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Over-expression of protein kinase C isoforms (α, δ, θ and ζ) in squamous cervical cancer

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Protein kinase C was found to be significantly over-expressed in cancer samples compared to adjacent normal cervical tissues by proteomics in our previous study. The aim of this study was to examine protein kinase C expression and to analyze the expression patterns of protein kinase C isoforms in squamous cervical cancer at the protein levels and their associations with clinical and pathologic factors of squamous cervical cancer. First, Western blotting was used to examine protein kinase C expression in the specimens of tumors and matched adjacent normal tissues which were collected from 12 patients with squamous cervical cancer. Protein kinase C isoforms (α, δ, θ and ζ) expression were then detected by immunohistochemistry in other 43 cases of squamous cervical cancer tissues, 32 cases of corresponding adjacent normal cervical squamous epithelial tissue and 31 cases of cervical intraepithelial neoplasia. Western blot analysis revealed that protein kinase C expression was positive in squamous cervical cancer while it was not expressed in normal cervical tissues. On the other hand, immunohistochemical analysis suggested that, protein kinase C isoforms (α, δ, θ and ζ) expression was significantly higher in squamous cervical cancer compared to cervical intraepithelial neoplasia, as well as in cervical intraepithelial neoplasia compared with normal tissues, respectively. High levels of protein kinase C α expression were associated with cellular differentiation ($P<0.05$). Protein kinase C δ was significantly associated with tumor stage ($P<0.05$) and protein kinase C ζ was associated with lymphatic metastasis ($P<0.05$). These findings indicate that protein kinase C isoforms expression in cervical lesions was associated with carcinogenesis and might play important roles throughout the process of cervical cancer development.

Key words: Cervical cancer, protein kinase C, isoforms

Protein kinase C (PKC) is a serine/threonine kinase that plays a key role in several steps of the signal transduction pathway, including cellular proliferation, differentiation, and apoptosis [1]. PKC consists of a family of at least 10 related isoforms that are classified into three subfamilies: conventional PKCs (α, βI, βII and γ), which are activated by phosphatidylserine, diacylglycerol and calcium; novel PKCs (δ, ε, η, θ and μ/PKD), which require phosphatidylserine and diacylglycerol, being independent of calcium for activation; atypical PKCs (ζ and ι/λ), which need only phosphatidylserine [2].

PKC isoforms have been shown to display variable expression profiles during cancer progression depending on the particular cancer type [3]. Several studies have shown that PKC α is over-expressed in urinary bladder cancers, endometrial cancers and hepatocellular carcinomas [4-6]. In contrast, colon cancer displays down-regulation of PKC α expression [7]. PKC θ expression has been shown to be up-regulated in gastrointestinal stromal tumors and breast cancers [8, 9] and down-regulated in pancreatic duct carcinomas [10]. Investigations also found that the expression of PKC δ increased in breast cancers [11] and PKC ζ increased in glioblastoma [12]. These studies provide supporting evidence for the role of a specific PKC isotype in different kinds of malignant tumors. However, to date, there is no report about PKC isoforms expression in the malignant progression of human squamous cervical cancer (SCC).

Cervical cancer is still one of the most common malignancies in women [13], and it is responsible for 471,000 annual cases and 233,000 deaths worldwide. SCC remains the most common histological type, which accounting for approximately 80%–85% of all cervical carcinomas. Its precursor is dysplasia, also described as cervical intraepithelial neoplasia (CIN) [14]. While the concept of a continuous spectrum of histopathological change from normal, stratified epithelium through CIN to invasive cancer has been widely accepted for

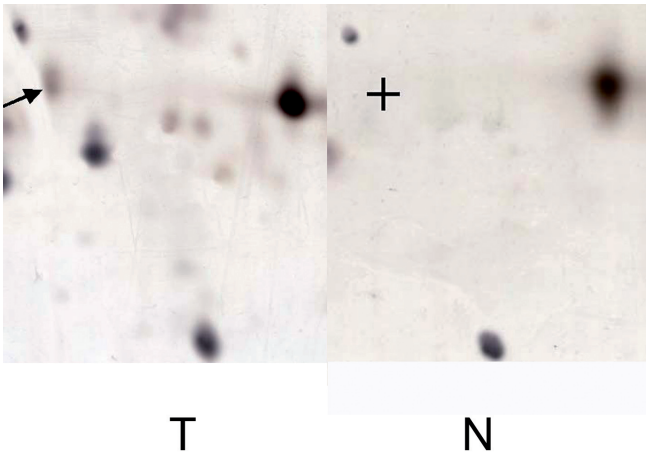

Fig. 1. PKC protein expression comparisons of squamous cervical cancer (T) with that of adjacent normal cervical tissue (N) in 2D gel electrophoresis. PKC protein spot was present in T (arrow) but absent in N (plus).

many years, the complete course of the normal cells become transformed has never been completely understood.

In our previous study, by using two-dimensional gel electrophoresis followed by matrix-assisted laser desorption/ionization-time of flight mass spectrometry, we examined the protein expression differences in human SCC and matching adjacent normal cervical tissues and revealed 55 proteins exhibiting a consistent, more than threefold expression regulation in squamous cervical cancer [15]. Of the 55 proteins PKC protein, which increased in SCC compared with adjacent normal cervical tissues (shown in Fig. 1 and Fig. 2) was identified.

In the present study, the expression of PKC in SCC and matched adjacent normal cervical tissues was confirmed by Western blotting and PKC isoforms (α, δ, θ and ζ) expression in SCC, CIN and normal cervical tissue was investigated using immunohistochemical technique. Our data provide the first definitive evidence of a correlation between PKC isoforms expression and clinicopathological features in patients with SCC.

Materials and methods

Tissue specimens. Tissue specimens were obtained with the informed consent of all patients and approval of the local research ethical committee. No patients had undergone any previous treatment. (1) Western blotting: twelve patients with cervical cancer of 42 to 59 years of age who underwent radical hysterectomy between July 2007 and March 2008 in our hospital were selected. From each cervix, two samples were collected: one from the central area of the cervical cancer and the other from the normal cervical tissue situated more than 2 cm away from the cervical cancer. The blood in samples was washed out and then samples were frozen in liquid nitrogen. (2) Immunohistochemistry: archival paraffin-embedded cervical tissue from forty-three patients with cervical cancer of 41 to 62 years old, which were treated by hysterectomy between 2006 and 2008, 43 squamous cervical cancer and 32 corresponding adjacent normal cervical tissue were obtained. According to the International Federation of Gynecology and Obstetrics classification, 30 patients had stage I (1 with stage Ia, 29 with stage Ib) and 13 patients had stage II (12 with stage IIa, 1 with stage IIb). Nine cases were histologically well differentiated, 32 cases were moderately

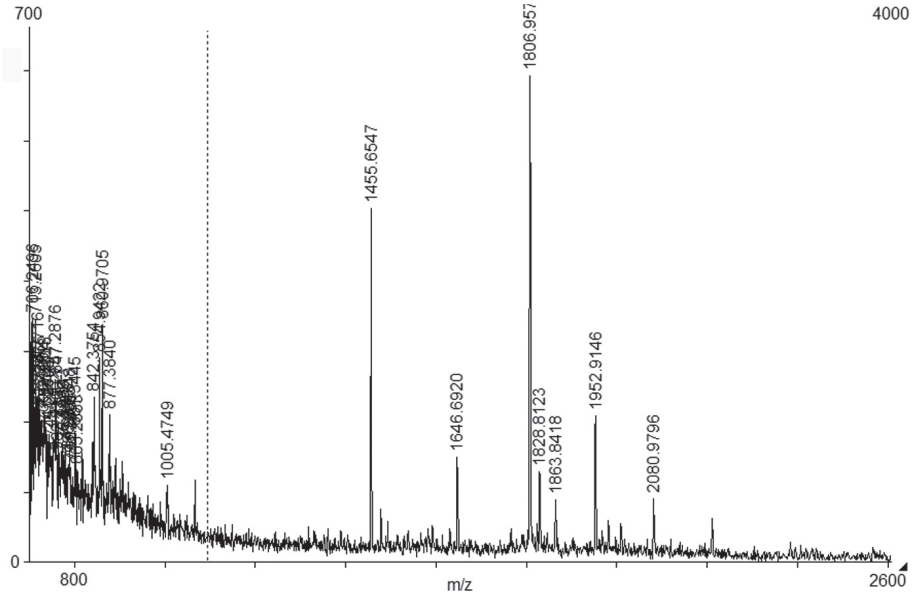

Fig. 2. Mass spectrometry of in-gel trypsin digests of this protein and analysis of the depicted peptide spectrum resulted in the identification of PKC.

differentiated and 2 cases were poorly differentiated. Eleven patients with lymphatic metastasis and 7 patients with vascular space involvement. The clinical and histopathological characteristics of 43 SCC patients are listed in Table 1. Thirty-one patients (mean age 44.9 years, range 36-54 years) diagnosed as dysplasia of different grades were selected (6 with CIN I, 13 with CIN II and 12 with CIN III).

Western blotting. Tissue samples (50–100 mg) were crushed in liquid nitrogen, and dissolved in the lysis buffer containing 8 M urea, 4% CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate), 20 mM Tris–HCl, pH 8.5, 2 mM ethylenediamine tetra-acetic acid (EDTA), 1 mM phenylmethylsulfonyl fluoride (PMSF), 60 mM dithiothreitol (DTT), and protease inhibitor cocktail. After sonication on ice for 5 min and centrifugation at 13,000 rpm for 30 min at 4°C, the protein concentration of the supernatants was quantified by the Bradford method. Equal amounts of protein (10 μg) of the extracted proteins were subjected to 12% sodium dodecyl sulfate-polyacrylamide gel at 20 mA until the bromophenol blue dye reaching the bottom of the gels and electro-transferred onto polyvinylidene fluoride (PVDF) membranes (Bio-Rad) at 350 mA for 1 h, using Bio-Rad Mini PROTEAN 3 system (Hercules, CA). The PVDF membranes were incubated in blocking buffer (Tris-buffered saline) containing 5% milk powder and 0.1% Tween 20 at room temperature for 2 h, and incubated with 1:3000 monoclonal mouse anti-human PKC (Santa Cruz, CA, USA) at 4°C overnight. The antibody against mouse IgG conjugated with horseradish peroxidase was adopted as secondary antibody. Peroxidase activity was visualized with the enhanced chemiluminescence reagents (Amersham Biosciences, Uppsala, Sweden). Equal protein loading was confirmed by parallel β-actin immunoblotting, and signal quantification was performed by densitometry scanning.

Immunohistochemistry. Immunohistochemistry staining was carried out by a standard streptavidin-peroxidase method. In brief, the 4 μm-thick sections from formalin-fixed, paraffin-embedded tissues, were paraffinized in xylene and rehydrated through a graded alcohol series after overnight incubation at 45-50 °C. For PKC α, δ, θ and ζ, the slides were heated in 10 mmol/L citrate buffer (pH 6.0) in a domestic pressure cooker for 20 min at 100 °C. Endogenous peroxidase activity was suppressed by a 20 min incubation with 0.3% hydrogen peroxide in methanol. The sections were incubated with normal goat serum to prevent nonspecific binding. The specific primary antibody, either 1:50 monoclonal mouse anti-human PKC α (Santa Cruz, CA, USA), 1:50 polyclonal rabbit anti-human PKC δ, PKC θ, or PKC ζ (Cell Signaling, CA, USA) were then applied for 2 h at room temperature. Biotinylated goat anti-mouse antibody (Santa Cruz, CA, USA) was used as secondary antibody (20 min), streptavidin peroxidase as label (20 min) and diaminobenzidine as chromogen. Counterstaining was performed with hematoxylin to enhance nuclear detection. Appropriate positive and negative control slides were stained in parallel.

Table 1. Clinical and histopathological characteristics of 43 SCC patients

Patient	Stage	Age	Dif	His	Lym	Vas
1	Ia	44	MD	II	-	-
2	Ib	43	MD	II	+	-
3	Ib	45	MD	II	-	-
4	Ib	61	MD	II	-	-
5	Ib	62	WD	I	-	-
6	Ib	58	WD	I	-	-
7	Ib	60	WD	I	-	-
8	Ib	52	MD	II	-	-
9	Ib	41	WD	I	-	-
10	Ib	49	WD	I	-	+
11	Ib	61	WD	I	+	-
12	Ib	43	MD	II	-	-
13	Ib	47	MD	II	-	-
14	Ib	50	MD	II	+	-
15	Ib	53	MD	II	-	-
16	Ib	53	MD	II	-	-
17	Ib	60	MD	II	-	-
18	Ib	44	MD	II	+	+
19	Ib	53	MD	II	+	-
20	Ib	60	MD	II	-	-
21	Ib	62	MD	II	-	-
22	Ib	52	WD	I	-	-
23	Ib	58	MD	II	-	-
24	Ib	61	MD	II	-	-
25	Ib	44	WD	I	-	-
26	Ib	59	MD	II	-	-
27	Ib	44	MD	II	-	-
28	Ib	55	MD	II	-	+
29	Ib	49	MD	II	+	+
30	Ib	45	MD	II	-	-
31	IIa	44	MD	II	+	-
32	IIa	53	MD	II	-	-
33	IIa	53	MD	II	+	-
34	IIa	58	MD	II	-	-
35	IIa	55	PD	III	-	-
36	IIa	40	MD	II	+	-
37	IIa	44	MD	II	-	-
38	IIa	62	MD	II	-	+
39	IIa	58	WD	I	+	+
40	IIa	58	MD	II	-	-
41	IIa	42	MD	II	-	+
42	IIa	53	PD	III	-	-
43	IIb	53	MD	II	+	-

Abbreviations: Dif, differentiation; WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated; His, histological grade; Lym, lymphatic metastasis; Vas, vascular space involvement.

PKCs-positive cells showed yellowish brown, cytoplasmic staining. All counting was done by two observers, without any knowledge of the diagnosis and results of the other observer's counts. All immunopositive cells were counted in at least 10 high-power fields (×40 objective, ×10 eyepiece) chosen

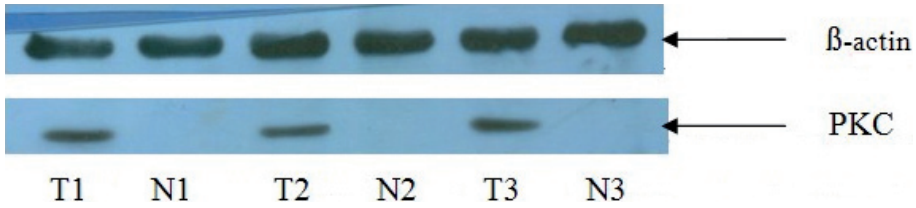

Fig. 3. Western blot analysis revealed markedly positive PKC expression in squamous cervical cancer (T) while PKC expression was not detected in normal cervical tissues (N). T1 patient at stage Ib was moderately differentiated and without metastasis. T2 patient at stage IIa was well differentiated and without metastasis. T3 patient at stage IIa was poorly differentiated, with lymphatic metastasis and vascular space involvement.

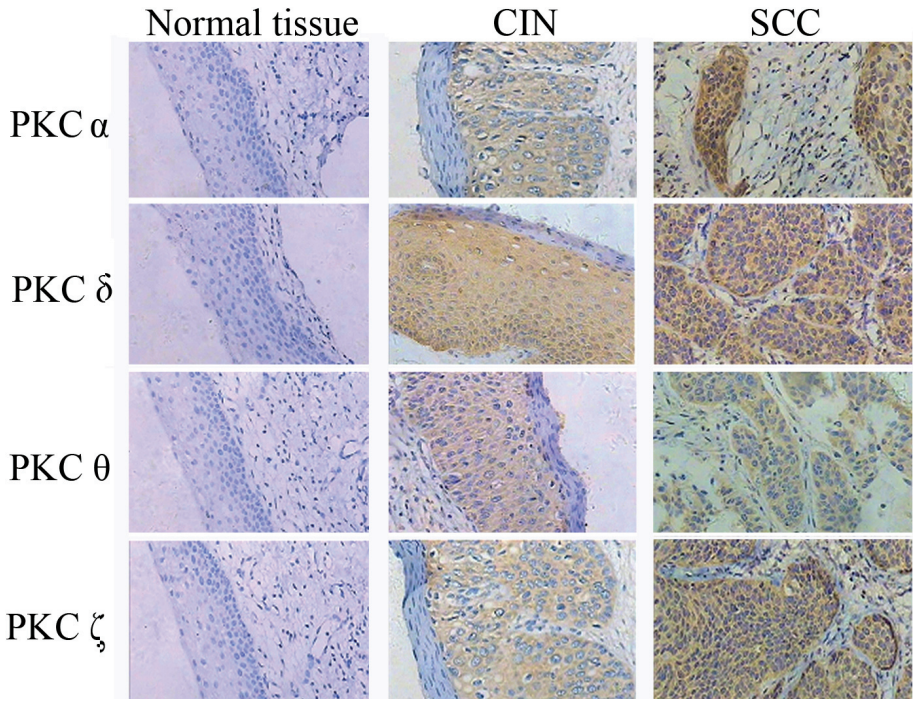

Fig. 4. PKC isoforms expression in normal tissue, CIN, SCC (original magnification ×200). PKCs-positive cells showed yellowish brown, cytoplasmic staining. Normal cervical tissues showed no staining of PKC α, δ, θ and ζ. CIN tissues showed moderate expression of PKC α, δ, θ and ζ. SCC tissues showed strong expression of PKC α, δ, θ and ζ.

at random. The number of PKCs-positive cells was given as a percentage for each case. Extent of immunostaining (based on the percentage of positive cells) was scored as 0 (0 to 5%), 1 point (6% to 24%), 2 points (25% to 49%), 3 points (50% to 74%), and 4 points (75% to 100%). Staining intensity was graded as 0 (negative), 1 point (weak), 2 points (moderate), and 3 points (strong). The immunohistochemical staining for PKCs was assessed according to the immunoreactive score (IRS) value by multiplying the individual scores of extent by intensity [16].

Statistical analysis. The chi-square test or Fisher's test was used for comparisons of categorical data. While Mann-Whitney U test was used to analyze the difference in PKC isoform expression intensity between cervical carcinoma and CIN tissue. *P* values less than 0.05 were considered statistically significant. Two-sided tests were used throughout all the analyses. All calculations were performed using SPSS 11.5 statistical software package (SPSS Inc., Chicago, USA).

Results

Confirmation of PKC protein expression by Western blotting. To confirm the different proteins identified by our previous proteomic approach, expression of PKC protein in 12 pairs of squamous cervical cancer and adjacent normal cervical tissues was detected by Western blotting.

The expression of PKC protein was detected in 9 out of 12 SCC tissues but 0 of 12 matched normal cervical tissues, as shown in Figure 3. Using Fisher's test, the expression of PKC was significantly positive in SCC versus normal cervix ($P<0.05$), which confirms the results of proteomic analysis.

PKC α, δ, θ and ζ expression in SCC, CIN and normal cervical tissues. Fig. 4 shows the expression of PKC α, δ, θ and ζ in the squamous epithelium. SCC tissues consistently showed diffuse and intense positive staining for PKC α, δ, θ and ζ, while CIN tissues consistently showed diffuse and moderate positive staining. In contrast, normal cervical tissues exhibited no staining or focal and weak positivity for PKC α, δ, θ and ζ.

Using chi-square test, PKC α, δ, θ and ζ showed lower positivity rate in normal cervical tissues than those in SCC and CIN tissues, respectively ($P<0.05$) (Table 2).

Using Mann-Whitney U test, the expression intensity of PKC α, δ, θ and ζ was statistically higher in SCC tissues in comparison with that in CIN tissues, respectively ($P<0.05$) (Table 3).

Association between PKC isoforms expression and clinicopathological parameters in SCC. As shown in Table 4, the expression of PKC α, δ, θ and ζ was noted in 36, 29, 35 and 41 specimens of 43 SCC, respectively. There was a significantly increased expression of PKC α in moderately and poorly differentiated tumors (91.1%) compared to that in well differentiated tumors (55.6%; $P<0.05$). PKC δ showed less frequent positivity in stage I (56.7%) than those in stage II (92.3%; $P<0.05$). There was a significantly increased expression of PKC ζ in tumors without lymphatic metastasis (100%) compared to that with lymphatic metastasis (81.8%; $P<0.05$). However, PKC θ expression was not related to the clinical stages, cellular differentiation, lymphatic metastasis and vascular space involvement ($P>0.05$).

Discussion

Accumulating data suggest that various PKC isoforms participate in the regulation of cell proliferation, differentia-

Table 2. The expression of PKC α, δ, θ and ζ in SCC, CIN and normal cervical tissues

Tissue	Cases	PKC α		PKC δ		PKC θ		PKC ζ	
		Positive cases	Positive rate(%)	Positive cases	Positive rate(%)	Positive cases	Positive rate(%)	Positive cases	Positive rate(%)
SCC	43	36	83.7*	29	67.4*	35	81.4*	41	95.3*
CIN	31	24	77.4*	20	64.5*	25	80.6*	28	90.3*
Adjacent normal cervical tissues	32	2	6.25	2	6.25	2	6.25	2	6.25

Table 2. The positivity rate of PKC α, δ, θ and ζ is higher in SCC and CIN tissues than normal cervical tissues, respectively ($P<0.05$)

Table 3 The expression of PKC α, δ, θ and ζ in SCC and CIN

Tissue	cases	PKCα	PKCδ	PKCθ	PKCζ
SCC	43	6.8±3.0*	4.8±3.0*	5.8±3.0*	7.6±3.4*
CIN	31	3.8±2.0	3.2±1.8	3.4±1.6	4.4±2.3

Table 3. The expression (IRS value) of PKC α, δ, θ and ζ is higher in SCC tissues than CIN tissues ($P<0.05$).

Table 4. Expression of PKC isoforms in SCC

Clinicopathological parameters	cases	PKCα	PKCδ	PKCθ	PKCζ
		Positive cases (Positive rate %)	Positive cases (Positive rate %)	Positive cases (Positive rate %)	Positive cases (Positive rate %)
stage					
Ia+Ib	30	25 (83.3)	17 (56.7)	24 (80.0)	28 (93.3)
IIa+IIb	13	11 (84.6)	12★ (92.3)	11 (84.6)	13 (100)
Differentiation					
Well-differentiated	9	5 (55.6)	5 (55.6)	8 (88.9)	8 (88.9)
Moderately-pooly differentiated	34	31* (91.1)	24 (70.6)	27 (79.4)	33 (97.1)
lymphatic metastasis					
absent	32	26 (81.3)	21 (65.6)	28 (87.5)	32 (100)
present	11	10 (90.9)	8 (72.7)	7 (63.6)	9▲ (81.8)
Vascular-space-involvement					
absent	36	30 (83.3)	24 (66.7)	30 (83.3)	34 (94.4)
present	7	6 (85.7)	5 (71.4)	5 (71.4)	7 (100)

Table 4. There was a significantly increased expression of PKC α in moderately and poorly differentiated tumors compared to well-differentiated tumors ($P<0.05$). PKC δ showed less frequent positivity in stage I than stage II ($P<0.05$). PKC ζ showed less frequent positivity in tumors with lymphatic metastasis than without lymphatic metastasis ($P<0.05$).

tion, survival and apoptosis [2]. These findings have enabled identification of abnormalities in PKC isoform function, as they occur in a variety of tumors [3-9]. And now, PKC is recognized as an important player in tumor carcinogenesis [3]. However, little is known about the specific role of each PKC isoform in the carcinogenesis of human SCC. To clarify the role of PKC isoforms in human SCC, we measured PKC isoforms expression in SCC, CIN, and normal cervical tissue and explored the relationship between the expression of PKC isoforms and clinicopathological parameters of the patients with SCC. Our results showed that the PKC isoforms (α, δ, θ and ζ) expressions were successively increased from normal to malignant cervical tissue. These findings indicate that PKC isoforms expression in cervical tissues associates with carcinogenesis and might play important roles throughout the process of cervical cancer development. Each isoform is considered in turn in the following discussion.

PKC α. The role of PKC α in the growth of cancer cells is more complex with variable responses depending on cancer type. PKC α has been linked to decreased [17] and increased proliferation of cancer cells [18]. Furthermore, PKC α has been shown to inhibit [17], or facilitate [19] apoptosis of cancer cells. Most of the studies favor the role of PKC α as inducer of proliferation and suppressor of apoptosis. Cameron et al. [20] demonstrated that PKC α mediates an essential pro-mitogenic and pro-survival signal in glioma cells.

Vast majority of studies have demonstrated that increased PKC α activity is associated with increased motility and invasion of cancer cells, and the inhibition of PKC α effectively reverses the phenotype. However, there appeared to be conflicting results regarding the PKC α expression in cancerous tissues and in normal tissues. Gökmen-Polar et al. [7] found that PKC α expression slightly decreased in aberrant crypt foci and dramatically reduced in colon tumors. In contrast, Varga et al. [4] demonstrated that PKC α expression was greater in superficial bladder cancerous tissues than in normal tissues. These findings were similar to those of Wu et al. [6], who found that PKC α mRNA was significantly increased in hepatocellular carcinomas as compared to the corresponding non-cancerous liver tissues. Our results also indicate that the expression intensity of PKC α was statistically higher in SCC tissues in comparison with that in CIN tissues, and PKC α showed higher positivity rate in CIN tissues than in normal cervical tissues.

In the present study, there was a significantly increased expression of PKC α in moderately and poorly differentiated SCC compared to that in well differentiated tumors, which was in agreement with the other study by Wu et al. [21]. They found that PKC α was highly expressed in the poor-differentiated hepatocellular carcinoma cell lines as compared with that in the well-differentiated cell lines. Thus, the results indicate that PKC α may inhibit differentiation of SCC cells.

PKC δ. Currently, it is unclear whether PKC δ is involved in promoting or inhibiting cancer formation/progression. Many authors reported that PKC δ increased apoptosis and was downregulated in malignancies [3]. At the same time, several clinical observations have shown that PKC δ expression was greater in cancerous tissues than in normal tissues [22]. According to a cDNA microarray analysis in pheochromocytomas, Tokuda et al. [22] found that PKC δ mRNA was significantly over-expressed in the tumor tissue in comparison to the adjacent non-tumor tissue. In the present study, the expression intensity of PKC δ was significantly higher in SCC tissues than in CIN tissues and PKC δ showed higher positivity rate in CIN tissues than in normal cervical tissues. In addition, PKC δ showed less frequent positivity in stage I than those in stage II. These findings parallel those reported by McKiernan et al. [11] in breast cancer, who found that PKC δ mRNA expression was significantly higher in estrogen receptor-positive compared with estrogen receptor-negative tumors and demonstrated that increasing concentrations of PKC δ mRNA associated with reduced overall patient survival. Our finding here of an association between elevated PKC δ expression and severe pathological changes suggests that alteration of PKC δ expression may be involved in progression of human SCC.

PKC θ. PKC θ is involved in the regulation of diverse cell functions such as cell proliferation, islet cell secretion, skeletal muscle differentiation, cancer cell-stroma interaction, transcription and apoptosis [23]. Evans et al. [10] revealed relative loss of PKC θ in pancreatic duct carcinomas compared with control normal minor ductular epithelial cells. However, vast of the functional studies have suggested that PKC θ upregulated in cancerous tissues. Kim et al. [8] showed that the expression of PKC θ was increased and it may play a role in the development of gastrointestinal stromal tumors. Belguish et al. [9] reported that PKC θ was elevated in transgenic mouse mammary tumors and in ER α-negative human breast cancers.

Our data revealed that the expression intensity of PKC θ was statistically higher in SCC tissues in comparison with CIN tissues and PKC θ showed higher positivity rate in CIN tissues than in normal cervical tissues, which showed a significant increase in PKC θ expression from normal to malignant tissue. It suggests that PKC θ may play a role in the carcinogenesis of human SCC.

The previous study by Kim et al. [8] found that PKC θ expression was not related to the clinical stages, histological types, degrees of cellularity and cellular atypia, the presence of necrosis, the presence of mucosal invasion, the locations of gastrointestinal stromal tumors and the risks of aggressive behavior. It was consistent with our results, which indicate that PKC θ expression not related to the clinicopathological parameters of SCC, such as clinical stages, cellular differentiation, lymphatic metastasis and vascular-space-involvement.

PKC ζ. Initial studies of PKC ζ were focused on its role in controlling cell growth and survival and it was found at the crossroad of NF-κ B and Jak/Stat signaling pathway [24], and later works identified its role at determining cell polarity [25]. PKC ζ binds to and phosphorylates Sp1, which changes with cell differentiation, transformation, and growth, and contributes to tumorigenesis [26].

PKC ζ levels and/or activities were often found elevated in cancer, that include breast, lung, liver, colon, and prostate cancer etc. as well as melanoma and glioblastoma [12]. Our study was consistent with previous studies. We found that the expression intensity of PKC ζ was significantly higher in SCC tissues than in CIN tissues and PKC ζ showed higher positivity rate in CIN tissues than in normal cervical tissues.

There appeared to be conflicting results regarding the role of PKC ζ in the metastasis of malignancies. Guo et al. [27] reported that knock-down of PKC ζ impaired CSF-1-induced migration of human acute monocytic leukemia cell line THP-1 as well as mouse peritoneal macrophages, emphasizing a potential and critical mechanism of PKC ζ in tumor metastasis which involved crosstalk between tumor and tumor-associated macrophage. In addition, Wu et al. [28] demonstrated that PKCzI257.3, a PKC ζ-specific small chemical inhibitor, inhibited epidermal growth factor – induced breast cancer cell line chemotaxis and migration. Both of them believed that, inhibition of PKC ζ might be an effective strategy at blocking both cancer cell chemotaxis and eventually preventing metastasis. Sanz-Navares et al. [29] demonstrated that PKC ζ suppressed the migration of metastatic melanoma cells. This finding was similar to our results, in SCC, there was a significantly increased expression of PKC ζ in tumors without lymphatic metastasis compared to that with lymphatic metastasis, which suggested that PKC ζ may suppress the migration of SCC. The discrepancy suggests that the role of PKC ζ in the metastasis of malignancies is strongly dependent on the type of cancer cells.

In summary, the present study has demonstrated characteristic alterations in PKC isoforms expression associated with human cervical carcinogenesis. The pattern of changes suggests that altered PKC isoforms expression, notably the successive increased PKC α, δ, θ and ζ expression in normal, dysplastic, and malignant cervical tissue, respectively, may be associated with deranged homeostatic mechanisms that normally regulate cell proliferation, differentiation, and apoptosis. Presently, there is considerable interest in the potential use of synthetic PKC inhibitors and activators as anticancer agents. Further studies are required to confirm our observations, which could enable targeting of specific PKC isoforms for therapeutic benefit in cervical neoplasia.

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