Clinical Trial/Experimental Study

Medicine

OPEN

Clinicopathological significances of PLOD2, epithelial–mesenchymal transition markers, and cancer stem cells in patients with esophageal squamous cell carcinoma

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Abstract

Background: To examine the expression level of procollagen-lysine2-oxoglutarate 5-dioxygenase 2 (PLOD2) in esophageal squamous cell carcinoma (ESCC) and analyze its correlation with clinicopathological parameters, in order to explore the mechanism of PLOD2 in regulating invasion and metastasis of ESCC.

Methods: Immunohistochemistry was used to detect the expression level of PLOD2 in tumor tissues and paired adjacent tissues of 172 patients with ESCC, and the relationship between PLOD2 expression and clinicopathological parameters was analyzed. The deposition of collagen fibers in tumor was detected by Sirius red staining. The correlation between tumor stem cells and epithelial–mesenchymal transition (EMT) markers ZEB1 was analyzed by multivariate logistic regression.

Results: The expression level of PLOD2 in tumor tissues of patients with ESCC (70.35%, 121/172) was significantly higher than that in paired adjacent tissues (29.65%, 51/172; $P < .01$). The positive expression rate of PLOD2 in ESCC was related to T classification, lymph node metastasis, and pathological tumor node metastasis of a tumor. The expression rates of ZEB1, CD44, and CD133 in ESCC were correlated with T classification, lymph node metastasis and pathological tumor node metastasis. Scarlet red staining showed that collagen fiber deposition in ESCC tissues with high expression of PLOD2 was significantly higher than that in tissues with low expression of PLOD2 ($P < .01$). A positive correlation was observed between the expression of PLOD2 and CD133, PLOD2 and CD44, and PLOD2 and N-cadherin ($P < .01$). Moreover, a negative correlation was noted between the expression of PLOD2 and E-cadherin ($P < .01$). The combined expression of PLOD2 and ZEB1 were independent prognostic factors for the total survival time of patients with ESCC.

Conclusion: PLOD2 is highly expressed in ESCC and is closely related to tumor invasion and metastasis. The mechanism of PLOD2 for promoting invasion and metastasis of ESCC may be related to activation of the EMT signaling pathway to promote EMT and tumor stem cell transformation.

Abbreviations: CSCs = cancer stem cells state, ECM = extracellular matrix, EMT = epithelial–mesenchymal transition, ESCC = esophageal squamous cell carcinoma, PLOD2 = procollagen-lysine2-oxoglutarate 5-dioxygenase 2.

Keywords: EMT, esophagus, PLOD2, squamous cell carcinoma, tumor stem cells

1. Introduction

Esophageal cancer is the most common malignant tumor of the digestive tract in China, and its incidence has significant regional differences. The incidence of esophageal cancer in China ranks first in the world, among which the incidence of esophageal cancer in people near Taihang Mountains is about 100 times higher than the world average, and >90% of them are esophageal squamous cell carcinoma (ESCC).[1,2] Although surgery, chemotherapy, radiotherapy, and biological targeting have improved the survival rate of patients with ESCC, tumor metastasis remains the main cause of death.[3]

Procollagen-lysine2-oxoglutarate 5-dioxygenase 2 (PLOD2) is a functional enzyme in the rough endoplasmic reticulum, and its main function is to hydroxylate lysine residues in the terminal peptide region of collagen, and form a pyridine chain structure with stable cross-linking and high tension resistance outside the cell, thus promoting the reconstruction of extracellular matrix

This study was supported by the Anhui Provincial University Natural Science Key Project (no. KJ2019A0386). We thank all colleagues in the Department of Pathology, the First Hospital Affiliated to Bengbu Medical College, for their help and support in this study.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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How to cite this article: Gong X, Wang A, Song W. Clinicopathological significances of PLOD2, epithelial–mesenchymal transition markers, and cancer stem cells in patients with esophageal squamous cell carcinoma. Medicine. 2022;101:34(e30112).

Received: 20 February 2022 / Received in final form: 28 June 2022 / Accepted: 1 July 2022

http://dx.doi.org/10.1097/MD.0000000000030112

(ECM). Studies have shown that PLOD2 can promote tumor invasion and metastasis by changing collagen fiber deposition and remodeling in ECM.[4–6] The expression of PLOD family in breast cancer,[7] biliary tract cancer,[8] gastric cancer,[9] colorectal cancer,[10] glioma,[11] and liver cancer[12,13] is higher than that in normal tissues. The expression of PLOD2 in ESCC and its involvement in invasion and metastasis of ESCC have been rarely reported.

Epithelial–mesenchymal transition (EMT) refers to the phenomenon wherein epithelial cells transform into mesenchymal cells under certain physiological and pathological conditions, which is mainly manifested by the loss of epithelial cell characteristics and the acquisition of mesenchymal characteristics.[14] The whole process is accompanied by loss of polarity and adhesion of epithelial cells, reorganization of cytoskeleton, remodeling of ECM, etc,[15,16] which leads to stronger migration and invasion of cells, inhibition of apoptosis, and promotion of metastasis and recurrence of tumors. Studies have shown that the stimulation of some key extracellular signals promotes the overexpression of EMT transcription factors such as SNAIL, TWIST, or ZEB1, which trigger EMT by inhibiting epithelial phenotype, enhancing mesenchymal characteristics and inducing the ability to degrade basement membrane and ECM.[17] At the molecular level, EMT leads to a decrease in the expression of E-cadherinherin, a surface marker of epithelial cells, or a loss of membrane localization, and an increase in mesenchymal markers,[18] which leads to functional changes in cell migration and invasion.

Some cancer cells will also induce the initial state of tumor during EMT, which is called cancer stem cells state (CSCs). The CSCs produces a type of cancer cell subgroup, which has the ability of self-regeneration, proliferation, and differentiation into various cancer cell lines through symmetric and asymmetric cell division. Based on these characteristics and the protection of tumor microenvironment, CSCs play an important role in regulating the occurrence and development of tumor and drug resistance.[19] At present, the widely accepted and applied tumor stem cell markers are CD44 and CD133. Some studies have identified some basic features of CSC,[20] including

1. A small amount of CSC can cause new tumors.
2. Self-renewal and differentiation.
3. Specific and distinguishable surface markers.
4. Transplant ability.
5. Resistance to conventional chemotherapy and radiotherapy.

Therefore, we hypothesized that PLOD2 protein promotes invasion and metastasis of ESCC, which further affects the prognosis of patients. However, the expression of PLOD2 protein in esophageal carcinoma and its correlation with prognosis remain unclear. Therefore, using immunohistochemistry, survival analysis and regression analysis, this study aimed to analyze the correlation between the expression of PLOD2 protein and the clinicopathological features, survival rate and prognosis of patients, as well as the correlation between PLOD2 protein and EMT and CSC-related markers, so as to provide a theoretical and experimental basis for the occurrence, diagnosis, prognosis and treatment of esophageal cancer.

2. Methods

2.1. Patients and clinical samples

A total of 172 specimens of ESCC were randomly selected from the Department of Clinical Pathology of the First Affiliated Hospital of Bengbu Medical College between January 2016 and December 2017. All patients presented complete clinical, pathological, and follow-up data but no distant metastasis before the surgery. Patients who received preoperative chemotherapy, radiotherapy, targeted therapy, or endocrine treatment were excluded from this study. This study was approved by the institutional ethical review board of First Affiliated Hospital of Bengbu Medical College. The patients were followed-up until September 2021 or death, and the survival time ranged from 6 to 69 months. None of the cases received radiotherapy or chemotherapy before operation. Normal tissues 3 cm away from the edge of tumor were selected as paired adjacent tissues, and all selected cases were diagnosed as ESCC by 2 pathologists. According to UICC latest pathological staging standard for esophageal cancer, the clinicopathological characteristics are listed in Table 1.

2.2. Immunohistochemical analysis

Immunohistochemistry was conducted according to the Elivision™Plus detection kit instructions (Lab Vision). After antibody coloring, the coloring reaction was terminated by running water for 1 minute, and the sections were oxidized in 0.5% high-iodized acid solution for about 10 minutes. Then, the sections were rinsed with running water for 2 minutes, then stained in Schiff solution for 15 minutes, and washed with distilled water for 3 times, 1 to 2 minutes each; Finally, the sections were counterstained with hematoxylin, dehydrated, air-dried, and mounted. PBS replaced the primary antibody that served as the negative control, and the corresponding protein-positive slice was a positive control.

2.3. Evaluation of staining

The immunohistochemical staining results were interpreted by 2 experienced pathologists using an independent double-blind method. PLOD2, CD44, and CD133 were positive, with brownish yellow granules in cell membrane or cytoplasm, and ZEB1 staining

Table 1

Patient clinicopathogical characteristics.

Patient characteristic	Frequency (n)	Percentage (%)
Gender		
Female	61	35.5
Male	111	64.5
Age (yrs)		
<60	81	47.1
≥60	91	52.9
Tumor location		
U	24	14
M	121	70.3
L	27	15.7
T classification		
T1	13	7.6
T2	40	23.3
T3	115	66.9
T4	4	2.3
Pathologic differentiation		
G1	35	20.3
G2	113	65.7
G3	24	14
Lymph node metastasis		
N0	120	69.8
N1	34	19.8
N2	12	7
N3	6	3.5
pTNM		
I	19	11
II	102	59.3
III	44	25.6
IV	7	4.1

pTNM = pathological tumor node metastasis.

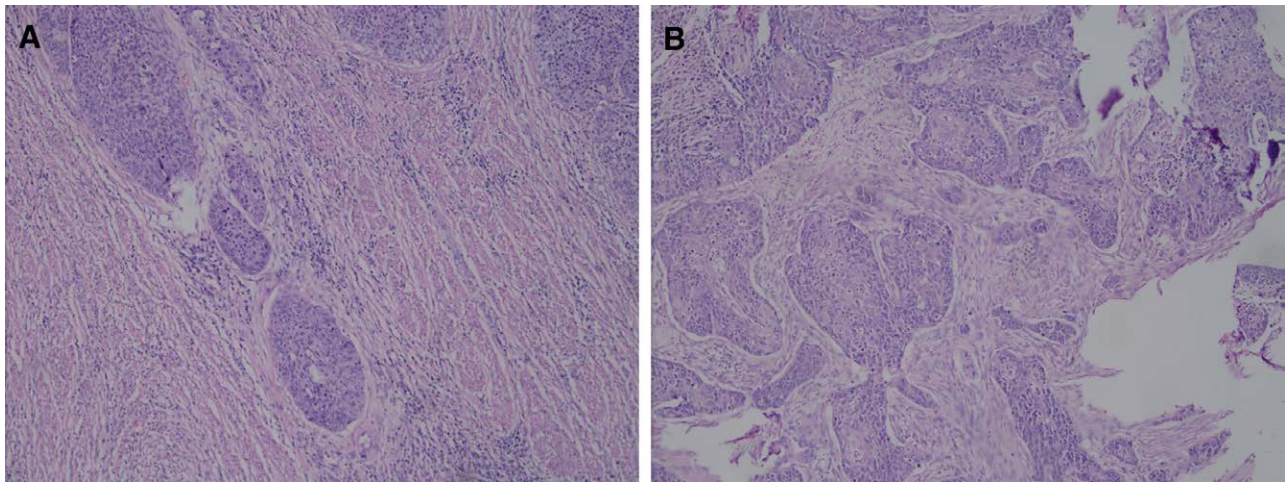

Figure 1. PLOD2 expression level on collagen fiber deposition in ESCC. (A) High expression of PLOD2 collagen fibers was thick, straight, and dense. (B) Low expression of PLOD2 collagen fibers was loose. ESCC = esophageal squamous cell carcinoma, PLOD2 = procollagen-lysine2-oxoglutarate 5-dioxygenase 2.

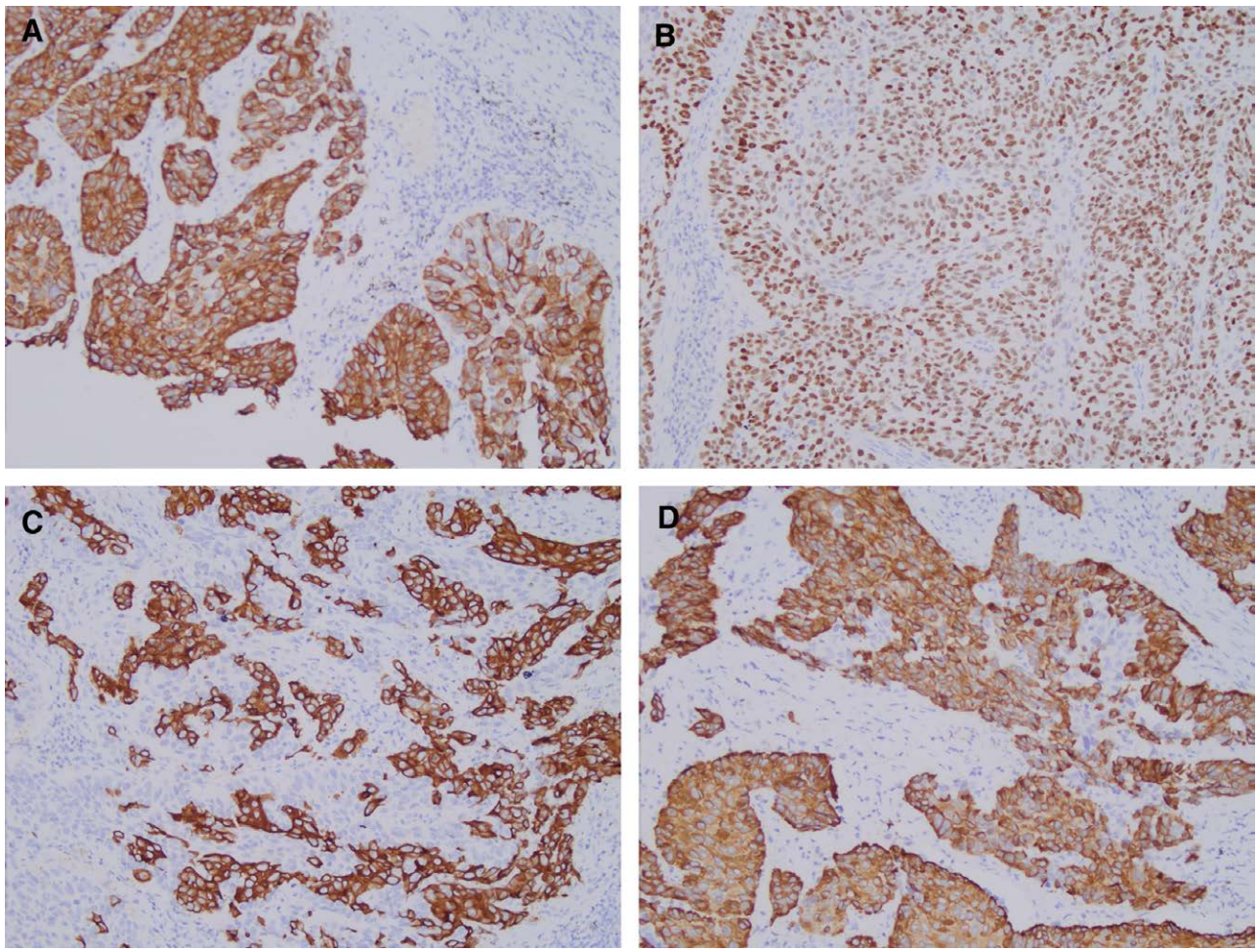

Figure 2. Immunostaining of PLOD2, ZEB1, CD133, and CD44 in ESCC. (A) Positive staining of PLOD2 in ESCC (100×). (B) Positive staining of ZEB1 in ESCC (100×). (C) Positive staining of CD133 in ESCC (100×). (D) Positive staining of CD44 in ESCC (100×). ESCC = esophageal squamous cell carcinoma, PLOD2 = procollagen-lysine2-oxoglutarate 5-dioxygenase 2.

Table 2

Correlations between PLOD2, ZEB1, CD133, CD44, and clinicopathological variables in esophageal squamous cell carcinoma.

	PLOD2			ZEB1			CD133			CD44		
Characteristics	**Negative**	**Positive**	***P***	**Negative**	**Positive**	***P***	**Negative**	**Positive**	***P***	**Negative**	**Positive**	***P***
Gender												
Female	18	43	.976	26	35	.760	26	35	.539	24	37	.668
Male	33	78		50	61		42	69		40	71	
Age (yrs)												
<60	25	56	.742	36	45	.949	34	47	.537	32	49	.557
≥60	26	65		40	51		34	57		32	59	
Tumor location												
U	7	17	.382	13	11	.184	10	14	.958	10	14	.340
M	33	88		48	73		47	74		41	80	
L	11	16		15	12		11	16		13	14	
T classification												
T1	10	3	.001	12	1	.001	13	0	<.001	9	4	.033
T2	10	30		18	22		13	27		16	24	
T3	31	84		46	69		40	75		39	76	
T4	0	4		0	4		2	2		0	4	
Pathologic differentiation												
G1	17	18	.023	24	11	.005	19	16	.110	17	18	.212
G2	28	85		43	70		39	74		37	76	
G3	6	18		9	15		10	14		10	14	
Lymph node metastasis												
N0	45	75	.004	61	59	.024	54	66	.065	57	63	<.001
N1	3	31		11	23		11	23		7	27	
N2	3	9		4	8		1	11		0	12	
N3	0	6		0	6		2	4		0	6	
pTNM												
I	14	5	<.001	14	5	.003	15	4	.001	11	8	<.001
II	31	71		47	55		39	63		46	56	
III	6	38		15	29		12	32		7	37	
IV	0	7		0	7		2	5		0	7	

PLOD2 = procollagen-lysine2-oxoglutarate 5-dioxygenase 2, pTNM = pathological tumor node metastasis.

was mainly located in the nucleus.[21] E-cadherin and N-cadherin were mainly expressed in the cell membrane and cytoplasm, and tumor cells that stained as strongly as normal epithelial cells were considered to have preserved expression, whereas those that exhibited weak staining were considered to have reduced expression.[22] The areas with positive staining were observed in at least 10 representative high-power visual fields (magnified 400 times) under the microscope. The results were evaluated by measuring both the staining intensity and the number of positively stained cells. The intensity of the positive reaction was scored as negative (0), weak (1), moderate (2), and intense (3). The reactivity was assessed by the percentage of positively stained cells as 0% to 5% (0), 6% to 25% (1), 26% to 50% (2), 51% to 75% (3), and 75% to 100% (4). The scores for the intensity and the percentage of positive cells were multiplied to obtain a weighted score for each patient, giving a minimum-to-maximum score of 0 to 12. The immunostaining was considered positive when the scores were ≥3.[23]

2.4. Statistical analysis

Statistical analysis was performed using SPSS 22.0 software package. The percentage of collagen deposition was analyzed by IPP analysis software. The comparison of the expression of PLOD2 between ESCC and noncancerous tissues was analyzed using the *t* test. The Fisher exact test or Pearson chi-square test was used to analyze the correlation between protein expression and clinicopathological indices. Spearman correlation analysis was used to evaluate the correlations between the expression levels of these factors. The Kaplan–Meier method was used to establish the survival curves, and the log-rank test was used for comparison. Univariate and multivariate analyses were performed to analyze the influence of various factors on overall survival using a Cox proportional hazards regression model. The hazard ratio and 95% confidence interval were used for analysis. A P value of <.05 was considered to be statistically significant.

3. Results

3.1. Effect of PLOD2 expression level on collagen fiber deposition in ESCC

Sirius red staining showed that collagen fibers were thick, straight and densely arranged in ESCC tissues with high expression of

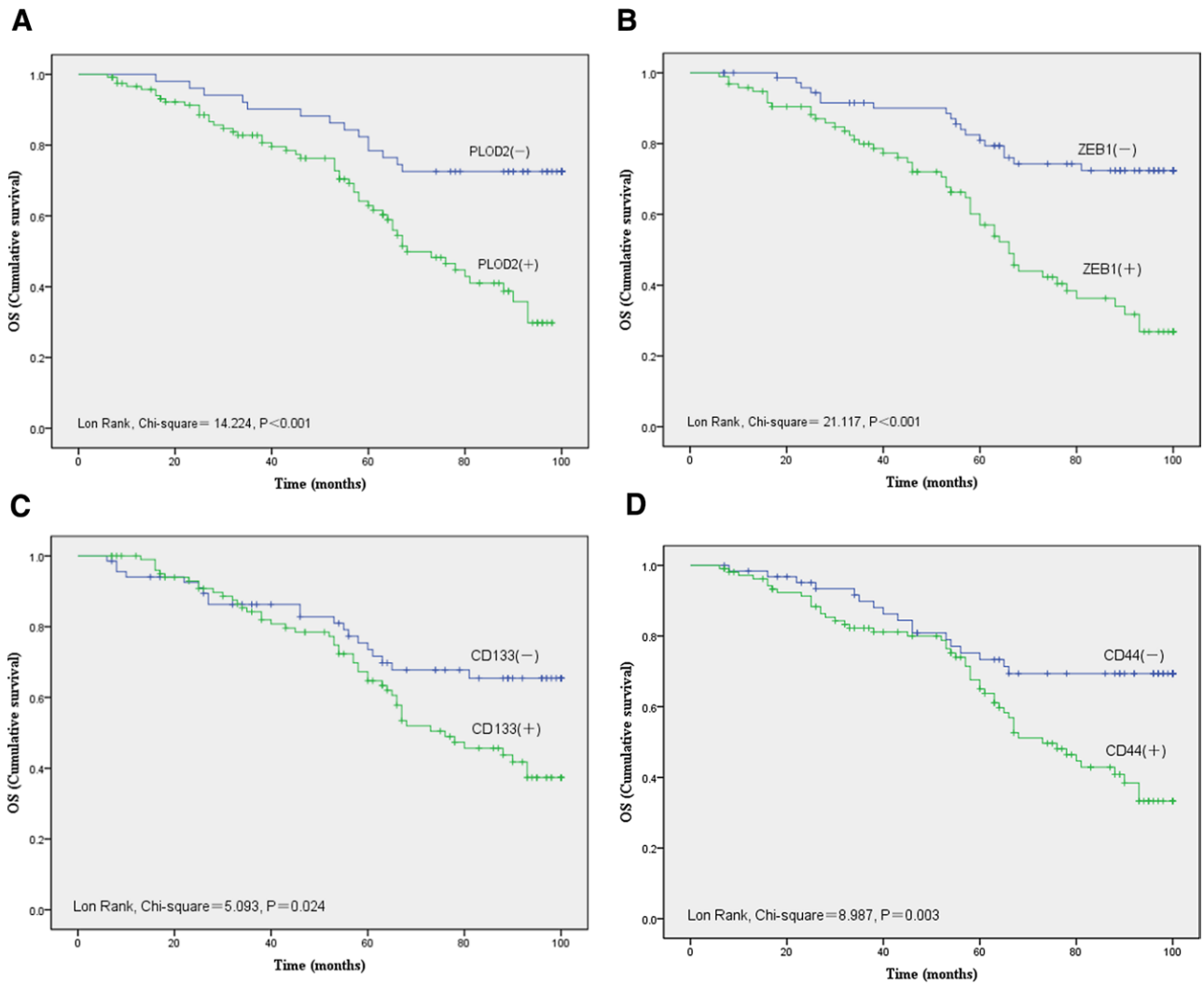

Figure 3. Kaplan–Meier analysis of the survival rate of patients with ESCC. (A) Overall survival of all patients in relation to PLOD2 expression (log-rank = 14.224, P < .001). (B) Overall survival of all patients in relation to ZEB1 expression (log-rank = 21.117, P < .001). (C) Overall survival of all patients in relation to CD133 expression (log-rank = 5.093, P = .024). (D) Overall survival of all patients in relation to CD44 expression (log-rank = 8.987, P = .003). ESCC = esophageal squamous cell carcinoma, PLOD2 = procollagen-lysine2-oxoglutarate 5-dioxygenase 2.

PLOD2 (Fig. 1A), with an average collagen fiber deposition percentage of (78.44% ± 7.25%), while collagen fibers were loosely distributed in cancer tissues with low expression of PLOD2 (Fig. 1B), with an average collagen fiber deposition percentage of (15.32% ± 3.18%).

3.2. Correlations between the expression levels of PLOD2, ZEB1, CD133 and CD44, and clinicopathological variables

Among the selected 172 ESCC cases, 121 (121/172, 70.35%) stained positive for PLOD2 (Fig. 2A), and 34 (34/172, 19.77%) stained positive among corresponding nontumor tissues. Furthermore, 96 ESCC cases (96/172, 55.81%) and 28 (28/172, 16.28%) corresponding nontumor tissues stained positive for ZEB1 (Fig. 2B). Also, 104 ESCC cases (104/172, 60.47%) and 23 (23/172, 13.37%) corresponding nontumor tissues stained positive for CD133 (Fig. 2C). Moreover, 108 ESCC cases (108/172, 62.79%) and 25 (25/172, 14.53%) corresponding nontumor tissues stained positive for CD44 (Fig. 2D). The differences were statistically significant ($P < .05$). The expression levels of PLOD2 and ZEB1 in ESCC correlated with T classification, pathological differentiation, lymph node metastasis, and pathological tumor node metastasis ($P < .05$), but not with age, gender and tumor location. The positive expression of CD133 and CD44 in ESCC correlated with T classification and pathological tumor node metastasis of the tumor ($P < .05$), but not with age, gender and location of the tumor. Detailed statistical results are shown in Table 2.

3.3. Univariate and multivariate analyses

The univariate analysis using the Kaplan–Meier method showed that the survival time of patients with positive expression of PLOD2 was significantly shorter than that of patients with negative expression of PLOD2 (log-rank = 14.224, $P < .001$) (Fig. 3A). The survival time of patients with positive expression of ZEB1 was significantly shorter than that of patients with negative expression of ZEB1 (Log-rank = 21.117, $P < .001$) (Fig. 3B). The survival time of patients with positive expression of CD133 was significantly shorter than that of patients with negative expression of CD133 (log-rank = 5.093, $P = .024$) (Fig. 3C). The survival time of patients with positive expression of CD44 was significantly shorter than that of patients with negative expression of CD44 (log-rank = 8.987, $P = .003$) (Fig. 3D). The multivariate analysis showed that PLOD2 and ZEB1 were independent prognostic factors for the total survival time of patients with ESCC after the surgery. The influence of clinicopathological factors on the postoperative survival time of these patients is shown in Table 3.

In this study, the results on the combined expression of PLOD2 and ZEB1, PLOD2 and CD133, and PLOD2 and CD44 were analyzed by univariate and multivariate analyses. The results showed that negative expression of the combination of PLOD2 and ZEB1 was associated with a better prognosis compared with the expression of other combinations (log-rank = 23.125, $P < .001$; Fig. 4A). Negative expression of the combination of PLOD2 and CD133 was not associated with a better prognosis compared with the expression of other combinations (log-rank = 2.397, $P = .122$; Fig. 4B). Furthermore, negative expression of the combination of PLOD2 and CD44 was related to a better prognosis compared with the expression of other combinations (log-rank = 6.645, $P = .010$; Fig. 4C). The multivariate analysis showed that the combined expression of PLOD2 and ZEB1 was an independent prognostic factor for the total survival time of patients with ESCC. Specific results are shown in Table 4.

3.4. Spearman correlation coefficient analysis

Spearman correlation coefficient analysis showed a positive correlation between the expression of PLOD2 and ZEB1 in 172 LSCC tissues ($r = 0.217$, $P = .004$). Also, a positive correlation

Table 3

Results of univariate analyses and multivariate analysis of overall survival time.

	Univariate analysis			Multivariate analysis		
Variables	**HR**	***P* value**	**95% CI**	**HR**	***P* value**	**95% CI**
PLOD2 expression						
Negative/positive	2.990	<.001	1.645–5.434	2.154	.026	1.098–4.223
ZEB1 expression						
Negative/positive	3.320	<.001	1.928–5.717	3.030	.002	1.519–6.045
CD133 expression						
Negative/positive	1.806	.027	1.070–3.045	0.929	.816	0.497–1.733
CD44 expression						
Negative/positive	2.269	.004	1.306–3.943	1.324	.395	0.693–2.529
Gender						
Female/male	1.048	.854	0.634–1.733	1.194	.506	0.708–2.016
Age (yr)						
<60/≥60	1.324	.256	0.816–2.147	1.308	.297	0.789–2.168
Tumor location						
U/M/L	0.920	.703	0.600–1.411	0.990	.968	0.603–1.625
T classification						
T1 + T2/T3 + T4	1.285	.347	0.762–2.165	1.023	.935	0.588–1.782
Pathologic differentiation						
G1/G2/G3	1.195	.392	0.795–1.797	0.863	.557	0.528–1.410
Lymph node metastasis						
Negative/positive	2.234	.001	1.378–3.620	1.059	.935	2.267–4.193
pTNM						
I + II/III + IV	2.276	.001	1.403–3.692	1.234	.770	0.301–5.055
E-cadherin expression						
Negative/positive	0.563	.027	0.338–0.935	1.179	.604	0.633–2.196
N-cadherin expression						
Negative/positive	1.078	.031	1.049–2.781	0.876	.633	0.507–4.233

95% CI = 95% confidence interval, HR = hazard ratio, PLOD2 = procollagen-lysine2-oxoglutarate 5-dioxygenase 2, pTNM = pathological tumor node metastasis.

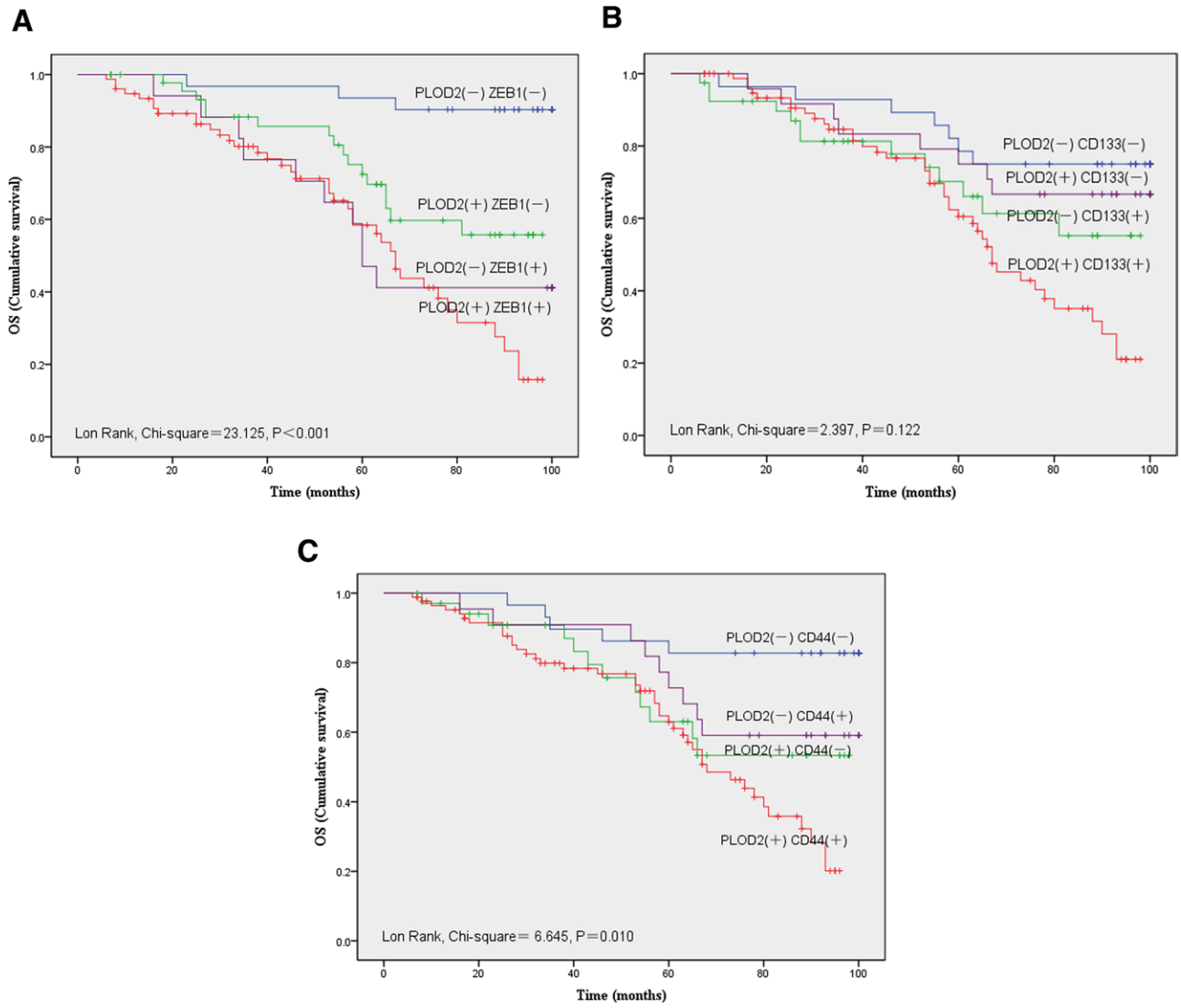

Figure 4. Kaplan–Meier analysis of the survival rate of patients with ESCC. (A) Overall survival of all patients in relation to the combined expression of PLOD2 and ZEB1 (log-rank = 23.125, $P < .001$). (B) Overall survival of all patients in relation to the combined expression of PLOD2 and CD133 (log-rank = 2.397, $P = .122$). (C) Overall survival of all patients in relation to the combined expression of PLOD2 and CD44 (log-rank = 6.645, $P = .010$). ESCC = esophageal squamous cell carcinoma, PLOD2 = procollagen-lysine2-oxoglutarate 5-dioxygenase 2.

Table 4

The combined expression results of univariate analyses and multivariate analysis of overall survival time.

Variables		PLOD2	ZEB1	CD133	CD44	E-cadherin	N-cadherin
PLOD2	*r*	1	0.217	0.178	0.264	−0.197	0.313
	P	–	.004	.019	<.001	.009	<.001
ZEB1	*r*		1	0.262	0.211	−0.384	0.320
	P		–	.001	.005	<.001	<.001
CD133	*r*			1	0.214	−0.035	0.176
	P			–	.005	<.001	.021
CD44	*r*				1	−0.225	0.199
	P				–	.003	.009
E-cadherin	*r*					1	−0.177
	P					–	.020
N-cadherin	*r*						1
	P						–

PLOD2 = procollagen-lysine2-oxoglutarate 5-dioxygenase 2.

was observed between the expression of PLOD2 and CD133 ($r = 0.178$, $P = .019$). Furthermore, a positive correlation was found between the expression of PLOD2 and CD44 ($r = 0.264$, $P < .001$). Moreover, a negative correlation was noted between the expression of PLOD2 and E-cadherin ($r = 0.197$, $P = .009$). Also, a positive correlation was found between the expression of PLOD2 and N-cadherin ($r = 0.313$, $P < .001$). The correlation among other factors is shown in Table 5.

4. Discussion

The etiology of ESCC is complex and remains unclear. Epidemiological studies have shown that vitamin deficiency, fungal infection, nitrite and genetic factors affect the occurrence of ESCC.[24] The incidence of ESCC differs among different regions and nationalities, and China has a high incidence of ESCC.[2] Because of the lack of specific clinical manifestations and effective diagnostic indicators in the early stage of ESCC, most patients are in the middle or late stage when they visit the hospital.[25–27] The main challenges in the treatment of ESCC are postoperative recurrence and metastasis, antiradiotherapy and chemotherapy, and nontargeted drugs. Therefore, it is critical to study the mechanism of occurrence and development of ESCC and find effective indicators for diagnosis and prognosis of ESCC.

Table 5

Results of Spearman correlation coefficient analysis.

	Univariate analysis			Multivariate analysis		
Variables	**HR**	***P***	**95% CI**	**HR**	***P***	**95% CI**
PLOD2 and ZEB1 expression	1.811	<.001	1.409–2.327	1.842	<.001	1.364–2.487
PLOD2 and CD133 expression	1.200	.125	0.951–1.514	1.073	.622	0.812–1.418
PLOD2 and CD44 expression	1.365	.011	1.074–1.736	1.162	.315	0.867–1.556
Gender						
Female/male				1.155	.593	0.681–1.956
Age (yr)						
<60/≥60				1.213	.445	0.739–1.992
Tumor location						
U/M/L				0.894	.653	0.549–1.457
T classification						
T1 + T2/T3 + T4				1.104	.725	0.635–1.919
Pathologic differentiation						
G1/G2/G3				0.963	.990	0.637–1.537
Lymph node metastasis						
Negative/positive				1.225	.791	0.273–5.509
pTNM						
I + II/III + IV				1.546	.572	0.341–7.015
E-cadherin expression						
Negative/positive				1.042	.889	0.585–1.857
N-cadherin expression						
Negative/positive				0.872	.621	0.507–1.501

95% CI = 95% confidence interval, HR = hazard ratio, PLOD2 = procollagen-lysine2-oxoglutarate 5-dioxygenase 2, pTNM = pathological tumor node metastasis.

The expression of PLOD2 gene increases with the progression of tumor, and the mode of collagen cross-linking changes under the catalysis of PLOD2. Collagen fibers change from coiled state around tumor cells to linear state, and tumor cells can transfer rapidly through this linear collagen fiber. Moreover, overdeposited collagen fibers combine with tumor cells and transfer them to vascular system, which leads to distant metastasis of tumor.[28–30] The results of our study Table 2 showed that the expression of PLOD2 in ESCC tissues was significantly higher than that in adjacent tissues, and was significantly correlated with lymph node metastasis and tumor invasion depth. Collagen fiber deposition in ESCC tissues with high expression of PLOD2 was significantly higher than that in tissues with low expression of PLOD2, which was consistent with the results of Di et al.[31]

In 2011, it was first discovered that the expression of PLOD2 in tumor tissues may be regulated by hypoxia.[32] Hypoxia is one of the important characteristics of tumor microenvironment. With the proliferation of tumor, hypoxia can appear in the tumor, and the expression of hypoxia-inducible factor HIF-1α can also increase. Studies have shown that HIF-1α plays a key role in regulating gene expression induced by hypoxia, it can be highly expressed in various tumors, and regulates the occurrence and development of tumors by promoting EMT and angiogenesis.[33] As we can see from Tables 4 and 5, the expression of PLOD2 was positively correlated with the expression of EMT pathway-related factors ZEB1, E-cadherin and N-cadherin, which suggests that PLOD2 may participate in tumor invasion and metastasis by regulating the EMT process through hypoxia-inducible factors.

In many human tumor types, the activation of EMT is closely related to entering CSCs. EMT not only causes malignant progression of tumor but also endows other epithelial tumor stem cells with many characteristics, including the expression of tumor stem cell-specific surface markers ($CD24^-/CD44^+$), and the increase of tumorigenicity of mice after inoculation.[34,35] In addition, the relationship between EMT and cancer stem cell production also involves the regulatory network control of EMT-related transcription factors and epigenetic regulatory factors. Studies have shown that ZEB1 can induce EMT and stem cell-like phenotype by directly inhibiting the expression of miR-200.[36] The relationship between EMT and stem cells in tumor enables cancer cells to switch between mesenchymal stem cell state and epithelial state of rapid proliferation and differentiation, which plays an important role in tumor recurrence, metastasis, and drug resistance.[37] This study found that the expressions of the transcription factors ZEB1, E-cadherin and N-cadherin related to EMT were positively correlated with the expressions of tumor stem cell markers CD44 and CD133, suggesting that activation of EMT may promote CSCs.

5. Conclusions

This study found that PLOD2 is closely related to clinicopathological factors such as ESCC and is an independent prognostic factor. Combined analysis of EMT-related factors and tumor stem cell-related markers showed that PLOD2 plays an important role in the progression of ESCC. However, due to the complexity and diversity of the microenvironment, the interaction among various substances and the overlapping of signaling pathways into a network, the interaction mechanism and regulation relationship between many substances and EMT and CSCs remain unclear. In the future, I hope that there will be opportunities to continue in-depth research in order to provide new ideas and theoretical basis for the optimization and innovation of esophageal cancer diagnosis and treatment technology.

Author contributions

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