Postprint: Expression of Fructose-Bisphosphate Aldolase A Before and After Targeted Drug Resistance in Lung Adenocarcinoma and Its Association with Patient Prognosis

Authors: Xiong Siyu, Lu Bing, Jiang Wenhong, YU Tingting, Yu Tingting

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Abstract

Background Lung cancer ranks first in both incidence and mortality among malignant tumors in China, with lung adenocarcinoma being closely associated with epidermal growth factor receptor (EGFR) gene mutations. Currently, relatively mature targeted therapeutic regimens are available for patients with EGFR-sensitive mutations, but the problem of drug resistance remains inevitable. Fructose-bisphosphate aldolase A (ALDOA) is a key glycolytic enzyme that is highly expressed in various cancers. The relationship between ALDOA and targeted drug resistance and prognosis in lung adenocarcinoma remains unclear.

Objective To investigate the expression of ALDOA in targeted drug resistance in lung adenocarcinoma patients and its relationship with patient prognosis.

Methods Based on the TCGA database, data from 483 lung adenocarcinoma tissues and 59 normal lung tissues were obtained and divided into high-expression and low-expression groups according to the median ALDOA expression level (239 cases each, 5 cases excluded due to missing data) to evaluate the relationship between ALDOA expression level and overall survival. Thirty lung adenocarcinoma patients who developed resistance after icotinib targeted therapy at the Affiliated Tumor Hospital of Xinjiang Medical University between October 2016 and May 2024 were included. Tumor tissue biopsy specimens and clinical data were collected from the patients. Follow-up was conducted until May 30, 2024, during which disease stability (PD), progression-free survival (PFS), and disease response were observed and recorded. ALDOA expression levels in specimens were detected by immunohistochemistry, and patients were divided into high-expression group (21 cases) and low-expression group (9 cases) based on ALDOA levels. Patient prognosis was analyzed.

Univariate Cox regression was used to analyze factors influencing PFS after targeted therapy resistance in lung adenocarcinoma patients. Survival analysis comparison was performed using the Log-rank test, and Kaplan-Meier method was used to plot PFS survival curves.

Results Analysis results based on the TCGA database showed that ALDOA expression level was higher in lung adenocarcinoma tissues than in normal lung tissues (P<0.05), and the overall survival (OS) of the high ALDOA expression group was lower than that of the low-expression group (HR=1.8, P<0.001). Comparison of ALDOA expression levels among patients with different clinical stages (stage I-IV) showed statistically significant differences (F=5.98, P<0.001). Immunohistochemistry results showed that ALDOA protein expression score in post-resistance lung cancer biopsy tissues was higher than that before resistance (paired t=4.104, P<0.001). Comparison of disease remission between low and high ALDOA expression groups revealed that the objective response rate (ORR) in the high ALDOA expression group was lower than that in the low-expression group (P=0.045). Survival analysis results showed that PFS of patients in the low ALDOA expression group was longer than that in the high-expression group. Log-rank test results showed statistically significant differences between the two groups (2=5.413, P=0.02). Univariate Cox regression analysis showed that low ALDOA expression was a protective factor for PFS after targeted therapy resistance in lung cancer (HR=0.066, 95%CI=0.007~0.648, P<0.05), while lymph node metastasis status (N2 and N3) was a risk factor for PFS after targeted therapy resistance in lung cancer (HR=14.015, 95%CI=2.017~97.379, P<0.05).

Conclusion Upregulated ALDOA expression after targeted drug therapy is a significant characteristic of targeted drug resistance, and its high expression is closely associated with poor prognosis in patients undergoing targeted therapy, suggesting that ALDOA may serve as a potential molecular marker for predicting failure of epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) therapy.

Full Text

Study on the Relationship Between Fructose-1,6-Bisphosphate Aldolase A Expression Before and After Targeted Drug Resistance in Lung Adenocarcinoma and Patient Prognosis

XIONG Siyu, LU Bing, JIANG Wenhong, YU Tingting*

Department of Pulmonary Oncology, Affiliated Cancer Hospital of Xinjiang Medical University, Urumqi 830011, China

*Corresponding author: YU Tingting, Chief Physician; E-mail: 13579910597@163.com

Abstract

Background: Lung cancer ranks first in both incidence and mortality among malignant tumors in China, with lung adenocarcinoma closely associated with epidermal growth factor receptor (EGFR) gene mutations. Currently, effective targeted therapies exist for patients with EGFR-sensitive mutations, yet drug resistance remains an unavoidable challenge. Fructose-1,6-bisphosphate aldolase A (ALDOA) is a key glycolytic enzyme highly expressed in multiple cancers, though its relationship with targeted drug resistance and prognosis in lung adenocarcinoma remains unclear.

Objective: To investigate ALDOA expression in lung adenocarcinoma patients before and after developing resistance to targeted therapy and its relationship with patient prognosis.

Methods: Using data from the TCGA database, we analyzed 483 lung adenocarcinoma tissues and 59 normal lung tissues. Samples were divided into high- and low-expression groups based on the median ALDOA expression level (239 cases each; 5 cases with missing data were excluded) to evaluate the relationship between ALDOA expression and overall survival. We also enrolled 30 lung adenocarcinoma patients who developed resistance to icotinib targeted therapy at the Affiliated Cancer Hospital of Xinjiang Medical University between October 2016 and May 2024. Tumor tissue biopsy specimens and clinical data were collected, with follow-up through May 30, 2024. Disease progression (PD), progression-free survival (PFS), and disease response were recorded. ALDOA expression levels in specimens were detected by immunohistochemistry, with patients categorized into high-expression (n=21) and low-expression (n=9) groups based on ALDOA levels. Prognosis was analyzed using univariate Cox regression to identify factors influencing PFS after targeted therapy resistance, with survival analysis performed using the Log-rank test and Kaplan-Meier curves.

Results: TCGA database analysis revealed that ALDOA expression was significantly higher in lung adenocarcinoma tissues than in normal lung tissues ($P<0.05$), with the high-expression group showing lower overall survival (OS) than the low-expression group (HR=1.8, $P<0.001$). ALDOA expression levels differed significantly across clinical stages (I-IV) ($F=5.98$, $P<0.001$). Immunohistochemistry showed that ALDOA protein expression scores were higher in post-resistance biopsy tissues than in pre-resistance tissues (paired $t=4.104$, $P<0.001$). The high-expression group had a lower objective response rate (ORR) than the low-expression group ($P=0.045$). Survival analysis demonstrated longer PFS in the low-expression group, with Log-rank test showing a statistically significant difference between groups ($^2=5.413$, $P=0.02$). Univariate Cox regression identified low ALDOA expression as a protective factor for PFS after targeted therapy resistance (HR=0.066, 95%CI=0.007-0.648, $P<0.05$), while lymph node metastasis status (N2 and N3) was a risk factor (HR=14.015, 95%CI=2.017-97.379, $P<0.05$).

Conclusion: Upregulation of ALDOA expression following targeted drug ther-

apy represents a significant feature of drug resistance, with high expression closely associated with poor prognosis during targeted treatment. This suggests ALDOA may serve as a potential molecular biomarker for predicting treatment failure with epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs).

Keywords: Lung cancer; Fructose-bisphosphate aldolase A; Targeted therapy; Drug resistance; Prognosis

1. Materials and Methods

1.1 Bioinformatics Analysis of ALDOA Expression in Lung Adenocarcinoma and Survival Analysis

We utilized the GEPIA2 online analysis platform (http://gepia.cancer-pku.cn) to analyze data from The Cancer Genome Atlas (TCGA) database. The dataset category was selected as "LUAD" with default parameters for all other settings, obtaining ALDOA expression data from 483 lung adenocarcinoma tissues and 59 normal lung tissues. Based on the median ALDOA expression level (cut-off=50%), samples were divided into high- and low-expression groups. Kaplan-Meier survival analysis was performed to evaluate the relationship between ALDOA expression levels and overall survival (OS).

1.2 Study Population

This study enrolled 30 patients from the Affiliated Cancer Hospital of Xinjiang Medical University who met the inclusion criteria: (1) histopathologically confirmed primary lung adenocarcinoma with EGFR-sensitive mutations (exon 19 deletion/exon 21 L858R); (2) first-line or subsequent treatment with first-generation icotinib (EGFR-TKI) until disease progression, with second tumor specimens obtained via image-guided percutaneous biopsy or endoscopic biopsy after resistance development. Exclusion criteria included: (1) missing clinical/pathological data or tissue specimens; (2) other tumor types with lung metastasis or concurrent malignancies; (3) severe cardiopulmonary/cerebral comorbidities; (4) expected survival <3 months. The study was approved by the Ethics Committee of the Affiliated Cancer Hospital of Xinjiang Medical University (Approval No.: K-2025104), with informed consent obtained from all patients.

1.3 Clinical Data Collection

Electronic medical records were reviewed to collect clinical data including gender, age, smoking history (defined as cumulative smoking $ $6 months, not quitting or quit <1 month; non-smoking defined as never smoked or <100 cigarettes total), pathological type, tumor differentiation, TNM stage, and EGFR mutation type (19del/21L858R).

1.4 Outcomes and Follow-up Indicators

Follow-up continued through May 30, 2024, with PD time and progression-free survival (PFS) recorded. Enhanced CT scans were performed every 8 weeks or when clinically indicated. PFS was defined as the time from initial icotinib treatment to disease progression or death from any cause.

1.5 Immunohistochemical Detection of ALDOA Expression in Biopsy Specimens Before and After Resistance

Paraffin-embedded lung adenocarcinoma biopsy specimens were obtained from the pathology department. Sections were baked, dewaxed in xylene, and rehydrated through graded ethanol. After heat-mediated antigen retrieval and cooling, sections were blocked with 3% hydrogen peroxide for 15 minutes, incubated overnight at 4°C with primary antibody (1:400 dilution), then with HRP-conjugated secondary antibody (1:200 dilution) at room temperature for 20 minutes. DAB chromogen was applied for visualization, followed by Harris hematoxylin counterstaining, dehydration, and mounting.

1.6 IHC Scoring

Specimens were evaluated by at least two senior pathologists using a standardized Olympus BX53 microscope platform. Five tumor-rich areas were randomly selected at 400× and 100× magnification (excluding necrotic and stromal regions). Staining intensity was scored as: 0 (negative), 1 (light yellow), 2 (brown-yellow), or 3 (dark brown). Positive cell proportion was scored as: 0 (<1%), 1 (1-25%), 2 (26-50%), 3 (51-75%), or 4 (>75%). The final score (0-12) was the product of intensity and proportion scores, categorized as: 0 (negative), 1-4 (weak positive), 5-8 (moderate positive), and 9-12 (strong positive). Scores of 0-4 were classified as low expression and $ $5 as high expression.

1.7 Statistical Analysis

SPSS 26.0 software was used for statistical analysis. Normally distributed continuous data were expressed as mean ± standard deviation and compared using independent samples t-test or one-way ANOVA. Categorical data were expressed as percentages and compared using ^{2} test or Fisher' s exact test. Paired t-test was used to compare ALDOA expression before and after icotinib treatment. Cox regression models identified factors influencing median PFS after targeted therapy resistance. Kaplan-Meier curves were plotted with Log-rank test for comparison. $P<0.05$ was considered statistically significant.

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2. Results

2.1 Comparison of ALDOA Expression Between Lung Adenocarcinoma and Normal Tissues and Across Different Stages

TCGA database analysis revealed significantly higher ALDOA expression in lung adenocarcinoma tissues compared to normal lung tissues ($P<0.05$). Using the median ALDOA expression level as cut-off (239 cases each; 5 samples excluded due to missing data), the high-expression group showed inferior OS compared to the low-expression group (HR=1.8, $P<0.001$). ALDOA expression differed significantly across clinical stages I-IV ($F=5.98$, $P<0.001$), indicating correlation with tumor stage [Figure 1: see original paper][Figure 2: see original paper].

2.3 ALDOA Expression in Cancer Tissues Before and After Resistance

Among the 30 enrolled patients (6 males [20%], 24 females [80%]; mean age 64.0$±$8.7 years), 3 had smoking history (10%) and 27 were non-smokers (90%). EGFR mutations included 19del in 19 cases (63.33%) and 21L858R in 11 cases (36.67%). TNM staging showed: T1-2 in 18 cases (60%), T3-4 in 12 cases (40%); N0-1 in 17 cases (56.67%), N2-3 in 13 cases (43.33%); M0 in 5 cases (16.67%), M1 in 25 cases (83.33%).

IHC revealed ALDOA protein primarily localized in tumor cytoplasm as brown-yellow granular deposits. Pre-resistance tissues showed lighter staining with lower expression intensity, while post-resistance tissues exhibited darker brown staining with broader expression [Figure 3: see original paper]. Quantitative analysis confirmed significantly higher ALDOA expression scores in post-resistance biopsies ($7.63\$±2.00) compared to pre-resistance ($5.59±\$1.39$; paired $t=4.104$, $P<0.001$).

2.4 Comparison of Baseline Characteristics Between ALDOA High- and Low-Expression Groups

Based on IHC scores, patients were divided into high-expression (n=21) and low-expression (n=9) groups. No significant differences were observed between groups in gender, age, TNM stage, smoking history, or EGFR mutation subtype ($P>0.05$) .

2.5 Prognosis and Survival Analysis

The high-expression group demonstrated significantly lower ORR compared to the low-expression group ($P=0.045$) . Median PFS was 15-24 months. Kaplan-Meier analysis revealed longer PFS in the low-expression group, with Log-rank test showing statistically significant difference between groups ($^{2}=5.413$, $P=0.02$) [Figure 4: see original paper].

2.6 Univariate Cox Regression Analysis of PFS Influencing Factors

Univariate Cox regression analysis (with variables assigned as shown in) identified low ALDOA expression as a protective factor for PFS after targeted therapy resistance (HR=0.066, 95%CI=0.007-0.648, P<0.05), while lymph node metastasis (N2 and N3) was a risk factor (HR=14.015, 95%CI=2.017-97.379, P<0.05) .

Discussion

Molecular targeted therapy represents a major focus in oncology, enabling precise identification and action on specific tumor cell targets. In NSCLC, EGFR mutations are the most common actionable alterations, offering superior clinical outcomes compared to platinum-based chemotherapy and improving patient quality of life and survival. However, resistance inevitably emerges, with most patients progressing after 10-14 months of first-line EGFR-TKI therapy. Tumor resistance, driven by genetic mutations, epigenetic modifications, and signaling pathway reprogramming, leads to treatment failure and profoundly impacts clinical decision-making and prognosis. First-generation EGFR-TKI resistance mechanisms include secondary mutations (e.g., T790M), bypass pathway activation, downstream signaling abnormalities such as PTEN loss, histological transformation to small cell lung cancer, and epithelial-mesenchymal transition. The tumor microenvironment may also contribute, though mechanisms remain unclear in many patients.

Our TCGA database and clinical cohort analyses demonstrate that high ALDOA expression correlates with reduced PFS in lung adenocarcinoma patients after targeted therapy resistance, establishing it as a key risk factor for poor prognosis. Previous studies identified age, gender, smoking history, mutation type, lymph node status, and distant metastasis as PFS correlates. Building on CONVINCE study data showing median PFS of 11.2 months with icotinib, we established this as our PD threshold for Cox regression modeling. Results confirmed low ALDOA expression as a protective factor and lymph node metastasis as a risk factor for PFS after resistance, providing potential indicators for precise prognostic assessment. These findings align with reports of ALDOA as an oncogenic factor across multiple solid tumors, suggesting it may be a pan-cancer driver of tumor progression and therapeutic resistance.

ALDOA promotes oncogenesis through multiple mechanisms. As a key glycolytic enzyme, high ALDOA expression drives the Warburg effect, accelerating glucose metabolism to provide biosynthetic precursors and energy for rapidly proliferating tumor cells, thereby enhancing survival under therapeutic pressure. Conversely, low ALDOA expression may weaken this metabolic advantage, rendering tumor cells more susceptible to drug clearance and improving patient outcomes. Recent research reveals that aldolase family members, including ALDOA, can directly promote proliferation by regulating cell cycle proteins such

as Cyclin D1. Thus, low ALDOA expression may slow cell cycle progression and enhance sensitivity to targeted agents, demonstrating non-metabolic pathways through which ALDOA mediates resistance.

This study has limitations. As a single-center study with a small sample size, our findings require validation in larger cohorts. The specific mechanisms by which ALDOA induces resistance remain unclear given tumor heterogeneity. Future studies should investigate the precise molecular pathways underlying ALDOA-mediated acquired resistance to first- and third-generation EGFR-TKIs, and develop specific ALDOA inhibitors to validate their efficacy in reversing resistance through in vitro and in vivo experiments, providing novel therapeutic strategies.

In conclusion, ALDOA plays a role in monitoring lung cancer targeted therapy efficacy, and its expression level may serve as an adjunct tool to identify patients with potentially poor prognosis at diagnosis. ALDOA represents not only a promising prognostic biomarker but also a potential therapeutic target in lung cancer.

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