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EXPRESSIONS OF sFRP1 AND β-CATENIN IN CERVICAL CANCER

EKSPRESIJA sFRP1 I β-KATENINA U KARCINOMU GRLIĆA MATERICE

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Summary: This study aimed to investigate the expressions of secreted frizzled-related protein 1 (sFRP1) and β-catenin in cervical cancer and cervical intraepithelial neoplasia (CIN), and to explore the relationship between both proteins and the prognosis of cervical cancer. Immunohistochemistry was performed to detect the protein expressions of sFRP1 and β-catenin in cervical cancer (n=78), CIN (n=30) and normal cervical tissues (n=20), and the relationships of sFRP1 and β-catenin with the clinicopathological characteristics and prognosis of cervical cancer were analyzed. The positive rate of sFRP1 was 100%, 70% and 33.3% in the normal cervical tissues, CIN and cervical cancer, respectively (P<0.05). The sFRP1 expression was positively correlated with the stage of cervical cancer and lymphatic metastasis (P<0.05). The 5-year survival rate was significantly higher in patients positive for sFRP1 than in those negative for sFRP1 (P<0.05). The rate of abnormal β-catenin expression in the normal cervical tissues, CIN and cervical cancer was 5%, 43.3% and 70.5%, respectively (P<0.05). The abnormal β-catenin expression was positively correlated with the stage of cervical cancer, lymphatic metastasis and pathological grade (P<0.05). The 5-year survival rate was markedly higher in patients with normal β-catenin expression than in those with abnormal β-catenin expression (P<0.05). The sFRP1 expression was negatively related to the β-catenin expression in cervical cancer (r = –0.557, P<0.001). Both sFRP1 and β-catenin play important roles in the initiation and development of cervical cancer, and both proteins can be used as indicators predicting the prognosis of cervical cancer.

Keywords: cervical cancer, secreted frizzled-related protein 1, β-catenin, immunohistochemistry, prognosis

Kratak sadržaj: U ovoj studiji ispitivana je ekspresija sFRP1 i β-katenina u karcinomu grlića materice i cervikalnoj intraepitelnoj neoplaziji (CIN), kao i odnos između ova dva proteina i prognoze u karcinomu grlića materice. Imunohistohemijskim putem utvrđena je ekspresija proteina sFRP1 i β-katenina u karcinomu grlića materice (n=78), CIN (n=30) i zdravom cervikalnom tkivu (n=20). Analizirana je povezanost sFRP1 i β-katenina sa kliničko-patološkim karakteristikama i prognozom u karcinomu grlića materice. Pozitivna stopa sFRP1 bila je 100%, 70% i 33,3% u zdravim cervikalnim tkivima, CIN i karcinomu grlića materice (p<0,05). Ekspresija sFRP1 pozitivno je korelisala sa stadijumom karcinoma grlića materice i limfnim metastazama (p<0,05). Stopa petogodišnjeg preživljavanja bila je značajno viša kod pacijenata pozitivnih na sFRP1 nego kod onih kod kojih je sFRP1 bio negativan (p<0,05). Stopa abnormalne ekspresije β-katenina u zdravim cervikalnim tkivima, CIN i karcinomu grlića materice bila je 5%, 43,3% i 70,5% (p<0,05). Abnormalna ekspresija β-katenina bila je u pozitivnoj korelaciji sa stadijumom karcinoma grlića materice, limfnim metastazama i patološkim stepenom (p<0,05). Stopa petogodišnjeg preživljavanja bila je izrazito viša kod pacijenata sa normalnom ekspresijom β-katenina u odnosu na one sa abnormalnom ekspresijom β-katenina (p<0,05). Ekspresija sFRP1 bila je u negativnom odnosu sa ekspresijom β-katenina u karcinomu grlića materice (r= –0,557, p<0,01). I sFRP1 i β-katenin igraju važne uloge u nastanku i razvoju karcinoma grlića materice i oba proteina se mogu upotrebljavati kao pokazatelji u predviđanju prognoze kod obolelih od karcinoma grlića materice.

Ključne reči: karcinom grlića materice, izlučeni protein-1 srodan ukovrdžanim receptorima, β-katenin, imunohistohemija, prognoza

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Introduction

Cervical cancer is the second most common malignancy threatening the health of women. Epidemiology shows the age of patients with cervical cancer is decreasing. Evidence has demonstrated that human papillomavirus (HPV) infection is a key pathogenic factor in cervical cancer and precancerous lesions. However, the HPV infection in the majority of patients may resolve spontaneously, and only a minority of patients positive for HPV develop cervical cancer (1). The findings suggest the HPV infection is not the unique cause of cervical cancer. In recent years, the role of the Wnt signaling pathway in the pathogenesis of tumors has been a focus in the studies on tumors. Secreted frizzled-related protein 1 (sFRP1) is a negative regulator of the Wnt signaling pathway and exerts a suppressive effect on the Wnt signaling pathway. β-catenin is a key element of the Wnt signaling pathway and plays an important role in its regulation. In the present study, the protein expressions of sFRP1 and β-catenin in cervical cancer, cervical intraepithelial neoplasia (CIN) and normal cervical tissues were determined and the role of both proteins in the initiation and development of cervical cancer as well as the relationship between both proteins and the prognosis of cervical cancer were analyzed.

Subjects and Methods

Subjects

The paraffin-embedded cervical tissues (n=128) were obtained from the First Affiliated Hospital of Zhengzhou University. These cervical tissues included 20 normal cervical tissues, 20 tissues of CIN and 78 cervical cancer tissues. In addition, patients with cervical cancer had complete follow-up data. The histological types of cervical cancer included squamous cell carcinoma (n=60) and adenocarcinoma (n=18). A total of 34 patients had stage I cervical cancer, 31 had stage II cancer and 13 had stage III ~ IV cancer. The pathological grades of these cervical cancers included G1–G2 (n=54) and G3 (n=24). Furthermore, 21 patients had lymph node metastases. The median age of these patients was 41 years (25–65 years). All patients were pathologically diagnosed and chemotherapy and radiotherapy were not administered before surgery.

Immunohistochemistry

The rabbit anti-human sFRP1 antibody, rabbit anti-human β-catenin antibody (Beijing Leagene Biotech, Co, Ltd), immunohistochemistry kit (SP method), diaminobenzidine (DAB) and antigen retrieval solution (Beijing Zhongshan Golden Bridge Biotech Co, Ltd) were used in the present study. Detection was performed according to manufacturer's instructions. The known tissues positive for sFRP1 and β-catenin served as positive controls and the primary antibody was replaced with PBS in the negative controls.

Assessment of results

(1) sFRP1: The positive cells had yellow granules in the cytoplasm and on the cell membrane. Five fields were randomly selected at a magnification of 400× under a light microscope and a total of 100 cells were counted in each field. The scoring according to the percentage of positive cells was performed as follows: 0, ≤2%; 1, 2–20%; 2, 21–50%; 3, ≥50%. The scoring according to the staining intensity was undertaken as follows: 0, not stained; 1, slightly stained; 2, highly stained. The sum of both scores was used as the protein expression: ≤1.9, negative (-); 2–2.9, weakly positive (+); 3–3.9, positive (++); ≥4, strongly positive (+++)(2). (2) β-catenin: Normally, β-catenin was found on the cell membrane. Five fields were randomly selected at a magnification of 400× under a light microscope and a total of 100 cells were counted in each field. The scoring according to the percentage of positive cells was performed as follows: 0, <25%; 1, 25–50%; 2, 51–75%; 3, ≥75%. The scoring according to the staining intensity was undertaken as follows: 0, not stained; 1, slightly stained (light yellow); 2, highly stained (dark yellow); 3, extremely stained (brown). The sum of both scores was used as the protein expression: 0, negative (–); 1–2, weakly positive (+); 3–4, positive (++); ≥4, strongly positive (+++). In addition, the fact that the β-catenin in >10% of cells was expressed in the cytoplasm and/or nucleus was regarded as ectopic expression. »–«, »+« and ectopic expression were classified as abnormal expression, and »++« and »+++« as normal expression (3).

Statistical analysis

Statistical analysis was performed with the SPSS version 16.0 statistic software package. Comparisons among rates were done with the chi square test and the relationship was tested using Spearman rank correlation analysis. Survival rate was analyzed using the Kaplan-Meier method and Log-rank analysis. A value of $P<0.05$ was considered statistically significant.

Results

sFRP1 expression and the clinicopathological characteristics of cervical cancer

sFRP1 was mainly expressed in the cytoplasm and on the cell membrane and characterized by brown granules. All the normal cervical tissues (n=20) were positive for sFRP1 with the positive rate of 100% (*Figure 1A*). Among 30 CIN tissues, 21 were

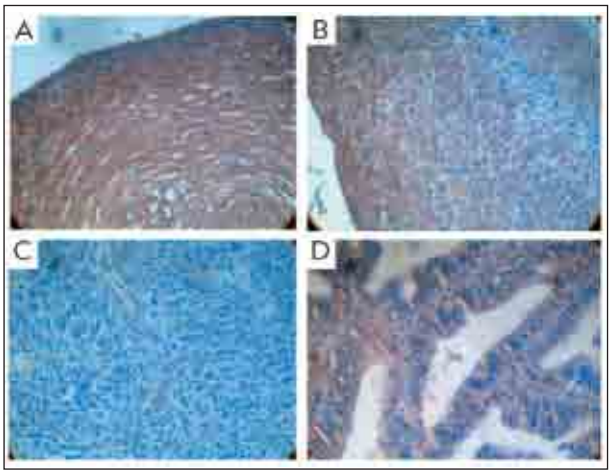

Figure 1 Expression of sFRP1 in different tissues (×400). A: normal cervical tissues; B: CIN; C: cervical squamous cell carcinoma; D: cervical adenocarcinoma.

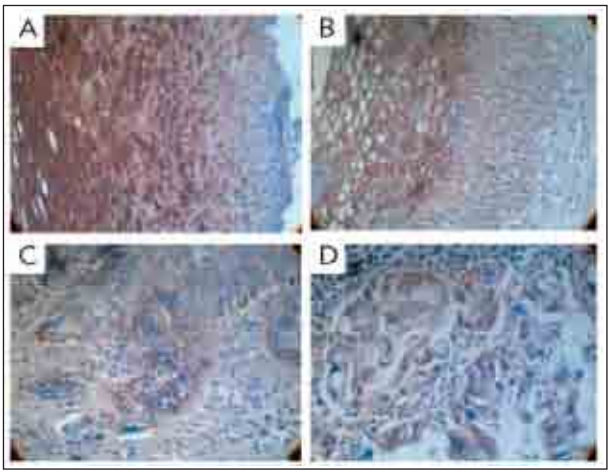

Figure 2 Expression of β-catenin in different tissues (×400). A: normal cervical tissues; B: CIN; C: cervical squamous cell carcinoma; D: cervical adenocarcinoma.

Table I Relationship between the expressions of sFRP1 and β-catenin and clinicopathological characteristics of cervical cancer.

Clinicopathological characteristics	n	Positive expression of sFRP1			Abnormal β-catenin expression		
		n (%)	χ^2	*P*	n (%)	χ^2	*P*
Stage			8.886	0.012[a]		9.550	0.008[a]
I	34	17 (50.0)	4.010	0.045[b]	18 (52.9)	5.558	0.018[b]
II	31	8 (25.8)	0.902	0.342[c]	25 (80.6)	0.263	0.608[c]
III – IV	13	1 (7.7)	5.446	0.020[d]	12 (92.3)	4.723	0.030[d]
Pathological grade							
G1–G2	54	19 (35.2)			32 (59.3)		
G3	24	7 (29.2)	0.271	0.603	23 (95.8)	10.689	0.001
Lymph node metastasis							
yes	21	14 (66.7)			19 (90.5)		
no	32	9 (28.1)	7.668	0.006	12 (37.5)	14.656	0.000
Pathological types							
squamous cell carcinoma	60	18 (30.0)			43 (71.7)		
adenocarcinoma	18	8 (44.4)	1.300	0.254	12 (66.7)	0.166	0.683

a: among three groups; b: between stage I and stage II; c: between stage II and stage III – IV; d: between stage I and stage III – IV.

positive for sFRP1 with a positive rate of 70% (21/30), but the sFRP1 was weakly expressed in these tissues (*Figure 1B*). Of the 78 cervical cancer tissues, only 26 were positive for sFRP1 with a positive rate of 33.3% (26/78), and the sFRP1 expression was very weak (*Figure 1C* and *1D*). Significant difference in the sFRP1 expression was noted among the three groups (2=33.26, P<0.05) and between any two groups (P<0.05).

In patients with stage I, II and III – IV cervical cancers, the positive rate of sFRP1 expression was 50%, 25.8% and 7.7%, showing significant difference (χ^2=8.89, P<0.05). The sFRP1 expression in stage I cervical cancer was markedly higher than that in stage II and III – IV cervical cancers (P<0.05). Moreover, the patients with lymph node metastases had a higher sFRP1 expression than those without metastases (66.7% vs 28.1%, P<0.05). The sFRP1 expression was not related to the clinicopathological grade and histological types (P>0.05) (*Table I*).

β-catenin expression and the clinicopathological characteristics of cervical cancer

In normal cervical epithelial cells, β-catenin was mainly expressed on the cell membrane and characterized by brown granules. In the present study, only one case had extremely weak staining in normal controls, but the nucleus was not stained (*Figure 2A*). In CIN and cervical cancer, β-catenin was abnormally expressed which was characterized by the weak staining or absence of staining of the cell membrane and obvious staining of the cytoplasm or nucleus (*Figure 2B*, *2C* and *2D*). The rate of abnormal expression had an increasing trend from normal cervical tissues, to CIN, to cervical cancer, and a significant difference in the abnormal expression of β-catenin was noted among the three groups (χ^2=29.56, P<0.05). Moreover, a marked difference was also found between any two groups (P<0.05).

In stage I, II and III – IV cervical cancer, the rate of abnormal expression of β-catenin was 52.9%, 80.6% and 92.3%, respectively, showing a significant difference (χ^2=9.55, P<0.05). Moreover, the rate of abnormal expression of β-catenin in stage I cervical cancer was markedly lower than that in stage II and III – IV cervical cancers (P<0.05). There was a pronounced difference in the abnormal expression of β-catenin between G1–G2 cancer and G3 cancer (P<0.05). A marked difference in the abnormal expression of β-catenin was also observed between patients with and without metastases (P<0.05). However, no relationship was found between the abnormal expression of β-catenin and the histological types of cervical cancer (P>0.05) (*Table I*).

Correlation between sFRP1 expression and β-catenin expression in cervical cancer

In tissues positive for sFRP1, 34.6% had an abnormal expression of β-catenin, but as high as 88.5% had an abnormal expression of β-catenin in the tissues negative for sFRP1 showing a marked difference (P<0.001). Correlation analysis showed there was a negative relationship between an sFRP1 expression and an abnormal expression of β-catenin (r=–0.557, P<0.001).

Relationship between sFRP1 and β-catenin expressions and prognosis of cervical cancer patients

Of the 78 patients with cervical cancer, 26 were positive for sFRP1 and 52 negative for sFRP1. The 5-year survival rate of patients positive for sFRP1 was markedly higher than that of patients negative for sFRP1 (79.8% vs 60.5%, P<0.05). In addition, 23 had a normal expression of β-catenin and 55 an abnormal expression of β-catenin. Moreover, the 5-year survival rate of patients having a normal expression of β-catenin was significantly higher than that of patients with an abnormal expression of β-catenin (83.7% vs 59.2%, P<0.05).

Discussion

The sFRP1 gene is mapped onto chromosome 8p12 and encodes a protein of 30 kDa. In the stage of embryonic development, sFRP1 is involved in the formation of the eyes, brain and vascular system and the cell differentiation. In adults, sFRP1 is mainly expressed in the choroids of the brain, the retina, lens and ciliary body and the endothelial cells, and participates in the metabolism of these tissues (4). The sFRP1 is a kind of secreted glycoprotein and serves as an inhibitor the of Wnt signaling pathway. sFRP1 has a cysteine-rich domain (CRD). The sequence at the N-terminal end is similar to that of the Wnt receptor (Frizzled, Frz). sFRP1 can competitively bind to the Wnt protein or bind to the Frz forming a non-functional complex which then suppresses the Wnt signaling pathway. The sFRP1 gene plays important roles in many biological processes, including anti-tumor processes and pro-apoptosis (5). Chunga et al. (6) found that the sFRP1 gene is a tumor suppressor gene in cervical cancer. The epigenetic silencing of sFRP1 may activate the oncogene in the classic Wnt signaling pathway. Overexpression of sFRP1 by transfection has been found to effectively inhibit the proliferation, metastasis and invasion of CaSki cells. Studies showed sFRP1 was not expressed in a variety of solid tumors including cancer, breast cancer and renal cell carcinoma (7–9).

In the present study, immunohistochemistry was done to detect the sFRP1 expression in the normal cervical tissues, CIN and cervical cancer. Our results showed the sFRP1 expression had a decreasing trend (100%→70%→33.3%) with the increase of tumor grade. Significant differences in the sFRP1 expression were found between any two groups (P<0.05). This suggests sFRP1 may be involved in the progression from CIN to cervical cancer. In addition, the positive rate of sFRP1 expression had a decreasing trend (50%→ 25.8%→7.7%) with the increase of tumor stage. Stage I cervical cancer had a markedly higher positive rate of sFRP1 expression than did stage II and III – IV (P<0.05). This finding implies sFRP1 may be involved in the invasion and metastasis of cervical cancer.

The β-catenin gene is one member of the catenin family and a multifunctional protein encoded by CTNNB1. The molecular weight of β-catenin is 90 kDa and the β-catenin gene is located on the chromosome 3P22. Evidence has demonstrated that β-catenin has dual functions of mediating cell adherence and signal transduction. β-catenin in the cytoplasm exists in two forms: one is bound to the E-cadherin involved in the regulation of intercellular adherence; the other is free β-catenin involved in the Wnt signal transduction. The free β-catenin can translocate from the cytoplasm to the nucleus and then bind to the transcription factor Tcf/Lef which then activates the target gene in the Wnt signaling pathway such as

c-myc, cyclin D1, etc. This process is involved in the regulation of cell proliferation and the occurrence of tumors (10).

Nakopoulou et al. (11) detected the β-catenin expression in the breast cancer of 141 patients. Their results showed the high β-catenin expression in the nucleus was related to the invasion and poor prognosis of breast cancer. Wanitsuwan et al. (12) investigated the β-catenin expression in the colon cancer of 163 patients. The results revealed that the rate of abnormal β-catenin expression (nucleus) was as high as 81.4%, which was also associated with the clinical stage and lymph node metastasis. Sangkhathat et al. (13) introduced siRNAs targeting β-catenin into the hepatoma cells which resulted in a decrease of β-catenin expression. Their results indicated the suppression of hepatoma cell proliferation. Chunga et al. (6) found that β-catenin abnormally aggregated in the cytoplasm and nucleus of CaSki cells (cervical cancer cells).

Our results showed the abnormal β-catenin expression level had an increasing trend from normal cervical tissues, to CIN, to cervical cancer, and that in cervical cancer it was markedly higher than in the other two groups. This finding implies the abnormal aggregation of β-catenin may be involved in the malignant transformation of cervical epithelial cells. Further experiments showed the abnormal expression of β-catenin was closely related to the clinical stage, pathological grade and lymph node metastasis, but not to histological types. This indicates the abnormal β-catenin expression may reflect the degree of malignancy and the potentials of invasion and metastasis. Chunga et al. (6) found β-catenin aggregation in the cytoplasm and nucleus of cervical cancer cells (CaSki cells) with a decreased sFRP1 expression. With the transfection technique, the overexpression of sFRP1 could significantly decrease the β-catenin in the nucleus. In the present study, with the decrease of the expression of sFRP1, an inhibitor of the Wnt signaling pathway, the abnormal β-catenin expression had an increasing trend. This result indicates the low expression of sFRP1 may be one of the causes of decrease of normal β-catenin expression and increase of abnormal β-catenin expression in cervical cancer. In addition, sFRP1 and β-catenin are unlikely to exert effects independently. Both proteins may play important roles in the initiation, development and metastasis of cervical cancer through co-ordination.

Our study also showed the 5-year survival rate in patients positive for sFRP1 was higher than that in patients negative for sFRP1, and the 5-year survival rate in patients with a normal β-catenin expression was higher than in those with an abnormal β-catenin expression. These findings reveal the absence of sFRP1 expression and an abnormal β-catenin expression may predict poor prognosis of cervical cancer patients. Therefore, we speculated that both proteins were associated with the prognosis of cervical cancer patients and could be used as predictors of cervical cancer.

Taken together, the expression of sFRP1, an inhibitor of the Wnt signaling pathway, was absent or weak in the cervical cancer, and the β-catenin expression was decreased in the cell membrane, but it abnormally aggregated in the cytoplasm and nucleus. These results indicate the Wnt signaling pathway is activated in cervical cancer and this pathway may be closely related to the occurrence and development of this type of cancer. The decreased expression of sFRP1 may be a cause of β-catenin aggregation in the cytoplasm and nucleus, and a cause of the activation of the Wnt signaling pathway. We postulate sFRP1 and β-catenin can be applied as predictors of cervical cancer and as targets in the biological therapy.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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