Translational Oncology 19 (2022) 101379

Contents lists available at ScienceDirect

Translational Oncology

journal homepage: www.elsevier.com/locate/tranon

Clinical significance of ALDH1A1 expression and its association with E-cadherin and N-cadherin in resected large cell neuroendocrine carcinoma

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ARTICLE INFO

Keywords
Aldehyde dehydrogenase 1A1 (ALDH1A1)
Cancer stem cell (CSC)
E- cadherin
N-cadherin
pulmonary large cell neuroendocrine carcinoma (LCNEC)

ABSTRACT

Background: The roles of cancer stem cells (CSCs) and epithelial-mesenchymal transition (EMT) in solid tumors are well established. However, the interaction between CSCs and EMT in pulmonary large cell neuroendocrine carcinoma (LCNEC) remains unknown. The aim of this study was to investigate the expression and clinical significance of a CSC marker (ALDH1A1) and its correlation with Epithelial-like phenotype marker (E-cadherin) and Mesenchymal-like phenotype marker (N-cadherin) in LCNEC patients.
Methods: Immunohistochemistry (IHC) for ALDH1A1, E-cadherin and N-cadherin expression was conducted on tissue microarrays made from 79 resected LCNEC patient samples. ALDH1A1 protein expression was evaluated by the IHC score, and its correlations with the expression of E-cadherin, N-cadherin and clinicopathological features were determined based on IHC data. Survival analyses were also performed.
Results: ALDH1A1 was positively expressed in 75.9% (60/79 cases) of LCNEC patients. No significant difference in clinicopathological variables was observed between the ALDH1A1-negative and ALDH1A1-positive groups. However, ALDH1A1 expression was positively correlated with E-cadherin (Spearman's rho = 0.229, p-value = 0.007), which represents the epithelial-like phenotype, but not with N-cadherin. Patients with expression of ALDH1A1 had significantly longer disease-free survival (DFS) and overall survival (OS) than those who were ALDH1A1 negative (median DFS: 52 vs 12 months, $p = 0.028$; median OS: not reached; $p = 0.027$). Multivariate analysis showed that ALDH1A1 was an independent favorable prognostic factor for DFS ($p = 0.032$, HR: 0.438, 95% CI: 0.206–0.932) and OS ($p = 0.025$, HR: 0.279, 95% CI: 0.091–0.852) in LCNEC patients.
Conclusion: This study suggests that ALDH1A1 can act as a favorable independent prognostic factor for LCNEC, which related to the epithelioid phenotype in EMT, and its internal mechanism needs further study.

Introduction

Pulmonary large cell neuroendocrine carcinoma (LCNEC) is one of the histological subtypes of lung cancer that is highly aggressive.[1] The incidence of this disease in lung cancer patients ranges from 2.1% to 3.5%.[2] Although the treatment strategy for pulmonary LCNEC includes surgery, radiotherapy and chemotherapy, the outcome remains poor, with 5-year survival rates ranging from 15% to 57%.[3,4] Unlike remarkable advances in other non-small cell lung cancer (NSCLC), no remarkable progress has been achieved in the treatment of LCNEC in recent decades. For this reason, the molecular mechanism of LCNEC urgently needs to be explored to guide individual therapy or find new targeted therapies.

Currently, two categories of NSCLC-like LCNEC and SCLC-like LCNEC have been promoted for pulmonary LCNEC, indicating the heterogeneity of this disease. SCLC-like LCNEC exhibits co-mutations of TP53 and STK11/KEAP1, and NSCLC-like LCNEC exhibits co-mutations of TP53 and RB1.[5] CSCs are a small subpopulation of tumor cells with

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https://doi.org/10.1016/j.tranon.2022.101379
Received 25 November 2021; Received in revised form 21 January 2022; Accepted 16 February 2022

stem or progenitor cell-like characteristics. These cells are responsible for tumor initiation, development, self-renewal, chemoresistance, tumor recurrence, and especially tumor heterogeneity.[6] Thus, we deduced that the specificity of cancer stem cells (CSCs) in lung LCNEC may be helpful for explaining this phenomenon. ALDH1A1 as a CSC marker has been demonstrated in previous studies and ALDH-expressing cells display stem-like features in various types of cancers, including breast, liver and colorectal cancers, and are associated with prognosis.[7] Therefore, we choose ALDH1A1 to explore the mechanism of CSCs in pulmonary LCNEC.

Studies have confirmed that the acquisition of CSC features of tumor cells requires the activation of EMT through the notch pathway [8] and EMT status is related to the invasion and metastasis ability of tumors. E-cadherin encoded by CDH1 is a marker of epithelial phenotype and its expression decreased during the epithelial-mesenchymal transition. N-cadherin encoded by CDH2 is a marker of Mesenchymal phenotype and its expression increased during the epithelial-mesenchymal transition. Therefore, their expression level can represent EMT state of the tumor to some extent.[9]

Hence, this study aims to investigate the Clinical significance of ALDH1A1 expression and its association with E-cadherin and N-cadherin.

Materials and methods

Patients and data collection

Seventy-nine resected LCNEC were retrieved from the archives of the department of pathology, Cancer Hospital, Chinese Academy of Medical Science between December 2011 and March 2017. The inclusion criteria were as follows: (1) surgically resected specimen with adequate pathological tissue; (2) pathologically confirmed LCNEC; (3) complete clinical and follow-up data. Patients in whom clinical information was lacking or with inadequate amounts of paraffin tissue and slides were excluded. The archival records were retrospectively reviewed and collected, including clinicopathological characteristics, treatment information and survival related data. Clinical TNM stage was based on the previously seventh edition of the American Joint Committee on Cancer (AJCC) staging system. This study was approved by the Ethics Committee of National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College (approval no. 20/234–2430). The requirement for individual consent for this retrospective analysis was waived. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Pathological examination and histologic reassessments

The archived slides of all patients were reviewed by one senior pulmonary pathologist (Lin Yang) and two junior pathologists (Xin Wang and Li Liu) according to the 2021 WHO classification criteria for lung tumors. Diagnosis was based on morphological or immunohistochemical features, such as organ-like structures, NSCLC cytological features, a high mitotic rate, and at least one positive neuroendocrine marker (CgA, CD56, Syn). Histopathological characteristics, including bronchial invasion, pleural invasion, lymph-vascular invasion, and lymph node metastasis, were also evaluated.

Tissue microarray construction and immunohistochemistry (IHC)

Representative paraffin tissues were selected by reviewing slides to construct a tissue microarray with a diameter of 1.5 mm (two cores/paraffin tissue). Tissue microarrays were sectioned consecutively for IHC staining (thickness of 3–5 mm). Rabbit anti-human monoclonal antibodies, including those specific to ALDH1A1 (1:200, CST, 54135S), E-cadherin (1:400, CST, 3195T) and N-cadherin (1:125, CST, 13116T), were obtained from Cell Signaling Technology (CST). IHC staining was performed on fully automated Roche immunohistochemical instruments (Roche Diagnostics, Shanghai, China). Positive controls were taken from colon adenocarcinoma, breast invasive ductal carcinoma and ovarian cancer sections. Blank Ig was used instead of the primary antibody as a negative control.

Evaluation of the immunohistochemical staining results

ALDH1A1 was expressed in the cytoplasm of tumor cells, while E-cadherin and N-cadherin were expressed in the membrane of tumor cells. The IHC score was evaluated by H-score by multiplying the percentage of positive cells and the intensity according to a four-point intensity scale (ranged 0–3).[10,11] We then translated the continuous H-score into the 4 gradations: 0 (H-score ranged 0–9), 1+ (H-score ranged 10–49), 2+ (H-score ranged 50–149) and 3+ (H-score ranged 150–300). The expression of ALDH1A1 was defined as negative when IHC score was 0, and positive when IHC score was 1+, 2+ and 3+. Low expression of E-cadherin and N-cadherin was defined as IHC score of 0 or 1+, while high expression of E-cadherin and N-cadherin positivity was defined as IHC score of 2+ or 3+ (Fig. 1).

Statistical analysis

Clinicopathological characteristics were analyzed by descriptive statistics. Continuous variables was presented as the mean ± standard deviation (SD), while categorical data was described as proportions. Comparisons of categorical variables were performed with the Chi-Square test, Fisher's exact test or the Student's *t*-test. Spearman analysis was performed to describe the relationship between markers. Disease-free survival (DFS) and overall survival (OS) were analyzed by Kaplan-Meier curves and log-rank tests with the 95% confidence interval (CI). Multivariate analysis was performed with the Cox proportional hazards regression model and was used to assess independent prognostic factors. All tests were two-sided, and a P value of less than 5% was considered statistically significant. Statistical analyses were performed using IBM SPSS statistic 25.0.

Results

Patient clinicopathological characteristics

Seventy-nine patients with resected limited-stage pulmonary LCNEC were retrospectively analyzed. The clinicopathological characteristics were summarized in Table 1. The average patient age was 62.6 years (range 43 to 79 years) with a male/female ratio 7.8 (70 cases/9 cases). Histologically, 40 cases (50.6%) were pure LCNECs without any combined components; 39 cases (49.4%) were combined with other components, including 18 with adenocarcinoma, 14 with small cell lung cancer, 2 with squamous cell carcinoma, 3 with squamous cell carcinoma and adenocarcinoma, and 2 with small cell lung cancer and adenocarcinoma. According to the seventh edition of the AJCC staging system, 55 cases (69.6%) were in stages I-II, and 24 cases (30.4%) were in stage III. The median follow-up time was 43 months (range 0–95 months) with an observational period from December 2011 to December 2019. The median DFS was 41 months, while the median OS was not reached. The 5-year DFS and OS rates were 42.9% and 74.6%,

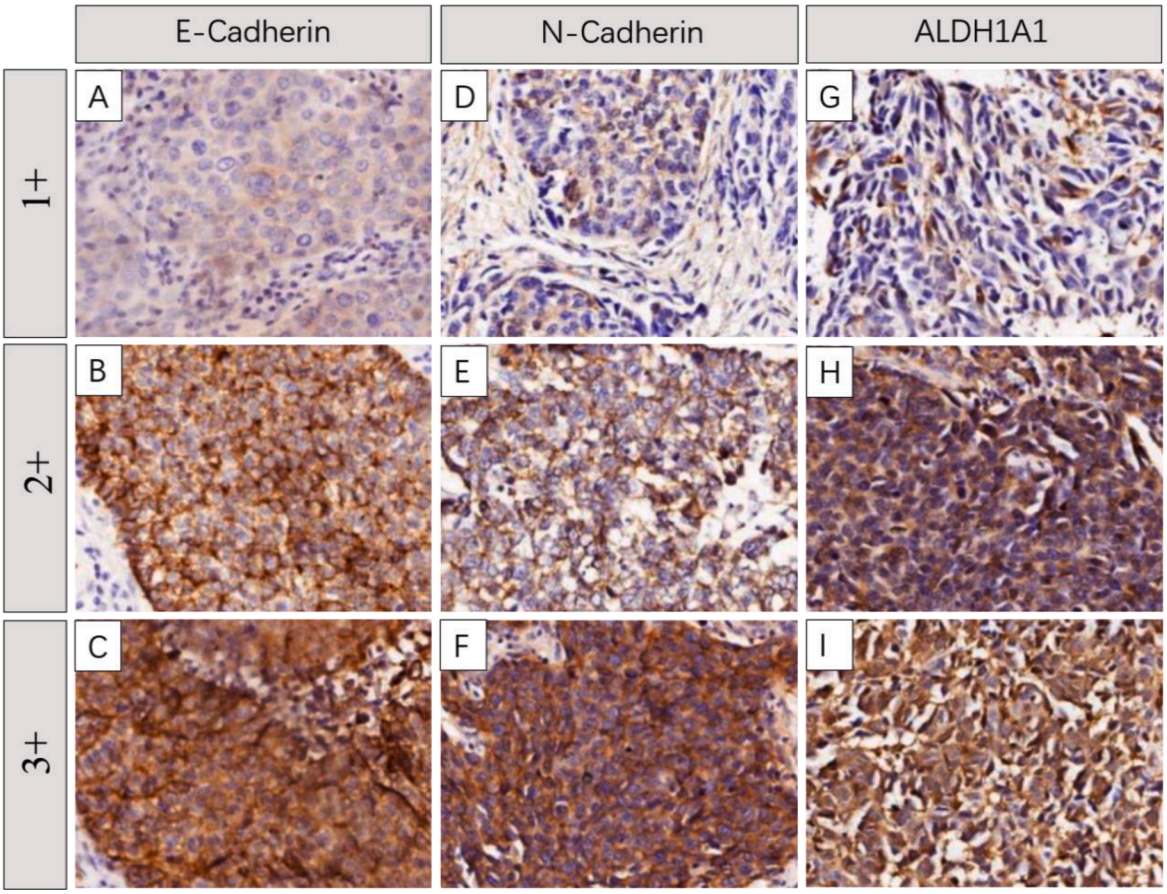

Fig. 1. (A I) Representative sections of LCNEC immunostained with E-cadherin, N-cadherin and ALDH1A1 antibody respectively in SCLC (× 100) with three gradations: 1+ (A,D,E), 2+ (B,E,H), 3+ (C,F,I).

respectively. Disease-free survival (DFS) was defined as the time from the start of surgery to the observation of tumor recurrence or distant metastasis confirmed by imaging or biopsy. If there was no recurrence during follow-up, the end point of DFS was last follow-up or death. Overall survival (OS) was defined as the time from the date of surgery to death or last follow-up (in the absence of death). The primary endpoint of this study was OS and the secondary endpoint was DFS.

Correlation analysis of ALDH1A1 with E-cadherin and N-cadherin

Among the 79 cases, ALDH1A1 positive expression was found in 60 (75.9%) patients. Using Chi-squared test to analyze its relationship with clinicopathological features (Table 1). There was no significant difference in these parameters, including age, gender, smoking and pathological features.

Up to 93.7% of the cases were detected high expression of E-cadherin while 20.3% of patients had high expression of N-cadherin. However, no correlation was observed between E-cadherin and N-cadherin (Spearman's rho = 0.122, p-value = 0.286). We investigated its relationships with clinicopathological characteristics, as shown in Table 2. As for histologic subtypes, the proportion of pure LCNEC in N-cadherin high expression group was significantly higher than that in N-cadherin low expression group (75% vs 44.4%, $p = 0.048$). However, the histologic subtypes did not differ between E-cadherin low and E-cadherin high expression groups. And the percentage of patients with age above 55 (82.4% vs 17.6%, $p = 0.054$) and pN0–2 stage (98.7% vs 1.3%, $p = 0.067$) in E-cadherin high expression group was higher than those in E-cadherin low expression group.

The results of the correlation analyses between ALDH1A1 and E-cadherin/N-cadherin using Spearman's rank correlation coefficients are shown in Table 3. The results showed that ALDH1A1 expression was positively correlated with E-cadherin expression (Spearman's rho = 0.229, p-value = 0.007) but not N-cadherin expression (Spearman's rho = −0.072, p-value = 0.530) .

Prognostic significance of the expression of ALDH1A1, E-cadherin and N-cadherin

Kaplan-Meier curve analysis showed a longer DFS and OS for patients with positive ALDH1A1 expression than that of negative expression (median DFS: 52 vs 12 months, $p = 0.028$; median OS: not reached; $p = 0.027$; Fig. 2). The relationship between E-cadherin and prognosis was also analyzed considering the correlation between ALDH1A1 and E-cadherin. However, E-cadherin was not associated with DFS or OS.

Univariate analysis revealed that DFS was associated with pathological stage ($p = 0.015$, HR: 2.170, 95% CI: 1.161–4.055), lymph nodes

Table 1
Clinicopathological feature in LCNEC patients stratified by ALDH1A1 expression.

Clinicopathological variables	Overall (N = 79)	ALDH1A1 negative (n = 19)	positive (n = 60)	P-value*
Age (mean (SD))	62.63 (7.51)	62.74 (5.49)	62.60 (8.09)	0.065
Age (%)				
≤55 years	16 (20.3)	2 (10.5)	14 (23.3)	0.332
>55 years	63 (79.7)	17 (89.5)	46 (76.7)	
Sex (%)				
Male	70 (88.6)	18 (94.7)	52 (86.7)	0.679
Female	9 (11.4)	1 (5.3)	8 (13.3)	
Pathological stage (%)				
Early stage (I-II)	55 (69.6)	12 (63.2)	43 (71.7)	0.570
Advanced stage (III)	24 (30.4)	7 (36.8)	17 (28.3)	
pT stage (%)				
T1	21 (26.6)	6 (31.6)	15 (25)	0.704
T2	48 (60.8)	10 (52.6)	38 (63.3)	
T3	10 (12.6)	3 (15.8)	7 (11.7)	
pN stage (%)				
N0	47 (59.5)	11 (57.9)	36 (60)	0.060
N1	10 (12.7)	1 (5.3)	9 (15)	
N2	20 (25.3)	5 (26.3)	15 (25)	
N3	2 (2.5)	2 (10.5)	0 (0)	
N metastasis (%)				
N0	47 (59.5)	11 (57.9)	36 (60)	0.871
N1-N3	32 (40.5)	8 (42.1)	24 (40)	
Pleural invasion (%)				
Yes	44 (55.7)	7 (36.8)	37 (61.7)	0.069
No	35 (44.3)	12 (63.2)	23 (38.3)	
Lymph-vascular invasion (%)				
Yes	19 (24.1)	5 (26.3)	14 (23.3)	0.767
No	60 (75.9)	14 (73.7)	46 (76.7)	
Histologic subtype (%)				
Pure LCNEC	40 (50.6)	10 (52.6)	30 (50)	1.000
Combined LCNEC	39 (49.4)	9 (47.4)	30 (50)	
Treatment				
Surgery	30 (37.9)	9 (47.4)	21 (35)	0.543
Surgery + chemotherapy	36 (45.6)	7 (36.8)	29 (48.3)	
Surgery + radio-chemotherapy	10 (12.7)	3 (15.8)	7 (11.7)	
Others/unknow#	3 (3.8)	0 (0)	3 (5)	
Recurrence site				
No	33 (41.8)	6 (31.6)	27 (45)	0.427
Yes	41 (51.9)			
Intra-thoracic	21 (26.6)	6 (31.6)	15 (25)	
Extra-thoracic	15 (19)	5 (26.3)	10 (16.7)	
Intra-thoracic + extra-thoracic	5 (6.3)	2 (10.5)	3 (5)	
Unknow	5 (6.3)	0 (0)	5 (8.3)	

LCNEC: large cell neuroendocrine carcinoma; ALDH1A1: aldehyde dehydrogenase 1A1; SD: standard deviation.

others/unknow include chemotherapy +surgery with/without chemotherapy, surgery + radiotherapy and surgery + unknow.

* $P<0.05$ is indicated by bold italics.

metastases ($p = 0.006$, HR: 2.391, 95% CI: 1.228–4.441) and ALDH1A1 expression ($p = 0.034$, HR: 0.489, 95% CI: 0.252–0.947); and OS was associated with ALDH1A1 expression ($p = 0.035$, HR: 0.375, 95% CI: 0.151–0.932) (Fig. 3). In the multivariate analysis, ALDH1A1 was an independent favorable prognostic factor not only for DFS ($p = 0.030$, HR: 0.470, 95% CI: 0.246–0.933) but also for OS ($p = 0.030$, HR: 0.360, 95% CI: 0.143–0.905) (Fig. 4).

Discussion

In this study, we explored the correlation between ALDH1A1 and EMT markers (E-cadherin and N- cadherin) expression and their relationships with LCNEC prognosis. It was observed that ALDH1A1 expression was positively associated with E-cadherin expression, and served as a favorable independent factor for DFS and OS in pulmonary LCNEC.

Previous studies have demonstrated that subpopulations with high ALDH1A1 enzyme activity in multiple types of cancers are enriched in stem-like cells. Hence, ALDH1A1 as a marker of CSCs has been widely used to isolate and identify CSCs. In our research, ALDH1A1 was commonly expressed in pulmonary LCNECs (75.9%), which is consistent with the findings of Morise et al.[12] According to Morise's study, ALDH1A1 was found to be positive in 73% of LCNEC patients and 67% of SCLC patients. Meanwhile, Gao et al. reported a positive rate of 41.28% for ALDH1A1 staining in NSCLC patients.[13] Furthermore, we explored the association of ALDH1A1 expression with prognosis. We found patients with high expression of ALDH1A1 had significantly longer disease-free survival (DFS) and overall survival (OS) than those who were ALDH1A1 negative. So ALDH1A1 is a favorable prognostic factor of LCNEC. However, Morise's study[12] got the completely opposite conclusion that positive ALDH1A1 expression was associated with shorter DFS and OS. The main reason for the discrepancies perhaps lies in that Morise's study combined SCLC and LCNEC as HGNEC due to the similarities in histological characteristics, which is different from ours. Because of the differences in the expression of neuroendocrine differentiation markers (ASH1, HES1)[14] and cancer stem-like cell (CSLC) markers (SOX2/CD116)[12] in LCNEC and SCLC, we inferred that the biological behaviors of the two types may be different. Therefore, it is more credible to analyze the effect of ALDH1A1 on the prognosis of LCNEC alone. Besides, antibodies we use are different. The sources of our ALDH1 antibodies are CST, while their antibodies from BD Transduction Laboratories. Moreover, their IHC scores were classified as positive (score ≥ 10) or negative (score < 10), so scoring system difference may be another reason for the opposite conclusion.

In addition, regarding the role of ALDH1A1 in tumor development or prognosis, many researches have been reported in recent years, but the conclusions are somehow inconsistent in different tumors. In our study, ALDH1A1 expression was found to be a favorable independent prognostic factor, which is consistent with that of ovarian cancer,[15] but different from other cancers, such as non-small-cell lung carcinoma (NSCLC),[16] breast,[17] pancreatic,[18] and gastric cancer primary tumors.[19] Besides, because the expression of ALDH1 in tumor cells is related to the stemness characteristics and poor clinical prognosis but that in mesenchymal cells is related to good prognosis, ALDH1A1 has been demonstrated to have dual role in prognosis in some cancers such as breast cancer[20,21] and prostate cancer.[22,23] The different roles in various kinds of cancers indicate that ALDH1A1 probably exhibit complicated biological effects in cancer development. Further in vivo *and vitro* experiment will be required to validate above hypothesis in future.

A number of studies have confirmed the correlation between CSC and EMT, and the molecular mechanisms was clarified in several carcinomas. In prostate cancer, it was found that the expression of ALDH1 in tumor stromal cells was related to the epithelial phenotype of primary prostate cancer, which improved the clinical outcome and reduced the incidence of prostate cancer metastasis.[24] In lung adenocarcinoma cell lines, correlation with E-cadherin and ALDH1 confirmed that CSC is associated with the epithelial phenotype in EMT.[25] ALDH1A1 was known to be active in the late steps of retinoic acid synthesis which is required for growth of epithelial cells.[26] And its function to mediate differentiation of epithelial cell was demonstrated in normal human mammary through retinoic acid metabolism.[27] Furthermore, retinoic acid converted by ALDH1A1 inhibits the EMT process through the

Table 2
Correlation between EMT markers and clinicopathological features.

Clinicopathological feature	E-cadherin low ($n = 5$)	high ($n = 74$)	P-value*	N-cadherin low ($n = 63$)	high ($n = 16$)	P-value*
Age (mean (SD))	57.80 (6.30)	62.96 (7.51)	0.683	62.75 (7.69)	62.19 (6.96)	0.558
Age						
≤55 years	3 (60)	13 (17.6)	0.054	13 (20.6)	3 (18.75)	1.000
>55 years	2 (40)	61 (82.4)		50 (79.4)	13 (81.25)	
Sex						
Male	4 (80)	66 (89.2)	0.463	56 (88.9)	14 (87.5)	1.000
Female	1 (20)	8 (10.8)		7 (11.1)	2 (12.5)	
Pathological stage						
Early stage (I-II)	3 (60)	52 (70.3)	0.637	44 (69.8)	11 (68.75)	1.000
Advanced stage (III)	2 (40)	22 (29.7)		19 (30.2)	5 (31.25)	
pT stage						
T1	0 (0)	21 (28.4)	0.102	18 (28.6)	3 (18.75)	0.714
T2	3 (60)	45 (60.8)		37 (58.7)	11 (68.75)	
T3	2 (40)	8 (10.8)		8 (12.7)	2 (12.5)	
pN stage						
N0	3 (60)	44 (59.5)	0.067	38 (60.3)	9 (56.25)	0.769
N1	0 (0)	10 (13.5)		8 (12.7)	2 (12.5)	
N2	1 (20)	19 (25.7)		16 (25.4)	4 (25)	
N3	1 (20)	1 (1.3)		1 (1.6)	1 (6.25)	
N metastasis						
No	3 (60)	44 (59.5)	1.000	38 (60.3)	9 (56.25)	0.782
Yes	2 (40)	30 (40.5)		25 (39.7)	7 (43.75)	
Pleural invasion						
Yes	2 (40)	42 (56.8)	0.650	35 (55.6)	9 (56.25)	1.000
No	3 (60)	32 (43.2)		28 (44.4)	7 (43.75)	
Lymph-vascular invasion						
Yes	1 (20)	18 (24.3)	1.000	13 (20.6)	6 (37.5)	0.194
No	4 (80)	56 (75.7)		50 (79.4)	10 (62.5)	
Histologic subtype						
Pure LCNEC	3 (60)	37 (50)	1.000	28 (44.4)	12 (75)	***0.048***
Combined LCNEC	2 (40)	37 (50)		35 (55.6)	4 (25)	
Treatment						
Surgery	1 (20)	29 (39.2)	0.221	25 (39.7)	5 (31.25)	0.886
Surgery + chemotherapy	2 (40)	34 (45.9)		28 (44.4)	8 (50)	
Surgery + radio-chemotherapy	1 (20)	9 (12.2)		8 (12.7)	2 (12.5)	
Others/unknow[#]	1 (20)	2 (2.7)		2 (3.2)	1 (6.25)	
Recurrence site						
No	1 (20)	32 (43.2)	0.217	26 (41.3)	7 (43.75)	0.960
Yes						
Intra-thoracic	3 (60)	18 (24.3)		16 (25.4)	5 (31.25)	
Extra-thoracic	0 (0)	15 (20.3)		13 (20.6)	2 (12.5)	
Intra-thoracic + extra-thoracic	0 (0)	5 (6.8)		4 (6.3)	1 (6.25)	
Unknow	1 (20)	4 (5.4)		4 (6.3)	1 (6.25)	

LCNEC: large cell neuroendocrine carcinoma; EMT: epithelial-mesenchymal transition.
[#] others/unknow include chemotherapy +surgery with/without chemotherapy, surgery + radiotherapy and surgery + unknow.
[*] $P<0.05$ is indicated by bold italics.

downregulation of IL-6 in cancer fibroblast cells,[28] making cells expressing ALDH1A1 more inclined to the epithelial-like phenotype. During the EMT process, tumor cells were observed to lose their epithelial phenotype and acquire a mesenchymal phenotype to obtain migratory ability and invasive properties.[29] Our study showed that ALDH1A1 was positively correlated with E-cadherin in pulmonary LCNEC. So it is reasonable to suppose that ALDH1A1 perhaps could inhibit EMT process by activation of retinoic acid. However, whether the impact of ALDH1A1 on the prognosis is related to EMT and its underlying mechanism need to be further studied.

In our study, ALDH1A1 expression was found to be predictive for prognosis. We speculated that it may have a certain relationship with the treatment effects or drug therapy selection. For example, T. Ishikawa used 66 triple negative breast cancer (TNBC) patients to compare the efficacy of FEC-D and TC6 as neoadjuvant chemotherapy, and found that the CSC subtype identified by ALDH1 which act no activity to these anticancer drugs may have different characteristics from other subtypes. [30] Besides, in our study, we found ALDH1A1 is related to epithelial phenotype markers in LCNEC. we guess that cells with high ALDH1A1 expression have an epithelial-like phenotype, which is less malignant and more differentiated than the mesenchymal-like phenotype,[31] and researches have proved that EMT promotes resistance to conventional chemotherapy such as paclitaxel, vincristine, and oxaliplatin.[32] Furthermore, in line with our study, a previous study showed that a high level of ALDH1A1 expression was positively associated with good prognosis of lung adenocarcinoma.[33] Thus, the ALDH1A1 protein may potentially distinguish different subtypes of lung LCNEC, such as epithelial-like and mesenchymal-like phenotypes, or hyper- and hypo-differentiation. Further investigation in a large-scale sample is needed.

But our research still has limitations. First, this is a retrospective study that only explores the relationship between a few markers and patient prognosis, but doesn't discuss the patient's follow-up chemotherapy regimen. Second, the exploration of the prognostic mechanism

Table 3
Correlations between ALDH1A1 and EMT markers according to immunohistochemical data on LCNEC.

Variable		ALDH1A1				rho[a]	P-value
		0	1+	2+	3+		
N-cadherin	0	12	15	22	0	−0.072	0.530
	1+	2	5	5	2		
	2+	3	7	3	0		
	3+	2	0	1	0		
E-cadherin	0	2	1	0	0	0.299	0.007*
	1+	1	1	0	0		
	2+	8	8	8	0		
	3+	8	17	23	2		

[a] Spearman rank test;.
* statistically significant; ALDH1A1: aldehyde dehydrogenase 1A1; EMT: epithelial-mesenchymal transition.

of ALDH1A1 on LCNEC is limited to EMT, with we lack of data support for other related mechanisms. Given we have considered these limitations, so next we will further explore the impact of other pathways related to ALDH1A1 on the prognosis and take other factors such as follow-up chemotherapy regimen et al. into account in order to further improve our research.

Funding

This work was supported by the Cancer Foundation of China, Beijing Hope Marathon Foundation [grant number LC2017A20] and the National Key Research and Development Program of China [grant numbers 2017YFC1308704, 2017YFC1311000 and 2017YFC1308700] and CAMS Innovation Fund for Medical Sciences[grant number 2021-I2M-C&T-B-062].

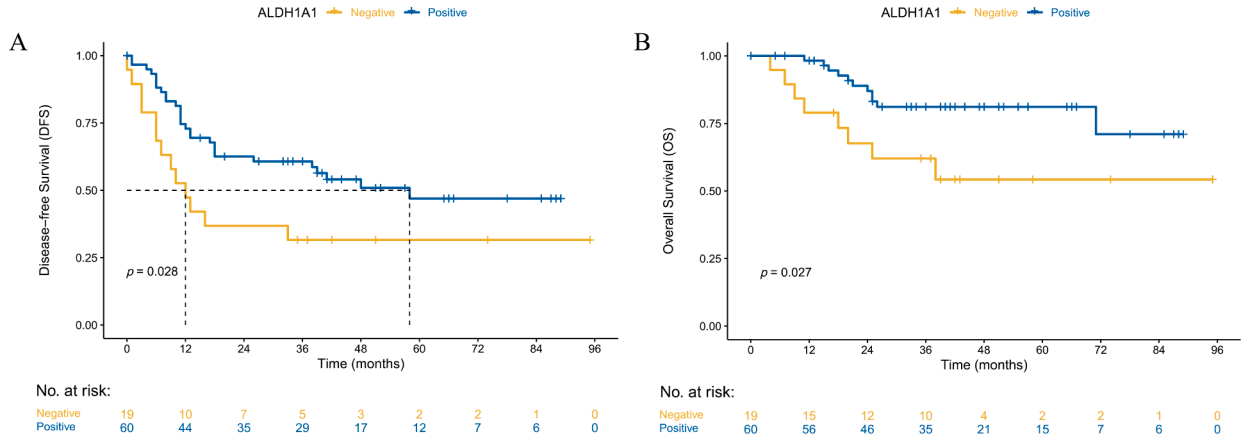

Fig. 2. Disease-free survival (DFS) (A) and overall survival (OS) (B) curves of 79 patients according to ALDH1A1 expression. ALDH1A1: Aldehyde dehydrogenase 1A1.

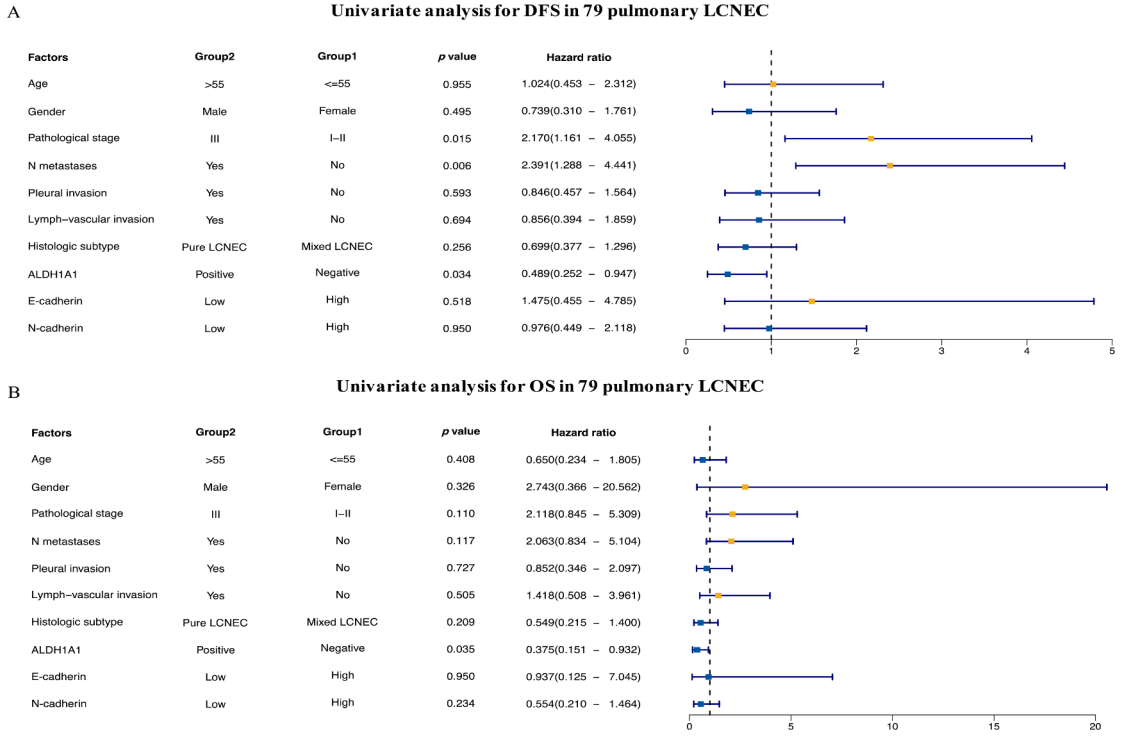

Fig. 3. Univariate analysis of disease-free survival (DFS) (A) and overall survival (OS) (B) in 79 pulmonary LCNEC by COX regression model. Univariate analysis showed that DFS was associated with pathological stage, lymph nodes metastases and ALDH1A1 expression (A), and OS was associated with ALDH1A1 expression (B).

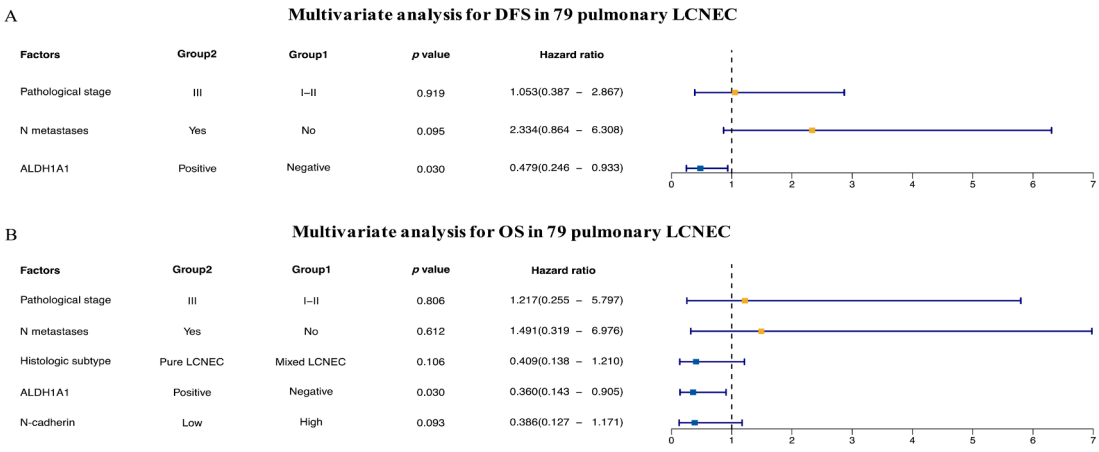

Fig. 4. Multivariate analysis of disease-free survival (DFS) (A) and overall survival (OS) (B) in 79 pulmonary LCNEC by COX regression model. Multivariate analysis showed that ALDH1A1 was an independent favorable prognostic factor not only for DFS ($p = 0.030$, HR: 0.470, 95% CI: 0.246–0.933) (A) but also for OS ($p = 0.030$, HR: 0.360, 95% CI: 0.143–0.905) (B).

Conflicts of interest

The authors state that they have no conflicts of interest.

CRediT authorship contribution statement

Jinyao Zhang: Investigation, Writing – original draft, Writing – review & editing. **Xujie Sun:** Investigation, Writing – original draft, Writing – review & editing. **Li Liu:** Methodology, Software, Project administration. **Jiyan Dong:** Methodology, Software, Project administration. **Lei Deng:** Methodology, Software, Project administration. **Xin Wang:** Methodology, Software, Project administration. **Yiying Guo:** Methodology, Software, Project administration. **Jianming Ying:** Conceptualization, Data curation, Funding acquisition, Supervision. **Puyuan Xing:** Conceptualization, Data curation, Funding acquisition, Supervision. **Junling Li:** Conceptualization, Data curation, Funding acquisition, Supervision. **Lin Yang:** Conceptualization, Data curation, Funding acquisition, Supervision.

Acknowledgments

The authors would like to thank all investigators and staff who participated in this study.

References

[1] F.G. Fernandez, R.J. Battafarano, Large-cell neuroendocrine carcinoma of the lung, Cancer Control 13 (2006) 270–275.
[2] F.G. Fernandez, R.J. Battafarano, Large-cell neuroendocrine carcinoma of the lung: an aggressive neuroendocrine lung cancer, Semin. Thorac. Cardiovasc. Surg. 18 (2006) 206–210.
[3] H. Takei, et al., Large cell neuroendocrine carcinoma of the lung: a clinicopathologic study of eighty-seven cases, J. Thorac. Cardiovasc. Surg. 124 (2002) 285–292.
[4] G. Veronesi, et al., Large cell neuroendocrine carcinoma of the lung: a retrospective analysis of 144 surgical cases, Lung Cancer 53 (2006) 111–115, https://doi.org/10.1016/j.lungcan.2006.03.007.
[5] J. George, et al., Integrative genomic profiling of large-cell neuroendocrine carcinomas reveals distinct subtypes of high-grade neuroendocrine lung tumors, Nat. Commun. 9 (2018) 1048, https://doi.org/10.1038/s41467-018-03099-x.
[6] M. Simple, A. Suresh, D. Das, M.A. Kuriakose, Cancer stem cells and field cancerization of oral squamous cell carcinoma, Oral Oncol. 51 (2015) 643–651, https://doi.org/10.1016/j.oraloncology.2015.04.006.
[7] G. Vassalli, Aldehyde dehydrogenases: not just markers, but functional regulators of stem cells, Stem Cells Int. 2019 (2019), 3904645, https://doi.org/10.1155/2019/3904645.
[8] I. Espinoza, R. Pochampally, F. Xing, K. Watabe, L. Miele, Notch signaling: targeting cancer stem cells and epithelial-to-mesenchymal transition, Onco. Targets Ther. 6 (2013) 1249–1259, https://doi.org/10.2147/OTT.S36162.
[9] F. van Roy, G. Berx, The cell-cell adhesion molecule E-cadherin, Cell Mol. Life Sci. 65 (2008) 3756–3788, https://doi.org/10.1007/s00018-008-8281-1.
[10] K.S. McCarty, L.S. Miller, E.B. Cox, J. Konrath, K.S. McCarty, Estrogen receptor analyses. Correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies, Arch. Pathol. Lab. Med. 109 (1985) 716–721.
[11] E. Specht, et al., Comparison of immunoreactive score, HER2/neu score and H score for the immunohistochemical evaluation of somatostatin receptors in bronchopulmonary neuroendocrine neoplasms, Histopathology 67 (2015) 368–377, https://doi.org/10.1111/his.12662.
[12] M. Morise, et al., Clinicopathological significance of cancer stem-like cell markers in high-grade neuroendocrine carcinoma of the lung, J. Cancer Res. Clin. Oncol. 141 (2015) 2121–2130, https://doi.org/10.1007/s00432-015-1985-3.
[13] F. Gao, et al., The role of LGR5 and ALDH1A1 in non-small cell lung cancer: cancer progression and prognosis, Biochem. Biophys. Res. Commun. 462 (2015) 91–98, https://doi.org/10.1016/j.bbrc.2015.04.029.
[14] R. Nasgashio, et al., The balance between the expressions of hASH1 and HES1 differs between large cell neuroendocrine carcinoma and small cell carcinoma of the lung, Lung Cancer 74 (2011) 405–410, https://doi.org/10.1016/j.lungcan.2011.04.012.
[15] B. Chang, et al., ALDH1 expression correlates with favorable prognosis in ovarian cancers, Mod. Pathol. 22 (2009) 817–823, https://doi.org/10.1038/modpathol.2009.35.
[16] X. Li, L. Wan, J. Geng, C.-.L. Wu, X. Bai, Aldehyde dehydrogenase 1A1 possesses stem-like properties and predicts lung cancer patient outcome, J. Thorac. Oncol. 7 (2012) 1235–1245, https://doi.org/10.1097/JTO.0b013e318257cc6d.
[17] E. Charafe-Jauffret, et al., Aldehyde dehydrogenase 1-positive cancer stem cells mediate metastasis and poor clinical outcome in inflammatory breast cancer, Clin. Cancer Res. 16 (2010) 45–55, https://doi.org/10.1158/1078-0432.CCR-09-1630.
[18] C. Kahlert, et al., Low expression of aldehyde dehydrogenase 1A1 (ALDH1A1) is a prognostic marker for poor survival in pancreatic cancer, BMC Cancer 11 (2011) 275, https://doi.org/10.1186/1471-2407-11-275.
[19] Y. Wakamatsu, et al., Expression of cancer stem cell markers ALDH1, CD44 and CD133 in primary tumor and lymph node metastasis of gastric cancer, Pathol. Int. 62 (2012) 112–119, https://doi.org/10.1111/j.1440-1827.2011.02760.x.
[20] E. Resetkova, et al., Prognostic impact of ALDH1 in breast cancer: a story of stem cells and tumor microenvironment, Breast Cancer Res. Treat. 123 (2010), https://doi.org/10.1007/s10549-009-0619-3.
[21] N. Bednarz-Knoll, et al., Stromal expression of ALDH1 in human breast carcinomas indicates reduced tumor progression, Oncotarget 6 (2015) 26789–26803, https://doi.org/10.18632/oncotarget.4628.
[22] C. Le Magnen, et al., Characterization and clinical relevance of ALDHbright populations in prostate cancer, Clin. Cancer Res. 19 (2013) 5361–5371, https://doi.org/10.1158/1078-0432.CCR-12-2857.
[23] P. Nastaly, et al., ALDH1-positive intratumoral stromal cells indicate differentiated epithelial-like phenotype and good prognosis in prostate cancer, Transl. Res. 203 (2019) 49–56, https://doi.org/10.1016/j.trsl.2018.08.007.
[24] R. Nasgashio, et al., The balance between the expressions of hASH1 and HES1 differs between large cell neuroendocrine carcinoma and small cell carcinoma of the lung, Lung Cancer 74 (2011) 405–410, https://doi.org/10.1016/j.lungcan.2011.04.012.
[25] V. Tiran, et al., Primary patient-derived lung adenocarcinoma cell culture challenges the association of cancer stem cells with epithelial-to-mesenchymal transition, Sci. Rep. 7 (2017) 10040, https://doi.org/10.1038/s41598-017-09929-0.
[26] G. Duester, Families of retinoid dehydrogenases regulating vitamin A function: production of visual pigment and retinoic acid, Eur. J. Biochem. 267 (2000) 4315–4324.
[27] G. Honeth, et al., Aldehyde dehydrogenase and estrogen receptor define a hierarchy of cellular differentiation in the normal human mammary epithelium, Breast Cancer Res. 16 (2014) R52, https://doi.org/10.1186/bcr3663.

[28] J. Guan, et al., Retinoic acid inhibits pancreatic cancer cell migration and EMT through the downregulation of IL-6 in cancer associated fibroblast cells, Cancer Lett. 345 (2014) 132–139, https://doi.org/10.1016/j.canlet.2013.12.006.
[29] C.O. Sung, C.-.K. Park, S.-.H. Kim, Classification of epithelial-mesenchymal transition phenotypes in esophageal squamous cell carcinoma is strongly associated with patient prognosis, Mod. Pathol. 24 (2011) 1060–1068, https://doi.org/10.1038/modpathol.2011.59.
[30] T. Ishikawa, et al., BRCAness is beneficial for indicating triple negative breast cancer patients resistant to taxane, Eur. J. Surg. Oncol. 42 (2016) 999–1001, https://doi.org/10.1016/j.ejso.2016.02.246.
[31] C. Faleiro-Rodrigues, I. Macedo-Pinto, D. Pereira, C.S. Lopes, Prognostic value of E-cadherin immunoexpression in patients with primary ovarian carcinomas, Ann. Oncol. 15 (2004) 1535–1542.
[32] T. Arumugam, et al., Epithelial to mesenchymal transition contributes to drug resistance in pancreatic cancer, Cancer Res. 69 (2009) 5820–5828, https://doi.org/10.1158/0008-5472.CAN-08-2819.
[33] R. Roudi, A. Korourian, A. Shariftabrizi, Z. Madjd, Differential expression of cancer stem cell markers ALDH1 and CD133 in various lung cancer subtypes, Cancer Invest. 33 (2015) 294–302, https://doi.org/10.3109/07357907.2015.1034869.