP-16	1	–	1
5-FU + CPA + ADR	–	1	1
CPA + CDDP + MMC	–	1	1

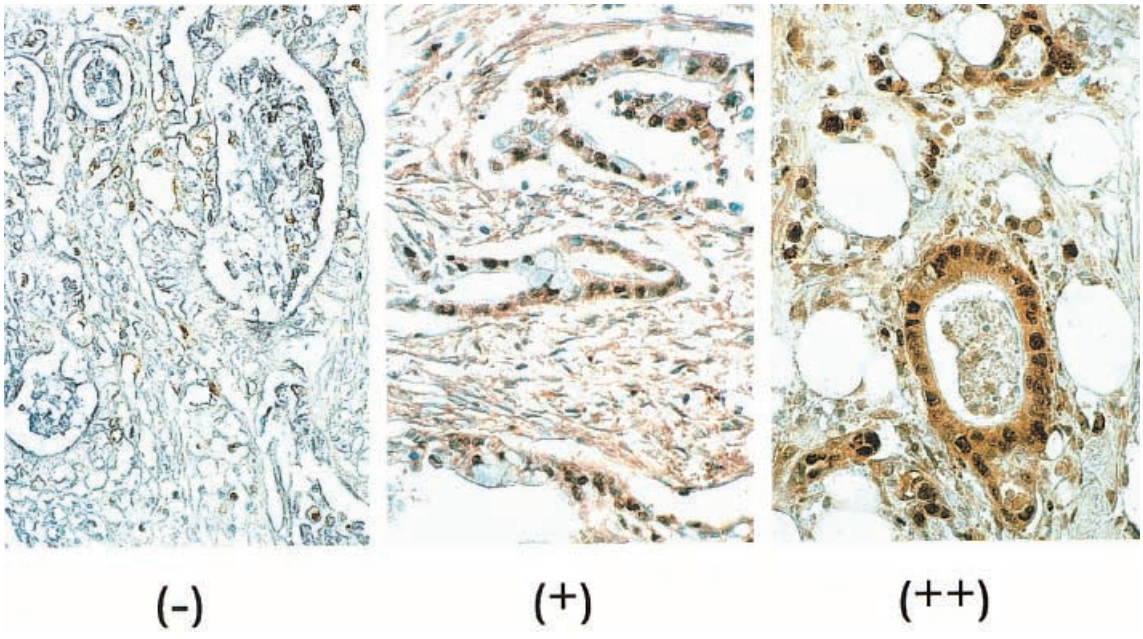

Fig. 1a–c Representative staining of TdRPase in various pancreatic diseases and grading of TdRPase immunoreactivity. Positive TdRPase expression was classified into the following three grades of immunoreactivity: 0–20% negative (–, grade 0), 21–50% low (+, grade 1), and 51–100% high (++, grade 2). **a** 61-year-old male, moderately differentiated adenocarcinoma, grade 0 immunoreactivity; **b** 63-year-old female, moderately differentiated adenocarcinoma, grade 1; **c** 65-year-old male, well differentiated adenocarcinoma, grade 2

ENTELLAN neu mounting medium (Merck, Rathway, N.J.), dried, and examined under an optical microscope.

For evaluation of TdRPase expression, staining was taken as positive only when an unequivocally strong cytoplasmic or nuclear staining was detected. The total number of stained or unstained cells was counted in three different fields of the specimen and the mean value was used for determining the rate of positivity of TdRPase expression. Immunoreactivity was classified into three grades: 0–20% negative (–, grade 0), 21–50% low (+, grade 1), and 51–100% high (++, grade 2) (Fig. 1).

Statistical analysis

The staining intensity of the TdRPase in four lesions was compared using the unpaired *t*-test, and TdRPase expression in primary lesions and involved nodes in the same patient with IDC was compared using the paired *t*-test. The survival curves of the patients were calculated by the Kaplan-Meier method and compared with the Cox-Mantel test. The degree of correlation between TdRPase staining and clinicopathological factors was determined using the Chi-squared test. *P*-values less than 0.05 were taken as significant.

Results

Comparative immunostaining of TdRPase between benign diseases and IDC of the pancreas

The staining intensity of TdRPase in various pancreatic lesions is summarized in Table 3. Stronger TdRPase

Table 3 Expression of TdRPase in various pancreatic diseases

Patient	Specimens	pTNM stage	Number	TdRPase immunoreactivity grade[a]			
				0	1	2	Mean ± SD
Invasive ductal carcinoma	Primary lesion		66	19 (29%)	23 (35%)	24 (36%)	1.076 ± 0.810**,***
		I	10	3	4	3	
		II	3	2	1	0	
		III	32	7	11	14	
		IV	21	7	7	7	
	Metastatic lesion		82	42 (51%)	17 (21%)	23 (28%)	0.768 ± 0.865**
	Involved node		46	25 (54%)	11 (24%)	10 (22%)	0.674 ± 0.818
	Distant metastasis		36	17 (47%)	6 (17%)	13 (36%)	0.889 ± 0.919
	Total		148	61 (41%)	40 (27%)	47 (32%)	0.905 ± 0.852*
Benign diseases	Hyperplasia		3	1 (33%)	2 (67%)	0 (0%)	
	Cystadenoma		4	3 (75%)	1 (25%)	0 (0%)	
	Pancreatitis		17	11 (64%)	4 (24%)	2 (12%)	
	Total		24	15 (63%)	7 (29%)	2 (8%)	0.458 ± 0.658*,***

*$P = 0.016$, **$P = 0.029$, ***$P = 0.002$
[a]Grade 0, 0–20% immunoreactivity; grade 1, 21–50%; grade 2, 51–100%

staining was seen in pancreatic IDC lesions (59%, 87/148) than in benign disease specimens (37%, 9/24) ($P = 0.016$). Moreover, immunoreactivity for TdRPase in primary lesions was significantly higher than in metastatic lesions ($P = 0.029$) or benign disease specimens ($P = 0.002$). There was no significant difference in TdRPase staining intensity between metastatic lesions and benign disease specimens. In benign disease specimens, weak TdRPase staining was seen, and there were no cases of grade 2 staining in hyperplasias or cystadenomas, whereas in pancreatitis only two cases of grade 2 staining were observed.

In patients with nodal involvement, TdRPase staining intensity was significantly lower in simultaneously involved nodes than in primary lesions ($P = 0.008$; Table 4). The immunoreactivity of TdRPase in primary lesions of IDC correlated significantly with the extent of nodal involvement ($r = 0.261$, $P = 0.0342$), but it showed no correlation with patient age, gender, stage or histological grade.

Table 4 Comparative expression of TdRPase between the primary lesion and the simultaneously involved node in the same patients

Staining intensity	TdRPase immunoreactivity grade[a]		Number ($n = 46$)
	Primary lesion	Nodal involvement	
Same	0	0	8
	1	1	5
	2	2	10
Different	1	0	11
	2	0	6
	2	1	5
	0	1	1
Mean ± SD	1.261 ± 0.773*	0.674 ± 0.818*	

*$P = 0.008$
[a]Grade 0, 0–20% immunoreactivity; grade 1, 21–50%; grade 2, 51–100%

TdRPase expression and patient survival

The relationship between TdRPase expression and patient survival is summarized in Table 5. There were

Table 5 Relationship between TdRPase expression, adjuvant chemotherapy (ACT) and survival

	TdRPase expression	Median survival (months) (number of specimens)			
		Resectable			Unresectable
		Overall	No nodal involvement	Nodal involvement	
Overall		13.4 (66)	19.2 (20)	11.0 (46)	4.0 (36)
ACT		15.8 (36)	24.6 (12)	11.0 (24)	7.0 (16)**
No ACT		10.5 (30)	11.0 (8)	10.0 (22)	2.0 (20)**
Primary lesion	Positive	12.4 (47)	17.4 (11)	11.0 (37)	–
	Negative	15.7 (19)	21.3 (9)	11.0 (9)	–
Involved node	Positive	–	–	9.0 (21)*	–
	Negative	–	–	13.0 (25)*	–
Distant metastasis	Positive	–	–	–	4.0 (19)
	Negative	–	–	–	4.0 (17)

*$P = 0.0438$, **$P = 0.0001$, Cox-Mantel test

no differences in the survival among patients grouped by grade of TdRPase expression in the primary lesion after pancreatectomy. Among patients with nodal involvement, however, survival was significantly higher in patients whose involved nodes expressed grade 0 TdRPase reactivity ($TdRPase^-$) than in patients with involved nodes expressing grade 1–2 reactivity ($TdRPase^+$) ($P=0.0438$), although there were no differences in survival when patients were grouped by TdRPase expression in primary lesions (Fig. 2). In addition, there were no differences in survival among the patients with unresectable pancreatic cancer grouped by the positivity of TdRPase expression.

Relationship between the efficacy of ACT and TdRPase expression

In patients who had undergone pancreatectomy, the survival rate of the ACT group was higher than that of the SA group, although the difference was not statistically significant (Table 5). In contrast, ACT was demonstrated to be effective in improving the survival of patients with unresectable cancer ($P=0.0001$; Table 5). TdRPase expression in the primary lesion of the IDC was not correlated with the efficacy of ACT after pancreatectomy (Fig. 3). In patients with $TdRPase^+$ or

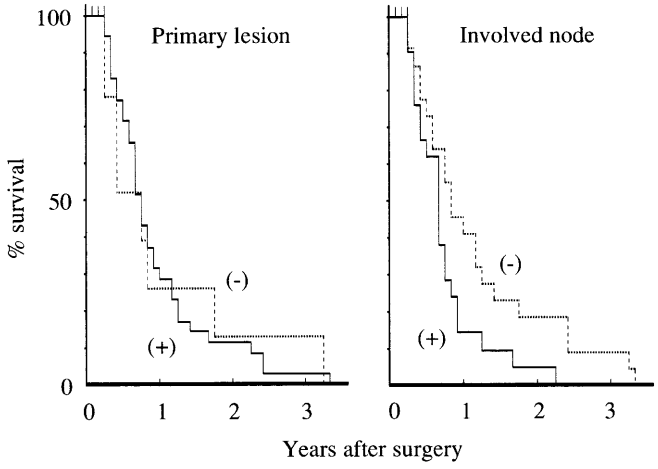

Fig. 2 Survival curves for patients with node-positive resectable pancreatic IDC. TdRPase expression in primary lesion: (+) grade 1–2, $n=37$; (–) grade 0, $n=9$. TdRPase expression in involved node: (+) $n=21$; (–) $n=25$. There was no significant difference in survival between the patients grouped by TdRPase expression in the primary lesion. In contrast, survival was significantly higher in patients with $TdRPase^-$ involved nodes than in those with $TdRPase^+$ involved nodes ($P=0.0438$)

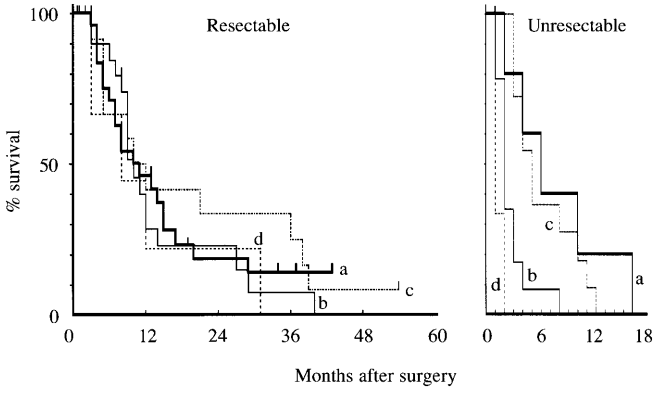

Fig. 3 Relationship between TdRPase expression and the efficacy of chemotherapy. Resectable IDC: *a* $TdRPase^+$ ACT group ($n=24$), *b* $TdRPase^+$ SA group ($n=23$), *c* $TdRPase^-$ ACT group ($n=12$), *d* $TdRPase^-$ SA group ($n=7$). There were no significant differences in survival rates among the four groups. Unresectable IDC: *a* $TdRPase^+$ ACT group ($n=5$), *b* $TdRPase^+$ SA group ($n=14$), *c* $TdRPase^-$ ACT group ($n=11$), *d* $TdRPase^-$ SA group ($n=6$). The survival rate in the ACT group was significantly higher than in the SA group amongst both $TdRPase^-$ and $TdRPase^+$ patients ($P=0.0001$ and $P=0.0323$, respectively)

Table 6 Multivariate analysis of the relationship between various clinicopathological and cytomolecular factors and the prognosis of patients with resectable pancreatic cancer by Cox's proportional hazard risk model (dependent variable, month; censoring variable, death due to pancreatic cancer)

	Variable	Parameter estimate (SE)	Conditional risk ratio (95% confidence limits)	*P*-value (chi-squared test)
Resectable (overall)	pTNM stage	0.635 (0.175)	1.887 (1.340–2.657)	0.0003
	Histological grade	0.674 (0.262)	2.002 (1.198–3.346)	0.0080
	Age	0.012 (0.016)	1.012 (0.980–1.045)	0.4698
	Adjuvant chemotherapy	–0.147 (0.281)	0.863 (0.498–1.497)	0.6006
	Gender	0.099 (0.316)	1.104 (0.594–2.052)	0.7551
	TdRPase expression, primary lesion	0.009 (0.178)	1.009 (0.712–1.430)	0.9617
Resectable (node-positive)	pTNM stage	1.007 (0.412)	2.739 (1.221–6.145)	0.0145
	Histological grade	0.665 (0.308)	1.945 (1.064–3.553)	0.0306
	TdRPase expression, involved node	–0.731 (0.366)	0.481 (0.235–0.986)	0.0457
	TdRPase expression, primary lesion	0.693 (0.509)	1.999 (0.738–5.416)	0.1732
	Adjuvant chemotherapy	0.389 (0.375)	1.475 (0.707–3.078)	0.3000
	Age	0.013 (0.017)	1.013 (0.979–1.049)	0.4517
	Gender	0.082 (0.363)	1.085 (0.533–2.209)	0.8217
Unresectable	Adjuvant chemotherapy	–2.030 (0.517)	0.131 (0.048–0.362)	0.0001
	TdRPase expression	–0.409 (0.268)	0.664 (0.392–1.124)	0.1271
	Histological grade	–0.636 (0.670)	0.529 (0.142–1.968)	0.3423
	Age	–0.002 (0.019)	0.998 (0.961–1.037)	0.9193
	Gender	–0.039 (0.446)	0.962 (0.402–1.037)	0.9311

TdRPase$^-$ primary lesions, the survival rate between the ACT group and the SA group was not significantly different. On the other hand, in patients with unresectable pancreatic TdRPase$^-$ or TdRPase$^+$ tumors, the survival rate was significantly higher in the ACT group than in the SA group, suggesting that TdRPase expression in distant metastases has no significant effect on the efficacy of ACT (Fig. 3).

Multivariate analysis

The implication of TdRPase expression was evaluated by multivariate analysis (Table 6). In patients with resectable IDC, stage and histological grade were found to be significant prognostic factors for IDC-related death, but TdRPase expression in the primary lesion was not a significant factor. In patients with resectable IDC involving nodes, TdRPase expression in involved nodes was found to be a significant low-risk factor, but it was not a significant factor in the primary lesion. On the other hand, in patients with unresectable IDC, only ACT was a significant low-risk factor.

Discussion

The present study demonstrated that the expression of TdRPase in pancreatic IDC was significantly higher than in benign diseases. The activity of TdRPase is higher in cancerous than in normal tissues of various organs such as breast, colon, stomach, urinary bladder, uterus and ovary [9, 11, 12, 13, 14, 20, 30], suggesting that the metabolism of TdR is more active in pancreatic IDC than in benign diseases. TdRPase is one of the key enzymes in the salvage pathway of DNA synthesis, where it metabolizes TdR into thymine and 2′-deoxy-D-ribose. Accordingly, in normal pancreatic duct cells or benign diseases of the pancreas expressing low levels of TdRPase, TdR monophosphate is only synthesized from deoxyuridine monophosphate (dUMP) and is utilized for de novo DNA synthesis. In contrast, in cancer cells expressing high levels of TdRPase, DNA may be synthesized mainly through the salvage pathway.

The present study also demonstrated that the expression of TdRPase was significantly lower in distant metastases than in primary lesions. Furthermore, in patients with both primary lesions and nodal involvement, TdRPase expression was significantly lower in the involved nodes than in the primary lesion. The activity of TdRPase in various cancers is higher in primary lesions than in liver metastasis [20]. Also the activity of TdRPase is lower in colon cancer xenografted into nude mice than in the original tumor, but the activity of TdR kinase and thymidylate synthase (TS) is not affected by transplantation into nude mice [13]. The lower activity of TdRPase in metastatic lesions than in primary lesions may not necessarily mean lower malignant potential in metastatic lesions than in primary lesions, because previous studies have demonstrated that DNA synthesis is sometimes higher in metastatic than in primary lesions [19, 21]. We speculate that these results may simply reflect heterogeneity in enzyme activity between the primary and metastatic lesions of pancreatic IDC. In other words, the major pathway of DNA synthesis may be different between primary and metastatic lesions. It is not clear, however, why the activity of TdRPase is lower in metastatic than in primary lesions. One possible explanation is that cancer cells whose DNA is synthesized mainly through a de novo pathway may have a biological potential to metastasize, and another is that metastasized cells may change their biological characteristics to accommodate environments different from that of the original lesion. This is a subject for future clarification.

Our study also demonstrated that the expression of TdRPase in primary lesions had no significant effect on the prognosis of patients with resectable IDC. In contrast, the expression of TdRPase in involved nodes was found to be significantly correlated with the prognosis of patients with node-positive IDC after pancreatectomy, as shown in the multivariate analysis. It is natural that the level of DNA synthesis in metastatic lesions should be more associated with prognosis than that in the primary lesions after resection of the primary lesion. In patients with unresectable IDC, TdRPase reactivity was not correlated with patient prognosis. One possible explanation for this discrepancy may be that the lesions assessed in patients with unresectable IDC included a variety of metastatic lesions of the liver, peritoneum and lymph nodes.

In the present study, ACT was found to improve significantly the survival of patients with unresectable IDC, as demonstrated in the multivariate analysis. In patients with resectable IDC, the survival was higher in the ACT group than in the SA group, but the difference was not significant. One possible explanation for this discrepancy is that the resectable IDCs consisted of various tumors at different stages, of different histologies, or with different surgical curabilities, and the effects of ACT might have been different. In contrast, unresectable IDCs consisted of only far-advanced stage 4 cancers with distant metastasis. On the other hand, TdRPase activity in the tumor was not significantly correlated with the efficacy of chemotherapy in patients with resectable or unresectable IDC. In the present study, the ACT of most patients included 5-FU or its derivative UFT. The mechanism of action of 5-FU is complex, and its effects on DNA, which are thought to be primarily responsible for its antitumor activity, have been investigated. TS is a major locus for 5-FU action [10]. It catalyzes the conversion of dUMP to deoxythymidine monophosphate (dTMP). 5-FU is phosphorylated to 5-fluoro-deoxyuridine monophosphate (FdUMP), and FdUMP inhibits TS activity by forming a ternary covalent complex with N5-N10-methylene tetrahydrofolate and TS. Accordingly, FdUMP blocks the pathway for the de novo synthesis of dTMP and

consequently inhibits DNA synthesis [23, 28]. If cancer cells have high TdRPase activity, however, they can synthesize DNA through the salvage pathway even if de novo synthesis is inhibited by 5-FU administration. In other words, TdRPase may function as an alternative pathway of DNA synthesis inhibited through the de novo pathway.

Although previous reports have suggested that TdRPase expression is involved in the efficacy of chemotherapy with 5-FU or its derivatives [11, 12, 14], the present study did not show a significant association between TdRPase expression and the efficacy of chemotherapy. All ACT regimens included FUs, and UFT was given in most cases. This also suggests that there was no relationship between TdRPase expression and the response to UFT-based chemotherapies. One hypothesis to explain this discrepancy is that TdRPase functions as an angiogenic factor, resulting in promotion of the growth of tumor cells, because TdRPase has been demonstrated to be identical to platelet-derived endothelial growth factor [16]. On the other hand, this discrepancy does not necessarily mean a lack of correlation between TdRPase expression and response to FUs (especially UFT). The lack of correlation could be due to the fact that 16 of 36 patients in the ACT group were treated with FU-based regimens that included other agents such as CPA, CDDP, MMC, ADR and VP-16, as shown in Table 2, and combining these agents 5-FU or its derivatives could alter its efficacy.

The present study showed, however, that the difference in the activity of TdRPase between the primary and metastatic lesions of pancreatic IDC may be one of the factors responsible for the ineffectiveness of chemotherapy with 5-FU or its derivatives. In other words, lesions with a high activity of TS, a target enzyme of 5-FU in de novo pathways of DNA synthesis, can be considered to be sensitive to 5-FU, but lesions with high activity of TdRPase, a key enzyme of the salvage pathway of DNA synthesis, can be considered to be resistant to 5-FU. Thus, if the primary lesion shows high TS activity and is sensitive to 5-FU and the metastatic lesion shows high TdRPase activity and is resistant to 5-FU, chemotherapy with 5-FU may result in no response. Such tumor heterogeneity in chemosensitivity has been one of the major issues in cancer chemotherapy [5, 7, 19, 21, 25], and the present study shows that the heterogeneity in TdRPase activity between primary and metastatic lesions may be one of the mechanisms responsible for the resistance to 5-FU-based chemotherapy. It is also reasonable that the efficacy of 5-FU or its derivatives may be dependent on the balance between the activities of TdRPase and TS.

In conclusion, the present study showed that TdRPase expression is different in primary lesions and metastases of pancreatic IDC, suggesting that DNA is synthesized through different pathways for the primary and the metastatic cancers. This heterogeneity in TdRPase activity may be one of the reasons for the resistance to 5-FU-based chemotherapy.

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