# ALDH3B1 Is an Independent Prognostic Biomarker of Lung Adenocarcinoma

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## Abstract

**Background:** Lung cancer is the leading cause of cancer-related death, and adenocarcinoma is the most common type of lung cancer. Although emerging evidence implicates the role of several aldehyde dehydrogenases in cancer progression, the expression and clinical significance of aldehyde dehydrogenase 3B1 in lung adenocarcinoma has never been studied. **Materials:** In our study, the expression of aldehyde dehydrogenase 3B1 in 250 cases of lung adenocarcinoma was detected with immunohistochemistry, and the patients were further divided into subgroups with different aldehyde dehydrogenase 3B1 expression. Using real-time polymerase chain reaction, we investigated the aldehyde dehydrogenase 3B1 messenger RNA in 20 lung adenocarcinoma and paired normal lung tissues. With the $\chi^2$ test, we evaluated the clinical significance of aldehyde dehydrogenase 3B1 by analyzing its correlation with the clinicopathological factors. Propensity score matching was performed to balance the baseline of cohort. With univariate and multivariate analyses, we screened the prognostic factors of lung adenocarcinoma and identified the independent prognostic factors before and after the propensity score matching. **Results:** Aldehyde dehydrogenase 3B1 expression was significantly associated with the sex and age of patients, tumor size, and histological grade. High expression of aldehyde dehydrogenase 3B1 predicted the poor prognosis ($P = .003$). Moreover, male patients ($P = .020$), large tumor size ($P = .009$), advanced T stage ($P = .001$), positive lymphatic invasion ($P < .001$), and advanced tumor–node–metastasis stage ($P < .001$) were all the prognostic factors for unfavorable outcome. Aldehyde dehydrogenase 3B1 was an independent prognostic biomarker of lung adenocarcinoma, indicating the poor prognosis. In addition, after balancing the baseline characteristics by propensity score matching, we also demonstrated that aldehyde dehydrogenase 3B1 was an independent prognostic biomarker of lung adenocarcinoma ($P = .007$). **Conclusions:** Aldehyde dehydrogenase 3B1 was an independent prognostic biomarker of lung adenocarcinoma, indicating the unfavorable prognosis. Postoperative detection of aldehyde dehydrogenase 3B1 would help stratify the high-risk patients with lung adenocarcinoma and guide individual treatment.

## Keywords
ALDH3B1, lung adenocarcinoma, overall survival rate, prognosis, biomarker

## Abbreviations
ALDH, aldehyde dehydrogenase; ALDH3B1, aldehyde dehydrogenase 3B1; ATS, American Thoracic Society; ERS, European Respiratory Society; HR, hazard ratio; IASLC, International Association for the Study of Lung Cancer; IHC, immunohistochemistry; LPO, lipid peroxidation; mRNA, messenger RNA; PSM, propensity score matching; qRT-PCR, quantitative real-time polymerase chain reaction; TNM, tumor–node–metastasis.

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## Introduction

Lung cancer is the leading cause of deaths caused by malignancy, accounting for more than 25% of all cancer-related deaths.[1] Histologically, lung cancer is categorized into the non-small-cell lung cancer and the small-cell lung cancer, which accounted for 85% and 15%, respectively, of all kinds of lung cancers. Non-small-cell lung cancers are further divided into 3 major histological subtypes: the adenocarcinoma, squamous cell carcinoma, and large cell lung cancer.[2] Among them, lung adenocarcinoma has the highest mobility of lung cancer and accounts for approximately 40% of all lung cancers.[3] Although the treatment options are much more plentiful than before and the prognosis of lung cancer is developing thanks to the improvement of comprehensive treatment especially the targeted therapy, lung cancer remains the leading cause of cancer-related death worldwide.[4] Since the developments of new therapeutic strategies are based on the discovery of new biomarkers, the need of new biomarkers of lung adenocarcinoma is still unmet.

Aldehyde dehydrogenases (ALDHs), consisting of 19 genes with distinct chromosomal locations, catalyze the aldehydes to nontoxic acids.[5] The aldehydes have a wide spectrum of generation, in both endogenous and exogenous ways.[5] There are more than 200 aldehyde species found to be involved in physical and pathological conditions, including lipid peroxidation (LPO), 4-hydroxy-2-nonenal (4-HNE), and malondialdehyde, which lead to many diseases.[5,6] The function of ALDHs is essential to maintain the homeostasis of cell and the whole organism by transferring the aldehydes to nontoxic acids. The dysfunctions of ALDHs are reported to correlate with the turbulence of aldehyde metabolism and consequent diseases, including Sjögren-Larsson syndrome, hyperprolinemia, hydroxybutyric aciduria, and pyridoxine-dependent seizures.[5] Among the 19 ALDHs, several members are widely recognized for its roles in tumorigenesis, progression, or drug resistance, such as ALDH1A1, ALDH2, and ALDH3A1.[7] However, the expressions and functions of many ALDH members in most cancer types have not been explored.

In the ALDH family, aldehyde dehydrogenase 3B1 (ALDH3B1) is a less studied member, and the distribution, expression pattern, and physiological function of ALDH3B1 have not been well investigated. Aldehyde dehydrogenase 3B1 is generally considered metabolically active with distinct specificity for various aldehyde substrates, especially the lipid-derived medium- and long-chain aliphatic aldehydes generated in the plasma.[8] Aldehyde dehydrogenase 3B1 is capable to protect cells from the damaging effects of oxidative stress.[9] A previous study investigated the expression of ALDH3B1 in normal human tissues and several types of tumors including lung, colon, breast, and ovary cancers and demonstrated that ALDH3B1 expression was overexpressed in a high percentage of human lung cancer.[10] In our study, we further verified the conclusion by detecting ALDH3B1 expression in 250 cases of lung adenocarcinoma and 20 pairs of adenocarcinoma/normal lung tissues. In addition, the clinical significance of ALDH3B1 was evaluated by analyzing its correlation with the clinicopathological factors and the overall survival rate. With multivariate analyses, we identified the independent prognostic factors of lung adenocarcinoma.

## Materials and Methods

### Patients and Follow-Ups

A total of 524 patients were diagnosed as lung adenocarcinoma in YIDU Central Hospital and 970 Hospital of Chinese PLA and underwent the radical surgery from 2007 to 2017, which was considered the primary cohort. From these patients, 250 patients were selected out if there were enough specimens for immunohistochemistry (IHC) and follow-up, constituting the validation cohort. Two hundred and seven patients were further selected from the validation cohort by propensity score matching (PSM). The flowchart of our study is given in Supplemental Figure 1. The paraffin-embedded specimens were obtained from the department of pathology in YIDU Central Hospital and Shandong Cancer Hospital and Institute, and the fresh lung adenocarcinoma tissues were obtained during operation without interference with routine pathology. All tissues were obtained with the prior consent of patients. The study was approved by the ethics board of YIDU Central Hospital and 970 Hospital of Chinese PLA. The tumor–node–metastasis (TNM) stage was referred to the Eighth American Joint Committee on Cancer/Union for International Cancer Control system. The International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society (IASLC/ATS/ERS) classification was referred to previous studies.[11,12]

### Messenger RNA Extraction and qRT-PCR

Total messenger RNA (mRNA) was extracted from frozen lung adenocarcinomas and paired normal lung tissues using the TRIzol reagent (Thermo Fisher), according to manual. Messenger RNA was reversely transcribed to complementary DNA with the ReverTra Ace qPCR RT kit (TOYOBO). SYBR Green Master (Roche) and Light Cycler Roche 480 PCR instrument were used to achieve the RT-PCR. $2^{-\Delta\Delta Ct}$ method was used to standardize the data with glyceraldehyde-3phosphate dehydrogenase (GAPDH) level as base. The primers of ALDH3B1 and GAPDH were as follows:

> ALDH3B1 forward: TTCTTAACAGCAGGGCCACC; reverse: GGGTCCATCCTGAGGCTCT.
> GAPDH forward: GAGTCAACGGATTTGGTCGT; reverse: GACAAGCTTCCCGTTCTCAG.

### Immunohistochemistry

Immunohistochemistry was carried out to visualize ALDH3B1 expression in the streptavidin-biotin immunoperoxidase method with reference to the previous study.[13] Specimens were
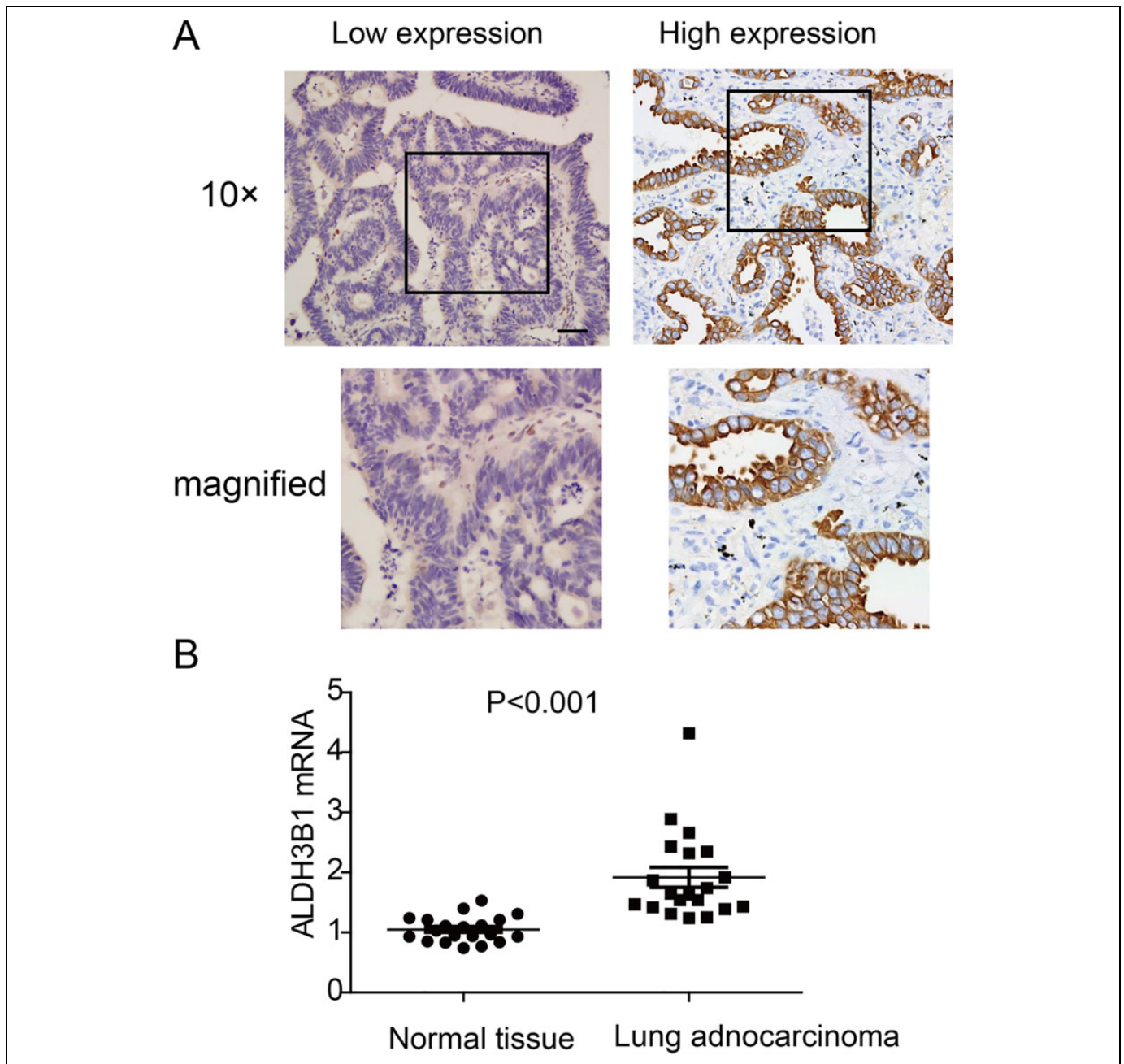
**Figure 1.** Expression of ALDH3B1 was detected with qRT-PCR and IHC. A, Representative images of ALDH3B1 low and high expression of lung adenocarcinoma. Scale bar: 50 μm. B, Quantitative real-time polymerase chain reaction in 20 pairs of lung adenocarcinoma and normal lungs revealed that ALDH3B1 in lung adenocarcinoma was higher than that in normal lung tissues. ALDH3B1 indicates aldehyde dehydrogenase 3B1; IHC, immunohistochemistry.

de-paraffinized with xylene and ethanol first and incubated in 3% $H_2O_2$ for 30 minutes to inactivate endogenous peroxidase and then in ethylenediaminetetraacetic acid (pH = 9.0) to get the optimal antigen retrieval. Phosphate-buffer saline supplemented with 1% bovine serum albumin was used to soak the specimens to block unspecific antigen binding. Primary antibody of ALDH3B1 (1:100, PA5-19328, Thermo Fisher Scientific) was used to incubate slides. After rinsed with phosphate-buffer saline, the corresponding secondary antibody

(Beyotime) and streptavidin-peroxidase complex (Beyotime) were used, and DAB solution (Beyotime) was finally applied for antigen visualization.

## Semiquantification of IHC

The results of IHC were semiquantified by IHC score by a senior pathologist. The IHC score consists of 2 constituent parts: the staining intensity and the percentage of positive cells.

**Table 1.** Information of Patients With Lung Adenocarcinoma.

| Factors | Number | Percentage |
|---|---|---|
| Sex | | |
|   Female | 112 | 44.80 |
|   Male | 138 | 55.20 |
| Age | | |
|   <60 | 96 | 38.40 |
|   ≥60 | 144 | 57.60 |
| Tumor diameter, cm | | |
|   ≤5 | 180 | 72.00 |
|   >5 | 70 | 28.00 |
| Histological grade | | |
|   I | 22 | 8.80 |
|   II | 145 | 58.00 |
|   III | 82 | 32.80 |
| T stage | | |
|   I | 55 | 22.00 |
|   II | 131 | 52.40 |
|   III | 47 | 18.80 |
|   IV | 17 | 6.80 |
| N stage | | |
|   N0 | 122 | 48.80 |
|   N1-N3 | 128 | 51.20 |
| Metastasis | | |
|   No | 246 | 98.40 |
|   Yes | 4 | 1.60 |
| TNM stage | | |
|   I | 89 | 35.60 |
|   II | 72 | 28.80 |
|   III | 85 | 34.00 |
|   IV | 4 | 1.60 |
| IASLC/ATS/ERS | | |
|   Acinar | 104 | 41.60 |
|   Papillary | 50 | 20.00 |
|   Solid | 30 | 12.00 |
|   Others[a] | 51 | 20.40 |
| EGFR mutation | | |
|   Negative | 154 | 61.60 |
|   Positive | 96 | 38.40 |
| KRAS mutation | | |
|   Negative | 210 | 84.00 |
|   Positive | 40 | 16.00 |

Abbreviations: ATS, American Thoracic Society; EGFR, epidermal growth factor receptor; ERS, European Respiratory Society; IASLC, International Association for the Study of Lung Cancer; KRAS, Kirsten rat sarcoma viral oncogene homolog; TNM, tumor–node–metastasis.
[a]Other IASLC/ATS/ERS histological subtype included lepidic, micropapillary, invasive mucinous and mixed mucinous/nonmucinous, and colloid histological type.

**Table 2.** Correlation Between ALDH3 and Other Factors.

| Factors | ALDH Low | ALDH High | $P^a$ |
|---|---|---|---|
| Sex | | | |
|   Female | 74 | 38 | .005 |
|   Male | 66 | 72 | |
| Age | | | |
|   <60 | 64 | 32 | .009 |
|   ≥60 | 66 | 78 | |
| Tumor diameter, cm | | | |
|   ≤5 | 108 | 72 | .047 |
|   >5 | 32 | 38 | |
| Histological grade | | | |
|   I | 16 | 6 | .039 |
|   II | 85 | 60 | |
|   III | 38 | 44 | |
| T stage | | | |
|   I | 30 | 25 | .948 |
|   II | 73 | 58 | |
|   III | 28 | 19 | |
|   IV | 9 | 8 | |
| N stage | | | |
|   N0 | 72 | 50 | .374 |
|   N1-N3 | 68 | 60 | |
| Metastasis | | | |
|   No | 136 | 110 | .133 |
|   Yes | 4 | 0 | |
| TNM stage | | | |
|   I | 50 | 39 | .352 |
|   II | 40 | 32 | |
|   III | 46 | 39 | |
|   IV | 4 | 0 | |
| IASLC/ATS/ERS | | | |
|   Acinar | 55 | 49 | .748 |
|   Papillary | 24 | 26 | |
|   Solid | 13 | 17 | |
|   Others[b] | 25 | 26 | |
| EGFR mutation | | | |
|   Negative | 75 | 79 | |
|   Positive | 52 | 44 | |
| KRAS mutation | | | |
|   Negative | 102 | 108 | |
|   Positive | 25 | 15 | |

Abbreviations: ALDH, aldehyde dehydrogenase; ATS, American Thoracic Society; EGFR, epidermal growth factor receptor; ERS, European Respiratory Society; IASLC, International Association for the Study of Lung Cancer; KRAS, Kirsten rat sarcoma viral oncogene homolog; TNM, tumor–node–metastasis.
[a]Calculated by the $\chi^2$ test.
[b]Other IASLC/ATS/ERS histological subtype included lepidic, micropapillary, invasive mucinous and mixed mucinous/nonmucinous, and colloid histological type.

The former one was defined as score 0 for negative staining, 1 for weak staining, 2 for moderate staining, and 3 for strong staining, while the latter one was categorized as 0 for 10% positive cells, score 1 for 10% to 30% positive cells, score 2 for 30% to 50% positive cells, and score 3 for 50% or more positive cells. The final IHC score was the product of these 2 parts multiplication and divided into subgroups with the cutoff, which was determined by the receiver operating characteristic curve.[14] The cutoff number was 3.5 in our study, meaning that score higher than 4 was set as the high-expression group.

## Statistical Analysis

All data without special illustration were reviewed and analyzed using the software SPSS version 25.0 by 2 senior pathologists. Statistical difference between lung adenocarcinoma and normal lung tissues was analyzed using Student $t$ test. The $\chi^2$ test was applied to evaluate the correlation between ALDH3B1
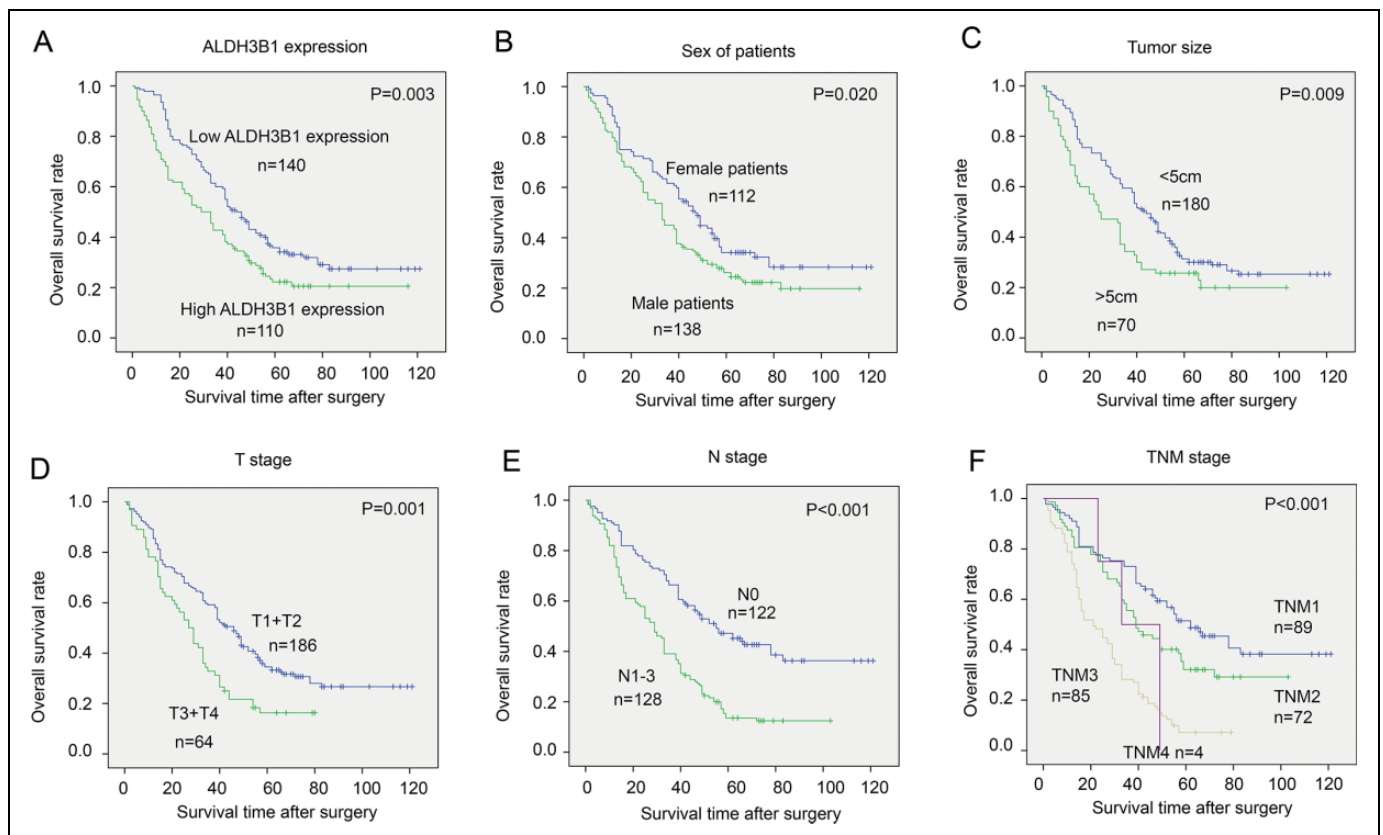
**Figure 2.** Survival curves of different clinicopathological factors. The overall survival curves were displayed according to (A) expression of ALDH3B1, (B) patients sex, (C) tumor size, (D) T stage, (E) N stage, and (F) TNM stage. The statistical significance was analyzed using the log-rank test. ALDH3B1 indicates aldehyde dehydrogenase 3B1; TNM, tumor–node–metastasis.

and clinicopathological factors. The Kaplan-Meier method was carried out to display the overall survival curve, and the log-rank test was performed to analyze the statistical difference between the groups. The multivariate analysis with the Cox proportional hazards regression model was performed to identify the independent prognostic factors. Propensity score matching of ALDH3B1 was applied to attenuate the bias and balance the baseline of cohort using SPSS version 25.0. Factors that had significant correlations with ALDH3B1 were enrolled into the model, and the match tolerance was set as 0.02. $\chi^2$ test was further performed to verify the result after PSM. A *P* value less than .05 was considered statistically significant.

## Results

### Expression of ALDH3B1 in Lung Adenocarcinoma

In our study, we collected 20 pairs of lung adenocarcinomas and the paired normal lung tissues and investigated the mRNA levels of ALDH3B1 in these tissues. These 20 tissue pairs were constantly collected during operation from 13 male and 7 female patients, with an average age of 47.8 years. Consequently, we demonstrated that ALDH3B1 in lung adenocarcinomas had a significantly higher level compared with the normal lung tissues (Figure 1A). The ALDH proteins can be expressed in all intracellular regions, including cytosol, nucleus, mitochondria, and endoplasmic reticulum, while one type of ALDH can be observed in more than 1 organelle, so that we performed IHC to evaluate the expression and location of ALDH3 in lung adenocarcinoma. In our study, ALDH3B1 was dominantly expressed in cell cytoplasm (Figure 1B). Patients were classified into low and high ALDH3B1 expression according to the IHC score, which accounted for 56.0% and 44.0%, respectively (Table 1).

### Aldehyde Dehydrogenase 3B1 Correlated With Patients' Sex, Age, Tumor Size, and Histological Grade

The cohort was categorized into low and high expression of ALDH3B1, and their correlation with clinical factors were evaluated using the $\chi^2$ test. In our study, ALDH3B1 expression was significantly associated with the sex and age of patients, tumor size, and histological grade (Table 2). These results suggested that ALDH3B1 expression may be influenced by the sex hormones or other relevant factors such as sex and age of patients and that ALDH3B1 may participate in tumor proliferation or differentiation process. Male patients ($P = .005$) and patients older than 60 years ($P = .009$) had high expression of ALDH3B1. In addition, high expression of ALDH3B1 was also

correlated with larger tumor size ($P = .047$) and high histological grade ($P = .039$), suggesting that ALDH3B1 may relate to tumor proliferation or differentiation. The IASLC/ATS/ERS histological type and mutations of epidermal growth factor receptor (EGFR) and Kirsten rat sarcoma viral oncogene homolog (KRAS) were detected to evaluate their relationship with ALDH3B1. In the cohort, patients with mutations of EGFR and KRAS accounted for 38.40% and 16.00%, respectively (Table 1), but no significant correlation between them and ALDH3B1 was observed (Table 2).

## Prognostic Value of ALDH3B1 and Other Clinical Factors

The prognostic significance of clinical factors and ALDH3B1 expression was evaluated with Kaplan-Meier method and statistical difference was analyzed with the log-rank test. In the univariate analysis, high ALDH3B1 expression was significantly associated with poor prognosis ($P = .003$; Figure 2A). The average survival time of low and high ALDH3B1 was 58.7 and 43.3 months, respectively. Moreover, the male patients tended to have a lower survival rate with the survival time as 45.6 months, which was less than the female patients as 58.8 months ($P = .020$; Figure 2B). In addition, large tumor size ($P = .009$), advanced T stage ($P = .001$), positive lymphatic invasion ($P < .001$), and advanced TNM stage ($P < .001$) were all correlated with unfavorable prognosis of patients with lung adenocarcinoma (Figure 2C-F and Table 3). Furthermore, the prognostic factors in univariate analysis were further selected for multivariate analysis to determine the independent prognostic factors (Table 3). In the Cox regression model, ALDH3B1 was confirmed as a prognostic biomarker of lung adenocarcinoma ($P = .027$). The hazard ratio (HR) of patients with ALDH3B1 high expression was 1.41-fold higher than those with low expression of ALDH3B1 (95% CI: 1.04-1.91). Moreover, both advanced T stage (HR = 1.50, 95% CI: 1.05-2.13, $P = .024$) and N stage (HR = 2.04, 95% CI: 1.49-2.79, $P < .001$) could indicate the unfavorable prognosis independently.

## Survival Significances After the PSM

In Table 2, ALDH3B1 expression was associated with other factors such as patients' sex, age, tumor size, and histological grade, indicating that the baseline characteristics between low and high levels of ALDH3B1 were not balanced and that ALDH3B1-relevant prognosis may be resulted from other factors such as sex. Although we confirmed the independent prognostic significance with multivariate analysis, we furthermore applied the PSM to attenuate the bias caused by other factors, such as sex or differentiation. Two hundred seven patients were further selected from the PSM and the baseline characteristics were significantly improved (Supplemental Table 1). After PSM, we further evaluated the prognostic value of ALDH3B1 by univariate and multivariate analyses. In the PSM cohort, the prognostic significance of ALDH3B1 was still available ($P = .004$; Figure 3A). Overall survival rates of low and high ALDH3B1 were 60.1% and

**Table 3.** Results of Univariate and Multivariate Analysis.

| Factors | Univariate analysis | | Multivariate analysis | | |
| --- | --- | --- | --- | --- | --- |
| | Average survival time | $P^a$ | HR | 95% CI | $P^b$ |
| Sex | | | | | |
| Female | 58.8 | .020 | 1 | | |
| Male | 45.6 | | 1.35 | 0.98-1.86 | .063 |
| Age | | | | | |
| <60 | 53.9 | .487 | | | |
| ≥60 | 49.8 | | | | |
| Tumor size, cm | | | | | |
| ≤5 | 55.9 | .009 | 1 | | |
| >5 | 38.7 | | 1.18 | 0.83-1.67 | .352 |
| Histological grade | | | | | |
| I+II | 56.6 | .580 | | | |
| III | 46.1 | | | | |
| T stage | | | | | |
| I+II | 56.7 | .001 | 1 | | |
| III+IV | 32.6 | | 1.5 | 1.05-2.13 | .024 |
| N stage | | | | | |
| N0 | 66.9 | <.001 | 1 | | |
| N1-N3 | 36.1 | | 2.04 | 1.49-2.79 | <.001 |
| Metastasis | | | | | |
| No | 52.4 | .511 | | | |
| Yes | 38.5 | | | | |
| TNM stage | | | | | |
| I | 70.1 | <.001 | | | |
| II | 52.3 | | | | |
| III | 27.3 | | | | |
| IV | 38.5 | | | | |
| ALDH3B1 | | | | | |
| Low | 58.7 | .003 | 1 | | |
| High | 43.3 | | 1.41 | 1.04-1.91 | .027 |

Abbreviations: ALDH3B1, aldehyde dehydrogenase 3B1; HR, hazard ratio; TNM, tumor–node–metastasis.
[a]Calculated by the log-rank test.
[b]Means calculated by the Cox regression model.

43.3%, respectively (Table 4). Besides ALDH3B1, tumor size ($P = .007$), T stage ($P < .001$), N stage ($P < .001$), and TNM stage ($P < .001$) were also determined as prognostic biomarkers (Figure 3B-E). In the multivariate analysis, the significance of ALDH3B1 as an independent prognostic factor was also confirmed (Table 4). Hazard ratio of high ALDH3B1 was 1.56. In addition, N stage was also identified as an independent prognostic factor (HR = 1.80, 95% CI: 1.27-2.54, $P = .001$).

## Discussion

As the most prevalent histological subtype of lung cancer, the molecular features of lung adenocarcinoma attracted more interests, resulting in many significant findings. Many mutations and ectopic activations were discovered in lung adenocarcinoma, such as *KRAS*, *EGFR*, *receptor tyrosine-protein kinase erbB-2*, and *MET*.[15,16] These findings lead to the application of many targeted drugs in lung adenocarcinoma, but a
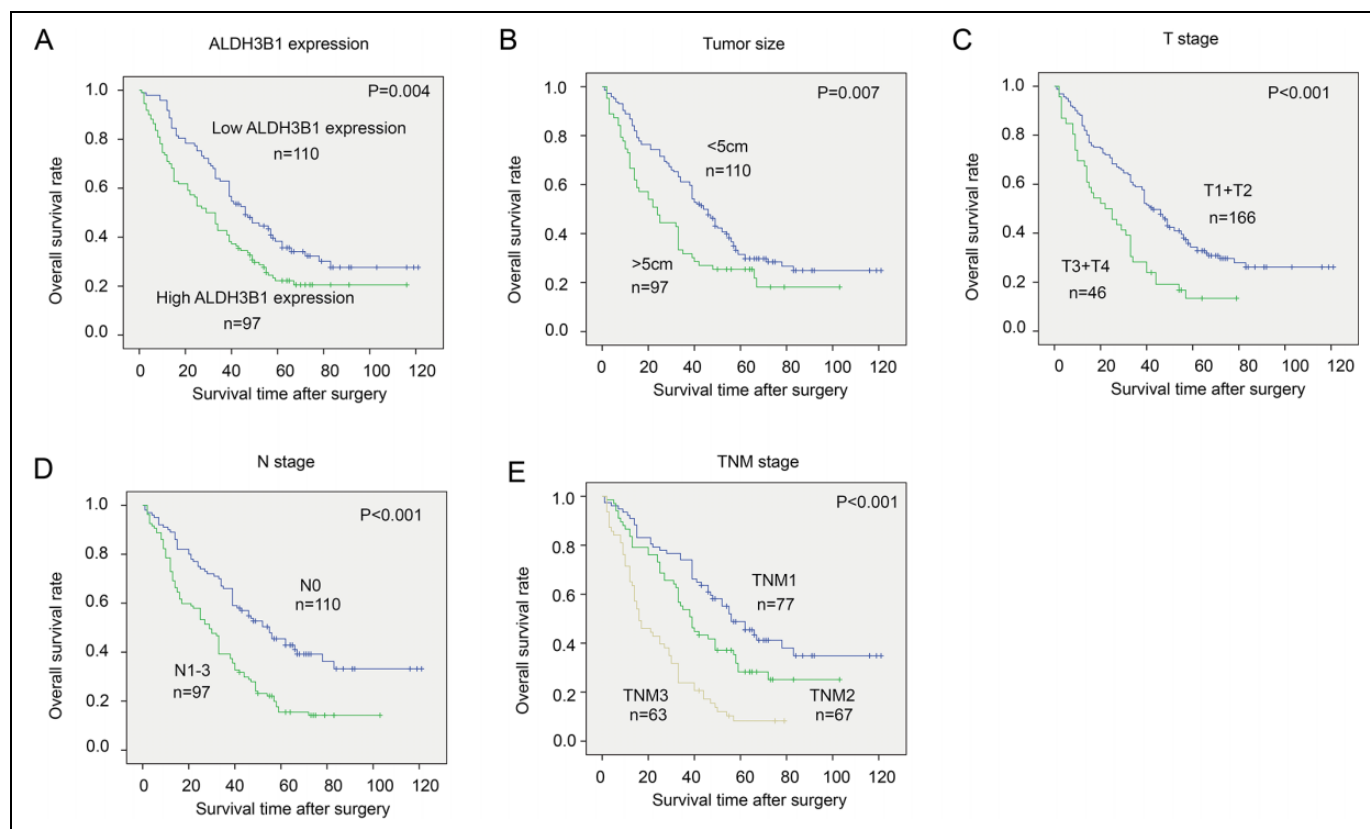
**Figure 3.** Survival curves of different clinicopathological factors after PSM. After PSM, a total of 207 patients were further selected. The overall survival curves of ALDH3B1 (A), tumor size (B), T stage (C), N stage (D), and TNM stage (E) were displayed. ALDH3B1 indicates aldehyde dehydrogenase 3B1; PSM, propensity score matching; TNM, tumor–node–metastasis.

certain proportion of patients benefit little from the targeted therapy because of the heterogeneity of lung cancer and the resistance to sustained medication. The discovery of new and effective biomarkers helps depict the overall molecular landscape of lung cancer and improve the new therapies. Here we demonstrated that ALDH3B1 was an independent prognostic biomarker of lung adenocarcinoma with 2 cohorts, which were before and after PSM. Analyses with or without PSM both verified the prognostic significance of ALDH3B1, indicating an important role of ALDH3B1 in predicting prognosis of lung adenocarcinoma. This is an interesting and significant study expanding the understanding of lung cancer biomarker. The sample size (250 cases) of patients who underwent radical surgery because of lung adenocarcinoma was large enough to get the conclusions, and the statistical meaning of ALDH3B1 as prognostic biomarker was very significant. All our results suggested that the detection of ALDH3B1 would help stratify the high-risk patients with lung adenocarcinoma more preciously and may help develop a potential targeted drug.

Production of aldehyde is everywhere in the body and the elimination of aldehyde is essential in numerous physiological processes. Accumulation of aldehydes plays a toxic function mainly by inactivating enzymes, and aldehydes participate in oxidative damage, the generation of reactive oxygen species,

and LPO.[17] As the main handler of aldehydes, the function of ALDHs is pivotal in both physiological and pathological functions and processes. For example, ALDH2 is the key enzyme that catalyzes acetaldehyde oxidation during ethanol metabolism and is involved in the process of many diseases such as atherosclerosis and myocardial infarction.[18,19] Emerging evidence implicated the important role of ALDH in cancer. People with ALDH2 mutations are more vulnerable to a variety of cancers, such as esophageal, stomach, colon, lung, head, and neck cancers.[20,21] Aldehyde dehydrogenases were considered as markers of cancer stem cells in several cancer types, such as colorectal cancer.[22,23] Moreover, ALDH1A was normally regarded to be expressed in chemotherapy- and radiotherapy-resistant cells and contribute to the drug resistance,[24] and ALDH inhibitors have been considered as potential anticancer drugs for a long time based on the emerging proofs.[25]

However, the function is little known about ALDH3B1 compared with the famous members of ALDH family, such as ALDH1A1 and ALDH2. A previous study reported that single-nucleotide polymorphism of ALDH3B1 was associated with paranoid schizophrenia.[26] The role of ALDH3B1 in cancer progression has never been reported. Our study was the first to prove that ALDH3B1 was associated with tumor size, differentiation, and prognosis of lung adenocarcinoma, which was

**Table 4.** Results of Univariate and Multivariate Analysis After PSM.

| Factors | Univariate analysis | | Multivariate analysis | | |
|---|---|---|---|---|---|
| | Average survival time | $P^{a}$ | HR | 95% CI | $P^{b}$ |
| Sex | | | | | |
| Female | 56.8 | .124 | | | |
| Male | 46.7 | | | | |
| Age | | | | | |
| <60 | 52.5 | .784 | | | |
| ≥60 | 50.2 | | | | |
| Tumor size, cm | | | | | |
| ≤5 | 55.9 | .007 | 1 | | |
| >5 | 37.1 | | 1.32 | 0.92-1.89 | .136 |
| Histological grade | | | | | |
| I+II | 51.7 | .606 | | | |
| III | 45.7 | | | | |
| T stage | | | | | |
| I+II | 56.3 | <.001 | 1 | | |
| III+IV | 29.2 | | 1.48 | 0.99-2.20 | .056 |
| N stage | | | | | |
| N0 | 64.6 | <.001 | 1 | | |
| N1-N3 | 36.9 | | 1.80 | 1.27-2.54 | .001 |
| TNM stage | | | | | |
| I | 68.1 | <.001 | | | |
| II | 49.4 | | | | |
| III | 25.6 | | | | |
| ALDH3B1 | | | | | |
| Low | 60.1 | .004 | 1 | | |
| High | 43.3 | | 1.56 | 1.13-2.17 | .007 |

Abbreviations: ALDH3B1, aldehyde dehydrogenase 3B1; TNM, tumor–node–metastasis.
[a]Calculated by the log-rank test.
[b]Means calculated by the Cox-regression model.

an important supplement to the function and clinical significance of ALDH3B1. However, the underlying molecular mechanisms of how ALDH3B1 expression affects the prognosis of lung adenocarcinoma were not elucidated in this study. As a member of ALDH family, ALDH3B1 has distinct substrate specificity on medium- and long-chain (6 carbons and longer) aldehydes, such as 4-HNE.[27] Based on our findings, we speculated that the upregulation of ALDH3B1 perhaps enhances the vanish of aldehyde and consequently increases the viability of tumor cells, but of course, our hypothesis certainly needs more experimental study to verify.

In summary, we investigated the expression of ALDH3B1 in 250 cases of lung adenocarcinoma and in 20 pairs of adenocarcinoma and paired normal lungs. Consequently, we demonstrated that ALDH3B1 expression was significantly associated with the patients' sex and age, tumor size, and the histological grade. High expression of ALDH3B1 could predict poor prognosis and ALDH3B1 was an independent prognostic biomarker of lung adenocarcinoma. Our results indicated that postoperative detection of ALDH3B1 may help stratify the high-risk patients with lung adenocarcinoma more preciously and may help develop a potential targeted drug.

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