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Original Article

HOXC6 expression is associated with a poor prognosis in early-stage cervical squamous cell carcinoma

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Abstract: Homeobox C6 (HOXC6) is involved in malignant progression in certain cancers. However, the expression profile of HOXC6 and its clinical/prognostic significance are unclear in early-stage cervical cancer. The mRNA and protein expression levels of HOXC6 were analyzed in five cervical cancer cell lines and four corresponding cervical cancer tumors by real-time PCR and Western blotting, respectively. Immunohistochemistry was performed to detect the expression of HOXC6 in paraffin-embedded tissues from 90 early-stage cervical cancer patients, 60 cervical intraepithelial neoplasia (CIN) patients and 19 normal cervical tissues (NCTs). Statistical analyses were used to evaluate the clinicopathological significance of HOXC6 expression. In this study, HOXC6 expression was differentially increased in cervical cancer cell lines and tissues than in normal tissues and ANTs. Moreover, its expression was significantly up-regulated at protein level in early-stage cervical cancer, compared to CIN and NCTs (P=0.004). Statistical analysis showed that HOXC6 expression was significantly correlated with tumor size (P=0.044), type of tumor growth (P=0.009), stromal invasion (P=0.030), lymph node metastasis (P=0.027), positive surgical margins (P=0.038), vaginal involvement (P=0.038), postoperative adjuvant therapy (P=0.012), recurrence (P=0.044) and survival (P=0.033). Kaplan-Meier analysis indicated that patients with higher HOXC6 expression had shorter overall survival duration than patients with lower HOXC6 expression. Cox-regression analysis demonstrated that HOXC6 expression was a factor significantly associated with the 5-year DFS and OS rates for 90 SCC patients. Our findings suggest that HOXC6 expression was associated with a poor prognosis in early-stage SCC, and it may serve as a prognostic biomarker.

Keywords: Cervical cancer, prognosis, HOXC6

Introduction

Cancer statistics indicated that an estimated 527,600 new cases and 265,700 deaths of cervical cancer would occur in the world annually [1], despite its decreasing incidence and mortality in several Western countries associated with wide implementation of Papanicolaou testing and vaccines [2-4]. And nearly 90% of cervical cancer occurred in developing countries, so that it remains a critical issue in China with 98,900 new cases and 30,500 deaths in 2015 [5]. The treatment strategy for cervical cancer depends on the FIGO stage and several traditional clinical pathological variables [6]. However, traditional pathological variables are not sufficiently reliable for predicting clinical outcomes or for guiding optimal treatment strategies. Therefore, there is still an urgent need for additional research to identify novel biomarkers to supply practical information for patient prognosis and suitable therapeutic options. It can provide the earliest and the most accurate forecast information and guide clinical individualized treatment.

The homeobox (HOX) genes, key factors in the regulation of embryogenesis, encode a group of transcription factors and regulate the expression of downstream target genes via specific DNA binding [7]. They are also involved in several processes such as cellular morphogenesis and differentiation [8-11]. There are 39 different human HOX genes clustered into four different groups (HOXA, HOXB, HOXC and HOXD) [8, 9]. Recent studies have found that the expression patterns of the HOXC6 are abnormal in a variety of tumors, such as those of the breast,

Table 1. Correlation between HOXC6 expression and clinicopathologic features of early-stage cervical cancer (N=90)

Variable	N	Percentage	HOXC6 expression		Chi-square value	*p* Value
			None or low (N=22)	High (N=68)		
FIGO stage						
Ia1	5	5.56	2	3	5.58	0.409
Ia2	7	7.78	2	5		
Ib1	33	36.67	11	22		
Ib2	21	23.33	2	19		
IIa1	12	13.33	3	9		
IIa2	12	13.33	2	10		
Age (years)						
≤40	35	38.89	9	26	0.050	0.823
>40	55	61.11	13	26		
Tumor size (cm)						
<4	53	58.89	17	38	4.065	0.044
≥4	37	41.11	5	32		
Types of tumor growth						
Unclear	8	8.89	4	4	9.448	0.009[a]
Endophytic	25	27.78	10	15		
Exophytic	57	63.33	8	43		
Differentiation grade						
G1	3	3.33	0	3	1.370	0.504[a]
G2	31	34.45	9	22		
G3	56	62.22	13	43		
Stromal invasion						
<1/2	40	44.44	15	25	7.046	0.030
≥1/2	50	55.56	7	43		
Lymphovascular space invasion (LVSI)						
Yes	15	16.67	2	13	1.203	0.273
No	75	83.33	20	55		
Pelvic lymph node metastasis (PLNM)						
Yes	43	47.78	6	37	4.907	0.027
No	47	52.22	16	31		
Positive surgical margins						
Yes	33	36.67	4	29	4.284	0.038
No	57	63.33	18	39		
Parametrial infiltration						
Yes	7	7.78	2	5	0.070	0.791
No	83	92.22	20	63		
Vaginal involvement						
Yes	33	36.67	4	29	4.284	0.038
No	57	63.33	18	39		
Postoperative adjuvant therapy						
Yes (total)	53	58.89	18	35	6.323	0.012
No	37	41.11	4	33		
Recurrence						
Yes	37	41.11	5	32	4.065	0.044
No	53	58.89	17	36		
Vital status at follow-up						
Alive	52	57.78	17	35	4.536	0.033
Death from SCC	38	42.22	5	33		

[a]Fisher's exact test.

lungs, and ovaries, oral squamous cell carcinoma and esophageal squamous cell carcinoma [10-17]. In a recent study, HOXC6 was shown to play an important role in several cellular events through the regulation of its functional biological targets such as Bcl-2 and JunD [18, 19]. Although HOXC6 is critical for various regulated cellular processes and is correlated with cancer progression, the function of HOXC6 in SCC is largely unknown.

In the present study, we investigated the clinical relationship between expression pattern of HOXC6 and early-stage cervical cancer progression. We found that the HOXC6 expression was significantly increased in cervical cancer cells and tissues, and was closely associated with tumor size, type of tumor growth, stromal invasion, lymph node metastasis, positive surgical margins, vaginal involvement, postoperative adjuvant therapy, recurrence and survival. In addition, using Cox-regression analysis, the effectiveness of HOXC6 as a factor significantly associated with the 5-year DFS and OS rates for SCC patients was assessed. Collectively, HOXC6 may be a prognostic biomarker in early-stage cervical cancer.

Materials and methods

Samples and patients

The current retrospective study enrolled 90 patients diagnosed with early-stage cervical squamous cell carcinoma (SCC) who underwent radical hysterectomy and lymphadenectomy in the Department of Obstetrics and Gynecology, the First Affiliated Hospital, Sun Yat-sen University from January 2006 to December 2009. All enrolled patients were in Ia2-IIa2 stage without preoperative radiotherapy or chemotherapy and with available clinical follow-up data. The mean patient age was 42.90±7.815 (ranging from 27 to 68). Clinical stages were determined according to the International Federation of Obstetrics and Gynecology, 2009 (FIGO). The last follow-up was carried out in December 2015, with the mean observation period of 56 months (6-92 months), and there were 38 cancer-related deaths. 19 samples of normal cervix from patients undergoing simple hysterectomy because of uterine leiomyomata were obtained as controls. In addition, samples from 20 patients with CINI and 17 with CINII undergoing biopsy, and 23 with CINIII undergoing hysterectomy were also selected as controls. Four fresh cervical SCC and their corresponding adjacent noncancerous tissues (ANTs) were collected from the cervical cancer patients without preoperative radiotherapy or chemotherapy, which were used for quantitative polymerase chain reaction (qPCR) and Western blotting analysis. Prior written consent of the patients for the use of these clinical materials for research purposes, and approval from the Institutional Ethical Board (IRB) in the First Affiliated Hospital of Sun Yat-sen University were obtained. The clinical information is summarized in **Table 1**.

Cell lines

The cervical cancer cell lines, including HeLa (HOXC6-high), SiHa, C33A and HCC94 (HOXC6-low), were cultured in Eagle's minimum essential medium (Gibco BRL, Rockville, MD) and Caski cells were cultured in RPMI-1640 medium (Gibco BRL). Media were supplemented with 10% fetal bovine serum (HyClone Laboratories, Logan, UT) and 1% antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) in a 5% CO_2-humidified atmosphere at 37°C. These five cell lines were purchased from American Type Culture Collection (ATCC, MD, USA).

RNA extraction and quantitative RT-PCR

Total RNA from cultured cells and fresh tissues were extracted using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions. 2 μg RNA from each sample was used for cDNA synthesis using random hexamer primers. qPCR was used to quantify HOXC6 mRNA level. Each sample was tested in triplicate. Expression data were normalized to the geometric mean with reference to the housekeeping gene GAPDH. The HOXC6 primer sequences were 5'-AAGAACTCCATCCGCCACAAC-3' (forward) and 5'-GCTTAAACACCTGGTCCAATGTC-3' (reverse). Primers for GAPDH were 5'-ACCACAGTCCATGCCATCAC-3' (forward) and 5'-TCCACCACCCTGTTGCTGTA-3' (reverse).

Western blotting

Cell lysates from cell lines and fresh tissue were obtained using cold RIPA buffer. Total proteins (50 μg) were fractionated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose membrane for Western blotting, as

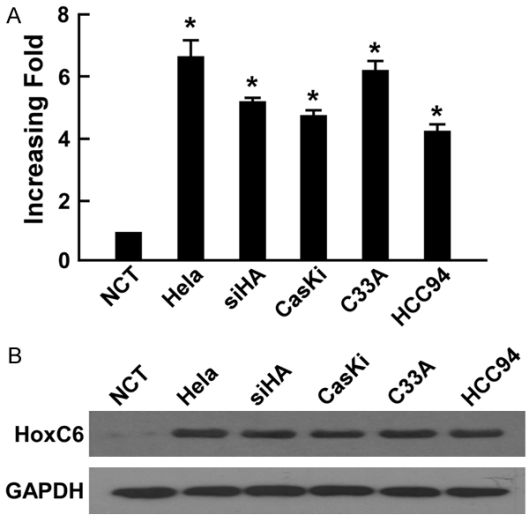

Figure 1. Overexpression of HOXC6 mRNA and protein in cervical cancer cell lines. qPCR (A) and Western blotting (B) examined the expression of HOXC6 mRNA and protein in cervical cancer cell lines (Hela, SiHa, CasKi, C33A, HCC94) and NCT. Expression levels were normalized against GAPDH. Error bars represent the standard deviation of the mean (SD) calculated from three parallel experiments. **P*<0.05.

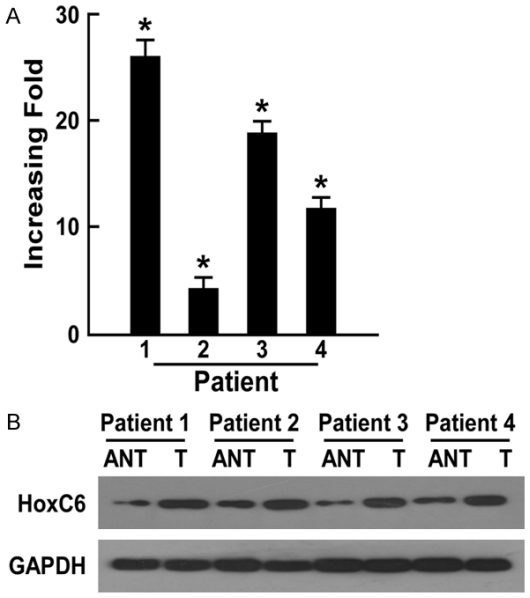

Figure 2. Overexpression of HOXC6 mRNA and protein in cervical cancer tissues. A. Average T/N ratios of HOXC6 mRNA expression in paired cervical cancer (T) and adjacent nontumor cervical tissues (ANT) were quantified using qPCR and normalized against GAPDH. Error bars represent the standard deviation of the mean (SD) calculated from three parallel experiments. B. Western blotting of HOXC6 protein expression in four pairs of matched cervical cancer (T) and adjacent nontumor cervical tissues (ANT). **P*<0.05.

described previously. Anti-HOXC6 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) antibody was used.

Immunohistochemical assay

IHC analysis was used to study altered protein expression in 90 human cervical cancer tissues, 60 cervical intraepithelial neoplasia patients and 19 normal cervical tissues. Briefly, 4-lm-thick paraffin sections of the cervical cancer tissue from the patient were baked at 65°C for 30 min and then deparaffinized with xylene and rehydrated. Submerging the sections into EDTA antigenic retrieval buffer and then microwaving were used for antigen retrieval. The samples were then treated with 3% hydrogen peroxide in methanol to quench endogenous peroxidase activity, followed by incubation with 1% bovine serum albumin to block nonspecific binding. Sections were then incubated with anti-HOXC6 antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) overnight at 4°C. Normal rabbit serum was used as a negative control. After washing, the tissue sections were then incubated with a biotinylated anti-mouse secondary antibody (Abcam), followed by further incubation with streptavidin-horseradish peroxidase complex (Abcam). The tissue sections were immersed in 3-amino-9-ethyl carbazole and counterstained with 10% Mayer's hematoxylin, dehydrated and mounted in Crystal Mount. Two observers who were blinded to the histopathological features and patient data of the samples evaluated the degree of immunostaining of formalin-fixed, paraffin embedded sections independently. The scores given by the two independent investigators were averaged and were based on both the proportion of positively stained tumor cells and the intensity of staining. The staining results were scored based on the following criteria: (i) percentage of positive tumor cells in the tumor tissue: 0 (0%), 1 (1-10%), 2 (11-50%), 3 (51-70%) and 4 (71-100%); (ii) staining intensity: 0 (none), 1 (weak), 2 (moderate), 3 (strong). The staining index was calculated as: staining intensity score × proportion of positive tumor cells (range from 0 to 12). A final score ≥7 was considered high expression.

Statistical analysis

Statistical analyses were carried out using the SPSS 13.0 software package (SPSS Inc., Chicago, IL, USA). The relationship between HOXC6

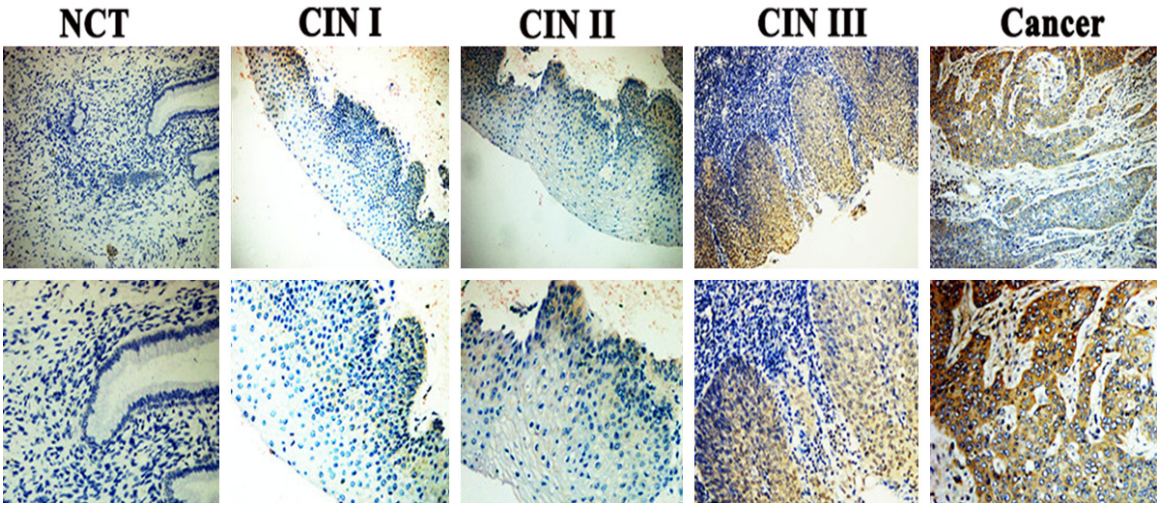

Figure 3. HOXC6 protein expression determined by immunohistochemical assay of cervical cancer patients. Among 90 patients, 68 showed positive HOXC6 staining mainly in the cytoplasm of cancer cells. HOXC6 expression gradually increased from CIN though to cervical cancer. HOXC6 expression was undetectable in NCTs, marginal in CINI, moderate in CINII and CINIII, and strong in cervical cancer tissues.

expression and clinicopathological characteristics was assessed using the chi-square test and Fisher's exact test. Bivariate correlations between study variables were calculated by Spearman's rank correlation coefficients. Survival curves were estimated using the Kaplan-Meier method and compared using the log-rank test. Multivariate survival analysis was carried out for all the parameters that were significant in the univariate analysis. The type of Cox model chosen by us was the forward method. In all cases, a *P* value of <0.05 was considered statistically significant.

Result

Elevated expression of HOXC6 in cervical cancer cell lines and cervical carcinoma

To investigate the characteristic of HOXC6 expression in cervical carcinoma, we comparatively analyzed the HOXC6 mRNA and protein profiles in different cervical cancer cell lines and samples. Western blotting and qPCR analyses revealed elevated expression levels of HOXC6 protein and mRNA, respectively, in all five cervical carcinoma cell lines compared to NCTs (**Figure 1**). Furthermore, comparative analysis showed that HOXC6 mRNA and protein levels were differentially up-regulated in all four cervical carcinoma samples compared with corresponding ANTs derived from the same patient (**Figure 2**). With these findings, our results demonstrated that HOXC6 is up-regulated in cervical cancer.

Association between HOXC6 expression and clinical features in cervical carcinoma

To further investigate the clinical significance of the above finding in cervical carcinoma, the expression of HOXC6 was examined by IHC in 90 paraffin-embedded, archived cervical cancer tissues, 20 cases of CINI, 17 cases of CINII, 23 cases of CINIII, and 19 NCTs. An overview of HOXC6 expression and clinicopathological parameters is given in **Table 1**. The positive expression rate of HOXC6 in CINI, CINII, CINIII and cervical cancer were 15.0% (3/20), 29.4% (5/17), 56.5% (13/23) and 75.6% (68/90). HOXC6 expression gradually increased from CINI through to cervical cancer, but was absent in NCTs ($P<0.001$), and the subcellular location of HOXC6 was primarily mainly in the cytoplasm of cancer cells (**Figure 3** and **Table 2**).

Statistical analyses were used to examine the correlation between HOXC6 expression and the clinicopathological characteristics of early-stage SCC patients. As shown in **Table 2**, analysis exhibited that HOXC6 expression was strongly correlated with tumor size ($P=0.044$), type of tumor growth ($P=0.009$), stromal invasion ($P=0.030$), lymph node metastasis (PLNM, $P=0.027$), positive surgical margins ($P=0.038$), vaginal involvement ($P=0.038$), postoperative

Table 2. HOXC6 expression in normal cervical tissue (NCT), CINI, CINII, CINIII and cervical squamous cell caner (SCC)

	N	HOXC6 expression		Chi-squre value	*P* value	Trend value	*P* value
		None or low (N=80)	High (N=89)				
Case							
NCT	19	19	0	55.262	0.000	54.537	0.000
CINI	20	17	3				
CINII	17	12	5				
CINIII	23	10	13				
SCC	90	22	68				

Table 3. Spearman analysis of correlation between HOXC6 and clinicopathological characteristics

Variables	HOXC6 expression level	
	Spearman correlation	*P* value
Types of tumor growth	0.324	0.002
Tumor size (cm)	0.213	0.044
Stromal invasion	0.238	0.024
Pelvic lymph node metastasis	0.233	0.020
Positive surgical margins	0.218	0.039
Recurrence	0.213	0.044
Vital status at follow-up	0.225	0.033

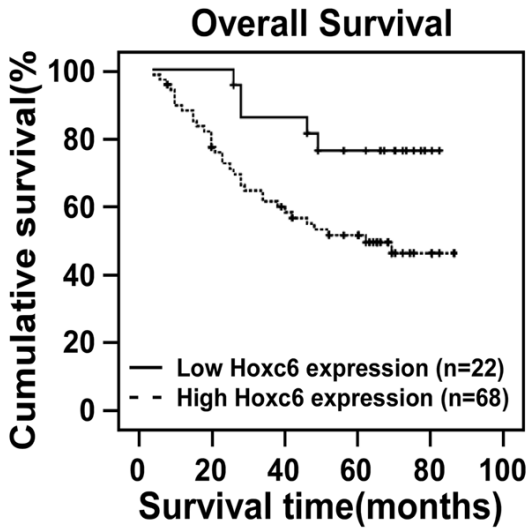

Figure 4. Kaplan-Meier curves with univariate analyses (log-rank) for cervical cancer patients with low versus high HOXC6 expression. The cumulative 5-year survival rate was 77.3% in the low HOXC6 protein expression group (n=22; thick line), but only 51.5% in the high-expression group (n=68; dotted line).

adjuvant therapy (*P*=0.012), recurrence (*P*=0.044) and survival (*P*=0.033). Moreover, Spearman correlation analysis (**Table 3**) confirmed that high HOXC6 expression level was correlated with tumor size (*R*=0.213, *P*=0.044), type of tumor growth (*R*=0.324, *P*=0.002), stromal invasion (*R*=0.238, *P*=0.024), PLNM (*R*=0.233, *P*=0.020), positive surgical margins (*R*=0.218, *P*=0.039), and survival (*R*=0.225, *P*=0.033). However, there were no significant associations between HOXC6 high expression and other clinical features including FIGO stage, age, differentiation grade, lymphovascular space invasion (LVSI), and parametrial infiltration.

Uni- and multi-variable analysis of clinicopathologic factors associated with 5-year OS and DFS rates of SCC patients

Kaplan-Meier analysis and the log-rank test were used to calculate the effect of HOXC6 expression on survival. The log-rank test revealed a significantly inverse correlation between high HOXC6 expression level and patient survival. Patients with low HOXC6 expression had longer survival times, whereas those with high HOXC6 expression had shorter survival times (**Figure 4**, log-rank, *P*=0.019). The cumulative 5-year OS rates for the patients with high levels of HOXC6 expression were 51.5%, whereas the rates were 77.3% for the patients with low or no HOXC6 expression.

Univariable analysis using log-rank tests showed that HOXC6 expression (*P*=0.001, *P*=0.012), FIGO stage (*P*<0.001, *P*<0.001), tumor size (*P*<0.001, *P*<0.001), stromal invasion (*P*=0.012; *P*=0.016), LVSI (*P*<0.001, *P*<0.001), PLNM (*P*<0.001, *P*<0.001) and parametrial infiltration (*P*=0.012, *P*=0.016) as factors significantly associated with the 5-year DFS and OS rate for 90 SCC patients. Multivariable analysis showed that tumor size (*P*=0.013, *P*=0.012), LVSI (*P*=0.025, *P*=0.013) and PLNM (*P*=0.017, *P*=0.027) were factors significantly associated with the 5-year DFS and OS rate for these patients. Thus, according to our findings that tumor size, LVSI and PLNM served as the independent prognostic factors significantly

Table 4. Cox-regression univariate and multivariate analyses of various prognostic parameters in patients with early-stage SCC

	Disease-free survival (DFS)				
		Univariate analyses		Multivariate analyses	
	N	*P*	Regression coefficient (95% CI)	*P*	HR (95% CI)
HOXC6					
High	68	0.017	2.670 (1.037-6.875)	0.268	0.571 (0.212-1.539)
None or low	22				
FIGO stage					
I stage	66	<0.001	2.052 (1.569-2.683)	0.052	0.395 (0.155-1.008)
II stage	24				
Tumor size (cm)					
<4	53	<0.001	5.171 (2.522-10-603)	0.013	0.256 (0.087-0.754)
≥4	37				
Stromal invasion					
<1/2	40	0.012	0.321 (0.133-0.776)	0.234	0.499 (0.159-1.567)
≥1/2	50				
Lymphovascular space invasion (LVSI)					
Yes	15	<0.001	0.252 (0.127-0.504)	0.025	2.835 (1.139-7.053)
No	75				
Pelvic lymph node metastasis (PLNM)					
Yes	43	<0.001	0.205 (0.096-0.436)	0.017	2.734 (1.194-6.261)
No	47				
Parametrial infiltration					
Yes	7	0.012	0.321 (0.133-0.776)	0.973	1.020 (0.316-3.295)
No	83				
	Overall survival (OS)				
		Univariate analyses		Multivariate analyses	
	N	*P*	Regression coefficient (SE)	*P*	HR (95% CI)
HOXC6					
High	68	0.012	2.912 (1.136-7.467)	0.187	0.516 (0.193-1.377)
None or low	22				
FIGO stage					
I stage	66	<0.001	2.006 (1.549-2.596)	0.052	0.417 (0.173-1.007)
II stage	24				
Tumor size (cm)					
<4	53	<0.001	5.407 (2.710-10.789)	0.012	0.239 (0.079-0.726)
≥4	37				
Stromal invasion					
<1/2	40	0.016	0.342 (0.142-0.821)	0.313	0.556 (0.178-1.737)
≥1/2	50				
Lymphovascular space invasion (LVSI)					
Yes	15	<0.001	0.269 (0.134-0.540)	0.013	3.154 (1.274-7.870)
No	75				
Pelvic lymph node metastasis (PLNM)					
Yes	43	<0.001	0.217 (0.105-0.451)	0.027	2.434 (1.107-5.352)
No	47				
Parametrial infiltration					
Yes	7	0.016	0.342 (0.142-0.821)	0.854	1.118 (0.340-3.683)
No	83				

Backward LR method was used for Cox Regression analysis and total cases were 90. Variables entered for analysis were: FIGO stage, age, types of tumor growth, tumor size, differentiation grade, stromal invasion, vaginal involvements, parametrial infiltration, PLNM, LVSI, postoperative adjuvant therapy, recurrence and HOXC6 expression.

associated with the 5-year DFS and OS for 90 SCC patients. Both analyses determined HOXC6 expression as being significant in affecting the 5-year DFS and OS rates (**Table 4**).

Discussion

In this study, we showed the overexpression of HOXC6 in cervical cancer cell lines and tissues at both the mRNA and protein levels. Furthermore, immunostaining analysis demonstrated HOXC6 expression was significantly up-regulated in early-stage cervical cancer, compared to CIN and NCTs. Kaplan-Meier analysis indicated that patients with higher HOXC6 expression had shorter overall survival duration than patients with lower HOXC6 expression. Cox-regression analysis demonstrated that HOXC6 expression was a factor significantly associated with the 5-year DFS and OS rates for SCC patients. Therefore, HOXC6 can be considered prognostic factors and provide a reference for clinical work.

Patients with PLNM always present higher mortality than patients without PLNM [20]. In our study, Cox-regression analysis similarly demonstrated that PLNM is an independent prognostic factor significantly associated with the 5-year DFS and OS for SCC patients. The previous study reported that 14.7% of patients with early-stage cervical cancer after standard treatment, which includes radical hysterectomy plus lymphadenectomy or co-chemoradiation, would have a recurrence and a suboptimal prognosis [21]. However, our study showed higher rates of recurrence and mortality, mainly because that the positive rate of PLNM was higher than the previous literature.

The HOX genes are a group of transcription factors related to developmental processes, embryonic morphogenesis, and differentiation [22-24]. HOXC6 has been shown to promote the proliferation of tumor cells and be poor prognostic factors for some human tumors, such as the squamous cell carcinoma of esophagus, oral, head and neck [12, 13, 19]. Recent studies described that elevated levels of HOXC6 mRNA have been demonstrated in CIN and cervical cancer [25, 26]. However, there is no study about profile of HOXC6 expression and its clinical/prognostic significance in cervical cancer. Our findings provided the first evidence that high expression of HOXC6 protein was associated with poor prognosis in early-stage SCC, and it may serve as a prognostic biomarker. First of all, HOXC6 expression gradually increased from CINI through to cervical cancer, but was absent in NCTs. Secondly, the cumulative 5-year DFS and OS rates for the patients with low or no levels of HOXC6 expression were higher than the patients with low or no HOXC6 expression. Thirdly, HOXC6 expression was a factor significantly associated with the 5-year DFS and OS rates for SCC patients. However, in the current study, multivariable analysis showed that tumor size, LVSI and PLNM as the independent prognostic factors, which not including HOXC6 expression. This is the limitation of our study, the most likely cause is that this retrospective study only enrolled 90 patients in our hospital and the sample size was relatively small. Thus, it is very necessary to organize a multicenter study in the future.

HOXC6 is a HOX family member that presumably functions by binding directly to DNA promoter elements via its homeodomain, which is a DNA binding domain located within its N terminus. Moreover, the previous study showed that HOXC6 binds to similar elements on the neural cell adhesion molecule (N-CAM) promoter [27]. Furthermore, an another study supported the new HOXC6 mechanism that involves direct binding of HOXC6 to specific promoter elements to activate Bcl-2 expression in HNSCC cells [19]. Hence, HOXC6 is critical for various regulated cellular processes and is correlated with cancer progression, and our future study needs to explore the related regulatory mechanism of tumorigenesis in SCC.

In conclusion, our study found HOXC6 was overexpressed and closely correlated with poor prognosis in early-stage cervical cancer. In addition, it may be a new therapeutic target and clinically novel indicator for predicting survival.

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Disclosure of conflict of interest

None.

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