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Original Article

Decreased vitamin D receptor protein expression is associated with the progression and prognosis of esophageal squamous cell carcinoma: a multi-ethnic cohort study from the Xinjiang, China

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Abstract: This study investigated VDR expression levels and evaluated its clinical significance in patients with esophageal squamous cell carcinoma (ESCC). VDR expression was quantified by immunohistochemical in 362 ESCC tissues, 393 adjacent normal esophageal tissue, and 129 precancerous lesion tissues from 393 patients with ESCC. The association between VDR expression and clinicopathological factors, including prognostic outcomes, were assessed. VDR expression in normal esophageal tissue was higher than that in ESCC both in Kazakh (59.6% versus 27.9%, $P = 5.807 \times 10^{-9}$) and Han (79.9% versus 39.8%, $P = 1.893 \times 10^{-15}$). VDR expression decreased along with the neoplastic transformation from normal esophageal tissue to low-grade intraepithelial neoplasia (LGIN) ($P = 0.0012$) and high-grade intraepithelial neoplasia (HGIN) ($P = 0.00039$). Low VDR expression was associated with differentiation ($P = 0.004$), lymph node metastasis ($P = 0.010$), and TNM stage ($P = 0.001$) in Han and with differentiation ($P = 0.010$) and TNM stage ($P = 0.008$) in Kazakh. Kaplan-Meier survival analysis showed that patients with low VDR expression levels showed worse prognosis and higher cumulative hazard than those with highexpression levels ($P = 0.034$). Cox regression analysis shows that VDR deficiency is an independent prognostic factor of the short overall survival (OS) to the ESCC (hazard ratio, 0.447; 95% CI, 0.204-0.978; $P = 0.044$). VDR low expression is associated with poor prognosis of ESCC, indicating that low VDR expression may become a valuable early diagnosis biomarker for the high-risk population. Targeting VDR may offer a promising therapeutic strategy for ESCC treatment.

Keywords: ESCC, VDR, progression, prognosis, multi-ethnic cohort

Introduction

Esophageal squamous cell carcinoma (ESCC) is responsible for significant morbidity and mortality in developing countries, and more than half of all new cases of ESCC worldwide are diagnosed in China, particularly among Chinese Kazakh ethnic population residing in Xinjiang, Northwest China [1-3]. Despite advances in treatment modalities, including surgery, chemoradiotherapy, and combination therapy, the prognosis for ESCC has not significantly improved, and the 5-year survival rate for patients with ESCC is 27%, which is largely attributable to the high rates of extensive local invasion and regional lymph node metastasis [4, 5]. Hence, molecular mechanisms of ESCC, as well as the biomarkers of tumor growth and development as new prognostic and therapy targets, must be investigated in the multi-ethnic population in the Xinjiang.

The active form of vitamin D, calcitriol, is regarded as a modulator of cell proliferation, differentiation, apoptosis, angiogenesis and metastasis [6-8]. These biological behaviors are generally regulated by the vitamin D receptor (VDR), which is a transcription factor that belongs to the nuclear receptor superfamily of steroid hormones [9]. VDR expression seems to have a great influence on the antineoplastic effects of calcitriol and analogues as well as a prognostic factor in some tumors, such as, breast cancer, urothelial bladder cancer, pancreatic cancer,

Table 1. Clinicopathological demographics for the 196 patients of Han ethnic and 166 patients of Kazakh ethnic with ESCC

	Han ethnic (N = 196)	Kazakh ethnic (N = 166)
Characteristic	No. (%)	No. (%)
Age at surgery, years		
Median	57	58
Range	47-68	49-67
Gender		
Male	132 (67.3)	116 (69.9)
Female	64 (32.7)	50 (30.1)
Differentiation[a]		
Well	76 (38.8)	13 (7.8)
Moderate	73 (37.2)	106 (63.9)
Poor	47 (24.0)	47 (28.3)
Invasion depth		
T1-T2	79 (40.3)	90 (54.2)
T3-T4	117 (59.7)	76 (45.8)
Lymph node metastasis		
No	105 (53.6)	83 (50.0)
Yes	91 (46.4)	83 (50.0)
TNM stage[b]		
I+II	110 (56.1)	112 (67.5)
III+IV	86 (43.9)	54 (32.5)

[a]Histologic grade was based on WHO classification published in 2010. [b]TNM stage was assessed according to the 7th Edition of the AJCC Cancer Staging Manual.

glioblastoma multiforme, non-small-cell lung cancer [3, 10-13]. With regard to esophageal cancer, There is one study demonstrated that VDR rs2107301 T>C polymorphism which significantly increase the risk of ESCC among patients who were drinking [14]. There is another study showing an increased VDR expression in low-grade intraepithelial neoplasia (LGIN) compared with esophageal adenocarcinoma (EAC), ESCC and normal esophageal tissue, and demonstrating that the overall survival rate of patients with VDR amplification was significantly worse than that of patients whose tumors were without amplification [15]. However, to our knowledge, there are no studies of VDR expression in a multi-ethnic ESCC cohort and no information concerning the role of VDR in the prognosis of this ESCC.

Normal esophageal squamous epithelium progress to ESCC, requiring genetic and pathological changes, which involves a multistage process from noninvasive precursor lesions. Esophageal squamous intraepithelial neoplasia (ESIN) has been shown to be a histologic harbinger of ESCC, which can be divided into 2 subtypes, LGIN and high-grade intraepithelial neoplasia (HGIN), based on the extent of cytological and architectural atypia [16]. Although many reports have putted forward that ESIN has prognostic significance for esophageal carcinoma, in that dysplastic lesions are frequent emerged in malignant transformed esophageal tissues, mechanisms regulating the malignancy and progression of ESCC remain under investigation [17]. Thus, the abnormal expressions of protein found in ESIN are likely the most promising candidate's method for predicting the risk of ESCC. However, very few researches using ESIN models have focused on protein expression changes in relationship to squamous cell carcinogenesis.

In our study, we detected the VDR expression in 196 and 166 ESCC patients and 184 and 110 adjacent normal tissues of Han and Kazakh ethnicity, respectively, and 129 esophageal precancerous lesions from Han. The correlation between the VDR expression of ESCC and the clinical features of the patients was analyzed. Kaplan-Meier survival analysis was used to detect the association between VDR lowexpression and the survival rate and the cumulative hazard of the patients. Cox regression analysis was used to find the independent risk factors for poor prognosis of ESCC. In doing so, we have found that VDR expression decreased gradually with the development of ESCC both in Kazak and Han ESCC patients. The mRNA levels of VDR were significantly elevated in human esophageal normal tissue in 106 ESCC specimens (NCBI/GEO/GSE23500). The VDR expression was positively correlated with tumor differentiation and TNM stage of Kazak ESCC, and positively correlated with tumor differentiation, lymph node metastasis and TNM of Han ESCC. The survival rate of ESCC patients with highexpression of VDR was higher than that of low VDR expression patients. VDR expression deletion is an independent prognostic factor for the short overall survival of ESCC patients. In summary, these data indicate that VDR performs an inhibitory function in the development of ESCC.

Materials and methods

Patients and tumor cases

Our study included 362 patients with ESCC after the radical resection of esophageal can-

cer, including 196 cases of Han and 166 cases of Kazakh from the Affiliated Hospital of Shihezi University, Yili Xinjiang Friendship Hospital, Xinjiang Autonomous Region People's Hospital from 1984 to 2013. All patients did not receive radiotherapy, chemotherapy, and immunotherapy. At the same time, we also collected the clinical data of all patients, such as age, histological grade, degree of differentiation, depth of invasion, lymph node metastasis and clinical stage (TNM stage) (**Table 1**). The independent diagnosis of ESCC was carried out by two pathological physicians according to the cancer staging manual of American Joint Committee on Cancer. All procedures are conducted in accordance with the ethical standards of the hospital. We conducted a follow-up on 70 cases of Han and Kazak ESCC patients after radical resection of esophageal cancer by the end of March 30, 2016. Moreover, we also analyzed the VDR mRNA expression between tumor tissue and adjacent normal tissue in NCBI/GEO/GSE23500.

Tissue chip and immunohistochemical evaluation method

Tissue microarray consists of a patient's esophagus squamous cell carcinoma, adjacent normal mucosa, and precancerous lesion of the esophagus. After being fixed in formalin and embedded in paraffin tissue sections were prepared for VDR immunohistochemical staining by Envision method. Each 4 μm section was dewaxed by a gradient dimethylbenzene gradient and dehydrated by an alcohol gradient. Antigen was repaired using microwave heating for 15 min with boiling citric acid buffer (PH = 6.0), cooling to room temperature for 30 min and then eliminating endogenous peroxidase. Each slice was incubated with a diluted 1:100 mouse monoclonal anti-VDR antibody (Santa Cruz, sc-13133) at 4°C overnight. Then, the slices were washed with 1 × Tris-buffered saline with Tween 20 and incubated in Envision Two antibody 30 min at 37°C. Finally, 3,3'-diaminobenzidine and hematoxylin were used to detect immuneactivity and displaying structure, respectively.

The positive standard was the cytoplasm or nucleus showing brown granules. The semiquantitative score was applied according to the product of VDR staining intensity and positive cell percentage. Scores were provided as follows: 0 (0% to 5% positive cells), 1 (6% to 25% positive cells), 2 (26% to 50% positive cells), 3 (51% to 75% positive cells), or 4 (75% positive cells). The tumor cell immunohistochemistry staining intensity score ranged from 0 to 3:0 (negative), 1 (buff), 2 score (yellow) and 3 (brown) [18]. Scores of 0 to 4 were defined as "low-expression levels", and 4 to 12 points indicate "highexpression levels", immunohistochemical staining was assessed by two independent pathology scientists without any clinical information.

Statistical analysis

Statistical analysis was implemented using SPSS 20.0 and GraphPad Prism 5.01. The relationship between the expression level of VDR and clinical pathological factors was analyzed by Chi-square test. The results are shown by standard deviation. The correlation between clinical prognosis research and expression were analyzed by Kaplan-Meier analysis. Cox proportional hazard test was used to evaluate multivariate hazard ratios for the variables. The *P*-value < 0.05 was regarded as statistically significant.

Results

VDR overexpressed in normal esophageal tissue compared with ESCC of Kazakh patients

The expression of VDR was detected in Kazakh ESCC tissues and adjacent normal tissues by immunohistochemical staining. Interestingly, VDR expression in tumor cells was predominantly localized to the cytoplasm and the cytomembrane. However, VDR was mainly present in the nuclei and cytoplasm of normal control cells. We evaluated VDR expression in 166 Kazakh ESCC samples (**Table 1**). VDR overexpression was examined in 40 of 166 (24.1%, IS = 3.500±3.634) tumor cases and 65 of 109 (59.60%, IS = 5.349±2.891) normal tissues (**Figure 1**). As a result, VDR staining of in Kazakh ESCC samples appeared at a significantly lower level compared with adjacent normal esophageal tissue ($P < 0.001$, **Figure 1D**). VDR positive expression was found in 66.06% of normal samples. However, VDR expression was lost to 39.16% in the ESCC samples ($P = 0.148 \times 10^{-3}$) (**Figure 1E**).

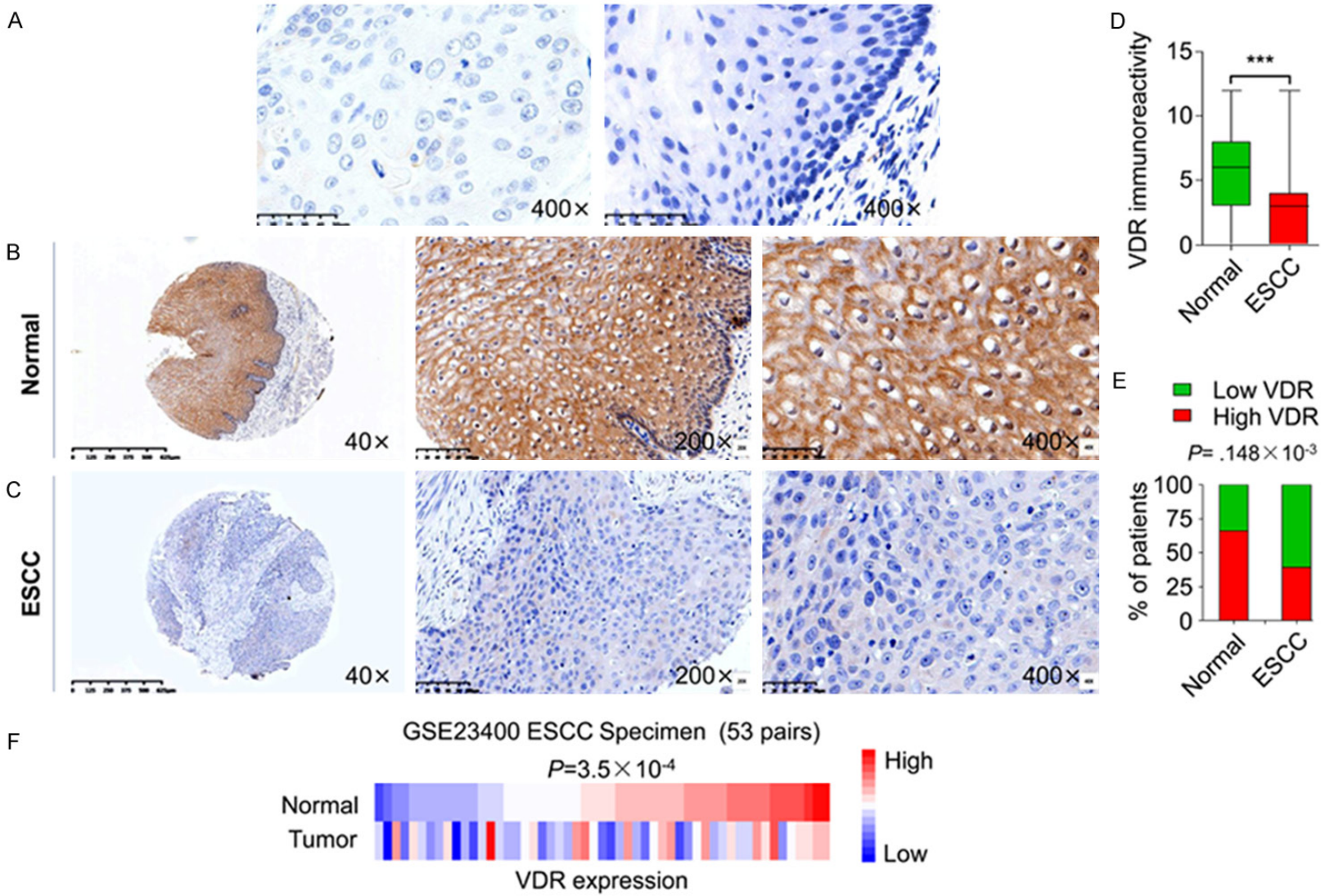

Figure 1. Immunohistochemical analysis of VDR protein in adjacent normal tissues and ESCC tissues from the Kazakh population. The negative controls for normal tissues (A left image) and esophageal cancer (A right image) specimens (magnification × 400). Typical VDR staining in normal tissues (B) and ESCC (C) from the Chinese Kazakh patients (left image magnification, × 40; middle image magnification, × 200; right image magnification, × 400). VDR staining was localized to the nuclei/cytoplasm. (D) (Box plot) Range of VDR expression score in normal and ESCC tissues (***P < 0.001). (E) VDR low expression associated with progression of ESCC (P = 0.148 × 10^{-3}). (F) The analysis of tumor tissues and adjacent normal tissues in NCBI/GEO/GSE23400 of VDR expression (P = 3.5 × 10^{-4}).

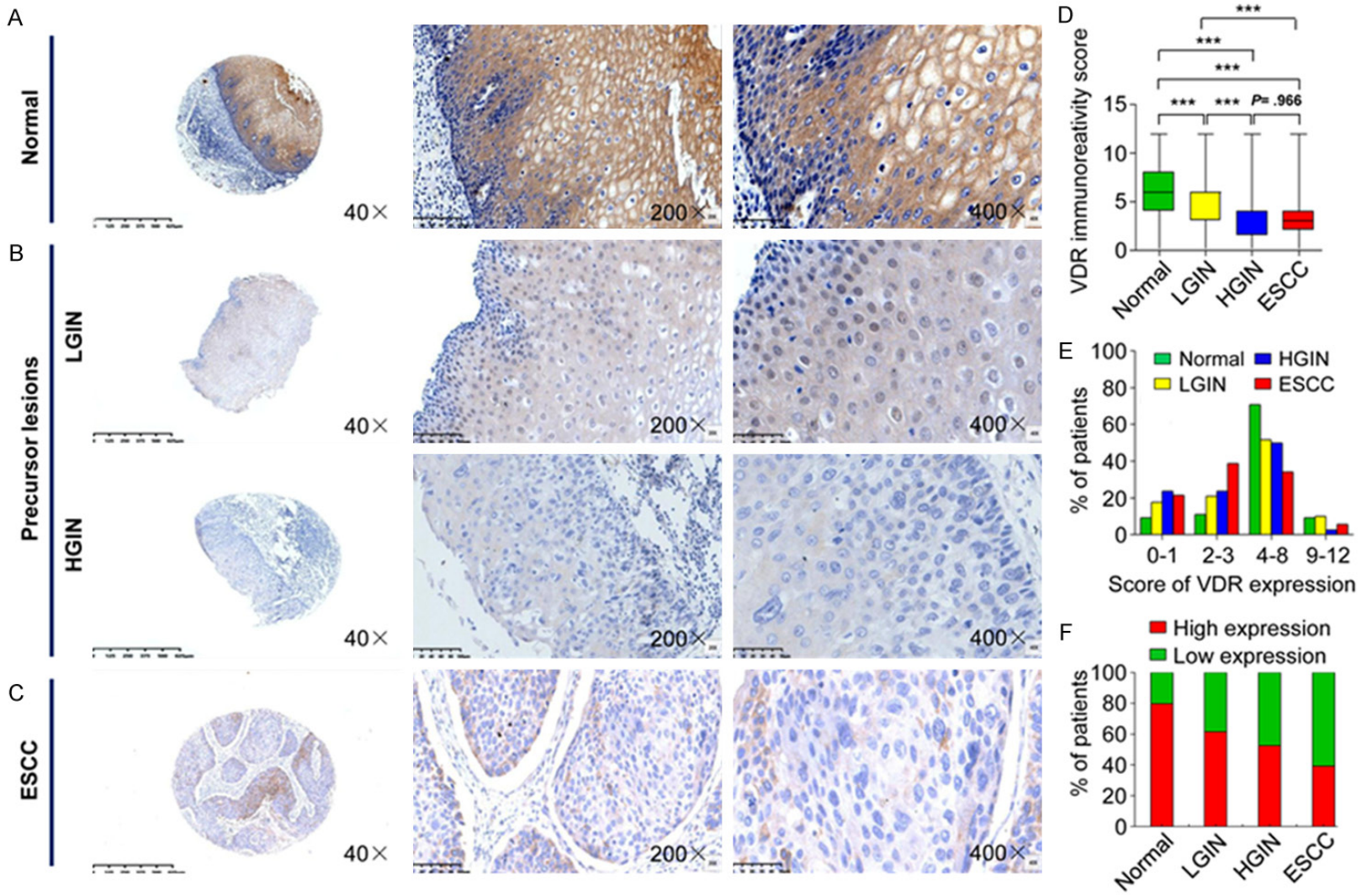

Figure 2. Immunohistochemical analysis of the VDR protein in adjacent normal tissues, precancerous lesions, and ESCC tissues from Chinese Han patients. Representative VDR immunostaining in upper panel normal tissues (A), middle-panel LGIN and HGIN (B), and bottom panel ESCC (C) in the Chinese Han population (left image magnification, × 40; middle image magnification, × 200; right image magnification, × 400). VDR staining was located in the nuclei/cytoplasm. (D) (Box plot) Range of VDR immunoreactivity score in normal tissues, LGIN, HGIN, and ESCC tissues (*P < 0.05. **P < 0.01. ***P < 0.001). (E) (frequency distribution histogram) frequency distribution of normal tissues, LGIN, HGIN, and ESCC tissues in four-level scores (0-1, 2-3, 4-8, and 9-12) of VDR expression. (F) (Positive distribution histogram) Positive and negative distribution of normal tissues, LGIN, HGIN and ESCC tissues (Normal: LGIN, P = 0.0012; Normal: HGIN, P = 0.00039; Normal: ESCC, P = 8.89×10^{-16} LGIN: ESCC, P = 0.00043).

Table 2. VDR protein expression during cancer progression by IHC analysis in Han population

Cancer progression	Immunostaining		*P*-value
	Low (%)	High (%)	
Normal[A]	37 (20.1)	147 (79.9)	A:B $P = 0.0012^{*}$; A:C $P = 0.00039^{***}$
LGIN[B]	35 (38.5)	56 (61.5)	A:D $P = 8.89 \times 10^{-16***}$; B:C $P = 0.349$
HGIN[C]	18 (47.4)	20 (52.6)	B:D $P = .00043^{***}$
ESCC[D]	119 (60.7)	77 (39.3)	C:D $P = 0.126$

$^{*}P < 0.05$, $^{***}P < 0.001$ as determined by Pearson's χ^2 test.

VDR overexpressed in normal esophageal tissue compared with ESIN and ESCC of Han patients

To explore the clinical relevance of VDR and reveal its function in the progression of ESCC, we detected the VDR expression by immunohistochemical staining in normal esophageal tissue, LGIN, HGIN and ESCC tissues collected from Kazakh and Han patients of Xinjiang China. Immunohistochemical staining results showed that VDR protein was mainly located in cytoplasm, as well as in the nucleus of several normal esophageal tissue mucosa cells. VDR staining was significantly different in the different stages of ESCC development. In Han populations, statistical analysis showed that the frequency of high VDR expression was the highest in normal esophageal tissues at 79.9% (147/184) and gradually decreased following the development of ESCC, with 61.5% (56/91) of LGIN and 52.6% (20/38) of HGIN, and the lowest at 39.3% (77/196) of ESCC. In normal esophageal tissue, VDR labeling was strong and highly expressed in the nuclei/cytoplasm of basal cells and suprabasal layer cells. However, VDR was weak and mainly expressed in HGIN and ESCC cells with the signal in nuclei/cytoplasm (**Figure 2**). Box-plot showed that the trend of VDR immunoreactivity score decreases in a stepwise manner from normal cells, LGIN, HGIN and ESCC, as shown by t-test (**Figure 2D**). In addition, the four level score (0-1, 2-3, 4-8, and 9-12) distribution of VDR protein expression in normal cells, precancerous lesions, and ESCC was significantly distinct (**Figure 2E**). Furthermore, as shown in **Table 2** and **Figure 2F**, Chi-square test showed that a significant difference exists in the comparison of VDR expression levels in different stages of cancer progression ($P < 0.001$). We conducted an in-depth analysis on VDR expression during cancer progression. VDR expression was significantly decreased in LGIN, HGIN and ESCC compared with normal esophageal tissue ($P < 0.05$). No significant difference was found between LGIN and HGIN (61.5% versus 52.6%, $P = 0.349$), but significant differences existed between LGIN and ESCC (61.5% versus 39.3%, $P = 4.3 \times 10^{-4}$). Although the decrease in frequency from HGIN to ESCC (52.6% versus 39.3%, $P = 0.126$) is not significant, the marginal differences in VDR expression between the two tissues was remain visible (**Table 2**; **Figure 2**). Further comparative analyses of 106 ESCC specimens (NCBI/GEO/GSE23500) also demonstrated that the mRNA levels of VDR were significantly elevated in human esophageal normal tissue ($P = 3.5 \times 10^{-4}$) (**Figure 1F**).

Low VDR expression presented association with differentiation, lymph node metastasis, and invasion depth in ESCC

To determine whether the level of VDR protein expression is related to the development of ESCC, we further explore the relationship between VDR expression and clinical pathological characteristics of ESCC patients from two different ethnic groups. As described in **Table 3**, in Han patients, VDR expression was significantly correlated with differentiation ($P = 0.004$), lymph node metastasis ($P = 0.010$), and clinical stage ($P = 0.001$). The result VDR expression may be involved in tumor aggressiveness, metastasis and differentiation. Meanwhile, in Kazakh patients, low VDR expression more frequently occurred in poorly differentiated tissue ($P = 0.010$). However, no significant correlation was found between VDR expression and other clinicopathological variables, such as gender, age and invasion depth, among the Chinese Han and Kazakh patients (**Table 3**).

Low VDR expression prompts poor prognosis in ESCC patients

To assess the value of VDR in the prognosis of ESCC patients, the relationship between VDR expression and the overall survival (OS) of 70 ESCC patients from Han or Kazakh was evaluated via Kaplan-Meier method. The analysis

Table 3. Correlations between VDR expression of ESCC and clinicopathological factors in Han and Kazakh ethnic

Variables	VDR expression in Kazakh ethnic				VDR expression in Han ethnic			
	Total Cases (%) (n = 166)	Low No. (%)	High No. (%)	*P*-value	Total Cases (%) (n = 196)	Low No. (%)	High No. (%)	*P*-value
Gender				0.243				0.062
Male	116 (69.88)	91 (78.45)	25 (21.55)		132 (67.3)	74 (56.1)	58 (43.9)	
Female	50 (30.12)	35 (70.00)	15 (30.00)		64 (32.7)	45 (70.3)	19 (29.7)	
Age (yrs)				0.624				1.000
≤ 60	105 (63.25)	81 (77.14)	24 (22.86)		117 (59.7)	71 (60.7)	46 (39.3)	
> 60	61 (36.75)	45 (73.77)	16 (26.23)		79 (40.3)	48 (60.8)	31 (39.2)	
Differentiation				0.010				0.004
Well	13 (7.83)	7 (53.85)	6 (46.15)		76 (38.8)	39 (51.3)	37 (48.7)	
Moderate	106 (63.86)	77 (72.64)	29 (27.36)		73 (37.2)	42 (57.5)	31 (42.5)	
Poor	47 (28.32)	42 (89.36)	5 (10.64)		47 (24.0)	38 (80.9)	9 (19.1)	
Invasion depth				0.399				0.992
T1-T2	90 (54.22)	66 (73.33)	24 (26.67)		79 (40.3)	48 (60.8)	31 (39.2)	
T3-T4	76 (45.78)	60 (78.95)	16 (21.05)		117 (59.7)	71 (60.7)	46 (39.3)	
Lymph node metastasis				0.147				0.010
No	83 (50.00)	67 (80.72)	16 (19.28)		105 (53.6)	55 (52.4)	50 (47.6)	
Yes	83 (50.00)	59 (71.08)	24 (28.92)		91 (46.4)	64 (70.3)	27 (29.7)	
TNM Stage				0.008				0.001
I+II	90 (67.47)	61 (67.78)	29 (32.22)		110 (56.1)	55 (50.0)	55 (50.0)	
III+IV	76 (32.53)	65 (85.53)	11 (14.47)		86 (43.9)	64 (74.4)	22 (25.6)	

shows that the median survival time for patients with high VDR expression was 60 (range, 27-92 months) and 35 months (range, 2-68 months) for patients with low VDR expression, Apparently, ESCC patients with high VDR expression presented longer OS rates and lower risk of death compared with those with low VDR expression. However, further analysis confirmed that VDR status is significantly associated with ESCC patients OS (log-rank $P = 0.034$; **Figure 3**).

To identify independent prognostic factors for ESCC survival, we used univariate and multivariate Cox regression models in all clinicopathological factors included in **Table 4**. Univariate Cox proportional hazard regression analysis revealed that VDR (hazard ratio, 0.449; 95% CI, 0.207-0.975; $P = 0.043$) was a significant prognostic predictor for OS of ESCC patients. Other clinicopathological parameters were not prognostic factors for OS in our study (**Table 4**). Multivariate Cox proportional hazard regression analysis indicated that VDR expression (hazard ratio, 0.447; 95% CI, 0.204-0.978; $P = 0.044$; **Table 4**) was a significant independent prognostic factor for favorable OS in ESCC. Our consequences suggest that VDR can be potentially viewed as a biomarker in ESCC.

Discussion

For the first time, we explore the expression of VDR in Han and Kazakh ESCC tissues, LGIN, HGIN, and normal esophageal tissues to further analyze the correlation between VDR expression and clinicopathological characteristics. Our result showed that the VDR expression in the ESCC tissues from Chinese Han and Kazakh ESCC patients both reduced compared with normal esophageal tissues. VDR expression was significantly decreased following malignant transformation from normal epithelium into LGIN and HGIN tissues in Han ethnic patients. Low VDR expression was associated with differentiation, lymph node metastasis, and TNM stage in Han and with differentiation and TNM stage in Kazakh. Moreover, VDR deficiency is an independent prognostic factor of the short overall survival rate of the ESCC, and patients with high VDR expression levels show longer survival time than those with low expression levels, which indicates that downregulation of VDR predicted the adverse prognosis. In

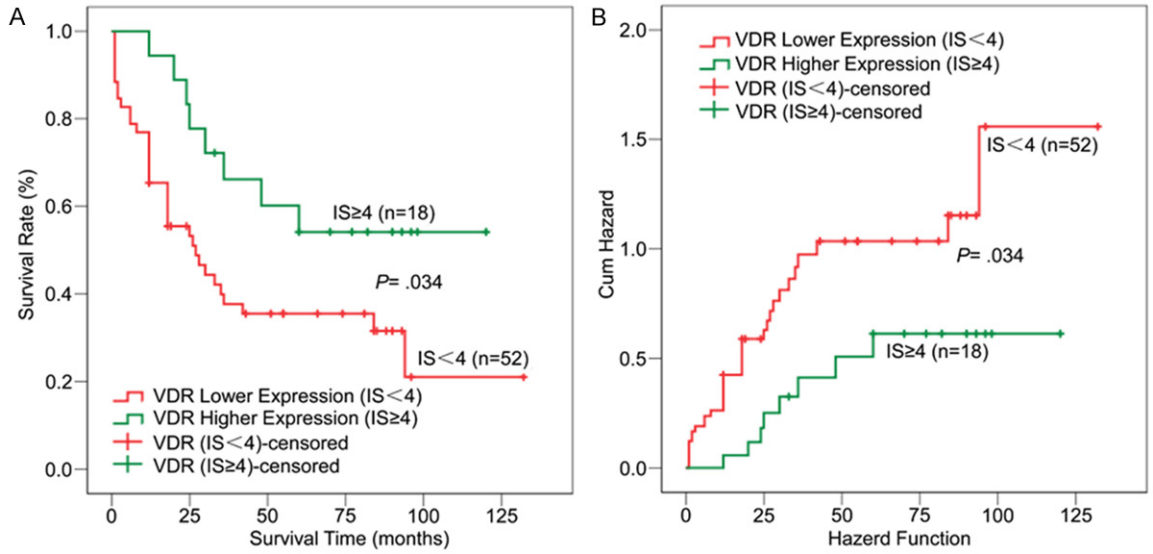

Figure 3. Kaplan-Meier survival curves of patients with high VDR expression and those with low expression. A: ESCC patients with high VDR expression (IS ≥ 4) show a significantly higher survival rate after surgery compared with those with low VDR expression (IS < 4) (P < 0.05). B: Patients with low VDR expression present higher risk of death compared with those with high VDR expression (P < 0.05).

Table 4. Univariate and multivariate Cox regression analyses of the prognostic variables in ESCC patients

Variables	Univariate analysis				Multivariate analysis			
	HR[a]	95% CI[b]		*P* value	HR[a]	95% CI[b]		*P*-value
VDR expression (IS < 4)	0.449	0.207	0.975	0.043*	0.447	0.204	0.978	0.044*
Gender (Female)	0.826	0.439	1.553	0.553	0.925	0.481	1.777	0.814
Age (≤ 60 years)	0.861	0.466	1.590	0.632	0.896	0.455	1.764	0.750
Differentiation (Poor)	1.188	0.754	1.872	0.457	1.135	0.714	1.806	0.592
Invasion depth (T2-T3)	1.286	0.700	2.361	0.418	1.259	0.640	2.476	0.505
Lymph node metastasis (Yes)	1.540	0.829	2.862	0.172	1.718	0.637	4.630	0.285
TNM Stage (III+IV)	1.441	0.784	2.649	0.240	0.916	0.342	2.452	0.861

[a]HR = hazard ratio, [b]CI = confidence interval, *$P < 0.05$.

summary, VDR might be a potential early biomarker for the diagnosis and a therapeutic target for the treatment of ESCC.

As a highly aggressive tumor, ESCC involves a multistage process, in which normal esophageal tissue follows a series of histological and genetic progression, goes through noninvasive precursor lesions, and finally becomes an invasive cancer [19]. Many reports indicate that precursor lesions have prognostic significance for ESCC in that dysplastic lesions are frequently emerged in ESCC tissues [20]. Therefore, the expression of proteins in precursor lesions is regarded as promising candidates of ESCC progression. We found that the expression of VDR negatively correlated with the progression of ESCC. In Han patients, we observed that the VDR protein expression progressively decreased from normal esophageal tissue to LGIN, to ESCC, peaking in normal esophageal tissue, which indicated that VDR would soon decrease once the esophageal squamous epithelium transformed into ESCC malignant progression. Patients with mild, moderate and severe dysplasia as well as in situ carcinoma have an increased risk of developing aggressive ESCC [3]. Similarly, the VDR protein expression was significantly lower in ESCC than in normal esophageal tissue of Kazakh. Although the two groups had different ethnic customs and dietary histories, reduced VDR protein expres-

sion was similarly observed in both Han and Kazakh ESCC tissues. Therefore, we confirmed the viewpoint that lowexpression of VDR is a good biomarker for the detection of precancerous lesion of esophageal.

Through our study, we found that the highexpression of VDR in ESCC was significantly lower than that of normal esophageal tissue both in Han and Kazakh population. There are also related reports of VDR expression in other tumors, but no uniform conclusion about VDR expression in different types of tumors. Wen et al. discovered that positive rate of VDR expression in gastric cancer tissue was obviously lower than the adjacent normal tissues and premalignant tissues [21]. Jóźwicki et al. observed that the highest VDR immunostaining was found in normal epithelium and was significantly lower in bladder cancer cells [12]. By contrast, expression of VDR was significantly increased in precancerous and oral squamous cell carcinoma compared with normal tissue [22]. A previous report identified the protein of VDR was highly expressed in EAC but rarely in normal squamous epithelium [15]. The difference between the two types of esophageal cancer may be related to different receptors for bile acid salt binding. FXR and VDR are both bile acid receptor, which have different distribution. FXR protein was mainly expressed in the nucleus of the basal layer of normal esophageal squamous epithelium and Barrett esophagus, negatively expressed in dysplasia and adenocarcinoma [23]. VDR protein expression was significantly increased in adenocarcinoma of esophagus and LGIN compared with normal esophageal squamous epithelium. Bile salts may be involved in the early stages of carcinogenesis through both FXR and VDR [15]. In future research, it is very important to explore the mechanism of VDR molecules in different tissues in a larger sample.

In addition, we also find that VDR expression was positively associated with differentiation, lymph node metastasis and TNM stage of Han ESCC patients, as well as differentiation and TNM stage of Kazakh ESCC patients. Our findings are consistent with those of several reports on VDR in other types of cancers. For instance, one research showed that VDR proteins were significantly down-regulated in gastric cancer tissues compared with normal and premalignant tissues, whilst VDR lowexpression strongly inhibited gastric cancer differentiation [21]. By contrast, another study reported that VDR mRNA expression in human well and moderately differentiated colon carcinoma is much more abundant than in epithelial cells of normal mucosa or of adjacent normal mucosa [24]. The effect of VDR expression on the differentiation of cancer may be related to the coactivator complexes. VDR binds to one of two coactivator complexes: DRIP or p160/SRC. Binding to DRIP occurs in the undifferentiated keratinocyte, but, as the cell differentiates, DRIP 205 levels fall and p160/SRC binding takes over as SRC3 expression increases. SCCs fail to respond to the prodifferentiating actions of 1,25(OH)2D3 [25]. Failure of VDR to induce differentiation in poor differentiated ESCC lies at least in part with its failure to induce the replacement of the DRIP complex with the SRC complex in the promoters of genes required for. In future research, we need to make an in-depth study of VDR in a larger multinations ESCC sample differentiation.

We demonstrated the association between VDR highexpression and long OS times of patients with ESCC. This has also been demonstrated for urothelial bladder cancers [12], pancreatic cancer [3] and hepatocellular carcinoma [26]. However, no survival difference is observed between VDR high-expression and low-expression groups in EAC [27]. Furthermore, decreased VDR expression in OSCC might be associated with tumor relapse [28]. These show that VDR lowexpression may be a new biomarker for the poor prognosis of patients with ESCC. At present, researchers have found that VDR affects the biological function of cancer cells through a variety of mechanisms. The functions of VDR in EAC were studied by Chiang et al. Those investigators activate VDR in HepG2 liver cancer cells with agonist MART-10, which significantly inhibit proliferation of HepG2 cell, as well as upregulate p21 and p27, that in turn arrested HepG2 cells at the G0/G1 phase profoundly [29]. VDR has an antiproliferative effect on many cancers, promoting apoptosis, potentiating differentiation, and inhibiting proliferation among a variety of tumor cells, such as those of the colorectal cancer, ovarian cancer, malignant melanoma, non-melanoma skin cancers and breast cancer [30-34]. In future research, we need to further explore the mech-

anism of the interaction between VDR and ESCC, in order to provide an important theoretical foundation for improving the prognosis of ESCC.

In conclusion, VDR lowexpression can be regarded as an early biomarker and prognosis marker for high ESCC risk population with the aim of early diagnosis. Targeting VDR may result in new therapeutic strategies in patients with ESCC. However, the specific mechanism of ESCC effect of lowexpression of VDR is unclear and requires further study.

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Disclosure of conflict of interest

None.

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