RESEARCH ARTICLE

HOXB7 Predicts Poor Clinical Outcome in Patients with Advanced Esophageal Squamous Cell Cancer

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Abstract

Background: Esophageal squamous cell carcinoma (ESCC) accounts for most esophageal cancer in Asia, and is the sixth common cause of cancer-related deaths worldwide. Previous studies indicated HOXB7 is overexpressed in ESCC tissues, but data on prognostic value are limited. Methods: A total of 76 advanced ESCC cases were investigated. Immunohistochemistry (IHC) was used to detect the expression levels of HOXB7 and Kaplan-Meier curves and Cox regression models to determine prognostic significance. Stratified analysis was also performed according to lymph node (LN) status. Results: Kaplan-Meier curve analysis indicated that HOXB7 positive patients had significantly shorter overall survival (OS) than HOXB7 negative patients. Multivariate analysis using the Cox proportional hazards model indicated only TNM stage and HOXB7 expression to be independent predictors of overall survival of advanced ESCC patients. HOXB7 indicated poor OS in both lymph node negative (LN−) and lymph node positive (LN+) patients. Conclusion: HOXB7 predicts poor prognosis of advanced ESCC patients and can be applied as an independent prognostic predictor.

Keywords: HOXB7 - prognosis - advanced ESCC cases

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Introduction

Esophageal cancer is the sixth common cause of cancer-related deaths worldwide. Esophageal squamous cell carcinoma (ESCC) is the major histological type of esophageal cancer (Jemal et al., 2011). More than half of the ESCC cases occurred in China and most of them are advanced ESCC when diagnosed. Despite of recent advances of diagnosis and treatment, advanced ESCC remains an immense harm to human health due to poor 5-year survival rate (Siegel et al., 2013). Smoking, nass use, hot tea drinking, opium consumption, poor oral health, low socioeconomic status, low intake of fresh fruit and vegetables have been found associated with increased risk of ESCC (Mao et al., 2013). Since traditional methods could not provide an accurate prediction for the prognosis of advanced ESCC patients, it is very necessary and worthy to develop a more sensitive and specific biomarker. Recently, researches of genetic biomarkers have identified a lot of ESCC prognositic factors, such as CYFRA21-1, CEA, hemoglobin and microRNAs (Zhang et al., 2013; Zhao et al., 2013). However, improvement of existing prognosis system and discovery of new prognositic factors are still important and full of clinical significance.

Homeobox genes (HOX) encode a large family of transcriptional factors, which are essential for embryonic development and tumorigenesis (Inamori et al., 1993; Corsetti et al., 1995). HOX genes are divided into 2 groups: Class I contains 39 members, which are respectively arranged in 2, 12, 17, 7 chromosomes and are divided into 4 paralogous clusters (HOXA-HOXD); Class II are scattered on other different chromosomes (Shah and Sukumar, 2010; Cillo et al., 2011). The expression of HOX genes keeps to temporal and spatial colinearity in embryonic development (Shah and Sukumar, 2010). Aberrant expression of HOX genes was found in various types of cancer, such as ovarian cancer (Yamashita et al., 2006), leukemia (Chang et al., 1997), breast cancer (Jin et al., 2012), cervical cancer (How et al., 2013), prostate cancer (Rubin et al., 2007), melanoma (Care et al., 1996), oral squamous cell cancer (De Souza Setubal Destro et al., 2010) and esophageal squamous cell cancer (Gu et al., 2009). However, the detailed relationship between HOX genes and tumorigenesis remains unclear (Shah and Sukumar, 2010).

It has been demonstrated that aberrant expression of HOXB7, one of class I HOX genes, plays a crucial role in tumorigenesis. HOXB7 was also found deregulated in myeloma (Novak et al., 2010), lung cancer (Yuan et al., 2014), oral squamous cell cancer (Xie et al., 2013) and many other types of cancer (Srebrow et al., 1998; Liao et al., 2011; Nguyen Kovochich et al., 2013; Braig et al., 2010). Moreover, overexpression of HOXB7 was associated with poor prognosis of breast cancer (Srebrow et al., 1998), colorectal cancer (Liao et al., 2011) and oral squamous cell cancer (Xie et al., 2013). Molecular and

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biochemical studies indicated that HOXB7 could promote proliferation of esophageal cancer cells (Xie et al., 2013), interact with proteins about DNA repair (Rubin et al., 2007), promote transforming ability in human breast epithelial cells (Srebrow et al., 1998).

Previously, two independent studies unanimously reported that HOXB7 was overexpressed in ESCC tissues other than normal esophageal tissues (Chen et al., 2005; di Pietro et al., 2012), suggested the potentiality of HOXB7 as a biomarker of ESCC. However, both the previous studies failed to further reveal the association of HOXB7 and prognosis of ESCC patients. In this study, we investigated the expression level of HOXB7 protein in 76 advanced ESCC tissues by immunohistochemistry and evaluated the prognostic significance of HOXB7 expression in advanced ESCC patients.

Materials and Methods

Samples and clinicopathological data

Seventy-six advanced ESCC cases were recruited in this study, including 62 males and 14 females, ages 44 to 84 years (median, 61years), with stage IIa (n=6), IIb (n=15), III (n=31), and IV (n=24) diseases. ESCC cases including in this study were all inoperable and received conventional chemotherapy in Zhongnan Hospital of Wuhan University. None of the cases had received radiotherapy or operative therapy. The tissues were obtained from the Department of Pathology of the hospital. ESCC specimens were staged according to American Joint Cancer Committee (AJCC) classification guidelines. The clinical data and follow-up were obtained from medical records and regular physical examination. Written informed consent was obtained from participants, and the process was approved by Ethics committees of Zhongnan Hospital of Wuhan University.

Immunohistochemistry

Tissue sections were treated with routine deparaffinization and hydration, and subsequently antigen retrieval was performed via microwave cooking in ethylene diamine tetraacetic acid (pH 8.0) for 30 min. Then treated with 3% hydrogen peroxide for 10 min at room temperature. Non-specific binding was blocked with 10% goat serum. Then the sections were incubated at 4 °C overnight with murine anti-HOXB7 monoclonal antibodies (Abcam, Cambridge, MA), followed by the incubation with biotin-conjugated antimouse antibody (Abcam, Cambridge, MA). After 3 washes with phosphate-buffered saline with 0.1% Tween-20, each slide was treated with diaminobenzidine (DAB) working solution at room temperature for 3–10 min, and then washed in distilled water and counterstained with hematoxylin.

Evaluation of Immunohistochemical Staining

All samples were inspected under light microscopy (APPLIED IMAGING at 200×) independently by two experienced pathologists who were blinded to clinicopathologic and outcome variables. Semiquantitative evaluation of nuclear HOXB7 protein was determined by histoscore (score from 0-3), the product of staining intensity (0: no staining; 1: weak staining; 2: moderate staining; 3: strong staining), and percent tumor cell staining (range from 0%-100%). Average histoscores from both pathologists were used, ≥2 was used to classify tumors with high HOXB7 expression, and <2 stands for low expression of HOXB7.

Table 1. Association of HOXB7 Expression with Clinicopathological Parameters

Characteristics	HOXB7 expression		χ^2	*p*-value
	-	+		
Age			0.957	0.328
<60 years	14	21		
≥60 years	21	20		
Sex			2.296	0.13
Male	26	36		
Female	9	5		
T stage			2.935	0.087
1-2	13	8		
3-4	22	33		
Lymph Node Status			0.093	0.76
Negative	14	15		
Positive	21	26		
Distant Metastasis			0.041*	0.84
Negative	32	38		
Positive	3	3		
TNM stage			0.607	0.436
I+ II	15	14		
III+IV	20	27		

*Chi-square test with Yates' continuity correction

Statistical analysis

The statistical analyses were performed using SPSS software, version 16.0 (SPSS, Chicago, IL, USA). The HOXB7 expression between cancerous and noncancerous tissue were compared using the chi square (χ^2) test. χ^2 test was also used to analyses the relationship between the clinicopathological factors and HOXB7 expression. Kaplan-Meier survival analysis was performed to evaluate the prognosis of advanced ESCC cases. Cox regression analysis was used to evaluate the association between survival and clinicopathological factors in all the advanced ESCC cases. $p<0.05$ was considered to be statistically significant.

Results

Association of HOXB7 expression and demographic features of advanced ESCC patients

A total of 76 advanced ESCC patients were included in IHC evaluation, and detailed demographic information and clinicopathological features of these cases are shown in Table 1. Overall, 41 of 76 patients (53.9%) were identified as high HOXB7 expression, similar to the previous reports. We found no significant associations between HOXB7 expression and any baseline clinicopathologic parameters of advanced ESCC patients.

Overexpression of HOXB7 indicated poor prognosis of advanced ESCC patients

Kaplan-Meier curve analysis suggested that the median survival time was 33.78 months for HOXB7-positive

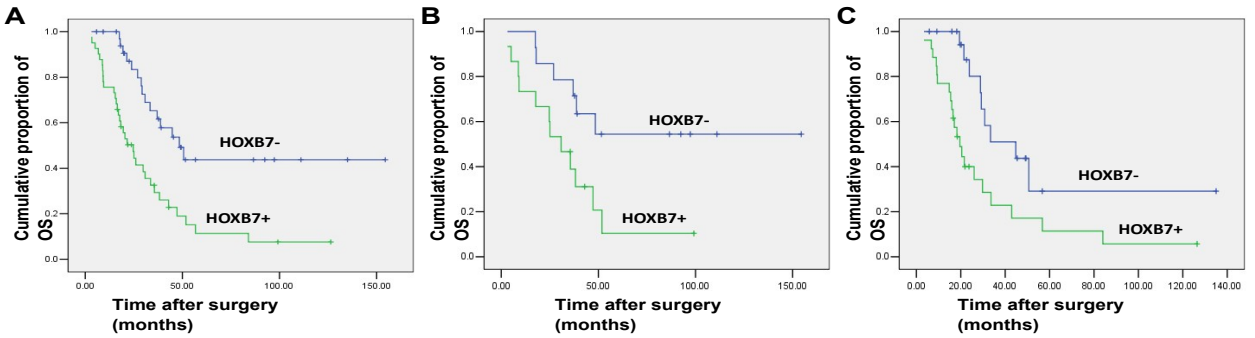

Figure 1. Kaplan-Meier Curve of OS in Advanced ESCC Patients with Different HOXB7 Status. A, All patients; B. LN- patients; C. LN+ patients

Table 2. Independent Predictors of OS in Multivariate Analysis of Advanced ESCC Patients

Characteristics	Overall Survival	
	RR (95% CI)	*p*-value
TNM stage		0.034
IIIIV vs.I, II	1.974 (1.053, 3.698)	
HOXB7		<0.001
Positive vs. Negative	3.407(1.812, 6.409)	

RR, Risk Ratio; CI, confidence interval

patients, which was significantly lower than the 85.90 months for HOXB7-negative patients (Figure 1A, log rank χ^2=13.59, p<0.001). Overexpression of HOXB7 indicated poor prognosis of advanced ESCC patients.

Univariate analysis was used to evaluate the association between clinicopathologic features and overall survival. T Stage (Wald χ^2=3.67, p=0.041), TNM stage (Wald χ^2=9.35, p<0.001), and HOXB7 expression (Wald χ^2=12.33, p<0.001) was statistically associated with poor prognosis. To rule out possible confounding factors, multivariate analysis with Cox regression model was applied. It is showed that TNM stage (Wald χ^2=4.51, p=0.034) and HOXB7 (Wald χ^2=14.47, p<0.001) expression were both independent poor predictors of overall survival time in advanced ESCC patients (Table 2).

Implication of HOXB7 expression for advanced ESCC patients, stratified by lymph node metastasis

For LN– patients, Kaplan-Meier curve analysis suggested that the median survival time was 34.59 months for HOXB7-positive patients, which was significantly lower than the 98.61 months for HOXB7-negative patients (Figure 1B, log rank χ^2=5.65, p=0.017). For LN+ patients, Kaplan-Meier curve analysis suggested that the median survival time was 31.16 months for HOXB7-positive patients, which was significantly lower than the 63.18 months for HOXB7-negative patients (Figure 1C, log rank χ^2=7.01, p=0.008).

For both LN– and LN+ advanced ESCC patients, overexpression of HOXB7 indicated poor prognosis.

Discussion

Transcriptional factors encoded by Homeobox genes, regulate cell cycle, proliferation, apoptosis and cell mobility (Shah and Sukumar, 2010). Previous studies indicated that Homeobox genes could have an essential influence on embryogenesis and tumorgenesis (Shah and Sukumar, 2010; Cillo et al., 2011). HOXB7, as one of the well studied Homeobox genes, were found involved in different types of cancer. However, two independent studies only reported its overexpression in ESCC tissues (Chen et al., 2005; di Pietro et al., 2012), but did not reveal the potential of HOXB7 as a prognostic factor of ESCC patients. Considering HOXB7 is also an essential prognostic factor of various types of cancer, we extrapolated that HOXB7 could also be used as a prognostic factor in ESCC patients.

In this study, we found that 53.9% advanced ESCC tissues were identified as high HOXB7 expression, which is in accordance with the results reported by other studies (Chen et al., 2005; di Pietro et al., 2012). Further investigation combined with clinical factors and follow-up data indicated that in advanced ESCC patients, HOXB7 overexpression was associated with poor prognosis and it is also an independent predictor of overall survival time. Additionally, stratified analysis indicated that the potential prognostic role of HOXB7 has no concern with lymph node metastasis status. Though we failed to investigate the potential molecular mechanism for the limited experimental conditions, the prognostic role of HOXB7 could be supported by several previous molecular studies suggesting its role in esophageal cancer proliferation (Xie et al., 2013), DNA repairing (Rubin et al., 2007), cell motility (Srebrow et al., 1998).

In conclusion, our results suggest that overexpression of HOXB7 could be a valuable prognostic marker of advanced ESCC, and it is also an independent prognostic predictor. However, our analyses are all based on a relatively small retrospective dataset. Thus, further investigation and validation is needed in a larger prospective cohort. Our cohort only consists of advanced ESCC patients. However, early staged ESCC patients have the same pathological mechanism as advanced ESCC patients (Xie et al., 2013), so it is also possible that HOXB7 could be prognostic in such patients, which also need further investigation.

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