Int J Clin Exp Pathol 2016;9(11):11820-11825
www.ijcep.com /ISSN:1936-2625/IJCEP0039217

Original Article

Expression of phospholipase A2 in breast cancer tissues and its significance

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Received August 31, 2016; Accepted September 24, 2016; Epub November 1, 2016; Published November 15, 2016

Abstract: Objectives: The relationship between the expression of secretory phospholipase A2 (sPLA2-II a) and cytosolic phospholipase A2 (cPLA2) and the onset, development, infiltration and metastasis of breast cancer was investigated. Methods: The protein expression of sPLA2-II a and cPLA2 in 57 cases of breast infiltrating ductal carcinoma tissues, 15 cases of breast fibroadenoma tissues and 15 cases of normal breast tissues was detected using immunohistochemical SP method. Results: The positive expression rates of sPLA2-II a (75.4%) and cPLA2 (68.4%) in breast infiltrating ductal carcinoma tissues were both significantly higher than those in breast fibroadenoma tissues (20.0% and 26.7%) and normal breast tissues (13.3% and 13.3%), both showing statistically significant difference (χ^2=27.585 and 18.989, P<0.05); the positive expression rates of sPLA2-II a and cPLA2 in breast infiltrating ductal carcinoma tissues were closely related to tumor clinical staging, histological grading and lymph node metastasis, all showing statistically significant difference (P<0.05); the protein expressions of sPLA2-II a and cPLA2 were in positive correlation with each other (γp=0.401, P<0.05). Conclusions: sPLA2-II a and cPLA2 play important roles in the infiltration, metastasis and mucous epithelium canceration of breast infiltrating ductal carcinoma. The joint detection of both sPLA2-II a and cPLA2 can be anticipated to be one of the molecular markers for early diagnosis and prognosis estimation of breast infiltrating ductal carcinoma.

Keywords: sPLA2-II a, cPLA2, breast infiltrating ductal carcinoma, immunohistochemistry, infiltration and metastasis

Introduction

Phospholipase A2 (cPLA2) is known as an enzyme family with glyceryl phosphatide degrading activity, which widely exists in multiple tissues, cells and secretes of bacteria, plants and mammals, and they are mostly located on animal cell and mitochondrial membrane [1]. The previous studies show that PLA2 can synthesize substances with different biological activities and play a pivotal role in the metabolism and substitution of phospholipase. Besides functioning as inflammatory mediators, PLA2 and its metabolites also engage in the regulation of cell growth and proliferation, showing a close relationship with malignancy [2]. In this study, the protein expression of sPLA2-II a and cPLA2 in 57 cases of breast infiltrating ductal carcinoma tissues, 15 cases of breast fibroadenoma tissues and 15 cases of normal breast tissues was detected using immunohistochemical SP method, and their role in the onset and development of breast infiltrating ductal carcinoma was investigated in hope of exploring the molecular markers for early diagnosis and prognosis estimation of breast infiltrating ductal carcinoma.

Materials and methods

Tissue sources

All the 57 cases of breast cancer tissues and 15 cases of breast fibroadenoma tissues were fresh samples cut in surgery in the First Affiliated Hospital of Zhengzhou University from March 2007 to February 2009. And 15 cases of normal breast tissues were collected from samples of breast adenosis or mastitis excision as the controls. All cases had no history of chemo-

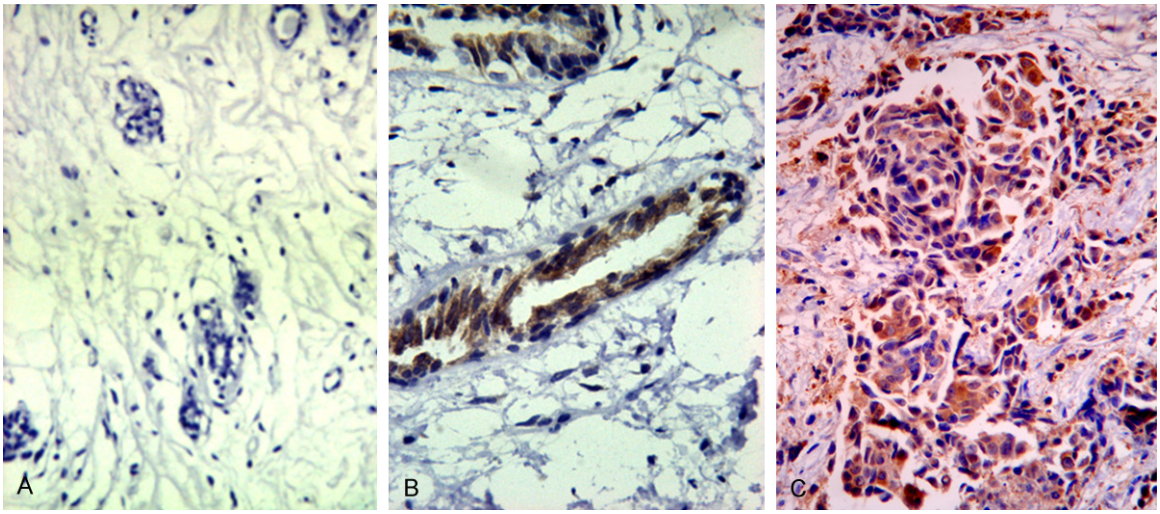

Figure 1. Expression of sPLA2-II a in normal breast tissues (A), breast fibroadenoma (B) and breast cancer tissues (C) SP×200.

therapy, radiotherapy and immunotherapy. All samples were confirmed as breast infiltrating ductal carcinoma, breast fibroadenoma and normal breast tissues by histopathological method. All samples were prepared in duplicate, one cryopreserved in liquid nitrogen for RT-PCR and the other fixed in 40 g/L paraformaldehyde for routine pathological sectioning and immunohistochemical detection. All patients were female, aged 29 to 87 years with an average of 50.34±11.25 years. Histological grading: nine cases were categorized as grade I, 34 cases as grade II and 14 cases as grade III; clinical staging: 21 cases were categorized as stage I, 23 cases as stage II and 13 cases as stage III; 21 cases were categorized in lymph-node-metastasis group in which tumor metastasis was detected in the lymph nodes adjacent to the breast cancer tissue excised in surgery, and 36 cases were categorized in no-lymph-node-metastasis group in which no tumor metastasis was detected in the adjacent lymph nodes where excision had been carried out; 23 cases had a tumor diameter of no longer than 2 cm, 27 cases 2~5 cm and seven cases no shorter than 5 cm.

Main reagents

Rabbit anti-human sPLA2-II a and cPLA2 PcAb were purchased from Abcam, UK. S-P immunohistochemical kit was purchased from Beijing Zhongshan Golden Bridge Biotech Co., Ltd.

Immunohistochemical staining

Using immunohistochemical S-P method, the tissue sections were incubated with anti-sPLA2-II a antibody (diluted at 1:200) and anti-cPLA2 antibody (diluted at 1:180) respectively, followed by DAB developing and hematoxylin counter-staining. The staining procedure was performed strictly according to the manual. The positively-stained gastric cancer sections were used as the positive controls, and the sections incubated with PBS in substitution for the first antibody were used as the negative controls.

Result evaluation of immunohistochemical staining

The positive signals of both sPLA2-II a and cPLA2 were reported by the presence of yellow granular substance in cytoplasm. Five visual fields were randomly selected under the high power lens (no less than 200 cells were observed in each visual field), and the result evaluation was carried out based on the percentage of positive cells and dying intensity in a nine-score system. The positive-cell percentage no higher than 10% scored 1 point, 10~50% scored 2 points and over 50% scored 3 points; negative staining scored 0 point, light-yellow staining scored 1 point, moderate-yellow staining scored 2 points and brown staining scored 3 points. The total score was calculated by positive-cell percentage score x staining intensity score. The total score of less than 3

Table 1. Expression of sPLA2-II a and cPLA2 in breast infiltrating ductal carcinoma tissues, breast fibroadenoma tissues and normal breast tissues

	sPLA2-II a				χ^2	P	cPLA2			χ^2	P
	n	–	+	Positive rate (%)			–	+	Positive rate (%)		
Breast infiltrating ductal carcinoma	57	14	43	75.4	27.585	0.000	18	39	68.4	18.989	0.000
Breast fibroadenoma	15	12	3	20.0			11	4	26.7		
Normal breast tissues	15	13	2	13.3			13	2	13.3		

Table 2. Relationship between the expression of sPLA2-II a and cPLA2 and the clinical biological behavior of breast infiltrating ductal carcinoma

Pathological features	n	sPLA2-II a			cPLA2		
		Cases of positive expression (%)	χ^2	P	Cases of positive expression (%)	χ^2	P
Clinical staging							
I	21	12 (57.1)	6.428	0.040	9 (42.9)	10.072	0.006
II	23	19 (82.6)			19 (82.6)		
III	13	12 (92.3)			11 (84.6)		
Histological grading							
I	9	2 (22.2)	16.336	0.000	3 (33.3)	7.140	0.028
II	34	29 (85.3)			24 (70.6)		
III	14	12 (85.7)			12 (85.7)		
Lymph node metastasis							
No	21	12 (57.1)	6.007	0.014	11 (52.4)	3.959	0.047
Yes	36	31 (86.1)			28 (77.8)		

points indicated negative and no less than 3 points indicated positive [3, 4].

Statistics

SPSS 13.0 software was adopted for χ^2 test and spearman correlation coefficient analysis, with the significant level set as α=0.05.

Results

Expression of sPLA2-II a protein in breast infiltrating ductal carcinoma and its relationship with clinical biological behavior

The positive expression of sPLA2-II a protein was primarily located in the cytoplasm of tumor cells, presented as light-to-deep-yellow granular substance (**Figure 1**). In the 57 cases of breast cancer tissues, 14 cases showed negative sPLA2-II a expression with another 43 showing positive results, with a positive expression rate of 75.4% (43/57); in the 15 cases of breast fibroadenoma tissues, 12 cases were negative with the remaining three positive, with a positive expression rate of 20.0% (3/15); in the 15 cases of normal breast tissues, 13 cases were negative with the remaining two positive, with a positive expression rate of 13.3% (2/15). The intergroup comparison of sPLA2-II a expression among breast cancer group, breast fibroadenoma group and normal breast tissue group showed statistically significant difference (χ^2=27.585, P<0.05) (**Table 1**). The expression of sPLA2-II a protein was closely related to the clinical staging, histological grading and lymph node metastasis of breast infiltrating ductal carcinoma (P<0.05) (**Table 2**).

Expression of cPLA2 protein in breast infiltrating ductal carcinoma and its relationship with clinical biological behavior

The positive expression of cPLA2 protein was primarily located in the cytoplasm of tumor cells, featuring brown or deep-yellow granular substance (**Figure 2**). In the 57 cases of breast cancer tissues, 18 cases showed negative cPLA2 expression with another 39 showing positive results, with a positive expression rate of 68.4% (39/57); in the 15 cases of breast

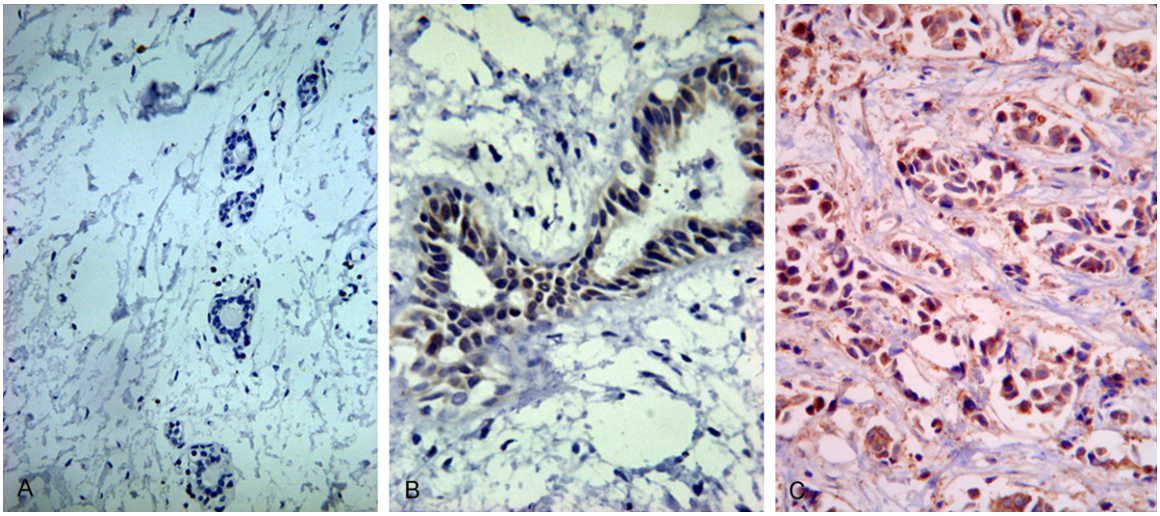

Figure 2. Expression of cPLA2 in normal breast tissues (A), breast fibroadenoma (B) and breast cancer tissues (C) SP×200.

Table 3. Correlation analysis on the protein expression of sPLA2-II a and cPLA2 in breast infiltrating ductal carcinoma tissues

sPLA2-II a protein expression	n	cPLA2 protein expression		γp	P
		+	–		
+	43	34	9	0.401	0.002
–	14	5	9		

fibroadenoma tissues, 11 cases were negative with the remaining four positive, with a positive expression rate of 26.7% (4/15); in the 15 cases of normal breast tissues, 13 cases were negative with the remaining two positive, with a positive expression rate of 13.3% (2/15). The intergroup comparison of cPLA2 expression among breast cancer group, breast fibroadenoma group and normal breast tissue group showed statistically significant difference (χ^2= 18.989, P<0.05) (**Table 1**). The expression of cPLA2 protein was closely related to the clinical staging, histological grading and lymph node metastasis of breast infiltrating ductal carcinoma (P<0.05) (**Table 2**).

Correlation analysis on the protein expression of sPLA2-II a and cPLA2

In the 43 sPLA2-II a-positive cases, there were 34 cases simultaneously presenting positive cPLA2 expression, while in the 39 cPLA2-negative cases, there were nine cases negatively showing sPLA2-II a expression. The protein expressions of sPLA2-II a and cPLA2 in breast infiltrating ductal carcinoma tissues were in positive correlation with each other (γp=0.401, P<0.05) (**Table 3**).

Discussion

Breast cancer ranks among the most common malignancies of the genital system that seriously jeopardizes women's health. With the improvement of people's living standard as well as social and economic development, its incidence has been on the annual rise with the affected population tending to be younger. In western countries and most part of China, the incidence of breast cancer has exceeded over other malignancies that haunt females. According to incomplete statistics, more than 1.2 million new-onset breast cancer patients emerge annually across the world, and approximately 400,000 females die of this disease each year, which has overwhelmingly impaired women's well-being and meanwhile done considerable harm to families and society. For a long period, how to hunt for the potential treatment targets of breast infiltrating ductal carcinoma on the molecular level has triggered substantial concern. In this study, the immunohistochemical technology system was adopted to investigate the varying patterns of sPLA2-II a and cPLA2 in normal breast tissues, breast fibroadenoma tissues and breast infiltrating ductal carcinoma tissues and their rela-

tionship with the clinical biological behavior of breast infiltrating ductal carcinoma.

Phospholipase A2 can be divided into three subtypes based on the site, homology of amino acid sequences and biochemical functions: secretory phospholipase A2 (sPLA2) and cytosolic phospholipase A2 (cPLA2) and Ca^{2+}-independent phospholipase A2 (iPLA2). sPLA2 includes sPLA2-I, II a, II b, III, IV, V, IX, X, etc., which reside in granules of cytoplasm or can be synthesized and then released extracellularly when cells receive certain stimuli, and they have no selective specificity to fatty acid at phospholipid Sn-2 site. cPLA2 extensively exists in various tissues and cells in vivo, the activation of which requires the participation of Ca^{2+} and the phosphorylation of kinase upstream, and they can preferentially hydrolyze phospholipid Sn-2 site to release AA in a selective manner. Both sPLA2 and cPLA2 display high sensitivity to Ca^{2+}. iPLA2 includes iPLA2-VI, VII and VIII, which are not of selective priority to AA, and besides, Ca^{2+} is not necessary in maintaining catalytic activity. iPLA2-VI was once believed to engage in the reestablishment of cytomembrane, and it was also considered to play a role in the release of AA after cells were stimulated [5]. Different PLA2 feature different structures and functions but also resemble in many physiological and biochemical aspects. PLA2 features thermostability, which is justified by its unaffected activity when placed at 65°C for 75 min or 90°C for 30 min, and high water-solubility. PLA2 is much more capable of hydrolyzing substrates in aggregation state (such as lipid micelles, single-layer and double-layer membrane lipid) rather than in dispersion state, and namely it primarily catalyzes heterogeneous reactions and its catalytic activity can be affected by the molecular arrangement of substrates. PLA2 can be activated by various physical, chemical and biological factors, such as mechanical damage, endotoxin, angiotensin II, prolactin, bradykinin, thrombin, oxygen radicals, antigens, etc. And some cytokines can also activate PLA2 such as IL-1, TNF, IFN and PAF.

sPLA2-II a widely resides in tissues and cells of mammals but has highly varying catalytic activity [6]. The enzyme activity of sPLA2-II a in the extracts of human tissue samples was measured by radiochemical method and the result showed that digestive tract, cartilage, parotid gland and prostate abounded in sPLA2-II a, in contrast with skeleton, skeletal muscle, heart, brain, kidney, liver, lung, spleen, pancreas, placenta and amnion where sPLA2-II a exerted low activity [7]. Study shows that sPLA2-II a can be activated by various physical, chemical and biological factors as well as certain cytokines. sPLA2-II a can hydrolyze the acyl-bond at Sn-2 site of membrane phospholipid, and then lysophosphatide and unsaturated fatty acid can be generated via epoxidase and lipoxygenase pathway, which can function as the downstream effectors of signal transduction as well as inflammatory mediators of lipid [8] that participate in a series of physiological and pathological processes. Extensively distributed in multiple human tissues, cPLA2 has a more consistent content compared with sPLA2-II a, though lung and hippocampal tissues show a slightly higher cPLA2 level. Likewise, the activation of cPLA2 can be regulated by various physical, chemical and biological factors as well as certain cytokines. And it can also be activated when Ca^{2+} is lacking. After activation, cPLA2 hydrolyzes phospholipid to generate arachidonic acid and other active substances which trigger inflammatory responses and then contribute to multiple diseases [9].

The results of this study showed that the expression of sPLA2-II a and cPLA2 in breast cancer tissues was significantly higher than that of normal controls, which indicated that the gene activation of sPLA2-II a and cPLA2 was related to the onset and development of breast cancer, and that sPLA2-II a and cPLA2 participated in the pathogenesis of breast cancer and may play an essential role. Based on our results, the expression of sPLA2-II a and cPLA2 in breast cancer tissues varies with the difference in tissue differentiation and clinical staging (the expression increased as the differentiation degraded); lymph-node-metastasis-positive group showed remarkably higher expression of sPLA2-II a and cPLA2 than the negative controls, indicating a correlation between sPLA2-II a and cPLA2 and the malignant degree of breast cancer. Both the malignant degree and the expression of sPLA2-II a and cPLA2 increased as the differentiation level of tissues lowered, which verified that sPLA2-II a and cPLA2 genes were related to cell differentiation and oncogenesis during the

development of breast cancer; furthermore, tumor prognosis can be affected by the differentiation degree of tumor, which suggested that sPLA2-II a and cPLA2 were associated with the prognosis and biological behavior of tumor. The author believed that sPLA2-II a and cPLA2 could be used as indicators to evaluate the infiltration behavior and prognosis of breast cancer. Besides, different types of breast cancer tissues possessed varying expression levels of sPLA2-II a and cPLA2, with breast infiltrating ductal carcinoma exceeding over the others, which indicated that the expression levels of sPLA2-II a and cPLA2 in tumor tissues could be of great significance to the clinical diagnosis and classification of breast cancer. However, the molecular mechanisms of their activities and their application potential remain to be further studied.

In conclusion, in-depth study on sPLA2-II a and cPLA2 genes can help us further understand the biological features of breast infiltrating ductal carcinoma and provide new perspectives into early diagnosis and treatment of this disease.

Acknowledgements

This work was supported by the National High-tech R&D Program of China (863 Program No. 2015AA020301), National Science Foundation of China (NSFC NO. 81372677) and Henan Province foundation and front engineering research project (No. 132300410073).

Disclosure of conflict of interest

None.

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