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Original Article

Correlation analysis between the protein expression of AKAP95, cyclinE, and Cx43 in breast cancer

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Abstract: Objective: To delve into the expressions of A-kinase anchor protein 95 (AKAP95), cell cycle protein E (cyclinE), and gap junction protein connexin 43 (Cx43) in breast cancer tissues, and to analyze the association between each of the proteins and pathological parameters, as well as the proteins' interrelationships. Methods: AKAP95, cyclinE, and Cx43 protein expression rates were evaluated by streptavidin-peroxidase immunohistochemistry in 50 breast cancer specimens and 10 pericarcinoma tissues. Results: The positive expression rate of AKAP95 was higher in breast cancer tissues (78%) than in pericarcinoma tissues (40%). The expression of cyclinE significantly increased in breast cancer tissues (80%) than in pericarcinoma tissues (20%). However, the Cx43 expression was significantly lower in breast cancer tissues (42%) than in pericarcinoma tissues (80%). There was a correlation between each two of AKAP95, cyclinE, and Cx43 expressions. Besides, protein expressions of AKAP95 and P53, Cx43 and P53, and Cx43 and estrogen receptor (ER) also correlated with each other. Conclusion: AKAP95 and cyclinE protein expression rates were significantly higher and Cx43 protein expression rates were significantly lower in breast cancer tissues compared with pericarcinoma samples, suggesting an association between these proteins and the development and progression of Breast Cancer. In addition, the correlations of these proteins also revealed that they may have a synergistic effect during the development of breast cancer. The high expression of P53 may be an antagonistic reaction to the cancer promoting effect of AKAP95 and cyclinE's high expression or a compensatory reaction to decreased tumor-suppressor effect of Cx43.

Keywords: Breast cancer, AKAP95, cyclinE, Cx43, P53, ER, correlation analysis

Introduction

AKAP95, a kind of nuclear AKAPs, regulates the protein kinase A (PKA) activity, participates in chromosome contraction, assists in gene expression, takes part in cell cycle regulation, and has many other functions [1]. At present, two subtypes of cyclinE, cyclinE1 and cyclinE2, exist. CyclinE1 regulates DNA synthesis by activating cyclin-dependent kinase 2 (CDK2), and cyclinE2 promotes cell G1/S phase transition by combining with CDK2/CDK3 [2]. AKAP95 can play a role after combining with cyclinE1 [3]. Cx43 protein is a transmembrane protein that constitutes a cell gap junction intercellular communication. The current study found that Cx43 protein can inhibit tumor growth by blocking G1/S phase transition [4]. The expression of AKAP95 in tumor tissues was less reported. This study introduced the AKAP95 protein expression in 50 cases of invasive ductal breast carcinoma, and the relationships between AKAP95, Cx43, cyclinE, P53, estrogen receptor (ER), and other proteins.

Materials and methods

Tumor sources

Tissue samples from 50 cases of invasive ductal breast carcinoma with definite pathological diagnosis were collected from breast carcino-

Table 1. Expression of AKAP95, cyclinE, and Cx43 in breast cancer tissues

Protein	Characteristics	Cancer	Pericarcinoma	x^2	*P*
AKAP95	Positive	39	4	5.926	0.024
	Negative	11	6		
CyclinE	Positive	40	2	14.286	0.001
	Negative	10	8		
Cx43	Positive	21	8	4.819	0.039
	Negative	29	2		

ma surgical specimens in the First Affiliated Hospital of Liaoning Medical University, China, between 2010 and 2011. Among them, 1 case was male and 49 cases were female. Age distribution was between 34 and 82 years, and the mean age of patients was 55.36 ± 10.64 years. Besides, 24 cases had lymph node metastasis, and 26 cases had no lymph node metastasis. Samples of some patients (n = 10) in the control group were collected from the tissues near the breast cancer tissues > 2.0 cm. All tissues were pathologically examined and no cancer cells were found. Informed consent forms were received from all patients. The experiment was approved by the Medical Ethics Committee of School of Public Health in Xiamen University, China.

Reagents and methods

The anti-AKAP8 monoclonal antibody (ab72-196) was purchased from Abcam (CA, USA). The anti-connexin43 (C-20) polyclonal antibody and anti-cyclinE monoclonal antibody (HE12) were purchased from Santa Cruz Biotechnology, Inc. (TX, USA), and the Ultrasensitive™ SP (Mouse/Rabbit) IHC Kit was purchased from Fuzhou Maixin Biotech. Co., Ltd. (FuZhou, FuJian, China). All samples were fixed in formalin, embedded in paraffin, and serially sliced to a thickness of approximately 4.0 μm. The streptavidin-peroxidase method was used for immunohistochemistry detection; the slices were stained with DAB and counterstained with hematoxylin.

Criteria for judging positive expression

A brown-yellow stain indicated positive expression of protein and no brown-yellow stain indicated negative expression of protein. Each section was randomly selected from 10 different points of view, and 200 tumor cells in each view were counted. The percentage of positive cells to total cells was used as the criteria for judging positive expression, which was shown as follows: "-", 0 to < 10% brown; "±", ≥ 10% and < 25% brown; "+", ≥ 25% and < 50% brown; "++", ≥ 50% and < 75% brown; and "+++", ≥ 75% brown. When the data were statistically processed, "+-" and "-" were regarded as negative expression, and "+", "++", and "+++" were regarded as positive expression.

Statistical analyses

The SPSS 13.0 software (SPSS Inc., IL, USA) was used to analyze the data for the x^2 test, Fishers exact test, and Spearman rank correlation analysis. The test level was α = 0.05.

Result

AKAP95, cyclinE, and Cx43 protein expression in breast cancer tissues and pericarcinoma tissues

As shown in **Table 1**, in the 50 cases of breast cancer tissues, AKAP95 and cyclinE protein expressions were 78% (39/50) and 80% (40/50), respectively, which were statistically significantly higher than that in pericarcinoma tissues, with 40% (4/10) and 20% (2/10), respectively (P < 0.05). And the positive expression of Cx43 in the cancer and pericarcinoma tissues was 42% (21/50) and 80% (8/10), respectively (P < 0.05). Both AKAP95 and cyclinE were weakly expressed in the cytoplasm, and highly in the nuclei of breast cancer tissues. However, Cx43 protein mainly expressed in the cytoplasm and few expressed in the nuclei of breast cancer tissues. The expressions of AKAP95, cyclinE, and Cx43 are shown in **Figure 1**.

Correlation between AKAP95, cyclinE, and Cx43 expressions with clinicopathological features

The positive expression rate of AKAP95 was higher in the P53 positive group than in the P53 negative group, while the positive expression rate of Cx43 protein was higher in the P53 negative group than in the P53 positive group. The differences between both of them were statistically significant (P < 0.05). At the same time, no significant difference of cyclinE expres-

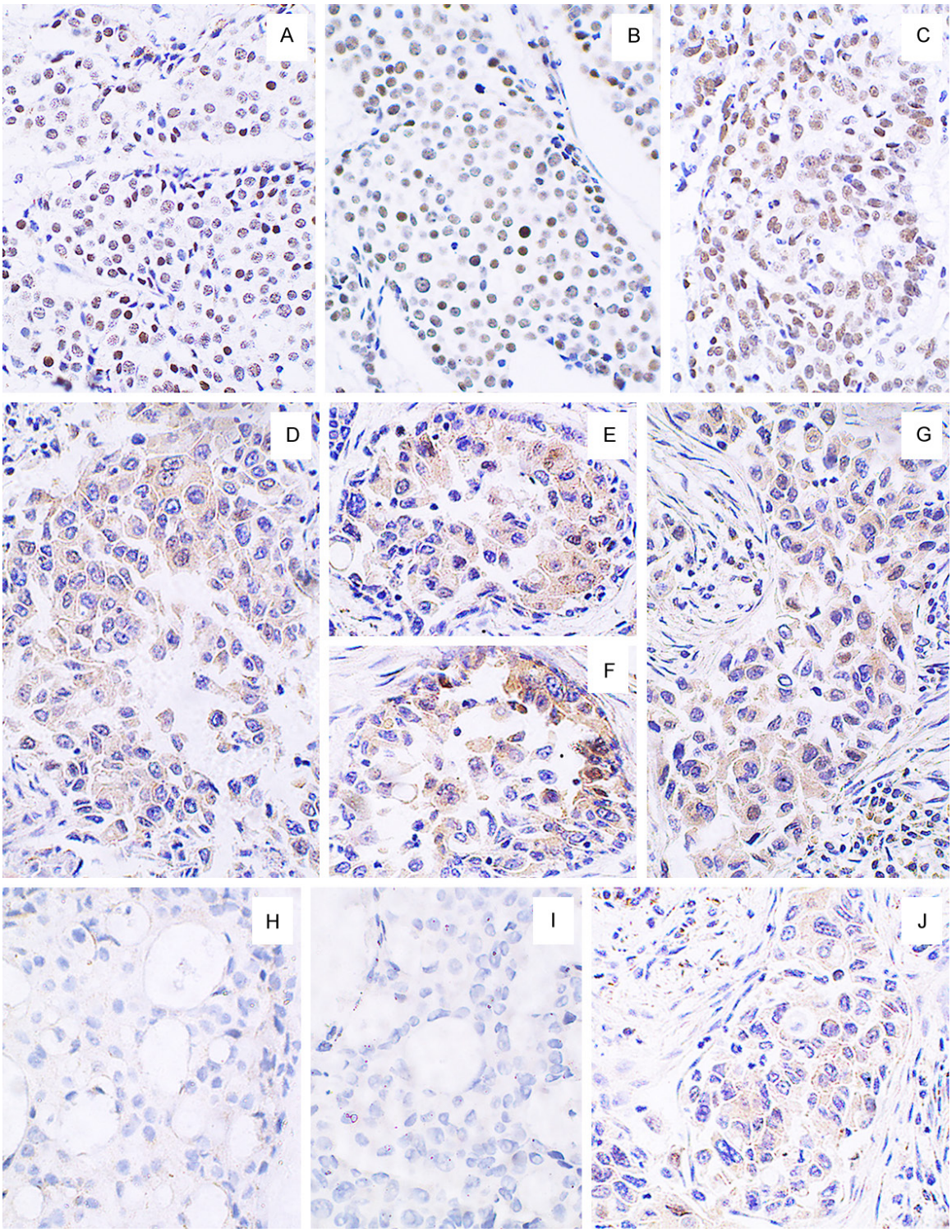

Figure 1. Expression of AKAP95, cyclinE, and Cx43 in breast cancer tissues (×400). A-C. AKAP95 highly expressed in the nuclei of breast cancer tissues. D-G. Expression of cyclinE in breast cancer tissues. D. CyclinE protein moderately expressed in the cytoplasm. E-G. CyclinE protein moderately expressed in the cytoplasm and highly expressed in the nuclei. H-J. Expression of Cx43 in breast cancer tissues. H, J. Cx43 protein moderately expressed in the cytoplasm. I. Cx43 highly expressed in the nuclei.

sion was noted between the P53 positive and P53 negative groups ($P > 0.05$), as shown in

Table 2. The positive expression of cyclinE was significantly increased while there was lymph

Table 2. Correlation between the expression of P53 and the expression of AKAP95, cyclinE, and Cx43

		P53		x^2	*P*
		Positive	Negative		
AKAP95	Positive	21	18	4.393	0.046
	Negative	2	9		
CyclinE	Positive	20	20	1.288	0.308
	Negative	3	7		
Cx43	Positive	6	15	4.428	0.035
	Negative	17	12		

Table 3. Correlation between the expression of cyclinE and lymph node metastasis

		Lymph node metastasis		x^2	*P*
		Positive	Negative		
CyclinE	Positive	21	19	5.864	0.029
	Negative	1	9		

Table 4. Correlation between the expression of ER and the expression of Cx43

		ER		x^2	*P*
		Positive	Negative		
Cx43	Positive	16	5	5.990	0.014
	Negative	12	17		

Table 5. Correlation analysis of AKAP95 and cyclinE in breast cancer tissues

AKAP95	Cyclin E					r_s	*P*
	-	+-	+	++	+++		
-	4	1	1	0	1	0.444	0.001
+-	0	0	4	0	0		
+	0	2	6	3	1		
++	1	2	5	5	5		
+++	0	0	4	1	4		

Note: r_s is the Spearman rank correlation coefficient.

node metastasis ($P < 0.05$), signed in **Table 3**, but not significantly increased in the AKAP95 or Cx43 expression ($P > 0.05$), without signed. Moreover, the positive expression rate of Cx43 was higher when ER was positive ($P < 0.05$), signed in **Table 4**, but no significant differences between AKAP95 and cyclinE expressions were observed with ER-positive expression ($P > 0.05$), without signed.

Correlation analysis of the expression of AKAP95, cyclinE, Cx43, P53, and ER

In this study, the relationships between the expressions of five proteins in breast cancer tissues were analyzed. The results suggest that AKAP95 correlated with cyclinE (**Table 5**, $P < 0.05$), Cx43 (**Table 6**, $P < 0.05$), and P53 (**Table 7**, $P < 0.05$); and Cx43 correlated with cyclinE (**Table 8**, $P < 0.05$), P53 (**Table 9**, $P < 0.05$), and ER (**Table 10**, $P < 0.05$).

Discussion

Arsenijevic and others found AKAP95 by yeast hybrid technology in dog thyroid tissue, and a further study showed that AKAP95 could combine with cyclinE [2]. Helga's research showed that AKAP95 could be PKA's anchored proteins in the nucleus to connect with PKA using PKA RII alpha T54's phosphorylation mediated by CDK1 [3]. With AKAP95, cyclins can combine with PKA RII subunits, but AKAP95 protein expression in the tumor research is rarely seen. Before that, AKAP95 high protein expression was found in the lung cancer and colorectal cancer tissues, so it was believed that protein AKAP95 may have carcinogenesis. With regard to the 50 cases of infiltrating ductal breast carcinoma, the present research showed that the expression of protein AKAP95 was still higher in cancer tissues than in pericarcinoma tissues, which is in accordance with the results of previous reports [5, 6].

CyclinE mainly affects the late G1 phase, regulating cells from G0 or G1 phase to S phase [7]. The combination of AKAP95 and cyclinE can be contended by CDK2, which phosphorylates cyclinE, then phosphorylates pRb, promoting cells to go through G1 phase to S phase [8, 9]. By means of inhibiting the expression of skp2 and reducing the degradation of p27, Cx43 could inhibit cell G1/S phase transformation [4, 10]. So it figures that Cx43 plays an important role in controlling cells as a tumor-suppressor role. Extracellular signal-regulated kinase 1/2 can make Cx43 immune coprecipitation down under the condition of ischemia or hypoxia. By adjusting the matrix metalloproteinase enzyme activity, mitogen-activated protein kinases (MAPK) will influence the expression of Cx43 protein [11]. After dealing with vascular smooth muscle cells using the platelet-derived growth factor, cyclinE can combine with Cx43 and its

Table 6. Correlation analysis of AKAP95 and Cx43 in breast cancer tissues

AKAP95	Cx43					r_s	*P*
	-	+-	+	++	+++		
-	2	4	0	1	0	0.282	0.048
+-	1	1	1	1	0		
+	5	4	1	2	0		
++	3	5	2	6	2		
+++	2	2	0	4	1		

Note: r_s is the Spearman rank correlation coefficient.

Table 7. Correlation analysis of AKAP95 and P53 in breast cancer tissues

AKAP95	P53					r_s	*P*
	-	+-	+	++	+++		
-	4	1	2	0	0	0.482	<0.001
+-	2	2	0	0	0		
+	7	3	4	0	0		
++	3	3	9	1	0		
+++	0	2	7	0	0		

Note: r_s is the Spearman rank correlation coefficient.

Table 8. Correlation analysis of cyclinE and Cx43 in breast cancer tissues

Cx43	Cyclin E					r_s	*P*
	-	+-	+	++	+++		
-	3	2	6	1	1	0.449	0.001
+-	1	3	5	5	2		
+	0	0	4	0	0		
++	1	0	5	2	6		
+++	0	0	0	1	2		

Note: r_s is the Spearman rank correlation coefficient.

Table 9. Correlation analysis of Cx43 and P53 in breast cancer tissues

Cx43	P53					r_s	*P*
	-	+-	+	++	+++		
-	3	1	8	1	0	-0.301	0.034
+-	4	4	8	0	0		
+	3	0	1	0	0		
++	8	2	4	0	0		
+++	0	2	1	0	0		

Note: r_s is the Spearman rank correlation coefficient.

combination amount could increase through high phosphorylation of Cx43 by MAPK, promoting the formation of new blood vessel lining [12]. P53 and Cx43 are both tumor-suppressor proteins, playing an important role in controlling the growth processing of cells [13-15]. The aforementioned studies suggest that cyclinE requires the participation of AKAP95 and Cx43 proteins to come into play. This study showed that AKAP95 and cyclinE, and Cx43 and P53 have correlated expression, and the positive expression of P53 and Cx43 provided the opposite result: when the Cx43 is highly expressed, P53 expression is very low or the other way around. All these data suggest that high expression of AKAP95 and cyclinE may promote cell cycle progression, and lower expression of Cx43 may reduce the inhibitory effect of cell cycle progression, which means it may induce high expression of P53 protein to antagonize AKAP95 and cyclinE protein's cancer promoting function or compensate Cx43 protein's suppressor function.

Table 10. Correlation analysis of Cx43 and ER in breast cancer tissues

ER	Cx43					r_s	*P*
	-	+-	+	++	+++		
-	11	5	3	2	1	0.460	0.001
+-	0	0	0	0	0		
+	2	8	1	9	1		
++	0	1	1	2	1		
+++	0	1	0	1	0		

Note: r_s is the Spearman rank correlation coefficient.

An ER protein is a ligand-dependent transcription-activating factor mediating the effects of estrogen on a target tissue, of which the high expression suggests a high degree of tumor differentiation, good effect on endocrine drug, and better prognosis [16, 17]. When it comes to the high expression of Cx43 on tumor tissue, it often shows a high degree of differentiation and low malignant degree [18, 19]. In the present-study survey of 50 cases of breast cancer tissues, Cx43 and ER protein expressions provide obvious relevance. If ER and Cx43 proteins could be detected clinically, it may help make appropriate judgments of breast malignant degree and prognosis. As previously reported, cyclinE is an important regulatory factor mediated by estrogen in breast cancer cells [20-22]. But this study did not detect the relevance of cyclinE and ER expressions in breast cancer.

In addition, the present study showed that no correlation existed between AKAP95, cyclinE, and Cx43 with some clinicopathological fea-

tures such as PR, Her-2, histologic grade, ki67, and so on.

Disclosure of conflict of interest

None.

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