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Association of serum PD-L1 and IGFBP-2 levels with prognosis in patients with esophageal carcinoma

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Abstract: This study investigates the prognostic value of serum biomarkers PD-L1 and IGFBP-2 in patients with esophageal carcinoma. It finds a significant positive correlation between these biomarkers and established tumor markers CEA and CYFRA21-1. The 3-year survival rate for the patient cohort was 45.10%, with deceased patients showing significantly higher levels of PD-L1 and IGFBP-2. ROC analysis indicates a strong predictive value for these biomarkers, with combined use potentially enhancing diagnostic accuracy. Kaplan-Meier curves reveal lower survival rates for patients with high biomarker expression. The study suggests that PD-L1 and IGFBP-2 could be valuable for clinical management and prognostic evaluation of esophageal carcinoma patients.

Keywords: Esophageal carcinoma; programmed death ligand 1; insulin-like growth factor-binding protein-2; prognosis.

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INTRODUCTION

Cancer is a globally recognized cause of death. In 2020, the cancer burden escalated to approximately 19.3 million new cases, with nearly 10 million fatalities (Sung, Ferlay *et al*., 2021). Esophageal carcinoma (EC), as a commonly seen digestive tract malignancy, maintains a consistently high incidence and mortality rate within China, presenting a significant threat to human health (Nachiappan and Kapoor, 2022, Wang, Yang *et al*., 2023). On a global scale, EC ranks as the eighth most prevalent cancer and the sixth major cause of cancer-related deaths (Ferlay, Colombet *et al*., 2019). A prominent feature of EC is that its onset process is often concealed, with many patients being diagnosed at the middle to advanced stage or having distant metastases (Luebeck, Curtius *et al*., 2013). Such circumstance not only enhances the difficulty of treatment but also severely impacts the patient's quality of life and survival expectation, with a 5-year survival rate of < 25% (Almatroudi, 2022). Hence, in-depth studies on the pathogenesis of EC and the exploration of effective prevention, diagnosis and treatment approaches hold substantial significance for enhancing the survival rate and improving the quality of life of patients.

Tumor immunosuppression not only promotes tumor growth but also reduces the effectiveness of anti-tumor therapy (Devalaraja, To *et al*., 2020). Programmed death ligand 1 (PD-L1), a member of the B7 superfamily, is widely expressed in various immune cells and tumor cells (Garrett-Thomson, Massimi *et al*., 2020). After PD-L1 binds to programmed death 1 (PD-1), it can send out inhibitory signals, thereby suppressing T cell activation and proliferation, accelerating T cell apoptosis and facilitating tumor cells to evade the attack of the immune system (He, Pan *et al*., 2020, Wlodarczyk, Durko *et al*., 2023). PDL1 has been detected in various solid tumors, and drugs targeting the PD1/PDL1 pathway have achieved remarkable therapeutic results in fields such as melanoma and non-small cell lung cancer (Nirmal, Maliga *et al*., 2022).

Insulin-like growth factor-binding protein-2 (IGFBP-2), as a component of the insulin-like growth factor family, is strongly associated with the onset and progression of various malignancies such as hepatocellular carcinoma, pancreatic cancer and glioma (Vocka, Langer *et al*., 2019). Research indicates that IGFBP-2 plays a key biological role in the malignant transformation processes of tumor cells, such as proliferation, migration, and invasion, either through an insulin-like growth factor ligand-dependent or -independent manner (Brandt, Grünler *et al*., 2015). Additionally, as a secreted protein, IGFBP-2 can be detected in the serum of tumor patients, demonstrating potential as a prognostic predictive biomarker (Guiot, Njock *et al*., 2021). Nonetheless, there is currently no study exploring the role of IGFBP-2 in the progression and prognosis of the EC. This study aims to investigate the potential of PD-L1 and IGFBP-2 as prognostic biomarkers for EC.

MATERIALS AND METHODS

Research subjects

102 EC patients, including 61 males and 41 females aged (69.38±9.27) years on average, who were admitted to our hospital from August 2019 to February 2021 were selected for this study. The basic information, clinic pathological features, tumor markers and levels of PD-L1 and IGFBP-2 were collected. The survival data were obtained through the hospital information system, the inpatient cause-of-death registration and reporting system,

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and telephone follow-up. Follow-up was conducted every six months and the 3-year follow-up data of all patients were statistically analyzed. This study was approved by the Medical Ethics Committee of our hospital.

Patient selection criteria

Inclusion criteria: All patients were diagnosed with esophageal squamous cell carcinoma in accordance with the 7th edition of the guidelines issued by the National Comprehensive Cancer Network (NCCN) (Strong, D'Amico *et al*., 2013) and underwent tests for tumor markers, PD-L1 and IGFBP-2, with complete follow-up and clinical data.

Exclusion criteria: Patients with other malignancies, severe impairments of important organs such as the heart, lungs, and kidneys, or mental disorders were excluded.

Detection methods

Five-milliliter venous blood samples were drawn from EC patients in the early morning on an empty stomach. After collecting, the blood samples were left to stand still and centrifuged for 5 minutes. Subsequently, the separated serum was transferred to a -80°C freezer for long-term preservation. In accordance with the guidelines of the enzyme-linked immunosorbent assay (ELISA) kits for PD-L1 and IGFBP-2 provided by Abcam, UK, we completed all the necessary experimental steps. Finally, through ADVIA2400, a micro plate reader from Siemens, Germany, we finished the sample detection and the result reading.

Ethical approval

This study was approved by the Ethics Committee of The First Hospital of Shanxi Medical University, Shanxi Province, China, in accordance with hospital regulations. The ethical approval number is not required as per the institutional guidelines.

STATISTICAL ANALYSIS

Data analyses were conducted using SPSS 21.0. Measurement data conforming to a normal distribution were presented in the form of the mean ± standard deviation, and t-tests were employed for comparisons between groups. The associations of PD-L1 and IGFBP-2 with tumor markers carcinoembryonic antigen (CEA) and Cytokeratin Fragment 19 (CYFRA 21-1) were detected through Pearson correlation. The value of PD-L1 and IGFBP-2 in predicting 3-year mortality was examined by receiver operating characteristic (ROC) curves and the patients were divided into high and low expression groups based on the cut-off value.

The Kaplan-Meier (K-M) survival curves were drawn to compare the difference in the 3-year survival rate between patients with high and low expression of serum PD-L1 and IGFBP-2 and the Log-rank test was utilized for between-group comparisons. A minimum significance threshold of $P<0.05$ was used.

RESULTS

Patient pathological features, PD-L1 and IGFBP-2 levels

The pathological features and levels of PD-L1 and IGFBP-2 of the 102 EC patients selected were statistically analyzed and presented in table 1.

Associations of PD-L1 and IGFBP-2 levels with tumor markers

Pearson correlation was carried out to determine the associations of PD-L1 and IGFBP-2 levels with tumor markers CEA and CYFRA21-1, revealing a positive correlation of both PD-L1 and IGFBP-2 with CEA and CYFRA21-1 ($P<0.001$, fig. 1).

Patient's 3-year survival status

We analyzed the 3-year survival status of 102 EC patients, with a total of 56 deaths and 46 survivors, resulting in a 3-year survival rate of 45.10%. The 3-year survival K-M curve is shown in fig. 2.

PD-L1 and IGFBP-2 levels in surviving and deceased patients

When comparing the PD-L1 and IGFBP-2 levels of patients who survived for 3 years and those who died, we found markedly higher PD-L1 and IGFBP-2 levels in the deceased than in survivors ($P<0.001$, fig. 3).

The predictive value of PD-L1 and IGFBP-2 levels for patient prognosis

By plotting ROC curves for predicting 3-year mortality of patients using PD-L1 and IGFBP-2, it was found that the AUC of PD-L1 and IGFBP-2 were 0.818 and 0.843, respectively, indicating high diagnostic value. By drawing the ROC curve of the combined diagnosis of PD-L1 and IGFBP-2, it was found that its AUC was 0.879, with a sensitivity higher than the individual prediction by PD-L1 and IGFBP-2. See fig. 4 and table 2 for the results.

Associations of PD-L1 and IGFBP-2 levels with patient prognosis

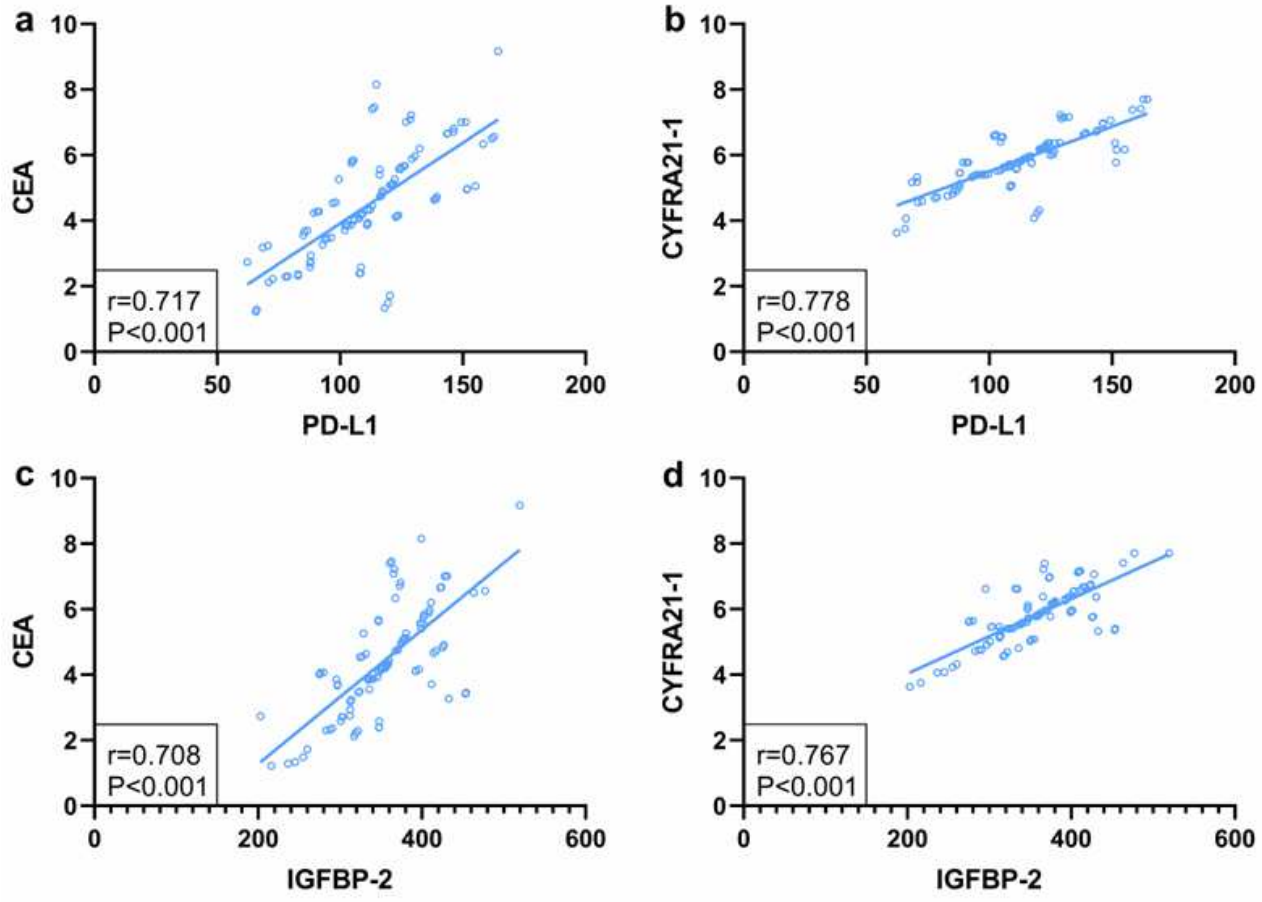
We classified patients into high and low expression groups based on the ROC curve Cut-off values of PD-L1 and IGFBP-2 for predicting 3-year mortality. Of them, 9 patients with high PD-L1 versus 37 patients with low PD-L1 survived for 3 years, while 8 patients with high IGF-BP-2 versus 38 patients with low IGF-BP-2 survived for 3 years. Then, K-M curves were plotted to show that the 3-year survival rate of patients with high levels of PD-L1 and IGFBP-2 was significantly lower than that of patients with low levels ($P<0.001$, fig. 5).

Table 1: Pathological features

	N=102
Age (years old)	69.38±9.27
Sex (male/female)	61/41
Pathological type (squamous cell carcinoma/adenocarcinoma)	84/18
Degree of differentiation (high and moderate/low)	58/44
TNM staging (I and II/III)	62/40
Tumor diameter (cm)	3.55±0.91
Lymph node metastasis (with/without)	28/74
CEA (ng/mL)	4.46±1.66
CYFRA21-1 (ng/mL)	5.82±0.84
PD-L1 (pg/mL)	111.41±23.69
IGFBP-2 (ng/mL)	3656.55±56.30

Table 2: ROC data

	AUC	Specificity	Sensitivity	Cut-off	95% CI
PD-L1	0.818	83.93%	80.43%	<108.50	0.732-0.904
IGFBP-2	0.843	85.71%	82.61%	<349.0	0.756-0.930
Joint diagnosis	0.879	87.50%	82.61%	<0.490	0.810-0.947



a. PD-L1 was positively correlated with CEA (r=0.717, P<0.001). b. PD-L1 was positively linked to CYFRA21-1 (r=0.778, P<0.001). c. There was a positive correlation between IGFBP-2 and CEA (r= 0.708, P<0.001). d. Positive correlation was determined between IGFBP-2 and CYFRA21-1 (r= 0.767, P<0.001).

Fig. 1: Associations of PD-L1 and IGFBP-2 levels with tumor markers.

DISCUSSION

The expression of PD-L1 in the tumor microenvironment holds a crucial position. Once it binds to the inhibitory receptor PD-1 on the surface of T lymphocytes, it can inhibit T cell proliferation and activation, resulting in T cell dysfunction (Ahmad, Borch *et al*., 2016). This mechanism is of vital significance in maintaining the immune tolerance of T cells and the evasion of immune surveillance by tumor cells (Wang, Li *et al*., 2021). In various malignant tumors, such as melanoma and breast cancer, the expression level of PD-L1 has been discovered to be abnormally elevated (Zhu, Xu *et al*., 2021).

In colorectal and renal cancer in particular, PD-L1 expressions have been shown to be an independent risk factor for patient outcomes (Sun, Chen *et al*., 2021, Zheng, Liu *et al*., 2022). In view of this, PD-L1 has emerged as a hotspot in the current field of tumor immunotherapy. Past studies have revealed that IGFBP-2 expression is significantly increased in serum and tumor tissues of patients with a variety of malignancies, a phenomenon that is closely related to malignant behaviors like tumor proliferation, invasion, metastasis and angiogenesis (Kaur, Balasubramaniam *et al*., 2020). The role of IGFBP-2 is not merely confined to promoting the malignant transformation process of tumors. It is also regarded as an important independent predictor that can affect the recurrence risk and long-term survival outcome of tumor patients after surgery (Espelund, Renehan *et al*., 2018). However, regarding PD-L1 and IGFBP-2 expression in EC and their associations with patient prognosis, there are still disputes in the academic community at present.

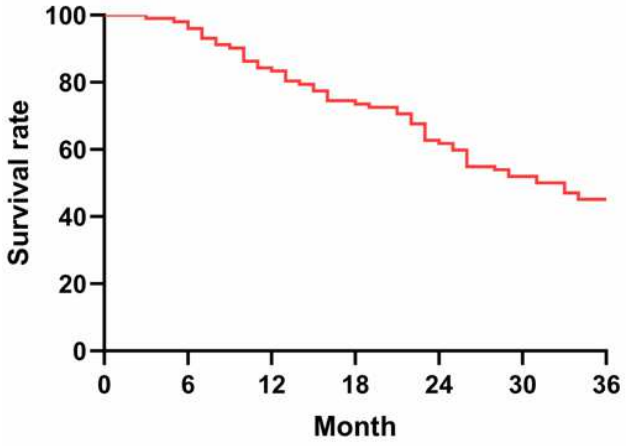
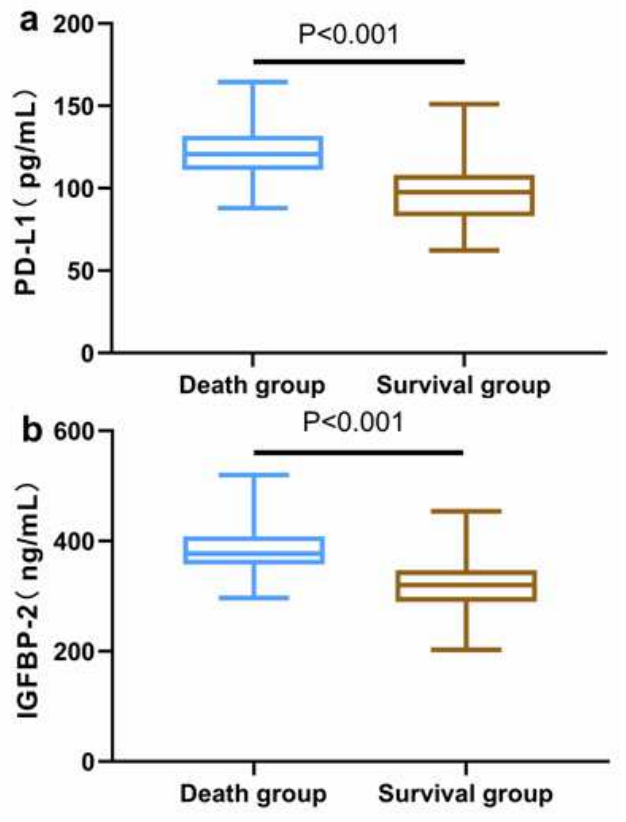


Fig. 2: K-M curves of patient's 3-year survival.

An increase in the levels of CEA and CYFRA21-1, as tumor markers, may suggest tumor invasiveness and poor patient prognosis (Yuan, Sun *et al*., 2022). The correlations of these biomarkers may assist in predicting the treatment response and survival outcome of patients and guide individualized treatment decisions, such as the selection of immunotherapy or targeted therapy (van den Heuvel, Holdenrieder *et al*., 2024). In this study, PD-L1 and IGFBP-2 were positively correlated with the tumor markers CEA and CYFRA21-1. This implies that when the levels of CEA and CYFRA21-1 rise, the levels of PD-L1 or IGFBP-2 also tend to increase. of the 102 EC patients enrolled, 46 survived at 3 years, with a 3-year survival rate of 45.10 percent, which is similar to previous studies (Ely, Alabaster *et al*., 2023). The comparison of PD-L1 and IGFBP-2 levels between surviving and deceased patients revealed that PD-L1 and IGFBP-2 were significantly higher in the deceased. This observation suggests that PD-L1 and IGFBP-2 may play important roles in the prognostic evaluation of EC. Low levels of PD-L1 may indicate weaker immune system escape ability of tumors, thereby helping to improve patient survival; while lower IGFBP-2 expression may be associated with slow tumor progression and lower invasiveness, helping to prolong patient survival. Gao and Zhang (2023) mentioned that IGFBP2 secreted by cancer-associated fibroblasts (CAFs) can promote the migration and invasion of esophageal squamous cell carcinoma cells. By using neutralizing antibodies to block the function of IGFBP2, the migration and invasion of esophageal squamous cell carcinoma cells can be inhibited.



a. The PD-L1 level of deceased patients was statistically higher than that of surviving patients (P<0.001). b. IGFBP-2 levels were significantly higher in the deceased than in survivors (P<0.001).

Fig. 3: PD-L1 and IGFBP-2 levels in surviving and deceased patients.

To validate the prognostic implications of PD-L1 and IGFBP-2, this study plotted ROC curves for predicting 3-year mortality in patients. The ROC curves plotted showed an AUC, specificity, and a sensitivity of 0.818, 83.93% and 80.43% for PD-L1, 0.843, 85.71%, and 82.61% for IGFBP-2 and 0.879, 87.50%, and 82.61% for their combined diagnosis, respectively. It can be seen that the combined diagnosis had higher AUC and specificity than PD-L1 and IGFBP-2 alone. This result also reflects the high predictive value of PD-L1 and IGFBP-2 as prognostic indicators and the ability of the combined diagnosis to further improve the accuracy of predicting the 3-year mortality risk of EC patients.

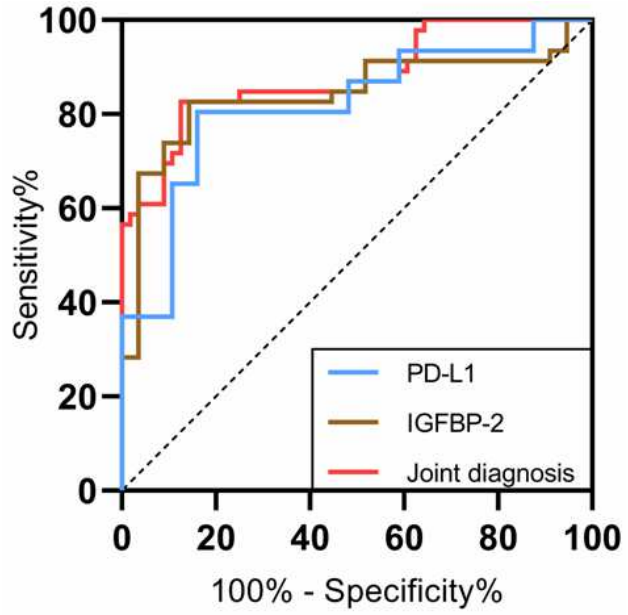
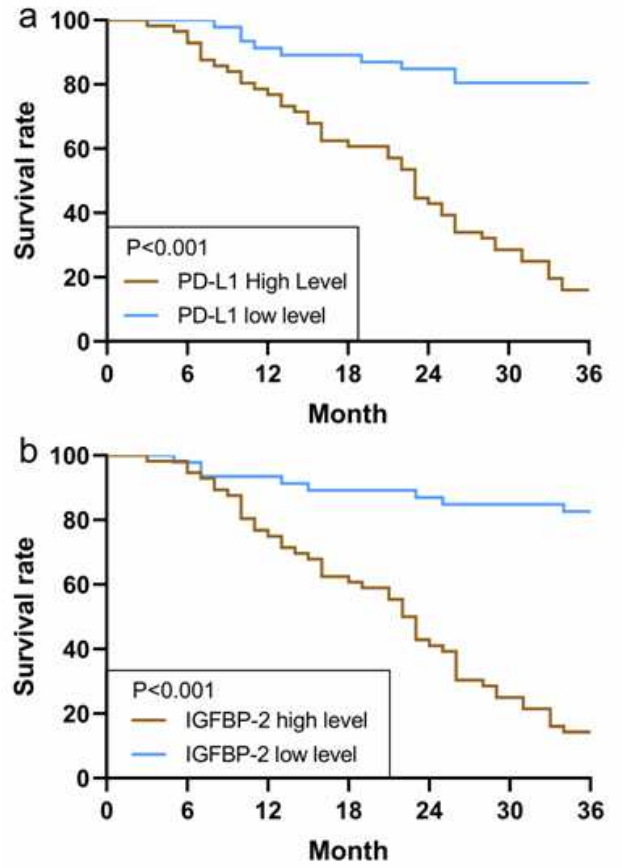


Fig. 4: ROC curves of PD-L1 and IGFBP-2 for predicting patients' 3-year mortality.

Finally, we drew the K-M curves of the 3-year survival of patients with high and low levels of PD-L1 and IGFBP-2 and discovered an evidently lower 3-year survival rate in patients with high levels of PD-L1 and IGFBP-2 compared to those with low levels. This result highlights the necessity of further research on PD-L1 and IGFBP-2 expressions in EC patients to guide clinical practice and facilitate the development of individualized treatment strategies. Further research can not only deepen our understanding of the biological mechanism of EC, but also provide crucial clues for the formulation of more effective treatment methods and offer more effective treatment choices for patients. In the study by Kawasaki *et al*. (Kawasaki, Noma *et al*., 2023), it was indicated that patients with PD-L1-positive EC had poorer survival rates and recurrence-free survival rates than those in the PD-L1-negative group, similar to our results. The study also mentioned that inhibiting PD-L1 might contribute to suppressing tumor progression and improving the tumor immunity of the host.



a. The 3-year survival rate of patients with high levels of PD-L1 was significantly lower than that of patients with low levels of PD-L1 (P<0.001). b. Patients with high levels of IGFBP-2 showed a lower 3-year survival rate than those with low levels of IGFBP-2 (P<0.001).

Fig. 5: 3-year survival K-M curves of patients with high and low levels of PD-L1 and IGFBP-2.

Certain deficiencies are present in this research. The final result may not be representative given the small sample size. Second, this study only included patients with EC and did not compare them with healthy individuals, so the differences in PD-L1 and IGFBP-2 between EC patients and healthy individuals are not yet clear. Finally, although the study found some relationships between PD-L1 and IGFBP-2 and prognosis, it did not explore the specific mechanism. Therefore, it is hoped that more samples can be included in the following research and basic research can be carried out to deepen our results.

CONCLUSION

The expression of PD-L1 in the tumor microenvironment is crucial as it can inhibit T cell function, contributing to

tumor immune evasion. PD-L1 is abnormally elevated in various cancers and is an independent risk factor for outcomes in colorectal and renal cancers. IGFBP-2, another biomarker, is increased in cancer patients and is linked to tumor malignancy and prognosis. In a study of 102 EC patients, higher levels of PD-L1 and IGFBP-2 were associated with worse survival rates. The study found that PD-L1 and IGFBP-2 levels correlated with tumor markers CEA and CYFRA21-1, suggesting their combined use could improve prognostic prediction. ROC curve analysis confirmed the predictive value of these biomarkers for 3-year mortality in EC patients. However, the study had a small sample size and did not compare patients with healthy individuals or explore the specific mechanisms behind the biomarkers' effects. Further research with larger samples and mechanistic studies is needed to validate these findings and guide clinical practice.

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