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Increased Expression of Prohibitin and its Relationship with Poor Prognosis in Esophageal Squamous Cell Carcinoma

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Abstract Prohibitin, a potential tumor suppressor, has been shown to be an anti- proliferative protein, a regulator of cell-cycle progression and in apoptosis. Recently, it was found to be over-expressed in breast cancer and gastric cancer, and it has been suggested as a biomarker in those diseases. To clarify the role and the prognostic significance of prohibitin expression in esophageal squamous cell carcinoma (ESCC), we analyzed the expression in ESCC and their corresponding nonneoplastic epithelia tissues by immunohistochemistry(IHC), Western blotting and real-time quantitative reverse transcription polymerase chain reaction(QRT-PCR).The relationship between prohibitin expression and clinicopathological variables was examined by statistical analysis. The findings suggested the up-regulation of prohibitin play an important role in the carcinogenesis of ESCC. The over-expression of prihibitin was significantly correlated with the depth of tumor, lymph node metastasis, distant metastasis, lymphatic invasion and vascular invasion of ESCC. These results suggested that prohibitin(+), lymph node metastasis and distant metastasis could be the independent risk factors for worse prognosis in ESCC patients.

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Keywords Prohibitin · Esophageal squamous cell carcinoma · Prognosis · Immunohistochemistry

Abbreviations

EC	esophageal cancer
ESCC	esophageal squamous cell carcinoma
IHC	immunohistochemistry
QRT-PCR	real-time quantitative reverse-transcription polymerase chain reaction
TNM	tumor, node, metastases
AJCC	American Joint Committee on Cancer
TMA	tissue microarray
PBS	phosphate buffer solution
DAB	diaminobenzidine
PMSF	phenylmethylsulphonyl fluoride
PVDF	polyvinylidene difluoride
HRP	horseradish peroxidase
ECL	enhanced chemiluminescence
RB	retinoblastoma tumor suppressor protein
BPH	Benign Prostatic Hyperplasia

Introduction

Esophageal cancer (EC) is the eighth most common cancer in the world [1]. Esophageal squamous cell carcinoma (ESCC) is the predominant histologic subtype of EC and characterized by high mortality rate in China [2]. Though great improvement has been achieved in the field of the molecular biology, the understanding of the pathogenesis, epidemiology and behavior of ESCC, survival rate of patients with ESCC has not been evidently improved during the past 3 decades [3]. To reduce the mortality and improve therapy, many studies had been done to find biomarkers for ESCC detection at the early stage, but very

few markers were put into clinical use. Prohibitin(also called PHB) is an evolutionarily conserved gene with homologues found in organisms ranging from yeast to man. It`s evolutionary conservation and ubiquitous expression indicate that it is a fundamentally important gene; and current data suggests that it has an important function in such dissimilar processes as development, senescence, and tumor suppression [4–6]. More recently, prohibitin was also found to be over-expressed in breast cancer and gastric cancer, and it is suggested as a biomarker in those diseases [7, 8]. However, the exact biological function of prohibitin in ESCC remains largely unclear and its prognostic significance also remains to be elucidated.

To our knowledge, this is the first report that shows a significant correlation between over-expression of prohibitin and tumor behavior of ESCC. In this study, we systemically investigated the expression of PHB in ESCC and adjacent nonneoplastic tissue at both protein and mRNA levels and found prohibitin was over-expressed in ESCC tissue compared to the latter. Moreover, we found that the elevated prohibitin expression patterns paralleled the carcinogenesis processes of ESCC and had close relation with the clinicopathological data and prognosis of ESCC patients.

Patients and Methods

Tissues and Patients History

The fresh tissues were obtained from 148 patients (95 female, 53 male; median age 59.5 years; range 41–72 years) who had undergone radical esophagectomy in the Surgery Department of the Peace Hospital Affiliated to the Changzhi Medical College (Changzhi, northern China) from 26 January to 14 August, 2003 in this study. The samples comprised both liquid nitrogen snap-frozen specimens obtained immediately after surgical resection and paraffin blocks. Each sample was matched with the adjacent nonneoplastic mucosa removed during the same surgery, usually 3–10 cm away from the border of the main tumor lesion. All patients were selected at their first diagnosis; and none had received radiotherapy, chemotherapy and/or immunotherapy before esophagectomy. All ESCC and the adjacent nonneoplastic mucosa tissues were confirmed by two independent pathologists who were blinded to the original diagnosis (Dr J-S. Wang and G-Q. Pan). During this process, the strict criteria was used to diagnose the nonneoplastic mucosa tissue as there was no carcinoma, metaplasia, dysplasia, and atypical hyperplasia in them, the chronic inflammation was allowed. We collected clinical parameters including gender, age, tumor site, tumor size, pathological TNM (tumor, node, metastases) stage, cell differentiation, lymphovascular invasion and survival rate. Primary tumor staging followed the sixth edition of TNM staging system of the American Joint Committee on Cancer (AJCC). Depth of infiltration was classified into four groups as follows: pT1 to submucosa ; pT2 to muscularis propria; pT3 to adventitia and pT4 to adjacent structures [9].

All of the survival status was regularly evaluated through letters and check-ups after surgery until December 31, 2008. The mean follow-up period was 42±7 months (range, 2–70 months). We considered as uncensored only the records of patients who had died of ESCC, we considered as censored record of all patients who were alive at the end of follow-up interval or patients who died of a cause not related to ESCC. Follow-up visits were scheduled at 1-mo intervals during the first year after surgery, every 3-mo period during the second year, and every 6-mo, there after. Overall survival was defined as the interval between the dates of surgery and death. The study was approved by the Medical Ethics and Human Clinical Trial Committee of the Changzhi Medical College. The clinicopathological factors and the expression level of prohibitin are shown in Table 1.

Construction of Tissue Microarray (TMA)

The tissue microarray was constructed as described previously by Kononen et al (in collaboration with Shanghai Biochip Company, Shanghai, China) [10]. After carefully choosing the morphologically representative region with their hematoxylin-eosin -stained slides on the chosen individual paraffin-embedded blocks (donor blocks), a core tissue biopsy of 2 mm was punched and transferred to the recipient paraffin- embedded block (receiver block). After this process, the recipient blocks were incubated twice for 5 min at 56°C to improve adhesion between cores and paraffin. To overcome tumor heterogeneity and the loss of tissue, for each case, the nonneoplastic tissues and cancer tissues were repeated thrice. Then, the TMA blocks of 148 pairs of formalin-fixed, paraffin-embedded ESCC and the corresponding nonneoplastic epithelia tissues were prepared.

Immunohistochemical Staining

The immunohistochemical reactions were performed on 4 μm thick sections obtained from the TMA blocks. For immunohistochemical analysis, the slides were deparaffinized, rehydrated, then immersed in 3% hydrogen peroxide solution for 10 min; heated in citrate buffer (pH 6.0) at 95°C for 10 min; and cooled at room temperature for 30 min. The slides were blocked by 10% normal goat serum at 37°C for 30 min and then incubated with mouse

Table 1 Correlation of prohibitin expression with clinicopathological parameters

Variable		n	Prohibitin expression (%)		χ^2	*P*-value
			+	–		
Overall frequency	Nonneoplastic	148	66(44.6)	82(55.4)	7.165	0.010*
	ESCC	148	89(60.1)	59(39.9)		
Gender	Male	96	59 (61.5)	37(38.5)	0.200	0.726
	Female	52	30(47.2)	22(42.3)		
Age(yr) at surgery	≥60	91	51(56.0)	40(44.0)	1.650	0.229
	<60	57	38(66.7)	19(33.3)		
Smoking	Smokers	109	69(63.3)	40(36.7)	1.731	0.253
	Nonsmokers	39	20(51.3)	19(48.7)		
Tumor site	Upper segment	26	17(65.4)	9(34.6)	1.104	0.576
	Middle segment	80	45(56.3)	35(43.7)		
	Lower segment	42	27(64.3)	15(35.7)		
Cell differentiation	Low-grade	75	41(54.7)	34(45.3)	1.897	0.183
	High-grade	73	48(65.8)	25(34.2)		
TNM classification						
T	T1	21	7(33.3)	14(66.7)	17.198	0.001*
	T2	36	16(44.4)	20(55.6)		
	T3	52	35(67.3)	17(32.7)		
	T4	39	31(79.5)	8(20.5)		
N	N0	74	35(47.3)	39(52.7)	10.175	0.002*
	N1	74	54(73.0)	20(27.0)		
M	M0	127	71(55.9)	56(44.1)	6.679	0.014*
	M1	21	18(85.7)	3(14.3)		
Lymphatic invasion	(–)	68	31(45.6)	37(54.4)	11.105	0.001*
	(+)	80	58(72.5)	22(27.5)		
Vascular invasion	(–)	79	40(50.6)	39(49.4)	6.382	0.012*
	(+)	69	49(71.0)	20(29.0)		

*$P<0.05$

monoclonal antibody of prohibitin (1:200 dilution, Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4°C. After washed with phosphate buffer solution (PBS), the sections were treated with corresponding streptavidin-peroxidase conjugated second antibody, then color reaction was detected by diaminobenzidine (DAB) reagent. Negative and positive controls were included for all experiments. Primary antibody was replaced by PBS in negative control, the breast infiltrating ductal carcinoma sections were utilized as positive control.

Slide Evaluation of Immunohistochemical Staining

Immunostaining for prohibitin was graded by a semiquantitative method based on a scale that takes into account the intensity and distribution of the staining. Evaluation of the IHC was performed by two pathologists (J-S. Wang and G-Q. Pan) under a light microscope (Olympus BX51; Tokyo, Japan), who were not aware of the original histological diagnosis. The result of the tissues was determined from at least 1,000 cells that were counted systematically at ×400 magnification in five visual fields. In the immunohistochemistry test for prohibitin, the presence of diffuse cytoplasmic staining was considered to be significant [7]. The intensity was scored as follows: 1 (weak), 2 (moderate), or 3 (strong). The percentage of positive cells was scored as follows: 0 (0%), 1 (1–25%), 2 (26–50%), 3 (51–75%), or 4 (76–100%). The two scores were combined to obtain the final one: negative (0–2), weakly positive (3–5), or strongly positive (6–7). For statistical analysis, positive cases included both weakly and strongly positive [11, 12].

RNA Extraction and QRT-PCR

We selected 20 frozen tissue tumor samples by microscopy to ensure that at least 90% of each sample composed of tumor and matched nonneoplastic esophageal mucosa specimens which were also obtained at the same time (included in the former 148 pairs of ESCC and their corresponding nonneoplastic epithelia tissues). Total RNA of every tissue was prepared by using Trizol (ShineGene, Shanghai, China) reagent and RNA quality was verified by spectrophotometry prior to use. Two μg RNA was reverse-transcribed at 25°C for 10 min, 40°C for 60 min, 70°C for 10 min in a 20 μl reaction mixture using the EnergicScript First Strand cDNA Synthesis Kits (ShineGene, Shanghai

China). Genomic copy number of prohibitin was evaluated by QRT-PCR on a FTC2000 Real-time PCR System (Funglyn, Toronto, Canada) with Shine Sybr Real Time qPCR Kit (Shine Gene, Shanghai, China), according to the manufacturer's protocol. β-actin was applied as the internal reference. The primer sequences were designed with Primer premier 5.0 software based on the GeneBank accession numbers NM_002634.2 (gene sequence of PHB, GeneID: 5245) and NM_001101.3 (gene sequence of β-actin, GeneID: 5245). The primers used for genomic PHB detection were: 5′- GCAGGACATTGTGGTAGGGG -3′ (nt208-227) (forward),5′-GCTGGTGAAGATGCGAG GAA -3′(nt 400-381)(reverse). β- actin:5′-TGA CGGGA CATCCGCAAA G-3′(nt 945-958)(forward), 5′-CTG GAAGG TGGACAG C AGG-3′(nt 1145-1124) (reverse). The size of the product was 193 bp and 205 bp respectively. Experiments were performed in triplicate in the same reaction. Results were analyzed and relative fold-changes between transcript levels per the manufacturer's instructions. Results of the real-time PCR data are presented as C(t), which is defined as the threshold PCR cycle number at which an amplified product is first detected. The average C(t) was calculated for both prohibitin and β-actin, and ΔC(t) was determined as the mean of the triplicate C(t)s for prohibitin minus the mean of the triplicate C(t)s for β-actin. The relative copy number of prohibitin for a sample compared with β-actin was expressed as $2^{-\Delta C(t)}$. Using this method, the data are presented as the folds change in the target sample of prohibitin relative to β-actin.

Western Blotting

The tissues selected in the QRT-PCR process were performed Western blotting analysis. Briefly, the frozen tissues were thawed in ice-cold lysis buffer (50 mmol/L Tris-HCl, pH 7.4, 100 mmol/L NaCl, 1 mmol/L $MgCl_2$, 2.5 mmol/L Na_3VO_4, 1 mmol/L phenylmethylsulphonyl fluoride [PMSF], 2.5 mmol/L EDTA, 0.5% Triton X-100, 0.5% NP-40, 5 g/mL of aprotinin, pepstatin A, and leupeptin), and the lysates were sonicated and centrifuged at 10,000 g for 15 min to sediment the particulate material. The supernatants were collected and the protein concentration was measured with the BCA protein assay (Pierce Chemical, Rockford, USA) according to the manufacturer's protocol. The proteins (60 mg/sample) were separated by SDS-PAGE and then transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, USA). Blots were blocked and then probed with antibodies against prohibitin monoclonal antibody (1:400 dilution, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and β-actin monoclonal antibody (1:2000 dilution, Santa Cruz Biotechnology, Santa Cruz, CA, USA), respectively. After washing, the signal was amplified by specific secondary antibodies which conjugated to horseradish peroxidase (HRP) (1:3000 dilution, Dako, Glostrup, Denmark). Prohibitin and β-actin protein was visualized with enhanced chemiluminescence (ECL) detection system (Amersham Pharmacia Biotech, Little Chalfont, U.K.) according to the manufacturer's instructions. The β-actin was taken as loading control. The protein quantity was analyzed by UTHSCSA Image Tool Version 3.0. The prohibitin protein expression was evaluated by the relative intensity ratio of prohibitin /β-actin protein.

Statistical Analysis

All analysis was performed with SPSS 13.0 for Windows (SPSS Inc., Chicago, USA). The Chi-square test was used to analyze the correlation between prohibitin expression and clinicopathological parameters. The data are expressed as the mean ± standard deviation (SD). The Wilcoxon signed-ranks test was used to evaluate the significance of

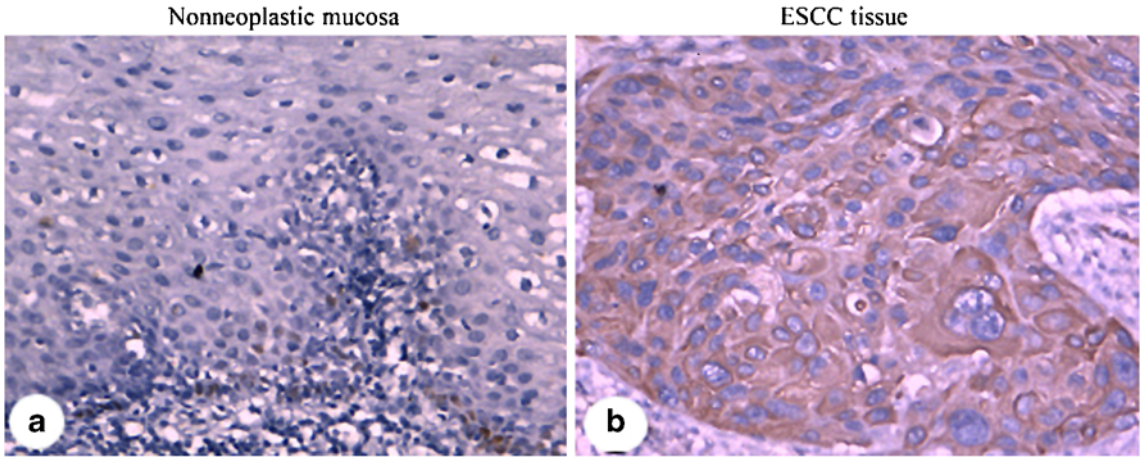

Fig. 1 Photographs of immunohistochemical staining for prohibitin in nonneoplastic mucosal tissue (**a** 400×) and its corresponding ESCC tissue (**b** 400×).

the difference in the expression level of prohibitin/β-actin mRNA and protein. Kaplan-Meier method was used to estimate survival; differences between subgroups were analyzed and compared with the log-rank test. Univariate proportional hazards regression was used to estimate the dependence of survival on each variable. Multivariate analysis was performed with the Cox proportional hazard regression model to test the variables selected by univariate analysis as having prognostic value. In all statistical analyses, $P<0.05$ was considered significant.

Results

Expression of the Prohibitin Protein and mRNA

As determined by IHC, 89 (60.1%) of 148 cases were positive for prohibitin protein expression in ESCC specimens, the positive rate for nonneoplastic epithelia group is 66/148(44.6%), significant up-regulation of prohibitin immunoreactivity was also found in ESCC tissues compared to the nonneoplastic esophageal mucosa samples ($\chi^2=7.165$,$P=0.010$).(Table 1)The predominant pattern of prohibitin staining was cytoplasm staining, typical results of ESCC tissues with positive (Fig. 1b). The nonneoplastic mucosa was mostly negative staining, at the same time few basal cells with nuclear positive reacting are shown. (Fig. 1a), but this was not calculated.

For further confirming prohibitin expression in these tissues, Western blotting and QRT-PCR were performed on 20 pairs of tumors and their nonneoplastic counterparts. The boxplots of Western blotting and QRT-PCR were shown in Fig. 2. Compared to the nonneoplastic esophageal mucosal samples, prohibitin expression in the ESCC specimens were highly variable. The analysis revealed that the levels of prohibitin/ β-actin mRNA expression in ESCC tissues were significantly higher than those in the corresponding nonneoplastic esophageal mucosal tissues (6.44±0.92 vs 4.86±0.89, $P=0.001$) (Fig.2a). Similarly, the Western blotting analysis revealed that the levels of prohibitin/β-actin protein were markedly up-regulated in ESCC tissues compared to the nonneoplastic esophageal mucosa (0.87±0.14 vs 0.46±0.12, $P<0.0001$) (Fig.2b).

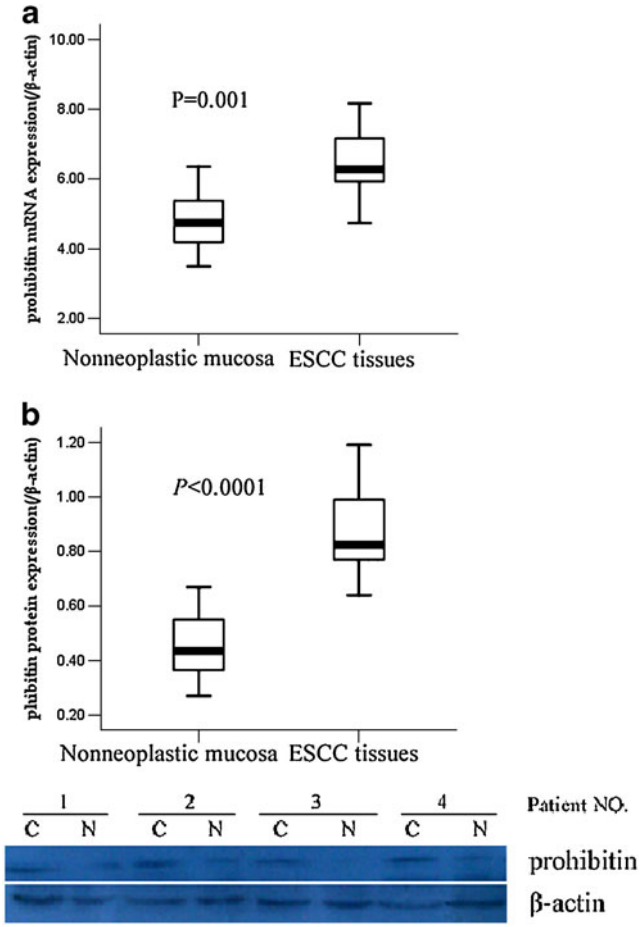

Fig. 2 **a** Prohibitin/β-actin mRNA expression level in ESCC tissues was significantly higher than that in the corresponding nonneoplastic esophageal mucosa tissues ($P=0.001$). **b** The Western blotting revealed that prohibitin/β-actin protein expression level was markedly up-regulated in ESCC tissues compared to the esophageal nonneoplastic mucosa ($P<0.0001$).The lower panel is representative of four paired ESCC tissue (signed"C") and their corresponding nonneoplastic esophageal mucosa (signed"N"), β-actin was used as a control

Relationship Between Prohibitin Expression and the Clinicopathological Findings in ESCC

The results in Table 1 also show the relationship between prohibitin expression and clinicopathological parameters. There were no statistically significant correlations between prohibitin expression and gender ($P=0.726$), age at surgery ($P=0.229$), smoking status ($P=0.253$), tumor site ($P=0.576$) and cell differentiation ($P=0.183$). The percentage of prohibitin positive expression rate increased as the tumor infiltration depth advanced; 33.3% was immunoreactive in T1 stage; 44.4% in T2; 67.3% in T3; 79.5% in T4, a significant difference was found among them ($P=0.001$). At the same time, the prohibitin positive expression rate in the group without lymph node metastasis was 35/74 (47.3%) and that of the lymph node metastasis group was 54/74 (73.0%), the positive expression rate of prohibitin increased as the lymph node metastasis rate advanced($P=0.002$). Histopathologically, evident distant metastasis was found in 3/59(5.1%) of prohibitin negative group, but a markedly higher rate 18/89(20.2%) in prohibitin positive group was detected ($P=0.014$). The rate of lymphatic invasion was also markedly higher in prohibitin positive group than that in prohibitin negative group (65.2% vs

37.3%) (P=0.001), similar to this, vascular invasion rate of prohibitin positive group was higher than the negative group (55.1% vs 33.9%) (P=0.012).These results indicated that the over-expression of prohibitin was associated with the progression of the disease.

Survival Analysis

The cumulative survival curves for the patients with positive or negative prohibitin expression are shown in Fig. 3. The median survival time for patients with negative prohibitin expression was 35 (95% CI: 24.4–45.6) months compared to only 19 (95% CI: 11.2–26.8) months for patients with positive prohibitin expression. The 1-year, 3-year and 5-year survival rates were 90.5%, 50.2%, and 38.3% in the prohibitin negative expression group (n=59) compared with 71.4%, 17.6%, and 8.1% in the positive expression group (n=89), respectively. The prohibitin negative expression group had significantly a better survival rate than the positive group (χ^2=13.281, P<0.0001).

To identify independent predictors for survival, univariate and multivariate Cox-regression analyses were performed. All the clinicopathological parameters including prohibitin protein expression level, age, gender, cell differentiation, smoking status, tumor site, invasion depth (T stage), lymph node metastasis rate (N stage), distant metastasis(M stage), lymphatic invasion rate and vascular invasion rate entered into the analysis. Univariate survival analysis of ESCC proved the following were poor prognostic factors for cancer-specific survival: prohibitin(+), T4 stage, N1 , M1 and positive for lymphatic invasion (P<0.05,Table 2). Factors with P<0.05 in univariate analysis were allowed entry into a stepwise multivariate proportional hazard model, which revealed that prohibitin (+), N1 and M1 individually contributed significantly to poor prognosis of ESCC patients.T4 stage is only significant compare to T1 stage, so it isn't an independent prognostic factor (P<0.05,Table 3).

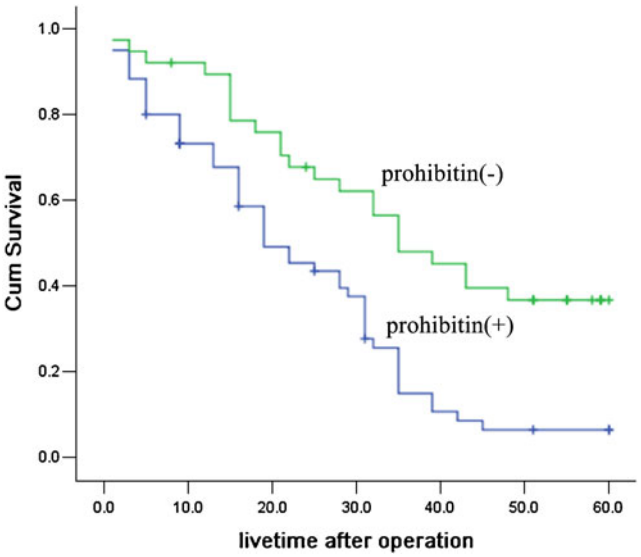

Fig. 3 Overall survival curves of patients with ESCC according to the status of prohibitin expression. (P<0.0001; χ^2=13.281)

Table 2 Univariate analysis of predictive factors for survival (Cox proportional hazards model)

Prognostic factors	Relative risk (95% CI)	P-value
Univariate		
Prohibitin(+)/(–)	1.938 (1.679–2.393)	0.003*
Gender (Male/Female)	1.109 (0.793–1.351)	0.461
Age (≥60 years/<60 years)	0.981 (0.737–1.233)	0.414
Smoking(Smoker/nonsmoker)	1.169 (0.875–1.327)	0.322
Cell differentiation (low-grade/ high-grade)	0.792 (0.473–1.019)	0.129
T stage		
T2/T1	1.237 (0.380–0.986)	0.169
T3/T1	1.219 (0.981–1.619)	0.083
T4/T1	2.016 (1.729–2.351)	0.021*
T4/T2	1.957 (1.285–2.327)	0.017*
T4/T3	1.349 (1.029–1.677)	0.031*
N stage (N1/N0)	2.595 (1.827–2.975)	0.022*
M stage (M1/M0)	2.329 (1.915–3.011)	0.006*
Lymphatic invasion (+)/(–)	1.621 (1.193–2.007)	0.039*
Vascular invasion (+)/(–)	1.473 (1.201–1.738)	0.056

CI confidence interval

*P<0.05

Discussion

ESCC of is an often-lethal disease that most commonly presents in an advanced stage with dysphagia. This might result from that the esophagus lacks a serosal covering and

Table 3 Multivariate analysis of predictive factors for survival (Cox proportional hazards model)

Prognostic factors	Relative risk (95% CI)	P-value
Prohibitin (+)/(–)	1.537 (1.129–1.907)	0.011*
T4/T1	1.735 (1.425–2.109)	0.020*
T4/T2	1.429 (1.103–1.779)	0.077
T4/T3	1.203 (0.987–1.437)	0.063
N stage (N1/N0)	2.031 (1.722–2.538)	0.035*
M stage (M1/M0)	1.875 (1.527–2.105)	0.014*
Lymphatic invasion (+)/(–)	1.327 (1.101–1.693)	0.073

CI, confidence interval

*P<0.05

thus early tumor growth causes the smooth muscles to dilate readily and the patient generally is asymptomatic [13]. So the rising incidence of ESCC and its quite poor prognosis have led to increasing interest in the research of the prognostic markers which might improving our diagnosis and evaluation of the disease. Prohibitin, a ubiquitous 32-kDa evolutionarily conserved protein, is found in a wide range of organisms such as bacteria, plants, yeast, protozoans, and mammals [14]. It was shown that prohibitin might affect the development of cancer by inhibiting the cell cycle traverse at the G1/S boundary [15]. Several studies have suggested prohibitin as a tumor suppressor gene and limiting cell proliferation [5, 16, 17]. Wang S et al. had showed that prohibitin functioned as a tumor suppressor through binding to RB family proteins and repressed E2F-mediated transcription, which played a play a major role in controlling mammalian cell-cycle progression [18].

Different to above, prohibitin was found to be over-expressed in gastric cancer tissues compared with noncancerous regions [19]. Several studies have indicated that the over-expression of prohibitin expression might play an important role in tumor development and/or progression in many types of cancers. Ummanni R's results revealed a significant up-regulation of prohibitin in prostate cancer compared to BPH [20]. Asamoto et al. found that the level of prohibitin mRNA was increased in all rat bladder tumors compared with that of normal rat bladder, but not mutations [21]. Jupe et al. detected that all breast cancer cell lines over-expressed the prohibitin mRNA and protein, and they proposed that the over-expression of prohibitin might be caused by the mutation in its 3′UTR [22]. Current evidence indicates that the role of prohibitin expression in different tumors is still controversial. And this is the exploring study to show the relationship between the alteration of prohibitin expression and the prognosis of patients with ESCC.

In our study of IHC, Western blotting and QRT-PCR, we found that ESCC tissues had a significantly higher prohibitin expression than the paired nonneoplastic mucosa at mRNA and protein levels. The further IHC study of the 148 paired tissues of ESCC revealed that the increase in prohibitin protein expression level corresponded to the differentiation degree of ESCC, a significant increase was detected in high grade ESCC tissues compared to low grade cancer tissues. Our study also found that the over-expression of prihibitin was significantly correlated with the depth of tumor invasion, lymph node metastasis, distant metastasis, lymphatic invasion and vascular invasion of ESCC. So, the over-expression the prohibitin might play an important role in the progression of ESCC.

Analysis of survival data revealed that the up-regulated expression of prohibitin was significantly associated with a poor prognosis of ESCC. In addition, by using univariate and multivariate Cox-regression analysis, we were able to demonstrate that prohibitin up-regulation, positive for lymph node metastasis and positive for distant metastasis were independent risk factors for worse prognosis in ESCC patients. These results also suggested that prohibitin immunohistochemical staining might provide clinically useful prognostic information in cases of ESCC.

The limitations of this study should also be considered when interpreting our results. The patients in our study were mainly from the Taihang area of China, a well known high-risk region of EC in the world. Cancers of the esophagus often exhibit a marked geographical location and specific race variation in incidence. And the basis for the evident disparity in EC might result from genetic factors [23]. So further studies including more patients from different areas are still needed to accommodate these findings with the function of prohibitin.

In conclusion, our results showed that the expression of prohibitin was significantly up-regulated at mRNA and protein level in ESCC tissues compared to the nonneoplastic esophageal mucosa. The over-expression of prohibitin was correlated with the progression and shorter survival of ESCC. This protein could be a useful indicator for prognosis of ESCC, as well as a potential target for more effective surveillance and treatment of the disease.

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