

ORIGINAL ARTICLE

Epac1, PDE4, and PKC protein expression and their association with AKAP95, Cx43, and cyclinD2/E1 in breast cancer tissues

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Keywords

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Abstract

Background: This study was conducted to investigate the exchange protein directly activated by cAMP (Epac1), PDE4, and PKC expression in breast cancer tissues, and the correlation between these proteins and AKAP95, Cx43, cyclin D2, and cyclin E1.

Methods: PV-9000 two-step immunohistochemistry was used to analyze protein expression.

Results: The positive rate of Epac1 protein expression in breast cancer tissues (58%) was higher than in para-carcinoma tissues (10%) ($P < 0.05$). There were no significant differences in the positive rates of PDE4 and PKC expression between breast cancer and para-carcinoma tissues ($P > 0.05$). The positive expression rate of PDE4 was higher in the P53 protein positive group compared to the P53 negative group ($P < 0.05$). Correlations between Epac1 and cyclin D2, PDE4 and cyclin D2, AKAP95 and PKC, Cx43 and PKC, and cyclin D2 and PKC proteins were observed ($P < 0.05$).

Conclusion: Epac1 expression in breast cancer tissues was increased, suggesting that the protein may be involved in the development of breast cancer. Correlations between Epac1 and cyclin D2, PDE4 and cyclin D2, AKAP95 and PKC, Cx43 and PKC, and cyclin D2 and PKC proteins suggested synergistic effects among these proteins in the development of breast cancer.

Introduction

Breast cancer is one of the most commonly encountered malignancies in women worldwide. In recent years, breast cancer incidence has been the highest among malignant tumors.¹

cAMP levels are directly related to several pathological events noted in different tissues, such as tumor cell proliferation and migration.^{2,3} The PDE4 enzyme specifically hydrolyzes cAMP and can reduce cAMP levels in the cell, to allow cAMP-dependent proteins to modulate cell signal transduction.⁴ The downstream protein of cAMP, namely, Epac1, plays an important role in several biological processes, including cell proliferation. The cAMP/Epac/Rapl signaling pathway has been shown to be involved in the regulation of the functions of several types of cells,

including cell secretion, apoptosis, proliferation, and differentiation. Epac1 and AKAP95 are cAMP dependent proteins involved in a variety of cellular functions.⁵ A correlation in breast cancer tissues between PDE4, Epac1, and AKAP95 is suspected. Cyclin D and cyclin E proteins can promote cell proliferation at the G1 phase in mammals, while AKAP95 as an intermediary can help cyclin D/E and protein kinase A RII subunits form the complex.⁶

We hypothesized that there may be a correlation between PDE4, Epac1, PKC, AKAP95, Cx43, and cyclin D/E; therefore, we analyzed the expression of Epac1, PDE4, and PKC proteins in 50 samples of breast cancer using an immunohistochemical method. The relationship between these proteins, and their relationship to AKAP95, Cx43, cyclin E1, and cyclin D2 was analyzed.

Methods

Tumor sources

Tissue samples with definite pathological diagnosis from 50 cases of invasive ductal breast carcinoma were collected from the First Affiliated Hospital of Liaoning Medical University between 2010 and 2011. The patients were aged between 34 and 82 years (mean 55.36 ± 10.64). Twenty-four cases exhibited lymph node metastases, while 26 had no lymph node metastases. The control group samples ($n = 10$) were collected from tissues over 3 cm distant from cancerous tissue in the 50 breast cancer patients. All adjacent tissues were pathologically examined and cancer cells were not found. All patients provided informed consent. The Medical Ethics Committee of the School of Public Health, Xiamen University, China, approved the study protocol.

Reagents and methods

All specimens were fixed in 10% neutral formaldehyde and paraffin embedded, and then cut into continuous sections 4 μm in diameter. A PV-9000 two-step immunohistochemical staining kit was used for expression analysis (Zhongshan Jinqiao Biotechnology Co. Ltd., Beijing, China) and the assay was conducted according to the manufacturer's instructions. The assay involved 3,3'-diaminobenzidine-tetrahydrochloride coloring and hematoxylin counterstaining. The rabbit anti-human Epac1 and PKC monoclonal antibody were purchased from Abcam (Cambridge, UK), while the mouse anti-human PDE4A monoclonal antibody was purchased from Santa Cruz Biotechnology Inc. (Dallas, TX, USA). Phosphate buffered saline was used as the negative control sample.

Criteria for judging positive expression

A brown–yellow stain indicated positive protein expression, whereas the absence of a brown–yellow stain indicated negative expression. Each section was randomly selected microscopically from 10 different points of view, and 200 tumor cells in each view were counted. The ratio of positive cells to total cells was used to evaluate positive expression, indicated as follows: “–” <10%; “ \pm ” \geq 10% and <25%; “+” \geq 25% and <50%; “++” \geq 50% and <75%; and “+++” \geq 75%. When the data were statistically processed, “ \pm ” and “–” were regarded as negative expression, whereas “+”, “++,” and “+++” were regarded as positive. Epac1 is located in the endomembrane system of cells, immunofluorescence staining of PDE4A of formalin-fixed A-431 cells shows membrane localization, and PKC is located in the cytoplasm, cell membrane, and nucleus.

Statistical analysis

Positive protein expression was analyzed using the χ^2 test method. The correlation between protein expressions was evaluated using Spearman's rank correlation analysis. The test level α was set at 0.05, and the analysis was carried out using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Epac1, PDE4, and PKC protein expression in breast cancer tissues

Our group has previously reported on AKAP95, cyclinE1, and Cx43 expression in breast cancer tissues.⁷ The positive rates of AKAP95 and cyclin E1 protein expression were 78% and 80%, respectively, which were higher than those noted in para-carcinoma tissues. The positive rate of the Cx43 protein was 42%, which was lower than that of the para-carcinoma tissues ($P < 0.05$). Concomitantly, our previous studies further demonstrated that the positive rate of cyclin D2 protein was 80% in breast cancer tissues (the present study only provides the positive rate of cyclin D2 protein in breast cancer tissue, as no para-carcinoma tissue sample was available).

The Epac1 protein expression levels in the 50 breast cancer samples are shown in Table 1. The positive rate of Epac1 expression in breast cancer was 58% (29/50), which was higher than that noted in the para-carcinoma tissues (10%, 1/10; $P < 0.05$). The positive rate of PDE4 expression in breast cancer was also 58% (29/50), lower than noted in the para-carcinoma tissues (70%, 7/10). The positive rate of PKC expression in breast cancer tissues was 76% (38/50), while the positive rate of PKC in para-carcinoma tissues was 40% (4/10). No statistical significance in PDE4 and PKC expression between breast cancer and para-carcinoma tissues was observed ($P > 0.05$) (data not shown). Epac1, PDE4, and PKC were mainly expressed in the cytoplasm of breast cancer tissues, and minimal expression was observed in the nuclei (Fig 1).

Relationship between Epac1, PDE4, and PKC protein expression and clinicopathological features

There were no significant differences in the positive rates of Epac1, PDE4, and PKC protein expression between the

Table 1 Epac1 protein expression in breast cancer tissues

Protein	Features	Breast cancer	Para-carcinoma tissues	χ^2	<i>P</i>
Epac1	Positive	29	1	7.680	0.006
	Negative	21	9		

breast cancer tissues with or without lymph node metastasis ($P > 0.05$) (data not shown). We further analyzed the differences between Epac1, PDE4, and PKC protein expression in breast cancer tissues that were negative and positive for estrogen receptor, progesterone receptor, P53, Her-2, and Ki67 markers. Only the positive rate of PDE4 protein in the group positive for P53 was higher compared to the rate in the P53 negative group ($P < 0.05$). No significant difference was found between the other proteins examined (Table 2).

Correlation between Epac1, PDE4, AKAP95, Cx43, cyclin E1, cyclin D2, and PKC protein expression in breast cancer tissues

We analyzed the correlation between Epac1, PDE4, and PKC protein expression and their correlation with

AKAP95, Cx43, cyclin E1, and cyclin D2 proteins in 50 samples of breast cancer tissues. The results indicated a correlation between Epac1 and cyclin D2 (Table 3), PDE4 and cyclin D2 (Table 4), AKAP95 and PKC (Table 5), Cx43 and PKC (Table 6), and cyclin D2 and PKC (Table 7) ($P < 0.05$). No statistical significance was noted between the other proteins examined (data not shown).

Discussion

Epac1 promotes the migration and invasion of pancreatic cancer cells,⁸ however, it regulates the growth of ovarian cancer cells⁹ and promotes the proliferation of prostate cancer via the human serine-threonine-protein/extracellular-signal-regulated-kinase and mammalian target of rapamycin signaling pathways.¹⁰ In the present study, the positive rate of Epac1 expression in breast cancer tissues

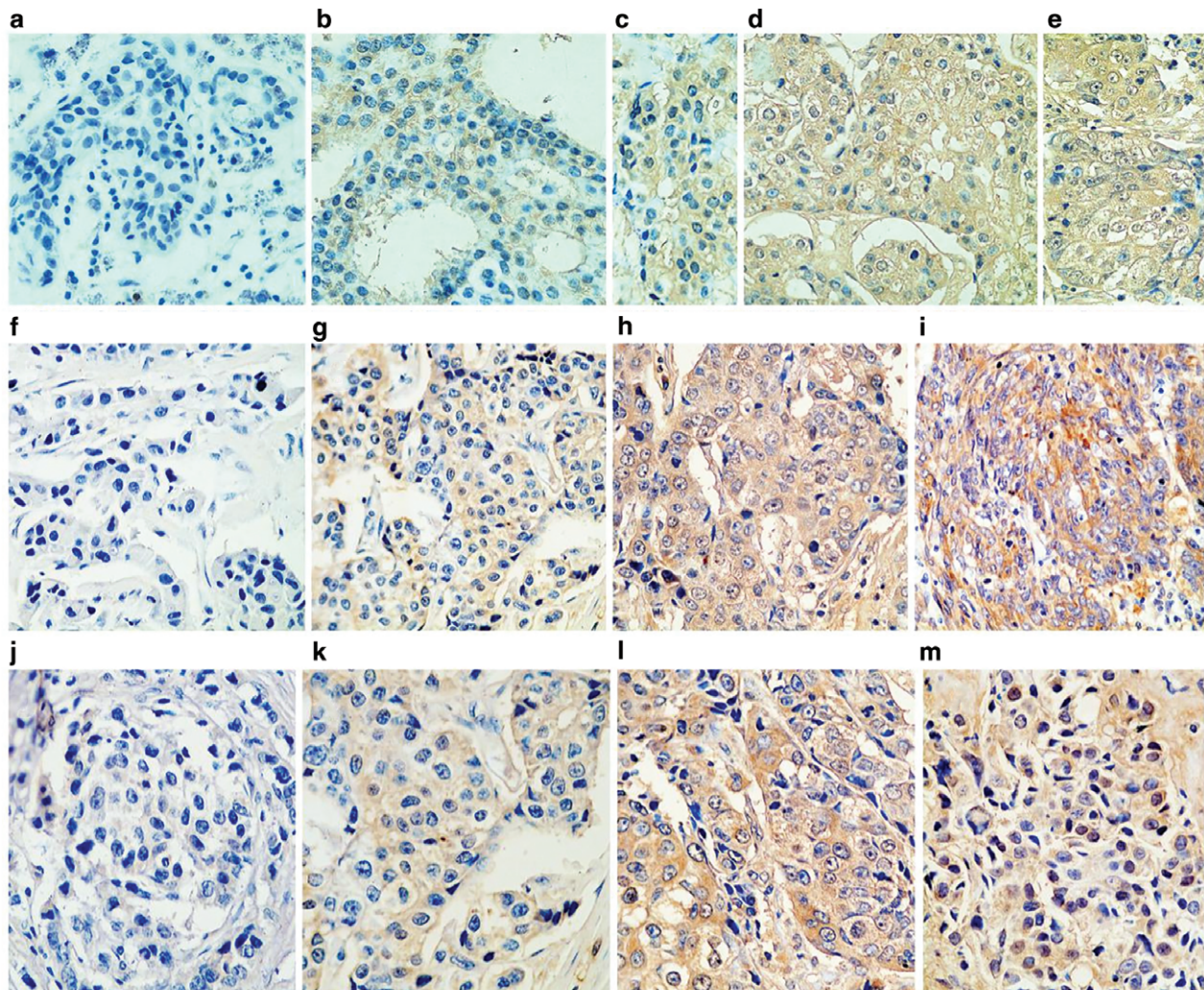


Figure 1 (a) Negative, (b,c) minimal and (d,e) high protein expression of Epac1, (f) negative, (g) minimal, and (h,i) high protein expression of PDE4, and (j) negative, (k) minimal, and (l,m) high protein expression of PKC in breast cancer tissues. Expression in (l) the cytoplasm and (m) nuclear regions.

Table 2 Relationship between P53 and PDE4, Epac1, and PKC proteins in breast cancer

		P53		χ^2	<i>P</i>
		Positive	Negative		
PDE4	Positive	22	7	4.748	0.029
	Negative	8	10		
Epac1	Positive	20	9	0.615	0.433
	Negative	11	8		
PKC	Positive	22	15	1.005	0.316
	Negative	9	2		

(58%) was higher than observed in para-carcinoma tissues (10%), suggesting that Epac1 might exert a breast cancer promoting effect, consistent with the results of previous studies.

PDE4 is a member of the cAMP-specific phosphodiesterase family and plays an important role in regulating intracellular cAMP concentration and its downstream signaling. The decreased expression of the *PDE4D* gene inhibited the proliferation of lung cancer cells with serine/threonine kinase 11 (*STK11*) mutations,¹¹ while as a tumor promoting factor, *PDE4D* played an important role in cancer cell progression.¹² We did not observe any difference in the positive rate of PDE4 protein expression between breast cancer and para-carcinoma tissues. We suspect that histological difference or fewer samples in the control group led to these results. P53 is a tumor suppressor protein.¹³⁻¹⁵ The positive rate of PDE4 was higher in the P53 protein positive group compared to the P53 negative group, suggesting that PDE4 overexpression may induce high expression of the P53 protein in order to balance the cancer promoting effects of the PDE4 protein.

PDE4 protein could regulate the level of cAMP by degrading cAMP. Epac1 and AKAP95 proteins are cAMP-dependent proteins; thus, we speculated that a synergistic effect is present between PDE4, Epac1, and AKAP95 proteins. However, no association between PDE4, Epac1, and AKAP95 proteins was observed in the tissues. Further studies are required in order to verify whether these findings are related to tumorigenesis. A correlation between

Table 3 Analysis of the correlation between Epac1 and cyclin D2 protein expression in breast cancer

Cyclin D2	Epac1					<i>rs</i> †	<i>P</i>
	-	+-	+	++	+++		
-	1	2	1	0	0	0.376	0.009
+-	2	0	2	0	1		
+	7	2	7	2	0		
++	1	1	5	3	0		
+++	2	1	0	4	3		

†*rs* is the Spearman rank correlation coefficient.

Table 4 Analysis of the correlation between PDE4 protein and cyclin D2 protein expression in breast cancer tissues

PDE4	Cyclin D2					<i>rs</i> †	<i>P</i>
	-	+-	+	++	+++		
-	0	2	1	1	0	0.396	0.006
+-	4	3	4	2	2		
+	1	0	10	4	5		
++	0	0	3	3	2		
+++	0	0	0	0	0		

†*rs* is the Spearman rank correlation coefficient.

Epac1 and PDE4 with cyclin D2 protein expression was noted, although cyclin D2 protein is a type of cytogenetic protein,^{16,17} suggesting that both Epac1 and PDE4 proteins were involved in cell cycle progression via cyclin D2.

A multitude of evidence has shown that PKC is involved in a variety of normal and abnormal cell proliferation and differentiation processes, thus confirming that PKC is associated with tumor progression. The deregulated expression of PKC subtypes and the increased activity of the corresponding enzymes exert significant effects in the regulation of cell growth, differentiation, tumor formation, and metastasis processes. Previous studies have shown that PKC- α is highly expressed in prostate, endometrium, advanced bladder, and liver cancer tissues.¹⁸ Although the positive rate of PKC protein in breast cancer tissues was lower than in the para-carcinoma tissues, our results did not indicate the function of breast cancer progression. PKC was associated with the expression of AKAP95, Cx43, and cyclin D2 (*P* < 0.05), while AKAP95 and cyclin D2 exhibited a cancer promoting effect.^{6,19-21} Cx43 has been shown to act as a tumor suppressor,²² suggesting that PKC may be involved in the phosphorylation of AKAP95, Cx43, and cyclin D2 proteins that affects their functional activity and, consequently, the cell cycle process.

There were some limitations to this study. Our sample size was small and we did not conduct any cytological experiments to verify our results.

Epac1 expression in breast cancer tissues was increased, suggesting that the protein may be involved in the

Table 5 Analysis of the correlation between AKAP95 protein and PKC protein expression in breast cancer

PKC	AKAP95					<i>rs</i> †	<i>P</i>
	-	+-	+	++	+++		
-	1	0	0	1	1	0.391	0.006
+-	2	3	2	2	0		
+	3	3	5	5	0		
++	0	2	4	8	4		
+++	0	0	0	2	0		

†*rs* is the Spearman rank correlation coefficient.

Table 6 Analysis of the correlation between Cx43 protein and PKC protein expression in breast cancer

PKC	Cx43					rs†	P
	-	+-	+	++	+++		
-	1	0	0	1	1	0.474	0.001
+-	2	2	3	2	0		
+	0	1	10	5	0		
++	0	0	6	5	7		
+++	0	0	0	1	1		

†rs is the Spearman rank correlation coefficient.

Table 7 Analysis of the correlation between cyclin D2 protein and PKC protein expression in breast cancer

PKC	Cyclin D2					rs†	P
	-	+-	+	++	+++		
-	1	0	1	0	1	0.300	0.036
+-	3	0	4	0	2		
+	1	3	9	2	2		
++	0	1	5	7	5		
+++	0	1	0	1	0		

†rs is the Spearman rank correlation coefficient.

development of breast cancer. The positive rate of PDE4 in the P53 protein positive group was higher compared to the P53 negative group, implying that PDE4 overexpression may induce high P53 protein expression. In addition, the correlations between Epac1 and cyclin D2, PDE4 and cyclin D2, AKAP95 and PKC, Cx43 and PKC, and cyclin D2 and PKC proteins suggested synergistic effects among these proteins in the development of breast cancer.

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Disclosure

No authors report any conflict of interest.

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