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Original Article

Aberrant expression of casein kinase 1δ (CK1δ) in cervical squamous cell carcinoma

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Abstract: Cervical cancer is one of the most common gynecological carcinoma, which seriously threaten the life and health of women globally. Previous studies have shown that β-catenin abnormalities in the expression and localization is closely related to the the pathogenesis and development of cervical cancer. In the Wnt/β-catenin signaling pathway, casein kinase 1 (CK1) protein family had both inhibitory and activated functions. As one of the seven isoforms of the CK1 family, CK1δ function is poorly defined. Here, by using tissue microarray, we found that, compared with control (chronic cervicitis tissue), CK1δ protein expression level was significantly elevated in 88 cervical squamous cell carcinoma (CSCC) tissues (7.7% vs. 58.0%, P<0.001). The increased CK1δ expression was associated with deep cervical stromal invasion in patients with cervical cancer (P=0.015). Besides, the expression levels of CK1δ and β-catenin in cervical cancer tissues was positively correlated in CSCC tissues (P<0.001). Therefore, we hypothesized that CK1δ overexpression may contribute to cervical cancer progression through activating the Wnt/β-catenin signaling pathway. Based on above experimental results, we can get a deeper understanding of the role of CK1δ in the Wnt/β-catenin signaling pathway and cervical cancer, and to find new biomarkers and therapeutic targets for the diagnosis of cervical cancer.

Keywords: Cervical cancer, CK1δ, β-catenin, tissue microarray, diagnosis

Introduction

Cervical cancer is the second most frequent women malignancy worldwide [1, 2]. Although the effective screening, diagnosis and treatment can greatly reduce the incidence and mortality of cervical cancer in developing countries, cervical cancer is still a great threat to women health. From the level of histology, normal cervical epithelium (NCE) become cervical squamous cell carcinoma (CSCC) is the need to go through a series of precancerous lesions, including low-grade squamous intraepithelial lesion (LSILs) evolution of high-grade squamous intraepithelial lesion (HSILs) [3]. At present, several biomarkers of diagnosing CSCC have been found by histological means, however, there is a great difference among different observers [4]. Therefore, it is urgent to find new and specific molecular markers for the diagnosis and treatment of cervical cancer.

The abnormal expression and localization of transcriptional factor β-catenin in the Wnt signaling pathway is closely related to the oncogenesis and development of cervical cancer [5, 6]. β-catenin can accelerate the formation of HPV-16 induced cervical cancer in mouse model [7]. Interestingly, the casein kinase1 (CK1) protein family plays the positive and negative role in Wnt/β-catenin signaling pathway. On the one hand, in the absence of Wnt, CK1α is able to phosphorylated β-catenin S45 site, and then prime events of GSK3β-mediated phosphorylation of T41, S37 and S45 sites [8]. On the other hand, CK1γ-mediated phosphorylation of Wnt receptor LRP6 in T1479 and T1493 sites, which can further promote the stability of β-catenin and transcriptional activity of Wnt/β-catenin downstream target genes [9-13].

The CK1 family consists of 7 members (α, β, γ1, γ2, γ3, δ and ε), but the role of CK1δ in Wnt pathway is not clear. In this study, to investigate the function of CK1δ in Wnt/β-catenin signaling pathway and cervical cancer, we performed tis-

Table 1. Statistic analysis of immunohistochemistry (IHC) staining of CK1δ and β-Catenin from the IHC staining results in chronic cervicitis tissue and CSCC tissue microarray

Biomarkers	Chronic cervicitis *n* (%)	Cervical cancer *n* (%)	*P* value
CK1δ	2/26 (7.7)	51/88 (58.0)	<0.001
β-Catenin	16/26 (61.5)	60/88 (68.2)	>0.05

sues microarray based-immunohistochemistry to detect the expression of CK1δ and β-catenin in 26 chronic cervicitis tissue and 88 CSCC tissue samples. Furthermore, we determine the correlation of CK1δ and β-catenin expression with tumor progression in patients with variable clinicopathological characteristics.

Materials and methods

CSCC and normal tissues

88 CSCC and 26 chronic cervicitis tissues, specimens for immunohistochemistry were obtained from patients without receiving any preoperative chemotherapy or radiotherapy at Jiangxi Provincial Maternal and Child Health Hospital between January 2008 and January 2013. Then multiple tissue microarrays were made from the paraffin-embedded sections.

Immunohistochemistry and judgment of results

The paraffin-embedded tissue microarrays sections were baked in the oven at 65°C for 12 h. After deparaffinization and blocking, the antigen-antibody reaction was incubated overnight at 4°C. The primary anti-CK1δ rabbit monoclonal antibody (Abcam) and anti-β-catenin (Abcam) were both used at a dilution of 1:100. Two independent pathologists who were blinded to the clinicopathological information and corresponding slides of patients evaluate the immunohistochemical staining of CK1δ and β-catenin. A semiquantitative scoring method was followed: based on the staining intensity (0, negative staining; 1, weak staining; 2, moderate staining; 3, strong staining) and the proportion of immunopositive cells (1, <25%; 2, 25-50%; 3, 50-75%; 4, ≥75%). The final score for CK1δ and β-catenin expression was the production of the two above-mentioned scores, ranging from 0 to 12. For the statistical analysis, a final staining index of 0-4 represented negative CK1δ and β-catenin expression, whereas a staining index of 5-12 represented positive CK1δ and β-catenin expression.

Statistical analyses

Statistical analyses were performed using SPSS 13.0 software. The results of immunohistochemistry were analyzed with χ^2 test and Spearman rank correlation test. All *P*-values were two-tailed, and *P*-values of 0.05 were considered to indicate statistical significance.

Results

Expression level and cellular distribution of β-catenin and CK1δ in tissue microarray

In this study, we first evaluated expression level and cellular distribution of β-catenin in 26 normal squamous epithelial samples and 88 CSCC samples by immunohistochemistry. In normal squamous epithelial samples, positive staining was strongly observed in majority cases (61.5%, 16 out of 26. **Table 1**) at the plasma membrane of both basal and parabasal cells (**Figure 1A-C**). In CSCC samples, positive staining of β-catenin (68.2%, 60 out of 88. **Table 1**) was found predominantly at the cytoplasm; however, nuclear expression was also rarely observed (**Figure 2A-D**). Moreover, these results are consisting with previous reports [5, 14].

Subsequently, we performed immunohistochemistry to investigate the expression level and cellular distribution of CK1δ in tissue microarray. Only 2 case out of 26 chronic cervicitis tissue samples (7.7%, 2 out of 26. **Table 1**) expressed CK1δ positive immunoreactivity (**Figure 1D-F**). By contrast, most CSCC cases (58%, 51 out of 88. **Table 1**) showed strong cytoplasmic staining, as well as the staining pattern of β-catenin in CSCC samples (**Figure 2E-H**). Besides, there was a strong correlation between the levels of β-catenin and CK1δ in CSCC tissues (**Table 2**). Taking together, these data further suggest that CK1δ is overexpression in CSCC tissues and is involved in activation of Wnt/β-catenin signaling pathway.

Relationship between clinicopathological variables and β-catenin and CK1δ in CSCC tissue microarray

Representative immunohistochemical staining of β-catenin and CK1δ in CSCC tissue microarray is shown in **Figure 2**. Positive staining of β-catenin and CK1δ in tumor cell were mainly localized within the cytoplasm. As shown in **Table 3**, the overexpression of β-catenin and

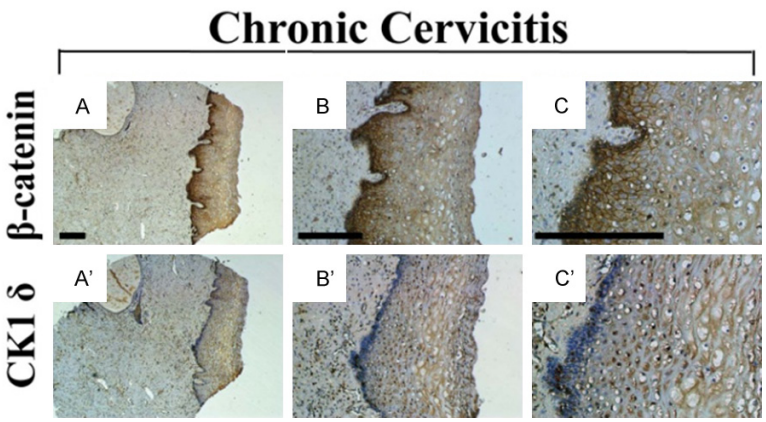

Figure 1. Low expression of CK1δ in human chronic cervicitis tissue microarray. A-C. Representative IHC staining results for β-Catenin in human chronic cervicitis tissue microarray. Scale bar =200 μm. A. Magnification is 5×; B. Magnification is 10×; C. Magnification is 20×. A'-C'. Representative IHC staining results for CK1δ in human chronic cervicitis tissue microarray. Scale bar =200 μm. A'. Magnification is 5×; B'. Magnification is 10×; C'. Magnification is 20×.

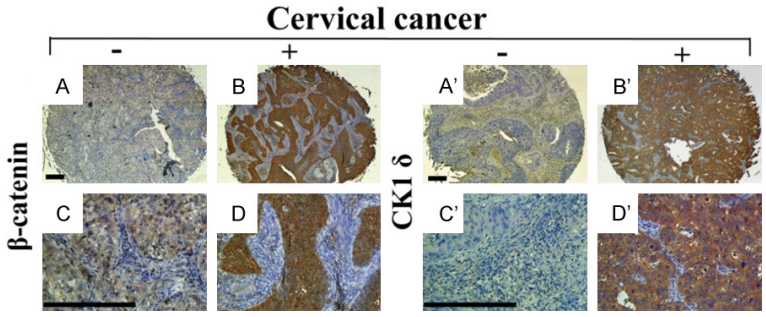

Figure 2. High expression of CK1δ in human CSCC tissue microarray. A-D. Representative IHC staining results for β-Catenin in human CSCC tissue microarray. Scale bar =200 μm. A, B. Magnification is 5×; C, D. Magnification is 20×; A, C. Representative negative staining; B, D. Representative positive staining. A'-D'. Representative IHC staining results for CK1δ in human CSCC tissue microarray. Scale bar =200 μm. A', B'. Magnification is 5×; C', D'. Magnification is 20×; A', C'. Representative negative staining; B', D'. Representative positive staining.

Table 2. Statistic analysis for CK1δ and β-Catenin correlation from the IHC staining results in CSCC tissue microarray

		CK1δ		Total
		Negative	Positive	
β-Catenin	Negative	22	6	28
	Positive	15	45	60
Total		37	51	88

rs=0.505, P<0.001.

CK1δ was correlated with depth of stromal invasion (P=0.014 and P=0.015, respectively), in addition, only the expression of β-catenin was correlated with vascular cancer embolus (P=0.031), However, no significant correlation was observed between β-catenin and CK1δ overexpression and other clinicopathological factors, including age (P=0.342 and P= 0.373 respectively), differentiation grade (P=0.866 and (P=0.285 respectively), Figo stage (P=0.080 and P=0.251 respectively), lymph node metastasis (P=0.234 and P= 0.513 respectively).

Discussion

The Wnt/β-catenin signaling pathway plays critical roles in tumorigenesis and metastasis in different types of cancer [15, 16]. Stabilization of β-catenin upregulates downstream target genes [17, 18]. Degradation of β-catenin is first regulated by phosphorylation of casein kinase 1α (CK1α), followed by GSK-3 [19, 20]. The CK1 family, which is evolutionarily conserved serine-threonine kinases, consists of seven isoforms in mammals (α, β, γ1, γ2, γ3, δ and ε). However, CK1δ as a member of CK1 family, its function in cancer, especially in cervical cancer remains unclear. In this study, we demonstrate that, unlike normal cervical tissues, CK1δ is overexpressed in CSCC tissue by using human tissue microarray. Moreover, CK1δ expression is associated with depth of stromal invasion. More importantly, high expression of CK1δ is correlated with β-catenin positive staining, which indicates that CK1δ promotes cervical tumorigenesis through activating Wnt/β-catenin signaling pathway.

CK1δ, Hrr25 human homologue, regulates multiple cellular processes, including DNA repair [21], microtubule dynamics [22], cell cycle [23],

Table 3. Relationship between CK1δ and β-catenin expression and clinic pathological characteristics of cervical cancer

Variables	No. of patients (n=88)	CK1δ	P*	β-catenin	P*
		Positive (%)		Positive (%)	
Age			0.342		0.373
<40	30	16 (53.3%)		19 (63.3%)	
≥40	58	35 (60.3%)		41 (70.7%)	
Differentiation grade			0.866		0.285
1	16	10 (62.5%)		9 (56.3%)	
2	69	39 (56.5%)		48 (69.6%)	
3	3	2 (66.7%)		3 (100%)	
Figo stage			0.080		0.251
I	77	42 (54.4%)		51 (66.2%)	
II	11	9 (81.8%)		9 (81.8%)	
Depth of stromal invasion			0.015		0.014
<1/2	37	16 (43.2%)		20 (54.1%)	
≥1/2	51	35 (68.6%)		40 (78.4%)	
Vascular cancer embolus			0.237		0.031
No	52	28 (53.8%)		31 (59.6%)	
Yes	36	23 (63.9%)		29 (80.6%)	
Lymph node metastasis			0.234		0.513
No	77	43 (55.8%)		52 (67.5%)	
Yes	11	8 (72.7%)		8 (72.7%)	

vesicle trafficking [24] and Wnt and Hedgehog signaling pathways [25, 26]. From our IHC results, how might activity of β-catenin regulated by CK1δ lead to activation of the Wnt pathway? Wnt ligand binds to its cell surface receptor, the seven-pass transmembrane protein Frizzled (Fz), the signal is transduced by Dishevelled (Dvl1-3), which interacts with Fz and is thought to activate Wnt signaling by recruiting GSK3-binding protein (GBP) to β-catenin destruction complex [27-29], thereby preventing GSK3 and inhibiting β-catenin degradation. Dvls have several potential phosphorylation sites and are substrates for CK1 and PKC [30]. Besides, several papers discover a functional connection between CK1 and Dvls [31, 32]. These previous observation indicate that CK1δ upregulates Wnt/β-catenin signaling pathway by activating Dvls.

In addition, CK1δ is overexpressed in several tumor types [33-35]. Intriguingly, our data also find that CK1δ expression is elevated in CSCC tissues. Taking together, these results indicate that aberrant expression of CK1δ is not limited to CSCC, and is worthwhile to be tested in other kinds of cancers in the future.

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Disclosure of conflict of interest

None.

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