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The Pattern of Expression of Phospholipase C-γ1 in Benign and Malignant Tumors of the Breast

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유방의 양성종양과 악성종양에서 Phospholipase C-γ1의 발현양상

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Purpose: The activation of phospholipase C (PLC) is one of the early events in various growth processes, including malignant transformation. Among PLC-isozymes, PLC-γ1 is activated through direct interaction with growth factor receptor tyrosine kinase. In this study, we evaluated the patterns of PLC-γ1 expression in benign and malignant tumors of the breast and compared the patterns with their normal counterpart.

Methods: Using immunoblot assay, we evaluated the patterns of expression in PLC-γ1 in 20 breast cancer tissues, 13 fibroadenoma tissues, and normal tissues. The level of expression was analyzed by densitometry.

Results: All of 20 breast cancer tissues and 13 fibroadenoma tissues showed overexpression of PLC-γ1 when compared with their normal counterparts. The level of the PLC-γ1 expression was 3.9-fold and 17.3-fold higher in fibroadenomas and breastcancers, respectively, than in normal tissues.

Conclusion: The result suggested that the level of PLC-γ1 expression increases as the normal tissue undergoes progression to fibroadenoma, and finally to carcinoma. The pattern of expression of PLC-γ1 in breast tissue implies that the PLC-γ1-mediated signal transduction may play a significant role in the progression of breast cancer from normal tissue. **(J Korean Surg Soc 2002;62:463-467)**

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중심 단어: Phospholipase C-γ1, 섬유선종, 유방암

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INTRODUCTION

Phospholipase C (PLC) plays a central role in transmembrane signal transduction pathway by hydrolyzing phosphatidyl inositol 4,5-bisphosphate to yield two second messenger molecules, inositol 1,4,5-triphosphate [Ins(1,4,5)P3] and diacylglycerol (DAG). Ins(1,4,5)P3 and DAG are well-known second messenger molecules, that is, Ins(1,4,5)P3 induces the release of calcium ion from intracellular stores and DAG is the physiologic activator of protein kinase C. (*1*) The activated PLC related to various growth factor receptors has a strong association with cellular signal transduction system that promotes tumor development. (*2*) So far, 10 mammalian PLC-isozymes have been characterized at the cDNA level; they can be subdivided into three types (PLC-β, PLC-γ, and PLC-δ) (*2,3*) on the basis of the relative locations of the X- and Y-domains in the primary structure. (*4,5*) The β type includes four PLCs (PLC-β1 through PLC-β4), the type includes two PLCs (PLC-γ1 and PLC-γ2), and type includes four enzymes (PLC-δ1 through PLC-δ4). (*4-6*) Among these, the two -type PLCs, PLC-γ1 and -γ2, but no β- or δ-type isozymes, are activated by phosphorylation through growth factor receptor tyrosine kinases or non-receptor tyrosine kinases. While PLC-γ1 shows a ubiquitous expression pattern, PLC-γ2 is mainly expressed in B-cells. (*7*)

The fact that PLC-γ1 is overexpressed in the benign proliferative disease, (*8*) familial adenomatous polyposis (*9*) and malignant tumors such as colorectal carcinoma (*10*) can support the hypothesis that PLC-γ1 is related to the cellular proliferation and malignant transformation. Based on the fact that the molecule that activates the PLC can promote the development of tumor, we can anticipate that the continuing activation of PLC may induce the development of tumor.

In 1998, the authors could reveal the overexpression of PLC-γ1 in 17 cases of human breast cancer tissues by immunoblot. (*11*) And we hypothesized that the PLC-γ1 overexpression might be a pathogenic trigger involved in the breast cancer. Now, this study aims to evaluate the pattern of expression of PLC-γ1 in benign and malignant tumors of the breast and to compare it with its normal counterpart.

METHODS

1) Materials

Breast tissues were obtained from 20 patients with breast cancer and 13 patients with fibroadenoma who underwent operation at Seoul National University Hospital, Seoul, Korea. The clinicopathologic features of the breast cancer patients are summarized in Table 1. The age of the breast cancer patients are ranged from 30 to 71 years with an average of 46.6 years. Most of them (16 of 20 cases) were operated by modified radical mastectomy and the others were operated by radical mastectomy, simple mastectomy, wide excision or quadrantectomy with axillary dissection. The TNM stages by the AJCC-UICC system were I in 2 cases, IIa in 8 cases, IIb in 5 cases and IIIa in 4 cases. Of breast cancer cases, 16 cases were infiltrative ductal carcinomas, 2 cases were microinvasive carcinomas, 1 case was a metaplastic carcinoma and 1 case was a malignant phyllodes tumor.

2) Immunoblot analysis

Tissues were obtained from surgical specimens, that is, breast cancer tissue and normal breast tissue were sampled from the same surgical specimen. Breast cancer tissues and their normal counterparts were homogenized with ice-cold lysis buffer (20

Table 1. Clinicopathologic findings and ratios of densitometry of patient with breast cancer

No.	Age	T	N	Node (+)	Stage	Hist.	Op	Ratio by densitometry (cancer/normal tissues)
1	44	3	0	0	IIb	IDC	MRM	13.2
2	43	2	0	0	IIa	IDC	MRM	19.1
3	47	2	1	3	IIb	IDC	MRM	16.9
4	40	2	0	0	IIa	Metp	MRM	13.8
5	55	2	0	0	IIa	IDC	MRM	16.0
6	41	3	2	5	IIIa	IDC	RM	15.6
7	57	2	0	0	IIa	IDC	MRM	17.9
8	30	2	1	1	IIb	IDC	MRM	16.7
9	58	2	2	19	IIIa	IDC	MRM	12.8
10	41	3	1	6	IIIa	IDC	MRM	13.6
11	39	1	0	0	I	MIC	SM	17.2
12	36	2	1	1	IIb	IDC	MRM	19.4
13	71	2	0	0	IIa	IDC	MRM	25.6
14	46	3	1	6	IIIa	IDC	MRM	30.2
15	42	2	0	0	IIa	IDC	MRM	29.1
16	47	2	0	0	IIa	IDC	MRM	9.9
17	30	2	1	10	IIb	IDC	MRM	12.3
18	58	2	0	0	IIa	IDC	MRM	12.2
19	55	1	0	0	I	MIC	QA	16.5
20	51	2	–	–	–	PT	WE	18.8

No. = number of patients; Node (+) = number of metastatic axillary lymph node; Hist. = histology; Op. = name of operation; IDC = infiltrating ductal carcinoma; MRM = modified radical mastectomy; Metp = metaplastic carcinoma; RM = radical mastectomy; MIC = microinvasive carcinoma; SM = simple mastectomy; PT = malignant phyllodes tumor; QA = quadrantectomy with axillary dissection; WE = wide excision.

mM HEPES, pH 7.2, 150 mM sodium chloride, 1% Triton X-100, 1 mM EDTA, 1 mM FGTA, 10 μg/ml leupeptin, 10 μg/ml aprotinine, 0.1 mM DTT and 1 mM phenylmethylsulfonyl fluoride). For immunoblot analysis, extracted proteins of 10 μg were denatured by heating at 95°C for 10 minutes with Laemmli cooking buffer and separated by 10% SDS-polyacrylamide gel electrophoresis. The resolved protein bands were transferred onto a nitrocellulose filter and blocking was performed in Tris-buffered saline containing 5% skimmed milk powder and 0.2% Tween 20. Membranes were probed with anti-PLC antibody (F7-2). The blotted membrane was then incubated using horseradish peroxidase-linked secondary antibody. Detection was performed with the ECL system.

Fibroadenoma tissues and their normal counterparts were processed by the same procedure.

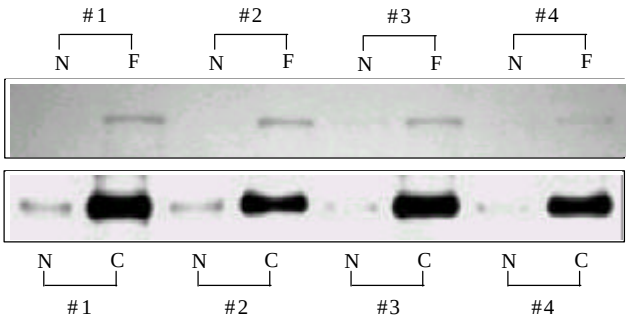

Fig. 1. Results of PLC-γ1 expression on Western blot. Lanes: N, normal breast tissue; F, fibroadenoma tissues; C, cancer tissue: 300 μg of whole cell extracts were separated by 8% SDS-polyacrylamide gel electrophoresis, transferred on a nitrocellulose membrane, and probed by monoclonal antibody (B-16-5).

RESULTS

1) The pattern of expression of PLC-γ1 in benign and malignant breast tissues investigated by Western blotting (Fig. 1)

Fig. 1 shows the expression of PLC-γ1 by immunoblot analysis. Whereas PLC-γ1 was vaguely detected in normal tissues, it was apparently expressed in fibroadenomas and breast cancer tissues. All of 20 breast cancer tissues showed overexpression of PLC-γ1 compared with normal breast tissues. The Western blotting on 13 fibroadenoma tissues also demonstrated overexpression of PLC-γ1 compared with normal breast tissues.

2) The densitometry of PLC-γ1 expression in normal, benign and malignant breast tissues

The level of PLC-γ1 expression was analyzed by densitometry and the results are shown in Table 1 and Fig. 2. The levels of PLC-γ1 in both breast cancer and fibroadenoma tissues were higher than their normal counterparts. The ratio of PLC-γ1 expression by densitometry in the breast cancer tissues to their normal counterpart ranged from 9.9 to 29.1, with an average about 17.3±5.4. In fibroadenomas, the ratio was lower than that of the breast cancer, and ranged from 1.7 to 6.1, with an average of 3.9±1.3.

Fig. 3 shows that the ratio in the breast cancer tissues was substantially higher than that in fibroadenoma tissues, suggesting that the level of PLC-γ1 increases as the normal tissue undergoes progression to fibroadenoma and to carcinoma and

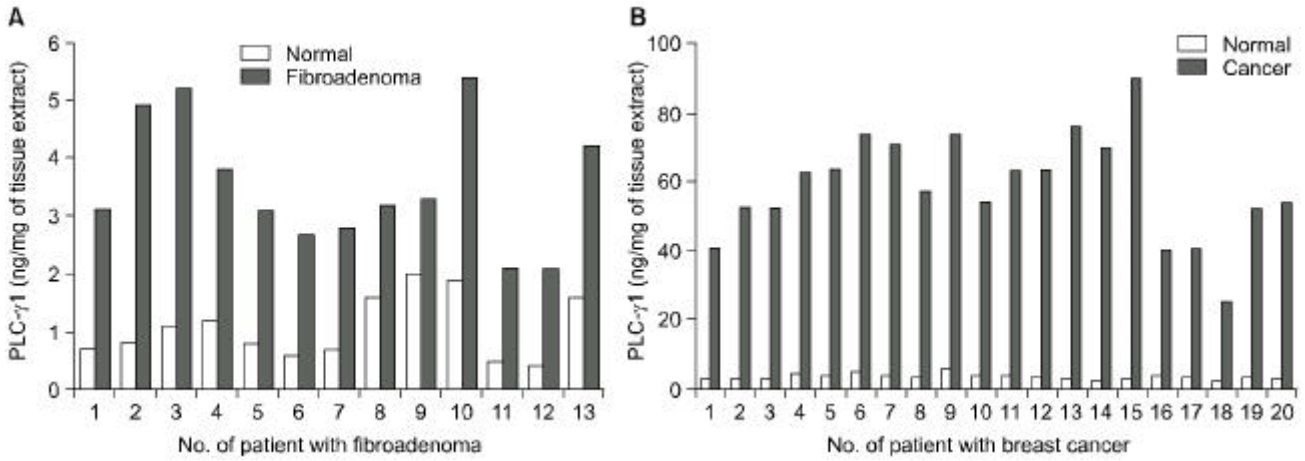

Fig. 2. Quantitation of PLC-γ1 by densitometry. (A) Comparison between fibroadenoma tissue and normal counterparts, (B) Comparison between breast cancer tissue and normal counterparts.

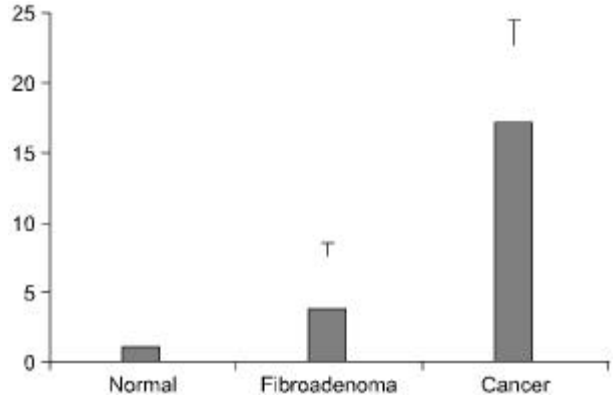

Fig. 3. Comparison of the level of PLC-γ1 expression determined by quantitative Western blot analysis with densitometry in normal, fibroadenoma and breast cancer tissues. The ratio (mean±s.d.) of the levels in fibroadenoma and breast cancer tissues to that in normal tissues were 3.9± 1.3 and 17.3± 1, respectively.

also indicates that neoplastic transformation of breast tissue is accompanied by an increase of the expression of PLC-γ1 in both benign and malignant tumors.

DISCUSSION

PLC-γ1 has been known to play a significant role in the malignant transformation through the signal transduction system. It has been reported that the expression level of PLC-γ1 was increased in various cancer tissues, including colorectal cancer, (*10*) breast cancer etc. (*8,12*) PLC-γ1 was also reported to be overexpressed in proliferative tissues (*8*) compared with normal tissues. The aim of this study was to evaluate the PLC-γ1 expressions in benign and malignant tumor tissues of the breast in comparison with that in their normal counterparts. In this study, we observed the overexpression of PLC-γ1 not only in the breast cancer tissues but also in the benign breast tumor tissues, that is, fibroadenoma tissues, although the level of PLC-γ1 expression was revealed to be higher in the former than in the latter (17.3 times vs 3.9 times). In other words, there was a progressive increase of the expression of PLC-γ1 from normal breast tissue to benign breast tumor tissue and to breast cancer tissue. Although fibroadenoma is not a premalignant lesion, but such a progressive increase of the expression of PLC-γ1 implies that PLC-γ1 may play a role in the proliferative disease in the breast.

Among multiple PLC-isozymes, only the PLC-γ form contains the Src-homology domains (SH domain) (two SH2 domains and one SH3 domain) that are also found in a number of proteins involved in the regulation of cell proliferation and differentiation. (*13*) The SH2 domains of PLC-γ1 are known to mediate the association between PLC-γ1 and phosphorylated tyrosines in the activated receptor tyrosine kinase or the src tyrosine kinase. The SH3 domain of PLC-γ1 is known to be responsible for the mitogenic effect of PLC-γ1. (*7*) Such a structural feature of PLC-γ1 is thought to be responsible for cell proliferation and maybe malignant transformation.

In 1999, Kassis et al. claimed that the motility-associated PLC-γ1 signaling pathway was a generalizable rate-limiting step for tumor cell progression. (*14*) Not only for breast carcinomas, PLC-γ1 is an important enzyme involved in the carcinogenesis and tumor progression for general malignant transformation. Furthermore, the reports that PLC-γ1 is important for motility signaling make us hypothesize that the overexpression of PLC-γ1 implies more invasiveness, therefore, more aggressiveness. We observed a progressive increase of the PLC-γ1 expression from normal breast tissue to fibroadenoma, and further to cacinoma, however no significant relationship between the value of PLC-γ1 and TNM stage, hormonal receptor, nuclear and histologic grades and other prognostic factors (i.e., p53, c-erbB-2, and bcl-2) in the breast cancer tissues (data not shown).

Among the implications of PLC-γ1, the potential of the molecule as a target of cancer therapy has been reported. Several studies (*14,15*) showed that the inhibition of PLC-γ1 could reduce cellular motility and invasion.

In conclusion, our study revealed a progressive increase of PLC-γ1 expression from benign to malignant tumor of the breast when compared with their normal counterpart. The pattern of PLC-γ1 expression in breast tissue implies that PLC-γ1-mediated signal transduction may play a significant role in the progression of normal tissue to breast cancer.

REFERENCES

1) Berridge MJ, Irvine RF. Inositol trisphosphate, a novel second messenger in cellular signal transduction. Nature 1984;312: 315-21.
2) Rhee SG, Choi KD. Regulation of inositol phospholipid-specific phospholipase C isozymes. J Biol Chem 1992;267:12393-6.
3) Rhee SG, Choi KD. Multiple forms of phospholipase C isoenzymes and their activation mechanism. Adv Second Messenger Phosphoprotein Res 1992;26:35-61.
4) Noh DY, Shin SH, Rhee SG. Phosphoinositide-specific phospholipase C and mitogenic signaling. Biochim Biophys Acta 1995;1242:99-113.
5) Rhee SG, Bae YS. Regulation of phosphoinositide-specific

phospholipase C isozymes. J Biol Chem 1997;272:15045-8.
6) Suh PG, Ryu SH, Moon KH, Suh HW, Rhee SG. Cloning and sequence of multiple forms of phospholipase C. Cell 1988;54: 161-9.
7) Kim MJ, Kim E, Ryu SH, Suh PG. The mechanism of phospholipase C-regulation. Exp Mol Med 2000;32:101-9.
8) Nanney LB, Gates RE, Todderud G, King LE, Carpenter G. Altered distribution of phospholipase C-1 in benign hyperproliferative epidermal diseases. Cell Growth Differ 1992;3:233-9.
9) Park JG, Lee YH, Kim SS, Park KJ, Noh DY, Ryu SH, Suh PG. Overexpression of phospholipase C-1 in familial adenomatous polyposis. Cancer Res 1994;54:2240-4.
10) Noh DY, Lee YH, Kim SS, Kim YI, Ryu SH, Suh PG, Park JG. Elevated content of phospholipase C-1 in colorectal cancer tissues. Cancer 1994;73:36-41.
11) Noh DY, Kang HS, Kim YC, Park IA, Youn YK, Oh SK, Choe KJ. The expression of phospholipase C-1 and its cellular characteristics. J Korean Cancer Assoc 1998;30:457-63.
12) Arteaga CL, Johnson MD, Todderud G, Coffey RJ, Carpenter G, Page DL. Elevated content of the tyrosine kinase substrate phospholipase C-γ in primary human breast carcinomas. Pro Natl Acad Sci USA 1991;88:10435-9.
13) Pawson T, Nah P. Protein-protein interactions define specificity in signal transduction. Genes Dev 2000;14:1027-47.
14) Kassis J, Moellinger J, Lo H, Greenberg NM, Kim HG, Wells A. A role for phospholipase C-γ-mediated signaling in tumor cell invasion. Clin Cancer Res 1999;5:2251-60.
15) Khoshyomn S, Penar PL, Rossi J, Wells A, Abramson DL, Bhushan A. Inhibition of phospholipase C-activation blocks glioma cell motility and invasion of fetal rat brain aggregates. Neurosurgery 1999;44:568-78.