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The role of LGR5 and ALDH1A1 in non-small cell lung cancer: Cancer progression and prognosis

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ABSTRACT

The Leucine rich repeat containing G protein coupled receptor 5 (LGR5), may be a candidate marker of non-small cell lung cancer (NSCLC) cells with stem cell-like properties. Aldehyde dehydrogenase 1A1 (ALDH1A1) is one of NSCLC stem cell markers. To identify the relationship of LGR5 and ALDH1A1 in NSCLC, we analyzed the expression of LGR5 and ALDH1A1 in NSCLC samples, and determined their clinical significance. We performed quantitative RT-PCR for LGR5 and ALDH1A1 expression in 24 NSCLC patients, and showed that LGR5 and ALDH1A1 mRNA were frequently increased in NSCLC tissues in comparison to that in adjacent normal tissues ($p = 0.0005$ and $p < 0.0001$, respectively). Besides, the expression of LGR5 and ALDH1A1 mRNA has a significant correlation ($r = 0.416$, $P = 0.0483$). The expression of LGR5 and ALDH1A1 in 109 NSCLC tumors and 50 adjacent normal tissues were detected by immunohistochemistry. Positive LGR5 and ALDH1A1 expression was defined in 28.4% and 41.3% of the NSCLC tumors, respectively. Further analysis indicated that 24 of these $LGR5^+$ (24/31) samples expressed ALDH1A1($r = 0.3883$, $p < 0.0001$), we also found co-localization of LGR5 and ALDH1A1 in tumor tissue samples. LGR5 and ALDH1A1 expression was significantly associated with higher pathological TNM stage of the disease (stage I + II and III + IV) ($P = 0.0311$ and $p = 0.0221$, respectively), the co-expression of LGR5 and ALDH1A1 was associated with nodal status ($p = 0.0424$). High expression of LGR5 or ALDH1A1 was related to poor prognosis ($P = 0.0125$ and $p = 0.0410$, respectively), and NSCLC patients with co-expression of LGR5 and ALDH1A1 had a poorer prognosis than the others ($P = 0.0011$). Both of them can be an independent risk factor of a poorer prognosis ($P = 0.016$ and $P = 0.024$, respectively). The expression of LGR5 and ALDH1A1 were closely associated with the tumorigenicity, metastasis and poor prognosis of NSCLC, and $LGR5^+$ cells in NSCLC were likely to be the cancer cells with stem cell-like properties due to the significant correlation between LGR5 and ALDH1A1.

1. Introduction

Lung cancer is one of the leading causes of cancer-related deaths in China and worldwide, of which more than 80% are NSCLC, mainly because of the lack of effective systemic treatment and rapid development of resistance to chemotherapy [1]. Development of effective therapeutics is urgently needed [2]. A putative explanation of an ineffective therapy is the presence of CSC [3].

Recent evidence suggests that human NSCLC, like other tumors, also harbor CSC populations [4,5]. NSCLC CSCs, a small subset of cancer cells maintain the capability of initiating tumors and more resistant to chemotherapy, lead to tumor recurrence, progression and ultimately patient death [6,7]. Identification of NSCLC CSCs has been hampered by the lack of robust specific surface markers [8]. Thus, it is urgent to identify novel markers that represent an effective therapeutic target for the disease.

LGR5, also known as GPR49, is a member of G-protein-coupled receptor superfamily with 18 leucine-rich repeat units and 7 transmembrane regions, which was reported as a cancer stem cell surface marker of colorectal carcinogenesis and the target gene of Wnt signaling pathway [9]. It was suggested to have a great

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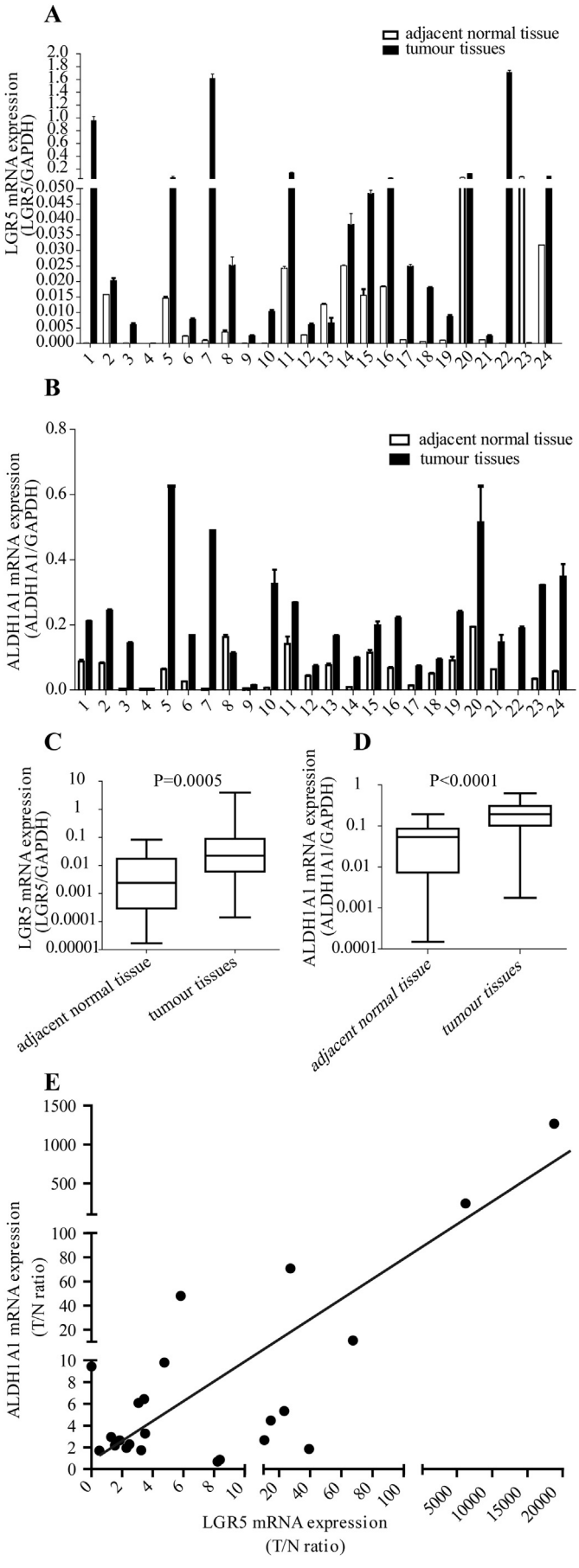

correlation with tumor growth, invasion and poor prognosis [10–12]. Recent research shows that LGR5 is expressed in a subset of lung adenocarcinoma [13]. ALDH1A1, which is a detoxifying enzyme that oxidizes intracellular aldehydes and thereby confers resistance to alkylating agents, is one of the classic NSCLC stem cell markers. ALDH1A1 has been used for selecting stem-like tumor cells in NSCLC cell lines [14,15]. Increased ALDH1A1 expression is associated with poor survival in a cohort of NSCLC patients [16–18]. The goal of the present study was to identify the relationship between LGR5 and ALDH1A1 in NSCLC, determine their clinical significance and lay the foundation for the further study of the role of LGR5 in NSCLC stem cells. To our knowledge, this is the first study concerning the relationship and prognostic value of these two proteins in NSCLC.

2. Materials and methods

2.1. Patients and tissue specimens

A total of 133 NSCLC cases which underwent complete resection from November 2009 and March 2012 at the First Affiliated Hospital, Soochow University (Suzhou, China) were included in this retrospective study. Those who received preoperative chemotherapy and/or radiotherapy were excluded.

Unfixed, fresh frozen NSCLC and corresponding non-malignant tissue (n = 24), formalin-fixed and paraffin-embedded NSCLC (n = 109) and adjacent normal tissues (n = 50) were retrieved and were pathologically confirmed. Formalin-fixed and paraffin-embedded samples were fixed with 10% neutral formalin, hematoxylin and eosin staining, pathologic analysis, and other medical records were used to confirm the diagnosis and clinical parameters, including age, gender, smoking habits, Tumor differentiation, Tumor subtypes, pathological TNM (p-TNM) and stage. These patients consist of 83 men and 26 women with age ranging from 42 to 82 y old.

2.2. Quantitative RT-PCR

Total RNA was isolated from NSCLC tissues using RNeasy Mini Kits (Qiagen, Tokyo, Japan), and RNAiso (Takara Bio, Shiga, Japan), with a slight modification of the DNase treatment. RNA quality was assessed in the BioDrop ulite PC (Bio-rad, USA). cDNA was synthesized using the PrimeScriptTM RT Master Mix Kit (Takara Japan). Quantitative RT-PCR analysis was performed on a c1000 Touch Thermal Cycler (Bio-rad, USA) System using SYBR Pre-mix Ex Taq (Perfect Real Time) (Takara). The eprimer sequences for GAPDH were 5′-ATCATCCCTGCCTCT ACTGG-3′ and 5′-TTTCTAGACGGCAGGTCAGGT-3′, those for LGR5 were 5′-GCAAACCTACGTCTGGACAA-3′ and 5′-TGATGCTGGAGCTGGTA AAG-3′, and those for ALDH1A1 were 5′-GCCAGGTAGAAGAAGGAGAT AAGGAGG-3′ and 5′-ALDH1A1-RT: TATAATAGTCGCCCCCTCTCGG AAG-3′. Fold-induction values were calculated using the $2^{-\triangle Ct}$ method and LGR5 and ALDH1A1 expression was normalized to

Fig. 1. LGR5 and ALDH1A1 mRNA expression in NSCLC and matched adjacent normal tissues. LGR5 and ALDH1A1 mRNA expression were estimated by qRT-PCR. (A, B) LGR5 and ALDH1A1 mRNA, in 24 cases of NSCLC and matched adjacent normal tissues, were evaluated. The majority of NSCLCs (79.2% and 58.3%, respectively) showed more than three times greater expression of LGR5 and ALDH1A1 compared with matched adjacent normal tissues. (C, D) Boxplots depicting overall distribution of LGR5 and ALDH1A1 in NSCLC versus adjacent normal tissue. The overall expression level of LGR5 and ALDH1A1 in NSCLCs were significantly higher than that in adjacent normal tissues (P = 0.0005 and P < 0.0001, respectively). (E) LGR5 and ALDH1A1 mRNA expression of NSCLC were normalized to that of adjacent normal tissues, the LGR5 and ALDH1A1 mRNA expression of NSCLC have a significant correlation (r = 0.416, P = 0.0483).

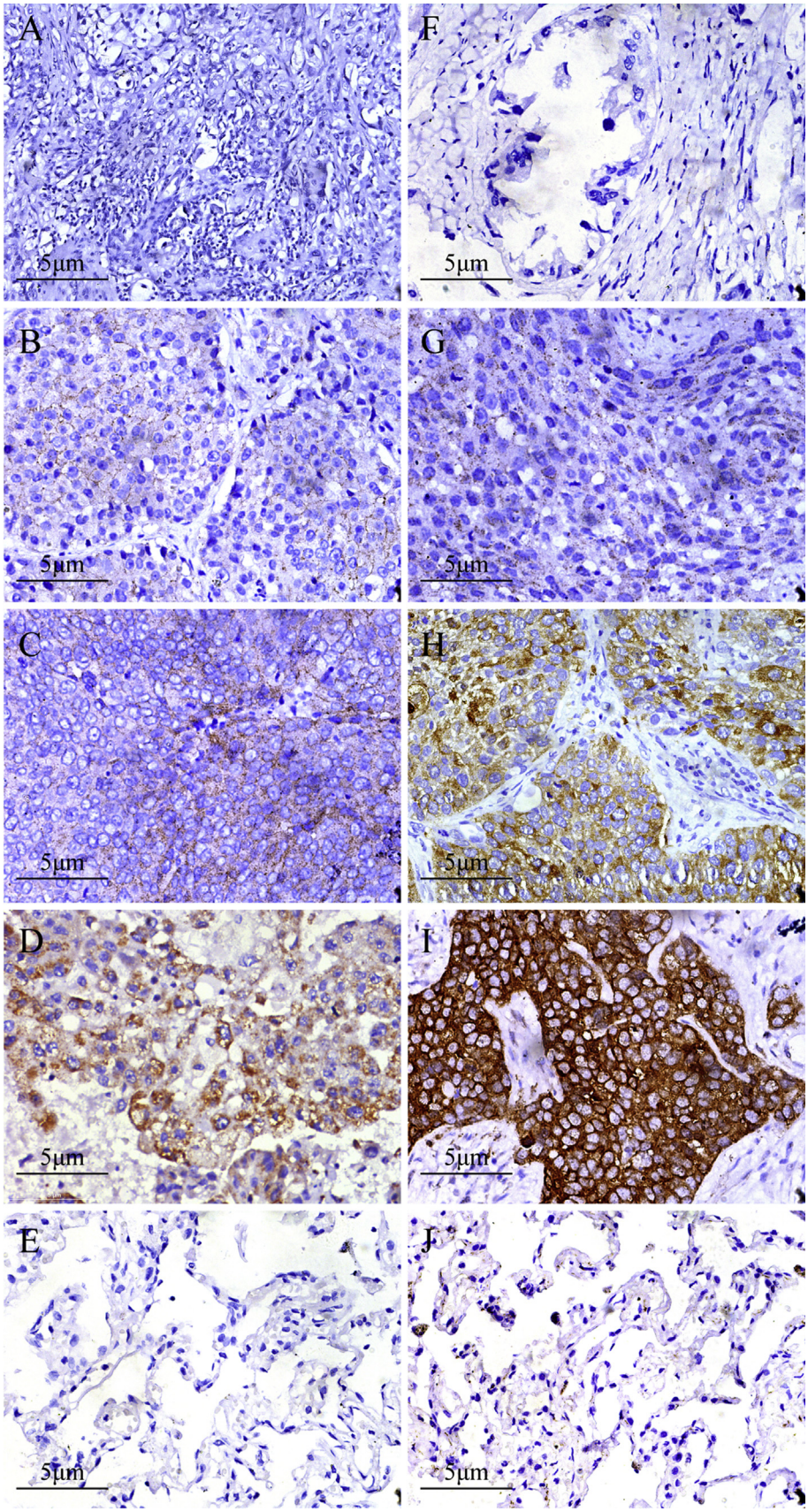

Fig. 2. Representative LGR5 and ALDH1A1 immunohistochemical staining in NSCLC and adjacent normal tissues in high-magnification (400×) images. (A–D) The LGR5 staining intensities were classified as negative (A score of 0), weak (B score of 1), moderate (C score of 2), or strong (D score of 3). (F–I) The ALDH1A1 staining intensities were classified as negative (F score of 0), weak (G score of 1), moderate (H score of 2), or strong (I score of 3). (E) Negative staining for LGR5 in adjacent normal tissues. (J) Negative staining for ALDH1A1 in adjacent normal tissues.

GAPDH. All experiments were performed in triplicate and repeated in at least three separate experiments.

2.3. Immunohistochemical and immunofluorescence staining of LGR5, ALDH1A1

For IHC and IF analysis, rabbit anti-human LGR5 polyclonal antibody (1:150) was purchased from Epitomics (USA), mouse anti-human LGR5 monoclonal antibody (1:10) was purchased from Mlitenyi Biotec (Germany), rabbit anti-human ALDH1A1 polyclonal antibody (1:100) was purchased from Abcam (UK). Control antibodies were purchased from eBioscience (SanDiego, CA).

Paraffin-embedded samples were cut into sections of 4 m with a Leica microtome (Germany). Individual expression of LGR5 and ALDH1A1 was examined in serial sections of 109 NSCLC tumor samples. IHC was performed on selected slides using the ChemMate Envision/HRP technique (Gene Tech Company Limited, Germany). The sections were deparaffinized in xylene, dehydrated in descending dilutions of ethanol, blocked endogenous peroxidase activity by treated with 3% hydrogen peroxide, antigen-retrieved by autoclaving for 2 min in 0.01 M citrate buffer (pH 6.0), blocked nonspecific staining with 3% bovine serum albumin, incubated with primary antibody followed by testing with a ChemMate Envision Detection kit and visualized with diaminobenzydine. One slide was incubated with an isotypemonoclonal antibody (negative control). Human colorectal cancer tissues were used as positive controls for LGR5 staining and mouse liver tissues were used as positive controls for ALDH1A1 staining. Finally, they were counterstained by hematoxylin.

Immunofluorescence staining was performed for LGR5 and ALDH1A1. Serial sections were deparaffinized and dehydrated as previously mentioned. The slides were simultaneously incubated with rabbit anti-ALDH1A1 polyclonal antibody, mouse anti-LGR5 monoclonal antibody and/or isotype immunoglobulin G (IgG) control for 1 h at room temperature. ALDH1A1 polyclonal antibody was detected with an Alexa Fluor 488 goat anti-rabbit IgG. LGR5 monoclonal antibody was detected using an Alexa Fluor 594 goat anti-mouse IgG. Images were captured by using a Leica DM2500 microscope (Germany).

2.4. Evaluation of immunohistochemical staining

LGR5 and ALDH1A1 expression were determined by two independent pathologists without knowledge of the patients' clinical details. The signal of LGR5 and ALDH1A1 were scored based on both the staining intensity and the percentage of stained cells. LGR5/ALDH1A1 staining intensities were scored as 0, 1, 2 and 3, respectively. We defined the intensity categories as follows: 0 = no appreciable staining (negative); 1 = barely detectable staining in epithelial cells compared with the stromal cells (weak); 2 = readily appreciable staining (moderate); 3 = dark brown staining of cells (strong). The percentage of cells that stained at a specific level was manually evaluated (0–100%). The final LGR5/ALDH1A1 staining score was calculated as the sum of the percentage of stained cells multiplied by the intensity scores, which resulted in an LGR5/ALDH1A1 IHC score that ranged from 0 (no LGR5/ALDH1A1-positive cell in the entire slide) to 300 (all cells showed strong LGR5/ALDH1A1 staining) [19]. And the cut-off value was the median LGR5/ALDH1A1 expression among all NSCLC samples. The ALDH1A1 staining score was classified as negative/weak (score$\leq$100) and moderate/High (score > 100).

2.5. Statistical analysis

Statistical analysis was performed using SPSS 13.0 software (SPSS Inc, Chicago). The relationships between LGR5, ALDH1A1

Table 1
Relationships between LGR5/ALDH1A1 expression and clinicopathological parameters.

Clinicopathological parameters	n	Lgr5 expression			ALDH1A1 expression			Lgr5 and ALDH1A1 expression		
		Positive	Negative	P-value	Positive	Negative	P-value	Positive	Negative	P-value
Total	109	31 (28.4)	78 (71.6)		45 (41.3)	64 (58.7)		24 (22.0)	85 (78.0)	
Age				0.6896			0.7631			0.4058
<65	60	18	42		24	36		15	45	
≥65	49	13	36		21	28		9	40	
Gender				0.0725			0.5633			0.2172
Male	83	20	63		33	50		16	67	
Female	26	11	15		12	14		8	18	
Smoking				0.7397			0.3023			0.9966
Never	50	15	35		18	32		11	39	
Smoker	59	16	43		27	32		13	46	
Tumor differentiation				0.4744			0.5412			0.5673
Well/moderately	69	18	51		30	39		14	55	
Poorly	40	13	27		15	25		10	30	
Tumor subtypes				0.2035			0.5866			0.0507
Adenocarcinoma	48	13	35		17	31		11	37	
Squamous-cell carcinoma	46	15	31		25	21		10	36	
Others	15	3	12		3	12		3	12	
Pathological T factor				0.6484			0.6631			0.2169
Siza <5 cm	56	17	39		22	34		15	41	
Size ≥5 cm	53	14	39		23	30		9	44	
Pathological N factor				0.1489			0.4368			0.0424
N0	47	10	37		17	30		6	41	
N1+N2	62	21	41		27	35		18	44	
Pathological M factor				0.4951			0.8005			0.3351
M0	107	30	77		44	63		23	84	
M1	2	1	1		1	1		1	1	
p-TNM stage				0.0311			0.0221			0.0087
Stage I + II	53	10	43		16	37		6	47	
Stage III + IV	56	21	35		29	27		18	38	

Abbreviations: LGR5, leucine rich repeat containing G protein coupled receptor 5; ALDH1A1, aldehyde dehydrogenase 1A1.

Table 2
The expression of LGR5 and ALDH1A1 in NSCLC.

LGR5 expression	ALDH1A1 expression	
	Negative/low (n, ratios [%])	Moderate/high (n, ratios [%])
Negative (n = 78)	66 (78.6)	12 (48.0)
Positive (n = 31)	18 (21.4)	13 (52.0)

Abbreviations: LGR5, leucine rich repeat containing G protein coupled receptor 5; ALDH1A1, aldehyde dehydrogenase 1A1.

expression and clinicopathological parameters were assessed with Pearson's χ^2 test or χ^2 test as appropriate. Cumulative survival of patients was estimated using the Kaplan–Meier method, and the significance of the survival differences was tested using the log-rank test. Multivariable analysis was performed by employing the Cox proportional hazards regression model to examine the interaction between LGR5 and ALDH1A1 expression and other clinicoathological variables, and estimate the independent prognostic effect of LGR5 and ALDH1A1 on survival by adjusting for

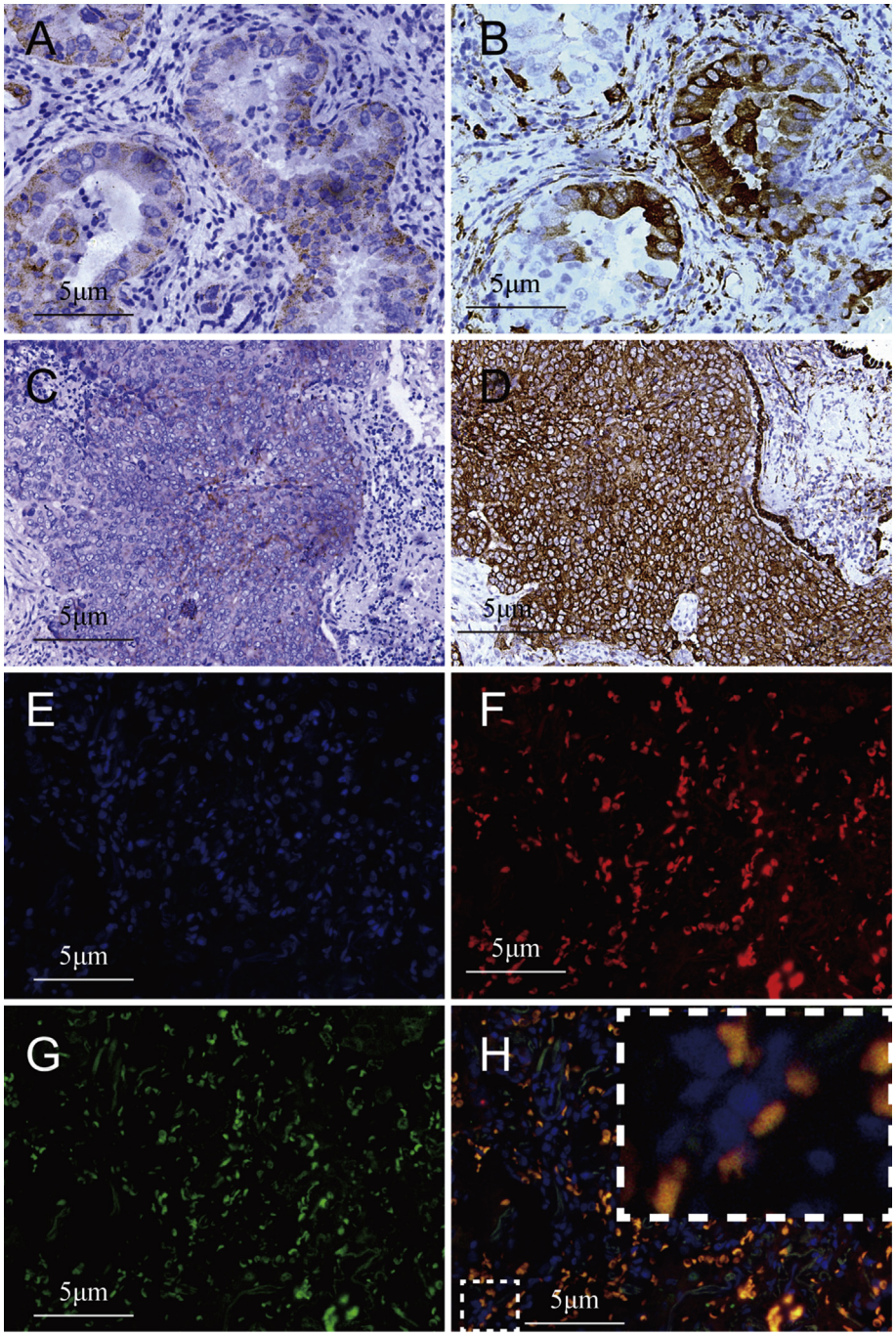

Fig. 3. Serial sections staining and IF double staining in NSCLC. (A–D) serial sections staining in NSCLC. The co-localization of LGR5 (A ×400)and ALDH1A1 (B ×400) in one serial section of NSCLC; The co-localization of LGR5(C ×200)and ALDH1A1 (D ×200) in another serial section of NSCLC. (E–G) IF double staining in NSCLC. NSCLC tissues were stained against DAPI (E blue ×400), LGR5 (F red ×400) and ALDH1A1 (G green × 400). (H) Merged images of E-G (×400). $LGR5^+/ALDHIA1^+$ cells were identified in NSCLC tissues (yellow). Inset shows a high-power view. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

confounding factors. All P values are two-sided, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. LGR5 and ALDH1A1 mRNA were differentially expressed in NSCLC and adjacent normal tissues

To investigate LGR5/ALDH1A1 expression and their relationship on transcriptional level in NSCLC, we evaluated their expression by qRT-PCR in surgical specimens from stage I/II/III NSCLCs. As shown in Fig. 1A, B, LGR5 and ALDH1A1 expression were markedly elevated in most tumor tissues compared with adjacent normal tissues. Of the 24 cases, 19 (79.2%) and 14 (58.3%) showed LGR5 and ALDH1A1 expression level in tumor tissues were more than three times higher than those in the matched adjacent normal tissues, respectively. The overall expression level of LGR5 and ALDH1A1 in NSCLC were significantly higher than that in adjacent normal tissues ($P = 0.0005$ and $P < 0.0001$, respectively) (Fig. 1C, D). Besides, the expression of LGR5 and ALDH1A1 mRNA had a significant correlation($r = 0.416$, $P = 0.0483$) (Fig. 1E).

3.2. Expression patterns of LGR5 and ALDH1A1 in NSCLC

To further study the differential expression of LGR5 and ALDH1A1 on translational level and their clinical implications in NSCLC, we performed an immunohistochemical examination of NSCLC ($n = 109$) and adjacent normal tissues ($n = 50$) (Fig. 2). As shown in Table 1, positive LGR5 staining was observed in 31 (28.44%) of the 109 cases according to the scoring criteria described in materials and methods section. Moreover, positive ALDH1A1 staining was detected in 45 (41.28%) of the 109 cases. We found no LGR5 expression was detected in non-neoplastic bronchial or alveolar epithelial cells and a few cases showed ALDH1A1 staining on normal bronchial epithelium at variable intensity, which was consistent with previous reports [13–18].

We found that higher LGR5 or ALDH1A1 expression levels were associated with a higher stage of the disease (stage III + IV) ($P = 0.0311$ and $P = 0.0221$, respectively). There was no significant association between LGR5/ALDH1A1 expression and age, gender, smoking habits, tumor differentiation, tumor subtypes, tumor size, nodal status and distant metastasis. But, the co-expression of LGR5 and ALDH1A1 was related to nodal status ($P = 0.0424$) (Table 1).

3.3. The relation of LGR5 and ALDH1A1 expression

We examined the ALDH1A1 expression in $LGR5^+$ tumor samples. Of these 31 tumors, 24 cases (77.42%, 24/31) also had ALDH1A1 expressed in the tumor cells. Indeed, data analysis showed that $LGR5^+$ was significantly correlated with ALDH1A1 expression ($r = 0.3883$, $p < 0.0001$). Moreover, expression of LGR5 was increased in $ALDH1A1^{mid/high}$ NSCLC tissues relative to $ALDH1A1^{-/low}$ NSCLC tissues (Table 2). To further determine whether LGR5 and ALDH1A1 were co-localized in tumor tissues, we observed LGR5 and ALDH1A1 expression in serial sections. As shown in Fig. 3A–D, LGR5 was co-localized with ALDH1A1 in some tumor tissues. Besides, we performed IF staining against LGR5 and ALDH1A1, LGR5 was co-localized with ALDH1A1 in tumor tissues (Fig. 3E–H).

3.4. Kaplan–Meier estimate of survival in LGR5-positive/negative and ALDH1A1-positive/negative patients

85 patients with NSCLC were included in the survival analysis. The overall follow-up periods ranged from 1 to 121 weeks (median, 61 weeks). Analysis of Kaplan–Meier survival curves and log-rank tests showed a poor survival rate in the LGR5-positive compared to LGR5-negative group ($P = 0.0125$, Fig. 4A). The prognostic impact of ALDH1A1 expression in NSCLC is the same to LGR5 ($P = 0.0410$, Fig. 4B). Moreover, patients with co-expression of LGR5 and ALDH1A1 had a poorer prognosis ($P = 0.0011$, Fig. 4C).

3.5. Effect of LGR5 and ALDH1A1 expression on survival with univariable and multivariable analyses

A Cox proportional hazards model was applied to estimate the effect of LGR5 and ALDH1A1 expression on survival. The crude hazard ratio (HR) of LGR5-positive compared to LGR5-negative was 2.444 (95% CI, 1.183–5.049; $P = 0.016$). HR of ALDH1A1-positive compared to ALDH1A1-negative was 2.296 (95% CI, 1.113–4.736; $P = 0.024$). HR of $LGR5^+/ALDH1A1^+$ compared to others was 3.293

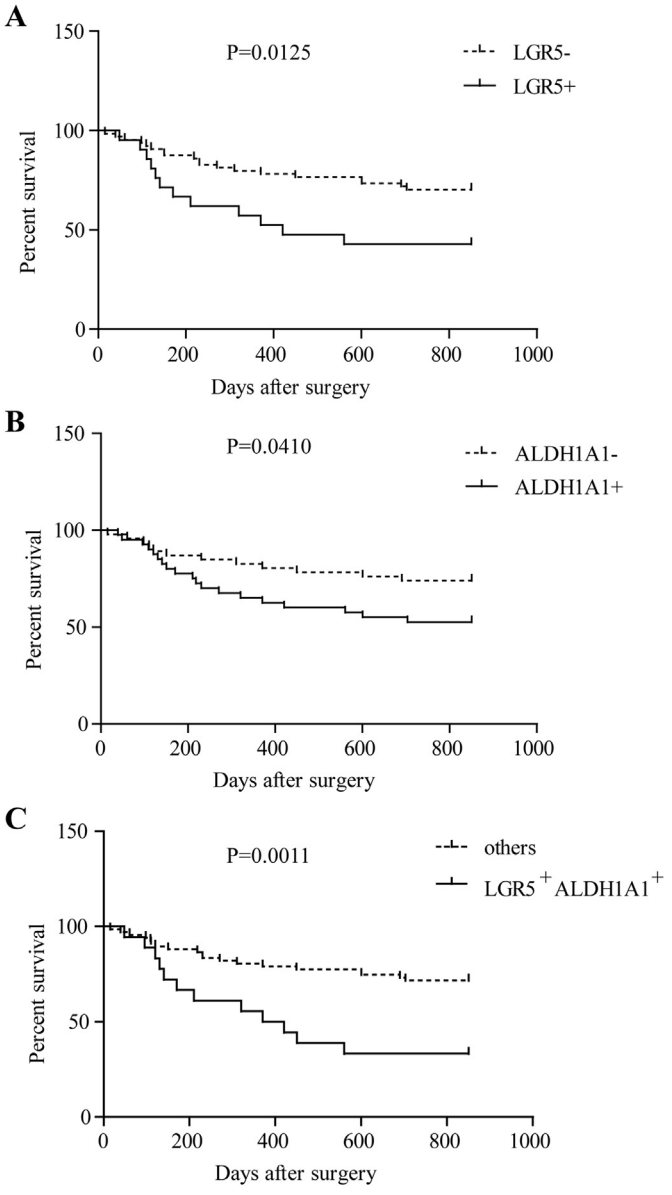

Fig. 4. High expression of LGR5 and ALDH1A1 were significantly associated with poorer survival in patients with NSCLC. Cumulative survival of NSCLC patients classified according to (A) $LGR5^+$and $LGR5^-$; (B) $ALDHIA1^+$ and $ALDHIA1^-$; (C) $LGR5^+/ALDHIA1^+$ and others.

Table 3
Univariable and multivariable analyses of the effect of LGR5 and ALDH1A1 expression on survival.

	n	Univariable analysis			Multivariable analysis		
		HR	95% CI	P-value	HR	95% CI	P-value
LGR5 expression		2.444	1.183,5.049	0.016	2.361	1.106,5.037	0.026
Positive vs. negative	21/64						
ALDH1A1 expression		2.296	1.113,4.736	0.024	2.306	1.101,4.830	0.027
Positive vs. negative	38/47						
LGR5/ALDH1A1 expression		3.293	1.251,10.902	0.018	2.918	1.076,10.353	0.037
LGR5+/ALDH1A1+ vs. others	18/67						
Age		0.772	0.375,1.591	0.484	0.838	0.401,1.751	0.639
≥65 vs. <65	38/47						
Gender		0.818	0.385,1.737	0.601	0.493	0.133,1.832	0.291
Male vs. female	61/24						
Smoking habits		1.184	0.580,2.417	0.643	1.946	0.568,6.663	0.289
Smoker vs. never smoker	46/39						
Tumor subtypes							
Large cell carcinoma		1 (ref)			1 (ref)		
Adenocarcinoma		0.728	0.342,1.550	0.410	0.658	0.286,1.515	0.325
Squamous carcinoma	8/43/34	0.795	0.795,0.368	0.558	0.758	0.332,1.729	0.510
Tumor differentiation			0.063,3.375	0.445	0.38	0.049,2.947	0.355
Moderately/poorly vs. well	80/5						
Tumor size		2.334	1.117,4.877	0.023	2.235	1.066,4.686	0.033
Siza <5 cm VS.size ≥ 5 cm	44/41						
Lymphatic invasion		6.01	2.099,17.207	0.001	6.081	2.117,17.467	0.001
no vs. n1 + n2	34/51						
p-TNM stage		4.66	1.908,11.381	0.001	4.437	1.798, 10.945	0.001
Stage I/II vs. stage III/IV	38/47						

Abbreviations: LGR5, leucine rich repeat containing G protein coupled receptor 5; ALDH1A1, aldehyde dehydrogenase 1A1.

(95% CI, 1.251–10.902; P = 0.018). Multivariable analysis was performed by employing the Cox proportional hazards regression model to examine the interaction between LGR5 and ALDH1A1 expression and other clinicoathological variables, and to estimate the independent prognostic effect of LGR5 and ALDH1A1 on survival by adjusting for confounding factors. After controlling for the effects of clinicopathological factors including age, gender, smoking habits, the adjusted HR of LGR5-positive group became 2.361 (95% CI, 1.106–5.037; P = 0.026) in comparison with LGR5-negative group and the adjusted HR of ALDH1A1-positive group became 2.306 (95% CI, 1.101–4.830; P = 0.027) in comparison with ALDH1A1-negative group, suggesting that LGR5 or ALDH1A1 expression is an independent risk factor of a poorer survival after controlling for clinicopathological factors (Table 3).

4. Discussion

In worldwide, the morbidity and mortality of lung cancer account for the first place in men and the second place in women [20]. Although with the progress of science and technology, overall treatment effect is still not satisfactory [21], so the research for effective diagnostic and therapeutic target of NSCLC has important clinical significance. In this study, we sought to evaluate the relationship between CSC-related proteins LGR5 and ALDH1A1, and their expression with the progression and prognosis in NSCLC. Our results supported the conclusion that the expression of LGR5 and ALDH1A1 were significantly correlated in NSCLC, and were associated with the progression of NSCLC and indicated poor prognosis. To the best of our knowledge, this is the first study supporting the relationship and prognostic value of these two proteins in NSCLC.

Our results indicated that co-expression of LGR5 and ALDH1A1 in NSCLC may predict greater possibility of lymph node metastasis, and NSCLC patients with dual overexpression of LGR5 and ALDH1A1 had a poorer prognosis than the others. Besides, since ALDH1A1 is a marker for lung cancer cells with stem cell-like properties and LGR5 expression was significantly correlated with ALDH1A1 expression, we speculated $LGR5^+$ cells in NSCLC were likely to be the cancer cells with stem cell-like properties. Whether LGR5 is the CSCs marker of NSCLC need further investigation.

In Shinichiro Ryuge's study, LGR5 was found to express in a subset of lung adenocarcinoma and was suggested to have a great correlation with a larger tumor size (>5 cm) (P = 0.033), higher pathological TNM stage of the disease (stage II and III) (P = 0.025), and poorer prognosis (P = 0.026) [13]. Not the same with this study, our study found that LGR5 was not only expressed in lung adenocarcinoma, but also expressed in squamous-cell carcinoma and large cell carcinoma, and we did not find the relationship between LGR5 expression with tumor size. We thought the discrepancies observed between the two studies due to the different sample sources and different clones of antibody.

Taken the above results together, we speculated that the expression of LGR5 and ALDH1A1 were closely associated with the tumorigenic, metastasis and poor prognosis of NSCLC. Since ALDH1A1 was aberrantly expressed in $LGR5^+$ NSCLC cells, LGR5 maybe a novel marker of NSCLC stem cells. Elucidation of the biological functions of LGR5 and ALDH1A1 may provide a better understanding of NSCLC and may also lead to the development of novel therapeutic strategies against NSCLC.

Conflict of interest statement

The authors have no conflict of interest.

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Transparency document

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