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RESEARCH

Overexpression of PTGIS Could Predict Liver Metastasis and is Correlated with Poor Prognosis in Colon Cancer Patients

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Abstract The purpose of this study was to evaluate the predictive ability of PTGIS for liver metastasis. Protein expression of PTGIS was analyzed on tissue microarray consisting of 117 CRC cases with liver metastasis (M1) and 104 cases of CRC without liver metastasis at least 5 years after resection of primary CRC (M0) by immunohistochemistry. Expression of PTGIS in 147 of 221 of primary lesions exhibited positive staining. Moreover, the PTGIS expression was significantly higher in CRC-M1 than CRC-M0 group. More importantly, the 87% (20/23) heterochronous metastatic cases showed positive staining for PTGIS. Collecting the primary and liver metastatic tumor samples from the same colon cancer patients, we tested the expression of PTGIS and revealed that the expression level of PTGIS in the hepatic metastases was noticeably higher than in the matched primary colon cancer tissues from the same patient in 9 out of 16 cases examined. Logistic regression analysis indicated that the expression of PTGIS and lymph node involvement were risk factors in colon cancer liver metastasis independent of the other variables. In leave-one-out validation model, the combination of PTGIS and lymph node involvement yielded the 89.7% satisfactory sensitivity and 83% specificity for detection of hepatic metastasis. Kaplan–Meier survival analysis revealed a correlation between higher PTGIS expression levels and shorter overall survival times. In conclusion, our results suggest that PTGIS combined with lymph node involvement may be used as accurate predictors of liver metastasis in colorectal cancer.

Keywords PTGIS · Colon cancer · Liver metastasis · Prognosis

Lichao Sun and Liang Peng contributed equally to this work.

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Introduction

Colorectal carcinoma (CRC) is one of the major causes of cancer death worldwide [1]. Liver is the most common target for metastasis in patients with this disease. It is estimated that approximately 50% of CRC patients develop liver metastases [2]. Liver metastasis is the most critical prognostic factor for CRC. Thus, early detection of liver metastasis is important for improving patient survival.

Cyclooxygenase-2 (COX-2) enzyme plays a causal role in the development of colorectal cancer, which is involved

in the conversion of arachidonic acid to prostaglandin H_2. Then prostaglandin I_2 synthase (PTGIS) could catalyze the rearrangement of prostaglandin H_2 to PGI_2. Intracellular PGI_2 have also been shown to interact with peroxisomal-proliferator-activated receptors (PPAR), which might contribute to colon cancer progression and angiogenesis [3–5]. Other studies also suggested that stromal cells including fibroblasts in cancer tissues could be induced the expression of PTGIS and PGI_2 may contribute to cancer cell growth [6]. Although the PTGIS promoter of hypermethylation has been reported in a limited number of clinical samples [7], previous study in our lab has implicated that PTGIS was highly expressed in colon cancer primary tissues. Until now little is known about the PTGIS expression level in colon cancer tissues and its predictive ability for liver metastasis.

Therefore, in this study we firstly examined the expression of PTGIS in primary colon cancer and paired liver metastatic samples, and analyzed the correlation between the expression of PTGIS and liver metastasis coupled with clinical pathological features. We propose that PTGIS expression is important for prognostic evaluation and suggest that PTGIS could be a novel predictive marker for colorectal cancer liver metastasis.

Materials and Methods

Colorectal Cancer Samples

Fresh surgical resection specimens from 221 CRC patients were collected from Cancer Institute/ Hospital, Chinese Academy of Medical Sciences (CAMS) & Peking Union Medical College (PUMC), Beijing. None of the patients received treatment before surgery. Primary tumor tissues and the matched histological normal mucosa from the same patients were separated by experienced pathologists and were fixed in 4% paraformaldehyde for paraffin embedding. Approval of the present studies was obtained from the Institutional Review Board of Cancer Institute/ Hospital, CAMS&PUMC. CRC cases with no history of liver metastasis at least 5 years after resection of primary CRC were named as CRC-M0. CRC cases with history of liver metastasis were designated as CRC-M1. Metastasis was considered as synchronous (94 cases) when it had been detected by computed tomography (CT) scan, ultrasonography, or by surgery at the time of initial diagnosis; and cases were considered as heterochronous (23 cases; mean colon cancer liver metastasis time, 25.9±15.5 mo) when liver metastases, confirmed by CT scan or ultrasonography, occurred after resection of the primary tumor.

Tissue Microarray Construction

Tissue microarrays were prepared from archival formalin-fixed, paraffin-embedded tissue blocks. For each tumor, a representative tumor area was carefully selected from a H&E-stain section. For each case, normal tissues were repeated twice, and the cancer tissues were repeated five times.

Immunohistochemistry and Scoring

The avidin-biotin-complex method was used for immunohistochemical analysis. Briefly, after deparaffinization in xylene and graded alcohols, heated antigen retrieval was done in citrate buffer (10 mM pH 6.0) by water-bath kettle heating for 30 min. Endogenous peroxidase was blocked in 0.3% hydrogen peroxide for 10 min. Nonspecific binding was blocked by incubation in 10% normal animal serum for 10 min. Sections were incubated at 4°C for 24 h with a primary antibody for PTGIS (sc-20933, Santa Cruz, 1:100). Biotinylated secondary antibody and horseradish-peroxidase-labeled avidin were subsequently used, and color was developed using the diaminobenzidine method. Expression levels of proteins were scored according to the stain pattern of malignant/epithelial cells. The stain of PTGIS is normally expressed in a homogenous manner. Immunostaining on the TMAs was graded semi-quantitatively considering both staining intensity and percentage of positive tumor cells by two study pathologists blinded to the clinicopathologic variables. The staining intensity was scored on a scale of four grades: 0, no staining of cancer cells; 1, weak staining; 2 moderate staining; 3, strong staining. The percentage of stained tumor cells was graded on a scale of 2 grades: no positive cells or positive in less than 25% of constituent carcinoma cells =0; and positive in greater than 25% of constituent carcinoma cells =1. PTGIS expression in the cancer tissue was defined as positive when the product of intensity score by percentage score is two or more, and negative when the score below 2.

Statistical Analysis

The SPSS 15 software package (SPSS, Inc., Chicago, IL) was used for statistical analysis. The PTGIS expression was first analyzed as continuous numeric data and the mean staining intensity between the primary tumors of CRC-M0 and CRC-M1 was compared with a two-sided t-test. According to an optimal cutpoint described in immunohistochemical analysis, PTGIS level was analyzed as a dichotomous variable for further evaluation. The association between the immunoreactive markers and clinicopathologic features was analyzed using χ^2-test or two-sided t-

test as appropriate. To estimate the variables of immunoreactive markers or clinicopathologic features that may contribute to the prediction of liver metastasis, those of significant difference between CRC-M0 and CRC-M1 were then evaluated by logistic regression analysis. All possible combinations of these factors were used to build up classifiers that were able to distinguish metastatic cases from non-metastatic cases were examined using Leave-one-out validation model. The survival rates were assessed by the Kaplan–Meier method and compared by the log-rank test. Statistical significance was set at $P<0.05$ (two-tailed).

Results

Patient Characteristics

The clinical backgrounds of the patients providing the 221 colon cancer samples including 104 CRC-M0 and 117 CRC-M1 were summarized in Table 1. There was significant difference in vascular invasion, TNM stage, depth of invasion, serum CEA level and lymph node metastasis between CRC-M1 and CRC-M0. And there was no significant difference in age, sex and size between these tumors.

PTGIS is Differentially Expressed in Human Primary Colon Carcinomas and Liver Metastatic Samples

To evaluate the expression of PTGIS in colon cancer and liver metastatic tissues, immunohistochemistry staining was performed on 221 paraffin-embedded primary colorectal cancer tumors with corresponding normal tissues and 16 paired liver metastatic samples. There was weak or no staining of PTGIS in the normal epithelium. The incidence of PTGIS expression in CRC-M1 was 80.3% (94/117). In the CRC-M0 cases, the incidence of PTGIS expression was 49% (51/104). More importantly, the 87% (20/23) heterochronous metastatic cases showed positive staining for PTGIS. In addition, about 39% cases of liver metastatic

Table 1 Clinicopathological characteristics of the 221colon cancer patients

		CRC-M0	CRC-M1	P
Gender (male:female)		51:53	64:53	0.19
	age	54.37±12.65	56.54±11.36	0.163
Tumor size(cm)	4.65±2.18	6.04±2.38	0.513	
Serum CEA level				0.000*
	0	66	37	
	1	23	23	
	2	11	32	
	3	4	25	
Differentiation				0.057
	Well	14	29	
	Moderate	82	70	
	poor	8	18	
Vascular invasion				0.020*
	No invasion	74	67	
	Invasion	29	51	
Depth of invasion				0.000*
	T1+T2	24	2	
	T3	27	11	
	T4	53	104	
Lymph node metastasis				0.000*
	N0	58	17	
	N1	33	50	
	N2	13	50	
Stage				0.000*
	I	18	2	
	II	40	4	
	III	46	17	
	IV	0	94	

CEA: ≤15 ng/ml, 0; >15-≤ 40 ng/ml, 1; >40-≤100 ng/ml, 2; >100 ng/ml, 3. *$p<0.05$

tissues and 19% cases of colorectal cancers also weakly expressed PTGIS in the stromal cells, respectively. Further analysis also showed that the expression of PTGIS was higher in M1 than the M0 (P=0.000) (Fig. 1a, Table 2).

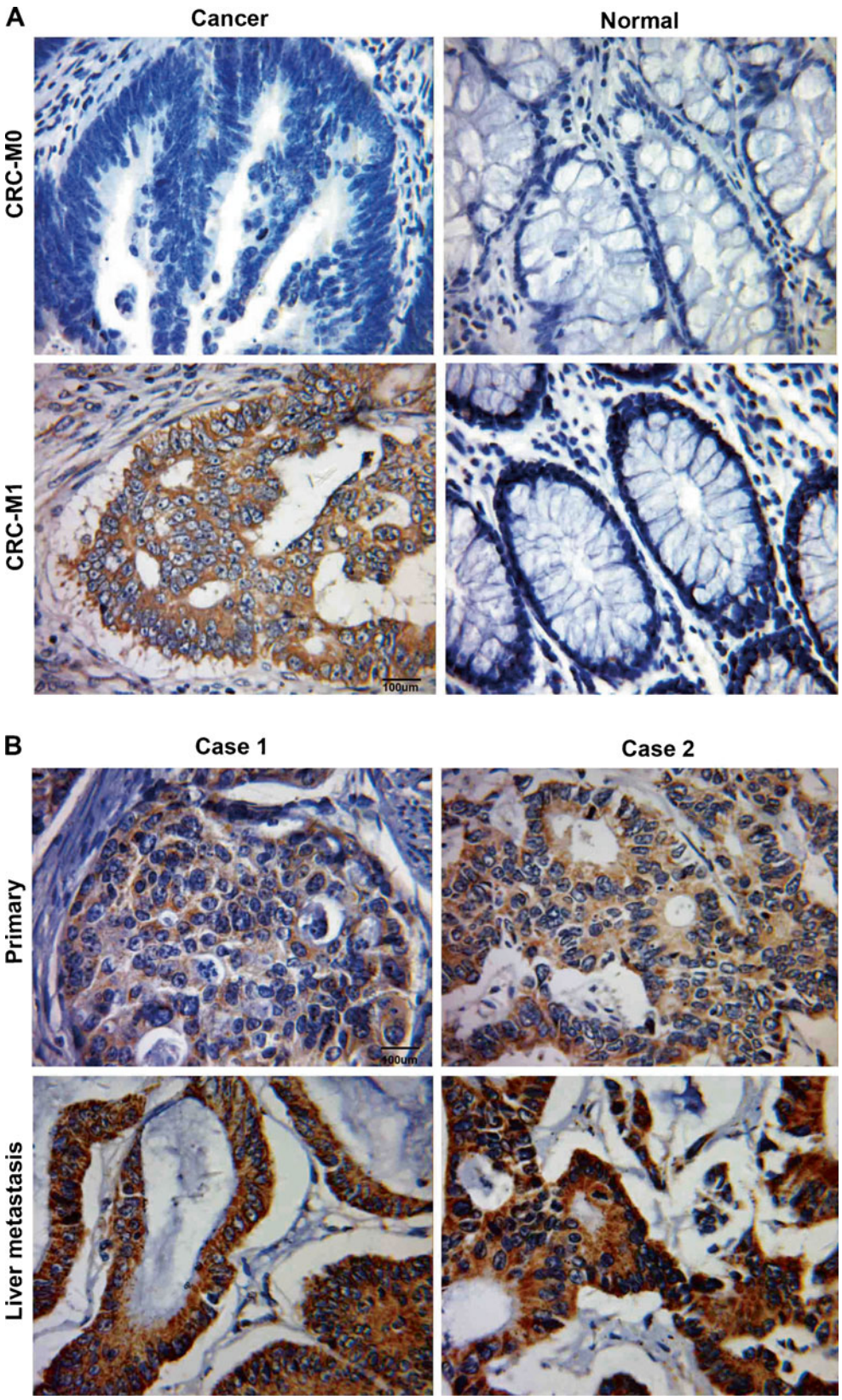

Fig. 1 Expression of PTGIS in human primary colon carcinomas and liver metastatic tissues. **a** Representative immunohistochemical staining of PTGIS in colorectal cancer samples and matched normal tissues. The expression of PTGIS is significantly greater in the metastasis group (CRC-M1) than in the control group (CRC-M0). **b** Immunohistochemical analysis of PTGIS expression in primary and metastatic colon cancers. (*scale bar*, 100 μm)

Table 2 The expression of PTGIS in 221colon cancer patients

Group	PTGIS expression		Total	*P* value
	Negative	Positive		
				0.000*
CRC-M0	51	53	104	
CRC-M1				
Synchronous	20	74	94	
Heterochronous	3	20	23	

Furthermore, we found that PTGIS was overexpressed in all cases of colon metastatic tumors in the liver. Importantly, the expression level of PTGIS in the hepatic metastases was noticeably higher than that in the matched primary colorectal tumors from the same patients in 9 out of 16 cases examined (Fig. 1b). These results suggested that PTGIS might play a key role in the colorectal cancer liver metastasis.

Higher Expression of PTGIS is Associated with Clinicopathological Parameters

Associations of PTGIS protein expression with other variables were shown in Table 3. PTGIS expression was significantly associated with serum CEA level (*P= 0.001*), clinical stage (*P= 0.000*), lymph node metastasis (*P= 0.000*) and depth of invasion (*P= 0.001*). While there was no significant correlation between PTGIS expression and vascular invasion, tumor size, differentiation, age and sex. Taken together, these observations indicated that over-

Table 3 Correlation PTGIS expression with clinicopathological characteristics in 221cases

		Negative	Positive	*p* value
Gender (Male: Female)		43:31	82:65	0.742
Age		54.5±12.7	56±11.6	0.393
Serum CEA level				0.001*
	0	42	61	
	1	17	29	
	2	13	30	
	3	2	27	
Tumor size(cm)		5.53±2.21	5.49±2.9	0.145
Differentiation				
	Well	10	9	0.064
	Moderate	59	117	
	poor	5	21	
Vascular invasion				0.261
	No invasion	51	90	
	Invasion	23	57	
Depth of invasion				0.001*
	T1 + T2	16	10	
	T3	17	21	
	T4	41	116	
Lymph node involvement				0.000*
	N0	42	33	
	N1	19	64	
	N2	13	50	
Stage				
	I	13	7	0.000*
	II	23	21	
	III	18	45	
	IV	20	74	
M				0.000*
	M0	51	54	
	M1	23	93	

Table 4 Stepwise logistic regression analysis of the clinicopathological and immunohistochemical factors

	Odd ratio	95% confidence intervals	*P* aluve
PTGIS	3.507	1.771–6.945	0.000*
Lymph node metastasis	2.769	1.386–5.535	0.004*
Vascular invasion	1.250	0.927–1.686	0.144
Serum CEA level	0.328	0.783–1.007	0.114
Tumor size(cm)	1.712	0.898–3.266	0.102
Differentiation	0.640	0.328–1.249	0.191
Depth of invasion	1.404	0.899–2.192	0.135
Sex	1.422	0.744–2.718	0.287
Age	0.988	0.617–1.582	0.959

expression of PTGIS was significantly associated with colon cancer liver metastasis.

PTGIS Expression Levels Predict the Risk of Metastasis of Colon Cancer to the Liver

Table 4 shows a stepwise logistic regression analysis of the clinicopathological and immunohistochemical factors. The incidence of lymph node metastasis and the expression of PTGIS were significantly greater in the CRCs with liver metastasis than in the CRCs without liver metastasis, and these risk factors were independent of the other variables. The odds ratio of the expression of PTGIS and lymph node involvement was 3.507 and 2.769, respectively. To further evaluate the predictors that reached statistical significance in logistic regression analysis, the two variables were selected for Leave-one-out validation analysis. This analysis revealed that the combination of PTGIS and lymph node involvement yielded the 89.7% satisfactory sensitivity and 83% specificity for detecting colorectal cancer liver metastasis (Table 5).

The Correlation of PTGIS Expression with Prognosis of Colorectal Cancer

The prognostic significance of PTGIS expression was determined by PTGIS staining and the corresponding clinical follow-up records. Kaplan–Meier survival analysis revealed a correlation between higher PTGIS expression levels and shorter overall survival times ($P=0.000$) (Fig. 2).

Discussion

Colorectal carcinoma (CRC) is one of the major causes of cancer death worldwide [8]. Liver metastasis is the most critical prognostic factor for CRC. Prediction of CRC liver metastasis may provide useful information for designing treatment strategy to improve patient survival.

Previous study in our lab has implicated that PTGIS was highly expressed in colon cancer primary tissues. Although one study has found the hypermethylation of the PTGIS promoter in 43 out of 100 colorectal cancers [7], the protein expression of PTGIS in colon cancer and liver metastatic tissues was seldom detected in present. To explore the roles of PTGIS in colon cancer liver metastasis, we collected 221 specimens with follow-up record and investigated the expression of PTGIS in colorectal cancer primary tissues coupled with paired the liver metastatic tissues. The result demonstrated that 147 of 221 cases exhibited positive staining of PTGIS. Further analysis showed that the PTGIS expression was significantly higher in M1 than M0 group ($p<0.05$). We also found that the expression level of PTGIS in the hepatic metastases was noticeably higher than that in the matched primary colon tumors from the same patients in 9 out of 16 cases examined. Clinicopathological analysis showed that the expression of PTGIS was significantly correlated with liver metastasis, serum CEA level, clinical stage, lymph node metastasis and depth of invasion. When were examined the ability to predict colon cancer liver metastasis using logistic regression analysis, the expression of PTGIS and lymph node involvement were found to be accurate predictors (The odds ratio 3.507 and 2.769, $p<$

Table 5 The predictive ability of the possible combinations of lymph node metastasis and PTGIS level in liver metastasis

	Sensitivity (%)	Specificity (%)
Liver metastasis		
Lymph node involvement	85.5	65.8
PTGIS	80.3	81.3
Lymph node involvement+PTGIS	89.7	83

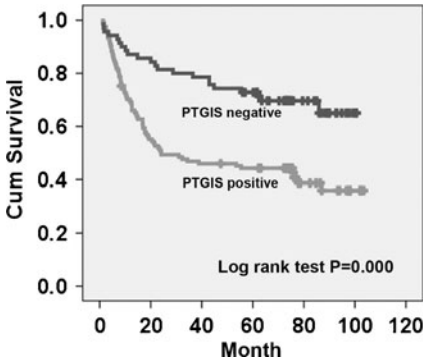

Fig. 2 Estimated overall survival according to the expression of PTGIS in 221 cases of colorectal cancer (CRC). Kaplan-Meier survival curve of colorectal cancer patients with PTGIS expression. Patients with expression of PTGIS showed significantly lower survival rates. (P=0.000)

0.05). Leave-one-out method has been used to identify marker in predicting cancer metastasis [9, 10]. The result showed that the combination of these two variables achieved 89.7% sensitivity and 83% specificity for distinguishing CRC-M1 from CRC-M0. Kaplan–Meier survival analysis revealed a correlation between higher PTGIS expression levels and shorter overall survival times. To our knowledge, this is the first study demonstrating that PTGIS expression was closely related to hepatic metastasis and poor prognosis of CRC.

We speculated the possible reasons for the contribution of PTGIS to colon cancer liver metastasis. For one thing, PTGIS is an enzyme involved in the synthesis of prostaglandin, whose physiological function is to catalysis PGH_2 into PGI_2, which has been implicated in colorectal cancer growth by preventing apoptosis [6, 11, 12]. PGI_2 also could activate nuclear receptor PPARδ, which is functionally active in human colorectal carcinoma cell lines [4]. PPARδ also was proposed as a downstream target of the APC/β-catenin pathway in colorectal carcinogenesis [12, 13]. For another, PTGIS might contribute to form a pre-metastatic microenvironment in colon cancer primary tissues, in which colon cancer cells might have got the potential of liver metastasis. Stromal cells including fibroblasts in cancer tissues could be induced the expression of PTGIS and PGI_2, which might contribute to cancer cell growth. Furthermore, PGI_2 plays an key role in vascular biology such as platelet aggregation, vascular permeability and vascular smooth muscle cell proliferation, which made the cancer cells extravasate faster in solid tumor [14, 15]. Also PTGIS coupled with production PGI_2 can contribute, at least in part, to tumor growth and metastasis via their role in the regulation of angiogenesis. PGI_2 has been shown to induce pro-angiogenic VEGF expression in rat intestinal epithelial cells, which is involved in the regulation of tumor-associated angiogenesis [16].

In conclusion, this study firstly shows that PTGIS is highly expressed in human CRC tissues, which is closely related to hepatic metastasis and poor prognosis of CRC. These high risk patients may benefit from strict surveillance or other effective treatment according to the expression of PTGIS. But this assumption must be verified in a prospective study.

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