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Original Article

Expression and clinical significance of HOXA5, E-cadherin, and β-catenin in cervical squamous cell carcinoma

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Abstract: Objectives: HOXA5 has been identified as a biomarker in pathogenesis of several cancers, such as non-small cell lung cancer (NSCLC) and breast cancer cells. The role has not been explored in cervical squamous cell carcinoma (CSCC). Methods: Tissues of 120 cases with CSCC and 30 controls with chronic cervicitis were constructed from our archived surgical pathology files and staining with HOXA5. Additional antibodies to E-cadherin and β-catenin were stained for comparison. For each marker, low expression was defined as staining score 0 to 3 points, whereas high expression referred to 4 points and above. Fifty-four patients in this research with cervical cancer were followed up for prognostic assessment. Result: HOXA5 had high expression in chronic cervicitis and low in CSCC (P=0.004). The positivity rates of HOXA5 in patients without muscular layer invasion (MLI) and lymphatic invasion (LI) was higher than that in metastasis (113 vs. 17; 117 vs. 3). Consistently, low expression of HOXA5 was more common in poorly differentiated carcinoma, CSCC subjects without MLI and LI. Expression of E-cadherin and β-catenin was parallel with the expression of HOXA5. Additionally, patients with higher expression of HOXA5 had much more favorable prognosis than those with lower expression among follow up of the 54 patients. Conclusion: In parallel with E-cadherin and β-catenin, low expression of HOXA5 was more common in CSCC patients with poor differentiation, without MLI and LI, among those which showed poor prognosis.

Keywords: HOXA5, E-cadherin, β-catenin, cervical squamous cell carcinoma

Introduction

Cervical cancer is the third most common malignancy in women worldwide, and the second leading cause of cancer deaths among females aged 20 to 39 years in less developed countries [1, 2]. Among the two types of cervical cancers, cervical squamous cell carcinoma comprises more than 90% of primary cervical cancers [3, 4]. Despite recent advances in cervical cancer prevention cervical squamous cell carcinoma remains a major cause of morbidity and mortality. Early detection and diagnosis of cervical squamous cell carcinoma greatly increases the chances for successful treatment. Therefore, it is important to find new biomarkers for early diagnosis and treatment of patients with cervical cancer.

Homeobox (HOX) genes, a subset of the HOX genes that contain a common 183-nucleotide sequence (homeobox) and encode a high conserved 60 amino-acid (homeoproteins) [5-7]. HOX genes constitute a family of regulatory genes that control embryonic development, and contribute to a variety of biological pathways [8-10]. HOX genes are found in clusters called A, B, C and D on four different chromosomes [11-13]. HOXA5 is a member of the HOX gene family, has been shown to play an important role in tumor development and progression [14-17]. Moreover, accumulating evidence suggests that misregulated expression of HOXA5 gene is associated with multiple cancers including non-small cell lung cancer, breast cancer, and acute myeloid leukemia [18-22]. However, the role of HOXA5 in cervical squamous cell carcinoma is still unclear. Therefore, we conducted this study to investigate the expression and clinical significance of HOXA5 in cervical squamous cell carcinoma development and progression.

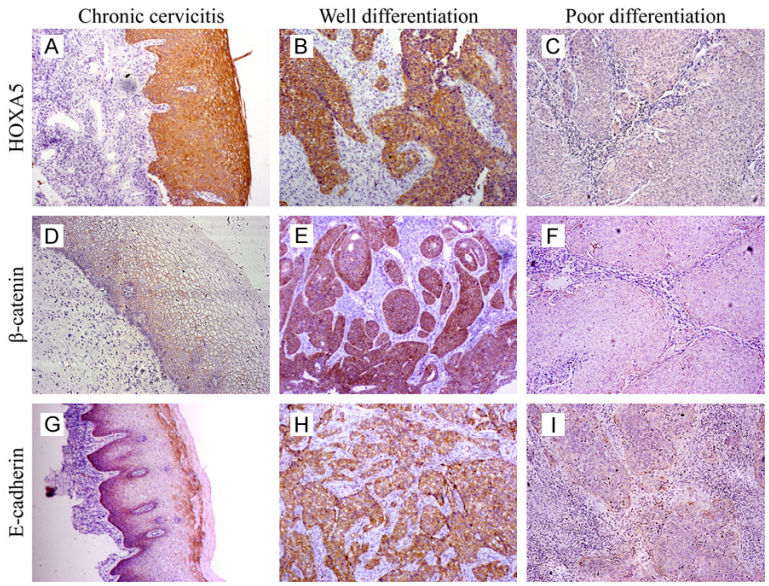

Figure 1. HOXA5 expression in chronic cervicitis tissues and cervical squamous cell carcinoma tissues (200 ×).

Materials and methods

Patients and tissue samples

Cervical squamous cell carcinoma tissues were obtained from surgical specimens resected from 120 patients who underwent surgery without any preoperative chemotherapy or radiotherapy, and 30 non-cancerous tissues from patients of chronic cervicitis at Affiliated Hospital of Guilin Medical University between April 2007 and October 2014. Histopathological diagnosis was performed according to the World Health Organization (WHO) criteria [23]. According to WHO classification, cervical squamous cell carcinoma tissues that belonged to well-moderately differentiated (n=61) and poorly differentiated (n=59) were taken. Clinicopathological staging was determined by the international TNM classification [24]. This investigation was approved by the Ethics Committee of Guilin Medical University. Written informed consent was obtained from all patients.

Immunohistochemistry

The paraffin-embedded tissue sections (4 μm) were immunostained for HOXA5, β-catenin, and E-cadherin using a polyclonal anti-HOXA5 antibody (Santa Cruz Biotechnology, CA; diluted at 1:200), a polyclonal anti-β-catenin antibody (Boster, Wuhan; diluted at 1:200) and a monoclonal anti-E-cadherin antibody (MXB, Fuzhou; diluted at 1:200), respectively. Immunohistochemistry was carried out according to the streptavidin biotin peroxidase methods. The paraffin-fixed sections were deparaffinized, hydrated, and then microwaved in EDTA buffer (PH 8.0). Subsequently, endogenous peroxidase was blocked by incubating with 0.3% hydrogen peroxide methanol for 10 min. The goat nonimmune serum (Solarbio, Beijing; diluted at 1:10) was used to remove non-specific binding. After that, the slides of samples were incubated with primary antibody at 4°C overnight. Next, horse radish peroxidase labeled primary antibody was incubated and DAB (MXB, Fuzhou, China) kits were used to visualize tantibody binding, followed by counterstaining with hematoxylin. Using PBS instead of primary antibody as a negative control, and the corresponding positive expression was positive control.

Evaluation of immunohistochemical results

Staining results were diagnosed by two pathologists who were not informed about the clinical information of the patients. E-cadherin and β-catenin are normally expressed on the cell membrane in cervical squamous epithelium, and HOXA5 is cytoplasmic staining [25-27]. Staining score evaluation criteria were as follow: A total of five representative magnifying fields (200 ×) were analyzed. (1) The percentage of positive cells fell into five categories: negative for 0, 1%-25% for 1, 26%-50% for 2, 51%-75% for 3, 76%-100% for 4; (2) The staining intensity was scored as follows: negative for 0, weakly for 1, moderately for 2, strong for 3 [28]. The final score of each slice was defined as (1) × (2). To facilitate statistical analysis, for E-cadherin, β-catenin, and HOXA5 staining score, 0 to 3 points was used for low expression, and 4 points indicated high expression.

Statistical analysis

Statistical comparisons were carried out by either X^2 test or Fisher's exact test. The overall

Table 1. Association of HOXA5, E-cadherin and β-catenin expression levels with cervical squamous cell carcinoma clinicopathological features

		HOXA5 expression			E-cadherin expression			β-catenin expression		
		High	Low	*P*-value	High	Low	*P*-value	High	Low	*P*-value
Clinicopathological features	Chronic cervicitis	20	10	P=0.004	28	2	P<0.0001	28	2	P<0.0001
	Cervical squamous cell carcinoma	45	75		49	71		41	79	
Age	<50 years	25	42		27	40		26	41	
	≥50 years	20	33		22	31		15	38	
Pathological grade	Well-moderately differentiated	31	30	P=0.002	33	28	P=0.003	28	33	P=0.006
	Poorly differentiated	14	45		16	43		13	46	
Muscular layer invasion (MLI)	MLI+	7	10		6	11		6	11	
	MLI-	38	65		43	60		35	68	
Lymphatic invasion (LI)	LI+	0	3		0	3		0	3	
	LI-	45	72		49	68		41	76	

Table 2. Relationship between the expression of HOXA5 and E-cadherin, β-catenin in cervical squamous cell carcinoma

		HOXA5 expression		*p*-value	r-value
		High	Low		
		46	74		
E-cadherin expression	High	25	24	P<0.05	0.217
	Low	21	50		
β-catenin expression	High	27	14	P<0.01	0.408
	Low	19	60		

survival time of cervical squamous cell cancer patients was evaluated by using the Kaplan-Meier method and log-rank test. All the analyses were carried out using SPSS 19.0 statistical software package (IBM, IL, USA).

Result

Expression of HOXA5 in cervical cervicitis and cervical squamous cell carcinoma

Expression of HOXA5 was detected in all patients with cervical cervicitis and CSCC by immunohistochemistry. However, the intensity of immunoreactivity showed significant difference between these two tissues (**Figure 1**). Low expression of HOXA5 was more common in cases with CSCC whereas high expression of HOXA5 among patients with chronic cervicitis (62.5 vs. 66.7%, P=0.004) (**Table 1**).

Subgroups were further established to identify the intensity of immunoreactivity of HOXA5 in cervical squamous cell carcinoma. Based on tumor differentiation, the proportion of cases with HOXA5 positivity exhibited no difference in poorly differentiated and well-moderately differentiated carcinoma. Low expression of HOXA5 was more common in poorly differentiated carcinoma tissue. According to the condition of muscular layer invasion (MLI), we found more patients with CSCC without MLI showed HOXA5 positivity compared with those with MLI (113 vs. 17). A similar finding was also observed between patients with and without lymphatic invasion (LI). Consistently, both CSCC without MLI and without LI showed low expression level of HOXA5.

Relationship between expression of HOXA5 and E-cadherin or β-catenin

E-cadherin and β-catenin have been confirmed as biomarkers in identifying cervical cancer. Therefore, we also conducted E-cadherin and β-catenin staining and further compared the relationship between expression of E-cadherin and β-catenin, and HOXA5 in patients with chronic cervicitis and CSCC (**Table 2**).

Consistent with HOXA5 staining, high expression of E-cadherin and β-catenin was detected in patients with chronic cervicitis whereas low expression was detected in CSCC. In terms of subgroups within CSCC, cases showing HOXA5 expression revealed no significant difference in well-moderately differentiated carcinoma and poorly differentiated carcinoma in both E-cadherin and β-catenin (61 vs. 59). Low expression of E-cadherin and β-catenin were more common than high expression in poorly differentiated carcinoma tissue, while well-

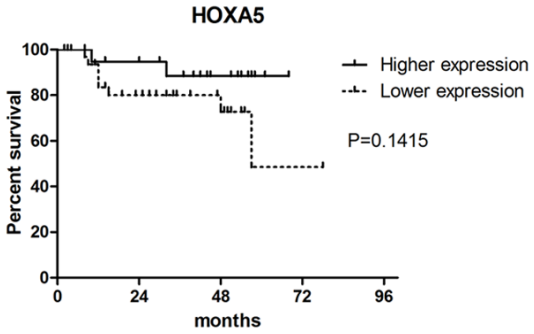

Figure 2. Kaplan-Meier curves of overall survival in cervical squamous cell carcinoma patients by HOXA5 expression level.

moderately differentiated carcinoma was on the contrary.

Follow-up study among 54 patients with cervical squamous cell carcinoma

Additionally, we followed up 54 patients with CSCC to estimate their survival rate for prognostic assessment. We found the survival rate for patients with higher expression of HOXA5 was more than 90%, contrasting to less than 50% in those with lower expression of HOXA5 (**Figure 2**).

Discussion

The occurrence of tumors, similar to early embryonic development, is an abnormal form of organogenesis. Whereas, HOX plays a key role in vertebrate embryonic development, research of HOX gene expression in tumors has become a hot spot in recent years. HOXA5, as a member of the HOX gene family, is an important transcriptional regulator which controls organismal morphogenesis [29-32]. HOXA5 have been found to have a critical role in tumorigenesis. For example, Strathdee G demonstrated that acute myeloid leukaemia (AML) samples often exhibited very high methylation levels, far greater than that seen in normal haematopoietic cells, which suggests that methylation of HOXA5 may play an important role in arrest of normal differentiation during the development of AML [33]. HOXA5 might act as a suppressor of metastasis during lung tumor progression, at least partly through inhibition of calcium-mediated actin cytoskeleton polymerization [34]. Lee pointed out that overexpression of HOXA5 alone induced very strong apoptotic response of human liposarcoma cells by caspase-dependent rather than p53-dependent [35]. Other research pointed out that during pathologic angiogenesis, sustained expression of HOXA5 regulated expression of several angiogenic effector molecules, notably increased expression of TSP-2, and reduced expression of VEGF. This leads to inhibition of pathological angiogenesis in tissues [36] and indicates that HOXA5 acts a complex role in regulating the occurrence and development of tumor.

To our knowledge, expression of HOXA5 in human cervical squamous cell carcinoma is poorly understood. Our study has, for the first time, contributed to unraveling the direct role of HOXA5 in development of cervical squamous cell carcinoma. Our results strongly indicate that HOXA5 plays an inhibitory role in cell differentiation, evidenced by immunohistochemical staining in chronic cervicitis and CSCC. In our study, HOXA5 had low expression in cervical squamous cell carcinoma, but high expression in squamous epithelium of chronic cervicitis. The expression level was related to the degree of tumor differentiation. Additionally, patients with high expression of HOXA5 had a better prognosis than those with low expression. So, we hypothesized that the low expression of HOXA5 plays a substantial role in the occurrence and development of cervical squamous cell carcinoma and it may become a new therapeutic target and diagnostic index. The relationship between HOXA5 low expression and CSCC may be related to abnormal DNA methylation at CpG island (small stretches of DNA with high frequencies of CpG sites), but the specific mechanism remains to be further studied.

Recently, Ordonez-Moran discovered that HOXA5 was suppressed by the Wnt pathway to maintain stemness and become active only outside the intestinal crypt where it inhibited Wnt signaling to enforce differentiation [37]. According to this, we proposed that HOXA5 may inhibit the occurrence and development of cervical cancer by regulating the Wnt/β-catenin signaling pathway. By immunohistochemical staining, we found that most cases with high HOXA5 expression were accompanied by E-cadherin and β-catenin membrane expression. Accordingly, cases with low HOXA5 expression, also exhibited low expression of E-cadherin and β-catenin. The possible mecha-

nism is during the process in tumorigenesis, the expression of HOXA5 was inhibited which led to abnormal Wnt signaling. Then, β-catenin lost the capability in binding with E-cadherin, and free in the cytoplasm. However, the specific mechanism of HOXA5 to inhibit the occurrence and development of cervical cancer by Wnt/β-catenin pathway remains to be elucidated.

In conclusion, this study is the first to demonstrate that HOXA5 can promote CSCC differentiation in cervical squamous cell cancer patients. In addition, the tumorous morphological change induced by abnormal expression of HOXA5 may be related with regulation of β-Catenin and E-cadherin expression. Furthermore, these results offer the possibility of using HOXA5 as a biomarker for CSCC diagnosis and treatment.

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Disclosure of conflict of interest

None.

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