Article

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G Protein-Coupled Estrogen Receptor Expression Can Predict Poorer Outcomes of EGFR-TKI Treatment for Lung Adenocarcinoma With Malignant Pleural Effusion: A Retrospective Study

Yuansheng Du[1,*,†], Yiguo Ai[1,†], Xingxing Wang[2], Chunling He[2], Xuqing Wen[1]

[1]Department of Cardiothoracic Surgery, The Second Affiliated Hospital of Shantou University Medical College, 515000 Shantou, Guangdong, China
[2]Department of Pathology, The Second Affiliated Hospital of Shantou University Medical College, 515000 Shantou, Guangdong, China
*Correspondence: Yuanshengdu0052@163.com (Yuansheng Du)
[†]These authors contributed equally.
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Background: Targeted therapy with epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) for lung adenocarcinoma still inevitably leads to drug resistance. The estrogen pathway interacts with the epidermal growth factor receptor (EGFR) signaling pathway, which is a potential combined therapeutic approach. However, findings from some previous preclinical studies evaluating the combination of EGFR-TKI with estrogen receptor antagonists have been inconsistent. The failure to stratify tumors based on the expression status of estrogen receptors may partly explain the inconsistency in the reported results. This study aims to explore the correlation between the expression status of G protein-coupled estrogen receptor (GPER), estrogen receptor α (ERα), and estrogen receptor β (ERβ) in lung adenocarcinoma with malignant pleural effusion (MPE) and the treatment outcomes of patients receiving EGFR-TKI.

Methods: The clinical data of 106 lung cancer patients with MPE at the initial diagnosis were retrospectively analyzed. The expression of GPER, ERα and ERβ in the malignant pleural effusion cell blocks of the patients was detected using immunohistochemistry, and the expression correlation among GPER, ERα and ERβ was analyzed using Spearman method. The Kaplan-Meier method and log-rank test were used to compare the effects of different expression levels of GPER, ERα, and ERβ on the progression-free survival (PFS) of patients. The Cox proportional hazards model was used to analyze the independent risk factors influencing the PFS rate of patients.

Results: Among these 106 cases, a total of 68 cases (64.2%) tested positive for GPER, 56 cases (52.8%) tested positive for ERα, and 60 cases (56.6%) tested positive for ERβ. The expression levels of GPER, ERα and ERβ were significantly correlated. Specifically, for ERα and GPER, $r_s = 0.515$, $p < 0.001$; ERβ and GPER, $r_s = 0.497$, $p < 0.001$; ERα and ERβ, $r_s = 0.469$, $p < 0.001$. The objective response rate (ORR) of GPER-positive patients was lower than that of GPER-negative patients (48.5% vs 73.7%, $p = 0.012$). The 12-month PFS rates of the GPER positive group and the GPER negative group were 41.0% and 57.9%, respectively, and the median PFS of the GPER positive group and the GPER negative group were 10.6 months and 13.2 months, respectively ($p = 0.035$). Cox multivariate analysis confirmed that positive GPER was significantly and independently associated with a shorter PFS (hazard ratio [HR] 2.003, 95% CI 1.231–3.259, $p = 0.005$).

Conclusion: High expression of GPER in advanced lung adenocarcinoma with malignant pleural effusion is a negative predictor of the efficacy of EGFR-TKI in these patients. Further prospective studies based on the stratification of GPER expression are warranted to understand the differences in the therapeutic effects of anti-estrogen treatment among patients with different levels of GPER expression.

Keywords: lung adenocarcinoma; G protein-coupled estrogen receptor; EGFR-TKI; malignant pleural effusion; estrogen

Introduction

At present, lung cancer remains the malignant tumor with the highest mortality rate worldwide, with 60% to 70% of patients already in the advanced stage at the time of diagnosis [1,2]. Lung adenocarcinoma is one of the common pathological types of lung cancer. In these patients, lesions are more frequently localized to the peripheral lungs, and the tumors are prone to invade the pleural cavity. More than half of the patients with advanced lung adenocarcinoma present with malignant pleural effusion [3,4]. Over the past decade, epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) targeted therapy, represented by gefitinib, has become an indispensable treatment method for EGFR-mutated lung adenocarcinoma. Multiple prospective clinical studies have confirmed that EGFR-TKI

plays a role in progression-free survival (PFS), disease-free survival (DFS), and overall survival (OS) in the palliative care of advanced EGFR-mutated non-small cell lung cancer (NSCLC) and adjuvant therapy after NSCLC surgery. It was significantly superior to the traditional platinum-containing two-drug combination regimen in terms of survival [5–8]. However, the emergence of drug resistance is still inevitable, and the mechanism of resistance to EGFR-TKI is complex. In addition to differences in therapeutic sensitivity among various EGFR mutation sites, resistance is also related to abnormalities at other nodes in the signaling pathway, activation of downstream signaling molecules of EGFR, and bypass activation [9]. In patients with EGFR gene mutations, it is necessary to further explore additional cytokine sites that affect the efficacy of EGFR-TKI treatment. The combination therapy of multi-target blocking is a potential therapeutic strategy to overcome resistance to EGFR-TKI.

The role of the estrogen pathway in the occurrence and development of lung adenocarcinoma and its influence on the efficacy of EGFR-TKI have attracted considerable attention. This is partly due to the significant gender differences in the pathological features of lung cancer: the most common tissue type of lung cancer in women is adenocarcinoma, with a high EGFR mutation rate [10]. Moreover, current research suggests that estrogen is a contributor to non-small cell lung cancer. As some prospective studies revealed, hormone replacement therapy (HRT) in menopausal women increases the incidence and mortality of lung cancer [11,12]. A recent retrospective study on Chinese patients with lung cancer undergoing surgical treatment showed that from 2006 to 2021, the proportion of female lung cancer patients soared from 29.9% to 59.5%, surpassing that of male patients [13]. Another epidemiological study showed a higher incidence of lung cancer among young women than among young men in the United States [14]. Cellular and animal model studies have confirmed that the activation of the estrogen receptor (ER) pathway plays an important role in the occurrence of lung cancer [15]. ERs become more highly expressed during the occurrence of lung cancer. Estrogen receptors can regulate genes involved in the progression of lung cancer and signal cascades related to invasion and angiogenesis [16]. The functional relationship between estrogen and the EGFR signaling pathway in lung cancer has been confirmed. Stimulating NSCLC with E2 can induce the production of epidermal growth factor (EGF) and lead to the rapid activation of the EGFR pathway [10,17]. Furthermore, EGFR signaling activation can increase the expression and activity of aromatase in NSCLC cells [18].

Based on these findings and the established effects of anti-estrogen therapy in breast cancer and prostate cancer, the combination of EGFR-TKI and anti-estrogen therapy has been explored in lung cancer, and more evidence has been obtained in basic research to support this. For instance, Stabile *et al.* [17] found that the combination of the estrogen receptor antagonist fulvestrant and EGFR-TKI demonstrated superior tumor suppression effects in lung cancer nude mouse xenograft models compared to the use of EGFR-TKI alone, indicating the efficacy of the dual inhibition of EGFR-TKI and estrogen receptor antagonists. However, previous preclinical studies on EGFR-TKI combined with estrogen receptor antagonists have inconsistent conclusions. A Phase I clinical trial tested the efficacy of gefitinib combined with fulvestrant, showing that NSCLC patients with higher ERβ tumor expression experienced an OS extension of 65.5 weeks [19]. However, a Phase II clinical study evaluating whether the addition of fulvestrant could enhance the anti-tumor efficacy of erlotinib (another EGFR tyrosine kinase inhibitor) indicated that, in unselected patients, PFS and response rates were similar between the two treatment groups [20]. A study also suggested that adding fulvestrant to EGFR-TKI is feasible, but it is not related to prolonged PFS [21]. Most of these preclinical studies did not stratify tumors based on the expression status of estrogen receptors, making it difficult to assess the efficacy of anti-estrogen drugs among patients with different expression profiles of estrogen receptors.

In this study, we examined the expression of estrogen-related receptors G protein-coupled estrogen receptor (GPER), estrogen receptor α (ERα), and estrogen receptor β (ERβ) in malignant pleural effusion cell blocks among patients with lung adenocarcinoma and analyzed the correlation between these markers and the treatment outcomes of EGFR-TKI in these patients.

Methods

Case Enrollment

We conducted a retrospective cohort study on patients with metastatic lung adenocarcinoma who underwent initial thoracic puncture with malignant pleural effusion (MPE) at the Second Affiliated Hospital of Shantou University Medical College from January 1, 2017, to January 1, 2021. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Second Affiliated Hospital of Shantou University Medical College (2017-46 and 2025-34). The ethics committee waived the requirement for informed consent because of the retrospective nature of the study.

Inclusion criteria: Aged over 18 years; The ethanol-fixed and paraffin-embedded MPE cell blocks were obtained from patients with cytologically confirmed lung adenocarcinoma (some patients were also histologically confirmed). All the cell blocks of the MPE samples contained adenocarcinoma cells. Lung adenocarcinoma was confirmed by either the biopsy pathological report of the primary tumor or by positive thyroid transcription factor-1 staining of the MPE cell blocks. These patients had measurable lung tumor lesions, tested positive for EGFR, and received EGFR-TKI treatment. The definition of EGFR

mutation-positive cases refers to those identified through genetic testing as harboring either an EGFR exon 19 deletion or an L858R mutation [22].

Exclusion criteria: The patient is under 18 years old, has other malignant tumors or tuberculous pleurisy, or other medical diagnoses that may lead to the development of pleural effusion (i.e., congestive heart failure, ascites, or pneumonia highly suspected clinically within 14 days after the first thoracentesis). Immediate loss to follow-up after the initial thoracentesis, no available chest X-ray within 14 days before the initial thoracentesis, and a previous history of thoracic drainage at an external institution.

Clinical Data Collection

MPE cell blocks of lung adenocarcinoma before the initiation of EGFR-TKI treatment were collected. Clinical features were obtained from medical records, including age, gender, smoking history (defined as having smoked more than 100 cigarettes over a lifetime), imaging features, time and drainage volume of the first and subsequent thoracentesis, Stage IV stage, ECOG PS, EGFR mutation, EGFR-TKI treatment and local intrathoracic treatment. EGFR-TKIswere classified into two groups based on the initially used EGFR-TKI drugs: (1) Gefitinib, icotinib or erlotinib were categorized as the first-generation EGFR-TKIs; (2) Osimertinib, Ameitinib and Furmonertinib were classified as third-generation EGFR-TKIs. Since fewer than five patients received the second-generation EGFR-TKI therapy, these patients were excluded to minimize statistical bias. The grade definition of pleural effusion is as follows: large, pleural effusion $>$1000 mL; moderate, pleural effusion 500 mL–1000 mL; small, pleural effusion $<$500 mL.

Evaluation Indicators of the Therapeutic Effect of EGFR-TKI Treatment

The size of the lung tumor lesion and the condition of pleural effusion were used as evaluation indicators. Before treatment, imaging examinations were conducted on the patients. The size of the lung tumor lesion was measured and recorded. Patients were followed up regularly at six-week intervals, with the lesion size assessed at each visit. According to the Response Evaluation Criteria for Solid Tumors (RECIST) [23], it was classified into complete response (CR), partial response (PR), stable disease (SD), and disease progression (PD). The objective response rate (ORR) was defined as (CR+PR)/N, and the disease control rate (DCR) was defined as (CR+PR+SD)/N. Follow-up visits were conducted via outpatient follow-up, inpatient follow-up, or telephone contact to collect tumor measurement data and survival status. Follow-up was completed on December 31, 2023. The primary endpoint, PFS, was defined as the time from initiation of EGFR-TKI therapy to tumor progression at any site or death.

Immunohistochemical Detection

The primary antibodies used were the NLS1183 GPER rabbit anti-human polyclonal antibody produced by Novus (Dilution: 1:200), the AF6058 ERα rabbit anti-human polyclonal antibody produced by Affinity (Dilution: 1:200), and the AF6469 ERβ rabbit anti-human polyclonal antibody produced by Affinity (Dilution: 1:200), with microwave antigen remediation. The positive control and negative control were, respectively, known positive sections, PBS was used instead of the primary antibody, and the secondary antibody was the AS-1107 immunohistochemical kit produced by Aspen Company (Dilution: 1:200). The expression levels of GPER, ERα and ERβ in the cell blocks were determined according to the manufacturer's instructions. The score was calculated based on the degree of staining and the number of positive cells: (1) Degree of staining: 0 points for all negative cells (-), 1 point for weakly positive cells (+), 2 points for moderately positive cells (++), and 3 points for strongly positive cells (+++). (2) Number of positive cells: Cells with a staining intensity higher than the background non-specific staining are considered positive. Six high-power fields in areas with a dense distribution of tumor cells were randomly selected for counting. At least 100 tumor cells should be counted to observe their positive expression. A positive expression rate of less than 1% was rated as 1 point, 1% to 10% as 2 points, 10% to 33% as 3 points, 34% to 66% as 4 points, and 67% to 100% as 5 points. If the sum of (1) and (2) is 5 to 8 points, the sample was determined positive for antigen expression; otherwise, it was negative. Immunohistochemical results were independently evaluated by two pathologists in a double-blind manner.

Statistical Analysis

Statistical analysis was performed using SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc. The sample differences of the patients' general information were analyzed using the χ^2 test or Fisher's exact test (e.g., expected counts $<$5). The expression correlations among GPER, ERα and ERβ were analyzed using the Spearman method. The comparisons of ORR and DCR were analyzed using the χ^2 test. A Kaplan-Meier survival curve was used for survival analysis, and the log-rank test was used to determine significant differences. Multivariate analyses of each factor were performed using the Cox proportional hazards model, and the results were considered statistically significant when the p-value was $<$0.05.

Results

Analysis of Clinical Case Data

A total of 106 lung cancer patients with MPE at the initial diagnosis were included. The median age was 65.3 years (ranging from 48 to 75 years). The ratio of men to women is 51:55, and 64 patients had a history of smoking.

Table 1. Patient characteristics at baseline.

Characteristics	Total (n = 106)	GPER		χ^2	p	ERα		χ^2	p	ERβ		χ^2	p
		Negative (n = 38)	Positive (n = 68)			Negative (n = 50)	Positive (n = 56)			Negative (n = 46)	Positive (n = 60)		
Gender				1.771	0.183			0.573	0.449			0.699	0.403
Male	51	15 (39.5%)	36 (52.9%)			26 (52.0%)	25 (44.6%)			20 (43.5%)	31 (51.7%)		
Female	55	23 (60.5%)	32 (47.1%)			24 (48.0%)	31 (55.4%)			26 (56.5%)	29 (48.3%)		
Age [median (IQR), yr]	66 (63–68)			0.664	0.415			0.175	0.676			0.968	0.325
<65	36	11 (28.9%)	25 (36.8%)			18 (36.0%)	18 (32.1%)			18 (39.1%)	18 (30.0%)		
≥65	70	27 (71.1%)	43 (63.2%)			32 (64.0%)	38 (67.9%)			28 (60.9%)	42 (70.0%)		
Smoking history				1.486	0.223			0.104	0.747			0.096	0.757
No	42	18 (47.4%)	24 (35.3%)			19 (38.0%)	23 (41.1%)			19 (41.3%)	23 (38.3%)		
Yes	64	20 (52.6%)	44 (64.7%)			31 (62.0%)	33 (58.9%)			27 (58.7%)	37 (61.7%)		
Stage IV				0.531	0.223			0.480	0.488			0.574	0.448
M1a	62	24 (63.2%)	38 (55.9%)			31 (62.0%)	31 (55.4%)			25 (54.3%)	37 (61.7%)		
M1b + M1c	44	14 (36.8%)	30 (44.1%)			19 (38.0%)	25 (44.6%)			21 (45.7%)	23 (38.3%)		
ECOG PS				0.029	0.864			0.013	0.911			0.439	0.508
0–1	63	23 (60.5%)	40 (58.8%)			30 (60.0%)	33 (58.9%)			29 (63.0%)	34 (56.7%)		
2–3	43	15 (39.5%)	28 (41.2%)			20 (40.0%)	23 (41.1%)			17 (37.0%)	26 (43.3%)		
Size of the pleural effusion				—	0.865			—	0.913			—	0.348
Small	9	4 (10.5%)	5 (7.4%)			5 (10.0%)	4 (7.1%)			6 (13.0%)	3 (5.0%)		
Moderate	60	21 (55.3%)	39 (57.4%)			28 (56.0%)	32 (57.1%)			24 (52.2%)	36 (60.0%)		
Large	37	13 (34.2%)	24 (35.3%)			17 (34.0%)	20 (35.7%)			16 (34.8%)	21 (35.0%)		
EGFR mutation				0.405	0.525			0.189	0.664			0.792	0.373
Deletion of exon 19	57	22 (57.9%)	35 (51.5%)			28 (56.0%)	29 (51.8%)			27 (58.7%)	30 (50.0%)		
L858R+ of exon 21	49	16 (42.1%)	33 (48.5%)			22 (44.0%)	27 (48.2%)			19 (41.3%)	30 (50.0%)		
TKIs				0.040	0.841			2.108	0.147			0.145	0.704
1th TKI	60	22 (57.9%)	38 (55.9%)			32 (64.0%)	28 (50.0%)			27 (58.7%)	33 (55.0%)		
3rd TKI	46	16 (42.1%)	30 (44.1%)			18 (36.0%)	28 (50.0%)			19 (41.3%)	27 (45.0%)		
Treatment				0.190	0.663			0.886	0.347			0.261	0.609
TKI	56	19 (50.0%)	37 (54.4%)			24 (48.0%)	32 (57.1%)			23 (50.0%)	33 (55.0%)		
TKI + local treatment	50	19 (50.0%)	31 (45.6%)			26 (52.0%)	24 (42.9%)			23 (50.0%)	27 (45.0%)		

GPER, G protein-coupled estrogen receptor; ERα, estrogen receptor α; ERβ, estrogen receptor β; IQR, interquartile range; ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

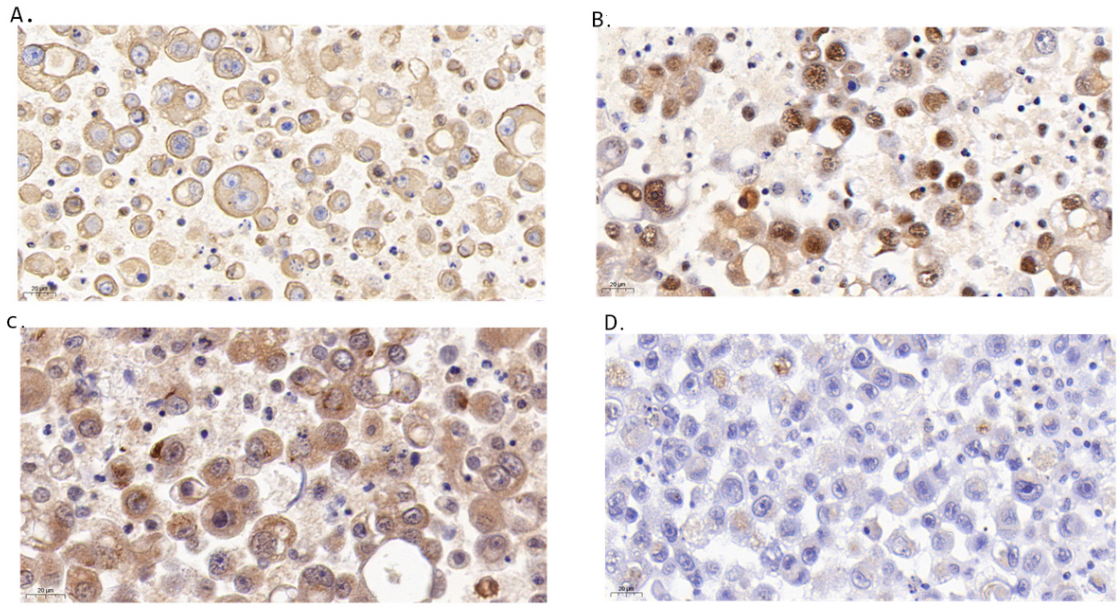

Fig. 1. Pathological detection. (A) GPER expression. (B) Expression of ERα. (C) Expression of ERβ. (D) Negative control. Scale bar = 20 µm. GPER, G protein-coupled estrogen receptor; ERα, estrogen receptor α, ERβ, estrogen receptor β.

At initial diagnosis, baseline pleural effusion volumes were categorized as follows: small in 9 cases, moderate in 60 cases, and large in 37 cases. All these patients were diagnosed with lung adenocarcinoma. The proportions of exon 19 deletion and exon 21 L858R mutation were 57 (53.8%) and 49 (46.2%), respectively. 56 (52.8%) patients underwent pleural effusion drainage and only received TKI treatment. 50 patients (47.2%) received TKIs, pleural effusion drainage and intrathoracic treatment. There were no statistically significant differences in gender, age, smoking history, Eastern Cooperative Oncology Group performance status (ECOG PS), Stage IV stage, pleural effusion volume, EGFR-TKIs type or treatment regimens among patients stratified according to different expression levels of GPER, ERα and ERβ. Table 1 summarizes the basic characteristics of the patients.

Pathological Detection

Among these 106 cases, a total of 68 patients (64.2%) tested positive for GPER, 56 patients (52.8%) tested positive for ERα, and 60 patients (56.6%) tested positive for ERβ. GPER staining was predominantly localized to the cytoplasm of tumor cells, ERα staining was primarily localized to the nucleus of tumor cells, while ERβ staining was distributed in both the nucleus and cytoplasm. The representative microscopic images of hormone receptor staining are shown in Fig. 1. There was a significant correlation in the expression levels of GPER, ERα and ERβ (see Table 2), ERα and GPER ($r_s = 0.515$, $p < 0.001$), ERβ and GPER ($r_s = 0.497$, $p < 0.001$), as well as ERα and ERβ ($r_s = 0.469$, $p < 0.001$).

Table 2. Spearman correlation coefficient between the results of IHC staining.

	ERα		ERβ	
	r_s	p value	r_s	p value
ERβ	0.469	<0.001		
GPER	0.515	<0.001	0.497	<0.001

IHC, immunohistochemical; ER, estrogen receptor; GPER, G protein-coupled estrogen receptor. $p < 0.05$ was considered statistically significant.

Overall Therapeutic Effect Analysis

The overall therapeutic effects of the patients in this study: CR, PR, SD, and PD were 0% (0 cases), 57.5% (61 cases), 33.0% (35 cases), and 9.4% (10 cases), respectively. The objective response rate (ORR) of GPER-positive patients was lower than that of GPER-negative patients (48.5% vs. 73.7%, $p = 0.012$). Although the results did not reach statistical significance, the ORR was lower in ERα (51.8% vs. 64.0%, $p = 0.204$) and ERβ (53.3% vs. 63.0%, $p = 0.316$) positive patients (See Table 3). The DCR of patients positive for GPER, ERα and ERβ was lower, which were (86.8% vs. 97.4%, $p = 0.290$), (87.5% vs. 94.0%, $p = 0.506$) and (88.3% vs. 93.5%, $p = 0.736$), respectively, and none of them reached statistical significance.

Table 3. Overall responses.

	Total (n = 106)	GPER				ERα				ERβ			
		Negative (n = 38)	Positive (n = 68)	χ^2	*p*	Negative (n = 50)	Positive (n = 56)	χ^2	*p*	Negative (n = 46)	Positive (n = 60)	χ^2	*p*
CR	0	0	0			0	0			0	0		
PR	61 (57.5%)	28 (73.7%)	33 (48.5%)			32 (64.0%)	29 (51.8%)			29 (63.0%)	32 (53.3%)		
SD	35 (33.0%)	9 (23.7%)	26 (38.2%)			15 (30.0%)	20 (35.7%)			14 (30.4%)	21 (35.0%)		
PD	10 (9.4%)	1 (2.6%)	9 (13.2%)			3 (6.0%)	7 (12.5%)			3 (6.5%)	7 (11.7%)		
ORR		73.7%	48.5%	6.314	0.012	64.0%	51.8%	1.613	0.204	63.0%	53.3%	1.005	0.316
DCR		97.4%	86.8%	-	0.290	94.0%	87.5%	-	0.506	93.5%	88.3%	-	0.736

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; DCR, disease control rate; GPER, G protein-coupled estrogen receptor; ERα, estrogen receptor α; ERβ, estrogen receptor β.

PFS Analysis

The PFS of patients was analyzed using the Kaplan-Meier survival curve and log-rank test (see Table 4 and Fig. 2). The median follow-up period for the PFS analysis was 11.5 months. The 12-month PFS rates of the GPER-positive group and the GPER- negative group were 41.0% and 57.9%, respectively, and those of the ER-positive group and the ER-negative group were 48.3% and 46.0%, respectively. The 12-month PFS rates of the ERβ-positive group and the ERβ-negative group were 43.2% and 52.2%, respectively. The median PFS of the GPER-positive group and the GPER-negative group was 10.6 months and 13.2 months, respectively ($p = 0.035$); The median PFS of Stage IV staging M1a and M1b + M1c was 12.6 months and 9.9 months, respectively ($p = 0.001$); The median PFS for PS scores ranging from 0 to 1 and from 2 to 3 were 12.6 months and 11.2 months, respectively ($p = 0.034$). The median PFS of the first-generation TKI and the third-generation TKI were 9.2 months and 17.5 months, respectively ($p < 0.001$). The above single-factor analysis showed statistically significant differences. There were no statistically significant differences in the log-rank test of the patients in terms of gender, age, smoking history, ERα expression status, ERβ expression status, Size of the pleural effusion, EGFR mutation type or local treatment of the thoracic cavity.

Multivariate Cox Proportional Hazards Analysis

Based on the results of the log-rank test, the expression status of GPER, ERα, ERβ, Stage IV stage, PS score, and the treatment status of TKI were used to construct a multivariate Cox proportional hazards model. The results showed (see Table 5) that positive GPER was significantly and independently associated with a shorter PFS (HR 2.003, 95% CI 1.231–3.259, $p = 0.005$), and among these stage IV lung cancers, M1b + M1c was independently associated with a shorter PFS (HR 1.861, 95% CI 1.213–2.855, $p = 0.004$), relative to M1a. The influence of PS score on PFS was statistically significant (HR 1.656, 95% CI 1.088–2.521, $p = 0.019$), and the third-generation TKI was significantly and independently associated with a longer PFS (HR 0.466, 95% CI 0.368–0.590, $p < 0.001$). The expression status of ERα and ERβ had no statistically significant effect on PFS ($p > 0.05$).

Discussion

In this study, we investigated the expression of GPER, ERα, and ERβ in malignant pleural effusion (MPE) from patients with lung adenocarcinoma and found that their expression levels were significantly correlated; however, in analyses evaluating EGFR-TKI efficacy, GPER showed greater predictive sensitivity than ERα or ERβ, and high GPER expression was associated with poorer EGFR-TKI treatment response.

GPER is one of the G protein receptor families and is mainly localized to the cell membrane. Previous studies have shown that compared with normal lungs, the expression of GPER in lung cancer cells and human lung cancer tissues is increased [24]. Among the 106 patients enrolled in this study, 64.2% were positive for GPER. GPER staining was predominantly localized to the cytoplasm of tumor cells, which was consistent with the expression rate and cellular localization of GPER in the above-mentioned literature. Since GPER is primarily localized to the cell membrane, its function depends on the cross-activation of EGFR. When GPER is stimulated by estrogen, it first activates matrix metalloproteinases through a tyrosine protein kinase (src kinase) encoded by the src gene. The latter can cause the precursor of heparin-bound epidermal growth factor (proHB-EGF) to undergo cleavage and generate HB-EGF. It then binds to EGFR and activates downstream signals [25], including changes in intracellular cyclic adenosine monophosphate (cAMP) concentration, activation of the PI3K/Akt axis, initiation of MAPK cascade amplification reactions, and intracellular calcium ion release [26–28]. By participating in these signaling mediators, GPER can also regulate the expression of certain genes that are associated with various cancer markers, including persistent proliferation, invasion and metastasis, as well as angiogenesis and tumor-promoting inflammation [29]. At present, clinical studies on the relationship between GPER and EGFR mutation status and the efficacy of EGFR-TKI remain scarce. Li *et al.* [30] reported that in patients who underwent tumor resection and were diagnosed with NSCLC, high expression of GPER was significantly associated with EGFR mutation status, tumor stage, lymph node metastasis, and poor postoperative prognosis. In this study, different expression levels of GPER in MPE from lung adenocarcinoma patients were found to affect multiple indicators of the therapeutic effect of EGFR-TKI. For example, the ORR of GPER-positive patients was lower than that of GPER-negative patients (48.5% vs. 73.7%), shorter median PFS (10.6 months vs. 13.2 months), and it was an independent risk factor for PFS (HR 2.003, 95% CI 1.231–3.259). In the overall response analysis, we also observed that the objective response rate (ORR) was significantly lower in GPER-positive patients than in GPER-negative patients, whereas the disease control rate (DCR) was numerically lower but did not reach statistical significance. This discrepancy may be partly explained by limited statistical power: the DCR values for GPER, ERα, and ERβ were 86.8% vs. 97.4%, 87.5% vs. 94.0%, and 88.3% vs. 93.5%, respectively. Given the high baseline DCR and small absolute differences, a substantially larger sample size would be required to achieve statistical significance, while the larger differences in ORR were sufficient to be detectable in the current cohort. Mechanistically, this may suggest that GPER positivity attenuates the depth of tumor shrinkage induced by EGFR-TKI. Through GPER-

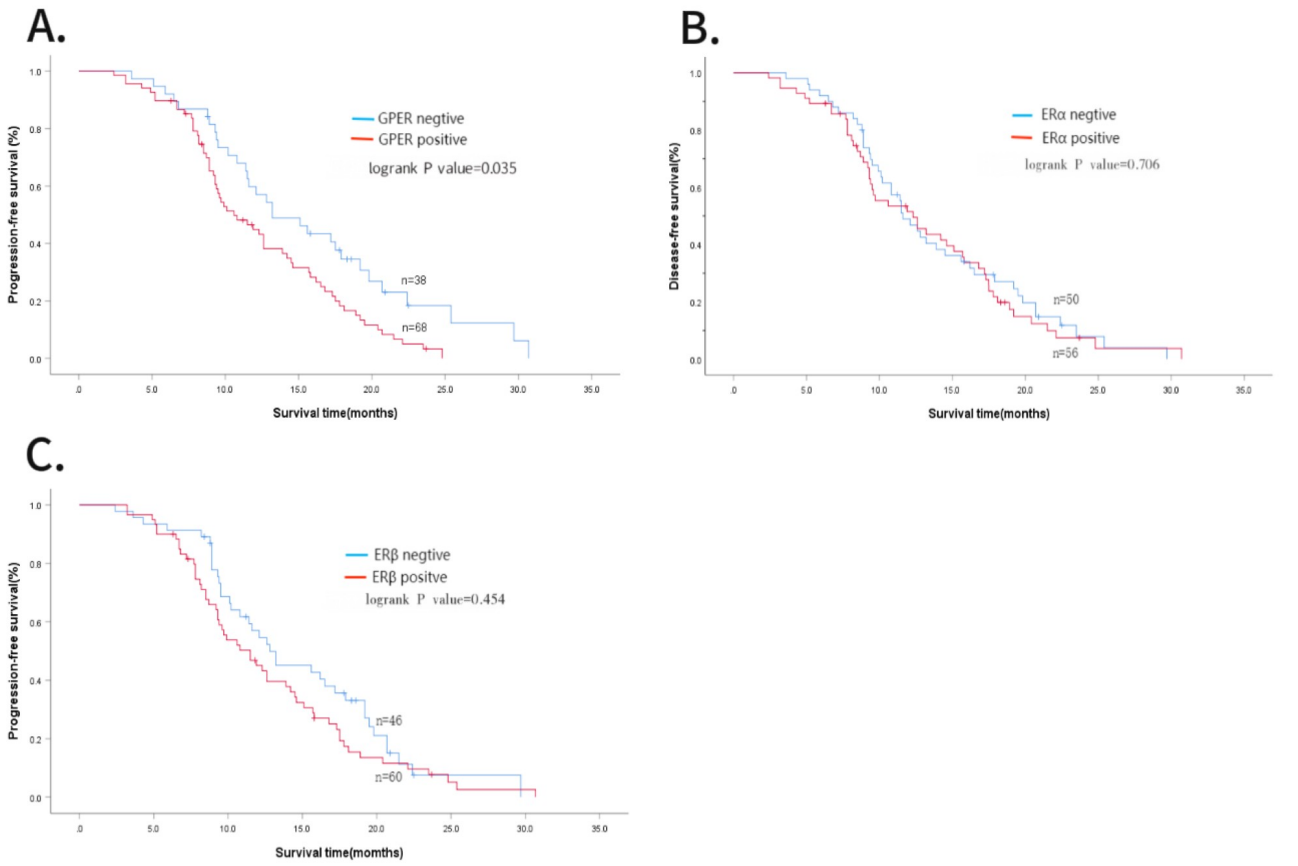

Fig. 2. mPFS curves for patients with different GPER, ERα, ERβ expression levels receiving EGFR-TKI therapy. (A) GPER expression. (B) ERα expression. (C) ERβ expression. mPFS, median progression-free survival; GPER, G protein-coupled estrogen receptor; ERα, estrogen receptor α; ERβ, estrogen receptor β.

mediated bypass activation, some patients who might otherwise achieve a partial response may instead remain in stable disease, resulting in a marked reduction in ORR but a relatively modest effect on DCR. Our research indicates that the high expression of GPER in advanced lung adenocarcinoma is significantly associated with reduced EGFR-TKI efficacy; however, given the retrospective design, this finding reflects an association rather than a causal relationship. Additionally, this is consistent with previous studies [24,29,30] not only suggesting GPER's potential driving role in lung cancer, but also suggesting that GPER may represent a potential target for EGFR-TKI–based combination strategies in lung adenocarcinoma.

Our research also suggested that GPER shows significant differences compared with ERα and ERβ in the survival analysis for predicting the efficacy of EGFRTKIs. There is a significant correlation in the expression of GPER, ERα and ERβ in advanced lung adenocarcinoma with malignant pleural effusion. However, only GPER yielded significant results in the survival analysis for predicting EGFR-TKI efficacy, whereas the expression levels of ERα and ERβ showed no statistically significant differences in both the log-rank test and the Cox proportional hazards model. GPER is more sensitive compared with ERα or ERβ. This difference may be attributed to fundamental distinctions in their cellular localization and signaling mechanisms. For a long time, ER α and ERβ were believed to be primarily localized to the cell nucleus. After estrogen combines with ERα and ERβ in the cell nucleus, complex macromolecular synthesis and protein modification are triggered, generating mRNA and corresponding protein expression. This process is referred to as the genomic pathway. However, subsequent research has indicated that both ERα and ERβ possess splicing variants capable of intracellular transport, constantly shuttling between the cytoplasm and the nucleus. Together with the newly discovered GPER, they activate the cAMP, MAPK and AKT signaling pathways, generating second messengers to activate ion channels and establishing cross-dialogue with other receptors, such as growth factors, which is called the non-genomic pathway [31–33]. ERα and ERβ and their splicing variants reflect the complex intracellular signaling processes of estrogen cells, which are influenced by numerous factors, and agonists or antagonists at each node regulate this highly dynamic process [34]. This may explain the inconsistency in previous studies related to ERα and ERβ.

Table 4. Log-rank test of PFS in 106 lung adenocarcinoma patients harboring EGFR mutations with pleural effusion at initial diagnosis.

Factor	n	mPFS (m)	χ^2	p	mPFS 95% CI
Gender			0.839	0.360	
Male	51	10.800			8.501–13.099
Female	55	12.600			10.771–14.429
Age (years)			3.237	0.072	
≤65	36	10.600			8.633–12.567
>65	70	12.600			10.625–14.575
Smoking history			1.115	0.291	
Yes	64	10.200			7.982–12.418
No	42	12.300			11.045–13.555
GPER			4.463	0.035	
Negative	38	13.200			8.675–17.725
Positive	68	10.600			8.614–12.586
ERα			0.142	0.706	
Negative	50	11.500			10.000–13.000
Positive	56	11.900			8.822–14.978
ERβ			0.560	0.454	
Negative	46	12.100			10.201–13.999
Positive	60	11.500			8.898–14.102
Stage IV			10.899	0.001	
M1a	62	12.600			8.551–16.649
M1b + M1c	44	9.900			8.715–11.085
ECOG PS			4.503	0.034	
0–1	63	12.600			9.270–15.930
2–3	43	11.200			10.077–12.323
Size of the pleural effusion			2.706	0.258	
Moderate	60	11.500			9.887–13.113
Large	37	12.800			10.297–15.30
Massive	9	8.900			8.608–9.192
EGFR mutation type			0.052	0.819	
Deletion of exon 19	57	11.800			9.290–14.310
L858R+ of exon 21	49	11.600			9.905–13.295
EGFR-TKIs			39.312	<0.001	
1th TKI	60	9.200			8.663–9.737
3rd TKI	46	17.500			16.079–18.921
Treatment			0.096	0.757	
TKI	56	10.800			8.524–13.076
TKI + local treatment	50	12.600			10.523–14.677

GPER, G protein-coupled estrogen receptor; ERα, estrogen receptor α; ERβ, estrogen receptor β; ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

GPER is localized to the cell membrane, where E2-GPER is involved in various rapid non- genomic processes occurring within a few seconds to a few minutes, and exhibits relatively close signal cross-response with EGFR [35]. Based on the different results of GPER, ERα and ERβ in our research, GPER, as an estrogen receptor with closer spatial and signaling crosstalk with EGFR, may exert a more direct impact on EGFR-TKI efficacy and could serve as a potential predictor of EGFR-TKI response in lung adenocarcinoma with malignant pleural effusion.

Pleural effusion is a common complication of advanced lung adenocarcinoma. In patients with EGFR mutations, pleural effusion exhibits a poor response to TKI treatment [36]. However, few reports have evaluated the correlation between the molecular and pathological conditions of cells in effusion and prognosis. This is because the limitations of conventional cytologic examination (CCE) of pleural fluid, such as low cell counts, cellular crowding, and the mixture of inflammatory cells or blood cells. Hypocellularity, cellular overcrowding, inflammatory infiltrate, and

Table 5. Multivariate analysis of mPFS in 106 lung adenocarcinoma patients.

Factor	*p*	HR	95% CI
GPER Positive vs. GPER Negative	0.005	2.003	1.231–3.259
ERα Positive vs. ERα Negative	0.939	0.982	0.623–1.549
ERβ Positive vs. ERβ Negative	0.649	1.119	0.690–1.814
M1b + M1c vs. M1a	0.004	1.861	1.213–2.855
ECOG PS 2–3 vs. ECOG PS 0–1	0.019	1.656	1.088–2.521
3rd EGFR-TKIs vs. 1th TKI EGFR-TKIs	$<$0.001	0.466	0.368–0.590

mPFS, median progression-free survival; GPER, G protein-coupled estrogen receptor; ERα, estrogen receptor α; ERβ, estrogen receptor β; ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

bloody effusions are frequent causes of a negative CCE. The preparation and detection of MPE cell blocks can effectively address the above shortcomings and reduce the need for invasive pleural biopsy or lung biopsy surgeries for patients [37]. Given a retrospective study, during the process of collecting relevant specimens from previous patients, we found that tissue specimens obtained from lung and pleural biopsies were limited in quantity and insufficient for research requirements. In contrast, MPE samples were relatively easy to obtain, and the preparation of cell blocks from MPE allowed preservation of a greater number of tumor cells. In recent years, the detection of molecular pathological indicators related to targeted therapy for lung cancer through MPE cell blocks has been widely recognized [37–39]. Therefore, we used MPE cell block samples to confirm the expression patterns of GPER, ERα and ERβ, highlighting the feasibility and clinical value of MPE cell blocks for biomarker assessment and therapeutic stratification. In addition, we found that Stage IV, PS score and different EGFR-TKIs were independent predictors of PFS in this patient population.

This study has several limitations. First, this was a single-center retrospective study with a relatively small sample size, which may introduce selection bias. Second, only immunohistochemical analyses were performed, and functional experiments remain lacking. Therefore, the molecular mechanisms by which high GPER expression influences EGFR-TKI efficacy require further validation in larger, multicenter cohorts and experimental studies.

Conclusion

This study indicates that the high expression of GPER in advanced lung adenocarcinoma with malignant pleural effusion is a negative predictor of the efficacy of EGFR-TKI treatment in these patients. There is a significant correlation in the expression of GPER, ERα and ERβ. However, compared with ERα and ERβ, GPER is more sensitive in predicting the PFS of patients treated with EGFR-TKI. The conclusions of some previous preclinical studies on EGFR-TKI combined with estrogen receptor antagonists were inconsistent. Most of these studies did not stratify tumors based on the expression status of estrogen receptors. Therefore, further prospective studies stratified by GPER expression are warranted to clarify differential responses to anti-estrogen treatment among patients with different GPER expression levels, and to explore potential treatment strategies to overcome resistance to EGFR-TKI treatment.

Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

YSD and YGA designed the research study. YSD and CLH performed the research. XXW and XQW analyzed the data. YSD and YGA drafted the article. All authors contributed to important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Second Affiliated Hospital of Shantou University Medical College (2017-46 and 2025-34). The ethics committee waived the requirement for informed consent because of the retrospective nature of the study.

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Conflict of Interest

The authors declare no conflict of interest.

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