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Retrospective Study

Serum Spondin-2 expression, tumor invasion, and antitumor immune response in patients with cervical cancer

Abstract

BACKGROUND

Cervical cancer is a gynecological malignancy common in middle-aged and older patients, with a high mortality rate. Spondin-2 is an extracellular matrix protein that involved in innate and acquired immune responses. Herein, we investigated the relationship between serum Spondin-2 expression, tumor invasion and infiltration, and immune response in patients with cervical cancer and provided a theoretical basis for clinical practice.

AIM

To investigate the relationship between serum Spondin-2 expression and cervical cancer-related indicators.

METHODS

Overall, 147 patients with cervical cancer who were admitted to our institution between January 2019 and August 2019 were assigned to the cervical cancer set, and 92 patients with benign uterine lesions and 86 healthy individuals were assigned to the benign and control sets, respectively. In each set, serum Spondin-2 expression was measured, and the receiver operating characteristic (ROC) curve was determined. Patients with cervical cancer were classified into high or low Spondin-2 sets depending on the Spondin-2 threshold value used for diagnosing cervical cancer. Patient's clinical data were

collected to compare the clinicopathologic characteristics, immune cytokine levels, and prognosis of patients with varying Spondin-2 expression levels.

RESULTS

The expression level of serum Spondin-2 was significantly higher in the cervical cancer set than in the benign and control sets ($P < 0.05$). According to the ROC curve, the cutoff value of Spondin-2 used in the diagnosis of cervical carcinoma was 25.68 ± 7.11 μg/L. The proportion of patients with Federation of Gynecology and Obstetrics stage III, nerve invasion, vascular invasion, and lymph node metastasis was higher in the high Spondin-2 set than in the low Spondin-2 set ($P < 0.05$). Interleukin-5 (IL-5) and IL-4 Levels were higher in the high Spondin-2 set than in the low Spondin-2 set. In contrast, IL-2 and tumor necrosis factor-α levels were lower in the high Spondin-2 set than in the low Spondin-2 set ($P < 0.05$). After 3 years of follow-up, progression-free survival and overall survival were significantly shorter in the high Spondin-2 set than in the low Spondin-2 set ($P < 0.05$).

CONCLUSION

The expression of serum Spondin-2 is upregulated in patients with cervical carcinoma and is related to tumor invasion and infiltration, antitumor immune response, and prognosis.

Key Words: Cervical cancer; Spinal protein 2; Invasion and infiltration; Immune response; Prognosis; Malignant tumor

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Core Tip: We measured the expression of Spondin-2 in the sera of patients with cervical cancer, patients with benign uterine lesions, and healthy individuals. Spondin-2 expression was regulated in patients with cervical cancer and was related to tumor invasion and infiltration, antitumor immune response, and prognosis.

INTRODUCTION

Cervical cancer is a gynecological malignancy that is common in middle-aged and older patients; in recent years, it reportedly occurs in younger patients and seriously endangers patient health[1]. Relevant studies have indicated that cervical cancer is associated with high-risk human papillomavirus (HPV) infection and abnormal immune response, among other factors. Among these, an abnormal immune response may not be helpful in HPV clearance. It may interfere with the body's normal antitumor response, leading to invasion and metastasis, which are important factors that cause high mortality[2,3]. Molecular markers that significantly affect the immune function in cervical cancer should be identified from the molecular biology perspective to guide patients' treatment and increase their survival. Spondin-2 is an extracellular matrix protein that plays a role in innate and acquired immune responses. Its expression is currently associated with the development and spreading of several malignant tumors, including prostate cancer[4] and lung adenocarcinoma[5]. This study aimed to examine the association between serum Spondin-2 expression, tumor invasion and infiltration, and immune response in patients with cervical cancer to provide a theoretical basis for clinical practice.

MATERIALS AND METHODS

General information

We enrolled 147 patients with cervical cancer who were admitted to our hospital between January 2019 and August 2019 for this study. Inclusion criteria included patients (1) diagnosed with cervical cancer by postoperative pathological examination

according to the clinical practice guidelines for cervical cancer proposed by the National Comprehensive Cancer Network in 2019[6]; (2) aged ≥18 years; (3) with serum samples obtained preoperatively; and (4) who signed an informed consent form. Exclusion criteria included patients (1) who did not receive chemoradiotherapy or antitumor treatment prior to the study enrollment; (2) who developed endometriosis, uterine fibroids, or other serious uterine lesions along with cervical cancer; (3) with other primary malignant tumors, immune system abnormalities, liver and kidney decompensation, or serious infection; and (4) with incomplete clinical data and follow-up data. Additionally, 92 patients with benign uterine disease and 86 healthy individuals were enrolled into the benign and control sets, respectively. Patients in the cervical cancer set were aged 37–69 (51.62 ± 5.87) years; had a body mass index (BMI) of 19–25 (22.75 ± 1.63) kg/m^2; had a disease duration of 1–3 (2.17 ± 0.65) years; had the following pathological types: squamous cell carcinoma in 101 cases, adenocarcinoma in 25 cases, and adenosquamous carcinoma in 21 cases; had the following Federation of Gynecology and Obstetrics (FIGO)[7] stages: stage I in 42 cases, stage II in 49 cases, and stage III in 56 cases; had nerve invasion in 92 cases and no nerve invasion in 55 cases; had vascular invasion in 69 cases and no vascular invasion in 78 cases; and had lymph node metastasis in 87 cases and no lymph node metastasis in 60 cases. Patients in the benign set were aged 40–65 (51.12 ± 6.21) years; had a BMI of 19–25 (22.61 ± 1.82) kg/m^2; had a disease duration of 1–3 (2.24 ± 0.59) years; and developed endometriosis in 42 cases, uterine fibroids in 36 cases, and adenomyosis in 14 cases. Patients in the control group were 35–65 (52.04 ± 5.48) years and had a BMI of 20–25 (22.84 ± 1.59) kg/m^2. Among the three groups, no significant differences were observed in age or BMI ($P > 0.05$).

Method

Detection of serum Spondin-2 and immune cytokine levels: Before surgery, blood samples were collected and centrifuged at 3,000 rpm for 10 min to separate the upper serum. An enzyme-linked immunosorbent assay was used to determine the serum

levels of Spondin-2, interleukin (IL)-2, IL-4, tumor necrosis factor (TNF-α), and IL-5. The human factor antibody was added to approximately 100 μL of the standard samples and 100 μL of the test samples, and the reaction was performed at 37 °C for 90 min. After the reaction was completed, horseradish peroxidase enzyme was added to each well and incubated at 37 °C for 30 min, followed by the addition of substrate solution and the termination of the reaction. The optical density values were measured at 450 nm using a microplate reader, and the factor levels to be measured were compared using the standard curve.

All patients with cervical cancer underwent extensive hysterectomy and bilateral pelvic lymphadenectomy. Their clinicopathological data, including pathological type, FIGO stage, nerve invasion, vascular invasion, and lymph node metastasis, were collected. A receiver operating characteristic (ROC) curve was used to analyze the cutoff value for serum Spondin-2 Levels used in the diagnosis of cervical cancer, and the patients were divided into high Spondin-2 and low Spondin-2 sets to analyze the differences in the clinicopathological characteristics of patients with different expression levels.

Follow-up: The patients were followed up every 3 mo until July 30, 2022 or death. Progression-free survival (PFS) and overall survival (OS) were assessed. PFS was defined as the time until confirmed tumor recurrence or metastasis, whereas OS was defined as the time until confirmed death.

Statistical analysis

The data were analyzed using SPSS 22.0, and the formula mean ± SD was used to represent the measurement data in accordance with the normal distribution. A one-way analysis of variance was applied for comparison between sets, the Student–Newman–Keuls-q test was applied for pairwise comparison between sets, and an independent sample t-test was used for comparison between two sets. The enumeration data were expressed as percentages (%). The χ^2 test was utilized for comparison between sets. The ROC curve was developed to determine the cutoff value of serum Spondin-2 expression

for diagnosing cervical cancer. The Kaplan–Meier curve was used to analyze the PFS and OS. A *P*-value of < 0.05 was considered statistically significant.

RESULTS

Serum Spondin-2 expression in each set

The serum Spondin-2 expression level was considerably higher in the cervical cancer set ($P < 0.05$) than in the benign and control sets (Table 1).

Determination of cutoff values for Spondin-2

ROC curves were established, and the maximum Youden's index was used as the cutoff value of Spondin-2 expression in patients with cervical cancer (21.20 μg/L). The sensitivity and specificity values were 71.1% and 95.3%, respectively, and the area under the curve value was 0.866 (95%CI,0.827–0.905; $P < 0.001$) (Figure 1).

Serum Spondin-2 expression in patients with different clinicopathological characteristics of cervical cancer

According to the cutoff value, patients with Spondin-2 expression levels greater than the cutoff value were assigned to the high expression set and those with lower levels were assigned to the low expression set. The proportion of patients with FIGO stage III, vascular invasion, nerve invasion, and lymph node metastasis was higher in the high Spondin-2 set than in the low Spondin-2 set ($P < 0.05$). No significant difference was observed in the pathogenic categories between the two sets ($P > 0.05$) (Table 2).

Relationship between serum Spondin-2 expression and immune response in patients with cervical cancer

The high Spondin-2 set had higher IL-4 and IL-5 levels than the low Spondin-2 set, whereas the low Spondin-2 set had higher IL-2 and TNF-α levels than the high Spondin-2 set ($P < 0.05$) (Table 3).

Relationship between serum Spondin-2 expression and prognosis in patients with cervical cancer

After 3 years of follow-up, PFS and OS were shorter in the high Spondin-2 set than in the low Spondin-2 set ($P < 0.05$) (Table 4, Figure 2A and B).

DISCUSSION

Cervical cancer has a complicated etiology, and HPV infection is a recognized risk factor. Disturbed immune responses not only facilitate HPV infection but also cause persistent infection and increase the risk of epithelial malignant transformation[8,9]. In addition, cervical cancer is prone to peripheral nerve invasion and invasive metastasis, which are important factors that result in a poor prognosis[10]. Therefore, it is imperative to analyze the factors related to invasion and infiltration and the antitumor immune response to improve cervical cancer prognosis.

Spondin-2 is a secreted protein, which was originally discovered in ovarian cancer cells, and is linked to innate and acquired immunity as a pattern-recognition molecule and integrin ligand for pathogenic microorganisms[11]. Spondin-2 is reportedly a critical indicator of the initial response to innate immunity, but its expression levels differ in tumor and non-tumor cells[12]. Moreover, Spondin-2 expression levels were elevated in the sera of patients with prostate cancer[13], hepatocellular carcinoma[14], and pancreatic cancer[15], and high levels were associated with bone metastasis and lymph node metastasis, suggesting that Spondin-2 was also involved in tumor invasion and metastasis. This study found that serum Spondin-2 expression was significantly higher in the cervical cancer set than in the benign and control sets, suggesting that Spondin-2 expression is elevated in the sera of patients with cervical cancer and may play a role in the development of cervical tumors and their microenvironment. An ROC curve was used to determine the cutoff value for serum Spondin-2 diagnosis, and patients with cervical cancer were divided into high Spondin-2 and low Spondin-2 sets. The results showed that the number of patients with FIGO stage III, nerve invasion, vascular invasion, and lymph node metastasis was higher in the high Spondin-2 set than in the

low Spondin-2 set, implying that serum Spondin-2 expression is related to these aspects progression of cervical cancer; thus, its high expression indicates disease progression. Nerve invasion is one of the routes of tumor metastasis and mainly refers to neurotropic invasion and peripheral spread of the tumor[16]. It involves the detachment of tumor cells into the stroma and vascular system and the formation of tumor thrombi around the lesion and other tissues and organs, forming the basis of tumor metastasis; these are high-risk indicators affecting the recurrence rate of cervical cancer and patient survival[17]. Spondin-2 influences the proliferation, invasion, and metastasis of cancer cells by regulating the Wnt signaling pathway, which may be related to the invasion and infiltration of cervical cancer cells[18]. Moreover, the susceptibility of cervical cancer to invasion and infiltration may be related to immune dysfunction[12], and Spondin-2 may influence cancer cell invasion and infiltration by participating in the body's specific and non-specific immune responses. T lymphocytes mediate the body's antitumor immune response, which is crucial for HPV elimination and monitoring of abnormal growing cells; when the immune function is abnormal, malignant cervical cells cannot be recognized and removed in time, which triggers the development and progression of cervical cancer. Spondin-2 acts as an integrin ligand in the extracellular matrix and can interfere with the body's normal immune response by recruiting inflammatory cells[19]. This study revealed that IL-4 and IL-5 in high Spondin-2 group were higher than those in low Spondin-2 group, while IL-2 and TNF- α level lower than low Spondin-2 group, suggesting that Spondin-2 is involved in the antitumor immune response. CD4+ is the primary cell type that exerts specific immune effects, including T helper type 1 (Th1) and Th2 subtypes, with the former secreting IL-2 and TNF-α. In contrast, the latter secretes IL-4 and IL-5, which play an immunosuppressive role. The findings of this study indicate that Spondin-2 can inhibit the antitumor immune response and promote the immune escape of tumor cells, thus affecting the progression of the disease. After 3 years of follow-up, PFS and OS were shorter in the high Spondin-2 set than in the low Spondin-2 set, indicating that high serum Spondin-2 expression is associated with a

poor prognosis of cervical cancer and further validating the involvement of Spondin-2 in the development of cervical cancer.

CONCLUSION

In conclusion, the serum level of Spondin-2 is elevated in patients with cervical cancer and is linked to tumor invasion and infiltration, antitumor immune response, and prognosis. Thus, it is a potential novel diagnostic marker and treatment target for cervical cancer. However, this study did not investigate its specific mechanism of action in depth, and further studies should be conducted.

ARTICLE HIGHLIGHTS

Research background

Cervical cancer is a gynecological malignancy that is common in middle-aged and older patients, with a high mortality rate; it seriously endangers the health of patients. Spondin-2 is an important molecular marker that is involved in innate and acquired immune responses. This study focused on the relationship between serum Spondin-2 expression, tumor invasion and infiltration, and immune response in patients with cervical cancer to provide a theoretical basis for clinical practice.

Research motivation

The motivation of this study was to investigate the differences in serum Spondin-2 expression levels in patients with cervical cancer, patients with benign uterine lesions, and healthy subjects, as well as the relationship between serum Spondin-2 Levels, tumor invasion and infiltration, and antitumor immune response.

Research objectives

This study aimed to investigate the relationship between serum Spondin-2 expression, tumor invasion and infiltration, and antitumor immune response in patients with cervical cancer.

Research methods

We detected Spondin-2 expression in the sera of patients with cervical cancer or benign uterine lesions and in those of healthy subjects. According to the threshold of Spondin-2 used in cervical cancer diagnosis, patients with cervical cancer were divided into high Spondin-2 and low Spondin-2 groups. Clinicopathological features, immune cytokine levels, and prognosis of patients with different levels of Spondin-2 expression were compared.

Research results

The serum Spondin-2 expression level was significantly higher in the cervical cancer group than in the benign and control groups. The proportion of patients with Federation of Gynecology and Obstetrics stage III, nerve invasion, vascular invasion, and lymph node metastasis was higher in the high Spondin-2 group than in the low Spondin-2 group. The levels of interleukin (IL)-5 and IL-4 were higher in the high Spondin-2 group than in the low Spondin-2 group, whereas the levels of IL-2 and tumor necrosis factor-α were lower in the high Spondin-2 group than in the low Spondin-2 group. After 3 years of follow-up, progression-free survival and overall survival were significantly lower in the high Spondin-2 group than in the low Spondin-2 group.

Research conclusions

The expression of Spondin-2 in patients with cervical cancer was upregulated, and it was associated with tumor invasion and infiltration, antitumor immune response, and prognosis.

Research perspectives

Serum Spondin-2 Levels can be used as a new diagnostic marker and therapeutic target for cervical cancer, providing a theoretical basis for clinical diagnosis and disease evaluation.

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Figure Legends

Figure 1 Receiver operating characteristic curve of Spondin-2 in the diagnosis of cervical cancer.

Figure 2 Comparison of progression-free and overall survival in patients with different serum Spondin-2 expression levels. A: Progression-free survival; B: Overall survival. PFS: Progression-free survival; OS: Overall survival.

Table 1 Serum Spondin-2 expression in each set (mean ± SD)

Set	*n*	Spondin-2 (μg/L)	*F*	*P* value
Cervical cancer set	147	25.68 ± 7.11	105.790	0.000
Benign set	92	17.52 ± 5.76		
Control set	86	14.16 ± 4.96		

Table 2 Serum Spondin-2 expression in patients with different clinicopathological features of cervical cancer

Clinical pathology	High Spondin-2 set (n = 101)	Low Spondin-2 set (n = 46)	χ^2 value	*P* value
Pathological type				
Squamous cell carcinoma	70	31	0.595	0.743
Adenocarcinoma	18	7		
Adenosquamous carcinoma	13	8		
FIGO stage				
Stage I–II	50	41	10.501	0.001
Stage III	51	5		
Nerve invasion				
Yes	72	20	10.438	0.001
No	29	26		
Vascular invasion				
Yes	57	12	11.688	0.001
No	44	34		
Lymph node metastasis				
Yes	66	21	5.075	0.024
No	35	25		

FIGO: Federation of Gynecology and Obstetrics.

Table 3 Relationship between serum Spondin-2 expression and immune response in patients with cervical cancer (mean ± SD)

Set	*n*	IL-2 (pg/mL)	TNF-α (ng/mL)	IL-4 (pg/mL)	IL-5 (pg/mL)
High Spondin-2 set	101	3.52 ± 0.83	10.62 ± 1.80	2.98 ± 0.53	4.43 ± 1.08
Low Spondin-2 set	46	4.12 ± 1.13	13.83 ± 2.56	2.45 ± 0.76	3.18 ± 0.72
t value	-	3.614	8.735	4.879	7.153
P value	-	0.000	0.000	0.000	0.000

IL: Interleukin; TNF: tumor necrosis factor.

Table 4 Relationship between serum Spondin-2 expression and prognosis in patients with cervical cancer (mean ± SD)

Set	*n*	PFS (mo)	OS (mo)
High Spondin-2 set	101	23.2 ± 9.3	26.8 ± 8.7
Low Spondin-2 set	46	28.9 ± 10.4	30.1 ± 9.7
t value	-	3.319	2.056
P value	-	0.001	0.042

PFS: Progression free survival; OS: Overall survival.

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