Phospholipase A2 Group III and Group X Have Opposing Associations with Prognosis in Colorectal Cancer

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Abstract. *Background: Although secretory phospholipase A2 (sPLA2) has been shown to be involved in various biological processes, its specific roles in sub-types of cancer development remain to be elucidated. Materials and Methods: We examined the expression of sPLA2 group III (GIII) in 142 patients with colorectal cancer using immunohistochemistry, and its correlation with clinicopathological features and outcomes. In addition, we examined the co-expression of sPLA2GIII and sPLA2GX using serial tissue sections to clarify the roles of both proteins in colorectal carcinogenesis. Results: In 66 cases, diffuse staining of sPLA2GIII was seen; this was defined as the group with high expression. High expression was associated with a significantly higher rate of lymph node metastasis (p=0.02) and poorer survival (p=0.03) compared with low expression. Patients with low sPLA2GIII and high sPLA2GX expression had a significantly higher survival rate than those with high sPLA2GIII and low sPLA2GX expression (p=0.038). Conclusion: sPLA2GIII expression may be used as a risk factor for lymph node metastasis and a prognostic marker in colorectal cancer. In addition, sPLA2GIII and sPLA2GX may play opposing roles in colorectal carcinogenesis.*

Colorectal cancer (CRC) is the third most common cancer in men and the second most common cancer in women, and is responsible for approximately 610,000 deaths annually worldwide (1). Despite surgical resection and combined chemotherapy, approximately 20% of patients with CRC develop distant metastases (2). The identification of new prognostic markers for distant metastasis may lead to improved treatment strategies and better outcomes for patients.

Phospholipase A2 (PLA2) is a key regulatory enzyme in arachidonic acid metabolism, catalyzing the hydrolysis of sn-2 fatty acyl ester bonds of phosphoglycerides, and releasing free fatty acids and lysophospholipids (3). One of the fatty acids released from membrane stores by PLA2 activity is arachidonic acid, a critical precursor in the biosynthesis of diverse eicosanoids, including prostaglandins, thromboxanes, and leukotrienes *via* the cyclo-oxygenase (COX)-1 and COX2 pathways (4). These lipid mediators are known to play a role in initiation and progression of CRC through cell proliferation, migration, and angiogenesis (5-9). In addition, COX2 modulates tumor growth by altering vascular endothelial growth factor expression (10). As a result, PLA2 is thought to play an important role in CRC. The PLA2 family of proteins includes calcium-independent PLA2 (iPLA2), high molecular weight cytosolic PLA2 (cPLA2), and low-molecular weight secretory PLA2 (sPLA2). sPLA2 proteins are calcium-dependent, secreted enzymes, containing a His/Asp catalytic dyad. To date, 11 sPLA2s (IB, IIA, IIC, IID, IIE, IIF, III, V, X, XIIA, and XIIB) have been identified in mammals (11-13).

Recently, various physiological functions of sPLA2s have been reported (14-27). The role of sPLA2GIII has been examined in several types of cancer (28-35). In stage II CRC, the expression of sPLA2GIIA was reported to be significantly correlated with disease recurrence (36). In addition, we demonstrated that the expression of sPLA2GX was inversely associated with hematogenous metastasis in CRC (37).

sPLA2GIII has the most unique structural property among mammalian sPLA2s, with prostaglandin E2-generating function in various cell types. sPLA2GIII is found in microvascular endothelial cells, and in uterine, breast, and

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Key Words: Phospholipase A2, colorectal cancer, immunohistochemistry, lymph node metastasis.

Table I. *Clinicopathological features in colorectal cancer.*

Characteristic		n (%)
Gender	Male	85 (59.9%)
	Female	57 (40.1%)
Mean age (years)±SD		62.8±11.2
Size (mm)±SD		47.6±22.3
Location	Cecum	5 (3.5%)
	Ascending colon	25 (17.6%)
	Transverse colon	12 (8.5%)
	Descending colon	3 (2.1%)
	Sigmoid colon	58 (40.8%)
	Rectum	39 (27.5%)
Histological type	Well	96 (67.6%)
	Moderately	40 (28.2%)
	Poorly	3 (2.1%)
	Mucinous	3 (2.1%)
T Stage*	T1	13 (9.2%)
	T2	21 (14.8%)
	T3	97 (68.3%)
	T4	11 (7.7%)
Lymphatic involvement	Absent	104 (73.2%)
	Present	38 (26.8%)
Venous involvement	Absent	68 (47.9%)
	Present	74 (52.1%)
Lymph node metastasis	Absent	81 (57.0%)
	Present	61 (43.0%)
Hematogenous metastasis	Absent	135 (95.1%)
	Present	7 (4.9%)
UICC Stage	I	27 (19.0%)
	II	53 (37.3%)
	III	53 (37.3%)
	IV	9 (6.2%)

SD, Standard deviation; N.S, not significant; UICC, the Union for International Cancer Control; *TMN Classification of malignant tumors (#39).

Table II. *PLA2 group III expression in colorectal cancer by clinicopathological features.*

			PLA2 group III expression		
Characteristic		n	High	Low	*p*-Value
Mean age (years)±SD		142	61.4±11.2	64.1±11.1	0.14
Gender	Male	85	40	45	0.87
	Female	57	26	31	
Size (mm)±SD		142	45.3±18.5	49.6±25.0	0.24
Location	Colon	103	48	55	0.96
	Rectum	39	18	21	
	Right sided	42	18	24	0.57
	Left sided	100	48	52	
Histological type	Well	96	48	48	0.48
	Moderately	40	15	25	
	Poorly	3	1	2	
	Mucinous	3	2	1	
T Stage*	T1	13	3	10	0.28
	T2	21	12	9	
	T3	97	46	51	
	T4	11	5	6	
Lymphatic involvement	Absent	104	45	59	0.2
	Present	38	21	17	
Venous involvement	Absent	68	29	39	0.38
	Present	74	37	37	
Lymph node metastasis	Absent	81	31	50	0.02
	Present	61	35	26	
Hematogenous metastasis	Absent	135	62	73	0.56
	Present	7	4	3	
UICC Stage	I	27	12	15	0.18
	II	53	19	34	
	III	53	30	23	
	IV	9	5	4	

SD, Standard deviation; N.S, not significant; UICC, the Union for International Cancer Control; *TMN Classification of malignant tumors (#39).

colonic tumor cells (9). Therefore, sPLA2GIII was suggested as a good candidate biomarker for CRC (38). However, detailed analysis of sPLA2GIII expression has not been carried out thus far, and the association between sPLA2GIII expression and prognosis in CRC remains to be elucidated.

Therefore, in the present study, we analyzed the expression pattern of sPLA2GIII in CRC, and its correlation with clinicopathological factors and outcomes. Furthermore, we examined the co-expression of sPLA2GIII and sPLA2GX using immunohistochemical (IHC) analysis of serial tissue sections, and analyzed the roles of both proteins in CRC carcinogenesis.

Materials and Methods

Patients and samples. One hundred and forty-two patients with CRC who were treated with curative resection and lymph node dissection at the University of Tokyo Hospital between January 1991 and December 1993 were enrolled in this study. There were 85 men and 57 women, with an age range of 38-90 years (mean=62±11 years). Patients with inflammatory bowel disease and familial adenomatous polyposis were excluded from this study. None of the patients had received preoperative chemotherapy or radiation therapy. All clinical and histopathological data regarding the patients and their tumors were collected from medical records. Clinicopathological features were analyzed using the Union for International Cancer Control (UICC) histological criteria, as defined in the seventh edition of the TNM Classification of Malignant Tumors (39). Patient follow-up to evaluate tumor recurrence was carried out for 5 years; serum carcinoembryonic antigen level was measured every months, computed tomography (CT) was performed every 6 months, and colonoscopy was performed every 12 months. The median observation period was 9.2 years. Patient consent was obtained for the use of clinical samples for research purposes, and approval for the study was obtained from the Ethics Committee of the University of Tokyo Hospital (2391-(1)).

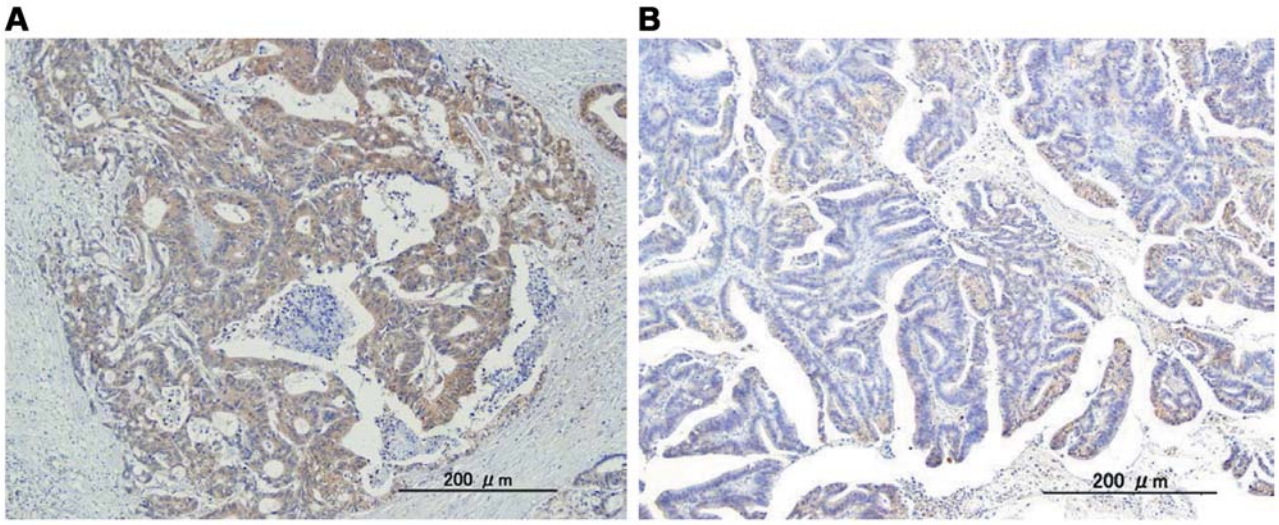

Figure 1. *Immunohistochemical staining for Phospholipase A2 Group III (PLA2GIII) in human colorectal cancer tissues. Diffuse (A) and focal (B) staining of tumor cells (original magnification, ×100).*

Surgically resected specimens were immediately fixed in 10% buffered formalin, and cross-sections of entire cancerous lesions were embedded in paraffin. Conventional pathological diagnosis of the primary lesion and dissected lymph nodes was performed using hematoxylin and eosin (HE)-stained sections.

sPLA2GIII and sPLA2GX IHC staining. IHC analysis was carried out using three 3 μm serial sections. One of the three sections was used for HE staining, and the other sections were used for IHC analysis with antibodies to sPLA2GIII and sPLA2GX. Rabbit polyclonal antibodies to sPLA2GIII and sPLA2GX were generated by immunization of a rabbit with the appropriate polypeptide at the Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan. The specificity and immunoreactivity of the antibodies were verified by immunoblotting with *sPLA2*-transfected cells (9). The sPLA2GIII and sPLA2GX antibodies were used at dilutions of 1:150 and 1:100, respectively.

The streptavidin-biotin immunoperoxidase method using a Histofine SAB-PO(R) kit was utilized (Nichirei, Tokyo, Japan). The sections were deparaffinized with xylene and dehydrated with 98% ethanol, placed in 0.01 M sodium citrate buffer (pH 6.0), and heated in an autoclave for 15 min. After washing twice in phosphate-buffered saline (PBS), endogenous peroxidase activity was inhibited by incubation with 0.3% hydrogen peroxide in methanol for 20 min. The sections were incubated with anti-sPLA2GIII and anti-sPLA2GX overnight at 4˚C, and secondary biotinylated goat anti-rabbit immunoglobulin was applied after washing in PBS. Color development was carried out using diaminobenzidine solution. Sections were then lightly counterstained with a mixture of Mayer's and Lillie-Mayer's hematoxylin, and mounted. For negative controls, the antibody was replaced with PBS.

Evaluation of immunostaining. Staining was independently evaluated by two observers who had received training in pathological diagnosis (S.K. and M.H.), and who were blinded to the clinical findings. Discrepancies between their findings were resolved by discussion. To evaluate protein expression regardless of intensity, results were graded as follows: none, not detected; focal, focally expressed; and diffuse, diffusely expressed. In the statistical analysis, none and focal were considered to be low expression, and diffuse was considered to be high expression.

Statistical analysis. The statistical significance of differences was evaluated using the chi-square, Fisher's exact, or non-paired Student's *t*-test, as appropriate. Overall survival was analyzed using the Kaplan-Meier method and log-rank comparison test. All statistical calculations were carried out using JMP Pro 11.0.0 statistical software (SAS Institute, Cary, NJ, USA). An association was considered significant when the *p*-value was less than 0.05.

Results

sPLA2GIII and sPLA2GX expression. Clinical features of the 142 patients are shown in Table I. The staining patterns of sPLA2GIII and sPLA2GX in cancerous lesions are illustrated in Figure 1. The normal colonic mucosa adjacent to cancerous lesions was almost absent for sPLA2GIII, and ranged from none to weak for sPLA2GX. In the tumoral lesions, the expression of sPLA2GIII and sPLA2GX was predominantly observed in the cytoplasm of neoplastic cells, with enhanced staining intensity compared to the normal mucosa. The stromal tissue showed no sPLA2GIII or sPLA2GX expression. IHC analysis of sPLA2GIII expression revealed that 66 (46.5%), 61 (43.0%), and 15 (10.6%) tumors stained diffusely, focally, and negatively, respectively. Therefore, the numbers of tumors with low and high expression of sPLA2GIII were 76 (53.5%) and 66 (46.5%), respectively. On the contrary, analysis of

Table III. *PLA2 group III expression in left-sided colorectal cancer and clinicopathological features.*

Characteristic		n	PLA2 group III expression		*p*-Value
			High	Low	
Lymphatic involvement	Absent	73	41	37	0.085
	Present	22	7	15	
Venous involvement	Absent	57	30	27	0.29
	Present	43	18	25	
Lymph node metastasis	Absent	60	24	36	0.049
	Present	40	24	16	
Hematogenous metastasis	Absent	93	44	49	0.62
	Present	7	4	3	

Table IV. *PLA2 Group X overexpression in colorectal cancers and clinicopathological features*

Characteristic		n	PLA2 group X expression		*p*-Value
			High	Low	
Mean age (years)±SD		142	62.5±11.6	63.4±10.6	0.64
Gender	Male	85	52	33	0.51
	Female	57	38	19	
Size (mm)±SD		142	47.6±24.5	47.6±18.0	0.99
Location	Colon	103	69	34	0.14
	Rectum	39	21	18	
	Right sided	42	29	13	0.83
	Left sided	100	61	39	
Histological type	Well	96	61	35	0.58
	Moderately	40	24	16	
	Poorly	3	2	1	
	Mucinous	3	3	0	
T Stage*	T1	13	9	4	0.17
	T2	21	16	5	
	T3	97	59	38	
	T4	11	6	5	
Lymphatic involvement	Absent	104	66	38	0.97
	Present	38	24	14	
Venous involvement	Absent	68	45	23	0.5
	Present	74	45	29	
Lymph nodes metastasis	Absent	81	56	25	0.1
	Present	61	34	27	
Hematogenous metastasis	Absent	135	89	46	0.006
	Present	7	1	6	
UICC Stage	I	27	21	6	0.03
	II	53	35	18	
	III	53	32	21	
	IV	9	2	7	

*TMN Classification of malignant tumors (#39).

sPLA2GX expression revealed that 90 (63.4%), 51 (35.9%), and 1 (0.7%) tumor showed diffuse, focal, and negative staining. The numbers of tumors with low and high sPLA2GX expression were 52 (36.6%) and 90 (63.4%), respectively.

Correlation of sPLA2GIII and sPLA2GX expression with clinicopathological features. Age, sex, tumor size, tumor location, histological type, and tumor stage had no significant relationship with the expression of either sPLA2GIII or sPLA2GX. The high expression of sPLA2GIII was associated with a significantly higher rate of lymph node metastasis than the low expression (35/66 *vs*. 26/76; $p=0.02$) (Table II). When the cases were restricted to those with left-sided CRC (100 cases), high sPLA2GIII expression was also associated with a significantly higher rate of lymph node metastasis than low expression (24/48 *vs*. 16/52; $p=0.049$) (Table III). However, there was no correlation between sPLA2GIII expression and hematogenous metastasis or TNM stage. In contrast, statistical analysis revealed an inverse relationship between sPLA2GX expression and hematogenous metastasis (6/52 *vs*. 1/90; $p=0.006$), and TNM stage ($p=0.03$)(Table IV).

Analysis of overall survival associated with sPLA2GIII and sPLA2GX expression in colorectal cancer. Patients with low sPLA2GIII expression had a significantly higher overall survival rate than those with high expression ($p=0.03$) (Figure 2A), whereas no statistically significant association was found between sPLA2GX expression and patient outcome ($p=0.35$) (Figure 2B). To confirm the relevance of the opposing associations of the expression of the two proteins with prognosis, we divided the patients into three sub-groups according to their sPLA2GIII and sPLA2GX expression: sPLA2GIII high/sPLA2GX low, sPLA2GIII low/sPLA2GX high, and others including those with both high and those both low. The sub-group with sPLA2GIII low/sPLA2GX high expression tended to have a longer overall survival, but statistical significance was not reached ($p=0.059$) (Figure 2C). However, analysis of overall survival between the subgroup with low sPLA2GIII and high sPLA2GX expression and the subgroup with high sPLA2GIII and low sPLA2GX expression found that low sPLA2GIII and high sPLA2GX expression was associated with a significantly higher survival rate than the latter ($p=0.038$). Multivariate analysis of significant prognostic factors from univariate analysis was performed (Table V). On multivariate analysis, sPLA2GIII expression was not found to be an independent risk factor for overall survival.

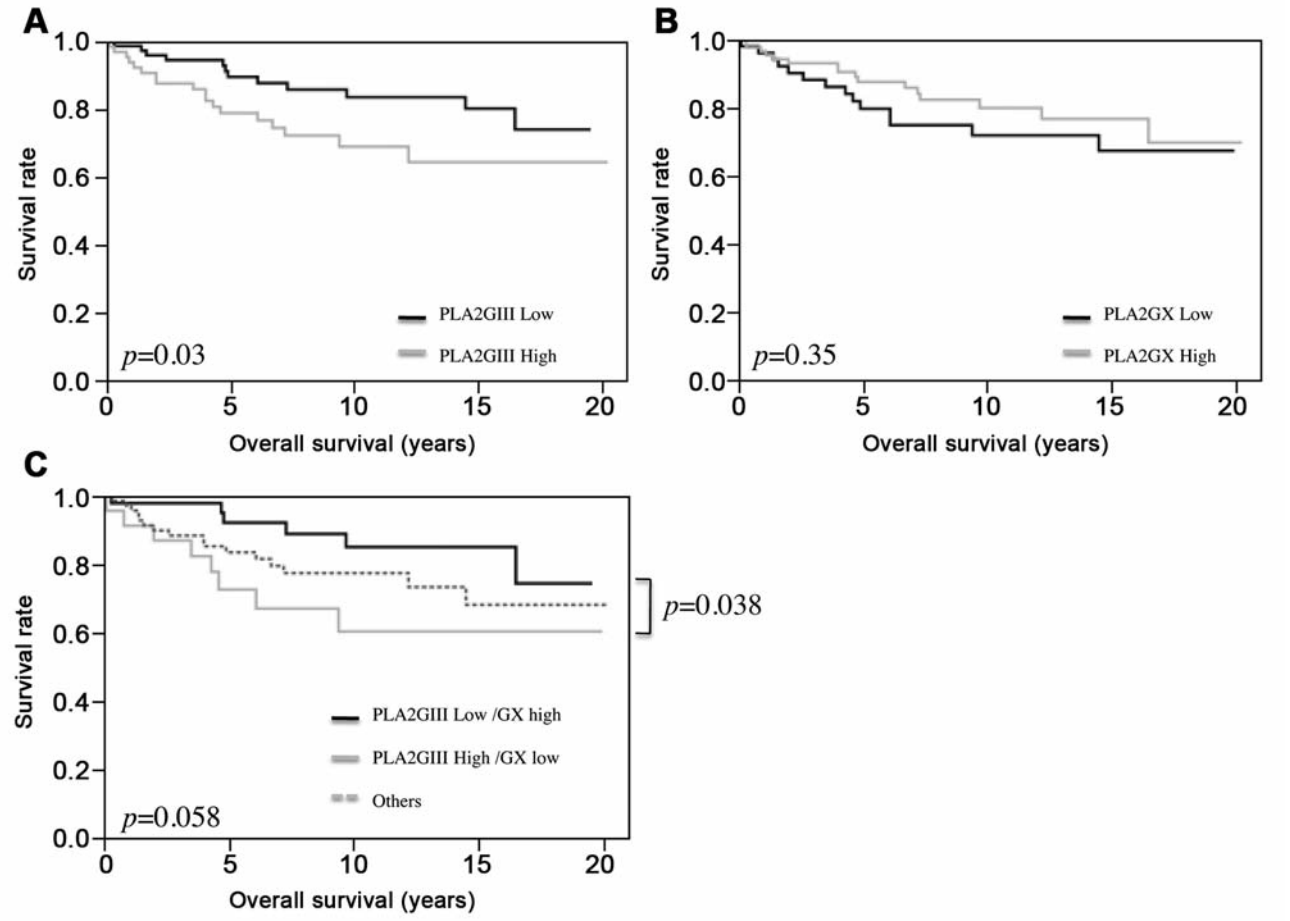

Figure 2. *Association of PLA2GIII (A) and PLA2GX (B) expression alone and combined (C) in colorectal cancer in relation to overall survival (OS).*

Discussion

In the present study, we demonstrated that high sPLA2GIII expression was significantly associated with lymph node metastasis and poorer prognosis in patients with CRC. Recently, the physiological functions of sPLA2s have been elucidated by examination of genetically manipulated mice (14, 15) with airway diseases (16, 17), arthritis (18), myocardial ischemia (19, 20), atherosclerosis (21), obesity (22), microbial defense (23), alopecia (24), reproduction (25), pain (26), and steatohepatitis (27). However, the roles of sPLA2, and in particular, those of their sub-types, have not been fully examined in tumor biology. To our knowledge, this is the first report of the prognostic significance of sPLA2GIII expression in patients with CRC.

We found that the normal mucosa adjacent to cancerous lesions was not stained using sPLA2GIII IHC, however, the cytoplasm of tumor cells was focally or diffusely stained. These results are in accordance with those of a previously reported study by Mounier *et al*., in which the expression of sPLA2GIII was significantly increased by up to 40-fold in tumoral tissue compared with that in normal mucosa at both the mRNA and protein levels (38). In addition, the high expression of sPLA2GIII that we found in 66 (46.5%) cases was significantly correlated with lymph node metastasis and poorer prognosis. High sPLA2 expression has been reported to correlate with prognosis in various types of cancer; overexpression of sPLA2GIIA was reported to be closely associated with malignant potential in breast and prostatic (31, 34), and Graff *et al*. showed that sPLA2GIIA expression was inversely related to 5-year survival in prostatic cancer (33). A significant difference was also found in both disease-free and disease-specific survival between patients with stage II CRC negative for sPLA2GIIA (longer survival) and those positive for sPLA2GIIA (36). These results are consistent with our findings of sPLA2GIII in CRC, suggesting that sPLA2GIII may play an important role in lymph node metastasis. In contrast, statistical analysis showed that sPLA2GX expression was inversely correlated with hematogenous metastasis and tumor grade. These results are

Table V. *Univariate and multivariate analyses of prognostic valuables for overall survival in colorectal cancer.*

	Univariate analysis	Multivariate analysis		
	p-Value	*p*-Value	Relative risk	95% CI
Gender (male *vs*. female)	0.1725			
Age (≥62 *vs*. <62)	0.1249			
Tumor size (≥47 *vs*. <47 mm)	0.0182	0.0194	2.55	1.16-5.97
Tumor location (colon *vs*. rectum)	0.9875			
Tumor location (right side *vs*. left side)	0.1376			
Degree of diff. (well/mod *vs*. poor/muc)	0.3808			
Depth of invasion (T1/2 *vs*. T3/4)	0.0511			
Lymphatic involvement (absent *vs*. present)	0.0045	0.065		
Venous involvement (absent *vs*. present)	0.0255	0.9839		
Regional lymph node metastasis (absent *vs*. present)	0.0161	0.7354		
Hematogenic metastasis (absent *vs*. present)	<0.0001	0.0029	6.64	2.05-18.52
PLA2 group III expression (high *vs*. low)	0.0299	0.1176		
PLA2 group X expression (low *vs*. high)	0.3511			

Diff, Differentiation, PLA2 group III, Phospholipase A2 Group III.

consistent with those of our previous study (37). Therefore, in order to carry out additional analysis, we divided the patients into three sub-groups according to sPLA2GIII and sPLA2GX expression status, and evaluated the overall survival in the three groups. Although significant differences were not detected among the three subgroups, an analysis of two subgroups demonstrated that the subgroup with low sPLA2GIII and high sPLA2GX expression had a significantly higher survival rate than that with high sPLA2GIII and low sPLA2GX expression, indicating that the expressions of sPLA2GIII and sPLA2GX might have opposing associations with prognosis in CRC. To the best of our knowledge, this is the first study showing IHC expression of sPLA2GIII and sPLA2GX in CRC, and its association with tumor metastasis and poor prognosis.

However, caution is required when interpreting our data. The study is limited by the small number of patients examined, and the retrospective nature of the study. Further prospective large-scale studies with long-term follow-up are needed.

In this study, we also evaluated the clinicopathological factors with regard to tumor location, by defining CRC as right- or left-sided. In CRC progression, two forms of genomic instability have been identified: microsatellite instability and chromosomal instability, and right- and left-sided colonic cancer exhibit different molecular profiles (40). Microsatellite instability and a methylator phenotype are prevalent in right-side tumors, and sporadic chromosomal instability tends to occur in left-side tumors (41, 42). In our previous study, we demonstrated that the rate of lymph node metastasis was significantly lower in left-sided CRC, correlating with sPLA2GX expression (37). In this study, we found no significant association of sPLA2GIII expression and tumor location. This result is consistent with the study by Mounier *et al*. that detected sPLA2G3 expression in both right- and left-sided adenocarcinoma (38). Additionally, in left-sided CRC, the rate of lymph node metastasis was significantly higher in the group with highs PLA2GIII expression than in the low-expression group (p=0.049), but no significant differences in overall survival were found (data not shown). These results are consistent with our suggestion that the expression of sPLA2GIII and sPLA2GX might have opposing associations with CRC prognosis.

In conclusion, we demonstrate that high sPLA2GIII expression significantly correlates with lymph node metastasis and a poorer prognosis. Our results also suggest that sPLA2GIII and sPLA2GX might play opposing roles in CRC carcinogenesis. Therefore, sPLA2GIII expression, in addition to sPLA2GX expression, might be useful prognostic markers for CRC, and further investigation of the role of sPLA2GIII expression in CRC is warranted.

References

1 Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D: Global cancer statistics. CA Cancer J Clin *61*: 69-90, 2011.

2 Siegel R, Ward E, Brawley O and Jemal A: Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA Cancer J Clin *61*: 212-236, 2011.

3 Vadas P and Pruzanski W: Role of secretory phospholipases A2 in the pathobiology of disease. Lab Invest *55*: 391-404, 1986.

4 Murakami M, Taketomi Y, Sato H and Yamamoto K: Secreted phospholipase A2 revisited. J Biochem *150*: 233-255, 2011.

5 Morioka Y, Ikeda M, Saiga A, Fujii N, Ishimoto Y, Arita H and Hanasaki K: Potential role of group X secretory phospholipase A(2) in cyclo-oxygenase-2-dependent PGE(2) formation during colon tumorigenesis. FEBS Lett *487*: 262-266, 2000.

6 Krause WF and DuBois RN: The molecular basis for prevention of colorectal cancer. Clin Colorectal Cancer *1*: 47-54, 2001.

7 Laye JP and Gill JH: Phospholipase A2 expression in tumours: A target for therapeutic intervention? Drug Discov Today *8*: 710-716, 2003.

8 Mills GB and Moolenaar WH: The emerging role of lysophosphatidic acid in cancer. Nat Rev Cancer *3*: 582-591, 2003.

9 Murakami M, Masuda S, Shimbara S, Ishikawa Y, Ishii T and Kudo I: Cellular distribution, post-translational modification, and tumorigenic potential of human group III secreted phospholipase A(2). J Biol Chem *280*: 24987-98, 2005.

10 Toomey DP, Murphy JF and Conlon KC: COX2, VEGF and tumour angiogenesis. Surgeon *7*: 174-180, 2009.

11 Murakami M, Taketomi Y, Girard C, Yamamoto K and Lambeau G: Emerging roles of secreted phospholipase A2 enzymes: Lessons from transgenic and knockout mice. Biochimie *92*: 561-582, 2010.

12 Murakami M, Taketomi Y, Miki Y, Sato H, Hirabayashi T and Yamamoto K: Recent progress in phospholipase A(2) research: from cells to animals to humans. Prog Lipid Res *50*: 152-192, 2011.

13 Lambeau G and Gelb MH: Biochemistry and physiology of mammalian secreted phospholipases A2. Annu Rev Biochem *77*: 495-520, 2008.

14 Grass DS, Felkner RH, Chiang MY, Wallace RE, Nevalainen TJ, Bennett CF and Swanson ME: Expression of human group II PLA2 in transgenic mice results in epidermal hyperplasia in the absence of inflammatory infiltrate. J Clin Invest *97*: 2233-2241, 1996.

15 Takaku K, Sonoshita M, Sasaki N, Uozumi N, Doi Y, Shimizu T and Taketo MM: Suppression of intestinal polyposis in Apc(delta 716) knockout mice by an additional mutation in the cytosolic phospholipase A(2) gene. J Biol Chem *275*: 34013-34016, 2000.

16 Masuda S, Murakami M, Mitsuishi M, Komiyama K, Ishikawa Y, Ishii T and Kudo I: Expression of secretory phospholipase A2 enzymes in lungs of humans with pneumonia and their potential prostaglandin-synthetic function in human lung-derived cells. Biochem J *387*: 27-38, 2005.

17 Ohtsuki M, Taketomi Y, Arata S, Masuda S, Ishikawa Y, Ishii T, Takanezawa Y, Aoki J, Arai H, Yamamoto K, Kudo I and Murakami M: Transgenic expression of group V, but not group X, secreted phospholipase A2 in mice leads to neonatal lethality because of lung dysfunction. J Biol Chem *281*: 36420-36433, 2006.

18 Boilard E, Lai Y, Larabee K, Balestrieri B, Ghomashchi F, Fujioka D, Gobezie R, Coblyn JS, Weinblatt ME, Massarotti EM, Thornhill TS, Divangahi M, Remold H, Lambeau G, Gelb MH, Arm JP and Lee DM: A novel anti-inflammatory role for secretory phospholipase A2 in immune complex-mediated arthritis. EMBO Mol Med *2*: 172-187, 2010.

19 Ishikawa Y, Komiyama K, Masuda S, Murakami M, Akasaka Y, Ito K, Akishima-Fukasawa Y, Kimura M, Fujimoto A, Kudo I and Ishii T: Expression of type V secretory phospholipase A in myocardial remodelling after infarction. Histopathology *47*: 257-267, 2005.

20 Fujioka D, Saito Y, Kobayashi T, Yano T, Tezuka H, Ishimoto Y, Suzuki N, Yokota Y, Nakamura T, Obata JE, Kanazawa M, Kawabata K, Hanasaki K and Kugiyama K: Reduction in myocardial ischemia/reperfusion injury in group X secretory phospholipase A2-deficient mice. Circulation *117*: 2977-2985, 2008.

21 Ivandic B, Castellani LW, Wang XP, Qiao JH, Mehrabian M, Navab M, Fogelman AM, Grass DS, Swanson ME, de Beer MC, de Beer F and Lusis AJ: Role of group II secretory phospholipase A2 in atherosclerosis: 1. Increased atherogenesis and altered lipoproteins in transgenic mice expressing group IIa phospholipase A2. Arterioscler Thromb Vasc Biol *19*: 1284-1290, 1999.

22 Li X, Shridas P, Forrest K, Bailey W and Webb NR: Group X secretory phospholipase A2 negatively regulates adipogenesis in murine models. FASEB J *24*: 4313-4324, 2010.

23 Nevalainen TJ, Graham GG and Scott KF: Antibacterial actions of secreted phospholipase A2. Review. Biochim Biophys Acta *1781*: 1-9, 2008.

24 Yamamoto K, Taketomi Y, Isogai Y, Miki Y, Sato H, Masuda S, Nishito Y, Morioka K, Ishimoto Y, Suzuki N, Yokota Y, Hanasaki K, Ishikawa Y, Ishii T, Kobayashi T, Fukami K, Ikeda K, Nakanishi H, Taguchi R and Murakami M: Hair follicular expression and function of group X secreted phospholipase A2 in mouse skin. J Biol Chem *286*: 11616-11631, 2011.

25 Masuda S, Murakami M, Matsumoto S, Eguchi N, Urade Y, Lambeau G, Gelb MH, Ishikawa Y, Ishii T and Kudo I: Localization of various secretory phospholipase A2 enzymes in male reproductive organs. Biochim Biophys Acta *1686*: 61-76, 2004.

26 Sato H, Isogai Y, Masuda S, Taketomi Y, Miki Y, Kamei D, Hara S, Kobayashi T, Ishikawa Y, Ishii T, Ikeda K, Taguchi R, Ishimoto Y, Suzuki N, Yokota Y, Hanasaki K, Suzuki-Yamamoto T, Yamamoto K and Murakami M: Physiological roles of group X-secreted phospholipase A2 in reproduction, gastrointestinal phospholipid digestion, and neuronal function. J Biol Chem *286*: 11632-11648, 2011.

27 Guan M, Qu L, Tan W, Chen L and Wong CW: Hepatocyte nuclear factor-4 alpha regulates liver triglyceride metabolism in part through secreted phospholipase A(2) GXIIB. Hepatology *53*: 458-466, 2011.

28 Leung SY, Chen X, Chu KM, Yuen ST, Mathy J, Ji J, Chan AS, Li R, Law S, Troyanskaya OG, Tu IP, Wong J, So S, Botstein D and Brown PO: Phospholipase A2 group IIA expression in gastric adenocarcinoma is associated with prolonged survival and less frequent metastasis. Proc Natl Acad Sci USA *99*: 16203-16208, 2002.

29 Aggarwal A, Guo DL, Hoshida Y, Yuen ST, Chu KM, So S, Boussioutas A, Chen X, Bowtell D, Aburatani H, Leung SY and Tan P: Topological and functional discovery in a gene coexpression meta-network of gastric cancer. Cancer Res *66*: 232-241, 2006.

30 Xing XF, Li H, Zhong XY, Zhang LH, Wang XH, Liu YQ, Jia SQ, Shi T, Niu ZJ, Peng Y, Du H, Zhang GG, Hu Y, Lu AP, Li JY, Chen S and Ji JF: Phospholipase A2 group IIA expression correlates with prolonged survival in gastric cancer. Histopathology *59*: 198-206, 2011.

31 Yamashita S, Yamashita J and Ogawa M: Overexpression of group II phospholipase A2 in human breast cancer tissues is closely associated with their malignant potency. Br J Cancer *69*: 1166-1170, 1994.

32 Kashiwagi M, Friess H, Uhl W, Berberat P, Abou-Shady M, Martignoni M, Anghelacopoulos SE, Zimmermann A and Buchler MW: Group II and IV phospholipase A(2) are produced in human pancreatic cancer cells and influence prognosis. Gut *45*: 605-612, 1999.

33 Graff JR, Konicek BW, Deddens JA, Chedid M, Hurst BM, Colligan B, Neubauer BL, Carter HW and Carter JH: Expression of group IIa secretory phospholipase A2 increases with prostate tumor grade. Clin Cancer Res *7*: 3857-3861, 2001.

34 Jiang J, Neubauer BL, Graff JR, Chedid M, Thomas JE, Roehm NW, Zhang S, Eckert GJ, Koch MO, Eble JN and Cheng L: Expression of group IIA secretory phospholipase A2 is elevated in prostatic intraepithelial neoplasia and adenocarcinoma. Am J Pathol *160*: 667-671, 2002.

35 Sved P, Scott KF, McLeod D, King NJ, Singh J, Tsatralis T, Nikolov B, Boulas J, Nallan L, Gelb MH, Sajinovic M, Graham GG, Russell PJ and Dong Q: Oncogenic action of secreted phospholipase A2 in prostate cancer. Cancer Res *64*: 6934-6940, 2004.

36 Buhmeida A, Bendardaf R, Hilska M, Laine J, Collan Y, Laato M, Syrjanen K and Pyrhonen S: PLA2 (group IIA phospholipase A2) as a prognostic determinant in stage II colorectal carcinoma. Ann Oncol *20*: 1230-1235, 2009.

37 Hiyoshi M, Kitayama J, Kazama S, Taketomi Y, Murakami M, Tsuno NH, Hongo K, Kaneko M, Sunami E and Watanabe T: The expression of phospholipase A2 group X is inversely associated with metastasis in colorectal cancer. Oncol Lett *5*: 533-538, 2013.

38 Mounier CM, Wendum D, Greenspan E, Flejou JF, Rosenberg DW and Lambeau G: Distinct expression pattern of the full set of secreted phospholipases A2 in human colorectal adenocarcinomas: sPLA2-III as a biomarker candidate. Br J Cancer *98*: 587-595, 2008.

39 Sobin LH, Gospodarowicz MK and Wittekind C: TNM Classification of Malignant Tumours (7th edn). Wiley-Blackwell: Chichester, 2009.

40 Bhalla A, Zulfiqar M, Weindel M and Shidham VB: Molecular diagnostics in colorectal carcinoma. Clin Lab Med *33*: 835-859, 2013.

41 Iacopetta B: Are there two sides to colorectal cancer? Int J Cancer *101*: 403-408, 2002.

42 Birkenkamp-Demtroder K, Olesen SH, Sørensen FB, Laurberg S, Laiho P, Aaltonen LA and Orntoft TF: Differential gene expression in colon cancer of the caecum versus the sigmoid and rectosigmoid. Gut *54*: 374-384, 2005.

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