Research Article

A Novel Three-Gene Model Predicts Prognosis and Therapeutic Sensitivity in Esophageal Squamous Cell Carcinoma

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To precisely predict the clinical outcome and determine the optimal treatment options for patients with esophageal squamous cell carcinoma (ESCC) remains challenging. Prognostic models based on multiple molecular markers of tumors have been shown to have superiority over the use of single biomarkers. Our previous studies have identified the crucial role of ezrin in ESCC progression, which prompted us to hypothesize that ezrin-associated proteins contribute to the pathobiology of ESCC. Herein, we explored the clinical value of a molecular model constructed based on ezrin-associated proteins in ESCC patients. We revealed that the ezrin-associated proteins (MYC, PDIA3, and ITGA5B1) correlated with the overall survival (OS) and disease-free survival (DFS) of patients with ESCC. High expression of MYC was associated with advanced pTNM-stage ($P < 0.011$), and PDIA3 and ITGA5B1 were correlated with both lymph node metastasis (PDIA3: $P < 0.001$; ITGA5B1: $P = 0.001$) and pTNM-stage (PDIA3: $P = 0.001$; ITGA5B1: $P = 0.009$). Furthermore, we found that, compared with the current TNM staging system, the molecular model elicited from the expression of MYC, PDIA3, and ITGA5B1 shows higher accuracy in predicting OS ($P < 0.001$) or DFS ($P < 0.001$) in ESCC patients. Moreover, ROC and regression analysis demonstrated that this model was an independent predictor for OS and DFS, which could also help determine a subgroup of ESCC patients that may benefit from chemoradiotherapy. In conclusion, our study has identified a novel molecular prognosis model, which may serve as a complement for current clinical risk stratification approaches and provide potential therapeutic targets for ESCC treatment.

1. Introduction

Esophageal cancer is the sixth leading cause of cancer-related deaths and the eighth most common type of malignant gastrointestinal cancer in the world [1, 2]. Adenocarcinoma and squamous cell carcinoma (ESCC) are the two major types of esophageal cancer, with the latter accounting for the 90% of cases worldwide [3]. In China, ESCC still remains the highest incidence and cancer-induced mortality rates, and the long-term prognosis of patients with ESCC is less than 20%, despite improvements in treatments such as surgical resection and adjuvant chemoradiation [4, 5]. This poor prognosis for ESCC patients is highly associated with the difficult nature of diagnosing early-stage ESCC and the frequent occurrence of local invasion and distant metastasis [5]. In addition, conventional chemotherapy and radiotherapy treatments are relatively ineffective [6]. Therefore, seeking novel molecular prognostic markers that can help
identify patients at high risk and improving their prognosis are urgent needs in the clinic.

However, signal molecular marker cannot meet the clinical requirements for biomarkers, such as high sensitivity and specificity, and it is more accurate than the current clinical staging system [7]. In the last few years, studies have demonstrated that combinations of multiple biomarkers were more sensitive and reliable than single molecular marker. Although several prognostic biomarkers for ESCC have been reported [8–12], there is still no ideal biomarker for clinical use.

Ezrin as a member of the ezrin/radixin/moesin (ERM) protein family plays an important role in regulating the growth and metastasis of cancer [13, 14]. In our previous studies, we showed that ezrin was upregulated in ESCC and promoted cellular proliferation and invasiveness of ESCC cells [15]. Furthermore, Ezrin might be a new prognostic molecular marker for ESCC patients [16]. Ezrin was also known as a key molecule connected with many other molecules in the biology of tumor development [17]. In these ezrin-related proteins, our previous studies identified that three proteins, i.e., MYC, PDIA3, and ITGA5B1, correlated with patients’ survival [11, 12]. MYC, a proto-oncogene, plays an integral role in a variety of normal cellular functions [18]. MYC amplification is a recurrent event in many tumors and contributes to tumor development and progression [19–22]. The progress of MYC-induced tumorigenesis in prostate cancer cells entails MYC binding to the ezrin gene promoter and the induction of its transcription [23]. Meanwhile, the induction of ezrin expression is essential for MYC-stimulated invasion [23], PDIA3 (protein disulfide isomerase family A, member 3), also known as ERp57, is one of the main members of the protein disulfide isomerase (PDI) gene family and is identified primarily as enzymatic chaperones for reconstructing misfolded proteins within the endoplasmic reticulum (ER) [24]. Several studies have linked PDIA3 to different types of cancer, including breast [25], ovarian [26], and colon [27] cancers. In ESCC, we found that PDIA3 interacted with ezrin, and it was not only involved in the development and progression of ESCC but also related to OS and DFS of ESCC patients [12]. ITGA5B1 is a member of the integrin family which plays a significant role in cell adhesion to the extracellular matrix (ECM) [28, 29]. In ESCC, ITGA5B1 upregulates the expression of ezrin through the L1CAM [30].

Although ezrin plays a pivotal role in ESCC progression, the clinical significance of ezrin-related proteins (MYC, PDIA3, and ITGA5B1) has not been thoroughly investigated in ESCC patients. Clinicopathological analyses of these ezrin-interacting proteins may further our understanding of the function of ezrin and provide therapeutic targets for ESCC. In the current study, we found that a three-gene signature comprised of MYC, PDIA3, and ITGA5B1 could independently predict ESCC patient survival.

2. Materials and Methods

2.1. Patients and Specimens. For this retrospective study, 284 cases of formalin-fixed, paraffin-embedded ESCC tissue were collected from the Shantou Central Hospital between November 2007 and January 2010. All patients underwent curative resection and were confirmed as having ESCC by pathologists in the Clinical Pathology Department of the Hospital. Information on age, gender, and histopathological factors was obtained from the medical records and shown in Table 1. An independent validation set (GSE53622 and GSE5364) was obtained from the publicly available GEO database (https://www.ncbi.nlm.nih.gov/). We excluded the ESCC patients without clinical survival information, and the clinicopathological information was shown in Table S1. Overall survival (OS) was defined as the interval between surgery and death from tumors or between surgery and the last observation taken for surviving patients. Disease-free survival (DFS) was defined as the interval between surgery and diagnosis of relapse or death. Ethical approval was obtained from the ethical committee of the Central Hospital of Shantou City and the ethical committee of the Medical College of Shantou University, and only resected samples from surgical patients giving written informed consent were included for use in research.

2.2. Tissue Microarrays (TMAs) and Immunohistochemistry (IHC). TMAs were constructed based on standard techniques as previously described [12]. IHC was performed using the PV-9000 2-step Polymer Detection System (ZSGB-BIO, Beijing, China) and Liquid DAB Substrate Kit (Invitrogen, San Francisco, CA) according to the manufacturer’s instructions and has been described in our previous studies [12]. The primary mouse monoclonal MYC antibody (1:100 dilution; Santa Cruz Biotechnology, USA), anti-PDIA3 antibody (polyclonal, 1:700 dilution; sigma, Saint Louis, MO), and anti-ITGA5B1 antibody (monoclonal, 1:50 dilution; millipore, USA) were used in this study.

2.3. Evaluation of IHC Variables. The protein expression was evaluated by an automated quantitative pathology imaging system (PerkinElmer, Waltham, MA, USA), as described previously [11]. Briefly, as shown in Figure S1, the automated image acquisition and color images were obtained using Vectra 2.0.8 software. Subsequently, the spectral libraries were constructed using Nuance 3.0 software. And then, the color images were evaluated by Inform 1.2 software as follows: (1) segmentation of the tumor region from the tissue compartments, (2) segmentation of the tumor region from the tumor region, and (3) H score calculation (= (% at 0) * 0 + (% at 1+) * 1 + (% at 2+) * 2 + (% at 3+) * 3) based on the optical density which produces a continuous protein expression value in the range of 0 to 300.

2.4. Construction of a Survival Predictive Model. Firstly, we used a univariate Cox proportional hazards regression analysis to evaluate the correlation between survival and each protein. Subsequently, we constructed a predictive model by the summation of the expression of each biomarker (high = 1, low = 0) multiplied by its regression coefficient, as described in the following equation: Y = (β1) × MYC + (β2) × PDIA3 + (β3) × ITGA5B1 [9]. Patients were then divided into three groups (high-risk, medium-risk, and low-risk) by the cut-off value generated by X-tile software [31].
2.5. Statistical Analysis. The SPSS v19.0 program was used for statistical analysis. Cumulative survival time was calculated by the Kaplan–Meier (K-M) method and analyzed by the log-rank test. The association of biomarkers and clinicopathological factors was evaluated by Fisher’s exact test. The Cox proportional hazards regression model was used for univariate and multivariate analyses. The predictive value of the parameters was determined by receiver operating characteristic (ROC) curve analysis. \( P < 0.05 \) was considered to be statistically significant.

3. Results

3.1. Immunohistochemical Characteristics of 3 Biomarkers. The expression levels of MYC, PDIA3, and ITGA5B1 protein in ESCC were examined by IHC. As shown in Figure 1(a), MYC, PDIA3, and ITGA5B1 were mainly localized in the cytoplasm. We further investigated the association between the expression of these 3 biomarkers and clinicopathological parameters. There was no significant correlation between the 3 markers and age, gender, tumor size, histologic grade, or invasive depth, etc. Nonetheless, low-expression of PDIA3 or high expression of ITGA5B1 significantly correlated with lymph node (LN) metastasis, whereas no correlation was found between MYC and LN metastasis (Table 2). In addition, PDIA3 had a negative correlation while MYC and ITGA5B1 had a positive correlation with \( pTNM \)-stage (Table 2). In support of these correlation analyses, MYC and ITGA5B1 showed increased expression in tumors with high clinical stage; in contrast, PDIA3 expression was down-regulated in stage III tumors compared with those with stages I and II (Figure 1(b)).

3.2. Prognostic Significance of MYC, PDIA3, and ITGA5B1 in Patients with ESCC. To further explore the clinical

### Table 1: The clinicopathological characteristics of generation dataset of patients with ESCC.

| Clinical and pathological indexes | Case no. | 5-year OS (%) | \( P^* \) | 5-year DFS (%) | \( P^* \) |
|----------------------------------|----------|---------------|-------------|---------------|-------------|
| Specimens                        | 284      |               |             |               |             |
| Mean age                         | 58.7     |               |             |               |             |
| Age (year)                       |          |               |             |               |             |
| \( \leq 58 \)                     | 148      | 48.1          | 0.036       | 43.4          | 0.207       |
| \( >58 \)                         | 136      | 39.1          |             | 35.8          |             |
| Gender                           |          |               |             |               |             |
| Male                             | 220      | 44.8          | 0.387       | 40.5          | 0.915       |
| Female                           | 64       | 40.2          |             | 37.2          |             |
| Therapies                        |          |               |             |               |             |
| Only surgery                     | 160      | 45.2          |             | 42.0          |             |
| Surgery + radiotherapy           | 39       | 53.6          |             | 51.3          |             |
| Surgery + chemotherapy           | 57       | 46.2          | 0.080       | 36.4          | 0.070       |
| Surgery + chemoradiotherapy      | 28       | 17.9          |             | 17.9          |             |
| Tumor size                       |          |               |             |               |             |
| \( \leq 3 \text{ cm} \)           | 67       | 55.6          |             | 54.4          |             |
| 3–5 cm                           | 134      | 43.5          | 0.057       | 37.9          | 0.021       |
| \( >5 \text{ cm} \)               | 83       | 34.7          |             | 31.1          |             |
| Tumor location                   |          |               |             |               |             |
| Upper                            | 16       | 33.5          |             | 25.0          |             |
| Middle                           | 122      | 45.6          | 0.463       | 44.8          | 0.127       |
| Lower                            | 146      | 43.3          |             | 37.2          |             |
| Histologic grade                 |          |               |             |               |             |
| G1                               | 45       | 57.7          |             | 57.7          |              |
| G2                               | 219      | 43.5          | 0.001       | 38.3          | <0.001      |
| G3                               | 20       | 15.0          |             | 15.0          |             |
| Invasive depth                   |          |               |             |               |             |
| T1                               | 13       | 84.6          |             | 84.6          | 0.013       |
| T2                               | 42       | 50.0          | 0.005       | 45.2          |             |
| T3                               | 229      | 40.2          |             | 36.2          |             |
| Lymph node metastasis            |          |               |             |               |             |
| N0                               | 141      | 58.1          |             | 53.5          |             |
| N1                               | 81       | 44.0          | <0.001      | 39.0          | <0.001      |
| N2                               | 46       | 15.2          |             | 13.0          |             |
| N3                               | 16       | 0.0           |             | 0.0           |             |
| pTNM-stage                       |          |               |             |               |             |
| I                                | 23       | 82.6          |             | 82.6          |             |
| II                               | 131      | 54.2          | <0.001      | 49.2          | <0.001      |
| III                              | 130      | 26.4          |             | 22.6          |             |

* Log-rank test of Kaplan–Meier method; \( P < 0.05 \) was considered significant. All patients underwent surgical treatment. OS: overall survival. DFS: disease-free survival.
significance of MYC, PDIA3, and ITGA5B1 in ESCC patients, Kaplan-Meier analysis and log-rank test were performed. As shown in Figure 2, high expression of MYC or ITGA5B1 was significantly associated with poor prognosis (MYC: OS, \(P = 0.024\); DFS, \(P = 0.024\); ITGA5B1: OS, \(P = 0.001\); DFS, \(P = 0.009\), Figures 2(a) and 2(c)). However, the overexpression of PDIA3 trended to predict a favorable OS (\(P = 0.002\)) and DFS (\(P = 0.003\), Figure 2(b)). Besides, because ITGA5B1 is a heterodimer of alpha and beta subunit, we used the expression level of ITGA5 instead of ITGA5B1 in microarray data, and the predictive value of MYC, PDIA3, and ITGA5 was further validated in an independent cohort (GSE53622 and GSE5364).

The results for validation set were in line with those in generation set (Supplementary Figure S2(a)). Univariate Cox regression analysis further identified that these 3 molecules were significantly associated with OS (MYC: \(P = 0.026\); PDIA3: \(P = 0.003\); ITGA5B1: \(P = 0.001\)) and DFS (MYC: \(P = 0.026\); PDIA3: \(P = 0.004\); ITGA5B1: \(P = 0.010\), Table 3).

3.3. A Molecular Prognostic Model of the 3 Biomarkers Signature. We then evaluated the prognostic value of a molecular model that takes consideration of all the 3 biomarkers. To this end, we calculated the risk score \(Y = (\beta_1) \times \text{MYC} + (\beta_2) \times \text{PDIA3} + (\beta_3) \times \text{ITGA5B1}\). In this dataset, the regression coefficients (\(\beta_1 = 0.347\), \(\beta_2 = -0.482\), \(\beta_3 = 0.501\) ) were calculated by univariate Cox proportional hazards analysis. All patients were divided into
low-, medium-, and high-risk groups based on the Y scores, and the optimal cut-off values were determined by the X-tile software based on patients’ prognosis [31]. Kaplan–Meier analysis further demonstrated that patients in the low-risk group indeed had markedly prolonged survival (OS: \(P < 0.001\); DFS: \(P < 0.001\), Figure 3(a)). The 5-year OS for low-, medium-, and high-risk groups was 62.9%, 41.3%, and 24.5%, respectively. Similar results were obtained for 5-year DFS in those groups, which were 56.0%, 37.4%, and 24.5%, respectively (Figure 3(a)). To validate whether this molecular prognostic model can serve as an independent predictor for OS and DFS, we carried out both univariate and multivariate analyses. As shown in Table 3, our newly defined molecular prognostic model, along with pTNM-stage and tumor size, was independent prognostic factors (Table 3). Moreover, receiver operating characteristic (ROC) analysis indicated that the predictive power of this molecular prognostic model was higher compared to each biomarker individually or the pTNM-stage (Figure 3(b)). The predictive value and power of molecular model for OS also yielded similar results from validation set as shown in Figure S2(b).

### 3.4. The Potential of the Molecular Prognostic Model in Identifying ESCC Patients Who Can Benefit from Chemoradiotherapy.

as shown in Table 1, chemoradiotherapy did not markedly prolong the OS and DFS of ESCC patients. To test the utility of the molecular prognostic model for predicting therapeutic efficacy, we performed K-M survival analysis. Our results showed that the OS and DFS of patients who were treated with surgery only were higher compared with those who received surgery + radiotherapy or surgery + chemotherapy in the low-risk group (Figure 4(a)). However, the opposite was true for patients in the high-risk group, in which ESCC patients who received only surgery had an unfavorable outcome (Figure 4(c)). Radiotherapy and chemotherapy tended to prolong patients’ survival as the risk went up as determined

### Table 2: The correlation between 3 markers and clinicopathological characteristics in ESCC.

| Variables                  | MYCa \(P^*\) | PDIA3b \(P^*\) | ITCA5SB1c \(P^*\) |
|----------------------------|--------------|----------------|-------------------|
| Age (year)                 |              |                |                   |
| \(\leq 58\)                | Low 67 High 81 | Low 84 High 64 | Low 92 High 56    |
| \(>58\)                    | Low 68 High 68 | Low 80 High 56 | Low 92 High 44    |
| Gender                     |              |                |                   |
| Male                       | Low 109 High 111 | Low 127 High 93 | Low 137 High 83 |
| Female                     | Low 26 High 38 | Low 37 High 27 | Low 47 High 17    |
| Therapies                  |              |                |                   |
| Only surgery               | Low 85 High 75 | Low 97 High 63 | Low 107 High 53 |
| Surgery + radiotherapy     | Low 14 High 25 | Low 20 High 19 | Low 25 High 14    |
| Surgery + chemotherapy     | Low 21 High 36 | Low 30 High 27 | Low 35 High 22    |
| Surgery + radiochemotherapy| Low 15 High 13 | Low 17 High 11 | Low 17 High 11    |
| Tumor size                 |              |                |                   |
| \(\leq 3\) cm              | Low 39 High 28 | Low 41 High 26 | Low 43 High 24    |
| 3–5 cm                     | Low 62 High 72 | Low 71 High 63 | Low 63 High 51    |
| \(>5\) cm                  | Low 34 High 49 | Low 52 High 31 | Low 58 High 25    |
| Tumor location             |              |                |                   |
| Upper                      | Low 6 High 10 | Low 9 High 7   | Low 8 High 8      |
| Middle                     | Low 64 High 58 | Low 65 High 57 | Low 82 High 40    |
| Lower                      | Low 65 High 81 | Low 90 High 56 | Low 94 High 52    |
| Histologic grade           |              |                |                   |
| G1                         | Low 25 High 20 | Low 20 High 25 | Low 32 High 13    |
| G2                         | Low 101 High 118 | Low 129 High 90 | Low 140 High 79   |
| G3                         | Low 9 High 11  | Low 15 High 5   | Low 12 High 8     |
| Invasive depth             |              |                |                   |
| T1 + T2                    | Low 32 High 23 | Low 37 High 18 | Low 34 High 21    |
| T3 + T4                    | Low 103 High 126 | Low 127 High 102 | Low 150 High 79   |
| Lymph node metastasis      |              |                |                   |
| N0                         | Low 73 High 68 | Low 64 High 77 | Low 105 High 36   |
| N1 + N2 + N3               | Low 62 High 81 | Low 100 High 43 | Low 79 High 64    |
| pTNM-stage                 |              |                |                   |
| I                          | Low 17 High 6  | Low 10 High 13 | Low 16 High 7     |
| II                         | Low 65 High 66 | Low 64 High 67 | Low 96 High 35    |
| III                        | Low 53 High 77 | Low 90 High 40 | Low 72 High 58    |

* Fisher’s exact test. \(P < 0.05\) was considered significant.
Figure 2: K-M survival analysis in ESCC patients based on the expression of MYC, PDIA3, and ITGA5B1. The H scores of each protein were divided into low and high groups as determined by X-tile, and the number of patients who were at risk at specific times was labeled under the x-axis (P < 0.05, log-rank test).
by our molecular prognostic model. In particular, patients treated with surgery + chemotherapy in the high-risk group had the most favorable OS and DFS compared with surgery alone and surgery + radiotherapy (Figure 4).

4. Discussion

ESCC is one of the most prevalent and lethal cancers in Asian [4]; however, there is no effective molecular signatures for predicting the effectiveness of adjuvant treatments and prognosis in the clinic. Previous studies demonstrated that the cytoskeleton changes are intimately associated with cancer invasion and metastasis [32]. In support of this notion, our research has confirmed that the membrane-cytoskeletal linking protein ezrin contributes significantly to ESCC progression [15]. In this study, we attempted to generate an effective molecular model based on ezrin-related proteins (MYC, PDIA3, and ITGA5B1) for potential clinical applications. Our data highlight that a molecular model elicited from MYC, PDIA3, and ITGA5B1 has superior prognostic values compared with pTNM-stage, which also facilitates the identification of ESCC patients who may benefit from chemoradiotherapy.

Ezrin, a membrane-cytoskeleton linker, plays a major role in promoting tumor progression [23, 33]. Our previous study has identified the mislocalization of ezrin during ESCC development, in which membranous ezrin in normal epithelial cells becomes cytoplasmic in ESCC [34]. "his abnormal localization changes the interacting proteins of ezrin, which has been shown to be critical for regulating tumor cell survival, invasion, and metastasis [12, 17]. "he expressions of MYC, PDIA3, and ITGA5B1 have been demonstrated to play critical roles in various malignant tumors and are independent prognostic factors in certain cancers [12, 35, 36].

It is important to note that although ESCC patients with higher risk predicted by our three-protein molecular model had poor prognosis, these patients might benefit from adjuvant therapies such as chemoradiotherapy, which improved their survival compared with surgical treatment alone. Compared with the model using three different genes (PPARG, MDM2, and NANOG), which we reported in 2015 [9], the current molecular model not only accurately predicts the OS of patients with ESCC but also predicts the DFS and sensitivity to chemoradiation. This makes it much more practical for clinical application. Our results are in line with

| Variables                          | Univariate analysis | Multivariate analysis |
|-----------------------------------|---------------------|-----------------------|
|                                   | HR (95% CI)        | P         | HR (95% CI)        | P         | HR (95% CI)        | P         |
| Age (>58 vs. ≤58)                 | 1.376(1.017 to 1.861) | 0.039 | 1.203(0.900 to 1.609) | 0.213 | 1.498(1.082 to 2.073) | 0.015 |
| Gender (female vs. male)          | 0.857(0.603 to 1.219) | 0.391 | 0.981(0.693 to 1.390) | 0.916 |
| Therapies (Surgery + radiotherapy vs. only surgery) | 0.918(0.642 to 1.423) | 0.025 | 0.981(0.693 to 1.390) | 0.916 |
|                                   | 0.035 | 1.701(1.087 to 2.662) | 0.020 |
| Tumor size (3–5 cm vs. ≤3 cm)     | 1.285(0.860 to 1.921) | 0.222 | 1.404(0.948 to 2.077) | 0.090 | 1.378(0.915 to 2.075) | 0.124 | 1.432(0.964 to 2.130) | 0.076 |
|                                   | 1.657(1.082 to 2.539) | 0.020 | 1.787(1.176 to 2.716) | 0.007 | 1.730(1.124 to 2.664) | 0.013 | 1.821(1.193 to 2.779) | 0.005 |
| pTNM-stage (III vs. I + II)       | 2.087(1.443 to 3.019) | <0.001 | 1.956(1.376 to 2.780) | <0.001 | 1.876(1.267 to 2.778) | 0.002 | 1.689(1.162 to 2.456) | 0.006 |
| MYC                               | 1.415(1.043 to 1.920) | 0.026 | 1.397(1.041 to 1.874) | 0.026 |
| PDIA3                             | 0.618(0.450 to 0.848) | 0.003 | 0.638(0.471 to 0.864) | 0.004 |
| ITGA5B1                           | 1.651(1.216 to 2.241) | 0.001 | 1.477(1.098 to 1.986) | 0.010 |
| Molecular prognostic model        | <0.001 | <0.001 | <0.001 | 0.006 |
| Medium-risk vs. ≤ low-risk         | 1.830(1.215 to 2.758) | 0.004 | 1.625(1.111 to 2.378) | 0.012 | 1.577(1.036 to 2.402) | 0.034 | 1.493(1.010 to 2.208) | 0.045 |
| High-risk vs. ≤ low-risk           | 2.914(1.828 to 4.680) | <0.001 | 2.457(1.580 to 3.823) | <0.001 | 2.539(1.556 to 4.141) | <0.001 | 2.122(1.338 to 3.367) | 0.001 |

Note. Multivariate analysis, Cox proportional hazards regression model. Variables were adopted for their prognostic significance by univariate analysis.
other clinical studies, which have shown that high expression and rearrangement of MYC are associated with better response to chemoradiotherapy compared with patients without these abnormalities [37, 38]. The mechanism behind this observation is probably related to the biological function of MYC in promoting DNA replication and cell cycle distribution [39]. As chemoradiotherapy utilizes the effects of DNA damage-induced cytotoxicity in neoplastic cells, it is not surprising to see an association between MYC and chemoradiosensitivity in ESCC patients. Indeed, overexpression of MYC has been shown to render tumor cells susceptible to chemotherapeutics, such as etoposide, doxorubicin, and camptothecin [40]. Nevertheless, MYC remains an attractive molecular target for therapy due to its high oncogenic properties [41]. Antisense oligonucleotides (ASOs) targeting MYC have been shown to block cell proliferation and induce apoptosis in solid and hematologic tumors [41, 42].

Compared with MYC, relatively little is known about the biological function of ITGA5B1 in carcinoma. Recent studies suggest that ITGA5B1 can prevent cell anoikis through suppressing inflammation- and oxidative stress-related genes [43, 44]. ITGA5B1 is especially more noticeable in regulating cell adhesion [45], and it can promote early peritoneal metastasis in serous ovarian cancer [46].

Figure 3: Predictive value of the molecular model. (a) K-M survival curves showing that the OS and DFS had a striking contrast between the ESCC patients in low-, medium-, and high-risk groups. (b) Receiver operating characteristic (ROC) curve was used to evaluate the ability of the molecular model for OS or DFS compared with each biomarker alone or the pTNM-stage.
| Therapy (P) | 2   | 3   |
|------------|-----|-----|
| 1          | 0.203 | 0.019 |
| 2          | 0.627 |     |

1 = surgery (n = 36)
2 = surgery + radiotherapy (n = 12)
3 = surgery + chemotherapy (n = 18)

| Therapy (P) | 2   | 3   |
|------------|-----|-----|
| 1          | 0.248 | 0.040 |
| 2          | 0.748 |     |

1 = surgery (n = 98)
2 = surgery + radiotherapy (n = 20)
3 = surgery + chemotherapy (n = 26)

**Figure 4: Continued.**
In line with the protumorigenic role of ITGA5B1, we are the first to uncover the high expression of this protein in more advanced and metastatic ESCC tumors with unfavorable prognosis. Further studies are needed to delineate the mechanisms behind the deregulation of ITGA5B1 and its biological function in ESCC. PDIA3 has been shown to confer chemoradioresistance to various types of tumor cells such as ovarian carcinoma [47, 48]. PDIA3 expression level is correlated with the clinical outcome of patients with ovarian carcinoma who receive chemoradiotherapy, and the sensitivity to paclitaxel can be enhanced by PDIA3 silencing [47, 48]. In ESCC, we found that PDIA3 decreased gradually with the progress of stage and related to favorable prognosis, which was in accord with the findings in gastric cancer [49], but contrary to those in hepatocellular carcinoma [50]. The favorable prognostic value of PDIA3 in ESCC implies that ESCC patients with high expression of PDIA3 may be more sensitive to chemotherapy such as paclitaxel, but further studies are warranted. These contrasting observations can be attributed to the differences in the carcinogenic machinery between ESCC and other carcinomas.

Taken together, these data suggest that MYC, PDIA3, and ITGA5B1 may serve as potential therapeutic targets for ESCC treatment, and cotargeting of these biomarkers might be more effective than targeting a single biomarker alone. Importantly, this study provides a clinically applicable molecular model that can more precisely predict clinical outcome than pTNM-stage, which may also facilitate the identification of ESCC patients who can benefit from radiotherapy or chemotherapy.

Data Availability

The clinical data and protein expression used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supplementary Materials

Figure S1: representative images showing the scoring process by the automated quantitative pathology imaging system. Figure S2: predictive value of three genes and the molecular model in validation dataset. Table S1: the clinicopathological
characteristics of validation dataset of patients with ESCC. 
(Supplementary Materials)

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