Screening and Evaluating Novel Autoantibodies Based on Proteomic Chips in Diagnosis of Esophageal Squamous Cell Carcinoma

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Primary research

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Abstract

Background

More and more studies have confirmed that TAAs could be used as potential biomarkers for tumor patients. The aim of this study is to identify novel TAAs through proteomic chips and construct a diagnostic model to discriminate esophageal squamous cell carcinoma (ESCC) cases from benign esophageal diseases cases and normal controls (NCs).

Methods

The human proteomic chips were used to screen TAAs. Enzyme-linked immunosorbent assay (ELISA) was adopted to verify and validate the candidate TAAs which were screened by the chips in verification phase (90 ESCC cases and 90 NCs) and validation phase (126 ESCC cases, 237 benign esophageal diseases cases and 126 NCs). Based on the candidate TAAs, then the diagnostic model for ESCC was constructed by logistic regression analysis in the training group and validated in the testing group.

Results

Firstly, thirteen potential candidate TAAs were identified by proteomic chips. In verification phase, the titers of six TAAs (anti-MAGEA1, anti-VCL, anti-PRKCZ, anti-TP53, anti-NFKB1 and anti-MAGEA4) in ESCC cases were higher than those in NCs while other seven TAAs showed no difference. Subsequently, six candidate TAAs were validated further in validation phase. Finally, the logistic regression model with 3 TAAs (anti-MAGEA1, anti-VCL, anti-TP53) could discriminate ESCC cases from NCs with area under curve(AUC) of 0.80 in the training group and 0.73 in the testing group, respectively. Meanwhile, the model could discriminate ESCC cases from benign esophageal diseases cases with AUC of 0.74.

Conclusion

The study has identified six novel TAAs through protein chips and constructed a diagnostic model. The panel showed great performance to distinguish ESCC cases from benign esophageal diseases cases and NCs.

1. Background

Esophageal cancer (EC) is one of the common malignant tumors in the world, and its incidence and mortality are relatively high. Global cancer statistics released in 2020 showed that EC has a higher incidence in East Asia, especially in China, and the incidence in males are significantly higher than that in females[1]. In 2020, the number of new cases and deaths of EC in China are 324,422 and 301,135, accounting for about 50% and 55% of the world[1]. According to the clinical histopathological type, EC was mainly divided into esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC) [1]. Importantly, the incidence of ESCC in China accounts for more than 70% of the world[2]. The onset of ESCC was insidious and there were no obvious clinical symptoms in the early stage[3]. At present, the pathogenesis of ESCC was not clear, and there were few effective treatment measures for advanced ESCC patients. Therefore, seeking a noninvasive diagnostic method for ESCC of early stage is crucial to improve the 5-year survival rate of patients. At present, the clinical diagnosis of ESCC was mainly based on clinical symptoms combined with cytological examination, endoscopy, other imaging and pathological cell techniques[4]. Due to the disadvantages which still existed such as low sensitivity, high cost and invasiveness, they were not suitable to use as screening tests for ESCC. So far, studies have found kinds of molecular biomarkers related to the diagnosis of ESCC, including cancer stem cells (CSCs) and serological markers CEA, CA199 and SCC etc., while there was still no consensus that a certain biomarker can be used in clinical practice[5, 6]. Therefore, actively exploring novel biomarkers for diagnosis of ESCC has important clinical significance for improving the prognosis of patients.

Previous studies have demonstrated that tumor-associated antigens (TAAs) can trigger immune response to produce autoantibodies against TAAs (TAAs) during the process of tumor growth[7, 8]. In recent years, more and more studies have confirmed that TAAs could be used as potential biomarkers for tumor patients[9-14].

Protein chip technology is playing an increasingly important role in the study of screening autoantibodies against tumor-associated antigens owing to its advantages of high-throughput, parallel and rapid analysis[15-19]. This study devoted to screen and validate

novel TAABs related to ESCC through the proteomic chips (containing more than 21,000 recombinant human proteins) and Enzyme-linked immunosorbent assay (ELISA), then a diagnostic model was constructed for evaluating the diagnostic value in ESCC.

2. Methods

2.1 Serum sample

Serum samples of ESCC cases and benign esophageal diseases (BED) cases were collected from Affiliated Tumor Hospital and the First Affiliated Hospital of Zhengzhou University (June 2013-December 2017). All enrolled ESCC cases were confirmed by pathological examination and did not receive any treatment. The benign esophageal diseases (BED) cases have no tumor-related diseases. The NCs came from the biological sample bank of the Key Laboratory of Tumor Epidemiology of Henan Province and did not have any digestive tract diseases or autoimmune diseases. This study was designed in three phases. In the discovery phase, 30 ESCC sera in 10 sera pools (three serum samples were mixed into one sera pool) and 21 NCs sera in 7 sera pools (three serum samples were mixed into one sera pool) and 3 individual sera were used to screen candidate TAABs. In the verification phase and validation phase, a total of 669 serum samples were used to verify and validate candidate TAABs through Enzyme-linked immunosorbent assay (ELISA), including 216 ESCC cases, 237 benign esophageal diseases (BED) cases and 216 normal controls (NCs). The verification phase included 90 ESCC cases and 90 NCs. The validation phase included 126 ESCC cases, 126 NCs and 237 benign esophageal diseases (BED) cases. All ESCC cases and NCs have no difference between age and gender after frequency matching. All serum samples were collected by standard methods and immediately taken to the laboratory for storage. This study has been approved by the Ethics Committee of Zhengzhou University and all participants have informed consent forms.

2.2 Proteomic chips

In this study, a small number of serum samples (10 ESCC serum pools vs 10 NC serum pools) were used for screening TAABs through the HuProt™ human proteomic chips. HuProt™ human proteomic chips were purchased from BCBio Biotechnology (Guangzhou, China). The chips are currently the largest collection of full-length human proteins available in the world. It contains 20,240 recombinant proteins, covering 81% of the human proteome. More detailed information about proteomic chips was available at <https://cdi.bio/huprot/>.

2.3 Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) was applied to detect the level of TAABs in serum samples. The protocol has been described in detail in our previous studies [20, 21]. In this study, the verification phase was used to verify the candidate TAABs that screened out from the proteomic chips, then the TAABs were validated in the validation phase. The 13 recombinant proteins were diluted in coating buffer at optimal concentrations of 0.25, 0.25, 0.25, 0.25, 0.25, 0.25, 0.25, 0.125, 0.125, 0.25, 0.25 and 0.125 µg/ml (MAGEA1, VCL, PPP1R15A, PDE4DIP, PRKCZ, TP53, SMARCC1, BASP1, SMARCE1, GABPA, ANKHD1, NFKB1, MAGEA4), respectively. Importantly, 6 same repeated serum samples and 2 blank controls were run on each plate. The mean value of the 8 samples was used to normalize the difference plates, and the blank controls were consulted for quality control for individual plates.

2.4 Statistical analysis

The human proteomic chips were used to screen TAABs, and the detected data were normalized based on signal-to-noise ratio (SNR) value. Whereafter, the SNR values of each sample were analyzed by the z-score method, and then the differentially expressed TAABs were found out. The FunRich website was applied to find proteins related to cancer pathways (GO, KEGG pathways), focusing on cell cycle regulation, apoptosis, signal transduction and expression locations. SPSS 26.0, GraphPad Prism 6.0 and MedCalc 11.0 were used to analyze the experimental data. PASS 15.0 software was used to estimate the sample size. Non-parametric tests were adopted to analyze whether there were differences between groups; ROC curve was used to evaluate the diagnostic value of a single TAAB. The sensitivity, specificity, accuracy, positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio and Youden index were calculated according to ROC curve (the cut-off value of single TAAB was determined according to the maximum Youden index and the specificity was greater than 90%). Logistic regression was used to construct the diagnosis model of ESCC. All *P* values were based on two-sided test, and *P* < 0.05 was considered to be statistically different.

3. Results

3.1 Baseline characteristics of the study population

The PASS software was used to calculate the sample size. The parameter was set as follows: $\alpha=0.05$; $\delta=0.05-0.1$; sensitivity =20%; specificity = 90%, then the range of sample size was 70-264. This study was designed in three phases. The first phase was the discovery phase, including 10 ESCC sera pools and 10 NCs sera pools, the high-throughput protein chips were used to screen candidate TAABs related to the diagnosis of ESCC. The second phase was the verification phase, including 90 ESCC cases and 90 NCs, ELISA was used to verify the 13 TAABs screened by the protein chips. The third phase was the validation phase, including 126 ESCC cases, 237 benign esophageal diseases (BED) cases and 126 NCs. Furthermore, the candidate TAABs screened out were validated by ELISA. The design of the present study was shown in **Figure 1**. The clinical baseline characteristics of the subjects in our studies were listed in **Table 1**. The sample size of verification phase and validation phase were within the range. And there was no significant difference in age and gender between the verification phase and validation phase.

3.2 The process of screening TAABs

Firstly, the data of chips was used to screen the TAABs related to the cancer pathway (GO, KEGG pathway) through the FunRich website with focusing on cell cycle regulation, apoptosis and signal transduction, expression position. The **figure S1** presented the GO and KEGG pathway. A total of 25 TAABs were screened out. Secondly, the fold change ≥ 2 and histogram were adopted to observe the TAAs with significant differences as a further step to screen the candidate TAAs. Subsequently, searched protein function and related literature through the Uniprot website and Pubmed. Finally, 13 TAABs (anti-PDE4DIP, anti-VCL, anti-TP53, anti-PPP1R15A, anti-SMARCC1, anti-BASP1, anti-SMARCE1, anti-GABPA, anti-NFKB1, anti-MAGEA4, anti-PRKCZ, anti-MAGEA1, anti-ANKHD1) were screened out. In addition, the mRNA expression level of 13 proteins of ESCA in GEPIA ($|\log_2FC|$ Cutoff=0.485, p-value Cutoff=0.01) were shown in **Figure S2**.

3.3 Autoantibodies in the verification phase and the validation phase

To verify the 13 TAABs screened out by the chips, the ELISA was carried out to verify and validate in the verification phase and the validation phase, respectively. The verification phase included 90 ESCC cases and 90 NCs. **Figure 2A** showed the optical density (OD) value of single TAAB in the verification phase. It indicated that the titers of six autoantibodies (anti-MAGEA1, anti-NFKB1, anti-VCL, anti-MAGEA4, anti-PRKCZ and anti-TP53) in sera were significantly higher than normal controls. However, the titers of anti-PPP1R15A, anti-SMARCC1, anti-SMARCE1, anti-GABPA, anti-PDE4DIP and anti-BASP1 in sera did not show significant differences compared with normal controls. **Figure 3** showed the diagnostic value of the single TAAB. The range of AUC was 0.51-0.68, the range of sensitivity was from 1.1% to 35.56%, and all of the specificity were greater than 90.0%.

The validation phase included 126 ESCC cases, 126 NCs and 237 benign esophageal diseases (BED) cases. **Figure 2B** showed the OD value of the six TAABs screened out by the verification phase. It indicated that the titers of six autoantibodies (anti-MAGEA1, anti-NFKB1, anti-VCL, anti-MAGEA4, anti-PRKCZ and anti-TP53) in the sera of patients with ESCC were also significantly higher than that in benign esophageal diseases (BED) cases and normal controls. **Figure 4** showed the diagnostic value of the six autoantibodies screened out by the verification phase. The range of AUC was 0.63-0.74, the sensitivity was from 32.5% to 40.5%, and the specificity were greater than 90.0%. Anti-MAGEA1 presented an outstanding diagnostic value, with an AUC of 0.74, sensitivity of 40.48%, specificity of 90.11% among six TAABs in the validation phase. Anti-TP53 achieved the favorable specificity of 91.45%. The diagnostic value of single TAAB in verification phase and validation phase that discriminate ESCC cases from NCs were shown in **Table 2**. Furthermore, the diagnostic value of single TAAB that discriminates ESCC cases from benign esophageal diseases (BED) cases were shown in **Table 3**. The range of AUC was 0.65-0.76, the sensitivity was from 32.54% to 43.65%, and the specificity were greater than 90.0%. However, the **Figure S3** showed that only anti-MAGEA1 could discriminate benign esophageal diseases (BED) cases from NCs among six TAABs, the AUC was 0.591 (95%CI:0.529,0.652).

3.4 Establishment of diagnostic model to discriminate ESCC from NC or BED

Validation phase served as a training group to construct diagnostic model for ESCC. The dependence variable was whether a participant was considered as ESCC or not. Six TAABs with different titers between ESCC cases and NCs were used as independence variables. The Logistic regression model was produced with three TAABs. The formula of the prediction model was $PRE(P=ESCC, 3TAABs) = 1 / (1 + \exp(-(-4.810 + 5.213 \times MAGEA1 + 4.699 \times VCL + 6.190 \times TP53)))$. In the training group, the AUC was 0.80, the sensitivity and the specificity were 61.1%, 90.1%, respectively (**Figure 5A**). Verification phase was used to test the model. The AUC of the testing group was 0.73, the sensitivity and the specificity were 65.6%, 72.2%, respectively (**Figure 5B**). The model was stable

by the Delong test ($P = 0.160$). Furthermore, the model had great performance to distinguish ESCC cases and benign esophageal diseases (BED) cases, with the AUC of 0.75, the sensitivity of 78.6% and the specificity of 59.0% (Figure 5C).

3.5 Subgroup analysis of diagnostic model

Patients with ESCC were divided into different subgroups according to clinical characteristics (Table 4). The results indicated that the diagnostic model did not perform well among the subgroups of clinical characteristics of ESCC (age, gender, family history, differentiated degree, TNM stage, degree of invasion). And the validation phase also showed similar results.

4. Discussion

According to the released global cancer data, the incidence of EC ranked seventh, and the mortality rate ranked sixth. East Asia had the highest incidence of EC, and China had the heaviest burden[1]. The current clinical treatment of EC included surgery, targeted drug therapy, adjuvant radiotherapy and chemotherapy[22-27]. However, only relying on current medical technology, the five-year survival rate was only between 15%-20% [3]. If EC can be detected at early stage, the 5-year survival rate could be increased by 80%-90%[4]. Therefore, improving the screening and diagnosis ability of EC was the most economical and effective means of prevention and treatment. Current diagnostic approaches may have various shortcomings in clinical diagnosis of EC, such as low sensitivity and traumatic[28-30]. The appearance of autoantibodies against tumor-associated antigens can make up these shortcomings and provide an opportunity for auxiliary diagnosis of esophageal cancer[9, 31-33].

In this study, thirteen tumor-associated antigens related to the diagnosis of EC were screened out through the protein chips. Then the corresponding thirteen autoantibodies were screened further through ELISA to confirm whether they have diagnostic value for ESCC. Finally, we constructed the logistic regression model for diagnosing ESCC, including anti-MAGEA1, anti-VCL and anti-TP53 in the model. The AUC of the diagnostic model in the training group was 0.80, and the sensitivity and specificity were 60.1% and 90.1%, respectively. The model was found to be stable by the Delong test. The results of this study confirmed the TAAbs were reliable as biomarkers for the diagnosis of ESCC.

Many researchers have performed similar studies. In 2014, Xu discovered a panel of autoantibodies against tumor-associated antigens (TP53, NY-ESO-1, MMP-7, HSP70, peroxiredoxin VI and Bmi-1) for ESCC with AUC of 0.78[34, 35]. In 2018, Zhang found that anti-TOPO48 could be used as a biomarker for early diagnosis and prognosis of ESCC, the area under the ROC curve was 0.863[36]. Our study produced consistent results that anti-TP53 could be a biomarker for detection of ESCC, meanwhile, the study also found other five potential TAAbs (anti-MAGEA1, anti-NFKB1, anti-VCL, anti-MAGEA4, anti-PRKCZ). A study found that a panel of three TAAbs (anti-HCCR, anti-C-myc and anti-MDM2) and three miRNAs (miR-21, miR-223 and miR-375) had a high diagnostic value for ESCC with AUC of 0.89, while too many biomarkers combined in the study may not be conducive to large-scale screening and cause more economic burden for patients[37]. Therefore, the author also discovered three TAAbs (anti-GNA11, anti-ACVR1B and anti-TP53) and constructed a model that the diagnostic value of ESCC was 0.88[38]. A protein chips customized by driver gene were used in the study, however, our study screen novel biomarkers through the comprehensive protein chips. Previous studies have indicated that the sensitivity of single TAAb was usually low, our study also adopted the approach of several combined TAAb(s) to improve the sensitivity, specificity and diagnostic value. Furthermore, our study also showed the TAAbs have the ability to differentiate ESCC cases from benign esophageal diseases (BED) cases.

As far as we know, the panel of the three TAAbs in the current study have not been studied in the diagnosis of ESCC. MAGEA1 mainly existed in cells and could play a role in embryonic development and tumor transformation or tumor progression. The related studies have showed that it was highly expressed in endometrial cancer, lung cancer and other cancers, and it may be also a prognostic factor in ESCC[39-41]. VCL mainly existed on the cell membrane and was mainly involved in cell adhesion. It was highly expressed in pancreatic cancer tissue and was related to the poor prognosis of cancer [33, 42]. TP53 was mainly present in the cytoplasm and nucleus, inducing growth arrest or apoptosis, regulating cell cycle. It was highly expressed in breast cancer, ovarian cancer, esophageal cancer, colorectal cancer tissues and cells and its autoantibodies were also found to be highly expressed in cancer[43-47].

The advantage of our studies lies in the screening of tumor-associated antigens related to the diagnosis of ESCC through the human proteomic chips. In addition, we constructed a diagnostic model combining three TAAbs to improve the efficiency of ESCC diagnosis and verified the stability of the model. Of course, this study is not flawless. More TAAbs have been screened out through the protein chips. This study may only verify a small number of them, and there are more potential markers waiting for our verification.

Conclusion

In conclusion, this study has identified six TAABs biomarkers as an auxiliary diagnostic method for ESCC and showed the diagnostic model constructed by the combination of three TAABs, which can distinguish ESCC cases from NCs and benign esophageal diseases (BED) cases. Furthermore, the related mechanism of these TAABs in ESCC is also worthy of our further exploration.

Abbreviations

ESCC: esophageal squamous cell carcinoma

NCs: normal controls

ELISA: enzyme-linked immunosorbent assay

TAABs: autoantibodies against the tumor-associated antigens

TAA: tumor-associated antigen

AUC: area under curve

EC:esophageal cancer

EAC: esophageal adenocarcinoma

CSCs: cancer stem cells

BED: benign esophageal diseases

OD: optical density

Declarations

Ethics approval and consent to participate

Informed consent forms have been signed by all participates before this study. The research protocol has been approved by the Ethics Committee of the Zhengzhou University and is based on the ethical principles of medical research involving human subjects in the Helsinki Declaration.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors declared that they have no conflict of interests.

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Authors' contributions

Zhuo Han, Guiying Sun and Peng Wang was responsible for study conception and design; Jinyu Wu and Zhuo Han were responsible forexperiment; Zhuo Han was responsible for data analysis, Keyan Wang, CuipengQiu, Chi Cui, Hua Ye, Liping Dai, Jianxiang Shi, and

Jianning Zhang were responsible for revision of the manuscript.

Acknowledgements

No Application.

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Tables

Table 1. Characteristics of all subjects in this study.

Variables	Verification phase (N=180)		Validation phase (N=252)		P
	ESCC(N=90)	NC(N=90)	ESCC(N=126)	NC(N=126)	
Age					
Median	65	64	65	64	0.201 ^a
(Q1, Q3)	(60.8,71.0)	(57.0,68.3)	(58.0,69.0)	(57.0,69.0)	
Range	41-88	47-84	45-87	43-83	0.809 ^b
Gender (n(%))					
Male	60(66.7)	60(66.7)	86(68.3)	86(68.3)	0.643 ^a
Female	30(31.3)	30(33.3)	40(31.7)	40(31.7)	0.806 ^b
Family history (n(%))					
Yes	15(16.7)		20 (15.9)		0.105 ^a
No	74(82.2)		53 (42.1)		
Unknown	1(1.1)		53 (42.1)		
Differentiated degree (n(%))					
Poorly	13(14.4)		14(11.1)		0.837 ^a
Moderately	32(35.6)		32(25.4)		
Highly	7(8.8)		14(11.1)		
unknown	38(42.2)		66(52.4)		
TNM stage (n(%))					
0	3(3.3)		8(6.3)		0.106 ^a
I	18(20.0)		25(19.8)		
II	14(15.6)		20(15.9)		
III	18(20.0)		18(14.3)		
IV	9(10.0)		5(4.0)		
Unknown	28(31.1)		50(39.7)		
Degree of invasion (n(%))					
T0-T1	22(23.7)		33 (26.2)		0.418 ^a
T2	8(8.6)		16 (12.7)		
T3	31(33.3)		28 (22.2)		
Unknown	29(31.2)		49 (38.9)		

Note: a: there was no difference between subgroups.

b: there was no difference between verification phase and validation phase.

ESCC, esophageal squamous cell carcinoma; NC, normal control; Q1,25% interquartile range; Q3,75% interquartile range.

Table 2. Diagnostic value of 13 TAAbs in verification phase and validation phase for (ESCC)

TAAbs	Verification cohort (90 ESCC vs 90 NC)						Validation cohort (126 ESCC vs 126 NC)					
	Se(%)	Sp(%)	AUC	95%CI	Accuracy (%)	P	Se(%)	Sp(%)	AUC	95%CI	Accuracy (%)	P
MAGEA1	33.3	91.1	0.670	0.591-0.749	62.2	<0.000	40.5	90.1	0.743	0.682-0.803	65.3	<0.000
NFKB1	35.6	91.1	0.675	0.596-0.754	63.3	<0.000	32.5	90.9	0.626	0.556-0.695	61.7	0.001
VCL	26.7	92.2	0.674	0.597-0.752	59.5	<0.000	34.9	90.1	0.673	0.607-0.739	62.5	<0.000
MAGEA4	25.6	91.1	0.609	0.526-0.692	58.3	0.012	37.3	90.1	0.640	0.573-0.708	63.7	<0.000
PRKCZ	26.7	92.2	0.648	0.567-0.728	59.5	0.001	36.5	90.6	0.674	0.606-0.741	63.6	<0.000
TP53	17.8	98.9	0.648	0.569-0.728	58.3	0.001	39.7	91.5	0.695	0.630-0.761	65.6	<0.000
ANKHD1	1.1	100.0	0.544	0.460-0.629	50.6	0.304	—	—	—	—	—	—
PPP1R15A	16.7	90.0	0.569	0.485-0.653	53.3	0.108	—	—	—	—	—	—
SMARCC1	12.2	93.3	0.542	0.458-0.627	52.8	0.329	—	—	—	—	—	—
BASP1	4.4	96.7	0.507	0.423-0.591	50.6	0.880	—	—	—	—	—	—
GABPA	15.6	90.0	0.508	0.422-0.593	52.8	0.856	—	—	—	—	—	—
SMARCE1	16.7	92.2	0.559	0.475-0.643	54.5	0.174	—	—	—	—	—	—
PDE4DIP	17.8	90.0	0.537	0.452-0.622	53.9	0.396	—	—	—	—	—	—

Note: ESCC, esophageal squamous cell carcinoma, NC, normal control, Se, sensitivity, Sp, specificity, AUC, area under the receiver operating characteristic curve, CI, confidence interval.

Table 3. Diagnostic value of 6 TAAbs for ESCC vs BED

TAAbs	126 ESCC vs 237 BED				
	Se(%)	Sp(%)	AUC	95%CI	Accuracy (%)
MAGEA1	37.3	90.7	0.681	0.620-0.743	72.2
NFKB1	32.5	91.1	0.651	0.589-0.713	70.8
VCL	38.9	90.3	0.704	0.646-0.761	72.5
MAGEA4	43.6	90.7	0.764	0.712-0.816	74.4
PRKCZ	38.1	91.6	0.740	0.686-0.793	73.0
TP53	37.3	92.4	0.700	0.642-0.758	73.3

Note: ESCC, esophageal squamous cell carcinoma, BED, benign esophageal disease, Se, sensitivity, Sp, specificity, AUC, area under the receiver operating characteristic curve; CI, confidence interval.

Table 4. Diagnostic value of the TAAbs panel for ESCC patients with different subtypes

	N	Se (%)	Sp(%)	Accuracy (%)	AUC	95%CI	P^1	P^2	PPV (%)	NPV (%)	+LR	-LR
Verification cohort												
All patients	90	34.4	92.2	63.3	0.729	0.656-0.802			81.5	58.4	4.4	0.7
Age												
≤65	48	22.9	94.4	69.5	0.681	0.588-0.774	0.422	0.109	68.6	69.7	4.1	0.8
>65	42	47.6	92.2	78.0	0.783	0.698-0.868	0.341		74.0	79.0	6.1	0.6
Gender												
Male	60	35.5	92.2	69.1	0.721	0.638-0.804	0.886	0.705	75.8	67.5	4.6	0.7
Female	30	35.7	91.1	78.0	0.746	0.646-0.845	0.787		55.5	82.0	4.0	0.7
Family history												
No	74	35.1	92.2	66.4	0.727	0.650-0.805	0.971	0.899	78.7	63.3	4.5	0.7
Yes	15	33.3	91.1	82.8	0.717	0.583-0.851	0.877		38.4	89.1	3.7	0.7
Differentiated degree												
Poorly	13	38.5	95.6	88.4	0.756	0.626-0.887	0.721	0.510	55.8	91.5	8.7	0.6
Highly and moderately	39	30.8	93.3	74.4	0.701	0.601-0.801	0.657		66.6	75.7	4.6	0.7
TNM stage												
I-II	35	28.6	93.3	75.2	0.702	0.603-0.801	0.668	0.190	62.4	77.1	4.3	0.8
III-IV	27	40.7	91.1	79.5	0.793	0.700-0.885	0.285		57.8	83.7	4.6	0.7
Degree of invasion												
T0-T1	24	25.0	94.4	79.8	0.650	0.521-0.780	0.296	0.130	54.3	82.5	4.5	0.8
T2-T3	39	38.5	91.1	75.2	0.770	0.684-0.855	0.476		65.2	77.4	4.3	0.7
Validation cohort												
All patients	126	61.1	90.5	75.8	0.795	0.739-0.851			86.5	69.9	6.4	0.4
Age												
	N	Se (%)	Sp(%)	Accuracy (%)	AUC	95%CI	P^1	P^2	PPV (%)	NPV (%)	+LR	-LR
≤65	68	63.2	90.5	80.9	0.784	0.710-0.858	0.818	0.651	78.2	82.0	6.7	0.4
>65	58	58.6	90.5	80.4	0.808	0.736-0.880	0.782		74.0	82.6	6.2	0.5

Gender												
Male	83	55.4	90.1	76.3	0.768	0.690-0.836	0.553	0.127	78.7	75.4	5.6	0.5
Female	43	72.1	90.5	85.8	0.848	0.771-0.925	0.276		72.1	90.5	7.6	0.3
Family history												
No	53	56.6	90.5	80.5	0.805	0.733-0.878	0.832	0.237	71.5	83.2	6.0	0.5
Yes	20	55.0	90.5	85.6	0.704	0.554-0.855	0.269		47.9	92.7	5.8	0.5
Differentiated degree												
Poorly	14	64.3	90.5	87.9	0.763	0.610-0.916	0.701	0.688	42.9	95.8	6.8	0.4
Highly and moderately	46	50.0	90.5	79.7	0.726	0.631-0.822	0.226		65.8	83.2	5.3	0.6
TNM stage												
I-II	53	54.7	90.5	79.9	0.743	0.657-0.830	0.315	0.731	70.8	82.6	5.8	0.5
III-IV	23	52.2	90.5	84.6	0.766	0.643-0.888	0.715		50.1	91.2	5.5	0.5
Degree of invasion												
T0-T1	33	48.5	95.2	85.5	0.710	0.596-0.825	0.190	0.316	72.6	87.6	10.1	0.5
T2-T3	44	54.5	90.5	81.2	0.783	0.697-0.869	0.820		66.7	85.1	5.7	0.5

Note: ESCC, esophageal squamous cell carcinoma Se, sensitivity, Sp, specificity, AUC, area under the receiver operating characteristic curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; +LR, positive likelihood ratio; -LR, negative likelihood ratio.

P^1 means comparison between TNM stage or family history or degree of invasion or distant metastasis or differentiated degree and all patients with the method of De Long et.

P^2 means comparison between ≤ 65 and >65 or TNM I-II and TNM III-IV stage or family history (-) and family history (+) or T0-1 and T2-3 or poorly differentiated degree and highly and moderately differentiated degree.

Figures

Figure 1

The design of this study. The first phase was the discovery phase, including 10 ESCC cases and 10 NCs, the high-throughput protein chips were used to screen candidate TAABs related to the diagnosis of ESCC. The second phase was the verification phase, including 90 ESCC cases and 90 NCs, ELISA was used to verify the 13 TAABs screened by the protein chips. The third phase was the validation phase, including 126 ESCC cases, 237 BED cases and 126 NCs. Furthermore, the candidate TAABs screened out were validated by ELISA. ESCC: esophageal squamous cell carcinoma; NCs: normal controls; TAABs: autoantibodies against tumor-associated antigens; ELISA: enzyme-linked immunosorbent assay.

Figure 2

The titer of 13 TAAs in ESCC cases and NCs. (A) Scatter plots of the titer of autoantibodies in the verification phase. (B) Scatter plots of the titer of autoantibodies in the validation phase. ESCC, esophageal squamous cell carcinoma; BED, benign esophageal diseases; NCs, normal controls. (□P<0.05, ▨P<0.01, ▩P<0.001).

figure 3

Figure 3

Diagnostic performance of 13 TAAbs in the verification phase for ESCC. ESCC, esophageal squamous cell carcinoma.

Figure 4

Diagnostic performance of 6 TAAs in the validation phase for ESCC and BED detection. ESCC, esophageal squamous cell carcinoma, BED, benign esophageal diseases, NC, normal control.

A

B

C

Figure 5

Receiver operating characteristic curve analysis of the prediction model with TAAbs panel in esophageal squamous cell carcinoma (ESCC) detection. (A) The prediction model with 3TAABs (anti-MAGEA1, anti-VCL, anti-TP53) in the training group. (B) The prediction model with 3TAABs (anti-MAGEA1, anti-VCL, anti-TP53) in the testing group. (C) The diagnosis performance of the prediction model for benign esophageal diseases (BED) cases.

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